Bile acid diarrhoea in post-cholecystectomy patients (BADCAP)

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Declaration

This thesis is submitted to the University of Warwick in support of my application for the degree of Doctor of Medicine by Research. It has been composed by myself and has not been submitted in any previous application for any degree.

The work presented (including data generated and data analysis) was carried out by the author except in the cases outlined below:

FGF19 plasma concentrations and C4 plasma concentrations have been provided by Tracy Neal at Affinity Biomarker labs from samples collected by the author

Parts of this thesis have been published by the author:

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Post-Cholecystectomy diarrhoea rate and predictive factors – a systematic review of the literature. (submitted)

Dedication

This dissertation is dedicated to Joseph and my parents for their never-ending patience, understanding and support.

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List of Abbreviations

ASBT	Apical sodium-dependent bile acid transporter
BAD	Bile acid diarrhoea
C4	7α-hydroxycholest-4-en-3-one
CA	Cholic acid
CDCA	Chenodeoxycholic acid
CYP7A1	cholesterol 7α-hydroxylase
CYP27A1	Sterol 27-hydroxylase
DCA	Deoxycholic acid
FGF19	Fibroblast growth factor 19
FGFR4	Fibroblast growth factor receptor 4
FXR	Farnesoid X Receptor
GIQLI	Gastrointestinal quality of life
HDL	High density lipoprotein
IBD	Inflammatory bowel disease
IBS	Irritable bowel syndrome
LCA	Lithocholic acid
LDL	Low density lipoprotein
OST	Organic solute transporter
PCD	Post-cholecystectomy diarrhoea
PPARα	Peroxisome proliferator-activated receptor alpha
PPI	Patient and public involvement
SCFA	Short chain fatty acids
SeHCAT	Selenium homocholic acid taurine test

SHP	Short heterodimer primer
SREBP1	Sterol response element binding protein 1
SREBP2	Sterol response element binding protein 2
UDCA	Ursodeoxycholic acid
VLDL	Very low-density lipoprotein

Abstract

Introduction

Bile acid diarrhoea (BAD) can occur due to disruption to the enterohepatic circulation e.g. following cholecystectomy. The mechanism behind BAD after cholecystectomy is as yet unknown. The aim of this work was to determine the rate of post-cholecystectomy diarrhoea and to assess whether FGF19 within the gallbladder was associated with the development of BAD.

Methods

The project was divided into two parts. The first part was a multicentre retrospective audit to assess the rate of investigation of post-cholecystectomy patients. The second part was a prospective case-control study in which patients were assessed pre- and post cholecystectomy (study group) and compared with patients also having keyhole surgery in the abdomen but not cholecystectomy (control group). Their bowel habits and a GIQLI questionnaire was performed to compare the two groups and to compare pre- and post-operative condition. A small subset of these patients also had blood tests.

Results

The multicentre audit found that only 2.1% of patients are investigated for diarrhoea post-cholecystectomy, which contrasts directly with our systematic review stating that 13.3% of patients have post-cholecystectomy diarrhoea.

In the case-control study, there were no significant results when assessing the effect of gallbladder FGF19 concentration on bowel habit, stool consistency, lipid levels, BMI or smoking. Gallbladder PPAR α was found to have a significant correlation with stool consistency, with the lower the PPARα concentration the higher the Bristol stool chart number (i.e. looser stool).

The study group showed a significant increase in triglycerides post-operatively, however there were no changes in cholesterol, HDL and LDL levels. Correlation of

these increased triglyceride levels and GIQLI, stool consistency and bowel habits showed no significant results.

Discussion

We have seen that a smaller percentage of patients is being investigated for diarrhoea than is expected. While there is a general improvement in post operative quality of life, we did not find any direct evidence that FGF19 levels within the gallbladder impact the development of post-cholecystectomy diarrhoea. While we have shown a significant increase in triglycerides postoperatively, there was also no correlation with PPARα. Further work is required particularly relating to the gut microbiome to further investigate this condition.

Chapter 1: Background and Basic Science

Bile acid synthesis and the enterohepatic circulation

Bile acids (BA) are synthesized from cholesterol in the liver. They are then stored in the gallbladder and secreted into the duodenum when stimulated by food intake, after which they travel along the small bowel to be re-absorbed in the terminal ileum. This creates a cycle of negative feedback by which bile acid synthesis is regulated.

The major bile acids in humans are cholic acid (CA), chenodeoxycholic acid (CDCA and deoxycholic acid (DCA), and the majority of these are synthesised by the neutral or 'classical' pathway (Chiang, 2004). Bile acid synthesis commences from cholesterol which undergoes 7α-hydroxylation by cholesterol 7α-hydroxylase (CYP7A1), a process which occurs in the hepatic microsomes. This is the rate-limiting step in bile acid biosynthesis, and CYP7A1 activity is subject to negative feedback from bile acids returning to the liver via the portal vein- the enterohepatic circulation. This step produces 7α-hydroxycholesterol which is then metabolized to 7α-hydroxycholest-4-en-3-one (C4), which may then be converted to 7α,12α-dihydroxycholest-4-en-3-one. α-hydroxycholest-4-en-3-one and 7α,12α-dihydroxycholest-4-en-3-one are then converted to the intermediates of 5β-cholestane-3α, 7α-diol and 5β-cholestane-3α, 7α, 12α-triol, with the mother nucleus of CDCA and CA respectively. 5β-cholestane-3α, 7α-diol is initially converted to 5β-cholestane-3α, 7α, 27α-triol while 5β-cholestane-3α, 7α, 12α-triol is converted to 5β-cholestane-3α, 7α, 12α 27α-tetrol by sterol 27-hydroxylase (CYP27A1) which are then oxidized to produce 3α,7α-dihydroxy-5β-cholestanoic acid and 3α,7α, 12α-trihydroxy-5β-cholestanoic acid respectively. The final step in this process is β-oxidation therefore producing CDCA and CA(Tazuma S, 2017). CA is then metabolized to DCA, while CDCA can be metabolised to both lithocholic acid (LCA) and ursodeoxycholic acid (UDCA).

Bile acids are then expressed into the duodenum to aid in digestion of fatty acids and are released by contraction of the gallbladder after stimulation by cholecystokinin, which is released from the stomach in response to a meal.

Primary bile acids in the ileum are absorbed via apical sodium-dependent bile acid transporter (ASBT) to activate ileal Farnesoid X Receptor (FXR), which induces

transcription of Fibroblast growth Factor 19 (FGF19). This is then released into the portal circulation and travels to the liver to activate hepatic FXR which acts on CYP7A1 via short heterodimer primer (SHP), thus decreasing bile acid synthesis (Zhou & Hylemon, 2014). Bile acids are also released back into the portal circulation via OSTα and β solute transporters to be transported to the liver to provide further negative feedback. FGF19 also binds to Fibroblast growth factor receptor 4 (FGFR4) in the hepatocytes, which interacts with β-klotho (KLB) to inhibit CYP7A1 leading to a decrease in bile acid synthesis via the classical pathway and activating hepatocyte FXR (Keely & Walters, 2016; Walters, 2014). Production of FGF19 therefore inhibits BA synthesis by these two negative feedback loops (Amigo *et al.*, 2011). These processes are shown in figures 1 and 2.

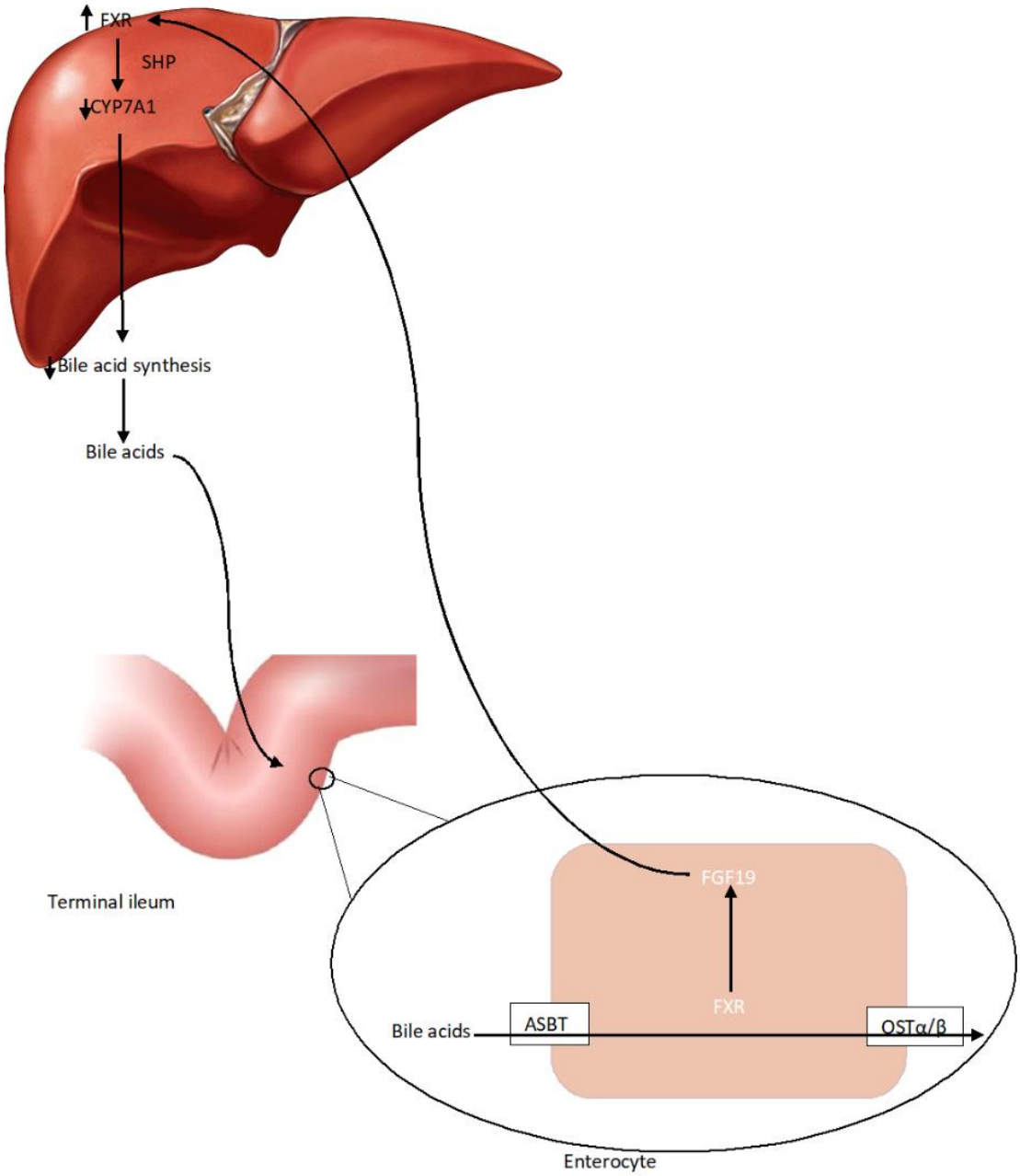

Figure 1: Primary bile acids in the ileum are absorbed via apical sodium-dependent bile acid transporter (ASBT) to activate ileal Farnesoid X Receptor (FXR), which induces transcription of Fibroblast growth Factor 19 (FGF19). This is then released into the portal circulation and travels to the liver to activate hepatic FXR which acts on CYP7A1 via short heterodimer primer (SHP), thus decreasing bile acid synthesis. Figure adapted from Farrugia, A. & Arasaradnam, R. (2020) Bile acid diarrhoea: pathophysiology, diagnosis and management. Frontline Gastroenterology, flgastro-2020-101436.

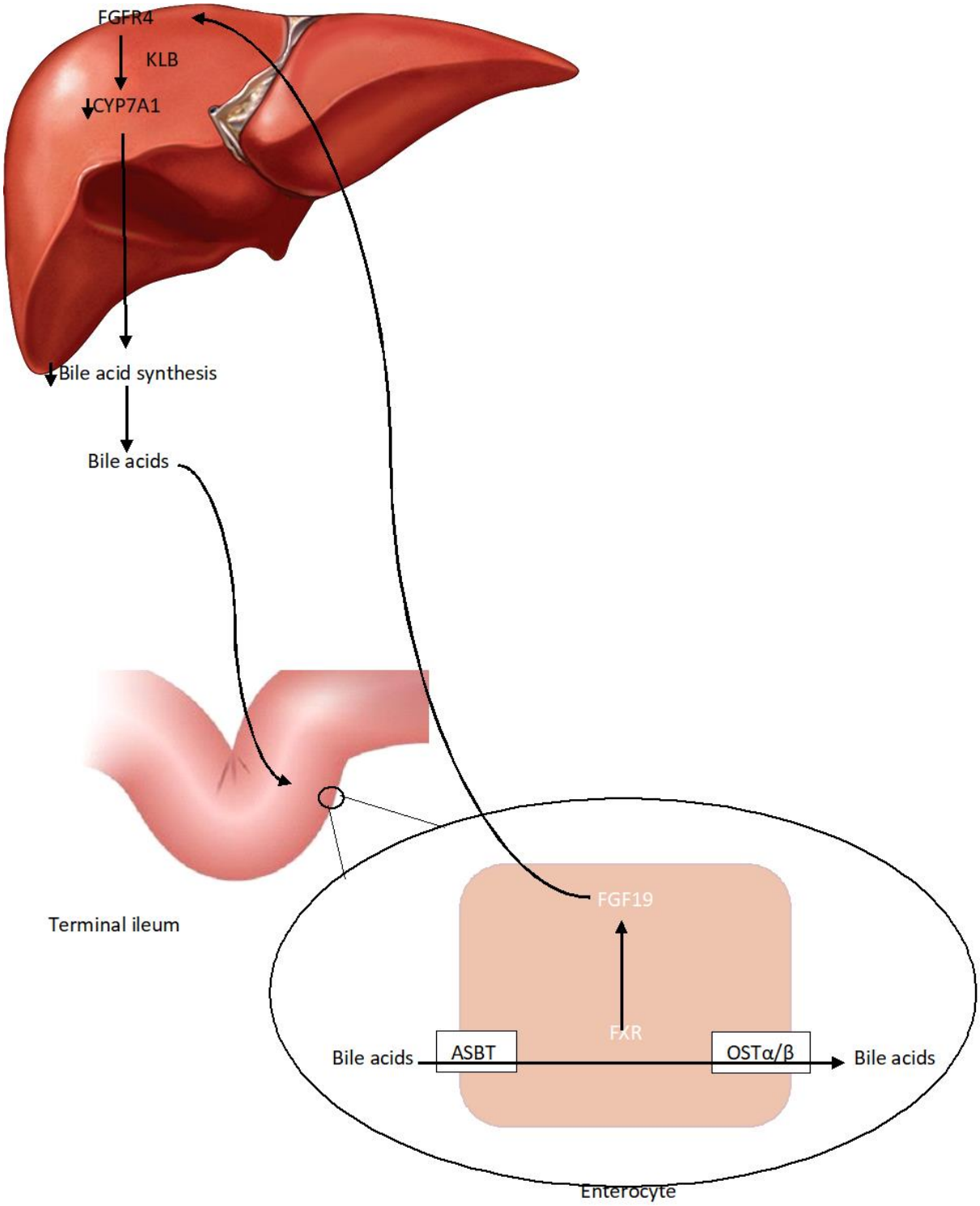

Figure 2: Bile acids are also released back into the portal circulation via OSTα and β solute transporters to be transported to the liver to provide further negative feedback. FGF19 also binds to Fibroblast growth factor receptor 4 (FGFR4) in the hepatocytes, which interacts with β-klotho (KLB) to inhibit CYP7A1 leading to a decrease in bile acid synthesis via the classical pathway and activating hepatocyte FXR. Figure adapted from Farrugia, A. & Arasaradnam, R. (2020) Bile acid diarrhoea: pathophysiology, diagnosis and management. Frontline Gastroenterology, flgastro-2020-101436.

