The diagnosis and management of biliary strictures

Thesis submitted by

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Declaration

I, George Goodchild, confirm that the research included within this thesis is my own work or that where it has been carried out in collaboration with, or supported by others, that this is duly acknowledged below, and my contribution indicated. Previously published material is also acknowledged below. I attest that I have exercised reasonable care to ensure that the work is original and does not to the best of my knowledge break any UK law, infringe any third party's copyright or other Intellectual Property Right, or contain any confidential material. I accept that the College has the right to use plagiarism detection software to check the electronic version of the thesis. I confirm that this thesis has not been previously submitted for the award of a degree by this or any other University.

Signature: George Goodchild, June 18th 2023

Acknowledgements and contributions

I want to acknowledge and give my warmest thanks to my supervisors Professor Pereira and the late Dr John Timms who made this work possible. I am also grateful to all those that I had the pleasure to work alongside including Dr Pauline Stassen who worked tirelessly as my co-investigators for chapter 3, Dr Alex Ney who stepped in to help me with my statistical analysis in chapter 4 after the unexpected passing of my fantastic and very patient lab supervisor Dr John Timms, Dr George Webster who was crucial in the design of our cholangioscopy study, Dr Nuno Nene who assisted me with cross-validation analysis and preparation of figures for chapter 4 and all of the co-authors that I worked alongside to publish as a result of work contained within this thesis.

Abstract:

Biliary strictures are considered indeterminate when the aetiology remains elusive despite standard diagnostic work-up. Misdiagnosis can have a dramatic impact on patient outcomes. Cholangiocarcinoma and IgG4 related disease (IgG4-RD) are two diseases which often mimic each other. Distinction can be challenging but is vital as treatment approaches and prognosis are very different. We describe the experience of the first inter-regional specialist IgG4-RD multidisciplinary team meeting (MDM) and demonstrate that the MDM improved awareness of a rare disease and enabled important diagnostic and management decisions as well as serving as a platform for patients to access third line treatment with Rituximab.

As part of the evaluation of indeterminate biliary strictures, digital single-operator cholangioscopy (d-SOC) with cholangioscopic biopsies is often used to provide both a visual assessment of the stricture and to obtain targeted biopsies. Some studies suggest a higher sensitivity for visual impression compared to histology, although in these studies endoscopists were unblinded to previous investigations. We investigated the diagnostic accuracy and inter-observer agreement (IOA) of d-SOC in the visual assessment of biliary strictures when blinded to background information. An international, multicentre, cohort study was performed. Cholangioscopic videos in patients with a known diagnosis were systematically scored. Anonymised videos were reviewed by 19 experts in 2 steps: 1: blinded for patients' history and investigations and 2: unblinded. Forty-four videos of 19 benign and 25 malignant cases were reviewed. The sensitivity and specificity for the diagnosis of malignancy was 74.2% and 46.9% (blinded) and 72.7% and 62.5% (unblinded). Cholangioscopic certainty of a malignant diagnosis led to over-diagnosis (sensitivity 90.6%, specificity 33%), especially if no background clinical information was provided. The IOA for the presence of malignancy was fair for both assessments (Fleiss' kappa (κ) 0.245 (blinded) and κ 0.321 (unblinded)). For individual visual features, the IOA ranged from slight to moderate for both assessments (κ 0.059 – 0.400 versus κ 0.031 – 0.452). Our study shows low sensitivity

and specificity for blinded and unblinded d-SOC video appraisal of indeterminate biliary strictures, with significant inter-observer variation. Whilst reaching consensus on the optical features of biliary strictures remains important, optimising visually-directed biopsies may be the most important role of cholangioscopy in biliary stricture assessment.

Whilst cholangioscopy and visually targeted biopsies remain an important tool in the assessment of patients with possible cholangiocarcinoma, this invasive test has considerable associated costs and carries some risks to the patient. Several non-invasive tests are currently available for clinical use, such as serum CA19-9 and CEA However, these lack sufficient sensitivity and specificity for standalone use. There is an urgent unmet need for further reliable non-invasive markers for the diagnosis of cholangiocarcinoma (CCA). Cytokeratin fragment 21-1 (CYFRA) and Mucin 5AC (MUC5AC) have reported utility for differentiating CCA from benign disease. Herein, serum levels of these markers were tested in 81 malignant cases verses 89 healthy controls regardless of time to diagnosis. Neither marker was significantly elevated in malignant cases versus benign disease (median 2.25ng/ml versus 1.95ng/ml p=0.89). Next, to assess the potential utility of both markers for early diagnosis of biliary tract cancer, levels were performed on a set of 89 pre-diagnosis serum samples taken from 55 cases of cancer and 91 matched benign controls obtained from the UKCTOCS biobank. The median time from sample collection to diagnosis was 31.5 months. Serum levels of CYFRA21-1 and MUC5AC in CCA samples, as measured by ELISA, were comparable to cancer free controls at 0-1 years, 1-2 years, 2-3 years and >3 years prior to diagnosis. When stratifying by time to diagnosis, both MUC5AC and CYFRA remained unchanged across all time groups. When combined into panels with previously described biomarkers, a panel combining CA19-9, ALP and CYFRA21-1 reached an AUC (cross validated) of 0.826 (95% CI 0.641-0.962) and sensitivity of 0.778 (95% CI 0.444-1) for the diagnosis of CCA.

Impact statement

The insight, analysis and discovery within this thesis will prove beneficial both within the clinical and research spheres surrounding the diagnosis and management of indeterminate biliary strictures. Chapter 1 gives an overview of the challenges and complexities around diagnosing and managing these patients and offers insight to clinicians and endoscopists alike. Its publication in a UK peer-reviewed journal aimed specifically at doctors managing such patients should mean that it leads to tangible improvements in practice. Chapter 2 gives a broad overview of a complex disease which is poorly understood by many clinicians. It also highlights the usefulness of referring these patients to a specialist multidisciplinary meeting (MDM) for their management. We published this in a general medicine journal so that it reached as many specialists as possible, therefore having maximum impact. In the article we also gave contact details for the MDM to ensure that these complex patients would benefit from its publication almost straight away. Indeed, following our IgG4 symposium we saw an increase in referrals to the MDM. Chapter 3 is a unique study which answered the important question of whether benign versus malignant biliary strictures could be reliably differentiated by visual impression alone. Given the importance of accurate diagnosis in these patients and the increasing use of cholangioscopy we felt that this was an important question to answer. Our results were published in a highly respected, international journal with a far-reaching audience and therefore will have maximum impact. Finally, in chapter 4 I demonstrated that CYFRA and MUC5AC are not useful serum biomarkers in the differentiation of PSC from CCA. Although this was a negative study, it is another piece of the puzzle in the search for reliable non-invasive biomarkers for the diagnosis of CCA. A challenge now is to publish these results in some form so that they are available to the wider scientific community.

List of related publications

Experience from the first UK inter-regional specialist multidisciplinary meeting in the diagnosis and management of IgG4-related disease

George Goodchild, Rory JR Peters, Tamsin N Cargill, Harry Martin, Adetokunbo Fadipe, Maria Leandro, Adam Bailey, Jane Collier, Louisa Firmin, Manil Chouhan, Manuel Rodriguez-Justo, Ross Sadler, Roger W Chapman, Helen Bungay, Eve Fryer, Joel David, Raashid Luqmani, Eleanor Barnes, George J Webster, Emma L Culver

Clin Med (Lond) 2020 May; 20(3): e32–e39. doi: 10.7861/clinmed.2019-0457

Immunoglobulin G4-related sclerosing cholangitis

George Goodchild, Stephen P. Pereira, George Webster

Korean J Intern Med. 2018 Sep; 33(5): 841–850. Published online 2018 Jul 27. doi: 10.3904/kjim.2018.018

Diagnostic accuracy and interobserver agreement of digital single-operator cholangioscopy for indeterminate biliary strictures

Pauline M.C. Stassen, MD, George Goodchild, BsC, MRCP, Pieter Jan F. de Jonge, MD, PhD

Marco J. Bruno, MD, PhD, George J.M. Webster, BSc, MD, FRCP

on behalf of theEuropean Cholangioscopy Group

Published:June 30, 2021DOI:https://doi.org/10.1016/j.gie.2021.06.027

Molecular Pathogenesis of Cholangiocarcinoma

Peter L. Labib, George Goodchild, Stephen P. Pereira

BMC Cancer. 2019; 19: 185. Published online 2019 Feb 28. doi: 10.1186/s12885-019-5391-

Biliary Strictures and Cholangiocarcinoma – Untangling a Diagnostic Conundrum

Alexander Ney, Andres Garcia-Sampedro, George Goodchild, Pilar Acedo, Giuseppe Fusai, Stephen P. Pereira

Front Oncol. 2021; 11: 699401. Published online 2021 Sep 30. doi: 10.3389/fonc.2021.699401

Clinical Manifestations and Long-term Outcomes of IgG4-Related Kidney and Retroperitoneal Involvement in a United Kingdom IgG4-Related Disease Cohort

Rhys D.R. Evans, Tamsin Cargill, George Goodchild, Ben Oliveira, Manuel Rodriguez-Justo, Ruth Pepper, John Connolly, Alan Salama, George Webster, Eleanor Barnes, Emma L. Culver

Kidney Int Rep. 2019 Jan; 4(1): 48–58. Published online 2018 Sep 1. doi: 10.1016/j.ekir.2018.08.011

List of acronyms

A1AG	Alpha-1-acid glycoprotein
AIDS	Acquired Immunodeficiency Syndrome
AIP	Autoimmune pancreatitis
AUC	Area under curve
BBS	Benign biliary stricture
BSEP	Bile salt export pump
C3/C4	Complement 3/4
CA19-9	Carbohydrate antigen 19-9
CCA	Cholangiocarcinoma
CD	Cluster of differentiation
CEA	Carcinoembryonic antigen
CI	Confidence interval
CT	Computed Tomography
CTC	Circulating tumour cell
CYFRA 21-1	Cytokeratin fragment 21-1
DAPI	4', 6'-diamidino-2-phenylindole
DNA	Deoxyribonucleic acid
dSOC	Digital single operator cholangioscopy
ECG	European Cholangioscopy Group
eCCA	Extrahepatic cholangiocarcinoma
EPCAM	Epithelial cell adhesion molecule
ERC	Endoscopic retrograde cholangiogram
ERCP	Endoscopic retrograde cholangiopancreatogram
ERCP-BD	Endoscopic retrograde cholangiopancreatogram-guided biliary drainage
ESGE	European Society of Gastrointestinal Endoscopy

ES	Endoscopic sphincterotomy
EUS	Endoscopic ultrasound
EUS-BD	Endoscopic ultrasound guided biliary drainage
EV	Extracellular vesicle
Fc-Feutin-A	Fucosylated Feutin-A
FCN2	Ficolin-2
fcSEMS	Fully covered self-expanding metal stent
FDG	Fluorodeoxyglucose
FIB	Fibrinogen binding protein
FISH	Fluorescent in-situ hybridisation
FNA	Fine-needle aspiration
FNB	Fine-needle biopsy
FPLD	Fibrocystic liver disease
GGT	Gamma-glutamyl transferase
GP	Glycophosophate
HCC	Hepatocellular carcinoma
HISORt	Histology, Imaging, Serology, Other, Response to therapy
HIV	Human immunodeficiency virus
HLA	Human leucocyte antigen
HPB	Hepatopancreatobiliary
HR	Hazard ratio
IBD	Inflammatory bowel disease
IDUS	Intraductal ultrasound
Ig	Immunoglobulin
IGF	Insulin-like growth factor
IgG4-RD	Immunoglobulin G4 related disease

IgG4-RP	Immunoglobulin G4 retroperitoneal fibrosis
IgG4-SC	Immunoglobulin G4 sclerosing cholangitis
IL	Interleukin
IOA	Interobserver agreement
iCCA	Intrahepatic cholangiocarcinoma
IPNB	Intra-papillary neoplasm of bile duct
ITIH4	Inter-alpha-trypsin inhibitor heavy chain 4
IQR	Interquartile range
kg	Kilogram
LAMP	Lysosomal-associated membrane protein 1
LRG1	Leucine-rich α2-glycoprotein
LSS	Light scattering spectroscopy
mg	Milligram
miRNA	MicroRNA
mo	Month
NBI	Narrow band imaging
MDM	Multidisciplinary meeting
MMP	Matrix metalloproteinase
MMR	Mismatch repair
MPD	Main pancreatic duct
MRI	Magnetic Resonance Imaging
MUC5AC	Mucin-5AC
NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis
NHS	National Health Service
OR	Odds ratio

OUH	Oxford University Hospital
pCCA	Perihilar cholangiocarcinoma
pCLE	Probe-based confocal laser endomicroscopy
PDT	Photodynamic therapy
PET	Positron emission tomography
PKM2	Pyruvate kinase muscle isozyme M2
PSC	Primary sclerosing cholangitis
PTD	Percutaneous transhepatic drain
RCT	Randomised controlled trial
RFA	Radiofrequency ablation
ROC	Receiver operator curve
RTX	Rituximab
RYGB	Roux-en-Y gastric bypass
SEER	Surveillance, Epidemiology and End Results
SRMA	Systematic Review Meta-Analysis
TB	Tuberculosis
Tfh	T follicular helper
TNFa	Tumour necrosis factor alpha
UCLH	University College London Hospital
ucSEMS	Uncovered self-expanding metal stent
UKCTOS	United Kingdom Collaborative Trial of Ovarian Cancer Screening
ULN	Upper limit of normal
WLI	White light endoscopy

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1. Introduction to biliary strictures: Background, aetiology and approach to diagnosis and management

The accurate diagnosis of biliary strictures based on imaging alone is frequently challenging, as there are a wide range of benign and malignant aetiologies(1) (Table 1). Due to different management algorithms, these conditions need to be diagnosed swiftly and accurately to guide appropriate therapy and optimise outcomes for patients(2). Because of the diagnostic difficulties sometimes encountered, definitive treatment such as surgery or chemotherapy may be delayed or given incorrectly, with potential consequences for patients. In one study of 238 patients undergoing surgical resection of suspected cholangiocarcinoma (CCA), 13% were found to have benign disease(3). Many of these patients have an autoimmune cholangiopathy (including IgG4-related disease, IgG4-RD) which can be effectively treated with steroids and other immunosuppressives. Pathologic confirmation of malignancy can be challenging and may require endoscopic techniques such as endoscopic ultrasound (EUS) and digital single operator cholangioscopy (dSOC). In those with unresectable malignant biliary obstruction, effective biliary decompression improves symptoms and enables patients to undergo palliative therapies, while in surgical candidates, routine preoperative biliary intervention may worsen outcomes(4). In the next part of this chapter, I expand on two causes of biliary strictures which both pose significant challenges in their diagnosis and management: CCA and IgG4 related sclerosing cholangitis (IgG4-SC).

	Condition
Malignant	Cholangiocarcinoma Pancreatic cancer Metastatic disease
Benign	Post-operative (following laparoscopic cholecystectomy or biliary anastomosis) Chronic pancreatitis Primary sclerosing cholangitis Autoimmune cholangiopathy, IgG4-related disease Post-radiation therapy Infections (TB, histoplasmosis, viral, parasitic, HIV cholangiopathy) Choledocholithiasis / Mirizzi syndrome Vasculitis Trauma Ischaemia Sphincter of Oddi dysfunction Post biliary sphincterotomy Extraluminal compression (lymph nodes, vascular)

Table 1. Differential diagnoses of biliary strictures

1.1 Cholangiocarcinoma: Epidemiology and overview

CCA is an aggressive cancer which originates from the biliary epithelium and can be classified as either intrahepatic (iCCA), perihilar (pCCA) or extrahepatic (eCCA), depending on the location within the biliary tree(5) (Figure 1). It accounts for 15% of all primary liver tumours and 3% of all gastrointestinal cancers(6). All subtypes of CCA have a poor prognosis, with 5-year survival rates below 20%(6). CCA can also be classified according to the tumour growth pattern (mass-forming, intraductal or periductal infiltrating) as well as the cell of origin (cholangiocytes, peribiliary glands, hepatic progenitor cells or hepatocytes). Although less commonly used in clinical practice, these alternative methods of classification may better predict tumour behaviour(7).

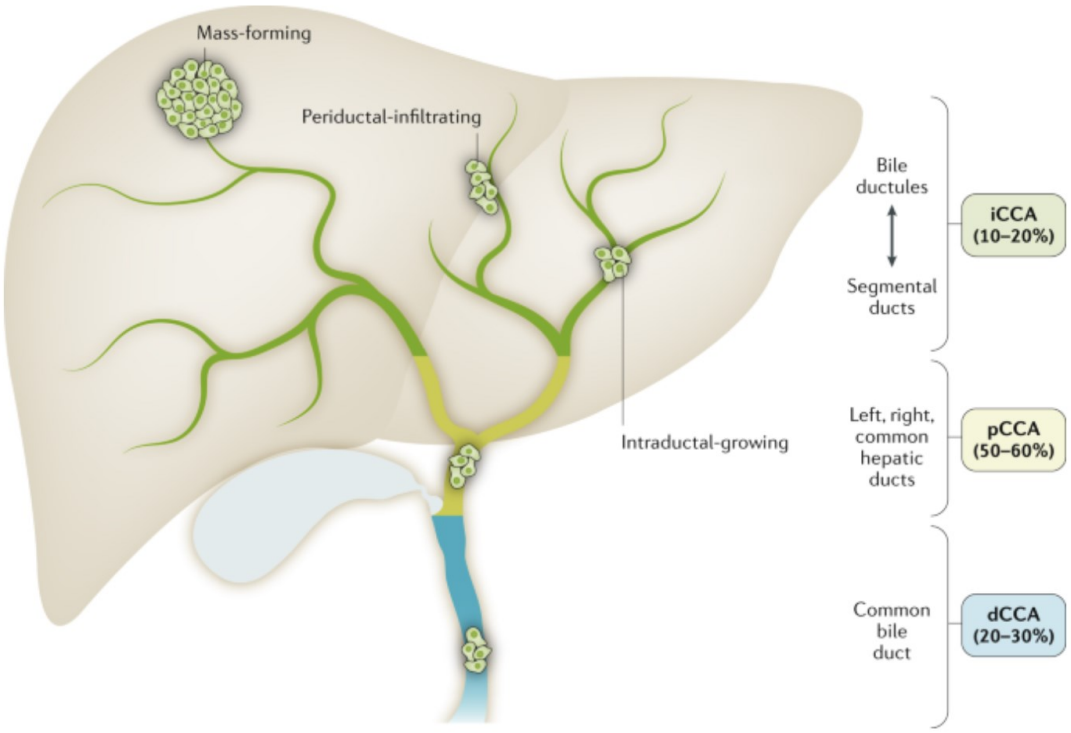


Figure 1. Anatomic classification of cholangiocarcinoma. (With permission, Nature Reviews Gastroenterology & Hepatology (*Nat Rev Gastroenterol Hepatol*)(6)

The overall global incidence of CCA is increasing. This is driven by an increase in the incidence of iCCA, whilst rates of pCCA and eCCA may have fallen slightly(8). Incidence rates show huge variation between countries, reflecting both geographical variations in risk factors as well as differing genetic susceptibility. The UK incidence rate was 4.3 per 100,000 in 2017, rising from 1.7 per 100,000 in 2002(9). However, in Thailand, where liver fluke infection is endemic, the incidence is 33 per 100,000 in men and 12 per 100,000 in women(10). Historical under-reporting of CCA and geographical variation in reporting means that incidence rates should be interpreted with caution and may be significantly higher than quoted(11). Figure 2 shows global variations in overall mortality from CCA. In the Western world, CCA is a rare disease in the general population,

but it is relatively common in those with primary sclerosing cholangitis (PSC); a chronic cholestatic liver disease which is characterised by progressive inflammation, fibrosis and stricturing of the biliary tree. Between 7-20% of patients with PSC will develop CCA, the majority of these in the first 5 years following their diagnosis(12). Resection, by surgical resection or liver transplantation is the only curative treatment. However, most cases of CCA are already advanced at the time of diagnosis meaning that treatment options are limited to palliative interventions. When palliative treatments, such as chemotherapy, are being considered, obtaining a histological diagnosis is paramount. However, because CCA often consists of small clusters of malignant cells surrounded by a desmoplastic stroma of cancer-associated fibroblasts, extracellular matrix and immune cells, confidentially making a pathological diagnosis can be challenging(13). Current diagnostic modalities include clinical, biochemical, radiological, and histological techniques – all of which have either low sensitivity or specificity, made worse in the presence of chronic stricturing and cholestasis associated with PSC(14). Most cases of CCA in the Western world are sporadic in nature however there are several known risk factors. It is postulated that these risk factors lead to the development of cancer through a well described pathway of cholestasis and chronic inflammation leading to cell proliferation, genetic and epigenetic mutations and subsequently carcinogenesis.

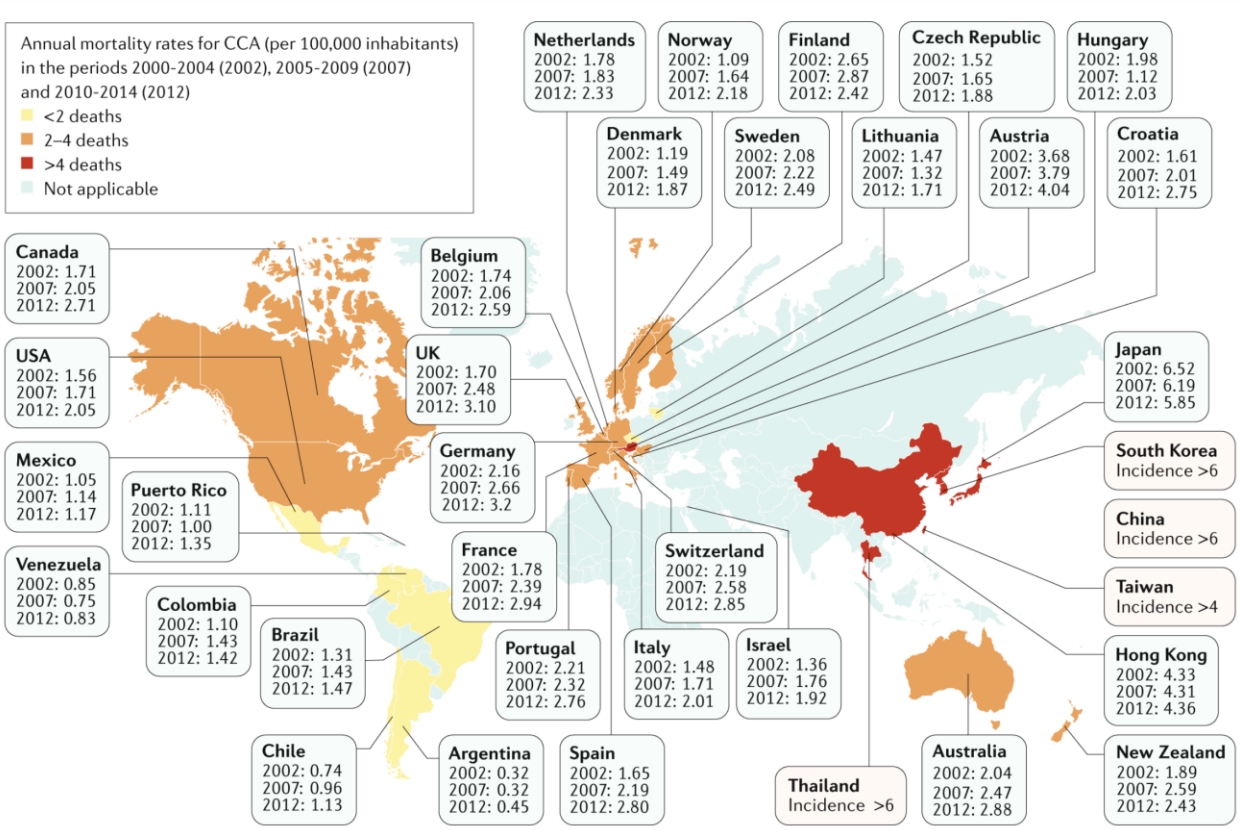


Figure 2: Mortality of cholangiocarcinoma worldwide. Global age-standardized annual mortality rates for cholangiocarcinoma (CCA) (deaths per 100,000 inhabitants, including intrahepatic CCA, perihilar CCA and distal CCA)(15). Data refer to the periods 2000–2004 (2002), 2005–2009 (2007) and 2010–2014 (2012). Yellow indicates countries/regions with low mortality (<2 deaths per 100,000 people), orange indicates countries/regions with mortality between 2 and 4 deaths per 100,000 people, and red indicates countries/regions with high mortality (>4 deaths per 100,000 people). Mortality in eastern countries/regions in which CCA is highly prevalent (that is, Thailand, China, Taiwan and South Korea) have not yet been reported and, therefore, CCA incidence is shown for these countries(16). (Source: Nature Reviews Gastroenterology & Hepatology (*Nat Rev Gastroenterol Hepatol*)(6).

1.2 Risk factors for cholangiocarcinoma

1.21 Cholestatic liver diseases

PSC is a chronic fibro-inflammatory disease leading to progressive stricturing of both the intra- and extrahepatic biliary tree. PSC is strongly associated with inflammatory bowel disease; up to 80% of patients with PSC have a diagnosis of ulcerative colitis (UC)(17). It is hypothesised that chronic cholestasis leads to overexposure of cholangiocytes to bile acids that cause uncontrolled cholangiocyte proliferation and eventual cholangiocarcinogenesis(18). As PSC causes longstanding cholestasis, often for many months before diagnosis, the prolonged exposure of cholangiocytes to bile is likely to be a significant factor in cholangiocarcinogenesis in this disease. Other chronic cholestatic conditions associated with the development of CCA include congenital hepatic fibrosis, choledochal cysts, biliary hamartomas and Caroli disease (collectively known as 'fibropolycystic liver disease, FPLD)(19). They arise because of abnormal development of the embryonic ductal plate that form the intrahepatic bile ducts and cholangiocytes. FPLD collectively confer a 15% lifetime risk of developing cholangiocarcinoma, although the incidence varies depending on the exact diagnosis; lifetime risk in patients with choledochal cysts is 15-20% whereas the development of CCA due to the presence of a biliary microhamartoma is very rare(20).

1.22 Liver cirrhosis

Liver cirrhosis results from progressive hepatic fibrosis and hepatocyte regeneration. The development of regenerating nodules and associated disordered angiogenesis leads to the development of portal hypertension. The most common aetiologies of liver cirrhosis include alcohol, non-alcoholic steatohepatitis (NASH), viral and autoimmune hepatitis(21). Irrespective of aetiology, liver cirrhosis has been associated with the development of iCCA. This may result from the formation of a similar microenvironment as that seen in PSC (cholestasis, chronic

inflammation, increased cell turnover and fibrosis)(22). Other studies have demonstrated an increased risk of both iCCA and eCCA in the presence of liver cirrhosis(23). The latter may be explained by dysregulated bile acid excretion into the common bile duct leading to a proinflammatory microenvironment within the extrahepatic biliary tree(24).

1.23 Gallstone disease

The prevalence of gallstone disease in the Western world is up to 20% and is the most common gastrointestinal-related reason for hospitalisation in Europe(25). Gallstones are most often formed of cholesterol or bile pigments. They usually form in the gallbladder (cholelithiasis) but can also form within the extrahepatic biliary tree (choledocholithiasis) or intrahepatic biliary tree (hepatolithiasis). Intraductal stone formation is more common in the setting of cholestatic liver disease, such as PSC. Gallstones are associated with an increased risk of both iCCA and eCCA(26). Several studies have suggested a reduced risk of CCA following cholecystectomy, an operation usually performed for gallstone disease. One recent retrospective study looked at 2096 patients with choledocholithiasis and no history of CCA or biliary intervention. A total of 12 (2.35%), 11 (0.74%), and 1 (1.00%) subsequent cholangiocarcinoma cases were diagnosed among 511 ES/endoscopic papillary balloon dilatation patients, 1485 patients with no intervention, and 100 ES/endoscopic papillary balloon dilatation and cholecystectomy patients, respectively. The incidence rates of recurrent biliary events were 527.79/1000 person-years and 286.69/1000 person-years in the subsequent cholangiocarcinoma and no cholangiocarcinoma group, showing a high correlation between subsequent cholangiocarcinoma risk and recurrent biliary events(27). The authors concluded that choledocholithiasis patients who undergo further cholecystectomy after ES/endoscopic papillary balloon dilatation have decreased subsequent cholangiocarcinoma risk due to reduced recurrent biliary events. Another recent study of 959,000 patients with gallstone disease confirmed that cholecystectomy reduced the risk of subsequent CCA by 30%, but only in those with complicated gallstone disease(28). The relationship between gallstone

disease and CCA remains incompletely understood. It is possible that the increased risk of CCA is secondary to gallstone disease itself rather than cholecystectomy per se. However, another explanation could be explained by the observed change in bile composition seen after cholecystectomy whereby there is a reduction in the circulating pool of primary bile salts but a preserved pool of deoxycholic acid, both of which are associated with increased cholangiocyte turnover and therefore potentially tumorigenesis. Hepatolithiasis is most often seen in the setting of Caroli disease and liver fluke infection, both of which are most common in parts of Asia. Hepatolithiasis is an established risk factor for CCA, and biliary carcinogenesis may involve chronic proliferative inflammation and the upregulation of cell-proliferating factors(29).

1.24 Chronic infections

Parasites are significant groups for carcinogenesis among which liver flukes are important pathogens in the development of CCA. Liver fluke infection is endemic in parts of East and Southeast Asia, including Thailand, China, Laos, Cambodia and Vietnam. Species including *Clonorchis sinensis* and *Opisthorchis viverrini* are associated with the development of CCA(30). Liver fluke infection is usually acquired by eating raw or undercooked freshwater fish (31). Liver fluke infection leads to CCA through a combination of physical damage to biliary epithelium from suckers, entrapment of eggs into small ulcers leading to granulomatous inflammation and subsequent cholestasis leading to DNA damage and eventual carcinogenesis(30).

Salmonella Typhi and Salmonella Paratyphi A are the pathogens of enteric (typhoid) fever; both can establish chronic carriage in the gallbladder(32). Chronic typhoid carriers carry a six-fold increase for cholangiocarcinoma(33). A retrospective analysis of 440 cases of hilar cholangiocarcinoma from a single centre in Egypt (1995-2004) found 52% of patients had a history of typhoid infection, although 54% of patients were also hepatitis C positive, another significant risk factor that could account for part of the increased risk observed(34).

Chronic infection with Hepatitis B and C viruses accounts for 38% of prevalent chronic liver disease globally(35). Several meta-analyses show an increased risk of ICC in both hepatitis B and hepatitis C infection(36,37). The association with hepatitis C is stronger in regions where hepatitis C is endemic, and likewise for hepatitis B. The largest meta-analysis (13 case-control studies and three cohort studies, n=202,135 and n=2,655,902 respectively) found hepatitis B to have an OR of 3.17 (95% CI 1.99-5.34) and hepatitis C an OR of 3.42 (95% CI 1.96-5.99)(38).

1.25 Inflammatory bowel disease

Inflammatory bowel disease (IBD) is a chronic inflammatory disorder, primarily involving, but not restricted to, the gastrointestinal tract. The association between IBD and PSC (and therefore CCA) is well established. CCA occurs at a younger age in IBD patients than in the general population (56 years vs 71 years, respectively). In the Western world, CCA occurring in patients < 40 years old is almost always associated with IBD(39,40). PSC-associated CCA in the presence of IBD appears to follow the dysplasia-carcinoma sequence. As well as arising from the established pathway of cholestasis, inflammation and altered DNA repair functions, malignancy associated with IBD may also occur in part because of the effects of immunosuppressive medications taken for IBD(41).

1.26 Toxins

Several studies have suggested that tobacco smoking and alcohol consumption may increase the risk of iCCA and eCCA, although the data is somewhat conflicted owing to the data coming from multiple study designs including population-based, cohort and case-control studies. A recent meta-analysis of 14 cohort studies (n=1,515,741 with 410 cases of iCCA) found heavy alcohol consumption (≥5 drinks/day) conferred a hazard ratio of 1.68, although the 95%CI was 0.99-2.86(42). In contrast, a meta-analysis in 2012 of 11 case-control studies (n=3374 iCCA, 394,774

controls) found heavy alcohol consumption (>80g/day or alcoholic liver disease) to confer an OR of 2.81 (95% CI = 1.52-5.21)(43). This discrepancy may be explained by the different designs of the included studies; alcohol consumption has been proven to be more strongly associated with hepatic malignancy in case-control studies(44). Whilst cohort studies tend to ask participants about recent alcohol consumption, case-control studies usually estimate lifetime alcohol intake(42). Although a meta-analysis in 2013 (six case-control studies, one cohort study) found no difference in cholangiocarcinoma risk between drinkers and non-drinkers (OR 1.09, 95% CI 0.87-1.37), the recent SEER analysis by Petrick et al. found patients with alcohol-related disorders to have an increased risk of cholangiocarcinoma (OR 2.60, 95% CI 2.23-3.04)(23,45). Smoking also increases the risk of both iCCA (OR 1.46, 95% CI 1.28-1.66) and eCCA (OR 1.77, 95% CI 1.59-1.96)(45). It has been suggested that carcinogenic tobacco components lead to changes in the biliary epithelium though direct exposure via the circulatory system.

Thorotrast (®) (thorium oxide) was a medical contrast agent widely in angiography and arteriography used up until 1950s(46). This compound conferred a 300-fold increased risk CCA development, with a latency period of up to 45 years after exposure(47). Although the mechanism has not been fully elucidated, it is known that Thorotrast is taken up into the reticuloendothelial system and contains an emitter of α-radiation(48). Combined with its exceptionally long half-life of 400 years, it is likely that chronic exposure to α-radiation lead to direct DNA damage and carcinogenesis.

1.27 Metabolic conditions

Diabetes mellitus is a risk factor for cancer of many organs and has been associated with both iCCA and eCCA. A meta-analysis in 2015 (15 case-control studies and 5 cohort studies, 10,362 patients with cholangiocarcinoma and 351,908 controls) found a combined OR of 1.74 (95% Confidence Interval (CI): 1.62–1.87)(49). The recent meta-analysis by Petrick et al. analysed the

risk of Type I and Type II diabetes separately and found raised ORs for both iCCA and eCCA (Type I diabetes OR 1.43 for iCCA and 1.30 for eCCA, Type II diabetes OR 1.54 for iCCA and 1.45 for eCCA. All lower values for 95% CI >1.0)(23). Obesity was also shown to be associated with iCCA and eCCA, although the OR was greater for iCCA (iCCA OR 1.42 (95% CI 1.21-1.66), eCCA OR 1.17 (95% CI 1.01-1.35)). These findings are consistent with a previous meta-analysis that found obesity to confer an OR of 1.37 (95 % CI 1.22–1.55) for CCA, although no sub-analysis between iCCA and eCCA was performed(50).

More recently, multiple meta-analyses have confirmed an association between Non-Alcoholic Fatty Liver Disease (NAFLD) and CCA(23,51,52). NAFLD is characterised by the presence of hepatic steatosis in the absence of other causes of hepatic fat accumulation. When NAFLD occurs in the presence of hepatic inflammation it is termed Non-Alcoholic Steatohepatitis (NASH). In one meta-analysis NAFLD conferred a 3-fold increase in the risk of iCCA (OR 3.52, 95% CI 2.87-4.32) and eCCA (OR 2.93, 95% CI 2.42–3.55)(23) although a very recent cumulative meta-analysis of all available studies did not show an increased risk of eCCA (OR (95% CI): 1.48 (0.93-2.36)(53).

There are several proposed pathological mechanisms for the interconnected risk factors of diabetes mellitus, obesity and NAFLD. Possible explanations may include; aberrant glucose metabolism and the formation of an immunosuppressive environment around normal biliary epithelium(54), obesity-related increases in leptin excretion causing increased cholangiocyte turnover(55), increased IL-6 and Tumour Necrosis Factor alpha (TNFα) from adipocyte-related low grade inflammation in obesity leading to hepatic fibrosis and cirrhosis(56) and hyperinsulinaemia and increased Insulin-like Growth Factor-1 (IGF-1) production in the liver leading to upregulation of genes involved in cell proliferation and survival(57).

1.28 Intraductal Papillary Neoplasms of the Bile Duct

Intraductal Papillary Neoplasms of the Bile Duct (IPNB) is a rare bile duct tumour characterised by papillary growths into the bile duct. The pathogenesis and natural history of IPNB is ill defined although they do share some pathological similarities to Intraductal Papillary Mucinous Neoplasms (IPMNs) of the pancreas(58). IPNBs are associated with hepatolithiasis and liver fluke infection in Asian countries (but not in Western countries) implying both genetic and environmental aetiologies. IPNBs have a high risk of malignant transformation to cholangiocarcinoma. At the time of surgical resection, approximately half of IPNBs show stromal invasion (IPNB associated with invasive carcinoma)(59). Figure 3 shows the cholangioscopic findings of IPNB.