Lipids and their relationship to bile acids

Bile acids are required for lipid absorption, as they emulsify dietary lipids which are then formed into chylomicrons. These are made of phospholipids, triglycerides, cholesterol esters, apolipoprotein B-48, apolipoprotein C-II and apolipoprotein E. Hydrolysis of triglycerides occurs, and residual chylomicrons, still containing triglycerides, are taken up by the liver to form very low-density lipoproteins (VLDLs). Triglyceride-rich VLDLs are taken up by the liver, resulting in lower serum triglyceride levels. Formation of primary bile acids (CDCA and CA) by hydroxylation of cholesterol via CYP7A1 leads to a hepatic cholesterol deficiency, resulting in upregulation of LDL receptor expression and higher LDL uptake. The end result of this process is reduced plasma LDL cholesterol levels (Sagar NM, 2016). This is shown in figure 3.

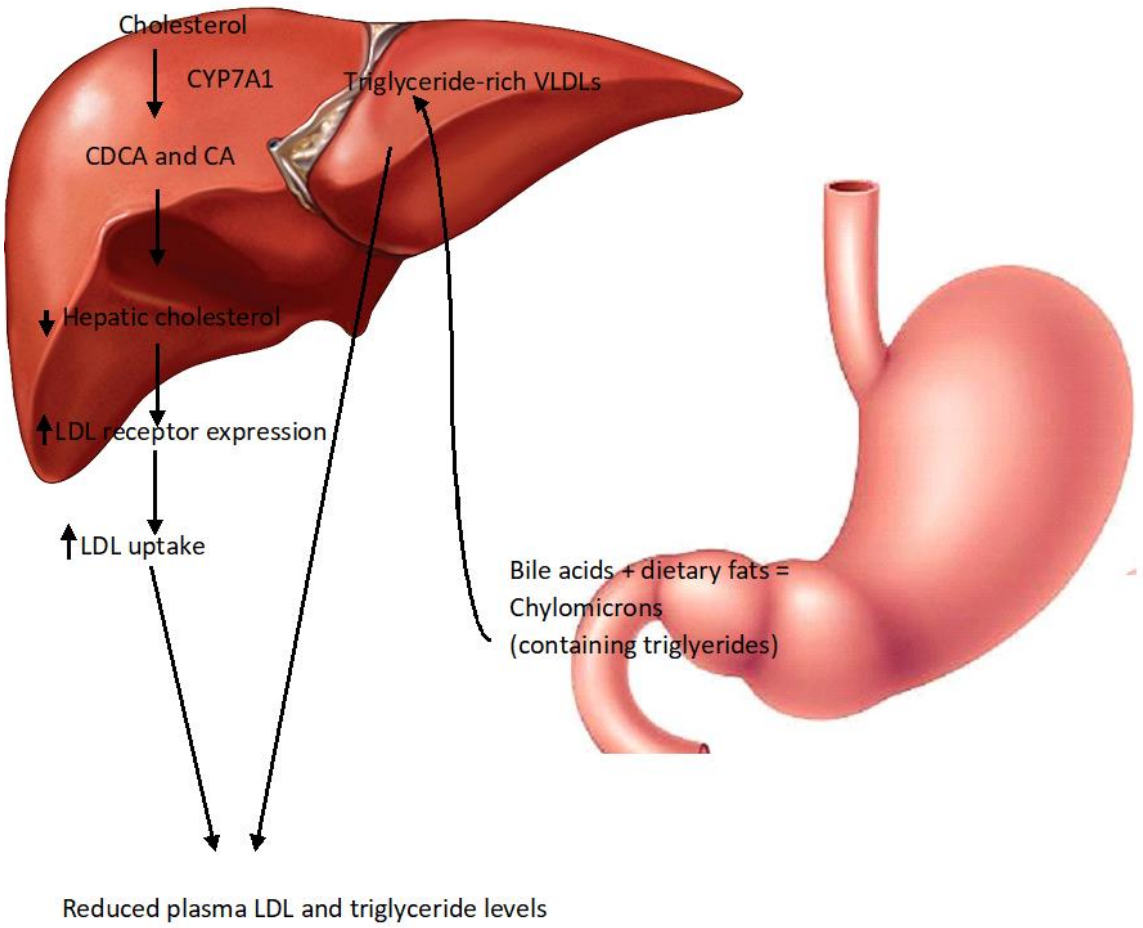

Figure 3: Triglyceride-rich VLDLs are taken up by the liver, resulting in lower serum triglyceride levels. Formation of primary bile acids (CDCA and CA) by hydroxylation of cholesterol via CYP7A1 leads to a hepatic cholesterol deficiency, resulting in upregulation of LDL receptor expression and higher LDL uptake. The end result of this process is reduced plasma LDL cholesterol and triglyceride levels.

ASBT levels in the terminal ileum will vary based on cholesterol levels to properly regulate the cholesterol level of the body. Low cholesterol levels result in cleavage of sterol response element binding protein-2 (SREBP2) into a mature transcription factor, and this induces ASBT expression. This upregulation, combined with an increase in bile acids enhances the negative feedback effect on CYP7A1, leading to decreased bile acid synthesis, and downregulation of ASBT due to the higher cholesterol levels (Xiao & Pan, 2017). FXR activation by bile acids (BAs) reduces hepatic triglyceride levels by decreasing SREBP1-stimulated lipogenesis via SHP. FXR also induces peroxisome proliferator-activated receptor alpha (PPARα) which regulates fatty acid metabolism by decreasing hepatic apo C-III production and increasing LPL-mediated lipolysis which increases triglyceride metabolism and decreased LDL secretion, thus causing increased free fatty acid oxidation and decreasing serum triglyceride levels. Overall, this implies that FXR activation leads to increased lipolysis and decreased lipogenesis. FXR also affects lipid transport by decreasing apolipoprotein expression and induces VLDL expression(Amigo *et al.*, 2011; Ferrebee & Dawson, 2015). In fact, administration of CDCA, a potent FXR agonist, leads to reduced synthesis of bile acids and cholesterol as well as reduced VLDL production thus reducing plasma triglyceride levels. This is shown in figure 4. However, serum cholesterol levels are increased. CDCA treatment also reduces C4 levels and increases FGF19 levels. FXR agonists also influence gene expression of apolipoproteins (Ghosh Laskar *et al.*, 2017). People treated with bile acid sequestrants, leading to overexpression of CYP7A1 due to lack of FXR stimulation, have hypertriglyceridaemia due to the induction of hepatic VLDL secretion, and indeed animal studies with mice have shown that CYP7A1 deficient mice have lower plasma triglyceride levels due to lower VLDL production, though plasma cholesterol levels were not affected (Post *et al.*, 2004).

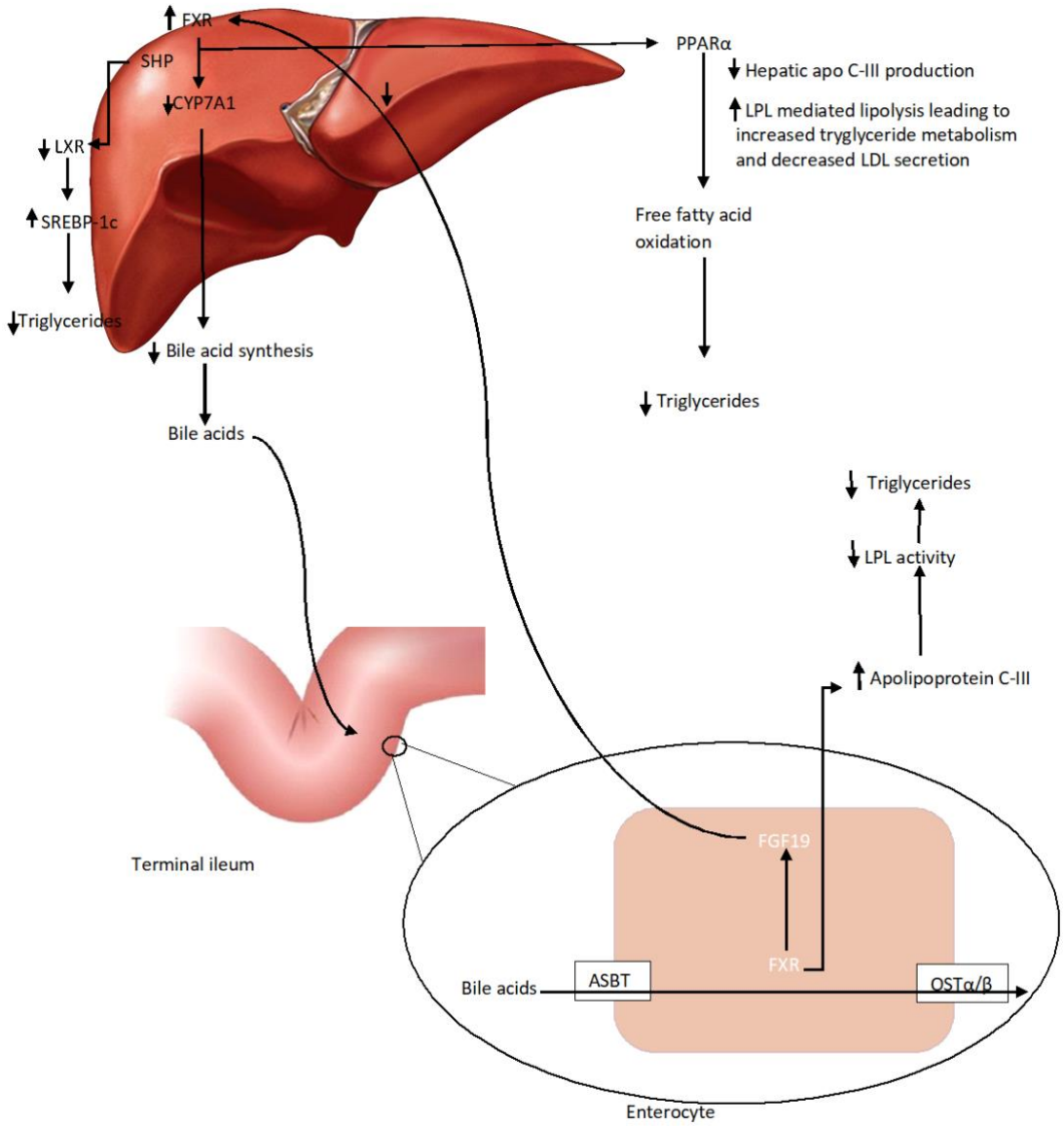

Figure 4: The relationship between FXR and lipid levels: Increased FXR causes three actions: activation of SHP, decrease in CYP7A1, and activation of PPARα. SHP activation leads to decreased triglycerides, decrease CYP7A1 leads to decreased bile acid synthesis leading to activation of FGF19 in the ileum and decreasing plasma triglycerides via apolipoprotein C-III, and PPAR activation causes free fatty acid oxidation leading to decreased triglycerides.

Bile acid diarrhoea

Types of bile acid diarrhoea

There are three main types of bile acid diarrhoea. Type 1 is caused by ileal pathology such as inflammation or resection. Type two is idiopathic or primary bile acid diarrhoea, while type 3 is secondary to other conditions where the ileum appears normal. An example of type 3 bile acid diarrhoea occurs after cholecystectomy, and this is characterised by large amounts of bile acids entering the terminal ileum, thus exceeding its normal absorptive capacity. This occurs either due to increased hepatic synthesis or defective bile acid regulation (Damsgaard *et al.*, 2018).

The ^{75}SeHCAT test is used to measure bile acid retention and a value of <15% retention is indicative of bile acid diarrhoea. This is the gold standard for measuring bile acid malabsorption, and has a high sensitivity and specificity (Damsgaard *et al.*, 2018). In one particular study, 34% of patients with a low ^{75}SeHCAT had either gallstones or a previous cholecystectomy (Appleby *et al.*, 2017). Other work has shown that having had a previous cholecystectomy implied that a patient was more likely to have a positive ^{75}SeHCAT test (Kurien *et al.*, 2011). C4 is a direct measure of bile acid synthesis and is increased in bile acid diarrhoea and can thus be used as a diagnostic tool in cases of ^{75}SEHCAT unavailability. ^{75}SeHCAT testing is recommended in patients presenting with chronic diarrhoea as part of the secondary clinical assessment, as per recent guidelines (Arasaradnam *et al.*, 2018). The use of an electronic nose to diagnose BAD is being investigated, as the gas signature profile of a patient with BAD demonstrated different gas fermentation profiles and this is due to gut dysbiosis. The main gases identified in BAD patients were 2-propanolol and acetamide (Covington *et al.*, 2013).

Mechanisms behind bile acid diarrhoea

The mechanism behind bile acid diarrhoea relates to the negative feedback mechanism in the rate-limiting step catalysed by CYP7A1. When the negative feedback mechanism is disrupted, as occurs in bile acid diarrhoea, the activity of CYP7A1 is increased and there is a six- to seven-fold increase in the synthesis of bile acids (Tazuma S, 2017).

Cholecystectomy, bile acid diarrhoea and relationship to lipid metabolism

Most patients with gallstone disease have raised triglycerides and cholesterol, and there was improvement in levels when serum levels were repeated after surgery (Gill & Gupta, 2017; Malik *et al.*, 2011). Post-cholecystectomy there is faster circulation of BA, resulting in negative feedback and therefore lower triglyceride levels (Amigo *et al.*, 2011). However, this is not seen in all studies as in some cases there is no change in lipid levels, including any difference between patients who develop post cholecystectomy diarrhoea and those who do not (Sauter *et al.*, 2002a).

Hypertriglyceridaemia has been linked to increased bile acid synthesis and higher triglyceride levels are associated with lower SeHCAT retention levels (Johnston IM, 2016). It has been demonstrated that primary bile acid diarrhoea was significantly associated with higher triglyceride levels (Appleby *et al.*, 2017).

In cases of lower FGF 19 levels, such as occurs in BAD, SHP is inhibited and thus SREBP-1 expression is not repressed leading to higher triglyceride levels. Due to decreased negative feedback, there is increased bile acid synthesis leading to increased LDL uptake. These are then converted into VLDLs during the bile acid synthesis process and released into the systemic circulation. Lack of FXR activation also means that LPL activity is increased leading to increased VLDL formation. All these factors work together resulting in hypertriglyceridaemia (Sagar NM, 2016).

Hypertriglyceridaemia leads to reduced ASBT expression, thus impairing intestinal bile acid absorption. This implies less bile acid uptake, therefore less FGF19 levels as ileal FXR is not activated, leading to reduced negative feedback on bile acid synthesis (Renner *et al.*, 2008). OCA treatment results in higher fasting total and LDL-cholesterol and a reduction in triglycerides (Walters *et al.*, 2015).

Cholecystectomy

Cholecystectomy is surgical removal of the gallbladder, and this is usually undertaken in the context of symptomatic gallstones as per NICE guidelines (NICE, 2014). Symptomatic gallstones may include infections (such as cholecystitis, infection of the gallbladder), pain often triggered by fatty food (known as biliary colic), or inflammatory disorders such as pancreatitis. Cholecystectomy is nearly always performed

laparoscopically (keyhole surgery) in a standard four-port approach (3 for instruments and one for the camera). After the gallbladder is retracted cephalad, Calot's triangle is dissected to expose and definitively identify the cystic duct and cystic artery. These are then clipped and divided (figures 5 and 6) after which the gallbladder is dissected from its bed on the undersurface of the liver and then removed. Care must be taken to avoid damage to the common bile duct (Novell).

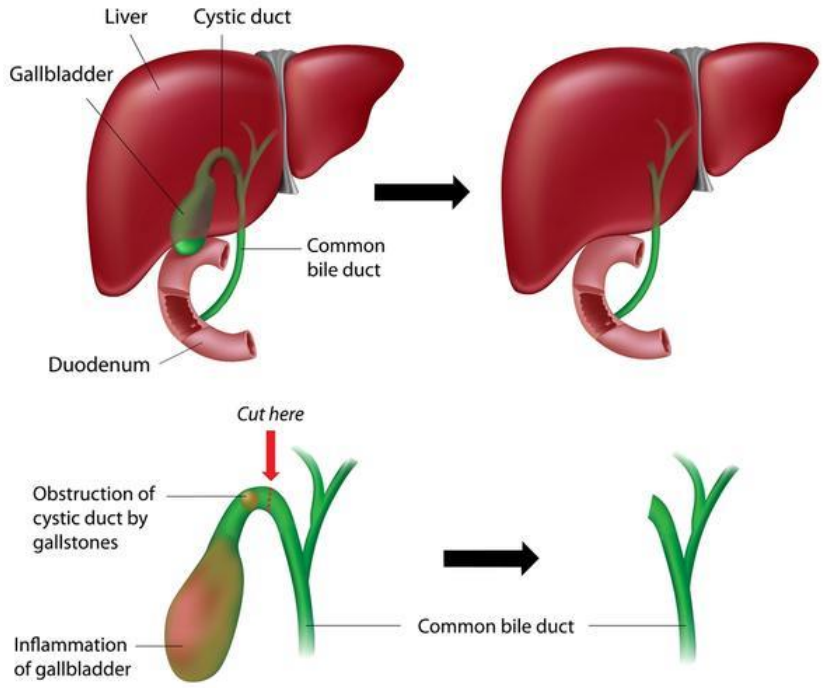

Figure 5: Cholecystectomy (taken from https://uppergisurgery.com.au)

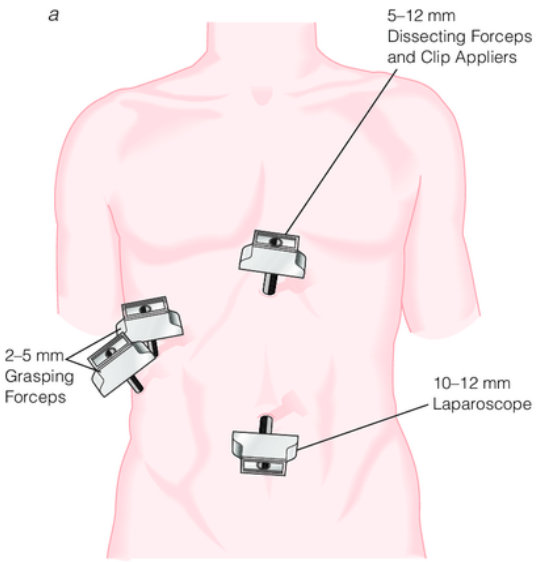

Figure 6: Port placement for laparoscopic cholecystectomy (taken from ACS Surgery: Principles and Practice, Chapter 5, unit 21: Cholecystectomy and common bile duct exploration ©2005)

Post-cholecystectomy diarrhoea – a systematic review of the literature

Methods

The review was registered on PROSPERO (CRD42019140444). A literature search was performed on multiple databases, which were PUBMED, EMBASE and MEDLINE, Cochrane, google scholar using the keywords ‘post-cholecystectomy’ ‘postoperative’ ‘cholecystectomy’ ‘diarrhoea’ and ‘predictive factors’. There were no language limitations or restrictions to the year of publication within the search. The last search date was 29th September 2020. The search strategy is outlined in figure 7.

The inclusion criteria consisted of cohort studies or randomised trials which specifically investigated the rate of post-cholecystectomy diarrhoea and predictive factors for it. Exclusion criteria consisted of case reports, case series, conference abstracts and expert opinion pieces, as well as systematic reviews since all the original articles from those reviews were included in this review. Studies were also excluded if they investigated symptoms which were present prior to operation and were then persistent postoperatively.

Data was extracted from the studies and entered into an electronic database. The results were subsequently collated. The data extracted included: patient numbers, age, gender, type of study, indication for surgery, preoperative symptoms, postoperative symptoms, predictive factors. The primary endpoint of the study was to identify the rate of post-cholecystectomy diarrhoea and the secondary endpoint was to identify potential predictive factors for post-cholecystectomy diarrhoea.

The systematic review was written according to preferred reporting systems for systematic reviews (PRISMA) guidelines (Moher *et al.*, 2009).

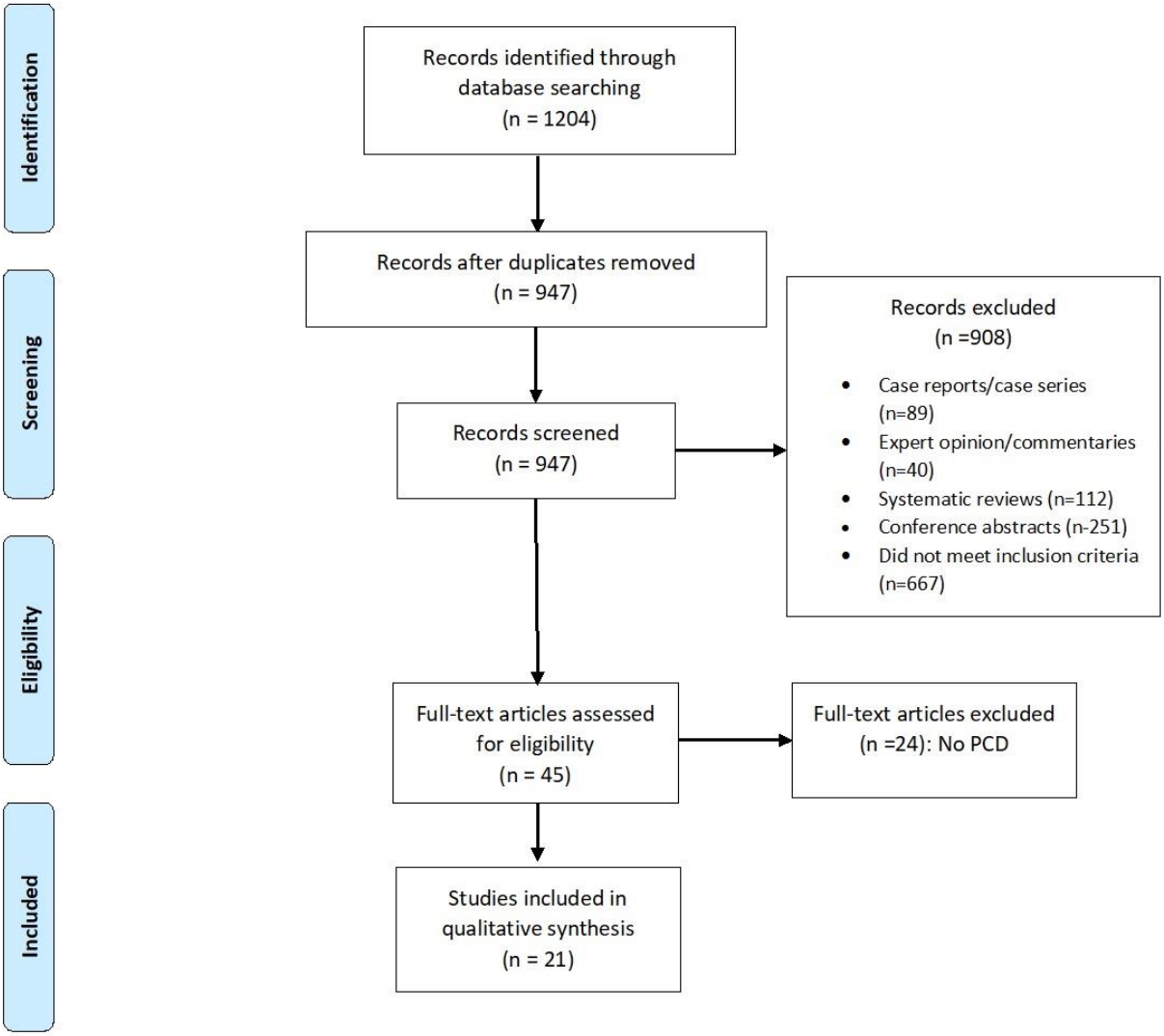

Figure 7: PRISMA flowchart for study selection

Results

Selected studies

A total of 1204 papers were identified in the initial search. Duplicates were removed which reduced the number to 947. Screening by title and abstract was undertaken and 45 papers were initially considered. Full-text review of these papers revealed that only 17 were relevant. These papers all described new-onset post-cholecystectomy

diarrhoea. A further 4 papers were found to fit the inclusion criteria after screening the reference lists of the 17 chosen articles. This is also shown in Figure 7. Two articles had to be excluded as full text could not be obtained despite contacting the authors.

Characteristics of included studies

Two of the studies included were randomised controlled studies. The other studies included were cohort, longitudinal, case-control or cross-sectional studies, of which 11 were prospective and 8 were retrospective. The studies and data obtained are shown in Table 1.

Level of evidence

The level of evidence was assessed as per the Oxford criteria for Evidence Based medicine. Due to the retrospective nature of the studies, and the fact that they were mostly cohort studies, the general level of evidence was low, classed at 3 or 4.

Demographics

Demographic data was not available in all studies. Five studies did not report sex, but from those that reported it there were 2250 women and 787 men. The age of the patients ranged from 18 to 85. 1855 cholecystectomies were performed laparoscopically and 378 were open, though once again there were five studies which did not provide this information.

Rate of PCD

3476 patients were included across all the studies, with 462 (13.3%) patients developing post-cholecystectomy diarrhoea. The individual rates of PCD in the studies vary between 2.1% and 57.2%. The greater majority of patients were assessed in the first few months postoperatively (mainly in the first three to six months), though there is also a large amount of variation in the timing of PCD diagnosis since patients were assessed between 6 weeks up to 4 years postoperatively. These are outlined in table 1 below. There was not enough data available to be able to calculate median time to development of PCD post-cholecystectomy.