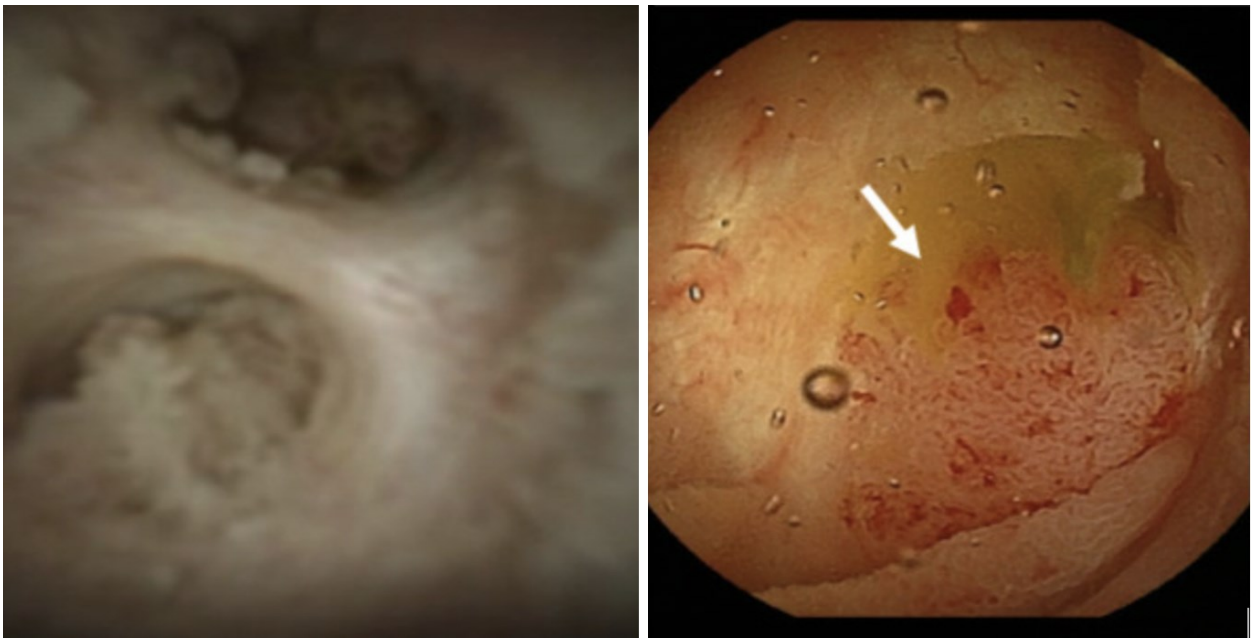


Figure 3: Per-oral cholangioscopic findings of intraductal papillary neoplasm of bile duct (IPNB). Left: Villous papillary tumour without mucin hypersecretion located in the bile duct hilum. Right: Villous papillary tumour (arrow) with mucin hypersecretion. (Source: Journal of Clinical Medicine)(59)

1.29 Genetic diseases and chromosomal factors

Lynch syndrome (previously known as hereditary non-polyposis colorectal cancer) is an autosomal dominant condition occurring due to a germline mutation of one DNA mismatch repair (MMR) gene. Patients with Lynch syndrome have an increased risk of multiple cancers, most commonly colorectal and endometrial cancers but also cancers of the upper gastrointestinal tract, hepatobiliary tree, urogenital tract, and brain. Lifetime risk of a pancreatic or biliary tract cancer is estimated at 2%, with one recent small study suggesting that MMR-deficient CCA occurring in the context of Lynch syndrome may have a better prognosis than those with non-Lynch associated CCA(60).

Several congenital abnormalities confer a higher risk for developing CCA. Defects in genes coding for bile salt transporter proteins (BSEP/ABCB11, FIC1/ATP8B1 and MDR3/ABCB4) cause cholestasis leading to the release of inflammatory cytokines, chronic inflammation and subsequent cholangiocarcinogenesis(61).

1.3 IgG4 related disease

1.31 Introduction

IgG4-RD is a recently discovered multisite fibroinflammatory condition that has been described in nearly every organ(62). Many diseases that have long been viewed as specific entities (e.g., Küttner's tumor, Mikulicz's disease, retroperitoneal fibrosis, and Riedel's thyroiditis) are now understood to relate to IgG4-RD(63). Typical disease sites include the pancreas, biliary tract, salivary and lacrimal glands, thyroid gland, lungs, kidneys, retroperitoneum, and aorta. The clinical features depend on the organ(s) involved, although the most typical presentation is the development of a mass lesion or swelling of one or more organs. For this reason, misdiagnosis as cancer is common in the setting of IgG4-RD(64). In one published series, 18/53 patients (34%) undergoing resection of presumed pancreatobiliary tumours were found to have IgG4-RD(65).

1.32 Pathogenesis

The precise pathophysiology underpinning IgG4-RD remains poorly understood. Current evidence suggests roles for both allergy and autoimmunity in disease development. It is probable that disordered innate and acquired immunity, reduced numbers of regulatory T-cells, and specific B-cell responses, are all involved in pathogenesis. Indeed, B-cell depletion following rituximab-induced disease remission was the first pathophysiological finding in IgG4-RD(66). Multiple human leukocyte antigen (HLA) association studies in the United Kingdom, Korea and Japan have identified region-specific HLA molecules and other immunoregulatory genes as determinants of disease susceptibility(67). Involved organs are richly infiltrated with both B and T lymphocytes, suggesting involvement of an antigen-mediated response(68). Supporting the existence of an antigen-driven immune response is the fact that oligoclonal IgG4 is present in the cerebrospinal fluid of patients with IgG4-related pachymeningitis(69). Later studies have identified

oligoclonal expansions of somatically hypermutated IgG4+ B-cell clones from tissues and blood in patients with IgG4-RD(70). The IgG4 antibody is thought to be involved in the pathogenesis of IgG4-RD however it remains unknown whether this antibody is directly involved (either as directly pathogenic or in a protective role) or whether it is simply an incidental marker of the underlying inflammatory response(71). Several other cells lines which have been investigated as drivers of inflammation in IgG4-RD, including CD4+ cytotoxic T lymphocytes, which are expanded in the tissue and blood of patients with IgG4-RD and which reduce in number following rituximab treatment, and T follicular helper cells (Tfh cells)(70). In healthy individuals, these cells control B-cell differentiation but, in IgG4-RD patients, they may contribute to class-switching and expansion of IgG4+ plasma cells(72–74).

1.33 Epidemiology and Diagnosis

IgG4-RD is a disease of predominantly older males with a male to female ratio of 8:3 and a mean age of onset of 60 years(75). This predilection for older males is particularly true for the archetypical form of IgG4-RD, type 1 autoimmune pancreatitis (AIP)/IgG4-related pancreatitis (IgG4-RP). The prevalence of type 1 AIP shows large geographic variation but is increasing globally. In 2016, the annual incidence of type 1 AIP in Japan was 3.1/100,000 of the population, a more than doubling from the previous national Japanese survey of 2011(76). The European incidence of type 1 AIP is much lower than in Asia, with one study suggesting the annual incidence in Germany to be <1/100,000(77). The accurate diagnosis of IgG4-RD remains challenging due to the diverse range of clinical presentations, multiple potential sites of organ involvement, poor disease recognition by clinicians, and high rates of misdiagnosis. It is thus difficult to give precise estimates of the disease prevalence across all organs.

Several diagnostic criteria are available to assist in the accurate diagnosis of IgG4-RD. The most widely used are the HISORt (Histology, Imaging, Serology, other ORgan involvement and response to therapy) criteria, originally described by the Mayo clinic(65). These criteria were initially designed to diagnose AIP but have since been adapted to aid in the diagnosis of IgG4-related sclerosing cholangitis (IgG4-SC)(78). The required components include characteristic cross sectional imaging findings, consistent histology, raised serum IgG4 levels, extra-biliary organ involvement, and a radiological or biochemical response to steroid treatment(79). Serum IgG4 levels are elevated (> 1.4 g/L) in up to 70% of all patients with IgG4-RD and may increase with the number of organs involved(80,81). However, it is well understood that raised serum IgG4 levels are of limited utility when used to distinguish IgG4-RD from inflammatory and malignant disease(81,82). Indeed, over reliance and incomplete understanding of the significance of serum IgG4 levels contributes to high rates of misdiagnosis in this condition. One UK cohort study found that only 22.4% of patients with raised serum IgG4 levels (> 1.4 g/L; n = 264) met the diagnostic criteria for IgG4-RD. Doubling the cutoff to > 2.8 g/L increased specificity from 85% to 96% but with an associated reduction in sensitivity (to 57% from 83%)(83). The positive predictive value of 45% was poor. Furthermore, serum IgG4 levels are elevated in up to 5% of healthy controls(81,82,84).

Histopathology remains the gold standard for accurately diagnosing IgG4-RD. The three classical features are: an IgG4-positive lymphoplasmacytic tissue infiltrate **(figure 4, below)**; storiform fibrosis (a whorled appearance); and obliterative phlebitis (partial or complete venous obliteration)(85). These hallmark histological findings (at least two of which are required to support a diagnosis of Igg4-RD) are often supported by increased numbers of tissue or circulating tissue IgG4-positive B-cells. The number of IgG4-positive plasma cells required to reach diagnostic significance is defined separately for each specific organ, however the IgG4:IgG ratio must be > 40%(85). It must be remembered that lymphoplasmacytic infiltrates in conjunction with elevated

numbers of IgG4-positive plasma cells are seen in a variety of inflammatory and malignant conditions(86,87). Therefore, the histopathology should be carefully interpreted in the context of the clinical presentation, serology, and radiology(88). Computed tomography (CT) and magnetic resonance imaging (MRI) are both useful in defining the extent of disease and assessing the response to treatment. Positron emission tomography (PET)/CT detects clinically silent organ involvement; a recent study found that 90% of patients with “single-organ involvement” initially enrolled in an IgG4-RD had multi-organ disease on PET/CT using the glucose analog 2-[(18)F]-fluoro-2-deoxy-d-glucose (FDG), highlighting the value of PET in detecting other organ involvement. Mention PET radiation here(89).

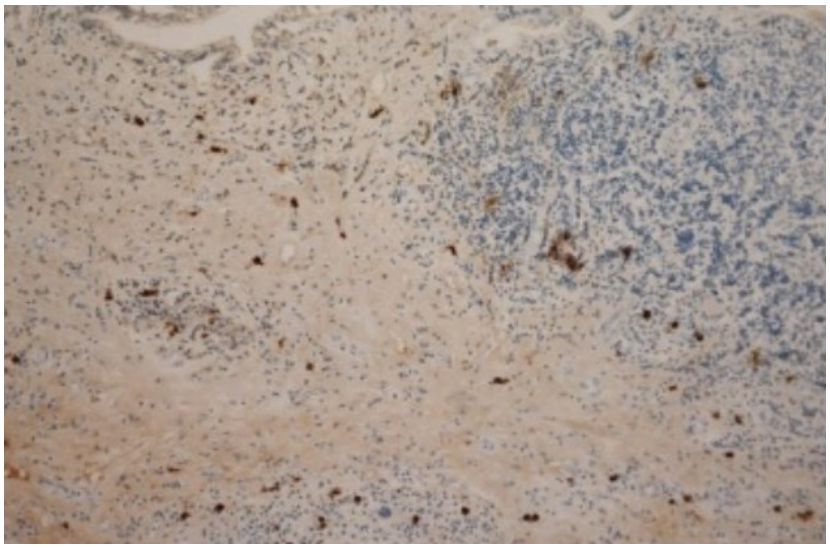


Figure 4: Abundant IgG4-positive plasma cells in bile duct wall (IgG4 staining x 200)

(Source: World Journal of Gastroenterology)(90)

1.34 Treatment

The goal of treatment in IgG4-RD is to alleviate symptoms, improve organ function and prevent disease progression from inflammation to irreversible fibrosis. Whilst treatment is usually required

in patients with organ involvement, those with minimal or mild disease for example, mild AIP or asymptomatic lymphadenopathy, a watch and wait approach may be appropriate. Corticosteroids are the cornerstone of treatment; in the first prospective, randomised, placebo-controlled trial of steroids in patients with AIP type 1 disease, remission was induced in both groups and corticosteroids were then withdrawn (at 26 weeks) in 19/49 patients but maintenance treatment continued for 36 months in 30/49. The relapse rate was significantly lower in the maintenance group, 7/30 patients (23.3%) relapsed compared with 11/19 (57.9%) in the cessation group (p = 0.011)(91). A typical adult induction regimen featured prednisolone 30 to 40 mg/day for 4 weeks, followed by tapering every 2 weeks (depending on the response) by 5 mg/week over 3 to 4 months. A response was usually evident within 4 to 6 weeks as evidenced by improved organ function, radiological improvement, a decrease in serum the IgG4 level, and improvement of symptoms. Once remission is achieved, those patients with either multiorgan involvement or who relapse off steroids, are generally considered for second line or maintenance treatment. Maintenance treatment options include single-agent low-dose corticosteroids (ideally at no more than 5 mg/day), with or without second-line immunosuppressive therapy(92) featuring either azathioprine 1.5 to 2 mg/kg/day or mycophenolate mofetil(93). One systematic review and meta-analysis (SRMA) of 62 studies involving 3,034 patients highlighted the key role of corticosteroids; 1,186 of 1,220 patients (97%) who received steroid monotherapy experienced a therapeutic response, however these were incomplete in 35% of patients. The study also evaluated the effectiveness of treatment for relapses of IgG4-RD. Relapses were reported in 464/1,395 patients (33%), despite the typically short follow-up time. Corticosteroids were effective in 219/231 (95%) of such patients, in 56/69 (81%) of those treated with azathioprine, in 16/22 (72%) of those treated with other immunosuppressive agents, and in all nine cases treated with rituximab (100%). In patients with AIP type 1, any associated proximal IgG4-SC predicted an increased risk of post-steroid relapse(94).

Rituximab (RTX), an agent that depletes CD20-positive plasmablasts, has recently been licensed for use by NHS England for those patients with steroid refractory disease and those where second line agents are ineffective or contraindicated. The drug has previously been shown to be effective in inducing and maintaining remission in IgG4-RD but was associated with high rates of serious infections(95,96). However, a recent European study of 46 patients with IgG4-RD treated with RTX between 2006-2019 also confirmed excellent clinical response rates to RTX but in this study 61% of patients had relapsed within 16 months. There were however high rates of clinical response when further RTX was prescribed to treat disease relapse(97). This small study may suggest that patients should be risk stratified after initial RTX and those most high risk offered a further treatment with RTX to prevent relapse of IgG4-RD.

1.35 Prognosis

IgG-RD was first described in 2001, therefore long-term data is lacking. Some patients recover from the disease spontaneously without treatment. Many patients experience a benign disease course however others exhibit an aggressive phenotype with multiorgan involvement and frequent relapses. There is limited evidence that early treatment may be associated with better outcomes, possibly due to fewer inflammatory and fibrotic complications(83). The risk of any malignancy (particularly lymphoma) appears to be increased by > 2-fold in patients with IgG4-RD compared with age- and sex-matched controls(98).

1.4 IgG4-related sclerosing cholangitis

When IgG4-RD affects the biliary tree, it is termed IgG4-SC. The biliary tree is the second most common site after the pancreas and estimates of the prevalence of Igg4-SC vary widely. In US and UK cohorts, the prevalence of isolated IgG4-SC was < 10% that of IgG4-RD, and 60% to 80% of AIP patients had evidence of IgG4-SC(65,68,80,99). IgG4-SC may exhibit a variety of presentations; a recent retrospective study of 527 patients with IgG4-SC in Japan found that 35%

presented with jaundice and 13% pruritus, whereas 28% were asymptomatic(100). Other presentations include weight loss and abdominal discomfort.

1.41 Diagnosis

The HISTORt criteria are useful in the diagnosis of IgG4-SC. Serum IgG4 levels are elevated in approximately 75% of cases(101). Initial imaging often includes a transabdominal ultrasound scan (TA-USS) which may demonstrate biliary dilation, a thickened gallbladder wall or a head of pancreas mass, the latter suggesting either AIP or pancreatic malignancy. An abnormality on TA-USS must prompt further investigation with cross sectional imaging to delineate strictures and stage masses(102). Magnetic resonance cholangiopancreatography (MRCP) and endoscopic retrograde cholangiography (ERC) are both useful in the evaluation of IgG4-SC. MRCP may reveal both intrahepatic (segmental) as well as long extrahepatic strictures and pre-stenotic dilatations; all of which occur more frequently in IgG4-SC than CCA(103). Any part of the biliary tree can be affected in IgG4-SC patients; however, the lower bile duct tends to be the most involved (often in conjunction with AIP type 1). IgG4-SC has been classified into four subtypes based on the anatomy of the strictures **(**Figure 5, below). MRCP also allows evaluation of the main pancreatic duct (MPD) in patients with suspected AIP; diffuse irregular narrowing of the MPD or a long stricture (more than one-third of the length of the MPD) with absence of pre-stenotic dilation are both indicative of AIP(103). IgG4-SC may present with no identifiable biliary stricture on imaging, in which case endoscopic ultrasonography may play a role in further evaluation(104).

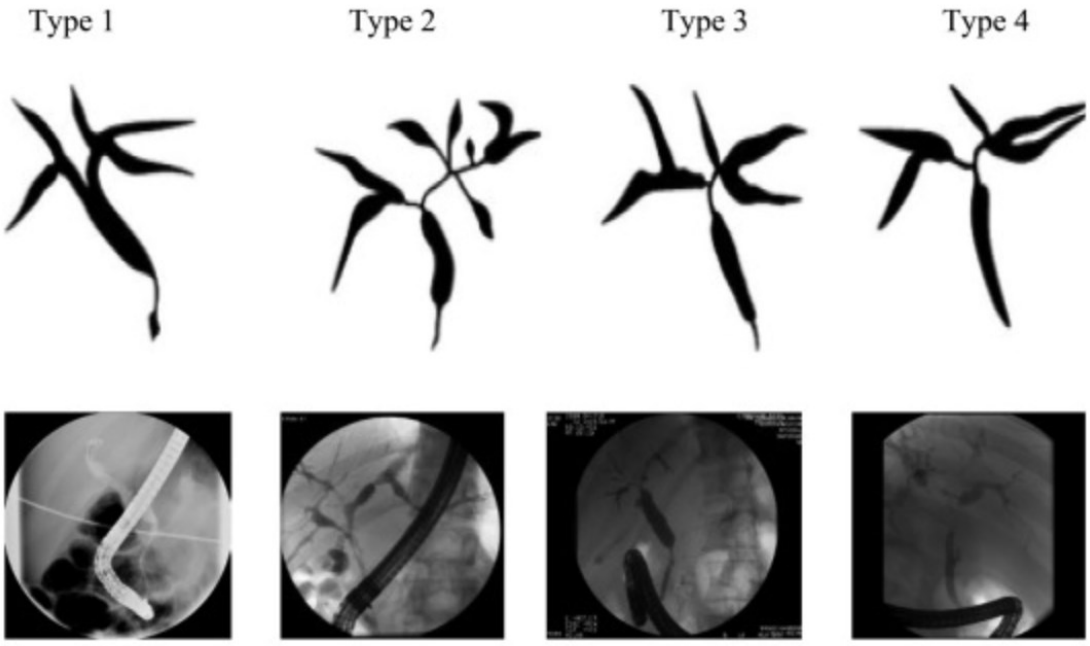


Figure 5: Classification of IgG4-SC by cholangiographic features. Type 1 characterised by low bile duct stricture. Type 2 includes both diffuse intrahepatic cholangiopathy and low bile duct stricture. Type 3 consists of a hilar and low bile duct stricture. Type 4 IgG4-SC is characterised by hilar stricturing alone. The type determines the differential diagnosis for IgG4-SC: type 1, pancreatic cancer, distal CCA, chronic pancreatitis; type 2, PSC and those for type 1; type 3, hilar CCA and those for type 1; and type 4, hilar CCA, which is the most difficult to differentiate. With permission from *Pancreas(105)*, copywrite 2006. Wolters Kluwer Health.

1.42 Endoscopic retrograde cholangiography in IgG4-SC

Endoscopic retrograde cholangiopancreatography (ERCP) is useful in the evaluation of IgG4-SC as it allows tissue acquisition for histological confirmation and facilitates therapy. Cytological examination of samples from biliary brushings are used to exclude malignancy in the setting of a dominant stricture but are not adequate to diagnose IgG4-SC(106). Ampullary biopsies should also be obtained as they are technically straightforward and often exhibit features diagnostic of

IgG4-RD. In one study, ampullary biopsies stained positive for IgG4 in 18/27 patients (67%) with symptomatic AIP, compared to none from the control group(107). Intraductal fluoroscopic biopsies may also reveal the characteristic histology of IgG4-RD. Recent advances in cholangioscopy have allowed the characteristic mucosal features of IgG4-SC to be evaluated via visually targeted biopsies(99). A targeted liver biopsy is sometimes useful in those with intrahepatic strictures or a pseudotumor. Biliary stenting should be considered but may not be required for all cases of obstructive jaundice secondary to known IgG4-SC and AIP. Corticosteroid therapy alone (without biliary stenting) has been shown to be a safe and effective treatment for obstructive jaundice attributable to major biliary strictures in Igg4-SC(108). Biliary stenting is desirable for patients with cholangitis or uncertain diagnoses.

1.43 Differential diagnosis

The imaging features of IgG4-SC are comparable to those of CCA and PSC; diagnosis based on cholangiographic appearances alone may be unreliable and misleading. These important differential diagnoses are described in further detail below.

1.431 Primary sclerosing cholangitis

Diffuse IgG4-SC is characterised by multifocal intrahepatic/proximal bile duct stricturing (type 2 disease) is similar in appearance to PSC. Studies evaluating expert opinion of IgG4-SC and PSC cholangiograms show that the cholangiographic features revealed by ERCP alone are associated with a high IgG4-SC diagnostic specificity (88%) but a low sensitivity (45%)(109). Cholangiographic features that might favor IgG4-SC over PSC include long strictures and the combined presence of both intra-hepatic and low bile duct strictures. In PSC the strictures tend to be shorter, and the biliary tree usually appears beaded. In addition to biliary stenosis, IgG4-SC has been associated with symmetrical thickening of the biliary ductal wall, smooth wall margins, and homogenous internal echoing on abdominal CT, MRI, endoscopic ultrasound, and intraductal

ultrasonography(99). These subtle features are apparent not only in stenotic areas but also in areas lacking stenosis (that therefore appear normal on cholangiograms). Dynamic stricturing over weeks or months, steroid responsiveness, and the presence of associated pancreatic changes, all favor a diagnosis of IgG4-SC over PSC. Serum IgG4 levels are raised in up to 15% of patients with PSC; therefore, the serum IgG4 level does not seem to be useful in differentiating PSC from IgG4-RD(82). Since there is some limited evidence that PSC and IgG4-SC may represent different ends of the same disease spectrum, it is considered reasonable to trial corticosteroids in patients with presumed PSC and elevated serum IgG4 levels, to assess for any reversibility in biliary stricturing(110).

1.432 Cholangiocarcinoma

Accurate and timely differentiation of CCA from Igg4-SC is paramount due to the vastly different treatment approaches. Type 1 IgG4-SC (isolated stenosis of the distal common bile duct) may be difficult to differentiate from eCCA, whereas IgG4-SC accompanied by stenosis of the hilar hepatic bile duct (types 3 and 4 disease) can be difficult to differentiate from pCCA (Figure 5, above)(99). One study of patients who underwent surgical resection of presumed pCCA (n=185) found that the resection specimen of 17% showed benign disease, with almost half of those having histopathological features diagnostic of IgG4-SC(111). IgG4-SC is particularly challenging to distinguish from pCCA in the absence of associated AIP, or when the pancreatic morphology is normal(112). In cases where CCA is suspected but when brush cytology is non-diagnostic, direct visualisation with cholangioscopy may have utility. Peroral cholangioscopy accompanied by visually targeted biopsies yields greater diagnostic accuracy than standard ERCP plus fluoroscopic biopsies(113). One SRMA (10 studies, n = 456) reported pooled sensitivity and specificity of 66% and 98% for CCA diagnosis by cholangioscopic biopsy(114). Thus, cholangioscopy has a role to play when ERCP fails to yield confirmatory cytology, and the diagnosis thus remains elusive. Neither serum IgG4 nor CA19-9 level accurately distinguish IgG4-

SC from CCA(115,116). Figure 6, below, shows a hilar stricture on MRCP (left) with corresponding cholangioscopic views demonstrating visual features of CCA.

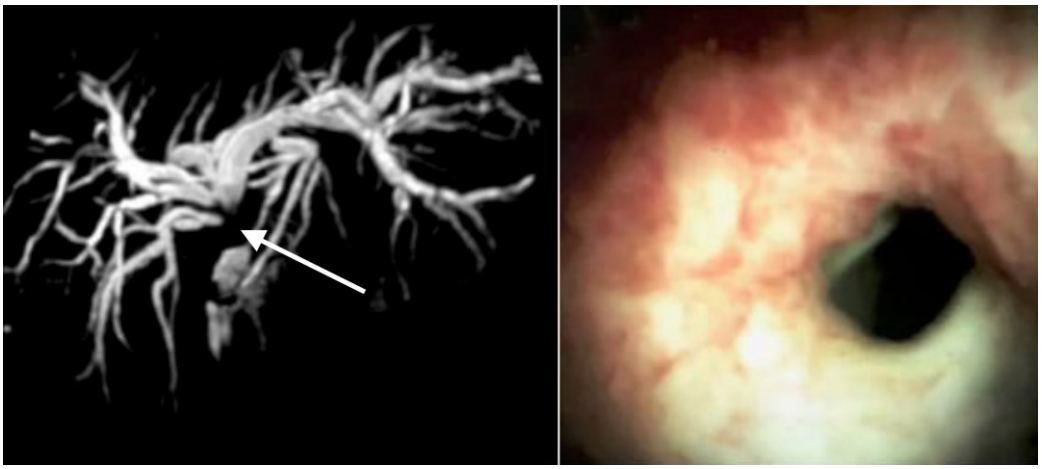


Figure 6: Left: MRCP scan showing a dilated intrahepatic biliary tree secondary to a stricture at the liver hilum (white arrow). Right: Cholangioscopy demonstrating mucosal nodularity and neovascularisation in the 7-3 o'clock position, secondary to pCCA.

1.433 Secondary sclerosing cholangitis

Secondary sclerosing cholangitis (SSC) is clinically and phenotypically like PSC and therefore Igg4-SC. The cholangiogram appearance may also resemble closely that of both PSC and IgG4-SC. SSC encompasses a range of diseases and aetiologies including infection (e.g., AIDS cholangiopathy), congenital conditions (e.g., Caroli's disease), sickle cell anemias, gallstones, infiltrative disease (e.g., histiocytosis X), and vascular aberrations (e.g., post-liver transplantation hepatic arterial thrombosis). The underlying diagnosis is usually revealed through clinical history taking.

1.44 Treatment

The aim of treatment in IgG4-SC is to prevent progression to irreversible fibrosis and prevent cholangitis. Unlike in AIP, spontaneous improvement in IgG4-SC is rare(93,117). As in other forms of IgG4-RD, a slowly tapering course of corticosteroids is the mainstay of treatment. A standard regimen would be prednisolone 30 to 40 mg daily for 4 weeks, with initial tapering over 2 weeks followed by tapering of 5 mg/week over 3 to 4 months, before maintaining a dose of 5 mg/day for 36-48 months. A response is usually apparent within 4 to 6 weeks, evidenced by normalisation of liver biochemistry and radiological improvement or resolution of biliary strictures. Patients with biliary obstruction should be carefully monitored during corticosteroid treatment for signs of cholangitis. Serum IgG4 levels often fall during corticosteroid treatment and may then normalise. One retrospective study of 527 patients with IgG4-SC found that 90% had an excellent response to corticosteroid therapy; the ALP levels fell to < 50% of the pre-corticosteroid level in 87% of cases and biliary strictures improved in 90%(100). Another study, which defined remission as complete resolution of strictures and/or normalisation of liver function tests, reported remission in 67% of IgG4-SC patients (vs. 99% of AIP cases)(118). A non-response to steroid therapy may be suggestive of advanced, fibrotic “burnt-out” IgG4-SC, or an alternative diagnosis.

1.45 Relapse

Relapse is common in patients with IgG4-SC. In an early cohort of patients from University College Hospital, London, with IgG4-SC and AIP, 57% (13/23) relapsed(92). Other studies have reported similar relapse rates(118,119). Factors predictive of relapse include proximal strictures (IgG4-SC of types 2 to 4) and very elevated serum IgG4 levels(65,83). Treatment options in those that relapse include up-titration or re-introduction of corticosteroids with or without the addition of second-line immunomodulators. Azathioprine (2mg/kg daily) is the most used second line agent followed by mycophenolate mofetil (750 to 1,000 mg twice daily). Evidence for second-line, immunomodulator therapy in IgG4-SC is limited, and it remains to be proven whether their use

reduces the time to further relapse. Factors that may predict resistance to immunomodulator therapy include other organ involvement (disease other than IgG4-SC) and retroperitoneal fibrosis(96). Rituximab is widely used as a third line agent in those with aggressive disease, those who are intolerant of immunomodulators and those that are steroid-dependent. A French study of 33 patients (including those with IgG4-SC) recorded clinical responses in 29/31 symptomatic patients (93.5%). Corticosteroid withdrawal was achieved by 17 patients (51.5%)(95). Another prospective open-label study that included patients with IgG4-SC given RTX found that 97% achieved a response, 77% were able to stop steroids and had not relapsed within 6 months, and 46% remained in remission at 12 months(120). A very recent SRMA looked at the effectiveness of RTX in patients with IgG4-related pancreatobiliary disease. Seven cohort studies were included with a total of 101 patients. Approximately 2/3 of patients (63.5%) were given rituximab for a flare after steroid response (i.e., relapse). The pooled rate of complete response to RTX was 88.9% with no heterogeneity with a median follow up of 19 months(121). As experience grows, a more top-down approach may be considered in high-risk patients (such as those with multi-organ disease); it may be that early, aggressive treatment of some patients prevents fibrotic complications(67).

1.5 Diagnosis of biliary strictures

1.51 Non-invasive biomarkers in the diagnosis of cholangiocarcinoma

Several reported cancer biomarkers have the potential to be incorporated into the diagnostic pathway for CCA, but all lack sufficient sensitivity and specificity limiting their possible use in screening and early diagnosis(122). The discovery and validation of new biomarkers are urgently needed to improve early diagnosis of a disease where surgical resection remains the only curative intervention.

1.511 Currently available tumour markers: Serum CA19-9, CEA and CA125

Carbohydrate antigen 19-9 (CA19-9) is the primary tumour marker used in the diagnosis of CCA, although its non-specificity and limited sensitivity hampers its clinical utility(123). A meta-analysis including 1,264 patients with CCA demonstrated a pooled sensitivity and specificity of 72% and 84%, respectively, for the differentiation of CCA from healthy controls and those with PSC (AUC 0.83)(124). Furthermore, in the 5-10% of the general population who are Lewis (a) antigen negative, CA 19-9 will remain undetectable(125). CA19-9 is elevated in a variety of hepatic and extra-hepatic conditions including cholangitis and cholestasis, further limiting its use in PSC, where these conditions commonly co-exist(126). A retrospective study of 79 patients with PSC found that more than one-third of patients with a dominant biliary stricture and CA 19-9 >129 IU/ml did not have CCA after a median follow-up of 30 months(127). Owing to the limited sensitivity of CA19-9 in the detection of CCA, any result should be cautiously interpreted considering the clinical picture and cross-sectional imaging. Current screening approaches for patients with PSC may include yearly MRCP and serum CA19-9, although this strategy is not currently recommended in the European Society of Gastroenterological Endoscopy (ESGE) (128) or British Society of Gastroenterology guidelines(129), because of a lack of supportive outcome data. Other readily available serum biomarkers associated with CCA include carcinoembryonic antigen (CEA) and cancer antigen 125. CEA is a cell-surface anchored glycoprotein that is involved in cell adhesion. Its elevation is most often associated with colorectal malignancy, however it is raised in up to 30% of patients with CCA showing a sensitivity and specificity of 72% and 84%, respectively(16). CA125 is a membrane associated glycoprotein encoded by the MUC16 gene which is often elevated in ovarian cancer, although it is also raised in up to 50% of CCA cases(130,131).

1.1512 Novel serum proteins and cytokines

Mass spectrometry-based proteomics is difficult to implement on serum samples due to abundant proteins such as globulins and albumin making the identification of low abundance proteins challenging(132). The development of methods for serum pre-fractionation prior to mass spectrometry profiling would aid the discovery of potential serum biomarkers for CCA(133). Despite these challenges, recently, high-throughput omics-based techniques have identified novel serum biomarkers that could be included in future, large, international validation studies(122). One study from the Mayo Clinic performed serum glycomic and proteomic analysis on 117 patients, 39 of whom had CCA and 39 had PSC. They identified multiple proteins that contained altered glycans in the sera of patients with CCA. One protein, fucosylated fetuin A, was able to differentiate CCA from PSC with reasonable performance (AUC 0.81 versus AUC 0.63 for CA19-9), suggesting it could have a role in the surveillance of patients with PSC(134). Anti-glycoprotein (GP)-2 immunoglobulin G autoantibodies have recently been found to be associated with CCA development in patients with PSC. In a European cohort of 250 patients, anti-GP2 positivity in PSC patients was associated with a significantly higher risk of developing CCA, independently of disease duration, bilirubin level and age(135). Osteopontin, a matricellular protein associated with multiple types of cancer, has been shown to be elevated in the sera of patients with CCA (n=27) compared to PSC (n=13) (*H* test $p = 0.001$)(135). Remarkably, elevated levels of circulating osteopontin were associated with poor survival after tumour resection. Another recent study characterised serum extracellular vesicles (EVs) from healthy controls and patients with CCA (n=43), PSC (n=30) and hepatocellular carcinoma (HCC), in addition to EVs derived from human CCA cell lines and normal cholangiocytes *in vitro.* Several differentially expressed proteins were identified in serum EVs of CCA versus PSC patients, including FIBG, A1AG1 and S100A8 (maximum AUC 0.80). Some candidates (including FIBG, FCN2 and ITIH4) also showed higher diagnostic values for early stage CCA (stages I-II) versus PSC than CA19-9(114), showing the potential usefulness of these serum EV proteomic signatures as early

diagnostic tools in CCA(132). However, validation studies are needed. To overcome the limited utility of individual markers, several studies have combined individual biomarkers into panels. A 2014 study aimed to determine the clinical usefulness of previously described biomarkers, including serum cytokeratin-19 fragment (CYFR21.1), matrix metalloproteinase-7 (MMP-7), CEA and CA19-9, both individually, and in combination, for the diagnosis of CCA. 24 patients with histologically confirmed CCA and 25 matched patients with benign liver disease underwent measurement of these biomarkers. The mean value of each marker was significantly higher ($p<0.01$) in CCA than in the controls and the combination of all serum markers afforded 92% sensitivity and 96% specificity in detecting CCA, with an overall diagnostic accuracy of 94% (137). More recently, our group evaluated several biomarkers with reported utility in the differentiation of CCA from benign biliary disease. In a cohort of 66 patients with CCA and 62 with PSC, a panel combining serum levels of PKM2, CYFR21.1, MUC5AC and GGT was able to differentiate CCA from PSC with a sensitivity of 82% and specificity of 90% (AUC 0.90 (138). Different cytokines have also been described as diagnostic and prognostic biomarkers in CCA patients. The pro-inflammatory cytokine interleukin 6 (IL-6) was found elevated in serum samples of patients with CCA compared to healthy individuals showing a sensitivity and specificity of 73% and 92%, respectively. However, further studies are needed to discover specific cytokines associated with CCA (139).

1.513 Serum cell-free non-coding RNA

In the last years microRNAs (miRNAs) have gained momentum as promising diagnostic markers for cancer. miRNAs are small, highly conserved RNA molecules involved in the post-transcriptional regulation of genes(140). One important feature of miRNAs is their presence and stability in biofluids, thus making them potentially suitable as non-invasive biomarkers. Several studies have demonstrated the role of miRNAs in the pathogenesis of various cancers, including CCA(141). Thus, blood borne miRNAs could be useful as early diagnostic tools in CCA. A number

of abnormal miRNA profiles have been reported in CCA. In one small study, miR-21 and miR-221 were found to be significantly overexpressed in the plasma of patients with iCCA (n=25) versus healthy controls (n=7) (AUC 0.94)(142). A similar study confirmed the utility of miR-21 in differentiating CCA from healthy controls (AUC 0.93), as well as CCA from benign biliary disease, including PSC (AUC 0.83)(143). Additionally, serum miR-150 had a sensitivity and specificity of 80.6% and 58.1%, respectively, for the diagnosis of iCCA versus healthy controls (AUC 0.764), with improved performance (AUC 0.92) when used in conjunction with CA19-9(144). A small study including 30 patients with PSC and 30 patients with CCA found that serum miR-483-p and miR-222 were differentially expressed between PSC and CCA(145). Whilst most reported diagnostic miRNAs appear to be up-regulated in CCA, others such as miR-150-5p or miR-106a may be down-regulated(146,147). Interestingly, miR-9 and miR-145 have been found overexpressed in bile samples from patients with CCA compared to healthy individuals while miR-150-5p levels, as found in blood, were downregulated(148). Table 1 summarises the utility of individual biomarkers in the diagnosis of CCA.

Table 2: Non-invasive biomarkers in cholangiocarcinoma

Biomarker	Change in CCA	Fluid	Sensitivity	Specificity	AUC	Study
CA 19-9	Upregulated in CCA	Serum	74.2%	74.2%	-	Cuenco, 2018
CEA	Upregulated in CCA	Serum	52%	55%	-	Lumachi 2014
miR-21	Upregulated in CCA vs healthy controls	Serum	87.8%	90.5%	0.908	Chusorn, 2013
miR-150	Upregulated in CCA vs healthy controls	Plasma	80.6%	58.1%	0.764	Wang 2015
miR-483-5p	Upregulated in CCA vs PSC	Serum	-	-	0.77	Bernuzzi , 2016
miR-222	Upregulated in CCA vs PSC	Serum	-	-	0.71	Bernuzzi , 2016
miR-150-5p	Downregulated in CCA vs PSC	Serum	-	-	0.74	Wu, 2016
Osteopontin	*Upregulated* in CCA vs healthy controls	Serum	87.5%	100%	-	Loosen, 2017
fc-Fetuin-A	Upregulated in CCA vs PSC	Serum	-	-	0.81	Betesh, 2017
MUC5AC	Upregulated in CCA vs PSC	Serum	60.6%	82.3%	0.72	Cuenco, 2018
CYFR21.1	Upregulated in CCA vs PSC	Serum	65.2%	75.5%	0.73	Cuenco, 2018
PKM2	*Upregulated* in CCA vs PSC	Serum	75.8%	82.3%	0.84	Cuenco, 2018
miR-21	Upregulated in CCA vs healthy controls	Urine	63.6%	71.4%	0.68	Silakit R, 2017
miR-192	Upregulated in CCA vs healthy controls	Urine	63.6%	66.7%	0.68	Silakit R, 2017

1.514 Serum circulating tumour cells

Circulating tumour cells (CTCs) are released into the blood stream by primary tumours, and have been proposed as diagnostic or prognostic tools for a number of malignancies including HCC, gastric, pancreatic, breast and colorectal cancers(149–153). The main challenge in the study of CTCs is their extreme rarity; even in patients with metastatic cancer, they have been shown to represent as little as one in 10^9 of the total circulating cells(154). However, due to their high potential, some studies have evaluated the presence of CTCs as a diagnostic tool for CCA. A number of technologies have been developed in an attempt to isolate CTCs from peripheral blood, including the CellSearch™ System, a clinically validated technology that is licenced by the US Food and Drug Administration. This semi-automated platform identifies, isolates and enumerates CTCs using cell specific EpCAM antibodies and immunofluorescent markers. One disadvantage of this method is that not all tumours overexpress EpCAM. In one study, up to 20% of CCAs did not overexpress this protein(155). Thus, Cellsearch™ further discriminates cell types based on their positivity to DAPI, cytokeratins (such as 8, 18 and 19) or negativity to CD45(156). One early study demonstrated the ability of this system to detect CTCs in patients with metastatic cancer versus healthy controls and those with benign disease. Of 344 patients that were either healthy or had benign disease, only 0.3% had >2 CTCs per 7.5ml of blood, compared to 36% of specimens collected from patients with metastatic disease, although it is not stated whether any had metastatic CCA(157). Several studies have suggested that CTCs may be associated with a poor prognosis in patients with advanced CCA(158,159), however it remains to be determined whether they have any diagnostic role in CCA. In one small study which included 13 patients with CCA, only 3 had significantly elevated CTCs (>2 per 7.5ml of blood)(156).