Predictive factors for PCD

Several potential risk factors for PCD were mentioned across the studies. Two studies mentioned age less than 45 or 50, as was a high BMI. Two studies suggested it was commoner in women, while another study suggested it was commoner in men. Two more two studies associated PCD with preoperative heartburn or gastritis, while two others still related this to high fat intake. The predictive factors identified in all studies are not consistent, and some other studies found no potential predictive factors including sex, age and preoperative symptoms.

Author	Year	Study type	PCD rate (%)	Investigative method	Predictive factors	Time post-op	Level of evidence
Ros and Zambon	1987	Prospective Cohort Study	8/93 (8.6)	Interview + own questionnaire	Not assessed	2 years	3
Wilson et al	1993	Retrospective case-controlled study	6/100 (6)	Own questionnaire	Not assessed	0-31 months	4
Heaton et al	1993	Retrospective cohort study	3/37 (9)	Questionnaire	Not assessed	3 months-26 years	4
McMahon et al	1995	Randomised controlled trial	62/233 (26.6)	Own Questionnaire; SF-36 and HADS	Not assessed	1 year	2
Fort et al	1996	Prospective Cohort Study	18/148 (12)	Own Questionnaire	Not assessed	4 years	3
Luman et al	1996	Prospective Cohort Study	2/97 (2.1)	Own Questionnaire	Not assessed	6 months	3
Gui et al	1998	Retrospective case control study	5/92 (5.4)	Questionnaire	Not assessed	12 months	4
Hearing et al	1999	Prospective cohort study	6/106 (5.7)	Telephone questionnaire +stool record form	Not assessed	2-6 months	3

Sauter et al	2002	Prospective cohort study	3/51 (5.9)	Interview	Not assessed	3 months	3
Topcu et al	2003	Retrospective case control study	8/200 (4)	SF36 and GIQLI	Not assessed	3-4 years	4
Finan et al	2006	Prospective cohort study	12/55 (21.8)	SF36	Not assessed	2-32 months	3
Fisher et al	2008	Prospective Cohort study	17/100 (17)	Telephone survey	High BMI, male, <50 years old	6-12 months	3
Mertens et al	2009	Prospective cohort study	17/129 (3.5)	Questionnaire	Preoperative flatulence and heartburn	6 weeks	3
Kim et al	2014	Prospective cohort study	13/65 (20)	SCL 90 R	Gastritis	3-6 months	3
Yueh et al	2014	Prospective longitudinal study	7/125 (5.7)	Questionnaire (internally validated)	High fat diet, age <45	3 months	3

Wanjura et al	2016	Retrospective cohort study	54/451 (12)	EQ-5D and GIQLI	Female, gallstone pain and pancreatitis/CBD stones	37-49 months	4
Talseth et al	2017	Retrospective cohort study	51/931 (5.47)	Questionnaire - HADS	women		3
Manriquez et al	2017	Retrospective cohort study	8/100 (8)	Telephone survey		4-6 months	3
Del Grande	2017	Retrospective cross-sectional study	39/111 (35.1)	Own questionnaire	Prior gastrointestinal symptoms	N/A	3
Kim et al	2018	Randomised controlled trial	79/138 (57.2)	EORTC-QLQ C-30	None found	3 months	2-3
Jasim et al	2018	Prospective cohort study	44/114 (38.59%)	Bristol stool chart	Age <40; increased BMI, fatty meals	10 days, 3 months, 6 months	3

Table 1: Included studies. PCD: post-cholecystectomy diarrhoea; SF-36: Short form 36 ; HADS: hospital anxiety and depression score; GIQLI: Gastrointestinal quality of life; MPQ: McGill pain questionnaire.

Diarrhoea is one of the commonest postoperative symptoms after cholecystectomy, and this can be either persistent or new postoperatively. It varies significantly between studies (Lamberts *et al.*, 2013a). Ros and Zambon (1987) were the first to mention this in the literature. They conducted a prospective cohort study to assess post-cholecystectomy symptoms two years postoperatively. At the time of assessment only 93 of the original 124 patients were available, eight of which developed loose stools and watery diarrhoea after surgery (Ros & Zambon, 1987). There have been multiple subsequent studies in which post-cholecystectomy patients were compared to patients having other surgeries such as inguinal hernia, laparoscopic sterilisation and hysterectomy, and bowel habit assessed and compared (Hearing *et al.*, 1999; Heaton *et al.*, 1993; Wilson & Macintyre, 1993). Some patients who developed diarrhoea resolved after a few weeks or months (Kim *et al.*, 2014; Manríquez *et al.*, 2017).

The question of whether laparoscopic or open cholecystectomy affected the symptoms which developed postoperatively was raised, and this was investigated by McMahon et al (1995) who performed a multicentre randomised controlled trial to assess the symptomatic outcome between minilaparotomy and laparoscopic cholecystectomy. However, they did not find any difference between open or laparoscopic surgery (McMahon *et al.*, 1995). Topcu et al (2003) also performed a study to evaluate gastrointestinal symptoms and quality of life, comparing open and laparoscopic cholecystectomy using the SF36 and GIQLI questionnaires, and once again found no difference in the PCD rate (Topcu *et al.*, 2003).

A variety of investigative tools have been used to investigate PCD and other post-cholecystectomy symptoms, including questionnaires (whether previously validated or designed anew by the researchers), telephone interviews, the Bristol stool chart and stool record forms, and the time frames ranged from six weeks up to four years postoperatively (Finan *et al.*, 2006; Gui *et al.*, 1998; Hearing *et al.*, 1999; Luman *et al.*, 1996). However, this wide range of investigative tools makes comparing studies, and their results, difficult. Most studies used validated questionnaires such as SF36, GIQLI and GSRS. However, they were often administered retrospectively thus limiting their objectivity. Some of the questionnaires were also aimed towards investigating general quality of life rather than gastrointestinal symptoms specifically. Non-validated

questionnaires were also used in some cases, and this limited their reproducibility. The other issue with the use of such questionnaires is that there is a lot of dependence on patient recall especially in the retrospective studies. In cases where a standardised tool, such as the Bristol Stool Chart, was not used, there were also differences in describing stool function and what is considered 'diarrhoea'. The main issue with patient recall is the perception of change when change is not always present.

The relationship between PCD and bile acids was first investigated in 1979, when a case series of three patients developing diarrhoea after cholecystectomy was published, demonstrating that two of them had elevated faecal bile acids and that in all three patients, cholestyramine treatment led to a resolution of symptoms, thus implying bile-acid mediation of such diarrhoea (Hutcheon *et al.*, 1979). Arlow et al. (1987) developed a 'choleric enteropathy' theory after they investigated eight patients with post-cholecystectomy diarrhoea, of which six had elevated faecal bile acids. They suggested that this diarrhoea may be due to increased production of dihydroxy bile acids and caused by the increase of enterohepatic cycles thus increasing daily turnover of primary bile acids, as well as continuous bile flux due to the loss of the gallbladder (Wilson & Macintyre, 1993). These patients also responded to treatment with cholestyramine (Arlow *et al.*, 1987).

Despite bile acid diarrhoea being described in case series in the 1980's, Fort et al were the first to investigate the prevalence and physiology of post-cholecystectomy diarrhoea (Fort et al., 1996). While cholecystectomy removes the storage area for the bile acid pool, studies have shown that the major effect of this on the enterohepatic cycle is that there is more bacterial dehydroxylation due to bile acid spending more time in the gut between meals (Phillips, 1996; Turumin et al., 2013). As an endogenous source of intestinal secretagogues, the theory that increased dehydroxylation of bile acids causes diarrhoea has been put forward, however it has been shown that the amount of secretion they cause is not enough to cause diarrhoea (Fromm et al., 1987). Fromm et al. investigated 25 patients with post-cholecystectomy diarrhoea (though the group was heterogenous and characterised by patients with other conditions that could also cause diarrhoea) and found that most of their patients failed to respond to cholestyramine therapy (Fromm et al., 1987).

Studies investigating bile acid metabolism after cholecystectomy have shown that there is an increase of secondary bile acids in the enterohepatic circulation. Patients who have undergone cholecystectomy have a higher total bile acid faecal excretion than those patients who have not. Deoxycholic acid (DCA), a secondary bile acid, concentrations is higher post-cholecystectomy (Breuer *et al.*, 1986). Deoxycholic acid induces net secretion of salt and water in the colon and thus this may be a factor in development of post-cholecystectomy diarrhoea, though this had been shown in studies using concentrations of DCA that are much higher than those found in the stool of normal patients (though not higher than DCA concentrations of patients with BAD). DCA was not found to increase basal rectal motility in a study by Edwards et al, though it was found to increase the sensitivity of the rectum by reducing the volume required to produce a desire to defecate, which may be another way in which DCA can effect postoperative diarrhoea (Edwards *et al.*, 1989).

Intestinal transit after cholecystectomy has been investigated when trying to identify the pathophysiology of post-cholecystectomy diarrhoea. Orocaecal and colonic transit have both been shown to increase after cholecystectomy (Fort *et al.*, 1996; Penagini *et al.*, 1988), though colonic transit remains technically within normal limits (Fort *et al.*, 1996). In some cases, though patients did not report actual diarrhoea after cholecystectomy, they did report an increase in bowel movements and a decrease in stool consistency (Moussavian *et al.*, 2000; Sauter *et al.*, 2002b). The investigators did not always define what they meant by diarrhoea the form of number of episodes per day and the use of the Bristol stool chart to; and some divided it into ‘mild’ and ‘severe’, again without defining what the parameters of these groups were (Kim *et al.*, 2014) (Del Grande *et al.*, 2017).

Levels of C4, which is a marker of bile acid synthesis, have been shown to increase after cholecystectomy, and this reflects increased synthesis postoperatively (Barrera *et al.*, 2015; Moussavian *et al.*, 2000). FGF19 and C4 levels show diurnal changes and typically peak at noon, however, this rhythm changes postoperatively and FGF19 levels are significantly less at noon, declining at three months after cholecystectomy. Despite FGF19 levels correlating to BA synthesis as measured by C4 levels preoperatively, this correlation was lost after cholecystectomy (Barrera *et al.*, 2015). Sauter et al, who investigated bile acid malabsorption after cholecystectomy by

measuring C4 levels and investigating changes in bowel habit, found that while most patients describe an increase in bowel motions after cholecystectomy, there was no correlation with C4 levels and the described changes in bowel habit, despite an overall increase in C4 levels after cholecystectomy (Sauter *et al.*, 2002b).

A total of 3476 patients were included across all the studies with 462 (13.3%) patients developing post-cholecystectomy diarrhoea, though the rates in the studies vary between 2.1% and 57.2% (Kim *et al.*, 2018; Luman *et al.*, 1996). A majority of patients were assessed in the first three to six months postoperatively, though there is also a large variation in the timing of assessments in these patients. These were between 6 weeks to 4 years postoperatively.

The difference in prevalence of diarrhoea across the studies could be attributed to factors such as study design, length of follow up, questionnaire wording (as some studies have used non-validated questionnaires), issues with patient recall and definitions of what is considered as diarrhoea. Unfortunately, most of the studies in this review do not have the statistical power to confirm an accurate incidence of post-cholecystectomy diarrhoea but have investigated post-cholecystectomy symptoms in general. In fact, most studies have focussed on dyspeptic symptoms and pain. Some studies were excluded as they did not specify whether diarrhoea was pre-existing or new onset following cholecystectomy.

Predictive factors for post-cholecystectomy diarrhoea

Predictive factors identified for post-cholecystectomy diarrhoea varied widely across the few studies that assessed such factors. Fisher et al (2008), Yueh et al (2014) and Jasim et al (2018) all concluded that it was associated with the male sex, age group younger than 50 and having a high BMI, (though Jasim et al (2008) actually had an age group of less than 40 years old) while Del Grande et al (2017) associated this with having gastrointestinal symptoms prior to surgery, though they did not specify which ones (Del Grande *et al.*, 2017; Fisher *et al.*, 2008; Jasim, 2018; Yueh *et al.*, 2014). Mertens et al (2009) clarified this by stating that it was preoperative flatulence and heartburn which predicted postoperative symptoms including diarrhoea. Yueh et al (2014) reported that not following a low-fat diet could be associated with PCD (Yueh *et al.*, 2014). Talseth et al's (2017) study found that PCD was more common when the

indication for cholecystectomy for biliary colic, while Manriquez et al (2017) asserted that it was more common in patients having cholecystectomy for asymptomatic cholelithiasis (Manríquez *et al.*, 2017; Talseth *et al.*, 2017). On the other hand, Kim et al (2018) identified no predictive factors including age, BMI, sex, ASA score, pre-operative ERCP, comorbidities, difficult laparoscopic cholecystectomy, open conversion or pathology(Kim *et al.*, 2018). Wanjura et al (2016) did not specifically correlate preoperative factors to diarrhoea, but did find the female gender, CBD stones or pancreatitis and gallstone pain as an indication for surgery were indicative of worse gastrointestinal symptoms postoperatively (Wanjura & Sandblom, 2016). Kim et al (2014) also asserted that gastritis was a preoperative predictive factor for developing post cholecystectomy symptoms however once again did not specifically relate this to diarrhoea (Kim *et al.*, 2014).

Changes in enterohepatic cycling post-cholecystectomy

Bile acids undergo enterohepatic cycling, meaning that any bile acids secreted from the liver are eventually returned to the liver from the terminal ileum. Bile acids are initially stored in the gallbladder and excreted upon ingestion of food. They travel to the distal small intestine, the ileum, where they are reabsorbed into the portal circulation and transported back to the liver. The whole bile acid pool in an adult is 2 to 4g and the bile acid pool circulates several times per meal. About 95% of bile acids are reabsorbed in each cycle, while the rest are eliminated with the faeces (Dawson, 2016).

In the first three to six months, CDCA kinetics are unaltered by cholecystectomy, however CA synthesis decreases post operatively by an average of 37%. This leads to an overall decreased hepatic synthesis of primary bile acids. The pool size and synthesis rate of DCA is also unaffected by cholecystectomy (Berr *et al.*, 1989). However, five years after cholecystectomy there is no significant change in the size and synthesis of the CDCA, CA and DCA pools. There is a slight increase in amount of CA transferred to the DCA pool (Kullak-Ublick *et al.*, 1995).

CDCA and CA fasting levels are higher in patients having undergone cholecystectomy patients, though not significantly different, and post-prandial peaks are earlier and

lower than patients still having their gallbladder. CA turnover rate is significantly higher after cholecystectomy (Roda *et al.*, 1978).

Incidence of bile acid diarrhoea

Emerging evidence over the last decade has shown that bile acid diarrhoea is not as uncommon as previously perceived. Up to 30% of patients with diarrhoea-predominant irritable bowel syndrome (IBS) have evidence of bile acid diarrhoea as determined by ^{75}SeHCAT testing (Smith, 2000; Kurien, 2011; Arasaradnam, 2012; Wedlake, 2009; Walters 2009; Pattni 2013). Compared to controls, patients with irritable bowel syndrome had lower ^{75}SeHCAT values and higher C4 levels but similar FGF-19 levels. >50% responded to bile acid sequestrant (colestipol) (Bajor *et al.*, 2015). In addition to patients with ileal disease (e.g. Crohn's disease, a disease of inflammation of the gastrointestinal tract)(Nyhlin *et al.*, 1994), bile acid diarrhoea has also been reported in those following cholecystectomy (Sciarretta *et al.*, 1992), and those with post-infectious diarrhoea (Niaz *et al.*, 1997). For those not responding to treatment, other additional causes should be sought, e.g. bacterial overgrowth, pancreatic insufficiency or microscopic colitis (Fernandez-Banares *et al.*, 2001), even if ^{75}SeHCAT testing has been abnormal. Another under recognised group are those with cancer especially those receiving pelvic chemoradiotherapy as >50% have BAD (Phillips *et al.*, 2015).

Gut microbiome and its relationship to bile acids

One aspect of post-cholecystectomy diarrhoea that has not been thus far investigated is the interplay between the gut microbiota, faecal bile acids and short chain fatty acids in the colon. Gut microbiota affect bile acids by causing deconjugation, dehydrogenation and dihydroxylation of primary bile acids in the distal small intestine and colon. This process causes a change in the bile acid pool composition therefore activating FXR and thus inhibiting bile acid synthesis. However, it is not known whether a change in the gut microbiota has any effect on bile acid diarrhoea. The amount of secondary bile acids (mainly DCA) in the bile acid pool depends on the rate of formation and absorption via the colon, the colonic transit time and the colonic pH, and there has been a correlation between high DCA levels and gallstones. Work from our group has shown that there is significantly reduced diversity of bacterial population in those with bile acid diarrhoea compared to those with diarrhoea predominant irritable

bowel syndrome. Specifically, there was increased in operational taxonomic units (OTUs) of 6 families: *Bifidobacteriaceae*, *Prevotellaceae*, *Lachnospiracheae*, *Prevotellaceae*, *Verrucomicrobiaceae* and *Bacteroidaceae* (Sagar et al., 2018*).*

These bacteria are involved in the digestion of complex carbohydrates into short chain fatty acids (SFCA), such as acetate, butyrate and proprionate. As both the levels of faecal bile acids in the colon and levels of SFCA are dependent on gut microbiota, any changes in faecal bile acids may affect SCFA and in turn effect the presence of diarrhoea. Patients with BAD also have a higher proportion of faecal bile acids, potentially due to decreased *Bifidobacteria* and *Leptum* species as well as an increased *E.Coli* in their gut microbiota. This may alter the affinity of BAs to FXR and TGR5, thus leading to decreased FXR activation therefore increased bile acid synthesis and may happen after cholecystectomy due to increased enterohepatic cycling leading to increased delivery of bile acids to the colon (Sayin *et al.*, 2013). In a study by Wang et al., an increase in *Bifidobacteria* showed a concurrent decrease in faecal bile acids while acetate and proprionate levels increased (Wang *et al.*, 2014). The relationship between the increase of SFCA with the decrease in total faecal bile acids has not been explored and it is unknown whether the increased enterohepatic cycling post-cholecystectomy affects it.

Investigations

There are several methods by which BAD can be diagnosed, all of varying reliability. The methods are compared in table 2.

Nuclear medicine

The BSG guidelines state that patients with chronic diarrhoea should all be investigated with a ^{75}SeHCAT scan as a first line in secondary care to exclude bile acid diarrhoea (Arasaradnam *et al.*, 2018). ^{75}SeHCAT (Selenium-75 homocholic acid taurine test) is a nuclear scan which was first described in 1982 and is used to determine the amount of bile acid malabsorption (Merrick *et al.*, 1982; Merrick *et al.*, 1985). Selenium-75 homocholic acid taurine is a synthetic analogue of the natural conjugated bile acid taurocholic acid. Its value in this test is that it behaves in the exact same way as bile acids, however is resistant to deconjugation by intestinal bacteria (Eusufzai *et al.*, 1993). A capsule of ^{75}SeHCAT is ingested after an overnight fast, and

three hours later a standard gamma camera is used to detect the baseline level. The scan is then repeated after seven days and the overall retention of ^{75}SeHCAT in the abdomen is measured. Degree of bile acid malabsorption is measured according to retention values, these being 10-15% for mild bile acid malabsorption, 5-10% for moderate bile acid malabsorption and less than 5% for severe bile acid malabsorption (NICE, 2012). This is the gold standard in the diagnosis of Bile acid diarrhoea (Ford *et al.*, 1992). However, its use is not widespread despite the ability to be used in any nuclear medicine department supporting a gamma camera and it is not licensed for use in the USA (Smith & Perkins, 2013). Sensitivity of ^{75}SeHCAT testing is 96% with a specificity of 100% at 7 days (Sciarretta *et al.*, 1986). ^{75}SeHCAT may also predict response to therapy.

The ^{75}SeHCAT test has been recognised as having potential for patient and system benefits given the prevalence of undiagnosed BAD by the NICE diagnostic guidance report on ^{75}SeHCAT in 2012. However, the report also suggested that more evidence is required to determine how cost effective this is, and has thus recommended further research to evaluate this technology and effects of treatment (Riemsma *et al.*, 2013) Its 2016 review, made no changes in light of lack of new evidence on ^{75}SeHCATs comparative diagnostic accuracy. It has been shown that ^{75}SeHCAT had a highest diagnostic yield of BAD to date, in a study comprising 36 studies and 5028 patients on bile acid diarrhoea biomarkers (limited by study heterogeneity) with 25% of patients previously diagnosed as having functional bowel disorders actually having primary BAD (Valentin *et al.*, 2015).

Pooled data from 15 studies show that there is a dose-response relationship between the severity of malabsorption and the effect of treatment with a bile acid sequestrant. Is has been shown that 96% of patients with less than 5% retention respond to colestyramine, while the clinical response was 80% at <10% retention and 70% at <15% retention (Wedlake *et al.*, 2009). In general the lower the ^{75}SeHCAT retention value the greater the likelihood of response to sequestrants.

Blood

Another method of diagnosing BAD, which is often used in the case of unavailability of ^{75}SeHCAT, is measuring blood C4 (7α-hydroxy-4-cholesten-3-one) levels. Patients

with BAD have raised baseline C4 levels secondary to impaired feedback by FGF19 (Walters *et al.*, 2009). This indicates increased bile acid synthesis and thus increased levels of bile acids in the colonic lumen (Eusufzai *et al.*, 1993). Other patients who may have increased C4 levels are those with disease of the terminal ileum, as this causes decreased reabsorption which may require more synthesis (Brydon *et al.*, 2011). C4 levels have been compared to ^{75}SeHCAT testing, and have been shown to have a negative predictive value of 98%, making it an attractive test to exclude BAD (Eusufzai *et al.*, 1993). If comparing to ^{75}SeHCAT <10%, fasting C4 level of >48.4ng/ml has a sensitivity of 90% and a specificity of 79%(Sauter *et al.*, 2002b). Standardisation for collection would be required, as timing of specimen collection has been shown to lead to significant variation. This is due to the diurnal variation of C4 levels as well as variation with hypertriglyceridaemia, ingestion of food, patients with liver disease and ethanol levels(Axelson *et al.*, 1991; Brydon *et al.*, 2011; Camilleri *et al.*, 2014; Duane, 1995; Galman *et al.*, 2005). C4 levels have been shown to correlate negatively with faecal bile acid excretion (Wong *et al.*, 2012) and they also have an inverse correlation with FGF19 levels(Pattni *et al.*, 2013).

Since FGF19 inhibits bile acid synthesis, fasting serum FGF19 levels are inversely correlated with C4 levels. Thus, lower FGF19 levels may indicate presence of BAD. FGF19 levels correlate well with ^{75}SeHCAT results, with a negative predictive value of 82% for ^{75}SeHCAT of <10%, with a sensitivity of 58% and specificity of 84% for a serum FGF19 level of <145pg/ml and 94% for ^{75}SeHCAT <5% (Pattni *et al.*, 2013). Using FGF19 levels as a diagnostic tool would also require standardisation as, much like C4, FGF19 levels also change rapidly after meals, and there is also a natural diurnal variation (Lundasen *et al.*, 2006). FGF19 levels are not yet routinely used in the diagnosis of bile acid diarrhoea however may be used in the future.

Stool

Another diagnostic test for BAD if ^{75}SeHCAT is unavailable would be measurement of faecal bile acids. This is a measure of the total excess bile acids exiting the colon. Patients with BAD have been shown to have higher amount of primary bile acids within he colon and this correlates with frequency and consistency of stool. (Shin *et al.*, 2013;

Wong *et al.*, 2012). Patients with BAD also have a higher stool weight. Studies have shown that total feacal bile acids of more than 2337 µmol/48 hours are diagnostic for BAD, however elevated primary faecal bile acids may also be used as a diagnostic test, as >4% primary bile acids are indicative of BAD when compared to healthy volunteers usually only have about 0.02% primary faecal bile acids. A 4% cut off may be used even when total faecal bile acids measure 1000µmol/48 hours (Vijayvargiya & Camilleri, 2019). However, this method may not be feasible in most cases due to being highly labour-intensive. It requires a 48-hour faecal collection taken during the last 2 days of a 4day 100g fat diet, as variation in dietary fat intake would lead to variation in bile acid levels. Faeces would than need to be homogenised, deconjugated and separated before performing either gas chromatography-mass spectrometry, liquid chromatography-tandem mass spectrometry and HPLC-mass spectrometry (Galman *et al.*, 2005; Griffiths & Sjovall, 2010; Mitchell *et al.*, 1973). This process is also complicated by the fact that there will also be diurnal variation in bile acid secretion. Thus, it requires a 48-hour collection period to ensure consistency (Camilleri *et al.*, 2015). It is also cumbersome and not commercially available in the UK.

There has also been some work using a percentage of primary faecal bile acids in a single stool sample combined with a serum C4 level, which has been shown to be a significant predictor of BAD. This study has shown that for every 10% increase of primary BAs in a single stool sample, there is a 2.5 higher change of BAD being diagnosed, whereas with every increase of 10ng/ml in C4 levels, there was a 2-fold increase in the chance of diagnosing BAD (Vijayvargiya, 2020).

Urine

A study using an electronic nose has shown that patients with bile acid diarrhoea have increased levels of volatile organic compounds in their urine, mainly 2-propanol and acetamide, when compared to healthy controls and patients with inflammatory bowel disease (Covington *et al.*, 2013). This may be a novel away to diagnose bile acid diarrhoea.

Therapeutic Trials

Occasionally, if no other diagnostic methods are available, bile acid sequestrants are used in a therapeutic trial. There has been a series of 264 patients where 53% had

BAD and 44% failed to respond to cholestyramine alone. However, half of these non-responders derived benefit from Colesevelam which is currently unlicensed but used with extended indication. Thus, in the case of a therapeutic trial, care must be taken as lack of response to cholestyramine does not necessarily constitute exclusion of BAD (Orekoya et al., 2015). Hence therapeutic trials of bile acid sequestrants (colestyramine or colesevelam) are not recommended. A summary of diagnostic methods is seen in table 2.

Diagnostic method	Advantages	Disadvantages
SeHCAT	Well established Predicts response to treatment	Involves radiation Not available in USA
C4	No radiation Simple	Diurnal variation Fasting sample Requires further validation
FGF19	No radiation Simple Commercial assay available	Diurnal variation Requires further validation
Faecal bile acids	No radiation	Cumbersome 48hr sample collection Not widely available
Urine	Easy collection	Experimental Not widely available
Therapeutic trial	Easily available	Unreliable

Table 2: comparison of diagnostic methods

A review of bile acid diarrhoea and the pathophysiology, diagnosis and management has been published (appendix 1).

Aims

The aims of the study were to determine the rate of bile acid diarrhoea in a prospective case-control study and whether there is a change in bowel habit and stool consistency after laparoscopic cholecystectomy. Further to this, we wanted to determine the role of gallbladder FGF19 in the development of post-operative BAD or change in bowel habit, as well as whether SHP had any role in this. Another part of the study was to determine the change in lipid levels (LDL, HDL and triglycerides) post-cholecystectomy and the mechanism behind this change, as well as its relationship to the development of bile acid diarrhoea, and whether gallbladder PPARα is associated with any change in lipid levels.

Chapter 2: Methodology and statistics

The study consisted of two parts. The first part was a multicentre audit on the investigation of diarrhoea in post-cholecystectomy patients. The second part was a prospective case-control study to aim to determine markers involved in the development of bile acid diarrhoea after cholecystectomy. While the overall methods are described in this chapter, each results chapter also has more detailed methodology and statistics as relevant to that chapter.