1.515 Urine biomarkers

Urine is an excellent sample matrix as it is non-invasively accessible, can be obtained in large quantities and is stable in its composition if handled correctly. Furthermore, since urine is an

ultrafiltrate of plasma, the urinary proteome is highly sensitive to changes in renal function and a wide range of non-renal diseases, including certain cancers. Urinary proteomic biomarkers have been described in many tumours including pancreatic, renal, prostate, bladder, lung, breast, ovarian cancer and CCA(160). Metzger and colleagues used capillary electrophoresis-mass spectrometry to evaluate the urinary proteome in early CCA(161). A 42-biomarker panel was initially identified based on the differentially excreted urinary peptides of 41 patients including 14 with CCA, 13 with PSC and 14 with other benign biliary disease. In a subsequent cross-sectional validation of 123 patients, the urinary peptide panel accurately diagnosed 35 of 42 CCA patients and 64 of 81 patients with benign biliary disease (including those with PSC), with an AUC of 0.87, 83% sensitivity and 79% specificity. Evaluation of 101 healthy controls gave 86% specificity. More recently, combined bile and urine proteome analysis was performed in a case-control phase II study on 87 patients (36 CCA, including 13 with CCA on a background of PSC, 33 PSC and 18 other benign disorders). A logistic regression model was developed and subsequently validated in a prospective cohort of 45 patients undergoing ERCP for the evaluation of biliary strictures(162). The combination of both urine and bile markers gave an accuracy of 92% in the detection of CCA (sensitivity 94%, specificity 76%, AUC 0.84). Other groups have suggested that urinary miRNAs may be useful in the diagnosis of CCA. In a study of 192 patients with either *Opisthorchis viverrini* infection, periductal fibrosis or CCA, miR-21 and miR-192 were found to be elevated in the urine of patients with CCA versus healthy controls (AUC 0.849). Of these two biomarkers, miR-21 discriminated CCA with most accuracy (AUC 0.682)(163). More recently, a small study showed lysosome associated membrane glycoprotein 1 (LAMP1), lysosome associated membrane glycoprotein 2 (LAMP2) and cadherin-related family member 2 (CDHR2) to be preferentially expressed in the urine of patients with CCA versus those with periductal fibrosis and healthy patients(164).

1.52 Optical techniques

1.521 ERCP with biliary brushings and fluoroscopic biopsies

Endoscopic retrograde cholangiopancreatography (ERCP) is usually performed following cross-sectional imaging to allow therapy, such as biliary stent insertion. It facilitates the acquisition of high-quality fluoroscopic cholangiograms providing information on stricture anatomy and the presence of associated mucosal irregularity or shouldering. Fluoroscopic images can distinguish malignant from benign strictures with an accuracy of at most 80%, thus tissue sampling is also required. Standard ERCP plus brush cytology has a variable sensitivity for malignancy of 26–73% (pooled sensitivity of 56% in a recent meta-analysis). Using ERCP plus intraductal fluoroscopic biopsies improves the sensitively to 67% whilst combing brush cytology with intraductal biopsies at the time of ERCP gives a pooled sensitivity of 74%(165). The addition of Fluorescence In Situ Hybridisation (FISH) and digital image analysis may further improve diagnostic yield. These techniques facilitate DNA analysis for chromosomal aneuploidy and nuclear DNA content - both of which relate to the risk of malignancy – and enhance tumour detection by up to 23%. A systematic review and meta-analysis (SRMA) of eight studies involving 828 patients showed a pooled sensitivity and specificity of FISH polysomy to detect CCA was 51% and 93%, respectively. The authors concluded that whilst FISH was highly specific, the limited sensitivity highlights the requirement for better markers in the early detection of CCA.

Novel imaging techniques, including narrow-band imaging (NBI), autofluorescence, confocal laser endomicroscopy (CLE) and elastic scattering spectroscopy allow augmented views of the visualised mucosa during ERCP. Supplementary approaches to standard ERCP include endoscopic ultrasonography with fine-needle aspiration (EUS-FNA), intraductal ultrasound (IDUS) or single operator cholangioscopy systems (Spyglass, Boston Scientific Corp, Natick, Massachusetts, USA), as described below.

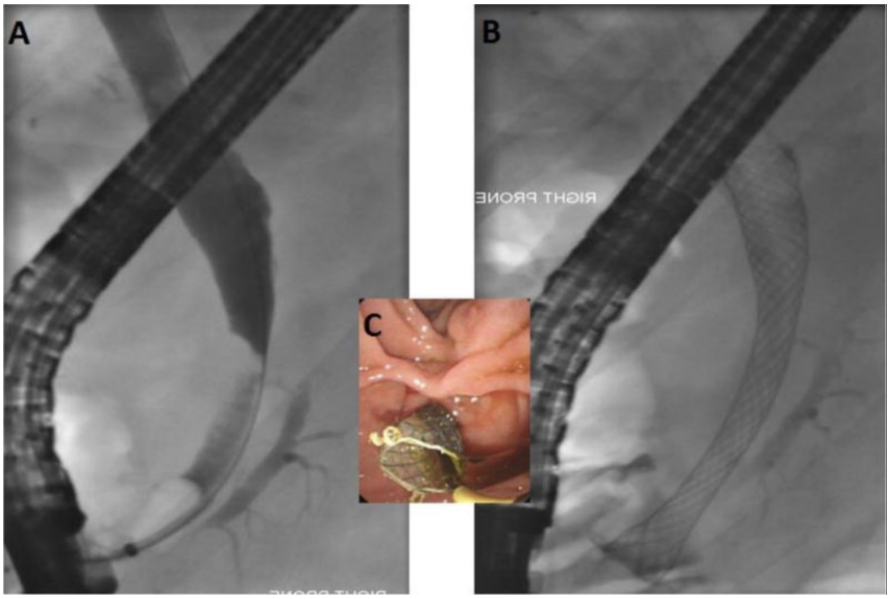


Figure 7: A: Cholangiogram showing distal bile duct stricture. B: Cholangiogram showing the stricture treated with a self-expanding metal stent. C: Endoscopic view of metal stent from duodenal lumen.

1.522 Endoscopic ultrasonography

Endoscopic ultrasound and fine needle aspiration (EUS-FNA) facilitates imaging and sampling of the pancreas and biliary tree. EUS-FNA is a well-established means of evaluating solid pancreatic tumours and is increasingly used in the assessment of biliary strictures. A SRMA involving 957 patients reported a pooled sensitivity and specificity of 80% and 97% for the diagnosis of cholangiocarcinoma by EUS-FNA. Limitations of biliary EUS-FNA include a small risk of tumour seeding. In one retrospective study, patients undergoing EUS-FNA prior to liver transplantation for pCCA were more likely to have peritoneal metastases at subsequent staging laparotomy (83% vs. 8%), although the number of patients who underwent EUS-FNA was small (n=16). Peritoneal

seeding has not been reported after transduodenal EUS-FNA for distal eCCA, where the biopsy tract is resected at the time of pancreatoduodenectomy. There have also been some case reports of peritoneal seeding after EUS-FNA of pancreatic cysts. The PIPE study evaluated the frequency of postoperative peritoneal seeding in patients with malignant and non-malignant intraductal papillary mucinous neoplasm (IPMN) who had undergone preoperative EUS-FNA (***n*** = 175) and compared it with that of patients with IPMN who had surgical resection without preoperative sampling (***n*** = 68). The frequency of postoperative peritoneal seeding was similar in the two groups (2.3% vs. 4.4%; p = 0.403). A recent systematic review of case reports identified 45 cases of post-EUS FNB needle tract seeding. The nodule was most often found to occur on the posterior gastric wall and appeared at a median of 19 months after primary resection. A low number of patients with needle tract seeding received neo-adjuvant chemotherapy., suggesting this may have a protective role(166). EUS-FNA may be combined with adjunct techniques including transient elastography and contrast agents when assessing pancreatic lesions and lymph nodes, which have been shown to improve the diagnostic accuracy.

1.523 Intraductal ultrasound

Intraductal ultrasonography (IDUS) provides real-time, cross-sectional views of the biliary tree and adjacent structures using a high-frequency ultrasound transducer introduced at the time of ERCP(167). In a retrospective study of 379 patients with indeterminate biliary strictures, IDUS could differentiate CCA from benign strictures with a both a sensitivity and a specificity of 98%. In a single centre study (n = 193), IDUS was better at diagnosing proximal than distal ductal strictures (98.1 vs 82.7%, p = 0.006).

1.524 Peroral cholangioscopy

Cholangioscopy plus visually targeted biopsies has been reported to have a greater diagnostic accuracy than standard ERCP with fluoroscopic biopsies. A single operator cholangioscopy system (Spyglass, Boston Scientific Corp, Natick, Massachusetts, USA) produces a 6000-pixel fibre optic image and facilitates visually directed biopsies via small disposable forceps. Cholangioscopy can be performed by peroral (POC), percutaneous transhepatic or intra-operative transcystic or transcholedochal routes(168). In a multicentre, prospective study of 105 patients, the sensitivity and specificity of the visual impression of malignancy at cholangioscopy was 90% and 96%, respectively. The sensitivity and specificity of cholangioscopy-guided biopsies for diagnosis of malignancy was 85% and 100%, respectively. A subsequent SRMA (15 studies, n=539) reported the pooled sensitivity of cholangioscopy-directed biopsies in diagnosing malignancy was 71.9% [95% confidence interval (CI): 66.1-77.1] and pooled specificity was 99.1% (95% CI: 96.9-99.9)(169). Cholangioscopy therefore has a particular role when ERCP alone fails to obtain confirmatory cytology and the diagnosis remains elusive.

1.525 Novel optical techniques

A) Chromoendoscopy, autofluorescence and narrow band imaging

Several techniques have been used to augment the visualised mucosa during cholangioscopy. Biliary NBI may facilitate in delineating tumours and distinguishing between malignant and benign vascular patterns(170)(171). A prospective study of 21 patients concluded that NBI was significantly better than white light at detecting the surface vascular pattern of strictures ($p < 0.05$)(172). A more recent prospective study evaluated the efficacy of NBI compared to conventional white-light imaging (WLI) during POC diagnosis of indeterminate biliary strictures. Seventy-one patients underwent POC plus NBI during targeted biopsy sampling. The strictures

were successfully interrogated, and adequate biopsy samples obtained in 67 of 71 patients (94.4%). In terms of visual impression, WLI and NBI gave 75.0% and 87.5% sensitivity, 82.9 and 91.4% specificity, and 82.8 and 91.3% accuracy, respectively. The areas under the WLI and NBI ROC curves were .80 and .96 (P = .01). Under NBI, the visualisation quality of surface structures, microvasculature, and tumour margins was higher than that under conventional WLI (P < .05)(173).

B) Light scattering spectroscopy

Light scattering spectroscopy (LSS) is a real time optical technique which detects cellular alterations via a specialised probe passed through down the endoscope. It allows a field assessment for malignancy via an "optical biopsy". Perelman *et al* developed a spatial gating fibre-optic probe that can rapidly acquire optical biopsies within pancreatic cysts during EUS. In a double-blind prospective study of 25 patients, this technique was able to distinguish neoplastic, mucinous, and serous lesions, thereby identifying the malignant potential in 21 out of 22 cysts, achieving 95% accuracy in the detection of malignancy, compared to 58% accuracy for cytology alone(174). In a more recent but small in vivo double-blind prospective study involving 29 patients undergoing routine ERCP, LSS detected malignant transformation with 97% accuracy(175).

C) Confocal laser endomicroscopy

Probe-based confocal laser endomicroscopy (pCLE) gives real-time images of individual cells during ERCP and EUS. A "cholangioflex" confocal probe (Mauna Kea Technologies, Paris, France) is placed through either a cholangioscope or standard duodenoscope. Following intravenous injection of fluorescein, a low-power laser directs light onto the biliary mucosa. Light emanating from this point is focused through a pinhole to a detector. The resultant images correlate with standard histology and can differentiate between malignancy, inflammation, and normal mucosa(176). A recent SRMA found the summary estimates for the pCLE diagnosis of

indeterminate biliary strictures were: sensitivity 0.88 (95% confidence interval (CI), 0.84-0.91); specificity 0.79 (95% CI 0.74-0.83); and Diagnostic Odds Ratio (DOR) 24.63 (95% CI 15.76-38.48). The summary estimates for tissue sampling by ERCP diagnosis for indeterminate biliary strictures were: sensitivity 0.54 (95% CI 0.49-0.59); specificity 0.96 (95% CI 0.94-0.98); and DOR 11.31 (95% CI 3.90-32.82). The area under the sROC curve of pCLE diagnosis of indeterminate biliary strictures is 0.90 higher than 0.65 of tissue sampling by ERCP(177). The authors concluded that pCLE is superior to tissue sampling by ERCP for the diagnosis of indeterminate biliary strictures. The American Society for Gastrointestinal Endoscopy guidelines state that CLE is a useful tool for differentiating benign from malignant biliary strictures in patients with suspected CCA. Small studies have combined CLE with cholangioscopy and shown the diagnostic accuracy can be improved from 78% to 82%(178,179).

1.6 Treatment of biliary strictures

1.61 Endoscopic decompression in malignant biliary obstruction

1.611 Pre-operative stenting

The goal of pre-operative biliary stenting is to improve both symptoms and liver function prior to either surgical resection (with curative intent) or neo-adjuvant chemotherapy. The benefits of pre-operative biliary drainage in patients with resectable disease is debated. In a landmark multicentre trial of 202 patients' obstructive jaundice due to pancreatic cancer randomised to either early surgery (<1 week from randomisation) or pre-operative biliary stenting, serious complications were reported in 39% of the early surgery group versus 74% in the biliary drainage group ($p<0.0001$). Overall mortality rates were similar across both groups. The authors concluded that pre-operative biliary drainage is not indicated in patients with a bilirubin <250umol/l(180). Other studies have attempted to address the issue of whether severely jaundiced patients should

undergo pre-operative biliary drainage. In this setting biliary drainage has been shown to reduce perioperative and postoperative complications(181). The optimal type of stent remains debated; one RCT found no significant difference between fully-covered self-expanding metal stents (fcSEMS) vs plastic stents (PS) (14.2% vs 16.3% re-intervention rate) although the mean interval from randomisation to surgery was only 13 days(182). In patients with a longer time to surgery, for example those receiving neo-adjuvant chemotherapy, fcSEMS provide prolonged patency over PS therefore reducing the need for re-intervention and minimising delays and interruptions to chemotherapy(183).

1.612 Palliative stenting

Self-expanding metal stents (SEMS) are generally superior to PS in the palliative setting. Using SEMS for distal extrahepatic or hilar malignant biliary strictures results in significantly longer median patency and lower re-intervention rates when compared to PS. In a recent meta-analysis of 19 studies involving 1989 patients, SEMS were associated with significantly lower rates of occlusion, less therapeutic failure (7% vs 13%), reduced need for re-intervention and lower rates of cholangitis (8% vs 21%) than PS(184). Whilst the cost of a single SEMS far exceeds that of a PS, several studies looking into the overall cost-effectiveness of SEMS vs PS have shown that SEMS are more cost effective than PS for patients with a life expectancy over four months(185). In distal malignant biliary obstruction, there is a choice between fcSEMS and uncovered SEMS (ucSEMS), each with their own advantages and disadvantages. A recent meta-analysis comparing the two groups found the fcSEMS cohort to have a lower incidence of adverse events (OR: 0.74, 95% CI: 0.57 to 0.97, P = 0.03) with no difference in stent dysfunction, stent patency or overall survival(186). Future developments in SEMS technology may include 3D printed stents tailored to the individual patient(187) and silver nano-particle loaded stents which may prolong stent patency and survival via antibacterial activity(188).

1.623 Stenting of malignant hilar strictures

Disconnection of the right and left liver tree, at the hilum, can occur from either intrinsic or extrinsic compression. Such hilar strictures are further characterised according to the Bismuth-Corlette classification. To relieve clinically apparent jaundice, it is estimated that at least one third of the liver parenchyma should be drained. Drainage options include endoscopic, percutaneous, or surgical techniques. Several studies have sought to define optimal stenting practice of hilar strictures based on the Bismuth-Corlette classification and stricture appearance on cross-sectional imaging. Most patients with malignant hilar strictures (pCCA) have unresectable disease. In those with a predicted survival of greater than four months, ucSEMS appear superior to PS for palliation with respect to clinical outcomes and cost-effectiveness(189). Sufficient biliary drainage can generally be achieved with unilateral, bilateral side-by-side or bilateral stent-in-stent approaches, with evidence currently lacking as to which of these approaches is superior. A recent SRMA aimed to assess the efficacy and safety of bilateral vs unilateral biliary drainage in inoperable malignant hilar obstruction. Nine studies were included (n=782). Bilateral stenting had significantly lower re-intervention rates compared with unilateral drainage (OR = 0.59, 95%CI: 0.40-0.87, P = 0.009). There was no difference in the technical success rate (OR = 0.7, CI: 0.42-1.17, P = 0.17), early complication rate (OR = 1.56, CI: 0.31-7.75, P = 0.59), late complication rate (OR = 0.91, CI: 0.58-1.41, P = 0.56) and stent dysfunction (OR = 0.69, CI: 0.42-1.12, P = 0.14) between bilateral and unilateral stenting for malignant hilar biliary strictures(190). In those with potentially resectable pCCA, several RCTs and meta-analyses have evaluated the value of pre-operative biliary drainage in this group(191), the results demonstrating significantly higher post-operative morbidity in the drained vs undrained groups (OR 1.67: 95% CI), therefore routine pre-operative stenting in hilar cholangiocarcinoma is not currently recommended(189).

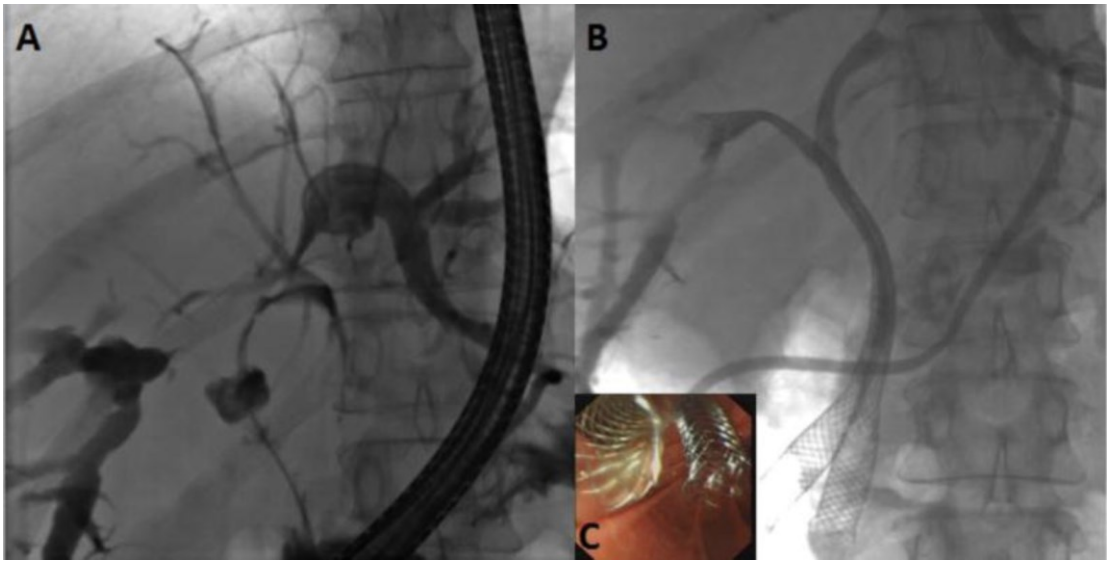


Figure 8: A: Cholangiogram showing hilar stricture. B: Cholangiogram showing the stricture treated with two self-expanding metal stents. C: Endoscopic view of metal stents from duodenal lumen.

1.62 Endoscopic decompression in benign biliary obstruction

Benign biliary strictures (BBS) have multiple potential aetiologies including post-surgical duct injury, PSC, chronic pancreatitis, trauma, and IgG4-related disease. Of these, post-cholecystectomy bile duct injury (0.5% incidence) and post-liver transplant anastomotic strictures (10–40% incidence) are the most common causes(192,193). The aim of treatment in BBS is to improve symptoms, relive obstruction, prevent cholangitis, and restore normal liver function. Endoscopic treatment of BBS has been shown to be safe, effective, and less invasive than surgery thus it is the first line therapeutic option in most patients with an accessible ampulla(194). Established therapeutic approaches include balloon dilation, placement of multiple PS with stent changes each 3–4 months (up to 12 months) or fcSEMS placement for 3–6 months, the aim of remodelling the stricture. The latter of these options has been shown to be safe, effective, technically straightforward and associated with fewer procedures(195). In one international

prospective study of 177 patients with BBS who underwent temporary fcSEMS placement, all stents were successfully removed without complication and stricture resolution was achieved in 76.3% of patients(196). More recently a SRMA of eight RCTs (n=524) looked at the safety and efficacy of multiple PS versus fc-SEMS in the management of BBS. The authors found no significant difference in the rate of stricture resolution (risk ratio, 1.02; 95% CI, 0.96-1.10), stricture recurrence (risk ratio, 1.68; 95% CI, 0.72-3.88) or adverse events (risk ratio, 1.17; 95% CI, 0.73-1.87) between the two groups. However, the average number of ERCPs required before BBS resolution was significantly lower in the fc-SEMS group than the multiple PS group (SMD, -1.99; 95% CI, -3.35 to -0.64)(197). Recently, the Kaffes™stent has been developed specifically for the management of BBS. This short stent has an anti-migration waist and is easily removable due to long retrieval wires left within the duodenum and may reduce the risk of post-ERCP pancreatitis by avoiding blockage of the pancreatic duct. Several studies have reported that this stent is safe and effective at resolving post-transplant BBS(198).

Dilation of BBS should preferably be delayed as early dilation (<4 weeks) has been shown to increase the risk of bile leak(199). Dilatation can be achieved with either a balloon or bougie. Balloon dilatation alone without combined stenting is associated with a high rate of stricture recurrence in anastomotic strictures and chronic pancreatitis(199). The exception is dominant strictures in PSC where repeated balloon dilatations without stent placement is associated with improved long-term outcomes including lower rates of cholangitis. Gotthardt *et al* prospectively studied 101 PSC patients with dominant strictures treated with either balloon dilatation or stenting. Long term follow-up showed high rates of bile duct patency with 5-year transplant-free and 10-year transplant-free survival rates of 81% and 52%, respectively(200). For patients with IgG4-related biliary strictures stenting is not required unless there is severe jaundice or cholangitis, in which case a stent may bridge the patient until steroid response(201). Insertion of ucSEMS in

BBS or indeterminate strictures is usually contraindicated due to the likelihood of tissue ingrowth and subsequent stent embedment(195).

1.63 Alternative biliary drainage techniques when ERCP is not possible

1.631 Percutaneous transhepatic drainage

In cases where endoscopic drainage is not possible percutaneous transhepatic drainage (PTD) with stent placement provides an alternative means of biliary drainage. PTD is useful for both pCCA where it allows for selective duct drainage and in cases of unsuccessful endoscopic access, for example due to gastric outlet obstruction, duodenal stents, or surgically altered anatomy such as Roux-en-Y hepaticojejunostomy(202).

1.632 Endoscopic ultrasound guided biliary drainage

Endoscopic ultrasound-guided biliary drainage is an alternative approach with outcomes favourable to percutaneous drainage(203). Passage of a guidewire through a tract from either the duodenum or stomach enables cannulation and stent placement using a ‘rendezvous’ technique, or alternatively a stent can be placed across the tract to allow bile to directly drain GI lumen. EUS-BD is a novel technique for patients who have failed endoscopic biliary stenting and may be considered as an alternative to PTD. Success rates have been shown to be above 90% in expert hands with rates of adverse events 17%(204). A SRMA (9 studies, 483 patients) compared EUS-BD with PTD. They concluded that whilst there was no difference in technical success, EUS-BD was associated with higher rates of clinical success, fewer adverse events, and lower rates of re-intervention. EUS-BD was also more cost effective(205). More recently, another SRMA compared traditional ERCP (ERCP-BD) and EUS-BD. Nine studies (n=634) were included. There were no

significant differences in clinical or technical success between EUS-BD and ERCP-BD (odds ratio [OR], 0.76; 95% CI: 0.30-1.91; OR, 1.45, 95% confidence interval [CI], 0.66-3.16, respectively). EUS-BD was associated with significantly less reintervention vs ERCP-BD (OR, 0.36, 95% CI, 0.15-0.86). The rates of adverse events were similar for EUS-BD and ERCP-BD (OR: 0.75, 95% CI, 0.45-1.24). There were no significant differences in the types of adverse events (stent migration, stent dysfunction, stent blockage, and duration of stent patency) between the two techniques. EUS-BD was associated with lower reintervention rates compared with ERCP-BD, with comparable safety and efficacy outcomes(203). The European Society for Gastroenterology guidelines recommend EUS-BD should be used over PTD in cases of failed ERCP in malignant biliary obstruction, where local expertise is available(206).

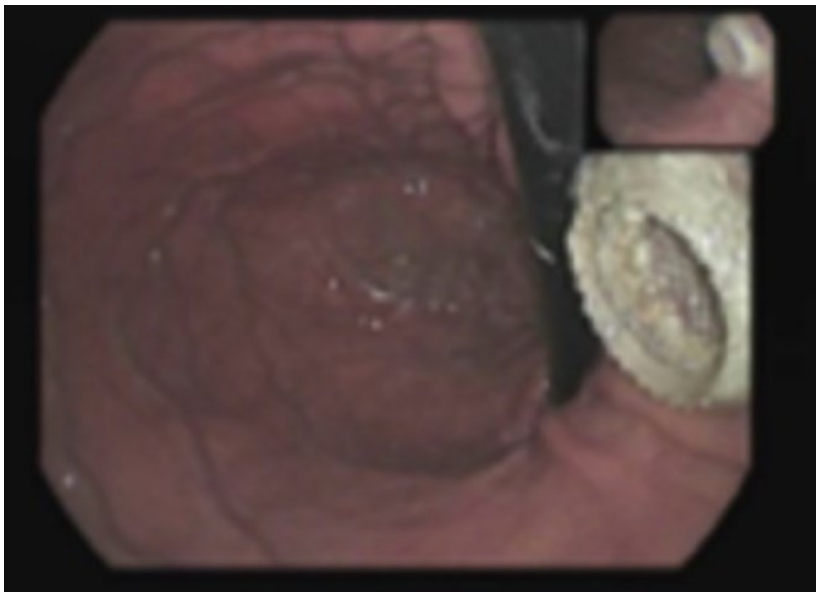


Figure 9: Lumen apposing metal stent (LAMS) as seen from the stomach with the endoscope.

1.633 Surgical bypass

When endoscopic and percutaneous fail or are deemed not possible, biliary decompression can been achieved through surgical biliary bypass procedures. A meta-analysis reported lower rates of recurrent biliary obstruction after surgical bypass when compared with endoscopic biliary stenting in the management of malignant biliary obstruction. Technical success rates and complication rates were also similar(207). Surgical bypass is only suitable for surgical candidates, limiting its use in frail patients and those with carcinomatosis.

1.64 Novel endoscopic treatment approaches

Patients with advanced CCA and pancreatic cancer have a very poor prognosis. Meta-analyses have not shown significant improvement with standard chemoradiation regimens(208), likely due to an advanced tumour stage at presentation coupled with aggressive cancer biology. However, two novel endoscopic interventions have shown promise in early studies: Photodynamic therapy (PDT) and radiofrequency ablation (RFA).

1.641 Photodynamic therapy

Photodynamic therapy (PDT) is a targeted light-based ablative technique involving intravenous injection of a photosensitising agent followed by endoscopic intraluminal laser irradiation. The agent concentrates within the cancer cells and once activated by the laser light forms reactive oxygen species. These lead to photodamage of intracellular structures and cell membranes causing cancer cell apoptosis and necrosis. A previous randomised controlled trial of PDT when compared to biliary stenting alone showed a significant increase in survival time from 98 days to 493 days(209). Another RCT showed median survival increasing from 210 days to 630 days(210). A recent meta-analysis appraised the currently available data for the use of PDT and RFA in unresectable distal CCA. A total of 55 studies (2146 patients) were included. In total, 1149 patients

underwent PDT (33 studies), 545 underwent RFA (22 studies), and 452 patients were treated with a stent-only approach. The pooled survival rate with PDT, RFA, and stent groups was 11.9 [95% confidence interval (CI): 10.7-13.1] months, 8.1 (95% CI: 6.4-9.9) months, and 6.7 (95% CI: 4.9-8.4) months, respectively. The pooled time of stent patency with PDT, RFA, and stent-only groups was 6.1 (95% CI: 4.2-8) months, 5.5 (95% CI: 4.2-6.7) months, and 4.7 (95% CI: 2.6-6.7) months, respectively. The pooled rate of 30-day mortality with PDT was 3.3% (95% CI: 1.6%-6.7%), with RFA was 7% (95% CI: 4.1%-11.7%) and with stent-only was 4.9% (95% CI: 1.7%-13.1%). The pooled rate of 90-day mortality with PDT was 10.4% (95% CI: 5.4%-19.2%) and with RFA was 16.3% (95% CI: 8.7%-28.6%)(211). The UK PHOTOSTENT-02 study randomised 92 patients with biliary tract cancer to either PDT plus stenting or stenting alone. Overall survival was worse in the PDT plus stenting group (6.2 versus 9.8 months). The authors suggested that there is no role for PDT in malignant biliary obstruction, outside of clinical trials (212)

1.642 Radiofrequency ablation

Endoscopic Radiofrequency Ablation (RFA) is a palliative locoregional treatment that allows destruction of tissue through heat. RFA can be used in malignant biliary obstruction either as a first line treatment to improve the bile duct calibre or to treat tumour in growth through ucSEMS(213). It has also been described for ablating residual adenomatous tissue after endoscopic ampullectomy. Currently there are two commercially available probes for use at ERCP over the wire; Habib™ EndoHBP and ELRA™. A catheter inserted into the biliary tree delivers a therapeutic heating zone leading to coagulation necrosis of the malignant stricture. After treatment, a stent should be placed to maintain biliary drainage. Existing studies on the effect on survival and stent patency with RFA are limited to small retrospective studies of mixed cohorts, therefore the exact utility remains largely unknown (214).

Several studies have specifically addressed biliary RFA in case of occluded metal stents. Kadayifci et al.(215) matched patients undergoing endobiliary RFA using a Habib™ endoprobe

inside the SEMS (n=25) to a control group in whom PS were inserted across the occluded SEMS (n=25). Biliary drainage was restored in all 50 patients. Stent patency was evaluated at 90 d, reaching 56% and 24% in the RFA and PS (control) groups, respectively. Additionally, stent patency was significantly longer in the RFA group compared to the control group (119.5 d vs 65.3 d, P = 0.03). There was no significant difference in the 30-d mortality rate and 3- and 6-mo survival rates between the RFA group and controls (P > 0.05). Another retrospective comparison of PDT vs RFA in the palliation of malignant biliary strictures compared results in 48 patients (16 RFA, 32 PDT) demonstrating similar median survival (9.6 mo in RFA, 7.5 mo in PDT)(216). More recently, a small study has shown the combination of RFA and iodine-125 (125I) seed strand brachytherapy (125I-BT) may have utility in the management of tumour ingrowth into SEMS, although larger studies are needed(217).

1.7 Hypotheses:

1) By discussing complex patients with IgG4-related disease in a specialist MDT, they will receive a faster and more accurate diagnosis, have a reduced risk of mis-diagnosis, receive the correct treatment more quickly and have better access to third line medicines i.e. Rituximab as well as access into clinical trials.

2) The utility of SpyGlass cholangioscopy in distinguishing between malignant and benign biliary strictures has been overestimated and when operators are blinded to the clinical history and previous investigations, cholangioscopy is not as useful as previous studies may have suggested, studies in which endoscopists were unblinded.

3) CYFRA and MUC5AC are circulating biomarkers that have been proven to be elevated in patients with CCA and therefore may be elevated in the months or years before a diagnosis of CCA and as such may have utility in the early diagnosis of CCA.

2. Initial experience from the first UK specialist IgG4-RD inter-regional multi-disciplinary meeting

2.1 Background

IgG4-RD is a poorly understood and rare fibro-inflammatory disorder that can occur in almost every organ(218). Accurate and early diagnosis is crucial to restore normal organ function and prevent progression to irreversible fibrosis(219,220). The diagnosis and management of IgG4-RD presents multiple challenges to healthcare professionals. Firstly, the archetypical presentation with mass-forming lesions or strictures is difficult to differentiate from cancer, whilst organ-specific disease patterns may mimic other immune-mediated chronic inflammatory pathologies(67,221). This can lead to inappropriate surgical resection for presumed cancer (18/53 (34%) underwent surgical resection for presumed pancreatobiliary malignancy in one series), delays in treatment (delayed corticosteroids in patients misdiagnosed with PSC) and misinformation regarding prognosis to patients and their families(65,222). Secondly, the clinical presentation varies depending on the organ(s) involved, thus patients may present to a wide range of physicians and/or surgeons who may find it difficult find a unifying diagnosis. Lastly, no single investigation can confirm or exclude the diagnosis, which depends on a combination of clinical, serological, radiological, and histopathological findings(85,223,224). Indeed, serum IgG4 levels are normal in 20-40% of IgG4-RD patients with insufficient sensitivity and specificity for use in isolation(83,225). Diagnostic guidelines often focus on a single organ and depend on biopsy and expert assessment to confirm the diagnosis, both of which can be difficult to obtain(85,223,226). Recent classification criteria developed by the international IgG4-RD classification criteria committee with a focus on diagnostic exclusion and less emphasis on histology may be more robust in many cases where tissue is unavailable or interpretation is equivocal(227).

As well as posing diagnostic challenges to clinicians, there is also considerable debate around the optimal treatment strategies in IgG4-RD(228). Observational and randomised studies have shown IgG4-RD to be highly corticosteroid responsive(91). However, relapse is common, occurring in up to 60% of cases(229,230). Furthermore, glucocorticoid-toxicity is frequent in IgG4-RD, with a recent study reporting 31/43 steroid treated patients experiencing steroid-related adverse events(231). Although immunomodulators including azathioprine, tacrolimus and mycophenolate can be prescribed as steroid-sparing agents, there is a paucity of published evidence regarding their efficacy(117). Rituximab, a B-cell depletion agent, has shown promise in patients intolerant of steroids and with refractory IgG4-RD(117). A recent SRMA of 7 cohort studies (101 patients) with IgG4 pancreatobiliary disease showed a pooled rate of complete remission at 6 months of 89%. The pooled relapse rate was 21% rising to 36% in those with multi-organ involvement(121). Another SRMA of 15 studies and 1159 patients indicated that rituximab maintenance therapy had the lowest relapse rate of all treatments (OR = 0.10, 95% CI [0.01, 1.63]), whereas glucocorticoids + immunomodulators was associated with a lower relapse rate compared with glucocorticoids alone (OR = 0.39, 95% CI [0.20, 0.80]). Further, patients treated with glucocorticoids + immunomodulators had a higher remission rate than those given glucocorticoids (OR= 3.36, 95% CI [1.44, 7.83]), IM (OR= 55.31, 95% CI [13.73, 222.73]) monotherapies or RTX induction therapy only (OR= 7.38, 95% CI [1.56, 34.94]). The rate of adverse events was comparable among the different treatment groups(232). Larger, randomised-controlled trials are needed to confirm these findings. NHS England has commissioned Rituximab as a third-line drug for IgG4-RD, with implementation of strict criteria including its prescription through a specialist IgG4-RD multi-disciplinary meeting (MDM). Novel therapies under evaluation include iguratimod, abatacept and reukimid, all currently registered for clinical trials in IgG4-RD with the US National Institutes of Health. A recent small (n=10) proof of concept study of abatacept, a T-cell modulator, found variable results, with only 50% of patients having a sustained treatment response in the absence of co-administration of glucocorticoids(233).

To address some of the challenges posed by this disease and to improve the care of patients with IgG4-RD across the UK, we established a cross-site specialist IgG4-RD MDM incorporating a diverse range of medical and surgical specialists to advise on the diagnosis and management of these often-complicated patients. By sharing resources, experience, and ideas into one functional team though group discussion and personalisation of care, MDMs have shown to be important in the diagnosis and management of both malignant and benign diseases(234). The role of MDMs seems increasingly important as the number of available immunomodulatory and biological medicines (and associated high costs) increases. Here I will summarise the overall experience from the first 12 months of our newly formed specialist IgG4-RD MDM, and describe how collective working can improve overall care for patients with IgG4-RD.

2.2 Methods

2.21 Background and formation of the IgG4-RD MDM

Both University College London Hospitals (UCLH) and Oxford University Hospitals (OUH) have extensive experience in managing patients with IgG4-RD, first describing patients with predominantly IgG4-related autoimmune pancreatitis (AIP) and sclerosing cholangitis (IgG4-SC) in 2007(235). Both centres receive referrals from not only their locality but also across the UK. Historically, complex cases have been discussed locally though gastroenterology and hepatopancreatobiliary medicine MDMs in each hospital. Informal discussion was held as required with colleagues from outside specialties. In response to increasing volumes and complexity of referrals as well as emerging controversies on optimal management, we formed a specialist combined UCLH-Oxford IgG4-RD MDM in November 2016. Referrals to the MDM were made directly through the clinical teams at OUH or UCLH or via a dedicated IgG4-RD website.