Retrospective audit

An initial local audit regarding the investigation of diarrhoea post-laparoscopic cholecystectomy was performed. Cross-referencing of a prospective electronic database of patients who underwent laparoscopic cholecystectomy (LC) and a ^{75}SeHCAT test at a tertiary centre between 2013 and 2017 was performed. A 7-day retention time of <15% was considered positive. Patient demographics were collected and compared for significance ($p<0.05$) using a Mann Whitney U test, due to the data being non-normally distributed. Difference in time from surgery to investigation between men and women was also investigated using the Mann Whitney U test. Multiple centres where ^{75}SeHCAT is performed were invited to take part in the same audit and five accepted. Therefore, the analysis was performed again with the larger national patient cohort. Further detail regarding the methodology and statistics of this audit is provided in Chapter 3.

Case control study

Approval was gained from the ethics committee and the Health research authority (appendices 2 and 3). Patients were recruited prospectively. A sample size of 110 was determined using a power calculation based on post-cholecystectomy diarrhoea rates from previous studies. This was based on a study of 80% power based on a prevalence of 12% to achieve a 95% confidence. The study group consisted of those undergoing laparoscopic cholecystectomy. The age-matched control group also had diagnostic laparoscopic surgery (keyhole surgery without removal or involvement of the gallbladder during surgery). These were mainly patients undergoing laparoscopic

Nissen fundoplications (surgery for heartburn), laparoscopic hernia repair, and laparoscopic bariatric surgery (for weight loss).

Patients were administered a GIQLI (gastrointestinal quality of life) questionnaire (appendix 4) as well as the Bristol stool chart before surgery and three months postoperatively. Symptoms were also assessed via the Rome IV criteria. They were also given the option to have blood tests taken for measurement of lipid levels, C4 and FGF19 again before and three months after surgery. Those patients having cholecystectomy were also asked for a gallbladder sample when it was removed as well as a liver biopsy (which was optional). Anyone who developed diarrhoea as per the BSG criteria was offered a ^{75}SeHCAT scan and a colonoscopy with ileal biopsy (Arasaradnam *et al.*, 2018).

The aims of the study were to determine the rate of bile acid diarrhoea in a prospective case-control study and whether there is a change in bowel habit and stool consistency after laparoscopic cholecystectomy. Further to this, we wanted to determine the role of gallbladder FGF19 in the development of post-operative BAD or change in bowel habit, as well as whether SHP had any role in this. Another part of the study was to determine the change in lipid levels (LDL, HDL and triglycerides) post-cholecystectomy and the mechanism behind this change, as well as its relationship to the development of bile acid diarrhoea, and whether gallbladder PPARα is associated with any change in lipid levels. More detail on methodology is provided in chapters 4, 5 and 6.

Symptom assessment: GIQLI and the Bristol stool chart.

The GIQLI questionnaire (Appendix 4) is a validated questionnaire developed to investigate the quality of life of patients with gastrointestinal symptoms. It consists of 36 items to which the patient has to select one of five answers. The answers are then scored, with ‘0’ points given to the least desirable answer and ‘4’ points given to the most desirable answer. A decision was made to use this questionnaire as it is a validated method of assessing gastrointestinal symptoms and thus a reliable method for internal comparison of patient symptoms pre- and post-operatively. It is also a reproducible method of investigating bowel symptoms such as diarrhoea.

The Bristol stool chart (figure 8) was also used as an investigative measure in this study (Lewis & Heaton, 1997). Patients were asked to quantify the number of times they opened their bowels daily and what their stool looked like as per the Bristol stool chart. This is another way of internally validating the patient's responses and comparing pre- and post-operative stool numbers and consistency.

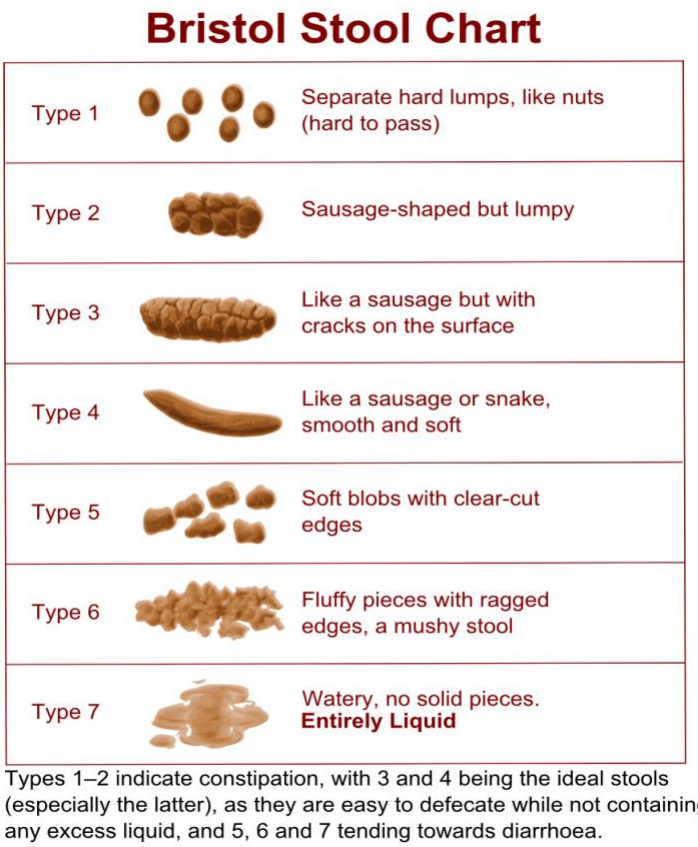

Figure 8: Bristol stool chart (Wikipedia commons/ CCBY-SA30)

ROME criteria

The ROME foundation created clear criteria for the diagnosis of irritable bowel syndrome (IBS). However it has been stated that 25 to 50% of patients with IBS-D (diarrhoea-predominant IBS) actually have bile acid diarrhoea (Camilleri, 2015). The criteria for diagnosis of IBS involve abdominal pain weekly for a minimum of 3 months, along with a minimum of two of the following criteria: increasing or improving pain related to defecation, associated with a change in stool frequency, or associated with a change in stool form. There are also subtypes associated with IBS, these being IBS-

C (constipation type) where more than 25% of bowel movements are Bristol Stool scale types 1 or 2 and less than 25% types 6 or 7; IBS-D (diarrhoea predominant) where >25% of bowel movements are classified as Type 6 and 7 on the Bristol stool chart and <25% are type 1 and 2; IBS-M (mixed type) where >25% of bowel movements are classified as Type 6 and 7 on the Bristol stool chart and >25% are type 1 and 2 and finally IBS-U (unclassified) where the diagnostic criteria for IBS are met however they do not fit any particular subtype (Drossman & Hasler, 2016).

Blood tests

Optional preoperative fasting serum and plasma samples to measure lipid levels and FGF19 were taken. These were repeated three months postoperatively for lipid levels and FGF19.

Gallbladder tissue

In the study group a sample of gallbladder tissue was also analysed for FGF19, SHP, and PPARα. There was an opt-in option for peri-operative liver biopsy. There were two main criteria for taking a liver biopsy if the patients consented and these were that the surgeon had to be a consultant specialist hepatobiliary surgeon and the laparoscopic cholecystectomy had to be done in the main theatres not the day surgery theatres due to staff experience and equipment availability in case of complications. Unfortunately, no patients consented for this.

^{75}SeHCAT test

^{75}SeHCAT (Selenium-75 homocholic acid taurine test) is used to determine the degree of bile acid malabsorption. Selenium-75 homocholic acid taurine is a synthetic analogue of taurocholic acid, which is a natural conjugated bile acid, and behaves in the exact same way except that it is resistant to deconjugation by intestinal bacteria (Eusufzai *et al.*, 1993). It is ingested in the form of a capsule a standard gamma camer is used to detect the baseline level after ingestion. The scan is repeated after seven days and the overall retention measured. Retention values of 10-15% are considered mild bile acid malabsorption, while 5-10% is moderate bile acid malabsorption and less than 5% is sever bile acid malabsorption (NICE, 2012). Patients were referred for ^{75}SeHCAT testing if they developed diarrhoea.

Colonoscopy

A colonoscopy is an endoscopic examination of the colon that can reach as far as the terminal ileum. As per the British Society of Gastroenterology (BSG) guidelines patients in this study with diarrhoea for more than four weeks will be offered a colonoscopy to exclude inflammatory bowel disease (IBD) or malignancy (Arasaradnam *et al.*, 2018).

Statistical analysis

To assess the GIQLI results pre- and post-operatively, the results were first tested for normal distribution. As the results were not normally distributed, a Wilcoxon signed rank test was used to assess for any differences in the pre- and post-operative period. This statistical test was chosen as the samples were related (the same patients pre- and post-operatively)

A Wilcoxon signed rank test was also used to analysis any changes in pre- and post-operative bowel habit and stool consistency, as well as to assess for any changes in lipid levels from pre- to post-operatively as this data was not normally distributed.

To analyse relationship of FGF19 concentrations with bowel habit, stool consistency and GIQLI scores a Spearman's relationship coefficient was used as these were two continuous variables which were non-parametric. The effect of smoking and BMI on change in bowel habit and stool type was investigated via Chi-squared test, which was selected as the variables were categorical and non-parametric. A multivariate model adjusted for age and sex was also performed to assess GIQLI differences pre- and post-operatively.

The data was analysed as a complete case analysis, with a missing at random model for incomplete cases. Statistical advice was sought for all the above.

Patient involvement

The BAD UK charity was approached to consider and represent the patient perspective. A presentation of the proposed study was given. Several patients who

developed BAD after cholecystectomy mentioned problems such as delayed diagnosis, being given an incorrect diagnosis such as IBS (irritable bowel syndrome), and lack of knowledge amongst health professionals about this condition. A patient and public involvement (PPI) letter was issued after the meeting to support the study (Appendix 5). Periodic updates about the study were provided at BAD UK support group meetings held in Coventry.

Recruitment process

Patients were recruited upon listing for laparoscopic cholecystectomy or other laparoscopic procedures. Once listed, the clinical team asked the patients whether they were happy to be contacted by the research team. If they were, contact was made by telephone to explain the purposes of the study and of those interested, the following were sent: participant information sheet, study documents and the GIQLI questionnaire. They were provided with contact details for any queries. Patients were then approached on the day of operation for a final decision. Those who agreed to participate in the study then proceeded with the consent process. A subset of patients also provided blood, urine and stool samples. Gallbladder tissue samples were taken intraoperatively. No patient consented to a liver biopsy.

Naturally, there were challenges to patient recruitment. Some patients who were recruited at preoperative clinic or surgical clinics which were then not listed for surgery for a long time and this negatively impacted on recruitment. Thus, the strategy was changed to include patients already listed for surgery. Those clinicians who agreed to facilitate recruitment were provided with information about the study, including a copy of the consent form and the patient information leaflet appropriate to their study group. This was mailed to patients in the week prior to their operation. They were then approached again prior to their operation to ask if there were any further questions and decide on participation in the study. They were informed that participation or not it would not impact their clinical care. They were also informed that they withdraw from the study at any time.

Another challenge to recruitment was the COVID-19 pandemic which meant that recruitment had to be cut short, as the surgeries these patients were undergoing were not cancer surgeries and therefore were reduced significantly during the pandemic.

Sample collection

Blood samples are collected in yellow top, purple top (EDTA) and green top (Heparin) sample bottles and centrifuged at 50k rpm for 15 minutes. The plasma layer from the lithium heparin bottle was pipetted into 1ml aliquots and the serum layer from the EDTA bottle was also aliquoted into 1mls. They were stored at the Arden tissue bank for freezing at –80C pending further analysis. The yellow top samples are sent to the UHCW pathology labs for fasting lipid profile determination.

The gallbladder samples were taken from theatre. Once the gallbladder was removed, it was opened by making a vertical incision on the serosal layer. It was then inverted to ensure that no tumours or polyps were present. A $1cm^2$ sample was then taken from the serosal aspect of the gallbladder and snap frozen in liquid nitrogen, then stored at –80C.

Impact of COVID-19

COVID-19 hit the UK in late March 2020 and impacted greatly on this study. Recruitment had to stop, therefore instead of the 55 patients that was our target sample size, recruitment had to stop at 40 patients for the study group. Non-emergency operations and non-cancer operations were also stopped during this time period (March 2020 to October 2020), thus none of the patients who were undergoing surgery could be suitable for enlisting to either group. Non-urgent surgery only re-started properly in March-April 2021. There were some patients who had initially agreed to come return for blood tests, however this was not possible as hospital visits were resticted to only those that were mandatory or medically indicated. Allied to this, I was recalled to full-time clinical activity. This severely impacted on my time for work on this study. However, some patients who were initially unable to be contacted for their questionnaire follow ups over the telephone were contacted during the lockdown period and thus more questionnaire follow-ups were obtained.

Patient demographics

Study group

The study group consisted of 40 patients, of whom 36 were followed up. The other four were lost to follow-up as the study team was unable make contact with them despite multiple and repeated attempts. Of these 36 patients, four developed diarrhoea and were sent for ^{75}SEHCAT test and colonoscopy. One patient did not attend for the tests. The median age was 48.5 (range 20 to 76) and 29 (72.5%) were female. All the patients who developed post-operative diarrhoea were female. The median BMI was 28.5 (range 20 to 41.8). Five patients were smokers, while 13 were ex-smokers. This is shown in table 3.

Control group

The control group consisted of 20 patients of whom 18 were followed up. Two patients were lost to follow up. The median age was 52 (range 32-76) and the group consisted of 11 men and nine women. 10 of these patients had a laparoscopic hernia repair, while five underwent laparoscopic Nissen fundoplication and five had a laparoscopic sleeve gastrectomy. The median BMI was 30.5 (23-54). This is also shown in table 3.