2.22 Aims of the IgG4-RD MDM

The main aims of the MDM were as follows:

- To set up the MDM infrastructure including rooms, communications, specialist input, administrative support, IT support.
- Establishing a diagnosis of definite or possible IgG4-RD. This required review of clinical, serological, radiological, and histopathological evidence. Where a diagnosis of IgG4-RD was not supported, advice was given for further steps required to achieve this and/or an alternative diagnosis was sought where possible, with referrals made to the appropriate speciality for ongoing care.
- Agreeing a management plan. This could include a watch-and-wait strategy, requesting further blood tests, imaging and/or biopsy, initiating first or second line treatment, discontinuation of treatment, and approving restricted third line treatment e.g., Rituximab.
- Assessment of treatment response
- Recruitment of patients into clinical and translational studies

2.23 Format of the IgG4-RD MDM

Referrals are made on a dedicated pro forma, sent to an MDM email address at each site, and details collected for audit purposes. A 75-minute teleconference meeting takes place once every 6 weeks via a video-link connecting the teams in OUH and UCL. External sites can also dial-in via phone or video-link. The MDM is chaired by consultant physicians at each site. Core members include consultant radiologists, histopathologists, gastroenterologists/hepatologists, rheumatologists and general physicians with an interest in IgG4-RD. Visiting specialists include clinical immunologists; neurologists; haematologists; respiratory physicians; nephrologists; ophthalmologists; ear, nose and throat and oral medicine physicians and surgeons. Research fellows, clinical registrars, junior doctors and students are all encouraged to attend.

All cases that were referred to the MDM coordinator were screened for urgency daily by a lead clinician at each site. In the case of urgent referrals for critically unwell patients for whom delayed discussion may cause harm (eg orbital mass encroaching the orbital nerve, biliary stricture causing symptomatic obstructive jaundice and risk of cholangitis, or lung mass which may be malignant), individual cases were discussed electronically via email and/or in person between the core members of the MDM at each referral site and a decision was reached regarding the need for further specialty input. This included referral for discussion to the relevant weekly local specialty cancer or benign MDM (eg lung mass to lung cancer MDM, pancreatic mass/hilar strictures to HPB MDM, or obstructive uropathy with hydronephrosis to urology MDM). All cases were then put on the IgG4-RD MDM to ensure that both patients and clinicians benefited from the IgG4 multidisciplinary approach.

2.24 Outcome data from the IgG4-RD MDM

MDM outcomes were agreed by consensus and documented on a dedicated proforma. All patients with IgG4-RD were invited to be included in a prospective IgG4-RD national database.

2.25 Diagnostic criteria for IgG4-RD

A diagnosis of IgG4-RD was made using the Japanese comprehensive diagnostic criteria (CDC) for systemic IgG4-RD, essentially incorporating a diffuse or localised mass/swelling and/or stricture in single or multiple organs; raised serum IgG4 levels; and histological findings of marked lymphoplasmacytic infiltration with abundance of IgG4-positive plasma cells, storiform pattern of fibrosis, obliterative phlebitis and variable eosinophils(224). The Boston consensus histopathological criteria for IgG4-RD with a focus on classical morphological findings and IgG4/IgG ratios were applied to all patients with biopsy and resection specimens available(85). In those with isolated HPB disease, the Mayo HISORt (histology, imaging, serology, other organ

involvement, and response to steroid therapy) criteria for AIP and IgG4-SC and the international consensus diagnostic criteria (ICDC) for AIP with a focus on imaging findings were used(223,236). Patients with type 2 AIP were excluded(237). While individual organ criteria have been developed (eg renal and orbital), all are based on the framework of the CDC with individual adjustments for laboratory values (eg hypocomplementaemia in IgG4-related renal disease), specific imaging findings (eg low attenuation cortical nodules and/or wedge-shaped lesions in the kidney), and histopathological findings (eg pulmonary lesions have an obliterative arteritis and fibrosis is not storiform, or orbital lesions have germinal centres)(238,239). Overall, as a team we had an awareness of individual organ manifestations, placed careful emphasis on organ-specific exclusions, with a strong push for tissue wherever possible.

2.26 Laboratory measurements

Routine haematology and biochemistry including differential blood count (eosinophils), liver function, renal function and inflammatory markers were assessed. Total serum IgG and IgG4 were measured by nephelometry. Elevated serum IgG and IgG4 were defined by institution range. Complement proteins (C3 and C4 levels) and serum IgE levels were requested to support diagnosis. Tissue-specific autoantibodies were requested to suggest alternative diagnoses, such as antineutrophil cytoplasmic antibodies, double stranded-deoxyribonucleic acid, anti-Sjögren's-syndrome-related antigen (anti-SS) A (Ro) and anti-SSB (La) antibodies, and cryoglobulins. Faecal elastase was measured in patients with symptoms of pancreatic exocrine insufficiency and requested in asymptomatic patients with pancreatic abnormalities on scan.

2.27 Radiological assessment

To assess multi-organ involvement and subclinical disease, radiological imaging is reviewed including CT of the chest, abdomen and pelvis, MRCP, ERCP, magnetic resonance imaging (MRI) of the head and neck, and PET-CT.

2.28 Histological assessment and tissue immunostaining

All biopsy and resection specimens were assessed for classical morphological features of IgG4-RD, specifically a lymphoplasmacytic infiltrate, storiform fibrosis and obliterative phlebitis (with variable presence of eosinophils) in accordance with consensus histological criteria(240). Tissues were immunostained with IgG and IgG4 monoclonal antibodies. The IgG4 count was reported as the average number of IgG4-positive plasma cells in three high-powered fields. An elevated IgG4 count in a biopsy specimen was defined in accordance with consensus criteria for each organ. In those with an elevated IgG4 count, an IgG4 to total IgG ratio was calculated; an elevated IgG4:IgG ratio was defined as >40%. Histological specimens were also assessed for any features to support an alternative diagnosis (e.g the presence of granulomas, necrosis or dysplasia).

2.3 Results

2.31 Referral patterns: speciality and geographical location

During the three-year observed period (2016-2019) there were 21 meetings including 156 patients referred for a total of 206 MDM discussions (some patients were discussed multiple times). The number of local cases discussed each year stayed constant however the total number of discussions increased year on year due to sharp increases in external referrals to the MDM. Referrals came from a broad range of medical and surgical specialities (Fig 11). Almost one in

three patients (49/156) were referred from either hepatology or pancreatobiliary medicine, representing both the specialist interests of the UCLH and OUH teams and the observed distribution of disease sites in IgG4-RD.

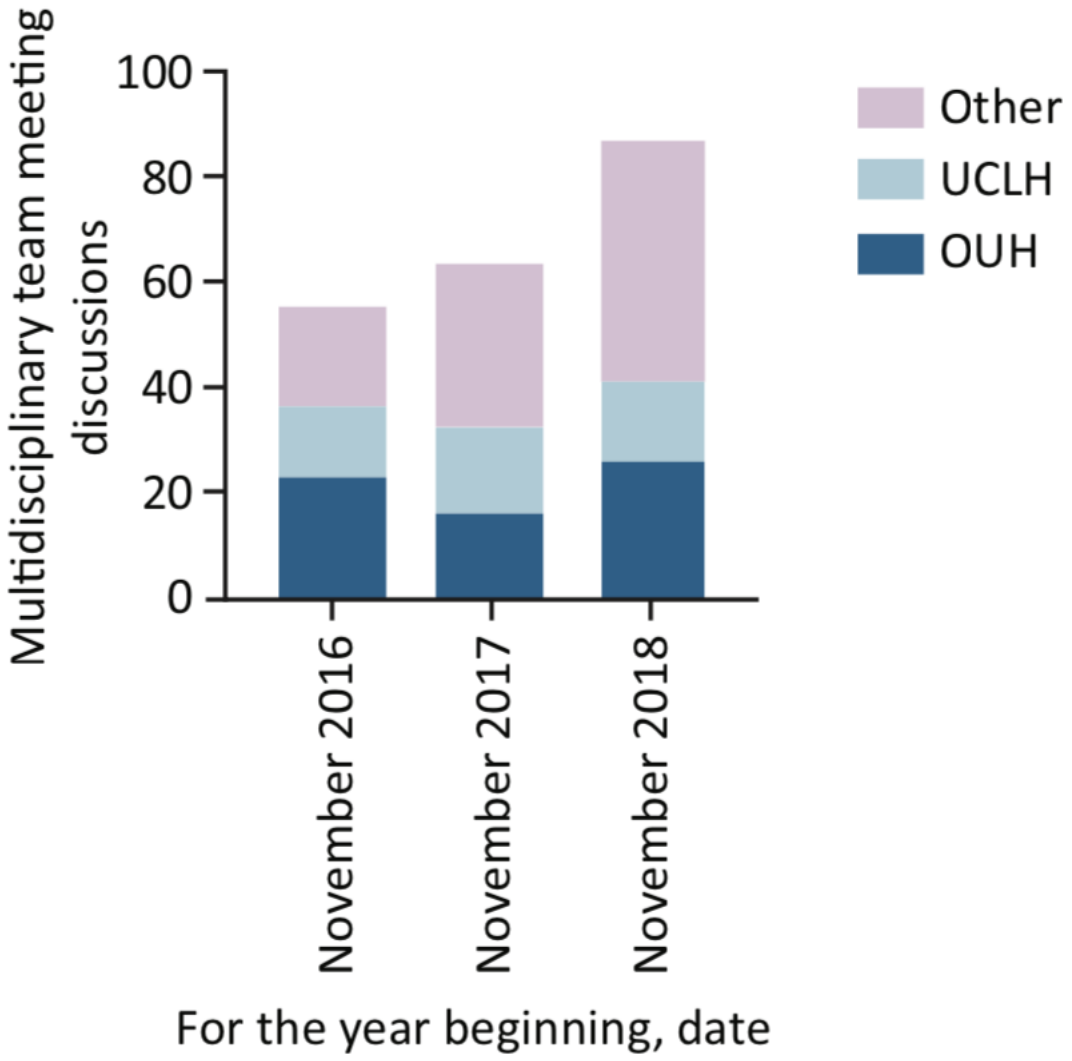


Figure 10. Referral sources to the IgG4-RD Multidisciplinary Meeting.

Referral by speciality. Bar chart with speciality referral on the x-axis and number of cases on the y-axis. Black bars: new referrals. Grey bars: re-discussion. Abbreviations: ENT: Ear, Nose and Throat; HPB: Hepatopancreatobiliary.

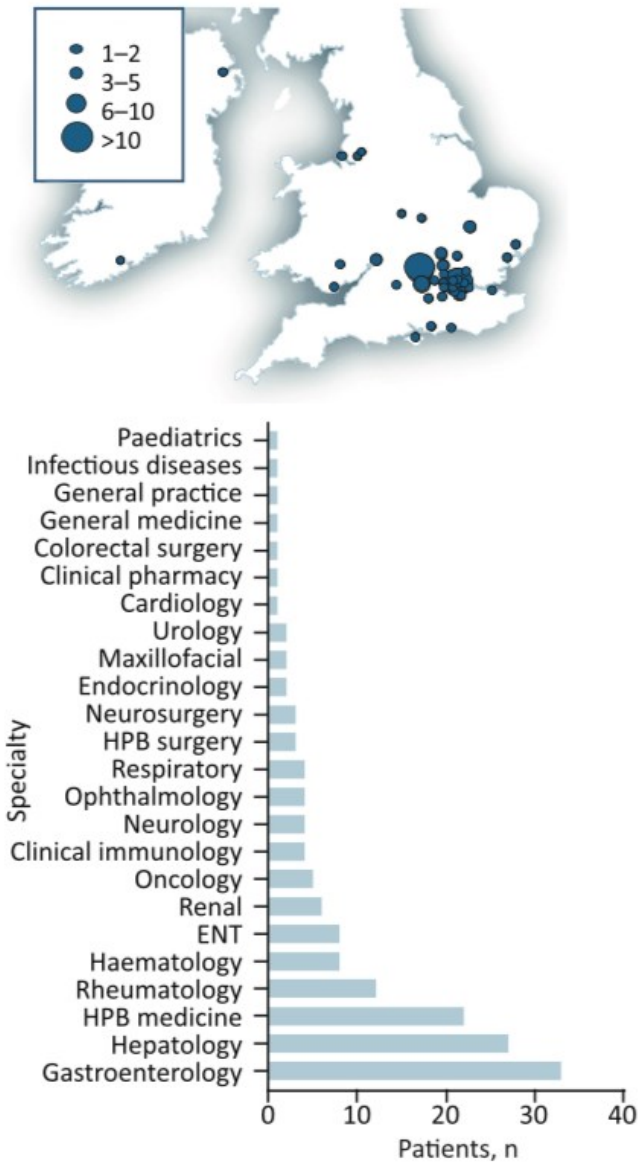


Figure 11: Above: UK map demonstrating geographic location of referral centres. Circles are proportional to referral numbers from each centre. Below: Number of multidisciplinary team meeting case discussions by referring specialty. ENT = ear, nose and throat; HPB = hepatopancreatobiliary.

2.32 Patient referrals: demographics and clinical characteristics

The median age of the 156 patients discussed was 60 years (range 11-90 years), male to female ratio 3.6:1. Serum IgG4 levels had been measured in 136 patients. The majority (n=91; 71%) had an elevated serum IgG4 (sIgG4) (>1 x ULN). 153 patients (98%) had cross-sectional imaging available for review at the MDM and 69% (n=107) had histopathology in the form of either biopsy or surgical resection specimen.

2.33 Referral pathway

Patients had been seen by an average of four specialities at the time of referral to the IgG4-RD MDM. 131 patients (84%) were new referrals to the MDM for either diagnostic clarification or management advice whilst 25 patients were for rediscussing.

2.34 Clinical diagnosis

Of the 156 patients discussed, 97 patients (62%) were given a diagnosis of possible or definite IgG4-RD. 60 met at least one of the diagnostic criteria for IgG4-RD, 37 did not meet the diagnostic criteria but were considered to have possible IgG4-RD by consensus, based on the presence of supportive features. The remaining 59 patients (38%) did not meet diagnostic criteria but were considered not to have IgG4-RD, with alternative diagnoses suggested or sought where possible.

A) Definite IgG4-RD

Sixty patients met diagnostic criteria and were diagnosed with IgG4-RD. Of these, 46 (77%) had an elevated serum IgG4 level. Forty-five (75%) had multiple-organ disease (≥2 organs) confirmed by a combination of clinical signs, laboratory results and radiology. Forty-two patients had a histological sample (resection and/or biopsy) available for review, which was felt to be sufficient to support a diagnosis in 39 cases. Of those with histological samples, 30/42 had ≥2 morphological criteria, IgG4 immunostaining and an IgG4:IgG ratio calculated to meet the Boston histopathological criteria.

B) Possible IgG4-RD

Thirty-seven patients did not meet diagnostic criteria but had supportive features and were diagnosed with possible IgG4-RD. Of these, 22 (59%) had an elevated serum IgG4 level and 20 (54%) had multiple-organ disease. Fifteen had a histological sample (resection and/or biopsy) that was felt to be sufficient to support a diagnosis. We recommended long-term clinical follow-up in all those within this category.

Organ involvement in IgG4-RD may be sub-classified into four broad phenotypic groups(227). Overall, there was good representation from all four groups in those with definite and possible IgG4-RD (Fig 12) with the majority falling into the HPB-dominant disease and systemic disease sub-groups.

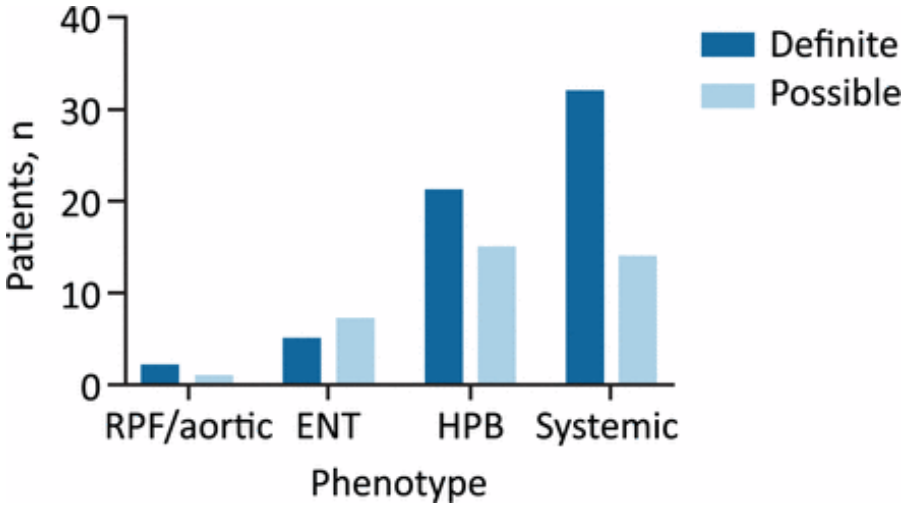


Figure 12: Multidisciplinary team meeting referrals by disease phenotype. Definite and possible immunoglobulin G4-related disease diagnoses. ENT = ear, nose and throat; HPB = hepatopancreatobiliary; RPF = retroperitoneal fibrosis.

C) Not IgG4-RD

Fifty-eight patients (37%) did not meet diagnostic criteria and were considered not to have IgG4-RD, with alternative diagnoses sought. Within this group, the MDM identified seven patients in whom malignancy was the likely diagnosis and, on this basis, further investigations/therapy was planned. This included one patient with inflammatory myelofibroblastic tumour; a disease that is well known to be challenging to differentiate histologically from IgG4-RD. Other notable non-IgG4-RD diagnoses include vasculitis (n=6), sarcoidosis (n=3), Crohn's disease (n=3) and primary sclerosing cholangitis (n=4). In 16 cases, the MDM felt there was not enough supportive evidence for a diagnosis of IgG4-RD but were unable to offer an alternative diagnosis.

2.35 Management advice

In all, there were 206 MDM discussions of the 156 patients and 116 patients were given management advice.

For those patients with definite or possible IgG4-RD, 61/97 had changes to their therapeutic strategy recommended as an outcome of the IgG4-RD MDM. There were 139 management discussions in 97 patients (Fig 13); in 80 (58%) of these, recommendations were made to change treatment. The majority (61/80) were escalations of therapy (addition of any treatment, increase in treatment dose or switch to an alternative treatment). In total, 19 patients were recommended for rituximab. Additional radiological imaging was recommended in 50 cases (36%) including fluorodeoxyglucose PET-CT, MRCP, orbital MRI and CT of the chest/abdomen/pelvis, primarily to investigate sub-clinical organ involvement and/or assess treatment response. A targeted biopsy was recommended to assist diagnosis in 20 cases (14%), with sites identified based on radiological assessment. Additional specialist opinion was sought outside of those present at the MDM in 12 cases (9%).

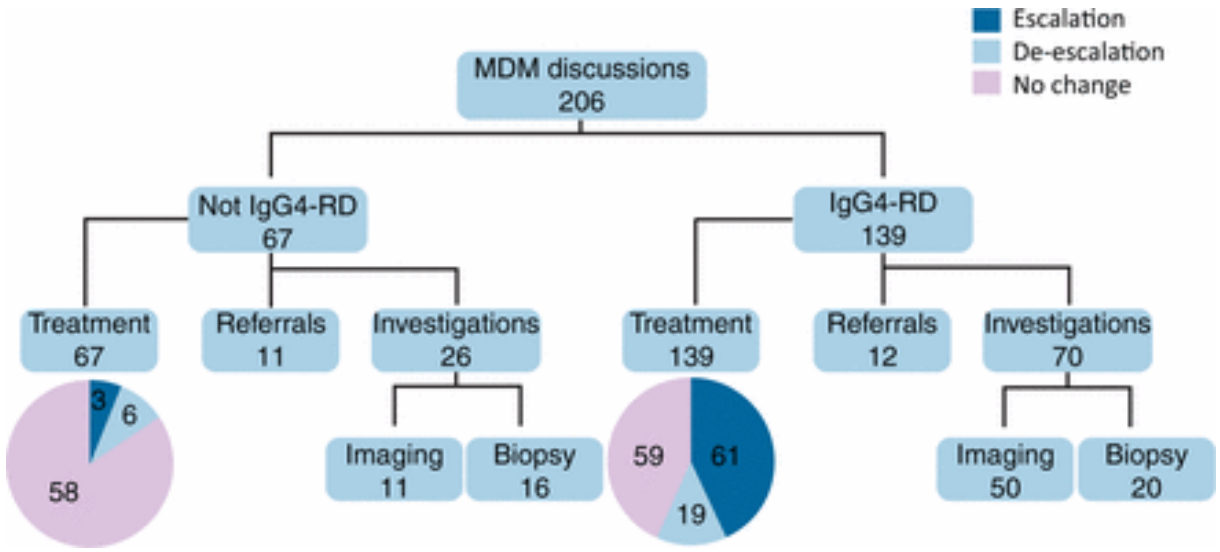


Figure 13: Multidisciplinary team meeting management recommendations for all 206 case discussions. Treatment demonstrates the proportion of patients in each group in which therapy was escalated, de-escalated, or unchanged. IgG4-RD = includes those patients given definite or possible immunoglobulin G4-related disease diagnoses; MDM = multidisciplinary team meeting

A small number of treatment recommendations were made for patients without a diagnosis of IgG4-RD (Fig 13). Primarily, these involved stopping inappropriate therapy, particularly corticosteroids (n=6) or, in a few cases, escalation of therapy (n=3), typically in active vasculitis. Where possible, the MDM recommended appropriate imaging (n=11), biopsy (n=15) or onward referral (n=11).

2.4 Discussion

Our initial experience of delivering a supra-regional IgG4-RD MDM is that it provides an invaluable forum in which to pool expertise to support diagnostic assessment and management. The surprising finding that the MDM was able to refute a suspected diagnosis of IgG4-RD in one-third of cases highlights the importance of such a service. Additionally, the trend of increasing numbers of external referrals to the MDM likely demonstrates an increased awareness of IgG4-RD as a condition with commensurate increase in demand for diagnostic and management advice. This is further demonstrated by looking at referrals before and after the inaugural UK IgG4-RD symposium, held in London in March 2018. The mean number of cases per MDM discussion prior to April 2018 was six rising to 13 between April 2018 and August 2019. With increased awareness of the condition, serum IgG4 testing is becoming more frequent, and yet interpretation of the result is key given the relatively low specificity of serum IgG4 as a diagnostic test. A number of inflammatory and malignant conditions can have an elevated serum IgG4, all of which are important clinical mimics of the disease(83). Furthermore, we demonstrated that 23% of patients given a definite or possible diagnosis of IgG4-RD at MDM had a normal serum IgG4 level. Referrals predominantly arose from pancreatobiliary medicine and hepatology. This reflects a specialty referral bias towards the founding clinicians of our MDM, but is also supported by recent published data sub-classifying clinical disease phenotypes in a multicentre cohort, whereby the pancreas was the most frequent organ involved in IgG4-RD(227). However, we demonstrated a broad coverage of all organs and specialties, incorporating cases from all four disease phenotypes (HPB; retroperitoneum and aorta; limited head and neck; and systemic disease). Indeed, as a more diverse range of specialists have become involved with our service, more detailed imaging to detect sub-clinical disease is performed, and recognition of this rare disease increases, we have seen a steady increase in the number of head and neck, retroperitoneal and aortic referrals over the last year. A particular diagnostic challenge relates to the 26% of new referrals in whom the condition was thought possible but did not meet diagnostic criteria and/or there was insufficient evidence (usually histology) to be certain of the diagnosis. Indeed, a

common scenario in the group of ‘possible’ cases were those patients in whom retrospective review of their clinical cases was highly consistent with IgG4-RD but who had received empirical treatment that rendered subsequent serological and histological results impossible to interpret. This highlights the importance of timely expert review with pre-treatment radiology, serology and histopathology to provide the best chance to reach an accurate diagnosis. The data from our MDM emphasised the importance and challenge of differentiating IgG4-RD from malignancy. In our series, two patients had undergone life-changing treatment for presumed cancer, but a diagnosis of IgG4-RD was eventually reached on the retrospective examination of histology specimens. However, IgG4-RD has also been associated with an increased risk of malignancy itself, meaning that malignancy should still be actively excluded even in the context of a positive IgG4-RD diagnosis(80,241,242). With broader clinician awareness of IgG4-RD, it is also vital that an assumption of IgG4-RD is not made without firm diagnostic grounds, and the pooled expertise of the IgG4-RD MDM may help with this. Once a diagnosis of IgG4-RD is made, treatment can be challenging. The morbidity associated with long-term steroid use is well known, yet data in IgG4-RD for steroid-sparing agents such as azathioprine is lacking, and it is still not clearly defined which patients should receive maintenance treatment and with what. The intent of the MDM is to minimise corticosteroid-related harm and promote second-line immunomodulatory agents when necessary. First-line induction treatment is often with oral corticosteroid therapy (prednisolone). Second-line immunomodulatory treatment includes azathioprine, methotrexate, mycophenolate, mercaptopurine and, in some cases, cyclophosphamide. The decision of which immunomodulator is guided by drug side effect profiles (eg azathioprine preferred in fertile females planning to conceive), familiarity in other disease areas (eg azathioprine in HPB disease, mycophenolate in those with multi-organ and in particular renal disease, and cyclophosphamide in critical orbital disease) and evolving clinical experience. The extensive therapeutic experience of rheumatologists within our MDM has been essential to establish this balance. Disease activity, specific organ ‘urgency’ and evidence of organ damage are important factors in deciding upon

appropriate medication and treatment duration. Critically, previous clinical experience in managing patients with IgG4-RD often influenced MDM treatment decisions among our patients. Thus, faced with a lack of high-quality evidence to guide choice of therapeutics in a rare disease, the collective experience of the MDM becomes increasingly valuable. Furthermore, management of such cases within a specialist MDM facilitates the development of rare disease registries, such as that maintained by our service and aligned with the MDM, which will provide evidence to inform future decisions.

A particular management issue surrounds the use of rituximab, which was recommended for patients following MDM discussion. This is a high-cost treatment with which many gastroenterologists are unfamiliar, but rheumatologists and haematologists are often experienced. The multispecialty involvement of the IgG4-RD MDM is well suited to advising on rituximab use, particularly in cases with HPB disease. It is imperative to ensure that the diagnosis is secure, and that due consideration has been given to all therapeutic options in order to ensure clinically appropriate and equitable access to this medication. In the UK, NHS England has recently commissioned the use of rituximab in IgG4-RD as a third-line therapy to reduce the risk of disease relapse and disease progression. Eligible patients are those with active disease that is not controlled with conventional therapies who either fail to respond to corticosteroids or have adverse reactions/contraindications to corticosteroids plus azathioprine or methotrexate or mycophenolate mofetil. The policy states that rituximab should only be prescribed after approval through a specialist IgG4- RD MDM and recommends data entry into a national registry database, such as ours. The advent of biosimilar agents will make their use more affordable. Our MDM therefore provides an effective forum for patient selection, treatment supervision and monitoring of this treatment. In the years since the MDM was set up, the case numbers have continued to grow, there has been an increase in the number of referral centres nationally and more specialists now dial in and attend the meetings in person. As experience with Rituximab has grown, there has been an increasing trend to move patients across from second line agents to Rituximab,

especially those with complex and multisystem disease in whom experience tells us that relapse is more likely.

3. Diagnostic accuracy and inter-observer agreement of digital single-operator cholangioscopy for indeterminate biliary strictures

3.1 Introduction and Aims

Indeterminate biliary strictures are those where the diagnosis remains elusive after patients have undergone evaluation with standard techniques such as routine biochemistry, tumour markers, cross-sectional imaging and ERCP plus brush cytology +/- biopsy(243). Most indeterminate biliary strictures are cholangiocarcinoma which can be difficult to detect(244). However, several important benign aetiologies do mimic CCA, such as PSC and IgG4-SC/AIP, and it is important to exclude these where possible. As discussed previously, standard diagnostic tools suffer from high specificity but low sensitivity for the diagnosis of CCA. Even combining fluoroscopic brush cytology with intraductal biopsies only results in a slight improvement from 45% to 59%(114). Performing digital single-operator cholangioscopy (d-SOC) may facilitate accurate diagnosis of CCA by allowing direct stricture inspection and furthermore allowing targeted biopsies from the most concerning areas of the stricture. Initial studies of visual impression at the time of dSOC have described several visual features of CCA including dilated, tortuous vessels, polypoid lesions, spontaneous bleeding from friable vessels and a disordered, irregular surface pattern(245–247). One systematic review reported sensitivity and specificity rates between 88.9-97% and 94.5-97.6%, respectively, for the diagnosis of CCA by visual inspection alone. When looking at the histological diagnosis by targeting intraductal biopsies, sensitivity and specificity rates ranged between 57.7-100% and 88.9-100%, respectively(248). These findings would imply that visual impression alone is superior at diagnosing indeterminate biliary strictures than biopsy.

However, this study had several limitations meaning that results and conclusions need to be interpreted with caution(109,245). Firstly, there were five studies included which between them had very few numbers of patients with PSC (the exact patients in whom evaluation of indeterminate biliary strictures can be most challenging). Secondly, there was over-representation of patients with anastomotic strictures post liver transplant. This cohort of patients have an extremely low pre-test probability of CCA and are not representative of patients undergoing dSOC for indeterminate strictures in routine practice. Finally, in none of the included studies were the endoscopists blinded to the clinical history or results of previous investigations. This significant limitation means that endoscopists would have held bias. Given this combination of limitations, we aimed to assess the diagnostic accuracy and inter-observer agreement (IOA) for the diagnosis of CCA by d-SOC in a representative patient population, with a high pre-test probability of cancer, while blinding the endoscopists to clinical information at the time of the performing the dSOC.

3.2 Materials and Methods

3.21 Study design and study population

An international, multicentre, retrospective cohort study was undertaken in participants undergoing d-SOC for the assessment of indeterminate biliary strictures, in 14 European tertiary centres. All participating units included members of the European Cholangioscopy Group (ECG), where each participating endoscopist had extensive experience of independently performing d-SOC.

Fully anonymised dSOC videos of those undergoing d-SOC for assessment of indeterminate biliary strictures were included for video assessment. Indeterminate biliary strictures were defined as: (1) a biliary stricture or filling defect of indeterminate nature after previous laboratory work-up, abdominal imaging (CT, MRI, EUS) or ERCP, with/without (2) negative or inconclusive cytology or histology after ERCP with brush cytology and/or intra-ductal biopsies; but with a persistent clinical suspicion of cancer. Videos without a known final diagnosis based on

cytopathology/histopathology reports and follow-up and of insufficient video quality were excluded from the study. This study was carried out in accordance with the Helsinki Declaration and was approved by the local ethics committee of each participating hospital.

3.22 D-SOC performance and video case selection

All ERCP and d-SOC procedures were carried out by an operator experienced in dSOC, with a minimum requirement of at least 30 independent dSOC procedures prior to the study. D-SOC was performed using the digital single-operator cholangiopancreatoscopy system (SpyGlassTM DS Direct Visualization System or SpyGlassTM DS II Direct Visualization System, Boston Scientific, Natick, Massachusetts, USA). The procedure was undertaken under either conscious sedation, propofol sedation or general anaesthesia, depending on the local protocols. In all patients' prophylactic antibiotics were given either before, during or after the procedure. During d-SOC, videos of the lesions were recorded, demonstrating both the pathological mucosa and the surrounding (normal) physiological biliary mucosa. A malignant biliary stricture was defined as positive histopathology obtained at the time of d-SOC or alternative sampling methods such as EUS-FNB, percutaneous biopsy, surgical resection specimens or autopsy. Benign disease was defined as negative histopathology or where there was an absence of definitive pathology, at least 12 months follow-up demonstrating no progression and no ongoing clinical concerns for cancer. Videos were anonymised to ensure that there was no accompanying cholangiography and no patient-specific or clinical data), with a duration of approximately two minutes per video. In order to help prevent bias of recognition of own procedures, each video was recorded into new study IDs before sending the whole set of videos to all endoscopists.

3.23 Study assessment

After assessing each video separately, a Case Record From (CRF) was filled in. A final diagnosis of malignant or benign was required for each individual case. The assessors were also required

to document the level of certainty of their diagnosis. A numerical scale (1-4) was used to define the level of certainty with '1' being absolutely uncertain and '4' being absolutely certain. Additionally, several cholangioscopic features that have been reported to be associated with malignant and benign strictures, was tabulated(113,249–253). The endoscopists were asked to document whether they thought each feature was present on visual inspection of the stricture.

Each assessor was required to review the full set of videos twice. The first time without knowledge of the past medical history and results of previous tests and results and brushings/biopsies. Once this 'blinded' assessment has been completed and submitted the CRFs a second assessment was carried out. This time, details of patient information, including demographics, medical history, cross-sectional imaging, and laboratory results (including tumour markers), was sent in order to complete the unblinded assessment using the same CRF. Time between blinded and unblinded video assessment varied between the participants and ranged from 1 day to 65 days, with ≥6 days for 15/19 reviewers.

3.24 Study outcomes

The primary outcomes were the diagnostic accuracy and IOA for the diagnosis of malignant biliary strictures by visual inspection at the time of d-SOC, for the blinded and unblinded video evaluation.

Secondary outcomes were the diagnostic accuracy rates when patients with a history of PSC and a previous history of biliary stent placement (prior to undergoing dSOC) were excluded with rates stratified for the level of diagnostic certainty (1-4) and the IOA. An additional outcome was the presence of individual cholangioscopic findings in malignant and benign strictures.

3.25 Statistical analysis

Statistical analysis contained descriptive statistics for patient demographic, serum tumour markers, previous test results, and stricture-related data, using frequencies (%) for categorical variables, mean (SD) for normally distributed continuous variables or median (interquartile range (IQR)) for non-normally distributed continuous variables. Logistic mixed models were used to estimate sensitivity and specificity when considering that measurements were not independent (the same reviewer evaluated all videos).

To estimate the sensitivity, we used only data from only those videos that were pathologically proven cancers with the reviewer's diagnosis (malignant versus benign) as the outcome. To obtain estimates for sensitivity dependent on whether the reviewer was blinded to additional clinical information, we included a fixed effect for the blinding status. The coefficient for the blinding status describes the differences in sensitivity between the blinded and unblinded review. Estimates (and corresponding 95% confidence interval (95% CI)) of the sensitivity under either scenario were obtained from the marginalised fitted values and their standard errors. To estimate specificity, we fitted an analogous model to the subset of videos that were diagnosed as benign (by pathology or follow-up (i.e. true diagnosis) and used the reviewer's diagnosis (benign versus malignant) as the outcome. To investigate sensitivity and specificity about the level of certainty the reviewer had about the diagnosis, we fitted logistic mixed models that additionally included the level of certainty as a categorical (fixed effects) covariate. Moreover, we performed sensitivity analyses for sensitivity and specificity (irrespective of the level of certainty) in the subset of videos of cases without PSC, and in the subset of videos of cases in which no plastic stent was placed prior to cholangioscopy.

The inter-observer agreement was measured using the Fleiss' kappa (κ) statistic along with 95% CIs . Intra-observer agreement was measured using the Cohen's kappa statistics along with 95% CIs. κ statistics were interpreted based on the Landis and Koch convention: poor agreement, ≤0.00; slight agreement, 0.01-0.20; fair agreement, 0.21-0.40; moderate agreement, 0.41-0.60; substantial agreement, 0.61-0.80; almost perfect agreement, 0.81-1.00.

All calculations were performed in R version 4.0.2 (2020-06-22) and using the package GLMMadaptive (0.7.15).

3.3 Results

3.31 Baseline characteristics

In total, 47 videos were submitted by 19 endoscopists. Forty-four videos were deemed eligible for video assessment by the coordinating investigators. Three videos were excluded due to insufficient visual quality.

Twenty-five patients (56.8%) were diagnosed with malignant disease and 19 patients (43.2%) were diagnosed with benign disease. In total, 26 patients (59%) were male, with a mean age of 62.7 years (± 13.8). Most patients had undergone EUS FNA, ERCP with intra-ductal biopsies and/or brush cytology for histopathological examination. The baseline characteristics of the included patients are summarised in table 3.

Table 1. Baseline characteristics of all 44 included patients		
	Definite malignant diagnosis (n=25)	**Definite benign diagnosis (n=19)**
Sex, male, n (%)	11 (44)	15 (78.9)
Age, mean (± SD), years	68 (± 9.8)	55.9 (±15.3)
Medical history		
PSC, n (%)	3 (12)	8 (42.1)
Chronic pancreatitis, n (%)	1 (4)	1 (5.3)
Gallstone disease, n (%)	1 (4)	4 (21.1)
Malignancy, n (%)	0 (0)	1 (5.3)
HPB surgery, n (%)	0 (0)	3 (15.8)
Placement of biliary stents, n (%)	13 (52)	10 (52.6)
Bile duct stone removal, n (%)	1 (4)	3 (15.8)
Stricture location		
Distal CBD, n (%)	4 (16)	3 (15.8)
Proximal CBD, n (%)	7 (28)	3 (15.8)
Hilar, n (%)	9 (36)	5 (26.3)
Intrahepatic left, n (%)	1 (4)	1 (5.3)
Intrahepatic right, n (%)	0 (0)	4 (21.1)
Cystic duct junction, n (%)	4 (16)	2 (10.5)
Multiple locations, n (%)	0 (0)	1 (5.3)
Previous diagnostic work-up		
CA19.9, n (%)	14 (56)	9 (47.4)
Level CA19.9, median (IQR)	72.5 (2 – 17752)	24 (5 – 127)
MRI, n (%)	21 (84)	13 (68.4)
CT, n (%)	22 (88)	15 (78.9)

EUS alone, n (%)	7 (28)	3 (15.8)
EUS with FNA/FNB, n (%)	7 (28)	4 (21.1)
ERCP with intraductal biopsies, n (%)	3 (12)	2 (10.5)
ERCP with brush cytology, n (%)	15 (60)	11 (57.9)
ERCP with intraductal biopsies and brush cytology, n (%)	2 (8)	3 (15.8)

Table 3. Baseline characteristics of all 44 included patients

3.32 Diagnostic accuracy

There were 12 observations with missing mucosal diagnosis. These were excluded from all sensitivity/specificity calculations. Estimated sensitivity and specificity rates are shown in figure 14. There was hardly any difference in sensitivity between blinded and unblinded review. Table 2 shows the odds ratio for a positive diagnosis of a malignant sample in the unblinded setting versus the blinded setting. It is close to 1, with a 95% CI spreading from 0.70 to 1.25, i.e. there was no evidence for a difference in sensitivity in both scenarios. Specificity differed under both scenarios and the odds ratio for a negative diagnosis in a benign sample in the unblinded versus blinded review was 2.09 with a 95% CI ranging from 1.52 to 2.87, meaning that in our data there was strong evidence that specificity is higher in unblinded review than in blinded review.

Figure 14 also shows the sensitivity and specificity rates when patients with PSC were excluded from analysis and when patients with a stent in situ prior to cholangioscopy were excluded. In both scenarios, the specificity increased after unblinding the reviewers for additional clinical information. The odds ratios for a negative diagnosis in a benign sample in the unblinded versus blinded review were 1.89 (95% CI [1.25 – 2.85]) (p = 0.003) and 2.23 (95% CI [1.39 – 3.57]) (p = 0.001), respectively. Again, this provides strong evidence that specificity is higher in the unblinded video review. This is shown in table 4.

Table 4. Comparison of sensitivity and specificity between blinded versus unblinded review

	OR unblinded / blinded	2.5%	97.5%	p-value
Overall				
Sensitivity	0.925	0.691	1.239	0.602
Specificity	2.078	1.511	2.859	0.001
Without PSC				
Sensitivity	1.080	0.789	1.479	0.630
Specificity	1.883	1.245	2.850	0.003
Without plastic stent				
Sensitivity	1.141	0.756	1.721	0.530
Specificity	2.225	1.392	3.559	0.001
Non-hilar strictures				
Sensitivity	1.053	0.732	1.515	0.781
Specificity	3.760	2.263	6.247	0.001

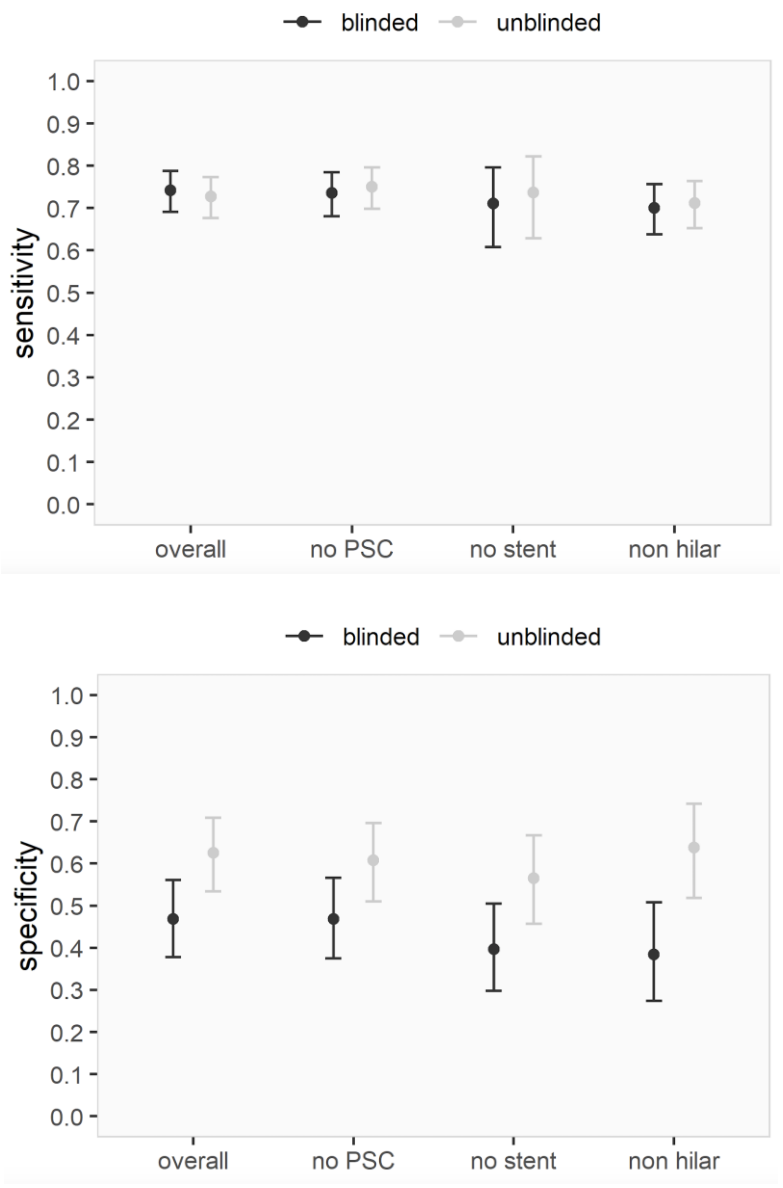


Figure 14. Diagnostic accuracy of d-SOC in the diagnosis of biliary strictures; overall, excluding patients with PSC, excluding patients with a stent in situ prior to d-SOC, and excluding patients with hilar strictures. Top, Sensitivity. Bottom, Specificity.

There were an additional 20 observations with missing level or certainty. They were excluded from the sensitivity/specificity calculations per level of certainty. The diagnostic accuracy rates are summarized in figure 15. Accordingly, when stratified for the reported level of certainty the sensitivity of the blinded video assessment increased from 47.8% to 90.6% (blinded) and from 39.8% to 91% (unblinded), and the specificity decreased from 49.2% to 33.6% (blinded) and from 68.1% to 61.7% (unblinded).

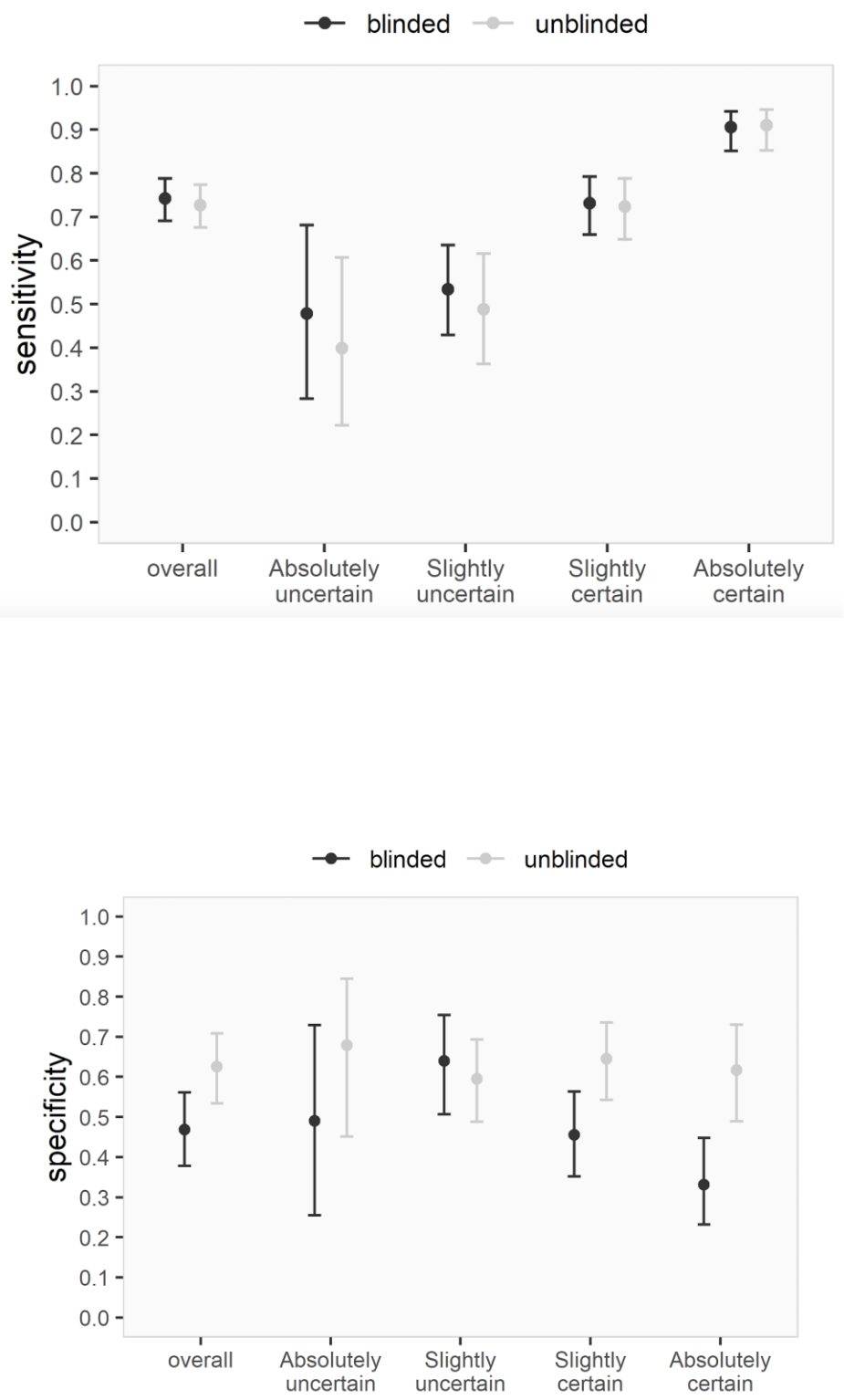


Figure 15. Diagnostic accuracy of d-SOC for the diagnosis of biliary strictures, stratified to the level of certainty. Top, Sensitivity. Bottom, Specificity.

3.33 Cholangioscopic features

Figure 16 shows the proportion of the videos of benign and malignant strictures in which the cholangioscopic features were identified as present by the reviewing endoscopists, for both assessments. Certain cholangioscopic features; focal lesion, raised lesion, irregular nodularity, easy oozing, and dilated tortuous vessels were more frequently identified in videos of malignant strictures as compared to videos of benign strictures.

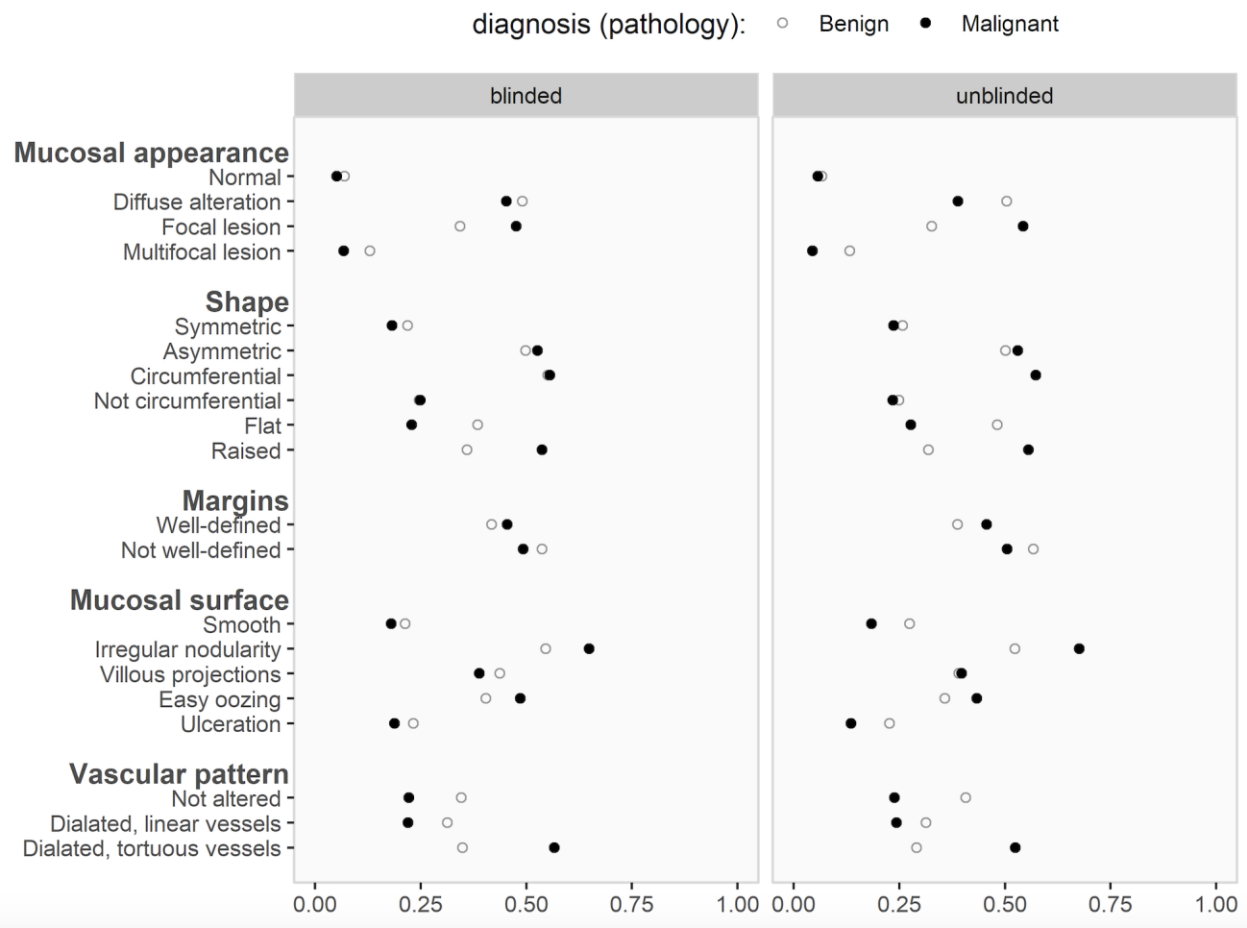


Figure 16. Cholangioscopic findings in benign and malignant strictures, for both blinded and the unblinded video assessment.

3.34 Inter-observer agreement and intra-observer agreement

The IOA for the visual diagnosis of a malignant biliary stricture was fair for both assessments (κ 0.243 (blinded) and κ 0.325 (unblinded)).

The inter-observer agreement for the individual cholangioscopic features ranged from slight to moderate (κ 0.058 – 0.401 (blinded) and κ 0.030 – 0.450 (unblinded). There was moderate agreement for circumferential lesions (κ 0.401) in the blinded assessment and for villous projections (κ 0.450) in the unblinded assessment.

The intra-observer agreement for the visual diagnosis of malignant biliary strictures was moderate (κ 0.454). For the cholangioscopic features this ranged between moderate and substantial (κ 0.464 – 0.700). Figures 17 and 18 show the inter-observer agreement and the intra-observer agreement for the visual diagnosis and for the cholangioscopic features.

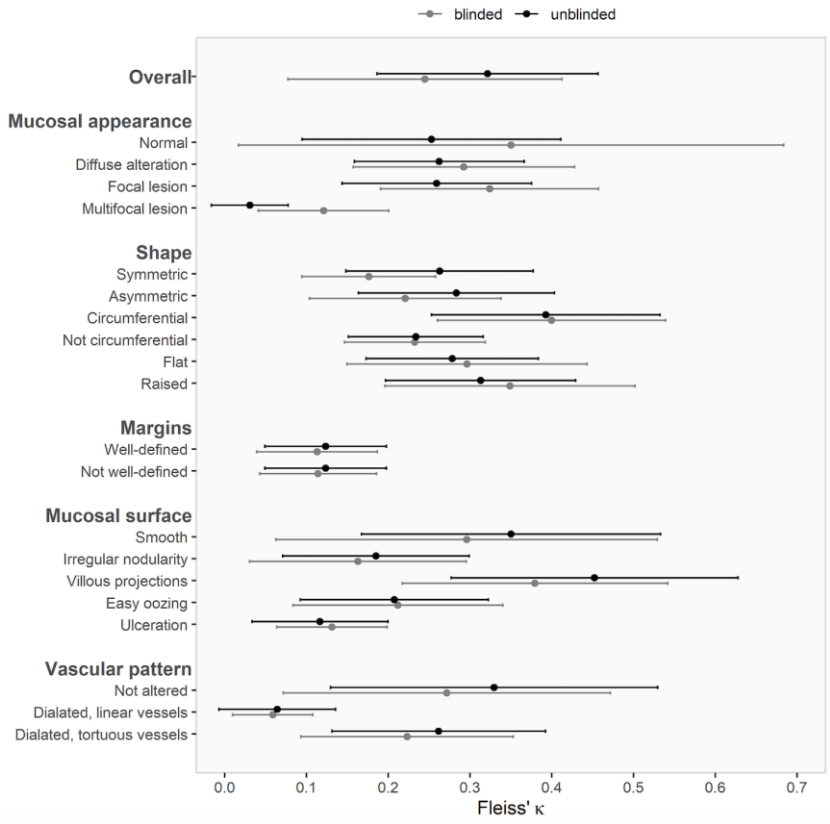


Fig 17. Inter-observer agreement on the diagnosis of malignant strictures and the cholangioscopic features (Fleiss' kappa and corresponding 95% confidence intervals). *Kappa* <0.20 slight agreement, 0.21-0.40 fair agreement, 0.41-0.60 moderate agreement, 0.61-0.80 substantial agreement, >0.81 almost perfect agreement.

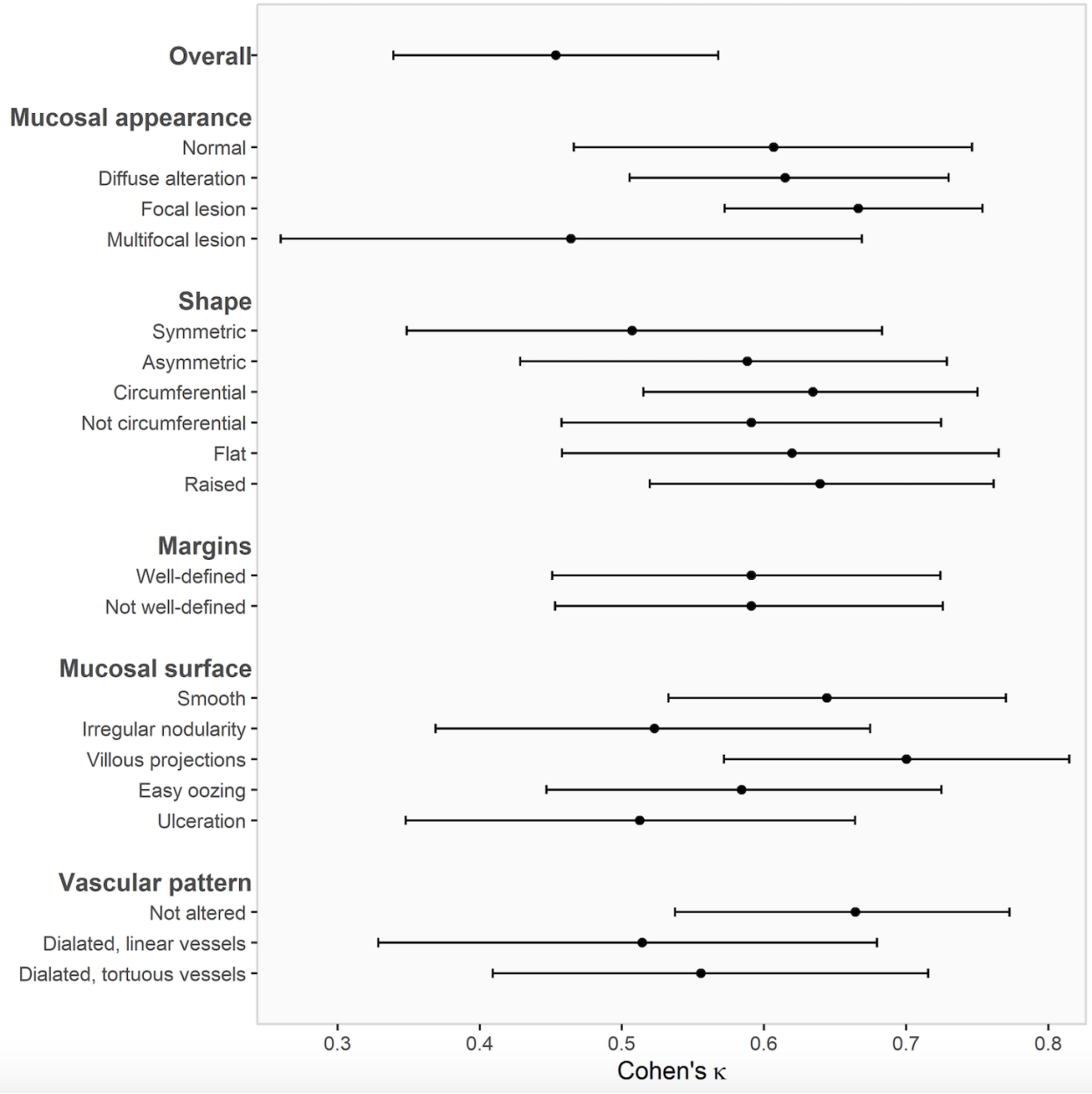


Figure 18. Intra-observer agreement on the diagnosis of malignant strictures and the cholangioscopic features (Cohen's kappa and corresponding 95% confidence intervals). *Kappa* <0.20 slight agreement, 0.21-0.40 fair agreement, 0.41-0.60 moderate agreement, 0.61-0.80 substantial agreement, >0.81 almost perfect agreement.

3.4 Discussion

Previous studies have suggested that dSOC may have utility in the assessment of indeterminate biliary strictures(114,248). However, these studies had several limitations such as study populations not being representative of real-life clinical dilemmas and endoscopists not being blinded to patients' histories and investigation results. This combination means that the real benefit of dSOC is likely to be less than previously described. Our aim was to investigate the true diagnostic utility of dSOC in the assessment of indeterminate biliary strictures.

In our study, we found a sensitivity and specificity of 74.1% and 47.1%, respectively, with blind assessment of cholangioscopic videos and of 72.8% and 63%, respectively, when endoscopists were unblinded to clinical information and results of previous investigations. These results are lower than previously published rates(248). It is our opinion that our results are more likely to reflect the real-world utility of d-SOC for evaluation of indeterminate biliary strictures. Our study demonstrates that when the only diagnostic tool available to endoscopists is the cholangioscopic video, it is challenging to distinguish between malignant and benign biliary strictures based on visual inspection alone. This is even though all endoscopists in our study were experienced in performing dSOC. Once the endoscopists were unblinded, the specificity improved but the sensitivity did not. Therefore, even when all the information is available at the time of dSOC, it is still very difficult to distinguish between benign and malignant biliary strictures. One explanation for this may be that in patients with PSC, the history, clinical information, and results of serological and imaging tests often do not help to discriminate between cancerous and benign strictures, particularly when previous tests have failed to make the diagnosis. The exception is in those patients with a clear history of benign stricture and a low pre-test probability of CCA. In these patients we found increased specificity after unblinding endoscopists with a significant odds ratio of 2.09.

In our study not only did we ask endoscopists to decide on benign versus malignant but also to state their level of certainty in coming to their judgement. This likely reflects real life clinical discussions that happen post-dSOC between endoscopists, surgeons, oncologists, and radiologists within HPB cancer MDMs.

Of interest, absolute certainty as to a cancer diagnosis was associated with a high sensitivity (>90%), but poor specificity (32.6%) when blinded. This means that in clinical practice there is likely an incorrect over-diagnosis of CCA based on dSOC visual impression alone. Unblinding endoscopists improved specify in these cases from 32.6% to 60.6%, reflecting a dampened suspicion of cancer in some cases when additional information was available. The question for us was why does this over diagnosis of CCA occur? We found that in patients with a final diagnosis of both benign and malignant strictures, endoscopists often identified morphological features that have been described in malignant strictures i.e., endoscopists felt that they saw individual features of cancer across both benign and malignant strictures. The presumption from this is that the experienced endoscopists were not good at identifying these features, or that the cholangioscopic features commonly attributed to malignancy may in fact also be observed in benign disease. The latter is supported by our finding that all cholangioscopic features were described in both benign and malignant strictures (figure 16), which suggests the possibility that morphological features may overlap between benign and malignant strictures. In both blinded and unblinded assessments, the endoscopists were asked to document the presence or absence of cholangioscopic features that had previously been described in the literature to be associated with either malignant or benign biliary strictures. Features that have been described more commonly in malignant strictures compared to benign include focal lesions, raised lesions, irregular margins, neovascularisation, easy bleeding, nodularity and dilated tortuous vessels(250,254). However, in our study we found these features often to be identified in benign strictures. Dilated, tortuous vessels, so called ‘tumour vessels’, are most described as predictive

for malignancy. In one prospective study, Kim et al. found a positive predictive value of 100% for malignancy, and crucially, these vessels were not found in benign strictures(250). Contrary to this study, we found that dilated, tortuous vessels were also present in benign strictures (in 33% of our cases). This has also been described by Vries et al(255). Importantly, previous studies did not distinguish between different morphological types of CCA: sclerosing type, nodular type, and papillary type. It is likely that visual features at dSOC do differ between these types therefore this should be considered when developing a classification system for the evaluation of bile duct strictures at dSOC.

Our study is not the first to report low sensitivity and specificity rates for d-SOC. Recently, de Vries et al. demonstrated in a non-blinded study a sensitivity and specificity of 64% and 62%, respectively(255). Like our study, a large proportion of study participants had either PSC or had a stent in situ at the time of d-SOC. In such patients, there is often diffuse mucosal change which can be challenging to distinguish from cancer. To evaluate the effect of including patients with PSC and stents in our study, we carried out further analysis with these patients excluded from analysis. When we did this, we found similar results as for the total group of patients (i.e. with PSC and stents included) which was only an increase in specificity once the assessors were unblinded. This confirms our suspicion that distinguishing between cancerous and non-cancers biliary strictures is challenging and often only possible after unblinding of endoscopists to additional clinical information. The study by de Vries et al. also included patients with stents prior to dSOC found that 71% of the patients with neovascularization were stented prior to dSOC and many did not have a final diagnosis of cancer. This confirms our findings that biliary stenting alters the bile duct mucosa and can lead to features that have previously been described in malignant strictures.

An important outcome of our study was the low inter-observer agreement. Prior to dSOC assessment we did not define the appearance of the previously described cholangioscopic features. For both the blinded and the unblinded assessments, the inter-observer agreement was slight to fair for most features. This shows that, when not predefined, there is no consensus on what is seen or how to describe the individual features. This was also reported by Sethi et al., who also found only slight to fair agreement for various cholangioscopic features and for the overall visual diagnosis(256,257). Given the recent advances in visual acuity at the time of dSOC (in the form of Spyglass DS - Boston Scientific Inc, Natick, Massachusetts, USA), and the quality control of submitted videos, our findings are unlikely to be due to suboptimal visualisation at the time fo dSOC.

Conclusion:

Our results, combined with several previous studies, demonstrate an urgent need for international consensus on the cholangioscopic features of malignant biliary strictures. We have shown that it is not appropriate to rely on visual impression alone to make a confident diagnosis and that good quality biopsies remain crucial in the diagnosis of cancer. Improve methods for taking larger, targeted biopsies may improve the diagnostic role of dSOC in the future.

The main strength of our study is the use of blinded videos with no information provided other than the dSOC video itself. Other strengths are including patients with indeterminate strictures in which distinguishing benign from malignant is known to be challenging, such as PSC and inviting only experienced endoscopists, both of which are likely to mean our study is most relevant to real life clinical practice.

We selected patients with indeterminate strictures, who had pathology that was difficult to define (even with prior ERCP and cytology). We excluded who had previously undergone dSOC earlier on in their pathway. In doing so, we may have inadvertently selected a study group with inherently

challenging strictures in which to make a diagnosis. This study did not address whether dSOC at the time of index ERCP (before stenting) would improve the diagnostic yield. Both contributed to, in our opinion, scenarios which reflect real-world clinical practice. Second, the international, multicentre design, including many participating endoscopists provided reliable inter-observer agreement, which can be translated into other clinical settings.

4. Investigation of two novel tissue biomarkers for the early diagnosis of cholangiocarcinoma

4.1 Introduction and background data

Several serum biomarkers have been shown to accurately distinguish between CCA and PSC, the most well-known of which is CA-19-9. As previously described, CA-19-9 has several limitations. Firstly, it is not useful in the 7% of the population who are Lewis A negative and secondly, it is elevated in the setting of biliary obstruction both in benign and malignant disease. One way to attempt to improve the diagnostic accuracy of CA-19-9 is to combine it with other serum biomarkers. In addition to CA19-9, leucine-rich α2-glycoprotein (LRG1), interleukin 6 (IL6), pyruvate kinase M2 (PKM2), cytokeratin 19 fragment (CYFRA21.1) and mucin 5AC (MUC5AC) have reported utility for differentiating CCA from PSC. Our group has previously demonstrated that CA19-9, PKM2, CYFRA21.1 and MUC5AC were significantly elevated in malignancy versus benign disease (figure 19). Area under the receiver operating characteristic curves for these individual markers ranged from 0.73–0.84, with the best single marker (PKM2) providing 61% sensitivity at 90% specificity. A panel combining PKM2, CYFRA21.1 and MUC5AC gave 76% sensitivity at 90% specificity, which increased to 82% sensitivity by adding gamma- glutamyltransferase (GGT) (figure 20).

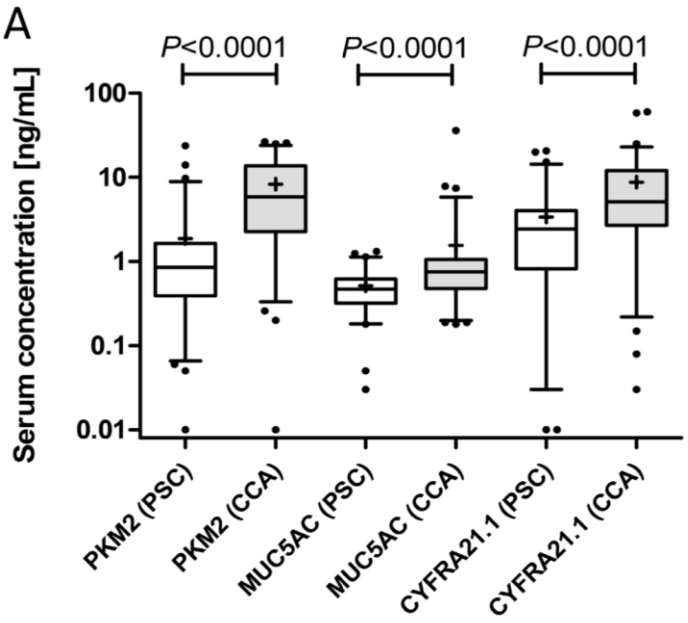


Figure 19. Box and whisker plots of serum levels of PKM2, CYFRA21-1 and MUC5AC in samples from PSC (n=62, white boxes) versus CCA (n=66, grey boxes) (138). Whisker limits represent the 5th and 95th percentiles, the box limits represent the interquartile range, the horizontal line the median and the + the mean. P values (Mann-whitney U test) are shown.

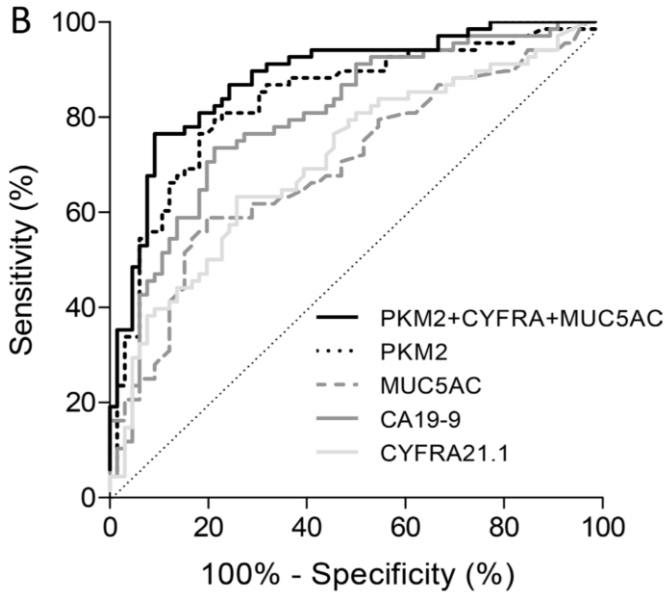


Figure 20. ROC curves of PKM2, CYRFA21-1, CA19-9 and MUC5AC, alone and in combination (138).

Our group have also previously studied the utility of serum biomarkers in the pre-diagnosis setting. To assess the performance of the markers for early diagnosis in CCA assays for ALP, TBIL, CRP, GGT, CA19-9, IL6, LRG1, and PKM2 were undertaken on a set of 89 pre-diagnosis serum samples obtained from 55 cases of CCA and 91 matched cancer-free controls identified from the UKCTOCS biobank (Table 5). The median time from serum collection to diagnosis was 31.5 months. When all samples were considered, CA19-9 (P = 0.002), ALP (P = 0.006), GGT (P = 0.039) and CRP (P = 0.0007) were significantly elevated in CCA pre-diagnosis samples compared to cancer-free controls up to 2 years before diagnosis. TBIL, LRG1, IL6 and PKM2 were not significantly elevated. This chapter is a continuation of this work by extending the study to look at the utility of two further proteins, CYFRA and MUC5AC, at various time points in the pre-diagnostic setting of CCA.

		Biliary tract cancer	Cancer-free controls	*P*-value
Number of cases		55	91	
Number of samples		89	91	
Median age and range (years)		65 (52–75)	62 (50–77)	0.0004
Median time to spin and range (hours)		22.8 (0.5–46.0)	22.6 (1.5–46.0)	0.965
Median time from sample collection to diagnosis and range (months)		31.5 (0.9–66.6)		
Tumour site:	Intrahepatic	29		
	Extrahepatic	4		
	Gall bladder	12		
	Overlapping	10		
Pre-diagnosis time group:	0–1 y	10		
	1–2 y	26		
	2–3 y	19		
	> 3 y	34		

Table 5: Clinical and sample characteristics of UKCTOCS CCA and control study set

4.12 CYFRA and MUC5AC

Cytokeratins are a group of filament proteins whose pattern of expression on malignant cells are usually retained from the cell or origin(258). For this reason, they have been widely used in tumour typing. CYFRA 21-1 is a cytokeratin-19 fragment that is soluble in serum and has been shown to be a clinically useful circulating tumour marker(259). Several studies have reported that it has utility both alone and in combination in the diagnosis and prognosis of several cancers including non-small cell lung cancer (NSCLC), squamous carcinoma of the head and neck and CCA(138,260,261).

Mucin 5AC (MUC-5AC) is a large, secreted gel-foaming glycoprotein. In health, one of its roles is performed in the large airways, where it binds to inhaled pathogens and facilitates mucociliary clearance(262). MUC-5AC is expressed in several cancer types suggesting a potential usefulness in differentiating tumour types(263,264). MUC-5AC is not usually expressed on healthy biliary mucosa but is upregulated in both pre-malignant and malignant biliary tract tumours and can be detected in the serum of some patients with CCA meaning there may be utility in early detection and prognostication(265).

4.2 Materials and methods

4.21 Patients and samples

Serum samples predating a diagnosis of biliary cancer plus matched controls came from patients recruited to the UK Collaborative Trial of Ovarian Cancer Screening(266) (UKCTOCS) and were collected according to a standard operating procedure. This nested case-control study within UKCTOCS was approved by the Joint UCL/UCLH Research Ethics Committee A (Ref. 05/Q0505/57). All volunteers gave informed consent and data was anonymised. Using volunteer NHS numbers, the Health and Social Care Information Centre cancer and death registers were interrogated for UKCTOCS participants who were subsequently diagnosed with CCA or gallbladder cancer (ICD10 codes C22.1/9, C23, C24.0/8). 55 cases of BTC were identified for which 89 samples obtained longitudinally prior

to diagnosis, at four different timepoints (i.e. time groups) (Table 5). Cases were matched with cancer-free controls (*n* = 91) by age (± 5 years), regional collection centre (same) and collection date (same).

4.22 Serum biomarker levels and Enzyme linked immunosorbent assays (ELISA) of candidate markers

The standard blood tests, which included liver biochemistry markers (total bilirubin (TBIL), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT)), C-reactive protein (CRP), and CA19-9 (Cobas CA19-9 CLIA; Roche and Fujirebio Diagnostics), were conducted at the Clinical Biochemistry service at UCLH.

For the measurements of the biomarkers of interest in patient serum samples the following commercial ELISA kits were applied, at respective dilutions and intra-assay coefficients of variation (CVs) as follows: human LRG1 ELISA kit (IBL International, Hamburg, Germany; dilution: 1:2000; CV = 5.5%), human IL-6 ELISA Kit (Pierce Biotechnology, Rockford, IL, USA; dilution: 1:5; CV = 6.7%), human PKM2 ELISA kit (Cloud-Clone Corp. Wuhan, China; dilution: 1:10; CV = 10.3%), human cytokeratin fragment antigen 21-1 (CYFRA21.1) ELISA Kit (Cusabio, Wuhan, China; dilution: 1:5; CV = 8.1%), and human MUC5AC ELISA Kit (Elabscience, Bethesda, MD, USA; dilution: 1:2; CV = 17.4%), and in accordance to manufacturer guidance.

4.23 Statistical analysis and biomarker modelling

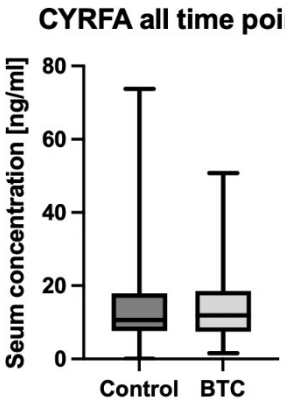
Univariate statistical analyses were performed using GraphPad Prism V5 and R 3.4.1. Continuous data between clinical groups were compared using the Mann- Whitney *U*-test for non-parametric data and the Student *t*-test when data was normally distributed.

Multivariable logistic regression analysis was used to examine the inter- relationship between serum biomarkers, liver function tests, biliary obstruction and cancer likelihood. The number

of variables in combined models was restricted to up to 4 to ensure that performance of models was representative and not suffering from over-fitting. The generalised linear models' glm function available with R (version 4.2.3) was used to create the models. All models were ranked according to their sensitivity at 90% specificity and ties were resolved by the respective ROC AUC leave-one-out cross-validation performance. ROC curves were generated with the *pROC* R package (version 1.18.0, https://cran.r-project.org/web/packages/pROC/index.html). 95% CIs for AUC and sensitivity were determined by stratified bootstrapping. All AUC confidence intervals crossing 0.5 were considered insignificant.

4.3 Results

The individual performance of CYFRA21.1 and MUC5AC in CCA early detection panels: As the first step, the performance of CYFRA21.1 and MUC5AC were individually tested in the study set of 81 malignant cases and 89 healthy control samples, regardless of time to diagnosis. Neither candidate was significantly elevated in malignant cases (median 2.25ng/ml) versus benign controls (median 1.95ng/ml) (p=0.89), figure 21 and 22 below.