	Study group	Control group
Total	40	20
Male:Female	11:29	11:9
Median BMI (range)	28.5(20-41.8)	32.7 (22-54)
Median age (range)	48.5 (20-76)	52 (32-76)
Smoker:ex-smoker:non-smoker	8:13:19	1:3:16
Comorbidities • GORD • IBS/colitis	24 • 5 • 2	13 • 3 • 0

Table 3: Patient demographics

The age, sex and BMI of the two groups were compared using the Chi-squared test as they are nominal (age and sex) vs continuous (BMI) variables. There was no significant different in the age, sex and BMI between the two groups, p=0.316, p=0.094 and p=0.279 respectively.

Chapter 3: Results - Rates of post-cholecystectomy diarrhoea in the local and national setting

Chapter Summary

An initial local audit regarding the investigation of diarrhoea post-laparoscopic cholecystectomy was performed. Cross-referencing of a prospective electronic database of patients who underwent laparoscopic cholecystectomy (LC) and a ^{75}SeHCAT test at a tertiary centre between 2013 and 2017 was performed. A 7-day retention time of <15% was considered positive. Patient demographics were collected and compared for significance ($p<0.05$) using a Mann Whitney U test.

34 of 2381 patients undergoing LC were investigated via endoscopy and ^{75}SeHCAT test for chronic diarrhoea postoperatively. 20 (59%) had a ^{75}SeHCAT retention of <15%. The mean time from surgery to ^{75}SeHCAT testing was 564 days (SD=371), and women were tested significantly later than men (660 vs 287 days, $p=0.006$).

Only a small proportion of post-cholecystectomy patients were investigated for BAD (1.4%), and of these 59% were positive. There was also a significant time delay to diagnosis. This may be partly due to the fact that cholecystectomies are now undertaken as a day case and routine follow-up is rarely offered.

The audit was then extended to other centres. The centres involved at the end of the audit were Coventry, Bath, Bristol, Oxford and Glasgow. A retrospective analysis of electronic databases from five large centres detailing patients who underwent laparoscopic cholecystectomy between 2013 and 2017 was cross-referenced with a list of patients who underwent ^{75}SeHCAT testing. A seven-day retention time of <15% was deemed to be positive. Patient demographics and time from surgery to investigation were collected and compared for significance ($p<0.05$).

A total of 9439 patients underwent a laparoscopic cholecystectomy between 1 January 2013 and 31 December 2017 in the five centres. 202 patients (2.1%) underwent investigation for diarrhoea via ^{75}SeHCAT, of which 64 patients (31.6%) had a ^{75}SeHCAT test result of >15% while 62.8% of those investigated were diagnosed with bile acid diarrhoea (BAD). 133 (65.8%) patients also underwent endoscopy and 74

(36.6%) patients had a CT scan. Median time from surgery to ^{75}SeHCAT test was 672 days (SD +/-482 days).

Only a small proportion of patients, post-cholecystectomy, were investigated for diarrhoea with significant time delay to diagnosis. The true prevalence of BAD after cholecystectomy may be much higher, and clinicians need to have an increased awareness of this condition due to its amenability to treatment. ^{75}SeHCAT is a useful tool for diagnosis of bile acid diarrhoea.

Methods – Local audit

A retrospective study was undertaken at University Hospitals Coventry and Warwickshire, England. Local approval was sought from the audit department (appendix 6). An electronic prospective database of patients undergoing laparoscopic cholecystectomy between 2013 and 2017 were cross-referenced with all the patients who underwent ^{75}SeHCAT testing during the same period of time. The data collected included age, sex, date of laparoscopic cholecystectomy and date of ^{75} SeHCAT test, reason for ^{75}SeHCAT test, results of ^{75}SeHCAT test, date of CT scan, date of endoscopy, endoscopy results, number of episodes of loose stool per day, and final diagnosis. A 7-day ^{75}SeHCAT retention of less than 15% was deemed to be positive. Statistical analysis was performed using SPSS. A Mann Whitney U test to analyse time to investigation differences between men and women as this data was not normally distributed. The time from surgery to ^{75}SeHCAT was tested using this methodology.

Results - Local audit

A total of 2381 patients had laparoscopic cholecystectomy between 2013 and 2017. Out of these, 38 had chronic diarrhoea and thus underwent ^{75}SeHCAT testing. The number of episodes per day (bowel frequency) ranged from 3 to 20. Five of these patients were excluded as testing occurred prior to surgery. Of the remaining 33, 20 (60.1%) had a ^{75}SeHCAT retention of <15%. 12 had a ^{75}SeHCAT retention of <5%, One patient had a ^{75}SeHCAT retention of >5-10%, and 7 had a ^{75}SeHCAT retention of 11-15%. This is shown in figure 9.

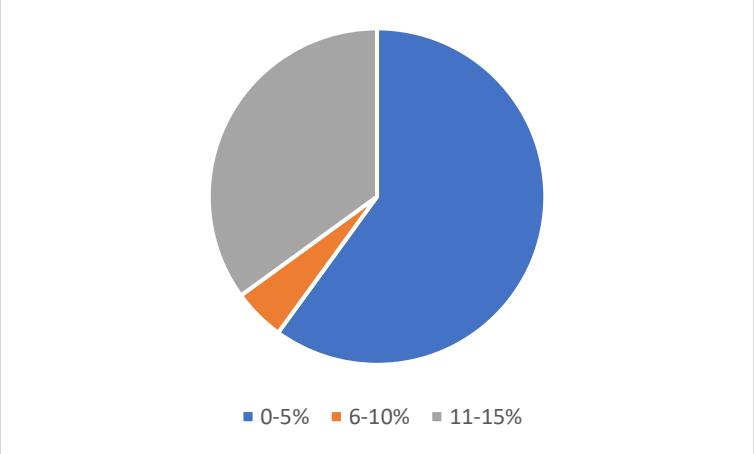

Figure 9: ^{75}SeHCAT retention <15%

The median age of the cohort was 47 years (17-66); 4 males and 16 females. The mean time from surgery to ^{75}SeHCAT testing was 564 days (SD +/-371 days), and women were tested later than men however this was not statistically significant (660 vs 287 days, p=0.072, using a Mann Whitney U test).

Twenty-nine of the patients who had ^{75}SeHCAT were also investigated via flexible sigmoidoscopy or colonoscopy, of whom 13 (44.85%) were normal, 8 (27.5%) had diverticulosis, 2 patients (6.9%) had mild inflammation and 5(17.2%) showed polyps which were shown to be tubular adenomas on histology. 10 of the patients who had ^{75}SeHCAT also had a CT scan of the abdomen and pelvis which were all normal.

Of the patients who had a negative ^{75}SeHCAT test, 3 were diagnosed as having IBS, one went on to have further investigations for Crohn's disease, one had dumping syndrome, one had complete resolution of symptoms after stopping omeprazole, and 7 remained unknown. This data was presented at the Association of Laparosocopic Surgeons of Great Britain and Ireland conference in December 2018 (appendix 7).

Methods - Multicentre audit

Multiple centres across the UK were contacted to take part in the multicentre audit. A protocol (appendix 8) was sent to these centres as well as a data collection sheet with drop down menus for answer selection to avoid mistakes as much as possible (appendix 9). Each centre was required to obtain individual audit department approval

prior to carrying out the study. Local approval from UHCW was also obtained (appendix 6).

The aim of the audit was to assess how many patients were being investigated for diarrhoea after laparoscopic cholecystectomy and whether this was congruent with the predicted number of patients who should be investigated according to the review performed in chapter 1, as well as how many of those investigated were eventually diagnosed with bile acid diarrhoea. Another point of interest were differences in any time to investigation between males and females.

The centres that registered to take part were provided with the study protocol, the data sheet and a deadline for these to be returned. They were also given a contact email for correspondence, which was set up specifically for the study. The data collected from each centre included age, sex, date of laparoscopic cholecystectomy and date of ^{75}SeHCAT test, results of ^{75}SeHCAT test, reason for ^{75}SeHCAT test, date of CT scan, date of endoscopy, endoscopy results, number of episodes of loose stool per day, and final diagnosis. The data sheet sent to the different centres consisted of drop-down menus with standardised answers for all the fields for internal validation. Once received, this data was analysed in terms of percentages. Differences in median time from surgery to investigation (the investigations in question being ^{75}SeHCAT, CT scan and endoscopy) between males and females were analysed using the Mann-Whitney-U test as the values obtained were not normally distributed. To further investigate, after obtaining statistical advice, a log of the time from cholecystectomy to investigation was taken to negate the non-normal distribution, and a T-test was used to determine whether there were still differences in investigation times. To further quantify this difference, a regression model of time to investigation adjusted for sex was also performed. The patients who had a ^{75}SeHCAT results <15% retention were divided into 3 groups, these being 0-5% (severe BAD), >5-10% (moderate BAD) and >10-15% (mild BAD) and any association between mild, moderate or severe BAD was investigated using the Chi-square test as they were categorical vs non-categorical data.

An electronic retrospective database of patients undergoing laparoscopic cholecystectomy between January 2013 and December 2017 was cross-referenced

with all the patients who underwent ^{75}SeHCAT testing during the same time period at these centres. A 7-day ^{75}SeHCAT retention of less than 15% was deemed to be positive. Patient demographics were collected and compared for significance ($p<0.05$) via the Mann Whitney U test. Time from surgery to investigation was also noted and any differences between men and women were compared using a Mann Whitney U test. To further investigate this a log of the time from cholecystectomy to investigation was taken and a student T-test was used to determine whether there were still differences in investigation times. To further quantify this difference, a regression model of time to investigation adjusted for sex was also performed.

Results - Multicentre audit

A total of 9439 patients underwent laparoscopic cholecystectomy between 1st January 2013 and 31st December 2017 in 5 centres: Oxford, Bristol, Bath and Coventry and Glasgow. Of these, 202 patients (2.14%) were investigated for bile acid diarrhoea via ^{75}SeHCAT.

Demographic data

There were 160 female patients (80%) and 42 male patients (20%), ranging in age from 2- to 90. The highest number of patients diagnosed with BAD was between the ages of 40-45. All patients younger than 35 were female, and the proportion of male patients increased after the age of 51. This is shown in table 4 below.

Ten patients had known inflammatory bowel disease, six having Crohn's disease, one having ulcerative colitis (one patient) and three having indeterminate colitis. Five patients had a previous terminal ileal resection, only one of which also had Crohn's disease.

Age	Number of patients (Male:Female)	Endoscopy n(%)	CT n(%)	Final Diagnosis of BAD n(%)	Final Diagnosis of IBD n(%)	Final Diagnosis of IBS n(%)	Final Diagnosis unknown n(%)
20-25	9 (0:9)	6(2.9%)	1(0.5%)	7(3.5%)	0	1(0.5%)	0
26-30	13 (0:12)	8(3.9%)	4(1.9%)	11(5.4%)	0	0	2(0.9%)
31-35	11 (0:11)	5(2.5%)	1(0.5%)	7(3.5%)	1(0.5%)	0	2(0.9%)
36-40	17 (2:13)	9(4.5%)	5(2.5%)	10(4.9%)	0	1(0.5%)	2(0.9%)
41-45	27 (4:23)	17(8.4%)	8(3.9%)	14(6.9%)	0	1(0.5%)	8(3.9%)
46-50	25 (3:22)	15(7.4%)	6(2.9%)	18(8.9%)	1(0.5%)	1(0.5%)	1(0.5%)
51-55	26 (7:19)	16(7.9%)	6(2.9%)	15(7.4%)	0	3(1.5%)	4(1.9%)
56-60	19 (4:15)	13(6.4%)	6(2.9%)	12(5.9%)	0	1(0.5%)	4(1.9%)
61-65	21(7:14)	14(6.9%)	12(5.9%)	9(4.5%)	1(0.5%)	0	8(3.9%)
66-70	11 (4:7)	11(5.4%)	6(2.9%)	8(3.9%)	0	1(0.5%)	1(0.5%)
71-75	15(5:10)	11(5.4%)	7(3.5%)	10(4.9%)	1(0.5%)	0	3(1.5%)
76-80	7(4:3)	2(0.9%)	5(2.5%)	4(1.9%)	0	0	1(0.5%)
81-85	2 (0:2)	2(0.9%)	2(0.9%)	1(0.5%)	0	0	1(0.5%)
86-90	2 (2:0)	1(0.5%)	0	1(0.5%)	0	0	1(0.5%)

Table 4: Demographics for multicentre audit

Indications for ^{75}SeHCAT testing

137 patients were referred for ^{75}SeHCAT with the indication being either, chronic diarrhoea or loose or watery stool, whil 21 patients were simply referred as “query of bile acid diarrhoea” or “bile acid malabsorption”. Seven patients were referred for

change in bowel habit while 17 patients were referred for abdominal pain, often accompanied by diarrhoea. Other reasons for referral included steatorrhea and bloating.

Other investigations

133 (65.8%) patients also had endoscopic examination, this being colonoscopy or flexible sigmoidoscopy. 86 of these were normal, while 29 revealed diverticular disease, 16 patients had polyps (all of which were tubular adenomas), and 2 patients had mild inflammation. Of those with a normal endoscopy, 43 were eventually diagnosed as having bile acid diarrhoea.

74 (36.6%) patients had CT scan of the abdomen and pelvis, of which 45 were normal, 11 showed diverticular disease, two confirmed inflammatory bowel disease and 15 showed non-bowel related pathology. These are shown in table 5.

Investigation	Number of patients and results
Endoscopy (colonoscopy/flexible sigmoidoscopy)	133 • 86: normal • 29: diverticular disease • 16: tubular adenomas • 2: mild inflamamtion
CT scan	74 • 45: normal • 2: IBD • 15: non-bowel related pathology

Table 5: Multicentre audit: other investigations and results

^{75}SeHCAT results and correlation with symptoms

The distribution of patients and their ^{75}SeHCAT results is shown in table 6 below. All of these patients had diarrhoea for more than four weeks. 106 patients had one to five episodes of diarrhoea per day, while 39 had six to ten episodes a day, 10 patients had eleven to fifteen episodes per day, and 3 patients had more than fifteen episodes per

day. The rest were not recorded by the assessing clinician. We found a significant correlation between the ^{75}SeHCAT result and the number of episodes of diarrhoea per day (p = 0.003, using chi-squared test, comparing <5%, >5% with <15%, >15%). This is also seen in table 6.