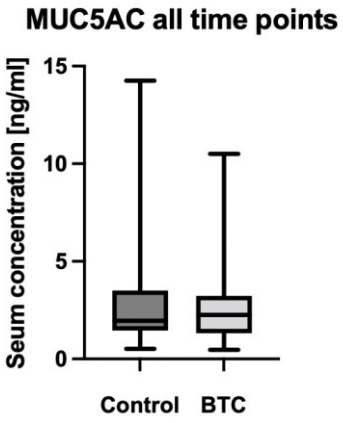
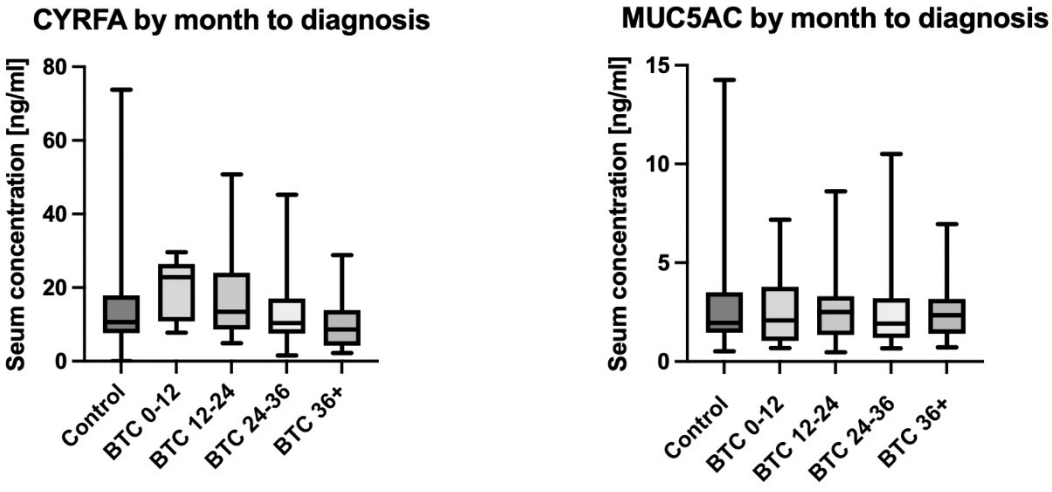


Figure 21 (left) and 22 (right) Serum levels of CYFRA 21.1 (L) and MUC5AC (R) in CCA versus controls. Serum levels of these markers were measured by ELISA and showed no significant differences across the two cohorts. Statistical analysis was performed using the Mann-Whitney test and levels plotted using prism.

Longitudinal performance of CYFRA 21.1 and MUC5A in pre-diagnosis samples

Next, to assess the potential of both candidate biomarkers for early diagnosis of CCA, assays for CYFRA21-1 and MUC5AC were performed on a set of 89 pre-diagnosis serum samples taken from 55 cases of CCA and 91 matched benign controls obtained from the UKCTOCS biobank (table 5). The median time from sample collection to diagnosis was 31.5 months. Serum levels of CYFRA21-1 and MUC5AC in BTC samples, as measured by ELISA (methods and materials section), were comparable to cancer free controls at 0-1 years, 1-2 years, 2-3 years and >3 years prior to diagnosis. When stratifying by time to diagnosis, both MUC5AC and CYFRA remained unchanged across all time groups (figures 23 and 24, below).



Figures 23 (left) and 24 (right): Longitudinal performance of CYFRA 21.1 (L) and MUC5AC (R) in pre-diagnosis samples. Serum levels of these markers were measured by ELISA. There was a significant increase in CYFRA from 36+ months to 0-12 months (Kruskal-Wallis statistic 11.75, p=0.0193). There was no significant difference in MUC5AC at different timepoints before the diagnosis of BTC (Kruskal-Wallis statistic 0.9941, p=0.9107).

3.2.3 Combined logistic regression models

To complement our previous work, combinations of up to 4 candidates using the following markers: TBIL, ALP, GGT, CRP, CA19-9, LRG1, IL6 and PKM2 were next tested in combination with CYFRA21.1 and MUC5AC, using logistic regression models with leave-one-out cross validation. Considering that CA19-9 levels were only significantly elevated 1-2 years before diagnosis but not beyond that point, we decided to determine the performance of our models up to 2 years prior to diagnosis. Panel AUCs and sensitivities at 80% and 90% specificity were determined for each model. Receiver operating characteristic (ROC) curves were constructed for each model to assess diagnostic performance. The AUC for the ROC curves as well as the sensitivities at the pre-determined specificities were used as ranking metrics. The 10 best models according to time to diagnosis are reported in Figure 25 and Tables 6 and 7. A full list of all combinations is provided as supplementary material (supplementary tables).

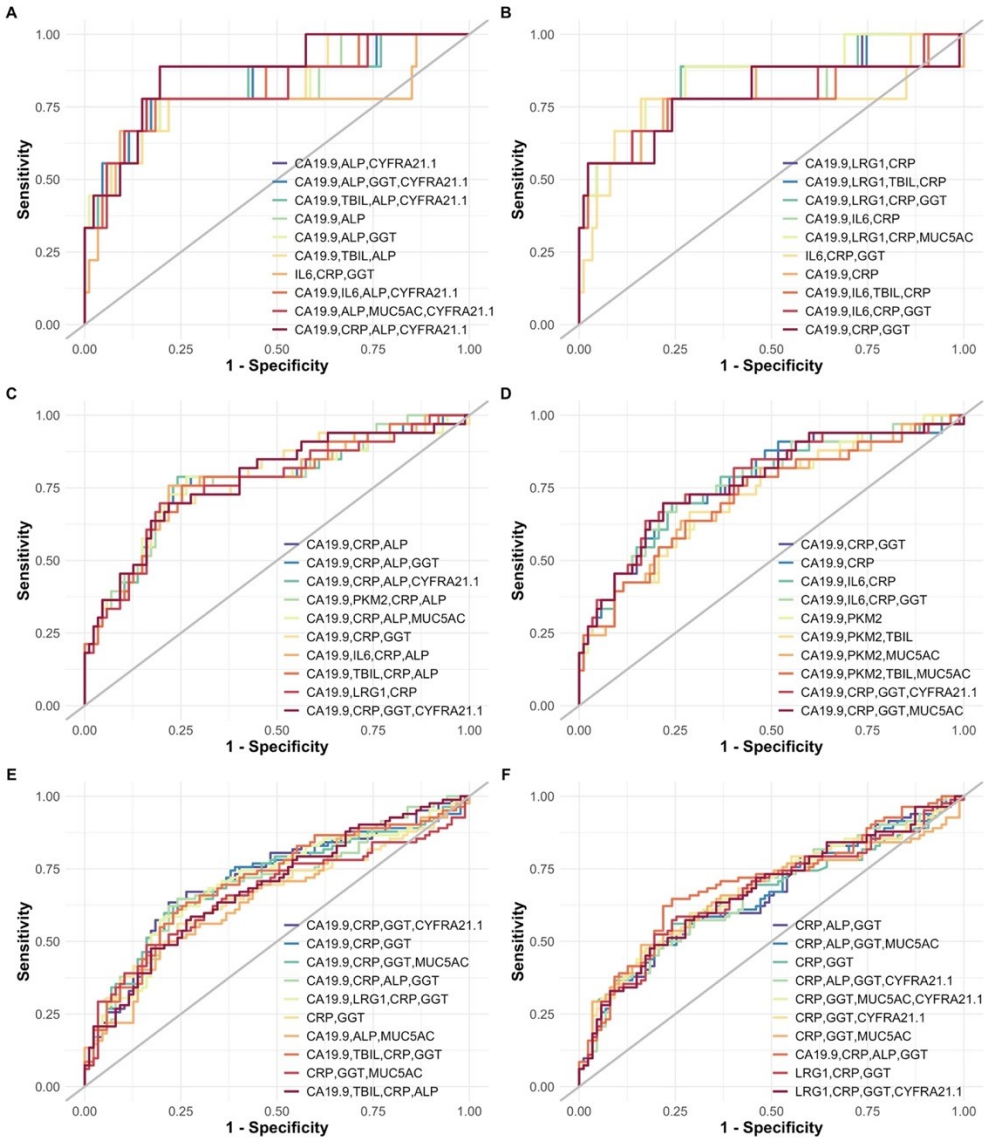


Fig 25. ROC curves for the 10 best logistic regression models according to sensitivity and time to diagnosis. 0-1 years to diagnosis **A**, 0-2 years to diagnosis **C** and all cases **E** models selected by sensitivity at 80% specificity and AUC. 0-1 years to diagnosis **B**, 0-2 years to diagnosis **D** and all cases **F** models selected by sensitivity at 90% specificity and AUC. The classifiers corresponding to this figure were developed by taking all available controls against the cases corresponding to the subgroups in A to F, but they were selected from the pool of all models by their AUC performance and sensitivity with leave-one-out cross validation (see also Supplementary Tables X and X). See also AUC and sensitivity values corresponding to the curves in Tables X and X.

Table 6. Top 10 logistic regression models performance, per time to diagnosis, ranked by leave one out cross validation AUC and sensitivity at 80% specificity.

Model	AUC	Sens	Spec
0-1 years vs All controls (80% specificity)			
CA19.9 , ALP , CYFRA21.1	0.826 (0.641-0.962)	0.778 (0.444-1)	0.8
CA19.9 , ALP , GGT , CYFRA21.1	0.826 (0.639-0.969)	0.778 (0.444-1)	0.8
CA19.9 , TBIL , ALP , CYFRA21.1	0.822 (0.644-0.966)	0.778 (0.333-1)	0.8
CA19.9 , ALP	0.806 (0.628-0.959)	0.778 (0.333-1)	0.8
CA19.9 , ALP , GGT	0.807 (0.624-0.963)	0.778 (0.333-1)	0.8
CA19.9 , TBIL , ALP	0.812 (0.639-0.954)	0.667 (0.333-1)	0.8
IL6 , CRP , GGT	0.762 (0.52-0.957)	0.778 (0.444-1)	0.8
CA19.9 , IL6 , ALP , CYFRA21.1	0.825 (0.646-0.967)	0.778 (0.444-1)	0.8
CA19.9 , ALP , MUC5AC , CYFRA21.1	0.817 (0.637-0.964)	0.778 (0.444-1)	0.8
CA19.9 , CRP , ALP , CYFRA21.1	0.87 (0.736-0.967)	0.889 (0.444-1)	0.8
0-2 years vs All controls (80% specificity)			
CA19.9 , CRP , ALP	0.766 (0.663-0.863)	0.667 (0.364-0.879)	0.8
CA19.9 , CRP , ALP , GGT	0.768 (0.662-0.862)	0.667 (0.394-0.879)	0.8
CA19.9 , CRP , ALP , CYFRA21.1	0.766 (0.657-0.865)	0.636 (0.364-0.879)	0.8
CA19.9 , PKM2 , CRP , ALP	0.77 (0.668-0.863)	0.667 (0.394-0.848)	0.8
CA19.9 , CRP , ALP , MUC5AC	0.763 (0.653-0.858)	0.667 (0.394-0.848)	0.8
CA19.9 , CRP , GGT	0.774 (0.666-0.868)	0.636 (0.364-0.818)	0.8
CA19.9 , IL6 , CRP , ALP	0.772 (0.664-0.865)	0.636 (0.393-0.879)	0.8
CA19.9 , TBIL , CRP , ALP	0.768 (0.661-0.86)	0.636 (0.364-0.848)	0.8
CA19.9 , LRG1 , CRP	0.761 (0.65-0.852)	0.697 (0.364-0.848)	0.8
CA19.9 , CRP , GGT , CYFRA21.1	0.775 (0.672-0.87)	0.636 (0.394-0.818)	0.8

All cases vs All controls (80% specificity)			
CA19.9 , CRP , GGT , CYFRA21.1	0.716 (0.636-0.794)	0.573 (0.329-0.732)	0.8
CA19.9 , CRP , GGT	0.706 (0.622-0.783)	0.537 (0.329-0.695)	0.8
CA19.9 , CRP , GGT , MUC5AC	0.704 (0.621-0.785)	0.573 (0.354-0.695)	0.8
CA19.9 , CRP , ALP , GGT	0.7 (0.621-0.78)	0.537 (0.341-0.72)	0.8
CA19.9 , LRG1 , CRP , GGT	0.702 (0.62-0.78)	0.573 (0.268-0.695)	0.8
CRP , GGT	0.662 (0.578-0.745)	0.488 (0.341-0.634)	0.8
CA19.9 , ALP , MUC5AC	0.656 (0.574-0.735)	0.476 (0.244-0.598)	0.8
CA19.9 , TBIL , CRP , GGT	0.706 (0.624-0.783)	0.549 (0.317-0.683)	0.8
CRP , GGT , MUC5AC	0.663 (0.58-0.748)	0.488 (0.341-0.622)	0.8
CA19.9 , TBIL , CRP , ALP	0.684 (0.606-0.764)	0.476 (0.305-0.622)	0.8

Table 7. Selected logistic regression model performance, per time to diagnosis, ranked by leave one out cross validation AUC and sensitivity at 90% specificity.

Model	**AUC**	**Sens**	**Spec**
0-1 years vs All controls (90% specificity)			
CA19.9 , LRG1 , CRP	0.844 (0.677-0.966)	0.556 (0.222-0.889)	0.9
CA19.9 , LRG1 , TBIL , CRP	0.843 (0.683-0.966)	0.556 (0.222-0.889)	0.9
CA19.9 , LRG1 , CRP , GGT	0.84 (0.663-0.96)	0.556 (0.222-0.889)	0.9
CA19.9 , IL6 , CRP	0.782 (0.549-0.964)	0.556 (0.222-0.889)	0.9
CA19.9 , LRG1 , CRP , MUC5AC	0.848 (0.693-0.971)	0.556 (0.222-0.889)	0.9
IL6 , CRP , GGT	0.762 (0.536-0.962)	0.667 (0.222-0.889)	0.9
CA19.9 , CRP	0.791 (0.55-0.964)	0.556 (0.222-0.889)	0.9

CA19.9 , IL6 , TBIL , CRP	0.779 (0.567-0.969)	0.556 (0.222-0.889)	0.9
CA19.9 , IL6 , CRP , GGT	0.785 (0.548-0.967)	0.556 (0.222-0.889)	0.9
CA19.9 , CRP , GGT	0.788 (0.576-0.958)	0.556 (0.222-0.889)	0.9
0-2 years vs All controls (90% specificity)			
CA19.9 , CRP , GGT	0.774 (0.673-0.869)	0.455 (0.212-0.636)	0.9
CA19.9 , CRP	0.764 (0.652-0.859)	0.455 (0.212-0.667)	0.9
CA19.9 , IL6 , CRP	0.764 (0.657-0.86)	0.455 (0.212-0.636)	0.9
CA19.9 , IL6 , CRP , GGT	0.772 (0.675-0.869)	0.455 (0.212-0.636)	0.9
CA19.9 , PKM2	0.722 (0.612-0.826)	0.394 (0.152-0.576)	0.9
CA19.9 , PKM2 , TBIL	0.723 (0.611-0.824)	0.394 (0.152-0.576)	0.9
CA19.9 , PKM2 , MUC5AC	0.721 (0.608-0.827)	0.394 (0.152-0.576)	0.9
CA19.9 , PKM2 , TBIL , MUC5AC	0.719 (0.603-0.826)	0.394 (0.152-0.576)	0.9
CA19.9 , CRP , GGT , CYFRA21.1	0.775 (0.667-0.869)	0.455 (0.212-0.636)	0.9
CA19.9 , CRP , GGT , MUC5AC	0.77 (0.666-0.86)	0.455 (0.212-0.636)	0.9
All cases vs All controls (90% specificity)			
CRP , ALP , GGT	0.66 (0.573-0.743)	0.366 (0.159-0.476)	0.9
CRP , ALP , GGT , MUC5AC	0.659 (0.575-0.739)	0.366 (0.171-0.476)	0.9
CRP , GGT	0.662 (0.572-0.743)	0.366 (0.232-0.5)	0.9
CRP , ALP , GGT , CYFRA21.1	0.666 (0.585-0.746)	0.354 (0.146-0.476)	0.9
CRP , GGT , MUC5AC , CYFRA21.1	0.676 (0.591-0.752)	0.354 (0.22-0.5)	0.9
CRP , GGT , CYFRA21.1	0.676 (0.587-0.755)	0.341 (0.207-0.5)	0.9
CRP , GGT , MUC5AC	0.663 (0.577-0.74)	0.354 (0.22-0.512)	0.9
CA19.9 , CRP , ALP , GGT	0.7 (0.619-0.778)	0.378 (0.146-0.5)	0.9
LRG1 , CRP , GGT	0.667 (0.586-0.746)	0.329 (0.183-0.452)	0.9
LRG1 , CRP , GGT , CYFRA21.1	0.671 (0.59-0.748)	0.329 (0.183-0.463)	0.9

When computed at 80% specificity, CYFRA21.1 was the marker that featured most in the top models in the 0-1 year to diagnosis group (figure x A, table x). The panel which included CA19-9, ALP and CYFRA21.1 was ranked on top in this group and reached an AUC (cross validated) of 0.826 (95% CI 0.641-0.962) and sensitivity of 0.778 (95% CI 0.444-1). The addition of GGT to this panel did not improve on its performance in a four-marker panel [AUC 0.826 (95% CI 0.639-0.969) and sensitivity 0.778 (95% CI 0.444-1). Similarly, a four-marker panel which included CA19-9, TBIL, ALP and CYFRA21.1 showed comparable performance with an AUC of 0.822 (95% CI 0.644-0.966) and sensitivity of 0.778 (95% CI 0.333-1). A panel which included both CYFRA21.1 and MUC5AC (in combination with CA19-9 and ALP) was ranked fifth in the 0-1 years to diagnosis with an AUC of 0.817 (95% CI 0.637-0.964) and panel sensitivity for CCA of 0.778 (95% CI 0.444-1). CA19-9 (as a single marker) predicted CCA with an AUC of 0.658 and a sensitivity of 0.55 (at 80% specificity).

In the 0-2 years to diagnosis, however, and when computed at 80% specificity, the top model included CA19-9, CRP and ALP [AUC 0.766 (95% CI 0.663-0.863) and sensitivity 0.667 (95% CI 0.364-0.879). The addition of CYFRA21.1 or MUC5AC did not result in significant improvement in performance in this group [AUC 0.766 (95% CI 0.657-0.865), sensitivity 0.636 (95% CI 0.364-0.879) and 0.763 (95% CI 0.653-0.858), sensitivity 0.667 (95% CI 0.394-0.848), respectively]. CA19-9 (as a single marker) predicted CCA with an AUC of 0.658 and a sensitivity of 0.55 (at 80% specificity). As a single marker, CA19-9 reached an AUC of

Across all samples regardless of time to diagnosis (and at 80% specificity), the top performing model included CA19.9 , CRP , GGT , CYFRA21.1 with an AUC 0.716 (95% CI 0.636-0.794) and panel sensitivity of 0.573 (95% CI 0.329-0.732). An AUC of 0.704 (95% CI 0.621-0.785) and sensitivity of 0.573 (95% CI 0.354-0.695) was reported when CYFRA21.1 was substituted with MUC5AC, yet no combination which included both these markers were ranked as top performing models.

Interestingly, when computing the multivariate logistic regression models at 90% specificity, CYFRA21.1 no longer featured in the top performing models (table x). The top model in the 0-1 years to diagnosis cohort included CA19-9, LRG1 and CRP. This three-marker panel reached an AUC of 0.844 (95% CI 0.677-0.966) and sensitivity of 0.556 (95% CI 0.222-0.889). The addition of MUC5AC to these biomarkers did not increase the performance of the panel [AUC 0.848 (95% CI 0.693-0.971) and sensitivity of 0.556 (95% CI 0.222-0.889)]. This combination was ranked 5th in its performance in this time group (0-1 years to diagnosis) (table x). In comparison, CA19-9 sensitivity was 0.444 (AUC 0.658) in this time group and at 90% specificity.

In the two years to diagnosis group, the combined panel of CA19-9, CRP and GGT was the top performer and predicted CCA with a sensitivity of 0.455 (0.212-0.636) with an AUC of 0.774 (0.673-0.869). The addition of CYFRA21.1 or MUC5AC to this panel, however, did not result in improvement in performance.

Regardless of time to diagnosis, the best predictor of CCA across all samples at 90% specificity was the panel which included CRP, ALP and GGT with an overall panel sensitivity of 0.366 (0.159-0.476) with an AUC of 0.66 (0.573-0.743). The addition of CYFRA21.1 and MUC5AC resulted in redundant model complexity with comparable performance to the three-marker model. No models which included both CYFRA21.1 and MUC5AC were ranked as top 10 performing at when computed at 90% specificity. The cross validated performance of CA19-9 in comparison showed a sensitivity of 0.485 with an AUC of 0.667 (80% specificity) and 0.30 (AUC 0.666) when computed at 90% specificity.

4.4 Discussion

In our past work we showed that CYRFA21-1 and MUC5AC are significantly elevated in malignant versus PSC serum samples(138). As single markers, CYFRA21.1 (at cut off level of >4 ng/ml) differentiated CCA from PSC with a sensitivity of 65% (95% CI 52.4–76.5) at 75% specificity (95% CI 63.3–85.8) and AUC of 0.732 (95% CI 0.645–0.819) while MUC5AC (at cut off level of >0.67 ng/ml) reached an AUC 0.72 (95% CI 0.631–0.809) and 60.6% sensitivity (95% CI 47.8–72.4) at 82.3 specificity (95% CI 70.5–90.8). In the same study, when using combined logistic regression models, the top performing panel in discriminating CCA from PSC patients included PKM2, CYFRA21.1 and MUC5AC in combination with PKM2 and the liver enzyme GGT. For this four-marker panel, a sensitivity of 81.8% was reached when computed at 90% specificity (AUC 0.903). Table 8 (below) details the performances reached for the single and multivariate logistic regression models.

Table 8: Performance of selected single and multivariate logistic regression models for discriminating CCA and PSC ranked in order of sensitivity at 90% specificity

Model	Sensitivity (%)	Specificity (%)	AUC
PKM2, MUC5AC, CYFRA21.1, GGT	81.8	90.0	0.903
PKM2, CYFRA21.1, TBIL	80.3	90.0	0.868
PKM2, MUC5AC, CYFRA21.1, CRP	77.3	90.0	0.907
PKM2, MUC5AC, CYFRA21.1, ALP	77.3	90.0	0.899
PKM2, MUC5AC, CYFRA21.1, TBIL	75.8	90.0	0.899
LRG1, PKM2, MUC5AC, CYFRA21.1	75.8	90.0	0.899
PKM2, MUC5AC, CYFRA21.1	75.8	90.0	0.899
CA19.9, PKM2, MUC5AC, CYFRA21.1	75.8	90.0	0.897
PKM2, MUC5AC, CRP, ALP	74.2	90.0	0.895
CA19.9, PKM2, TBIL	74.2	90.0	0.867
CA19.9, PKM2, CYFRA21.1	72.7	90.0	0.87
PKM2, CYFRA21.1	71.2	90.0	0.869
CA19.9, PKM2, MUC5AC, CRP	69.7	90.0	0.891
LRG1, PKM2, MUC5AC, GGT	69.7	90.0	0.888
PKM2, CYFRA21.1, ALP	69.7	90.0	0.87
PKM2, CRP, GGT	69.7	90.0	0.866
PKM2, GGT	68.2	90.0	0.866
PKM2, CRP	68.2	90.0	0.85
PKM2, TBIL	68.2	90.0	0.848
PKM2, MUC5AC, CRP	66.7	90.0	0.889
CA19.9, PKM2	66.7	90.0	0.854
CA19.9, PKM2, MUC5AC	65.2	90.0	0.882
PKM2, MUC5AC	63.6	90.0	0.88
PKM2	60.6	90.0	0.842
CA19.9	50.0	90.0	0.801
CA19.9, CYFRA21.1	47.0	90.0	0.765
MUC5AC, CYFRA21.1	45.5	90.0	0.78
CYFRA21.1	40.9	90.0	0.73
CA19.9, LRG1, IL6	36.4	90.0	0.637
MUC5AC	30.3	90.0	0.728

Under the same work, our group also studied and reported on the potential of TBIL, the liver enzymes ALP and GGT, the inflammatory marker CRP, the tumours marker CA19-9, LRG1, IL6 and PKM2 in panels for early diagnosis of BTC, in pre-diagnostic samples (55 cases of BTC and 91 matched cancer-free controls) obtained from the UKCTOCS cohort and sampled at 0-1 years, 1-2 years, 2-3 years and >3 years before a diagnosis of CCA was made. In a comprehensive analysis of all samples, the levels of CA19-9, ALP, GGT, and CRP were significantly elevated in pre-diagnosis samples of biliary tract cancer (BTC) when compared to cancer-free controls (CA19-9: P = 0.002, ALP: P = 0.006, GGT: P = 0.039, CRP: P =

0.0007). Moreover, there was a noticeable increase in the proportion of samples with CA19-9 levels surpassing 37 U/mL (P = 0.038) (Supplementary Table 2). However, the levels of TBIL, LRG1, IL6, and PKM2 did not exhibit a significant elevation. Using the standard cut-off value of > 37 U/mL, CA19-9 demonstrated a sensitivity of 17% with a specificity of 93%. By optimising the cut-off value, the sensitivity increased to 53%, while the specificity decreased to 69%. Upon further analysis, when the samples were categorised based on the time to diagnosis, TBIL, LRG1, IL6, and PKM2 showed no significant changes across the different time groups. ALP levels were elevated only within 1 year before diagnosis, while CA19-9, CRP, and GGT exhibited increasing levels as the diagnosis approached. However, the differences between the cases and controls for GGT narrowly missed reaching statistical significance (Figure 3). CRP and CA19-9 were also significantly elevated 1-2 years before the diagnosis but not beyond that point. Combining LRG1, IL6, and PKM2 with CA19-9 did not yield a significant improvement in the AUC (Area Under the Curve) compared to using CA19-9 alone, considering all samples or only those collected within 1 year before diagnosis (Table 4). Similarly, combining CRP and ALP with CA19-9 did not significantly enhance the AUC compared to CA19-9 alone, whether considering all samples or only those collected within 1 year before diagnosis.

As part of this thesis and to complement our past research, we therefore sought to study the value of CYFRA21.1 and MUC5AC alongside the markers we previously reported on in our publication from 2018(138) in a pre-diagnostic setting, as biomarkers of CCA early detection. This study aimed to investigate the utility of previously proposed biomarkers for early cancer detection in the pre-diagnosis setting. MUC5AC was not able to differentiate malignant from benign disease across all samples nor was it significantly elevated at any time point prior to diagnosis. CYFRA21-1 was significantly elevated in the 12-month prior to a diagnosis of CCA meaning that it may have utility in the early diagnosis of CCA.

5. Conclusion

Indeterminate biliary strictures remain a challenge to clinicians. Two of the most important conditions to recognise promptly are CCA and IgG4-RD, where the only cure for CCA is surgery as opposed to IgG4-RD which responds to immunosuppression. Misdiagnosing and confusing these conditions may carry serious consequences for the patient. There are several novel tools to aid detection of CCA where early diagnosis is crucial. The identification of novel non-invasive diagnostic biomarkers is promising and now requires further evaluation in large, prospective trials. The re-emergence of peroral cholangioscopy alongside several other diagnostic techniques has led to improvements in the diagnostic accuracy of endoscopic assessment of indeterminate biliary strictures, although some previous studies may have over-estimated the ability of visual impression at dSOC to accurately diagnose CCA. Our study showed that using dSOC in the diagnosis of indeterminate biliary strictures gave poor accuracy rates and with significant inter-observer variation. Cancer was over-diagnosed with visual features previously associated with malignant strictures being seen across both benign and malignant strictures. This demonstrates a possible overlap between morphological features in benign and malignant biliary strictures and raises doubt of their ability to distinguish between inflammatory and malignant strictures at the time of dSOC. Our findings show that there remains a need to find international consensus on visual characteristics of malignant biliary stricture but also that optimising visually-directed biopsy techniques may be even more important in improving the diagnostic accuracy of dSOC. In cases where CCA is excluded and/or IgG4 disease is suspected, we have shown that the collective experience and multidisciplinary decision making within an IgG4-RD MDM provides an invaluable forum for ensuring accurate diagnosis and consistency in management. Improvements in the range of biliary access techniques, endobiliary stents and novel ablative treatments along with local therapies have led to significant improvements in the palliation of both pancreaticobiliary malignancy and management of benign aetiologies such as PSC and IgG4-RD.

6. References

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7 Supplementary Information

Supplementary Table 1 10 best multivariate logistic regression models according to sensitivity at 80% specificity and AUC, determined with leave-one-out cross-validation. 95% CIs for AUC and sensitivity were determined by stratified bootstrapping. All AUC confidence intervals crossing 0.5 were considered insignificant.

Model	AUC_LOOCV	Sens_LOOCV	Spec_LOOCV
0-1 years vs All controls (80% specificity)			
CA19.9 , ALP , CYFRA21.1	0.734 (0.504-0.926)	0.667 (0.222-1)	0.8
CA19.9 , ALP , GGT , CYFRA21.1	0.728 (0.481-0.925)	0.667 (0.222-1)	0.8
CA19.9 , TBIL , ALP , CYFRA21.1	0.725 (0.507-0.921)	0.667 (0.111-1)	0.8
CA19.9 , ALP	0.722 (0.484-0.926)	0.667 (0.222-1)	0.8
CA19.9 , ALP , GGT	0.719 (0.483-0.92)	0.667 (0.222-0.889)	0.8
CA19.9 , TBIL , ALP	0.716 (0.474-0.912)	0.667 (0.222-0.889)	0.8
IL6 , CRP , GGT	0.706 (0.466-0.916)	0.667 (0.222-1)	0.8
CA19.9 , IL6 , ALP , CYFRA21.1	0.706 (0.483-0.898)	0.667 (0.222-0.889)	0.8
CA19.9 , ALP , MUC5AC , CYFRA21.1	0.699 (0.409-0.922)	0.667 (0.222-1)	0.8
CA19.9 , CRP , ALP , CYFRA21.1	0.699 (0.452-0.912)	0.667 (0.222-0.889)	0.8
0-2 years vs All controls (80% specificity)			
CA19.9 , CRP , ALP	0.731 (0.622-0.835)	0.636 (0.303-0.818)	0.8
CA19.9 , CRP , ALP , GGT	0.723 (0.601-0.83)	0.636 (0.303-0.818)	0.8
CA19.9 , CRP , ALP , CYFRA21.1	0.722 (0.603-0.83)	0.636 (0.273-0.788)	0.8
CA19.9 , PKM2 , CRP , ALP	0.72 (0.598-0.827)	0.636 (0.273-0.818)	0.8
CA19.9 , CRP , ALP , MUC5AC	0.715 (0.591-0.828)	0.636 (0.303-0.788)	0.8
CA19.9 , CRP , GGT	0.726 (0.61-0.83)	0.606 (0.333-0.818)	0.8
CA19.9 , IL6 , CRP , ALP	0.722 (0.61-0.826)	0.606 (0.303-0.818)	0.8
CA19.9 , TBIL , CRP , ALP	0.717 (0.6-0.823)	0.606 (0.273-0.788)	0.8

CA19.9 , LRG1 , CRP	0.714 (0.595-0.82)	0.606 (0.303-0.788)	0.8
CA19.9 , CRP , GGT , CYFRA21.1	0.711 (0.597-0.816)	0.606 (0.333-0.788)	0.8
All cases vs All controls (80% specificity)			
CA19.9 , CRP , GGT , CYFRA21.1	0.676 (0.589-0.758)	0.537 (0.293-0.671)	0.8
CA19.9 , CRP , GGT	0.669 (0.588-0.751)	0.5 (0.256-0.646)	0.8
CA19.9 , CRP , GGT , MUC5AC	0.652 (0.57-0.738)	0.5 (0.28-0.659)	0.8
CA19.9 , CRP , ALP , GGT	0.659 (0.57-0.743)	0.476 (0.293-0.671)	0.8
CA19.9 , LRG1 , CRP , GGT	0.654 (0.571-0.735)	0.476 (0.232-0.634)	0.8
CRP , GGT	0.618 (0.528-0.706)	0.476 (0.305-0.61)	0.8
CA19.9 , ALP , MUC5AC	0.614 (0.53-0.698)	0.476 (0.195-0.573)	0.8
CA19.9 , TBIL , CRP , GGT	0.656 (0.569-0.738)	0.463 (0.256-0.634)	0.8
CRP , GGT , MUC5AC	0.605 (0.514-0.69)	0.463 (0.305-0.585)	0.8
CA19.9 , TBIL , CRP , ALP	0.642 (0.557-0.723)	0.451 (0.22-0.585)	0.8

Supplementary Table 2 10 best multivariate logistic regression models according to sensitivity at 90% specificity and AUC, determined with leave-one-out cross-validation. 95% CIs for AUC and sensitivity were determined by stratified bootstrapping. All AUC confidence intervals crossing 0.5 were considered to be insignificant.

Model	AUC_LOOCV	Sens_LOOCV	Spec_LOOCV
0-1 years vs All controls (90% specificity)			
CA19.9 , LRG1 , CRP	0.736 (0.526-0.911)	0.444 (0.111-0.778)	0.9
CA19.9 , LRG1 , TBIL , CRP	0.719 (0.503-0.904)	0.444 (0.111-0.778)	0.9
CA19.9 , LRG1 , CRP , GGT	0.713 (0.499-0.89)	0.444 (0.111-0.778)	0.9
CA19.9 , IL6 , CRP	0.709 (0.443-0.926)	0.444 (0.111-0.778)	0.9
CA19.9 , LRG1 , CRP , MUC5AC	0.708 (0.462-0.897)	0.444 (0.111-0.778)	0.9
IL6 , CRP , GGT	0.706 (0.458-0.911)	0.444 (0.111-0.778)	0.9

CA19.9 , CRP	0.704 (0.433-0.931)	0.444 (0.111-0.778)	0.9
CA19.9 , IL6 , TBIL , CRP	0.702 (0.451-0.926)	0.444 (0.111-0.778)	0.9
CA19.9 , IL6 , CRP , GGT	0.701 (0.429-0.922)	0.444 (0.111-0.778)	0.9
CA19.9 , CRP , GGT	0.699 (0.451-0.923)	0.444 (0.111-0.778)	0.9
0-2 years vs All controls (90% specificity)			
CA19.9 , CRP , GGT	0.726 (0.61-0.825)	0.455 (0.212-0.636)	0.9
CA19.9 , CRP	0.729 (0.618-0.834)	0.424 (0.182-0.606)	0.9
CA19.9 , IL6 , CRP	0.726 (0.613-0.83)	0.424 (0.152-0.606)	0.9
CA19.9 , IL6 , CRP , GGT	0.704 (0.584-0.815)	0.424 (0.212-0.606)	0.9
CA19.9 , PKM2	0.68 (0.56-0.783)	0.394 (0.121-0.545)	0.9
CA19.9 , PKM2 , TBIL	0.657 (0.538-0.774)	0.394 (0.121-0.545)	0.9
CA19.9 , PKM2 , MUC5AC	0.656 (0.53-0.761)	0.394 (0.121-0.545)	0.9
CA19.9 , PKM2 , TBIL , MUC5AC	0.639 (0.516-0.755)	0.394 (0.121-0.545)	0.9
CA19.9 , CRP , GGT , CYFRA21.1	0.711 (0.601-0.816)	0.364 (0.182-0.576)	0.9
CA19.9 , CRP , GGT , MUC5AC	0.701 (0.572-0.809)	0.364 (0.182-0.606)	0.9
All cases vs All controls (90% specificity)			
CRP , ALP , GGT	0.621 (0.537-0.704)	0.341 (0.11-0.439)	0.9
CRP , ALP , GGT , MUC5AC	0.604 (0.517-0.689)	0.329 (0.098-0.451)	0.9
CRP , GGT	0.618 (0.529-0.706)	0.329 (0.195-0.463)	0.9
CRP , ALP , GGT , CYFRA21.1	0.621 (0.533-0.703)	0.329 (0.098-0.439)	0.9
CRP , GGT , MUC5AC , CYFRA21.1	0.612 (0.526-0.696)	0.329 (0.134-0.463)	0.9
CRP , GGT , CYFRA21.1	0.629 (0.54-0.719)	0.329 (0.183-0.476)	0.9
CRP , GGT , MUC5AC	0.605 (0.516-0.689)	0.317 (0.159-0.463)	0.9
CA19.9 , CRP , ALP , GGT	0.659 (0.569-0.739)	0.305 (0.122-0.439)	0.9
LRG1 , CRP , GGT	0.619 (0.528-0.707)	0.305 (0.11-0.427)	0.9
LRG1 , CRP , GGT , CYFRA21.1	0.615 (0.526-0.702)	0.305 (0.134-0.415)	0.9

Supplementary table 2: the performance (AUC, Sensitivity, Specificity) of all panels computed at 80% specificity (CV; Cross validated)

Biomarkers	AUC	Sens	Spec	AUC_CV	Sens_CV	Spec_CV
CA19.9 , CRP , GGT , CYFRA21.1	0.716	0.573	0.8	0.676	0.537	0.8
CA19.9 , CRP , GGT	0.706	0.537	0.8	0.669	0.5	0.8
CA19.9 , CRP , GGT , MUC5AC	0.704	0.573	0.8	0.652	0.5	0.8
CA19.9 , CRP , ALP , GGT	0.7	0.537	0.8	0.659	0.476	0.8
CA19.9 , LRG1 , CRP , GGT	0.702	0.573	0.8	0.654	0.476	0.8
CRP , GGT	0.662	0.488	0.8	0.618	0.476	0.8
CA19.9 , ALP , MUC5AC	0.656	0.476	0.8	0.614	0.476	0.8
CA19.9 , TBIL , CRP , GGT	0.706	0.549	0.8	0.656	0.463	0.8
CRP , GGT , MUC5AC	0.663	0.488	0.8	0.605	0.463	0.8
CA19.9 , TBIL , CRP , ALP	0.684	0.476	0.8	0.642	0.451	0.8

CA19.9 , CRP , ALP , MUC5AC	0.681	0.488	0.8	0.64	0.451	0.8
CA19.9 , ALP	0.654	0.476	0.8	0.625	0.451	0.8
CA19.9 , GGT	0.663	0.524	0.8	0.618	0.451	0.8
CA19.9 , GGT , CYFRA21.1	0.671	0.549	0.8	0.616	0.451	0.8
TBIL , CRP , GGT , MUC5AC	0.667	0.476	0.8	0.59	0.451	0.8
CA19.9 , IL6 , CRP , GGT	0.706	0.5	0.8	0.662	0.439	0.8
CA19.9 , CRP , ALP , CYFRA21.1	0.697	0.463	0.8	0.658	0.439	0.8
CA19.9 , CRP	0.68	0.463	0.8	0.645	0.439	0.8
CA19.9 , TBIL , CRP , CYFRA21.1	0.68	0.5	0.8	0.634	0.439	0.8
CRP , GGT , CYFRA21.1	0.676	0.476	0.8	0.629	0.439	0.8
CA19.9 , ALP , GGT	0.667	0.5	0.8	0.626	0.439	0.8
CA19.9 , ALP , CYFRA21.1	0.662	0.476	0.8	0.622	0.439	0.8
TBIL , CRP , GGT	0.67	0.476	0.8	0.607	0.439	0.8
CA19.9 , CRP , ALP	0.685	0.476	0.8	0.649	0.427	0.8
CA19.9 , PKM2 , CRP , GGT	0.701	0.512	0.8	0.647	0.427	0.8
CA19.9 , PKM2 , CRP , ALP	0.679	0.451	0.8	0.637	0.427	0.8

CA19.9 , ALP , GGT , CYFRA21.1	0.674	0.5	0.8	0.621	0.427	0.8
LRG1 , CRP , GGT , CYFRA21.1	0.671	0.488	0.8	0.615	0.427	0.8
CRP , GGT , MUC5AC , CYFRA21.1	0.676	0.488	0.8	0.612	0.427	0.8
CA19.9 , ALP , MUC5AC , CYFRA21.1	0.663	0.463	0.8	0.612	0.427	0.8
IL6 , CRP , GGT	0.666	0.463	0.8	0.611	0.427	0.8
CA19.9 , LRG1 , CRP , MUC5AC	0.67	0.488	0.8	0.607	0.427	0.8
IL6 , CRP , GGT , CYFRA21.1	0.683	0.488	0.8	0.619	0.415	0.8
LRG1 , CRP , GGT	0.667	0.524	0.8	0.619	0.415	0.8
CA19.9 , ALP , GGT , MUC5AC	0.669	0.512	0.8	0.613	0.415	0.8
CA19.9 , PKM2 , CRP , MUC5AC	0.675	0.488	0.8	0.612	0.415	0.8
CA19.9 , PKM2 , ALP , GGT	0.663	0.512	0.8	0.612	0.415	0.8
CA19.9 , PKM2 , ALP	0.652	0.5	0.8	0.612	0.415	0.8
IL6 , CRP , GGT , MUC5AC	0.669	0.5	0.8	0.599	0.415	0.8
CA19.9 , CRP , CYFRA21.1	0.678	0.451	0.8	0.643	0.402	0.8

CA19.9 , CRP , MUC5AC , CYFRA21.1	0.677	0.463	0.8	0.63	0.402	0.8
CA19.9 , CRP , MUC5AC	0.67	0.488	0.8	0.624	0.402	0.8
CRP , ALP , GGT	0.66	0.476	0.8	0.621	0.402	0.8
LRG1 , CRP , ALP , GGT	0.67	0.427	0.8	0.612	0.402	0.8
TBIL , CRP , GGT , CYFRA21.1	0.675	0.451	0.8	0.611	0.402	0.8
PKM2 , CRP , ALP , GGT	0.665	0.463	0.8	0.607	0.402	0.8
PKM2 , TBIL , CRP , GGT	0.674	0.451	0.8	0.604	0.402	0.8
IL6 , TBIL , CRP , GGT	0.67	0.451	0.8	0.596	0.402	0.8
CA19.9 , GGT , MUC5AC	0.665	0.476	0.8	0.593	0.402	0.8
CA19.9 , IL6 , CRP , ALP	0.696	0.463	0.8	0.65	0.39	0.8
CA19.9 , IL6 , CRP , MUC5AC	0.677	0.463	0.8	0.621	0.39	0.8
CRP , ALP , GGT , CYFRA21.1	0.666	0.451	0.8	0.621	0.39	0.8
PKM2 , CRP , GGT	0.672	0.439	0.8	0.616	0.39	0.8
CA19.9 , IL6 , ALP , GGT	0.676	0.439	0.8	0.615	0.39	0.8
CA19.9 , TBIL , CRP , MUC5AC	0.673	0.476	0.8	0.611	0.39	0.8

CA19.9 , PKM2 , ALP , CYFRA21.1	0.662	0.476	0.8	0.607	0.39	0.8
CA19.9 , LRG1 , PKM2 , CRP	0.673	0.488	0.8	0.606	0.39	0.8
CRP , ALP , GGT , MUC5AC	0.659	0.488	0.8	0.604	0.39	0.8
CA19.9 , PKM2 , GGT	0.66	0.439	0.8	0.602	0.39	0.8
CA19.9 , PKM2 , GGT , MUC5AC	0.657	0.439	0.8	0.584	0.39	0.8
CA19.9 , IL6 , GGT , MUC5AC	0.668	0.451	0.8	0.583	0.39	0.8
CA19.9 , LRG1 , CRP , ALP	0.685	0.439	0.8	0.639	0.378	0.8
CA19.9 , IL6 , CRP , CYFRA21.1	0.685	0.427	0.8	0.634	0.378	0.8
CA19.9 , PKM2 , CRP	0.678	0.476	0.8	0.625	0.378	0.8
CA19.9 , PKM2 , CRP , CYFRA21.1	0.674	0.415	0.8	0.624	0.378	0.8
CA19.9 , LRG1 , CRP , CYFRA21.1	0.675	0.476	0.8	0.621	0.378	0.8
CA19.9 , IL6 , ALP	0.659	0.439	0.8	0.617	0.378	0.8
IL6 , CRP , ALP , GGT	0.672	0.415	0.8	0.611	0.378	0.8
CA19.9 , TBIL , ALP , GGT	0.667	0.5	0.8	0.611	0.378	0.8
CA19.9 , IL6 , ALP , MUC5AC	0.661	0.463	0.8	0.608	0.378	0.8
LRG1 , TBIL , CRP , GGT	0.675	0.488	0.8	0.606	0.378	0.8

CA19.9 , PKM2 , ALP , MUC5AC	0.657	0.476	0.8	0.605	0.378	0.8
CA19.9 , TBIL , ALP , CYFRA21.1	0.659	0.451	0.8	0.604	0.378	0.8
CA19.9 , IL6 , GGT , CYFRA21.1	0.676	0.463	0.8	0.603	0.378	0.8
IL6 , PKM2 , CRP , GGT	0.673	0.463	0.8	0.602	0.378	0.8
CA19.9 , LRG1 , GGT , CYFRA21.1	0.668	0.476	0.8	0.596	0.378	0.8
CA19.9 , GGT , MUC5AC , CYFRA21.1	0.675	0.512	0.8	0.595	0.378	0.8
CRP	0.642	0.415	0.8	0.576	0.378	0.8
IL6 , CRP	0.656	0.439	0.8	0.575	0.378	0.8
TBIL , CRP	0.679	0.427	0.8	0.625	0.366	0.8
CA19.9 , IL6 , PKM2 , CRP	0.682	0.451	0.8	0.624	0.366	0.8
PKM2 , CRP , GGT , CYFRA21.1	0.676	0.439	0.8	0.622	0.366	0.8
CA19.9 , IL6 , LRG1 , CRP	0.679	0.415	0.8	0.621	0.366	0.8
CA19.9 , IL6 , ALP , CYFRA21.1	0.665	0.463	0.8	0.611	0.366	0.8
CA19.9 , TBIL , ALP	0.653	0.476	0.8	0.61	0.366	0.8
TBIL , CRP , ALP , GGT	0.663	0.402	0.8	0.606	0.366	0.8

CA19.9 , IL6 , TBIL , ALP	0.659	0.439	0.8	0.603	0.366	0.8
LRG1 , CRP , GGT , MUC5AC	0.665	0.524	0.8	0.6	0.366	0.8
CA19.9 , LRG1 , GGT , MUC5AC	0.661	0.427	0.8	0.584	0.366	0.8
CA19.9 , IL6 , CRP	0.684	0.402	0.8	0.638	0.354	0.8
CA19.9 , LRG1 , CRP	0.675	0.402	0.8	0.623	0.354	0.8
CRP , ALP , CYFRA21.1	0.66	0.415	0.8	0.62	0.354	0.8
CA19.9 , PKM2 , TBIL , CRP	0.678	0.463	0.8	0.617	0.354	0.8
PKM2 , CRP , ALP	0.654	0.378	0.8	0.614	0.354	0.8
IL6 , CRP , ALP , CYFRA21.1	0.661	0.366	0.8	0.612	0.354	0.8
IL6 , TBIL , CRP	0.683	0.451	0.8	0.611	0.354	0.8
TBIL , CRP , ALP , CYFRA21.1	0.656	0.39	0.8	0.611	0.354	0.8
IL6 , LRG1 , CRP , GGT	0.672	0.415	0.8	0.61	0.354	0.8
CA19.9 , LRG1 , ALP	0.653	0.39	0.8	0.608	0.354	0.8
CA19.9 , LRG1 , GGT	0.665	0.415	0.8	0.607	0.354	0.8
CRP , ALP , MUC5AC , CYFRA21.1	0.661	0.39	0.8	0.606	0.354	0.8
LRG1 , PKM2 , CRP , GGT	0.667	0.463	0.8	0.605	0.354	0.8

IL6 , PKM2 , CRP , ALP	0.664	0.366	0.8	0.605	0.354	0.8
CA19.9 , IL6 , GGT	0.663	0.451	0.8	0.604	0.354	0.8
PKM2 , TBIL , CRP , ALP	0.66	0.378	0.8	0.604	0.354	0.8
PKM2 , CRP , GGT , MUC5AC	0.675	0.439	0.8	0.602	0.354	0.8
CA19.9 , TBIL , GGT	0.664	0.524	0.8	0.599	0.354	0.8
PKM2 , CRP , ALP , MUC5AC	0.656	0.39	0.8	0.599	0.354	0.8
CA19.9 , TBIL , ALP , MUC5AC	0.656	0.476	0.8	0.598	0.354	0.8
LRG1 , PKM2 , CRP , ALP	0.653	0.402	0.8	0.598	0.354	0.8
IL6 , LRG1 , TBIL , CRP	0.676	0.415	0.8	0.589	0.354	0.8
CA19.9 , IL6 , TBIL , GGT	0.665	0.451	0.8	0.587	0.354	0.8
CA19.9 , LRG1 , PKM2 , GGT	0.656	0.451	0.8	0.586	0.354	0.8
ALP , GGT , MUC5AC	0.624	0.378	0.8	0.564	0.354	0.8
IL6 , PKM2 , ALP , GGT	0.633	0.39	0.8	0.561	0.354	0.8
CA19.9 , MUC5AC	0.619	0.354	0.8	0.554	0.354	0.8
GGT , CYFRA21.1	0.611	0.39	0.8	0.521	0.354	0.8
CA19.9 , TBIL , CRP	0.684	0.415	0.8	0.637	0.341	0.8

CRP , ALP	0.655	0.39	0.8	0.625	0.341	0.8
LRG1 , TBIL , CRP	0.673	0.39	0.8	0.603	0.341	0.8
CA19.9 , LRG1 , ALP , MUC5AC	0.655	0.39	0.8	0.599	0.341	0.8
CA19.9 , PKM2 , GGT , CYFRA21.1	0.672	0.39	0.8	0.597	0.341	0.8
CA19.9 , TBIL , GGT , CYFRA21.1	0.672	0.549	0.8	0.596	0.341	0.8
CA19.9 , IL6 , LRG1 , GGT	0.665	0.415	0.8	0.595	0.341	0.8
CA19.9 , IL6 , PKM2 , GGT	0.661	0.415	0.8	0.592	0.341	0.8
TBIL , CRP , CYFRA21.1	0.648	0.378	0.8	0.592	0.341	0.8
CRP , CYFRA21.1	0.629	0.341	0.8	0.587	0.341	0.8
CA19.9 , PKM2 , TBIL , GGT	0.658	0.439	0.8	0.585	0.341	0.8
ALP , GGT	0.624	0.378	0.8	0.583	0.341	0.8
IL6 , TBIL , CRP , CYFRA21.1	0.656	0.39	0.8	0.579	0.341	0.8
LRG1 , ALP , GGT	0.634	0.366	0.8	0.578	0.341	0.8
CA19.9 , TBIL , GGT , MUC5AC	0.663	0.476	0.8	0.575	0.341	0.8
IL6 , ALP , GGT	0.628	0.39	0.8	0.567	0.341	0.8
LRG1 , CRP , CYFRA21.1	0.619	0.39	0.8	0.567	0.341	0.8

PKM2 , ALP , GGT , CYFRA21.1	0.637	0.378	0.8	0.564	0.341	0.8
GGT	0.597	0.354	0.8	0.51	0.341	0.8
LRG1 , CRP , ALP	0.665	0.415	0.8	0.615	0.329	0.8
IL6 , CRP , ALP	0.663	0.366	0.8	0.614	0.329	0.8
CA19.9 , LRG1 , ALP , GGT	0.67	0.415	0.8	0.611	0.329	0.8
LRG1 , CRP , ALP , CYFRA21.1	0.663	0.378	0.8	0.611	0.329	0.8
PKM2 , CRP , ALP , CYFRA21.1	0.655	0.341	0.8	0.608	0.329	0.8
CA19.9 , PKM2 , TBIL , ALP	0.651	0.463	0.8	0.6	0.329	0.8
CA19.9 , LRG1 , TBIL , ALP	0.65	0.378	0.8	0.593	0.329	0.8
TBIL , CRP , MUC5AC	0.66	0.366	0.8	0.588	0.329	0.8
IL6 , TBIL , CRP , MUC5AC	0.671	0.39	0.8	0.578	0.329	0.8
TBIL , CRP , MUC5AC , CYFRA21.1	0.647	0.378	0.8	0.575	0.329	0.8
LRG1 , CRP	0.649	0.402	0.8	0.573	0.329	0.8
IL6 , LRG1 , CRP	0.656	0.415	0.8	0.571	0.329	0.8
ALP , GGT , CYFRA21.1	0.631	0.378	0.8	0.571	0.329	0.8
PKM2 , ALP , CYFRA21.1	0.629	0.366	0.8	0.571	0.329	0.8

PKM2 , ALP , GGT	0.626	0.378	0.8	0.571	0.329	0.8
LRG1 , TBIL , CRP , MUC5AC	0.659	0.415	0.8	0.569	0.329	0.8
IL6 , CRP , MUC5AC	0.652	0.402	0.8	0.568	0.329	0.8
LRG1 , TBIL , CRP , CYFRA21.1	0.638	0.39	0.8	0.568	0.329	0.8
CRP , MUC5AC , CYFRA21.1	0.633	0.39	0.8	0.568	0.329	0.8
CA19.9 , CYFRA21.1	0.611	0.366	0.8	0.563	0.329	0.8
IL6 , LRG1 , CRP , CYFRA21.1	0.629	0.415	0.8	0.557	0.329	0.8
LRG1 , PKM2 , ALP , GGT	0.628	0.378	0.8	0.556	0.329	0.8
IL6 , ALP , GGT , CYFRA21.1	0.634	0.354	0.8	0.554	0.329	0.8
ALP , GGT , MUC5AC , CYFRA21.1	0.631	0.366	0.8	0.552	0.329	0.8
PKM2 , TBIL , ALP , GGT	0.627	0.378	0.8	0.552	0.329	0.8
IL6 , ALP , GGT , MUC5AC	0.624	0.39	0.8	0.55	0.329	0.8
CA19.9 , MUC5AC , CYFRA21.1	0.617	0.402	0.8	0.548	0.329	0.8
TBIL , ALP , GGT , MUC5AC	0.623	0.378	0.8	0.545	0.329	0.8
TBIL , GGT	0.596	0.366	0.8	0.48	0.329	0.8
CRP , ALP , MUC5AC	0.654	0.39	0.8	0.61	0.317	0.8

CA19.9 , IL6 , PKM2 , ALP	0.662	0.366	0.8	0.606	0.317	0.8
PKM2 , TBIL , CRP	0.664	0.402	0.8	0.605	0.317	0.8
CA19.9 , LRG1 , ALP , CYFRA21.1	0.66	0.463	0.8	0.604	0.317	0.8
IL6 , CRP , ALP , MUC5AC	0.659	0.366	0.8	0.601	0.317	0.8
PKM2 , CRP	0.647	0.341	0.8	0.594	0.317	0.8
CA19.9 , LRG1 , TBIL , GGT	0.664	0.415	0.8	0.588	0.317	0.8
IL6 , PKM2 , CRP	0.654	0.378	0.8	0.585	0.317	0.8
PKM2 , TBIL , CRP , MUC5AC	0.66	0.415	0.8	0.584	0.317	0.8
IL6 , CRP , CYFRA21.1	0.638	0.378	0.8	0.577	0.317	0.8
ALP , MUC5AC	0.617	0.354	0.8	0.576	0.317	0.8
IL6 , PKM2 , ALP	0.624	0.317	0.8	0.572	0.317	0.8
CA19.9	0.617	0.329	0.8	0.57	0.317	0.8
PKM2 , ALP , MUC5AC	0.622	0.354	0.8	0.567	0.317	0.8
CRP , MUC5AC	0.636	0.402	0.8	0.563	0.317	0.8
TBIL , ALP , GGT	0.623	0.378	0.8	0.562	0.317	0.8
LRG1 , ALP , GGT , MUC5AC	0.634	0.366	0.8	0.56	0.317	0.8

IL6 , CRP , MUC5AC , CYFRA21.1	0.643	0.366	0.8	0.559	0.317	0.8
PKM2 , ALP , MUC5AC , CYFRA21.1	0.628	0.402	0.8	0.557	0.317	0.8
LRG1 , TBIL , ALP , GGT	0.634	0.366	0.8	0.556	0.317	0.8
PKM2 , ALP , GGT , MUC5AC	0.626	0.39	0.8	0.556	0.317	0.8
CA19.9 , IL6	0.612	0.366	0.8	0.556	0.317	0.8
PKM2 , GGT	0.608	0.366	0.8	0.555	0.317	0.8
LRG1 , CRP , MUC5AC , CYFRA21.1	0.622	0.39	0.8	0.551	0.317	0.8
IL6 , TBIL , ALP , GGT	0.628	0.39	0.8	0.549	0.317	0.8
CA19.9 , IL6 , MUC5AC	0.619	0.39	0.8	0.547	0.317	0.8
CA19.9 , TBIL	0.61	0.329	0.8	0.545	0.317	0.8
CA19.9 , TBIL , CYFRA21.1	0.605	0.366	0.8	0.545	0.317	0.8
CA19.9 , LRG1 , CYFRA21.1	0.612	0.329	0.8	0.542	0.317	0.8
CA19.9 , TBIL , MUC5AC	0.602	0.354	0.8	0.533	0.317	0.8
PKM2 , GGT , MUC5AC	0.613	0.341	0.8	0.53	0.317	0.8

CA19.9 , LRG1 , MUC5AC , CYFRA21.1	0.614	0.341	0.8	0.526	0.317	0.8
CA19.9 , IL6 , TBIL , CRP	0.685	0.427	0.8	0.631	0.305	0.8
CA19.9 , LRG1 , TBIL , CRP	0.68	0.451	0.8	0.619	0.305	0.8
TBIL , CRP , ALP	0.659	0.354	0.8	0.612	0.305	0.8
IL6 , LRG1 , CRP , ALP	0.671	0.366	0.8	0.609	0.305	0.8
CA19.9 , LRG1 , PKM2 , ALP	0.651	0.415	0.8	0.598	0.305	0.8
PKM2 , ALP	0.618	0.341	0.8	0.579	0.305	0.8
CA19.9 , PKM2	0.616	0.354	0.8	0.574	0.305	0.8
PKM2 , CRP , MUC5AC	0.652	0.366	0.8	0.572	0.305	0.8
ALP , MUC5AC , CYFRA21.1	0.628	0.341	0.8	0.568	0.305	0.8
IL6 , PKM2 , CRP , MUC5AC	0.662	0.366	0.8	0.566	0.305	0.8
LRG1 , PKM2 , ALP	0.619	0.341	0.8	0.566	0.305	0.8
CA19.9 , PKM2 , MUC5AC	0.625	0.354	0.8	0.564	0.305	0.8
IL6 , LRG1 , ALP , GGT	0.643	0.354	0.8	0.562	0.305	0.8
LRG1 , ALP , GGT , CYFRA21.1	0.642	0.366	0.8	0.56	0.305	0.8

IL6 , PKM2 , ALP , MUC5AC	0.626	0.317	0.8	0.559	0.305	0.8
LRG1 , PKM2 , ALP , CYFRA21.1	0.629	0.366	0.8	0.557	0.305	0.8
LRG1 , GGT	0.619	0.366	0.8	0.554	0.305	0.8
LRG1 , PKM2 , ALP , MUC5AC	0.621	0.354	0.8	0.553	0.305	0.8
CA19.9 , LRG1 , PKM2	0.616	0.366	0.8	0.552	0.305	0.8
TBIL , ALP , GGT , CYFRA21.1	0.633	0.378	0.8	0.551	0.305	0.8
CA19.9 , IL6 , CYFRA21.1	0.614	0.39	0.8	0.551	0.305	0.8
LRG1 , CRP , MUC5AC	0.635	0.402	0.8	0.548	0.305	0.8
CA19.9 , LRG1	0.608	0.329	0.8	0.547	0.305	0.8
CA19.9 , LRG1 , PKM2 , MUC5AC	0.621	0.39	0.8	0.541	0.305	0.8
CA19.9 , IL6 , MUC5AC , CYFRA21.1	0.619	0.402	0.8	0.539	0.305	0.8
CA19.9 , LRG1 , MUC5AC	0.61	0.354	0.8	0.532	0.305	0.8
LRG1 , GGT , MUC5AC	0.618	0.366	0.8	0.519	0.305	0.8
IL6 , PKM2 , GGT , MUC5AC	0.616	0.341	0.8	0.517	0.305	0.8
GGT , MUC5AC	0.605	0.329	0.8	0.504	0.305	0.8

LRG1 , CRP , ALP , MUC5AC	0.663	0.39	0.8	0.6	0.293	0.8
IL6 , LRG1 , PKM2 , CRP	0.65	0.39	0.8	0.564	0.293	0.8
PKM2 , TBIL , ALP	0.617	0.354	0.8	0.561	0.293	0.8
CA19.9 , IL6 , PKM2 , MUC5AC	0.634	0.366	0.8	0.56	0.293	0.8
CA19.9 , PKM2 , TBIL	0.611	0.329	0.8	0.558	0.293	0.8
CA19.9 , IL6 , PKM2 , CYFRA21.1	0.632	0.317	0.8	0.556	0.293	0.8
IL6 , LRG1 , PKM2 , ALP	0.625	0.305	0.8	0.555	0.293	0.8
CA19.9 , PKM2 , TBIL , CYFRA21.1	0.618	0.341	0.8	0.55	0.293	0.8
LRG1 , PKM2 , TBIL , ALP	0.618	0.354	0.8	0.548	0.293	0.8
IL6 , LRG1 , CRP , MUC5AC	0.65	0.402	0.8	0.546	0.293	0.8
CA19.9 , PKM2 , TBIL , MUC5AC	0.616	0.329	0.8	0.546	0.293	0.8
LRG1 , PKM2 , GGT	0.611	0.341	0.8	0.543	0.293	0.8
IL6 , LRG1 , GGT	0.62	0.378	0.8	0.542	0.293	0.8
IL6 , PKM2 , GGT	0.61	0.354	0.8	0.537	0.293	0.8
CA19.9 , TBIL , MUC5AC , CYFRA21.1	0.607	0.354	0.8	0.532	0.293	0.8

LRG1 , GGT , CYFRA21.1	0.619	0.402	0.8	0.531	0.293	0.8
CA19.9 , LRG1 , TBIL	0.607	0.329	0.8	0.531	0.293	0.8
IL6 , LRG1 , PKM2 , GGT	0.614	0.366	0.8	0.528	0.293	0.8
CA19.9 , LRG1 , TBIL , CYFRA21.1	0.604	0.317	0.8	0.526	0.293	0.8
LRG1 , TBIL , GGT	0.619	0.354	0.8	0.524	0.293	0.8
IL6 , LRG1 , TBIL , GGT	0.62	0.378	0.8	0.517	0.293	0.8
LRG1 , PKM2 , GGT , MUC5AC	0.613	0.329	0.8	0.517	0.293	0.8
IL6 , LRG1 , GGT , MUC5AC	0.62	0.366	0.8	0.51	0.293	0.8
IL6 , GGT , CYFRA21.1	0.618	0.378	0.8	0.506	0.293	0.8
GGT , MUC5AC , CYFRA21.1	0.614	0.402	0.8	0.499	0.293	0.8
IL6 , GGT	0.599	0.378	0.8	0.499	0.293	0.8
LRG1 , TBIL , GGT , MUC5AC	0.616	0.366	0.8	0.493	0.293	0.8
TBIL , GGT , CYFRA21.1	0.611	0.39	0.8	0.492	0.293	0.8
TBIL , CRP , ALP , MUC5AC	0.654	0.366	0.8	0.601	0.28	0.8
IL6 , TBIL , CRP , ALP	0.664	0.341	0.8	0.599	0.28	0.8
PKM2 , TBIL , CRP , CYFRA21.1	0.641	0.329	0.8	0.583	0.28	0.8

ALP , CYFRA21.1	0.625	0.305	0.8	0.58	0.28	0.8
IL6 , ALP	0.618	0.305	0.8	0.577	0.28	0.8
IL6 , PKM2 , CRP , CYFRA21.1	0.645	0.341	0.8	0.573	0.28	0.8
CA19.9 , PKM2 , CYFRA21.1	0.626	0.305	0.8	0.565	0.28	0.8
IL6 , ALP , MUC5AC	0.616	0.329	0.8	0.563	0.28	0.8
IL6 , PKM2 , ALP , CYFRA21.1	0.63	0.305	0.8	0.562	0.28	0.8
TBIL , ALP , MUC5AC	0.615	0.366	0.8	0.556	0.28	0.8
CA19.9 , PKM2 , MUC5AC , CYFRA21.1	0.631	0.317	0.8	0.555	0.28	0.8
PKM2 , GGT , CYFRA21.1	0.627	0.354	0.8	0.551	0.28	0.8
PKM2 , TBIL , ALP , CYFRA21.1	0.625	0.366	0.8	0.55	0.28	0.8
PKM2 , TBIL , ALP , MUC5AC	0.619	0.354	0.8	0.548	0.28	0.8
CA19.9 , LRG1 , PKM2 , CYFRA21.1	0.624	0.305	0.8	0.543	0.28	0.8
CA19.9 , LRG1 , PKM2 , TBIL	0.614	0.341	0.8	0.536	0.28	0.8
CA19.9 , IL6 , TBIL , CYFRA21.1	0.605	0.378	0.8	0.536	0.28	0.8

PKM2 , TBIL , GGT	0.607	0.366	0.8	0.529	0.28	0.8
IL6 , LRG1 , GGT , CYFRA21.1	0.627	0.366	0.8	0.519	0.28	0.8
CA19.9 , LRG1 , TBIL , MUC5AC	0.602	0.305	0.8	0.516	0.28	0.8
PKM2 , TBIL , GGT , MUC5AC	0.611	0.341	0.8	0.508	0.28	0.8
LRG1 , TBIL , GGT , CYFRA21.1	0.618	0.402	0.8	0.505	0.28	0.8
LRG1 , GGT , MUC5AC , CYFRA21.1	0.62	0.378	0.8	0.502	0.28	0.8
TBIL , GGT , MUC5AC	0.602	0.354	0.8	0.476	0.28	0.8
TBIL , GGT , MUC5AC , CYFRA21.1	0.613	0.402	0.8	0.469	0.28	0.8
LRG1 , TBIL , CRP , ALP	0.663	0.402	0.8	0.606	0.268	0.8
IL6 , PKM2 , TBIL , CRP	0.67	0.366	0.8	0.593	0.268	0.8
ALP	0.615	0.293	0.8	0.591	0.268	0.8
PKM2 , CRP , CYFRA21.1	0.639	0.366	0.8	0.584	0.268	0.8
LRG1 , PKM2 , CRP	0.644	0.366	0.8	0.571	0.268	0.8
CA19.9 , IL6 , PKM2	0.624	0.341	0.8	0.566	0.268	0.8
LRG1 , ALP , MUC5AC	0.621	0.28	0.8	0.565	0.268	0.8

IL6 , ALP , MUC5AC , CYFRA21.1	0.625	0.305	0.8	0.555	0.268	0.8
IL6 , PKM2 , TBIL , ALP	0.624	0.329	0.8	0.552	0.268	0.8
TBIL , ALP , MUC5AC , CYFRA21.1	0.629	0.317	0.8	0.55	0.268	0.8
LRG1 , PKM2 , CRP , MUC5AC	0.644	0.378	0.8	0.549	0.268	0.8
CA19.9 , IL6 , PKM2 , TBIL	0.619	0.317	0.8	0.549	0.268	0.8
CA19.9 , IL6 , LRG1 , PKM2	0.621	0.354	0.8	0.546	0.268	0.8
IL6 , TBIL , ALP , MUC5AC	0.617	0.354	0.8	0.545	0.268	0.8
CA19.9 , IL6 , LRG1	0.613	0.329	0.8	0.539	0.268	0.8
CA19.9 , IL6 , LRG1 , CYFRA21.1	0.615	0.341	0.8	0.534	0.268	0.8
PKM2 , GGT , MUC5AC , CYFRA21.1	0.627	0.366	0.8	0.529	0.268	0.8
CA19.9 , IL6 , LRG1 , MUC5AC	0.616	0.39	0.8	0.529	0.268	0.8
LRG1 , PKM2 , TBIL , GGT	0.608	0.341	0.8	0.518	0.268	0.8
IL6 , PKM2 , TBIL , GGT	0.611	0.354	0.8	0.514	0.268	0.8
IL6 , GGT , MUC5AC	0.61	0.366	0.8	0.494	0.268	0.8
IL6 , GGT , MUC5AC , CYFRA21.1	0.623	0.341	0.8	0.489	0.268	0.8

IL6 , TBIL , GGT , CYFRA21.1	0.618	0.378	0.8	0.478	0.268	0.8
LRG1 , PKM2 , TBIL , CRP	0.667	0.378	0.8	0.583	0.256	0.8
LRG1 , ALP	0.622	0.28	0.8	0.578	0.256	0.8
PKM2 , CRP , MUC5AC , CYFRA21.1	0.642	0.329	0.8	0.57	0.256	0.8
IL6 , LRG1 , ALP	0.625	0.293	0.8	0.568	0.256	0.8
LRG1 , PKM2 , CRP , CYFRA21.1	0.636	0.341	0.8	0.567	0.256	0.8
IL6 , ALP , CYFRA21.1	0.623	0.28	0.8	0.566	0.256	0.8
IL6 , LRG1 , ALP , CYFRA21.1	0.627	0.268	0.8	0.558	0.256	0.8
IL6 , LRG1 , ALP , MUC5AC	0.622	0.28	0.8	0.556	0.256	0.8
IL6 , TBIL , ALP	0.617	0.329	0.8	0.556	0.256	0.8
LRG1 , TBIL , ALP , MUC5AC	0.62	0.293	0.8	0.542	0.256	0.8
LRG1 , PKM2 , GGT , CYFRA21.1	0.625	0.317	0.8	0.535	0.256	0.8
CA19.9 , IL6 , TBIL	0.606	0.305	0.8	0.535	0.256	0.8
IL6 , PKM2 , GGT , CYFRA21.1	0.628	0.341	0.8	0.533	0.256	0.8
PKM2 , TBIL , MUC5AC	0.56	0.341	0.8	0.471	0.256	0.8

IL6 , TBIL , GGT , MUC5AC	0.61	0.366	0.8	0.465	0.256	0.8
CA19.9 , IL6 , LRG1 , ALP	0.66	0.366	0.8	0.603	0.244	0.8
LRG1 , ALP , CYFRA21.1	0.628	0.256	0.8	0.57	0.244	0.8
TBIL , ALP	0.613	0.317	0.8	0.569	0.244	0.8
LRG1 , ALP , MUC5AC , CYFRA21.1	0.631	0.293	0.8	0.556	0.244	0.8
LRG1 , TBIL , ALP	0.62	0.293	0.8	0.556	0.244	0.8
IL6 , TBIL , ALP , CYFRA21.1	0.624	0.305	0.8	0.546	0.244	0.8
PKM2 , TBIL , GGT , CYFRA21.1	0.625	0.329	0.8	0.526	0.244	0.8
IL6 , TBIL , GGT	0.6	0.378	0.8	0.474	0.244	0.8
PKM2	0.533	0.268	0.8	0.473	0.244	0.8
TBIL , ALP , CYFRA21.1	0.626	0.305	0.8	0.562	0.232	0.8
LRG1 , TBIL , ALP , CYFRA21.1	0.625	0.268	0.8	0.55	0.232	0.8
IL6 , LRG1 , TBIL , ALP	0.622	0.293	0.8	0.549	0.232	0.8
CA19.9 , IL6 , TBIL , MUC5AC	0.608	0.378	0.8	0.527	0.232	0.8
PKM2 , MUC5AC	0.549	0.293	0.8	0.469	0.232	0.8
CA19.9 , IL6 , LRG1 , TBIL	0.609	0.305	0.8	0.526	0.22	0.8

IL6 , PKM2 , MUC5AC	0.559	0.293	0.8	0.453	0.195	0.8
LRG1 , PKM2 , TBIL	0.551	0.256	0.8	0.458	0.183	0.8
PKM2 , TBIL	0.551	0.28	0.8	0.484	0.171	0.8
PKM2 , TBIL , CYFRA21.1	0.56	0.268	0.8	0.477	0.171	0.8
PKM2 , TBIL , MUC5AC , CYFRA21.1	0.565	0.268	0.8	0.462	0.171	0.8
LRG1 , PKM2 , MUC5AC	0.548	0.293	0.8	0.44	0.171	0.8
TBIL , MUC5AC , CYFRA21.1	0.524	0.268	0.8	0.394	0.171	0.8
IL6 , PKM2 , TBIL	0.555	0.268	0.8	0.464	0.159	0.8
IL6 , PKM2	0.541	0.244	0.8	0.451	0.159	0.8
TBIL , CYFRA21.1	0.523	0.244	0.8	0.405	0.159	0.8
TBIL	0.5	0.22	0.8	0.365	0.159	0.8
IL6 , TBIL	0.515	0.293	0.8	0.354	0.159	0.8
LRG1 , PKM2	0.534	0.207	0.8	0.446	0.146	0.8
TBIL , MUC5AC	0.5	0.232	0.8	0.381	0.146	0.8
PKM2 , CYFRA21.1	0.56	0.244	0.8	0.481	0.134	0.8

IL6 , LRG1 , PKM2	0.541	0.207	0.8	0.426	0.134	0.8
PKM2 , MUC5AC , CYFRA21.1	0.569	0.22	0.8	0.473	0.122	0.8
IL6 , PKM2 , CYFRA21.1	0.564	0.195	0.8	0.467	0.122	0.8
IL6 , PKM2 , TBIL , CYFRA21.1	0.563	0.232	0.8	0.462	0.122	0.8
IL6 , TBIL , CYFRA21.1	0.534	0.293	0.8	0.396	0.122	0.8
IL6 , TBIL , MUC5AC	0.517	0.28	0.8	0.369	0.122	0.8
IL6	0.555	0.244	0.8	0.117	0.122	0.8
IL6 , PKM2 , MUC5AC , CYFRA21.1	0.573	0.22	0.8	0.46	0.11	0.8
IL6 , PKM2 , TBIL , MUC5AC	0.568	0.244	0.8	0.456	0.11	0.8
LRG1 , PKM2 , MUC5AC , CYFRA21.1	0.568	0.244	0.8	0.445	0.11	0.8
LRG1 , TBIL , MUC5AC	0.535	0.207	0.8	0.402	0.11	0.8
LRG1 , MUC5AC	0.536	0.232	0.8	0.391	0.11	0.8
LRG1 , PKM2 , CYFRA21.1	0.559	0.195	0.8	0.454	0.085	0.8
IL6 , LRG1	0.559	0.232	0.8	0.413	0.085	0.8
LRG1 , TBIL	0.562	0.207	0.8	0.448	0.073	0.8

IL6 , LRG1 , TBIL	0.567	0.183	0.8	0.423	0.073	0.8
LRG1	0.546	0.171	0.8	0.416	0.073	0.8
LRG1 , CYFRA21.1	0.524	0.22	0.8	0.398	0.061	0.8
LRG1 , TBIL , CYFRA21.1	0.536	0.159	0.8	0.411	0.049	0.8
MUC5AC , CYFRA21.1	0.517	0.195	0.8	0.376	0.049	0.8
IL6 , MUC5AC , CYFRA21.1	0.531	0.268	0.8	0.375	0.049	0.8
IL6 , LRG1 , CYFRA21.1	0.534	0.171	0.8	0.391	0.037	0.8
IL6 , LRG1 , MUC5AC	0.55	0.232	0.8	0.386	0.037	0.8
LRG1 , MUC5AC , CYFRA21.1	0.53	0.195	0.8	0.383	0.037	0.8
IL6 , CYFRA21.1	0.533	0.244	0.8	0.375	0.037	0.8
CYFRA21.1	0.517	0.22	0.8	0.376	0.012	0.8
IL6 , MUC5AC	0.542	0.28	0.8	0.33	0.012	0.8
MUC5AC	0.506	0.293	0.8	0.315	0	0.8

Supplementary table 3: the performance (AUC, Sensitivity, Specificity) of all panels computed at 90% specificity (CV; Cross validated)

Biomarkers	AUC	Sens	Spec	AUC_CV	Sens_CV	Spec_CV
CRP , ALP , GGT	0.66	0.366	0.9	0.621	0.341	0.9
CRP , ALP , GGT , MUC5AC	0.659	0.366	0.9	0.604	0.329	0.9
CRP , GGT	0.662	0.366	0.9	0.618	0.329	0.9
CRP , ALP , GGT , CYFRA21.1	0.666	0.354	0.9	0.621	0.329	0.9
CRP , GGT , MUC5AC , CYFRA21.1	0.676	0.354	0.9	0.612	0.329	0.9
CRP , GGT , CYFRA21.1	0.676	0.341	0.9	0.629	0.329	0.9
CRP , GGT , MUC5AC	0.663	0.354	0.9	0.605	0.317	0.9
CA19.9 , CRP , ALP , GGT	0.7	0.378	0.9	0.659	0.305	0.9
LRG1 , CRP , GGT	0.667	0.329	0.9	0.619	0.305	0.9

LRG1 , CRP , GGT , CYFRA21.1	0.671	0.329	0.9	0.615	0.305	0.9
IL6 , CRP , CYFRA21.1	0.638	0.341	0.9	0.577	0.293	0.9
LRG1 , CRP , GGT , MUC5AC	0.665	0.341	0.9	0.6	0.293	0.9
PKM2 , CRP , GGT , CYFRA21.1	0.676	0.341	0.9	0.622	0.293	0.9
CA19.9 , CRP , GGT	0.706	0.341	0.9	0.669	0.28	0.9
CA19.9 , PKM2 , CRP , CYFRA21.1	0.674	0.341	0.9	0.624	0.28	0.9
CRP , CYFRA21.1	0.629	0.341	0.9	0.587	0.28	0.9
CRP , MUC5AC , CYFRA21.1	0.633	0.329	0.9	0.568	0.28	0.9
CA19.9 , PKM2 , CRP , GGT	0.701	0.317	0.9	0.647	0.28	0.9
IL6 , CRP , GGT	0.666	0.317	0.9	0.611	0.28	0.9
CA19.9 , LRG1 , CRP , GGT	0.702	0.305	0.9	0.654	0.28	0.9
LRG1 , CRP , CYFRA21.1	0.619	0.305	0.9	0.567	0.28	0.9
CA19.9 , CRP , GGT , MUC5AC	0.704	0.354	0.9	0.652	0.268	0.9
PKM2 , CRP , GGT , MUC5AC	0.675	0.354	0.9	0.602	0.268	0.9
TBIL , CRP , GGT , CYFRA21.1	0.675	0.341	0.9	0.611	0.268	0.9
LRG1 , CRP , MUC5AC	0.635	0.329	0.9	0.548	0.268	0.9

PKM2 , CRP , GGT	0.672	0.329	0.9	0.616	0.268	0.9
TBIL , CRP , GGT	0.67	0.329	0.9	0.607	0.268	0.9
TBIL , CRP , MUC5AC	0.66	0.329	0.9	0.588	0.268	0.9
CA19.9 , PKM2 , ALP , GGT	0.663	0.305	0.9	0.612	0.268	0.9
PKM2 , TBIL , CRP , GGT	0.674	0.366	0.9	0.604	0.256	0.9
LRG1 , CRP	0.649	0.329	0.9	0.573	0.256	0.9
IL6 , CRP , GGT , CYFRA21.1	0.683	0.317	0.9	0.619	0.256	0.9
IL6 , CRP , MUC5AC , CYFRA21.1	0.643	0.317	0.9	0.559	0.256	0.9
CA19.9 , ALP , GGT	0.667	0.293	0.9	0.626	0.256	0.9
CA19.9 , TBIL , ALP , GGT	0.667	0.293	0.9	0.611	0.256	0.9
TBIL , CRP	0.679	0.293	0.9	0.625	0.256	0.9
CRP , ALP , MUC5AC	0.654	0.268	0.9	0.61	0.256	0.9
CRP , ALP	0.655	0.256	0.9	0.625	0.256	0.9
IL6 , PKM2 , CRP , GGT	0.673	0.354	0.9	0.602	0.244	0.9
CA19.9 , TBIL , CRP , GGT	0.706	0.341	0.9	0.656	0.244	0.9
LRG1 , PKM2 , CRP , GGT	0.667	0.329	0.9	0.605	0.244	0.9

LRG1 , TBIL , CRP , GGT	0.675	0.329	0.9	0.606	0.244	0.9
TBIL , CRP , MUC5AC , CYFRA21.1	0.647	0.329	0.9	0.575	0.244	0.9
CA19.9 , IL6 , CRP , GGT	0.706	0.317	0.9	0.662	0.244	0.9
CRP	0.642	0.305	0.9	0.576	0.244	0.9
TBIL , CRP , CYFRA21.1	0.648	0.305	0.9	0.592	0.244	0.9
CA19.9 , CRP , ALP	0.685	0.293	0.9	0.649	0.244	0.9
CA19.9 , CRP , ALP , MUC5AC	0.681	0.293	0.9	0.64	0.244	0.9
GGT , CYFRA21.1	0.611	0.28	0.9	0.521	0.244	0.9
CA19.9 , CRP , GGT , CYFRA21.1	0.716	0.268	0.9	0.676	0.244	0.9
CRP , ALP , CYFRA21.1	0.66	0.268	0.9	0.62	0.244	0.9
CRP , ALP , MUC5AC , CYFRA21.1	0.661	0.268	0.9	0.606	0.244	0.9
CRP , MUC5AC	0.636	0.268	0.9	0.563	0.244	0.9
GGT , MUC5AC , CYFRA21.1	0.614	0.268	0.9	0.499	0.244	0.9
ALP	0.615	0.256	0.9	0.591	0.244	0.9
CA19.9 , CRP , ALP , CYFRA21.1	0.697	0.256	0.9	0.658	0.244	0.9
GGT	0.597	0.256	0.9	0.51	0.244	0.9

IL6 , CRP , GGT , MUC5AC	0.669	0.366	0.9	0.599	0.232	0.9
TBIL , CRP , GGT , MUC5AC	0.667	0.366	0.9	0.59	0.232	0.9
IL6 , TBIL , CRP , GGT	0.67	0.354	0.9	0.596	0.232	0.9
PKM2 , CRP , ALP , GGT	0.665	0.341	0.9	0.607	0.232	0.9
CA19.9 , LRG1 , ALP , GGT	0.67	0.317	0.9	0.611	0.232	0.9
CA19.9 , PKM2 , TBIL , CRP	0.678	0.28	0.9	0.617	0.232	0.9
PKM2 , CRP , ALP , CYFRA21.1	0.655	0.28	0.9	0.608	0.232	0.9
ALP , GGT	0.624	0.268	0.9	0.583	0.232	0.9
ALP , GGT , MUC5AC	0.624	0.268	0.9	0.564	0.232	0.9
CA19.9 , PKM2 , CRP , ALP	0.679	0.268	0.9	0.637	0.232	0.9
CA19.9 , PKM2 , TBIL , ALP	0.651	0.268	0.9	0.6	0.232	0.9
ALP , CYFRA21.1	0.625	0.256	0.9	0.58	0.232	0.9
ALP , MUC5AC	0.617	0.256	0.9	0.576	0.232	0.9
PKM2 , CRP , ALP	0.654	0.256	0.9	0.614	0.232	0.9
PKM2 , CRP , ALP , MUC5AC	0.656	0.256	0.9	0.599	0.232	0.9
PKM2 , TBIL , ALP	0.617	0.256	0.9	0.561	0.232	0.9

CA19.9 , LRG1 , CRP , ALP	0.685	0.317	0.9	0.639	0.22	0.9
CA19.9 , IL6 , PKM2 , GGT	0.661	0.305	0.9	0.592	0.22	0.9
CA19.9 , PKM2 , GGT	0.66	0.305	0.9	0.602	0.22	0.9
CA19.9 , PKM2 , GGT , MUC5AC	0.657	0.305	0.9	0.584	0.22	0.9
IL6 , LRG1 , CRP , CYFRA21.1	0.629	0.305	0.9	0.557	0.22	0.9
IL6 , LRG1 , CRP , MUC5AC	0.65	0.305	0.9	0.546	0.22	0.9
LRG1 , CRP , MUC5AC , CYFRA21.1	0.622	0.305	0.9	0.551	0.22	0.9
CA19.9 , ALP , GGT , MUC5AC	0.669	0.293	0.9	0.613	0.22	0.9
CA19.9 , LRG1 , PKM2 , GGT	0.656	0.293	0.9	0.586	0.22	0.9
CA19.9 , PKM2 , GGT , CYFRA21.1	0.672	0.293	0.9	0.597	0.22	0.9
CA19.9 , PKM2 , TBIL , GGT	0.658	0.293	0.9	0.585	0.22	0.9
PKM2 , GGT , MUC5AC	0.613	0.293	0.9	0.53	0.22	0.9
CA19.9 , PKM2	0.616	0.28	0.9	0.574	0.22	0.9
PKM2 , ALP , GGT , CYFRA21.1	0.637	0.28	0.9	0.564	0.22	0.9
PKM2 , GGT	0.608	0.28	0.9	0.555	0.22	0.9
TBIL , GGT , CYFRA21.1	0.611	0.28	0.9	0.492	0.22	0.9

TBIL , CRP , ALP	0.659	0.268	0.9	0.612	0.22	0.9
TBIL , GGT , MUC5AC , CYFRA21.1	0.613	0.268	0.9	0.469	0.22	0.9
ALP , GGT , CYFRA21.1	0.631	0.256	0.9	0.571	0.22	0.9
CA19.9 , GGT	0.663	0.256	0.9	0.618	0.22	0.9
CA19.9 , GGT , MUC5AC	0.665	0.256	0.9	0.593	0.22	0.9
CA19.9 , PKM2 , ALP	0.652	0.256	0.9	0.612	0.22	0.9
CA19.9 , TBIL , GGT	0.664	0.256	0.9	0.599	0.22	0.9
CA19.9 , TBIL , GGT , MUC5AC	0.663	0.256	0.9	0.575	0.22	0.9
GGT , MUC5AC	0.605	0.256	0.9	0.504	0.22	0.9
IL6 , PKM2 , GGT	0.61	0.256	0.9	0.537	0.22	0.9
PKM2 , GGT , CYFRA21.1	0.627	0.256	0.9	0.551	0.22	0.9
TBIL , ALP , GGT , MUC5AC	0.623	0.256	0.9	0.545	0.22	0.9
CA19.9 , LRG1 , GGT	0.665	0.244	0.9	0.607	0.22	0.9
CA19.9 , MUC5AC , CYFRA21.1	0.617	0.232	0.9	0.548	0.22	0.9
IL6 , PKM2 , GGT , CYFRA21.1	0.628	0.232	0.9	0.533	0.22	0.9
CA19.9 , ALP , MUC5AC	0.656	0.22	0.9	0.614	0.22	0.9

CA19.9 , TBIL , ALP , MUC5AC	0.656	0.22	0.9	0.598	0.22	0.9
TBIL , CRP , ALP , GGT	0.663	0.341	0.9	0.606	0.207	0.9
CA19.9 , CRP , MUC5AC , CYFRA21.1	0.677	0.317	0.9	0.63	0.207	0.9
LRG1 , CRP , ALP , GGT	0.67	0.317	0.9	0.612	0.207	0.9
CA19.9 , CRP , MUC5AC	0.67	0.305	0.9	0.624	0.207	0.9
CA19.9 , IL6 , GGT	0.663	0.305	0.9	0.604	0.207	0.9
CA19.9 , IL6 , TBIL , GGT	0.665	0.305	0.9	0.587	0.207	0.9
CA19.9 , CRP , CYFRA21.1	0.678	0.293	0.9	0.643	0.207	0.9
CA19.9 , IL6 , GGT , MUC5AC	0.668	0.293	0.9	0.583	0.207	0.9
PKM2 , TBIL , CRP , ALP	0.66	0.293	0.9	0.604	0.207	0.9
PKM2 , TBIL , GGT , MUC5AC	0.611	0.293	0.9	0.508	0.207	0.9
CA19.9 , TBIL , CRP , ALP	0.684	0.28	0.9	0.642	0.207	0.9
IL6 , CRP , MUC5AC	0.652	0.28	0.9	0.568	0.207	0.9
PKM2 , TBIL , GGT	0.607	0.28	0.9	0.529	0.207	0.9
TBIL , CRP , ALP , MUC5AC	0.654	0.28	0.9	0.601	0.207	0.9

CA19.9 , ALP , GGT , CYFRA21.1	0.674	0.268	0.9	0.621	0.207	0.9
IL6 , GGT	0.599	0.268	0.9	0.499	0.207	0.9
IL6 , GGT , MUC5AC	0.61	0.268	0.9	0.494	0.207	0.9
IL6 , PKM2 , TBIL , GGT	0.611	0.268	0.9	0.514	0.207	0.9
TBIL , ALP , GGT	0.623	0.268	0.9	0.562	0.207	0.9
CA19.9 , GGT , MUC5AC , CYFRA21.1	0.675	0.256	0.9	0.595	0.207	0.9
IL6 , CRP	0.656	0.256	0.9	0.575	0.207	0.9
IL6 , GGT , MUC5AC , CYFRA21.1	0.623	0.256	0.9	0.489	0.207	0.9
IL6 , PKM2 , GGT , MUC5AC	0.616	0.256	0.9	0.517	0.207	0.9
LRG1 , PKM2 , CRP	0.644	0.256	0.9	0.571	0.207	0.9
LRG1 , PKM2 , CRP , ALP	0.653	0.256	0.9	0.598	0.207	0.9
PKM2 , GGT , MUC5AC , CYFRA21.1	0.627	0.256	0.9	0.529	0.207	0.9
TBIL , GGT	0.596	0.256	0.9	0.48	0.207	0.9
ALP , GGT , MUC5AC , CYFRA21.1	0.631	0.244	0.9	0.552	0.207	0.9
ALP , MUC5AC , CYFRA21.1	0.628	0.244	0.9	0.568	0.207	0.9

CA19.9 , CYFRA21.1	0.611	0.244	0.9	0.563	0.207	0.9
CA19.9 , LRG1 , TBIL , GGT	0.664	0.244	0.9	0.588	0.207	0.9
PKM2 , ALP	0.618	0.244	0.9	0.579	0.207	0.9
CA19.9 , GGT , CYFRA21.1	0.671	0.232	0.9	0.616	0.207	0.9
CA19.9 , IL6 , GGT , CYFRA21.1	0.676	0.232	0.9	0.603	0.207	0.9
CA19.9 , TBIL , GGT , CYFRA21.1	0.672	0.232	0.9	0.596	0.207	0.9
CA19.9 , TBIL , ALP	0.653	0.22	0.9	0.61	0.207	0.9
PKM2 , ALP , GGT	0.626	0.317	0.9	0.571	0.195	0.9
CA19.9 , IL6 , ALP , GGT	0.676	0.305	0.9	0.615	0.195	0.9
LRG1 , PKM2 , ALP , GGT	0.628	0.293	0.9	0.556	0.195	0.9
CA19.9 , PKM2 , ALP , MUC5AC	0.657	0.28	0.9	0.605	0.195	0.9
CA19.9 , PKM2 , MUC5AC	0.625	0.28	0.9	0.564	0.195	0.9
LRG1 , ALP , GGT	0.634	0.28	0.9	0.578	0.195	0.9
LRG1 , PKM2 , GGT , MUC5AC	0.613	0.28	0.9	0.517	0.195	0.9
TBIL , CRP , ALP , CYFRA21.1	0.656	0.28	0.9	0.611	0.195	0.9
CA19.9 , PKM2 , ALP , CYFRA21.1	0.662	0.268	0.9	0.607	0.195	0.9

CA19.9 , TBIL , CRP	0.684	0.268	0.9	0.637	0.195	0.9
CA19.9 , TBIL , CRP , MUC5AC	0.673	0.268	0.9	0.611	0.195	0.9
IL6 , GGT , CYFRA21.1	0.618	0.268	0.9	0.506	0.195	0.9
LRG1 , ALP , GGT , MUC5AC	0.634	0.268	0.9	0.56	0.195	0.9
LRG1 , PKM2 , GGT	0.611	0.268	0.9	0.543	0.195	0.9
LRG1 , ALP , GGT , CYFRA21.1	0.642	0.256	0.9	0.56	0.195	0.9
LRG1 , GGT , MUC5AC	0.618	0.256	0.9	0.519	0.195	0.9
LRG1 , PKM2 , CRP , CYFRA21.1	0.636	0.256	0.9	0.567	0.195	0.9
PKM2 , CRP , CYFRA21.1	0.639	0.256	0.9	0.584	0.195	0.9
PKM2 , CRP , MUC5AC , CYFRA21.1	0.642	0.256	0.9	0.57	0.195	0.9
TBIL , ALP , GGT , CYFRA21.1	0.633	0.256	0.9	0.551	0.195	0.9
TBIL , GGT , MUC5AC	0.602	0.256	0.9	0.476	0.195	0.9
CA19.9 , IL6 , LRG1 , GGT	0.665	0.244	0.9	0.595	0.195	0.9
CA19.9 , LRG1 , ALP	0.653	0.244	0.9	0.608	0.195	0.9
CA19.9 , LRG1 , GGT , MUC5AC	0.661	0.244	0.9	0.584	0.195	0.9
LRG1 , GGT	0.619	0.244	0.9	0.554	0.195	0.9

LRG1 , PKM2 , GGT , CYFRA21.1	0.625	0.244	0.9	0.535	0.195	0.9
PKM2 , ALP , MUC5AC	0.622	0.244	0.9	0.567	0.195	0.9
PKM2 , CRP	0.647	0.244	0.9	0.594	0.195	0.9
PKM2 , TBIL , GGT , CYFRA21.1	0.625	0.244	0.9	0.526	0.195	0.9
CA19.9 , LRG1 , GGT , CYFRA21.1	0.668	0.232	0.9	0.596	0.195	0.9
CA19.9 , ALP	0.654	0.22	0.9	0.625	0.195	0.9
CA19.9 , LRG1 , TBIL , ALP	0.65	0.22	0.9	0.593	0.195	0.9
LRG1 , TBIL , CRP , CYFRA21.1	0.638	0.317	0.9	0.568	0.183	0.9
PKM2 , ALP , GGT , MUC5AC	0.626	0.317	0.9	0.556	0.183	0.9
PKM2 , TBIL , ALP , GGT	0.627	0.305	0.9	0.552	0.183	0.9
CA19.9 , PKM2 , CRP , MUC5AC	0.675	0.293	0.9	0.612	0.183	0.9
IL6 , LRG1 , ALP , GGT	0.643	0.293	0.9	0.562	0.183	0.9
CA19.9 , PKM2 , CRP	0.678	0.28	0.9	0.625	0.183	0.9
LRG1 , PKM2 , TBIL , GGT	0.608	0.28	0.9	0.518	0.183	0.9
IL6 , LRG1 , PKM2 , GGT	0.614	0.268	0.9	0.528	0.183	0.9
IL6 , PKM2 , CRP , ALP	0.664	0.268	0.9	0.605	0.183	0.9

IL6 , TBIL , GGT	0.6	0.268	0.9	0.474	0.183	0.9
LRG1 , CRP , ALP , CYFRA21.1	0.663	0.256	0.9	0.611	0.183	0.9
CA19.9 , CRP	0.68	0.244	0.9	0.645	0.183	0.9
CA19.9 , IL6 , CRP , ALP	0.696	0.244	0.9	0.65	0.183	0.9
IL6 , PKM2 , CRP , MUC5AC	0.662	0.244	0.9	0.566	0.183	0.9
IL6 , LRG1 , GGT , CYFRA21.1	0.627	0.232	0.9	0.519	0.183	0.9
CA19.9	0.617	0.22	0.9	0.57	0.183	0.9
CA19.9 , MUC5AC	0.619	0.22	0.9	0.554	0.183	0.9
CA19.9 , TBIL , CRP , CYFRA21.1	0.68	0.22	0.9	0.634	0.183	0.9
LRG1 , GGT , CYFRA21.1	0.619	0.22	0.9	0.531	0.183	0.9
PKM2 , TBIL , ALP , CYFRA21.1	0.625	0.22	0.9	0.55	0.183	0.9
CA19.9 , TBIL	0.61	0.207	0.9	0.545	0.183	0.9
PKM2 , ALP , CYFRA21.1	0.629	0.207	0.9	0.571	0.183	0.9
LRG1 , TBIL , CRP , MUC5AC	0.659	0.317	0.9	0.569	0.171	0.9
IL6 , LRG1 , CRP , GGT	0.672	0.305	0.9	0.61	0.171	0.9
CA19.9 , LRG1 , TBIL , CRP	0.68	0.28	0.9	0.619	0.171	0.9

LRG1 , TBIL , ALP , GGT	0.634	0.28	0.9	0.556	0.171	0.9
CA19.9 , LRG1 , PKM2 , TBIL	0.614	0.268	0.9	0.536	0.171	0.9
IL6 , ALP , GGT	0.628	0.268	0.9	0.567	0.171	0.9
IL6 , ALP , GGT , CYFRA21.1	0.634	0.268	0.9	0.554	0.171	0.9
IL6 , LRG1 , GGT	0.62	0.268	0.9	0.542	0.171	0.9
IL6 , TBIL , GGT , CYFRA21.1	0.618	0.268	0.9	0.478	0.171	0.9
LRG1 , TBIL , GGT , MUC5AC	0.616	0.268	0.9	0.493	0.171	0.9
CA19.9 , LRG1 , ALP , MUC5AC	0.655	0.256	0.9	0.599	0.171	0.9
CA19.9 , LRG1 , CRP , CYFRA21.1	0.675	0.256	0.9	0.621	0.171	0.9
CA19.9 , LRG1 , PKM2 , ALP	0.651	0.256	0.9	0.598	0.171	0.9
CA19.9 , PKM2 , TBIL , MUC5AC	0.616	0.256	0.9	0.546	0.171	0.9
IL6 , TBIL , GGT , MUC5AC	0.61	0.256	0.9	0.465	0.171	0.9
PKM2 , CRP , MUC5AC	0.652	0.256	0.9	0.572	0.171	0.9
PKM2 , TBIL , ALP , MUC5AC	0.619	0.256	0.9	0.548	0.171	0.9
PKM2 , TBIL , CRP	0.664	0.256	0.9	0.605	0.171	0.9
TBIL , ALP , MUC5AC	0.615	0.256	0.9	0.556	0.171	0.9

CA19.9 , IL6 , PKM2 , CRP	0.682	0.244	0.9	0.624	0.171	0.9
CA19.9 , LRG1 , TBIL , MUC5AC	0.602	0.244	0.9	0.516	0.171	0.9
CA19.9 , PKM2 , TBIL	0.611	0.244	0.9	0.558	0.171	0.9
CA19.9 , TBIL , CYFRA21.1	0.605	0.244	0.9	0.545	0.171	0.9
IL6 , CRP , ALP	0.663	0.244	0.9	0.614	0.171	0.9
IL6 , CRP , ALP , MUC5AC	0.659	0.244	0.9	0.601	0.171	0.9
IL6 , LRG1 , GGT , MUC5AC	0.62	0.244	0.9	0.51	0.171	0.9
LRG1 , ALP	0.622	0.244	0.9	0.578	0.171	0.9
LRG1 , ALP , MUC5AC	0.621	0.244	0.9	0.565	0.171	0.9
LRG1 , GGT , MUC5AC , CYFRA21.1	0.62	0.244	0.9	0.502	0.171	0.9
LRG1 , PKM2 , CRP , MUC5AC	0.644	0.244	0.9	0.549	0.171	0.9
TBIL , ALP	0.613	0.244	0.9	0.569	0.171	0.9
TBIL , ALP , CYFRA21.1	0.626	0.244	0.9	0.562	0.171	0.9
IL6 , LRG1 , CRP	0.656	0.232	0.9	0.571	0.171	0.9
IL6 , PKM2 , ALP , GGT	0.633	0.232	0.9	0.561	0.171	0.9
LRG1 , CRP , ALP	0.665	0.232	0.9	0.615	0.171	0.9

LRG1 , CRP , ALP , MUC5AC	0.663	0.232	0.9	0.6	0.171	0.9
LRG1 , TBIL , GGT , CYFRA21.1	0.618	0.22	0.9	0.505	0.171	0.9
CA19.9 , ALP , CYFRA21.1	0.662	0.207	0.9	0.622	0.171	0.9
CA19.9 , ALP , MUC5AC , CYFRA21.1	0.663	0.207	0.9	0.612	0.171	0.9
CA19.9 , LRG1 , ALP , CYFRA21.1	0.66	0.207	0.9	0.604	0.171	0.9
CA19.9 , TBIL , ALP , CYFRA21.1	0.659	0.207	0.9	0.604	0.171	0.9
CA19.9 , IL6 , ALP , CYFRA21.1	0.665	0.195	0.9	0.611	0.171	0.9
IL6 , CRP , ALP , GGT	0.672	0.354	0.9	0.611	0.159	0.9
CA19.9 , LRG1 , CRP , MUC5AC	0.67	0.293	0.9	0.607	0.159	0.9
CA19.9 , LRG1 , PKM2 , CRP	0.673	0.293	0.9	0.606	0.159	0.9
PKM2 , TBIL , CRP , MUC5AC	0.66	0.28	0.9	0.584	0.159	0.9
IL6 , ALP , GGT , MUC5AC	0.624	0.268	0.9	0.55	0.159	0.9
IL6 , TBIL , ALP , GGT	0.628	0.268	0.9	0.549	0.159	0.9
IL6 , TBIL , CRP , ALP	0.664	0.268	0.9	0.599	0.159	0.9
CA19.9 , PKM2 , CYFRA21.1	0.626	0.256	0.9	0.565	0.159	0.9

IL6 , PKM2 , CRP	0.654	0.256	0.9	0.585	0.159	0.9
PKM2 , TBIL , CRP , CYFRA21.1	0.641	0.256	0.9	0.583	0.159	0.9
LRG1 , TBIL , CRP , ALP	0.663	0.244	0.9	0.606	0.159	0.9
LRG1 , TBIL , GGT	0.619	0.244	0.9	0.524	0.159	0.9
CA19.9 , IL6 , CRP , CYFRA21.1	0.685	0.232	0.9	0.634	0.159	0.9
CA19.9 , LRG1 , CRP	0.675	0.232	0.9	0.623	0.159	0.9
CA19.9 , LRG1 , MUC5AC , CYFRA21.1	0.614	0.232	0.9	0.526	0.159	0.9
CA19.9 , TBIL , MUC5AC	0.602	0.232	0.9	0.533	0.159	0.9
CA19.9 , IL6 , ALP	0.659	0.22	0.9	0.617	0.159	0.9
CA19.9 , IL6 , ALP , MUC5AC	0.661	0.22	0.9	0.608	0.159	0.9
CA19.9 , IL6 , TBIL , CRP	0.685	0.22	0.9	0.631	0.159	0.9
CA19.9 , LRG1 , CYFRA21.1	0.612	0.22	0.9	0.542	0.159	0.9
CA19.9 , LRG1 , TBIL	0.607	0.22	0.9	0.531	0.159	0.9
CA19.9 , PKM2 , TBIL , CYFRA21.1	0.618	0.22	0.9	0.55	0.159	0.9
CA19.9 , LRG1 , MUC5AC	0.61	0.268	0.9	0.532	0.146	0.9

IL6 , LRG1 , TBIL , GGT	0.62	0.268	0.9	0.517	0.146	0.9
LRG1 , PKM2 , TBIL , ALP	0.618	0.256	0.9	0.548	0.146	0.9
CA19.9 , IL6 , PKM2 , ALP	0.662	0.244	0.9	0.606	0.146	0.9
CA19.9 , PKM2 , MUC5AC , CYFRA21.1	0.631	0.244	0.9	0.555	0.146	0.9
LRG1 , ALP , CYFRA21.1	0.628	0.244	0.9	0.57	0.146	0.9
LRG1 , PKM2 , ALP	0.619	0.244	0.9	0.566	0.146	0.9
LRG1 , PKM2 , ALP , MUC5AC	0.621	0.244	0.9	0.553	0.146	0.9
TBIL , ALP , MUC5AC , CYFRA21.1	0.629	0.244	0.9	0.55	0.146	0.9
CA19.9 , LRG1 , TBIL , CYFRA21.1	0.604	0.232	0.9	0.526	0.146	0.9
LRG1 , ALP , MUC5AC , CYFRA21.1	0.631	0.232	0.9	0.556	0.146	0.9
PKM2 , ALP , MUC5AC , CYFRA21.1	0.628	0.232	0.9	0.557	0.146	0.9
CA19.9 , IL6 , TBIL , ALP	0.659	0.22	0.9	0.603	0.146	0.9
LRG1 , PKM2 , ALP , CYFRA21.1	0.629	0.22	0.9	0.557	0.146	0.9
CA19.9 , IL6 , LRG1 , ALP	0.66	0.207	0.9	0.603	0.146	0.9
IL6 , CRP , ALP , CYFRA21.1	0.661	0.207	0.9	0.612	0.146	0.9

IL6 , PKM2 , CRP , CYFRA21.1	0.645	0.207	0.9	0.573	0.146	0.9
LRG1 , TBIL , ALP	0.62	0.207	0.9	0.556	0.146	0.9
LRG1 , TBIL , CRP	0.673	0.207	0.9	0.603	0.146	0.9
CA19.9 , IL6 , PKM2 , TBIL	0.619	0.195	0.9	0.549	0.146	0.9
LRG1 , TBIL , ALP , CYFRA21.1	0.625	0.195	0.9	0.55	0.146	0.9
IL6 , PKM2 , ALP	0.624	0.159	0.9	0.572	0.146	0.9
CA19.9 , LRG1 , PKM2	0.616	0.28	0.9	0.552	0.134	0.9
CA19.9 , LRG1 , PKM2 , MUC5AC	0.621	0.28	0.9	0.541	0.134	0.9
CA19.9 , IL6 , CRP , MUC5AC	0.677	0.268	0.9	0.621	0.134	0.9
IL6 , PKM2 , TBIL , CRP	0.67	0.256	0.9	0.593	0.134	0.9
CA19.9 , IL6 , TBIL , MUC5AC	0.608	0.244	0.9	0.527	0.134	0.9
CA19.9 , LRG1 , PKM2 , CYFRA21.1	0.624	0.244	0.9	0.543	0.134	0.9
CA19.9 , TBIL , MUC5AC , CYFRA21.1	0.607	0.244	0.9	0.532	0.134	0.9
LRG1 , TBIL , ALP , MUC5AC	0.62	0.244	0.9	0.542	0.134	0.9
CA19.9 , IL6 , CRP	0.684	0.232	0.9	0.638	0.134	0.9

CA19.9 , IL6 , MUC5AC	0.619	0.232	0.9	0.547	0.134	0.9
LRG1 , PKM2 , TBIL , CRP	0.667	0.232	0.9	0.583	0.134	0.9
CA19.9 , IL6 , LRG1 , CRP	0.679	0.207	0.9	0.621	0.134	0.9
CA19.9 , IL6 , PKM2 , CYFRA21.1	0.632	0.207	0.9	0.556	0.134	0.9
CA19.9 , IL6 , PKM2 , MUC5AC	0.634	0.171	0.9	0.56	0.134	0.9
IL6 , PKM2 , TBIL , ALP	0.624	0.171	0.9	0.552	0.134	0.9
IL6 , PKM2 , ALP , CYFRA21.1	0.63	0.159	0.9	0.562	0.134	0.9
CA19.9 , IL6 , PKM2	0.624	0.146	0.9	0.566	0.134	0.9
IL6 , LRG1 , PKM2 , CRP	0.65	0.256	0.9	0.564	0.122	0.9
CA19.9 , IL6 , MUC5AC , CYFRA21.1	0.619	0.244	0.9	0.539	0.122	0.9
CA19.9 , LRG1	0.608	0.244	0.9	0.547	0.122	0.9
CA19.9 , IL6 , LRG1 , MUC5AC	0.616	0.207	0.9	0.529	0.122	0.9
CA19.9 , IL6	0.612	0.195	0.9	0.556	0.122	0.9
CA19.9 , IL6 , TBIL	0.606	0.195	0.9	0.535	0.122	0.9
CA19.9 , IL6 , LRG1 , PKM2	0.621	0.159	0.9	0.546	0.122	0.9
TBIL	0.5	0.159	0.9	0.365	0.122	0.9

CA19.9 , IL6 , TBIL , CYFRA21.1	0.605	0.244	0.9	0.536	0.11	0.9
IL6 , TBIL , CRP	0.683	0.232	0.9	0.611	0.11	0.9
CA19.9 , IL6 , LRG1	0.613	0.22	0.9	0.539	0.11	0.9
IL6 , TBIL , ALP	0.617	0.22	0.9	0.556	0.11	0.9
CA19.9 , IL6 , LRG1 , TBIL	0.609	0.207	0.9	0.526	0.11	0.9
IL6 , ALP	0.618	0.207	0.9	0.577	0.11	0.9
TBIL , MUC5AC	0.5	0.171	0.9	0.381	0.11	0.9
IL6 , TBIL , CRP , MUC5AC	0.671	0.305	0.9	0.578	0.098	0.9
CA19.9 , IL6 , CYFRA21.1	0.614	0.232	0.9	0.551	0.098	0.9
CA19.9 , IL6 , LRG1 , CYFRA21.1	0.615	0.232	0.9	0.534	0.098	0.9
IL6 , PKM2 , ALP , MUC5AC	0.626	0.232	0.9	0.559	0.098	0.9
IL6 , TBIL , ALP , CYFRA21.1	0.624	0.22	0.9	0.546	0.098	0.9
IL6 , ALP , CYFRA21.1	0.623	0.207	0.9	0.566	0.098	0.9
IL6 , ALP , MUC5AC , CYFRA21.1	0.625	0.183	0.9	0.555	0.098	0.9
IL6 , LRG1 , PKM2 , ALP	0.625	0.183	0.9	0.555	0.098	0.9
PKM2 , MUC5AC	0.549	0.122	0.9	0.469	0.098	0.9

IL6 , ALP , MUC5AC	0.616	0.244	0.9	0.563	0.085	0.9
IL6 , TBIL , ALP , MUC5AC	0.617	0.232	0.9	0.545	0.085	0.9
IL6 , TBIL , CRP , CYFRA21.1	0.656	0.232	0.9	0.579	0.085	0.9
IL6 , LRG1 , CRP , ALP	0.671	0.22	0.9	0.609	0.085	0.9
IL6 , LRG1 , ALP	0.625	0.195	0.9	0.568	0.085	0.9
IL6 , LRG1 , ALP , MUC5AC	0.622	0.195	0.9	0.556	0.085	0.9
IL6 , LRG1 , TBIL , CRP	0.676	0.195	0.9	0.589	0.085	0.9
IL6 , LRG1 , TBIL , ALP	0.622	0.183	0.9	0.549	0.085	0.9
LRG1 , PKM2	0.534	0.146	0.9	0.446	0.085	0.9
LRG1 , PKM2 , MUC5AC	0.548	0.146	0.9	0.44	0.085	0.9
IL6 , PKM2	0.541	0.134	0.9	0.451	0.085	0.9
PKM2	0.533	0.134	0.9	0.473	0.085	0.9
IL6 , PKM2 , MUC5AC	0.559	0.122	0.9	0.453	0.085	0.9
IL6 , TBIL	0.515	0.171	0.9	0.354	0.073	0.9
TBIL , MUC5AC , CYFRA21.1	0.524	0.171	0.9	0.394	0.073	0.9
IL6 , LRG1 , ALP , CYFRA21.1	0.627	0.159	0.9	0.558	0.073	0.9

IL6 , LRG1 , PKM2	0.541	0.146	0.9	0.426	0.073	0.9
IL6 , TBIL , MUC5AC	0.517	0.11	0.9	0.369	0.073	0.9
IL6 , PKM2 , CYFRA21.1	0.564	0.073	0.9	0.467	0.073	0.9
PKM2 , CYFRA21.1	0.56	0.073	0.9	0.481	0.073	0.9
IL6	0.555	0.159	0.9	0.117	0.061	0.9
LRG1 , PKM2 , TBIL	0.551	0.159	0.9	0.458	0.061	0.9
IL6 , PKM2 , TBIL	0.555	0.134	0.9	0.464	0.061	0.9
PKM2 , TBIL	0.551	0.134	0.9	0.484	0.061	0.9
TBIL , CYFRA21.1	0.523	0.122	0.9	0.405	0.061	0.9
IL6 , TBIL , CYFRA21.1	0.534	0.11	0.9	0.396	0.061	0.9
LRG1 , PKM2 , CYFRA21.1	0.559	0.085	0.9	0.454	0.061	0.9
IL6 , PKM2 , MUC5AC , CYFRA21.1	0.573	0.073	0.9	0.46	0.061	0.9
PKM2 , MUC5AC , CYFRA21.1	0.569	0.073	0.9	0.473	0.061	0.9
IL6 , PKM2 , TBIL , MUC5AC	0.568	0.159	0.9	0.456	0.049	0.9
PKM2 , TBIL , MUC5AC	0.56	0.134	0.9	0.471	0.049	0.9
PKM2 , TBIL , CYFRA21.1	0.56	0.11	0.9	0.477	0.049	0.9

LRG1 , PKM2 , MUC5AC , CYFRA21.1	0.568	0.085	0.9	0.445	0.049	0.9
PKM2 , TBIL , MUC5AC , CYFRA21.1	0.565	0.134	0.9	0.462	0.037	0.9
IL6 , PKM2 , TBIL , CYFRA21.1	0.563	0.11	0.9	0.462	0.037	0.9
LRG1	0.546	0.073	0.9	0.416	0.037	0.9
MUC5AC , CYFRA21.1	0.517	0.159	0.9	0.376	0.024	0.9
LRG1 , TBIL	0.562	0.122	0.9	0.448	0.024	0.9
LRG1 , TBIL , MUC5AC	0.535	0.122	0.9	0.402	0.024	0.9
LRG1 , TBIL , CYFRA21.1	0.536	0.098	0.9	0.411	0.024	0.9
IL6 , LRG1 , MUC5AC	0.55	0.073	0.9	0.386	0.024	0.9
LRG1 , MUC5AC	0.536	0.073	0.9	0.391	0.024	0.9
LRG1 , MUC5AC , CYFRA21.1	0.53	0.061	0.9	0.383	0.012	0.9
LRG1 , CYFRA21.1	0.524	0.049	0.9	0.398	0.012	0.9
CYFRA21.1	0.517	0.171	0.9	0.376	0	0.9
IL6 , CYFRA21.1	0.533	0.11	0.9	0.375	0	0.9
IL6 , MUC5AC	0.542	0.11	0.9	0.33	0	0.9

MUC5AC	0.506	0.11	0.9	0.315	0	0.9
IL6 , LRG1 , TBIL	0.567	0.098	0.9	0.423	0	0.9
IL6 , MUC5AC , CYFRA21.1	0.531	0.098	0.9	0.375	0	0.9
IL6 , LRG1	0.559	0.061	0.9	0.413	0	0.9
IL6 , LRG1 , CYFRA21.1	0.534	0.037	0.9	0.391	0	0.9