^{75}SeHCAT results	**<5%**	**6-10%**	**11-15%**	**>15%**
Total	72	40	26	64
Male	17	11	4	10
Female	55	29	22	54
1-5 episodes/day	28	19	16	41
6-10 episodes/day	20	6	2	6
11-15 episodes/day	5	3	1	1
>15 episodes/day	2	1	0	0

Table 6: ^{75}SeHCAT results and correlation to bowel habits

Time to investigation

There was no significant difference between men and women when assessing time from laparoscopic cholecystectomy to referral for investigation such as ^{75} SeHCAT scan or endoscopy. There was a significant difference between referral time for men and women for CT scan (p = 0.022, Mann Whitney U test), however this does not hold up on taking a log and performing a t-test, or on performing a regression analysis adjusting for sex. The regression analysis was performed using SPSS and adjusting for sex to assess whether this impacted time from surgery to investigation. This is shown in table 7 and figures 10, 11 and 12.

Median time from cholecystectomy	Total /days (SD)	Female/days (SD)	Male/days (SD)	p-value (Mann Whitney U test)	p-value(log and T-test)	Regression analysis p-value (Hazard ratio with 95%CI)
^{75}SeHCAT	672 (482)	726 (461)	539 (548)	0.139	0.212	0.55 (0.901;0.63.-1.277)
Endoscopy	696 (545)	723 (517)	545 (623)	0.290	0.66	0.739 (1.078;0.691-1.682)
CT	778 (595)	938 (531)	388 (709)	0.022	0.41	0.323 (1.39; 0.723-2.674)

Table 7: Median time from cholecystectomy to investigation

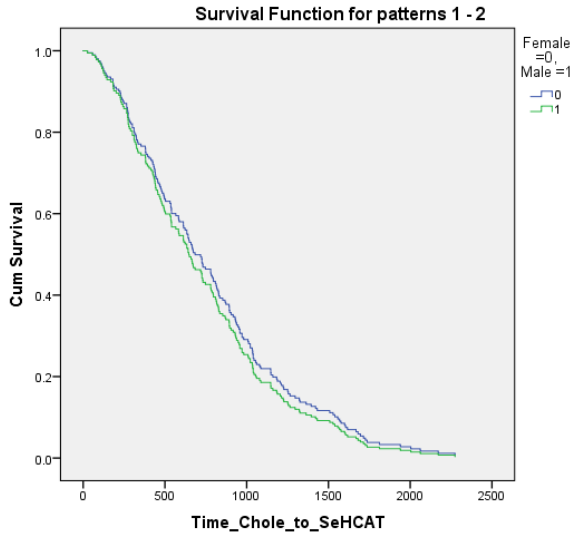

Figure 10: regression analysis for time to 75SeHCAT adjusted for sex

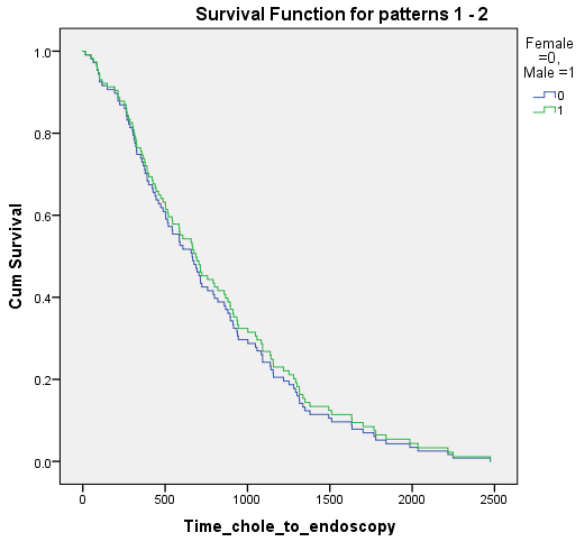

Figure 11: regression analysis for time to endoscopy adjusted for sex

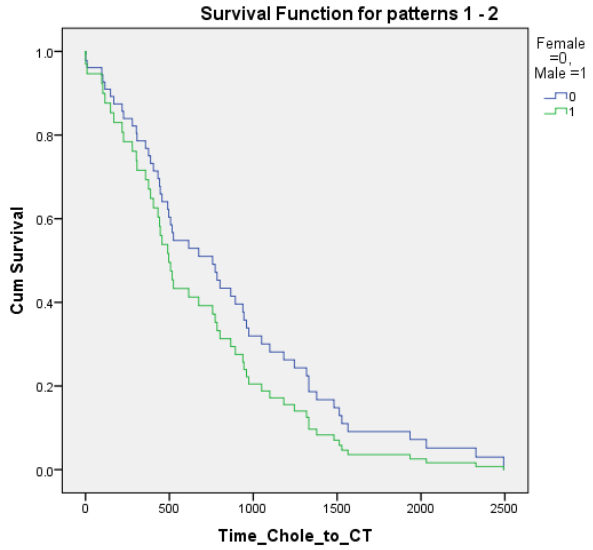

Figure 12: regression analysis for time to CT adjusted for sex

Final diagnosis

130 patients were diagnosed with bile acid diarrhoea (64.3%). 4 patients were diagnosed with IBD, 9 with IBS, and 37 remained unknown. The list of diagnosis is shown in figure 13 below.

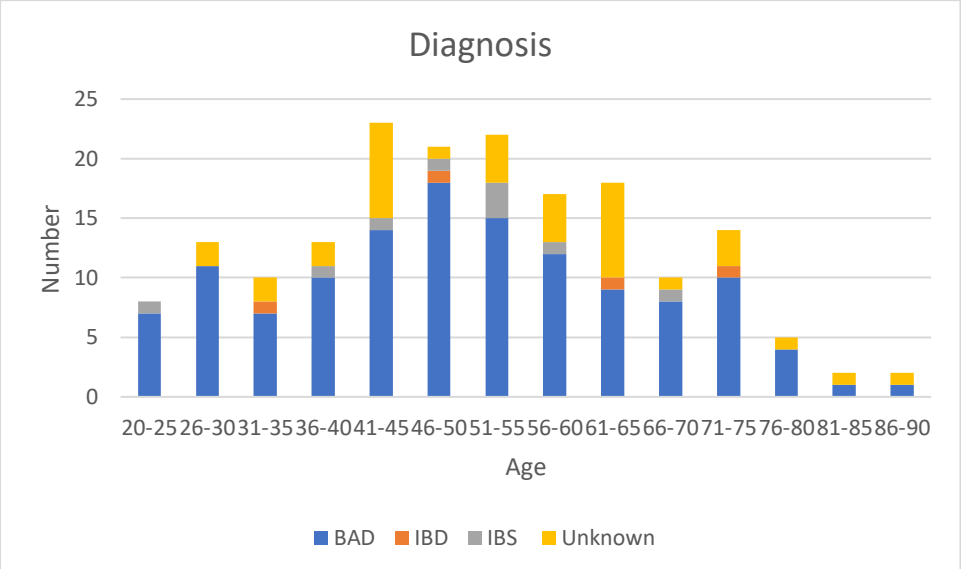

Figure 13:Final diagnosis by age group. 64.3% of patients were diagnosed with BAD. 4 patients were diagnosed with IBD, 9 with IBS, and 37 remained unknown. The rest were classified as other.

Discussion

Locally, only a small proportion of post-cholecystectomy patients were investigated for BAD (1.4%), and in those that were investigated 60.1% were positive. There was also a significant time delay to diagnosis. There is an obvious delay in initiating investigations, with a mean of 564 days between surgery and ^{75}SeHCAT test, implying that there is not much awareness of the possibility of developing bile acid diarrhoea after cholecystectomy. The difference in time to investigation between men and women may also imply that complaints are not taken seriously, and in fact there has been a study that there is perception of increased bowel frequency in women after cholecystectomy but no real diarrhoea (Hearing *et al.*, 1999).

In the study involving five tertiary centres, a small number of patients were investigated for diarrhoea, with only 2.14% of the entire post-cholecystectomy population being investigated. The published rate of post-cholecystectomy diarrhoea is highly variable, ranging from 2.1% to 57.2% (Fisher *et al.*, 2008; Fort *et al.*, 1996; Kim *et al.*, 2018; Lamberts *et al.*, 2013a; Luman *et al.*, 1996; Sauter *et al.*, 2002b),. Our own review of the literature showed a post-cholecystectomy diarrhoea rate of 13%. Thus, the number of patients investigated does not correlate with the known rate of post-cholecystectomy diarrhoea reported in the literature implying there is a large number of patients who are either not being investigated or are not seeking medical attention. This may be due to a lack of awareness that diarrhoea may develop post-cholecystectomy due to deficiencies in the pre-operative consent process, with up to 70.3% of patients not being consented for the possibility of developing diarrhoea after laparoscopic cholecystectomy(Hussain *et al.*, 2016). It could also mean that the rest of the cohort either did not develop diarrhoea or if they did it was self-limiting and therefore did not warrant investigation.

There is an obvious delay in initiating investigations, with a median of 672 days between surgery and ^{75}SeHCAT test, possibly implying that there is not much awareness of the possibility of developing bile acid diarrhoea after cholecystectomy within the medical community. While the difference in time to investigation between men and women was not statistically significant (p=0.139), there is still an obvious difference, with median time to testing for female patients being 726 days while median time to testing for male patients was 539 days. Thus, there is a median difference of

187 days between men and women being investigated. This may imply that complaints from females are taken less seriously, and in fact there has been a study that suggests that while there is perception of increased bowel frequency in women after cholecystectomy, there is no real diarrhoea (Hearing *et al.*, 1999). However, this is not really the case as patients have had positive ^{75}SeHCAT tests after developing diarrhoea post-cholecystectomy, as seen in our results.

Not all patients had endoscopic investigation as well as ^{75}SeHCAT testing, as recommended by the British Society of Gastroenterology guidelines (Arasaradnam *et al.*, 2018), implying that inflammatory bowel disease was not ruled out in all patients. As inflammatory bowel disease is one of the causes of bile acid diarrhoea, mainly ileal Crohn's disease, this is a confounding factor in our study. Another confounding factor is that some patients were known to have Crohn's disease prior to laparoscopic cholecystectomy whilst others had a right hemicolectomy for various conditions. As both of these states affect the terminal ileum and may lead to malabsorption of bile acids, for such patients it is unclear what the cause of bile acid diarrhoea was for these patients, as it could have been due to the malabsorption from the terminal ileum, or the overproduction following cholecystectomy, or a mixture of both. With endoscopic investigations there was once again a delay in investigation of 178 days between men and women (median of 723 days for women and 545 days for men). While again this was not significant (p=0.29) one can appreciate a time difference in investigation which will affect quality of life (Bannaga *et al.*, 2017).

A large proportion of patients had a CT scan as part of their investigation, despite the CT scan being more useful in the investigation of structural rather than functional disorders. In this we can see a significant difference between referral time for men and women (p=0.022), this being a median of 938 days for women and 388 days for men. We can see that for all investigations, median time for investigation of female patients was longer. This is a pattern which has been previously observed in healthcare, resulting in higher morbidity and mortality for female patients (Colella *et al.*, 2015; Li *et al.*, 2018).

Despite men being investigated quicker than women in all aspects (^{75}SeHCAT, endoscopy and CT scan) there is a significant delay in initiating investigations after

laparoscopic cholecystectomy with a median time to investigation being longer than 18 months for each of these. As symptoms tend to develop within the first three months after cholecystectomy, we can see that patients are not being investigated in a timely manner (Wilson & Macintyre, 1993). This would negatively affect patients' quality of life (Bannaga *et al.*, 2017). However, there may be other issues in play here such as social factors preventing some patients from seeking help or attending for tests, as well as delays resulting from local processes such as referral practices and waiting list times for tests such as ^{75}SeHCAT which is not found in all centres, as well as endoscopy waiting times. Thus, it is difficult to say what effect this has on time from cholecystectomy to testing. There may also be differences in practice between regions to take into account, since this study was conducted across the UK.

This study has shown that severity of bile acid diarrhoea as seen on the ^{75}SeHCAT result could correlate with symptoms ($p=0.003$). However, all patients were investigated after having diarrhoea for 4 weeks and the majority had a up to 10 episodes per day, as per guideline advice (Arasaradnam *et al.*, 2018).

Another interesting point to note is that while 64.3% of the cohort was diagnosed with bile acid diarrhoea and 14.4% had another diagnosis, 20.2% of patients were not formally diagnosed with any condition. Thus, it seems there is further work to be done in diagnosing post-cholecystectomy diarrhoea. We also noted that all patients younger than 35 years of age were female, and there are generally less males in each age group under the age of 50. This seems to imply that younger women are possibly at higher risk of developing PCD. This correlates with some studies (Wanjura & Sandblom, 2016) but not with others who state that it was younger males who were more at risk (Fisher *et al.*, 2008; Jasim, 2018; Yueh *et al.*, 2014).

This study dealt with real-time clinical data thus showing a true perspective of patients who were investigated post-laparoscopic cholecystectomy for diarrhoea. However, if the patients had a previous laparoscopic cholecystectomy in a separate hospital they were missed in our dataset. However, some patients are empirically started on bile acid sequestrants rather than being investigated via ^{75}SeHCAT amd these were also missed in this study. Another disadvantage is that not all patients who develop diarrhoea are investigated via ^{75}SeHCAT. Bile acid diarrhoea is not yet a well-known

condition and thus the only patients who were referred for ^{75}SeHCAT testing were those seen by the clinicians who are aware of the condition or referred to specialists award of the condition. We can also see a difference in number between male and female patients within the group. This is the largest study of its kind to date, further studies involving more direct comparison between patients investigated for diarrhoea post-cholecystectomy and those who were not would be required for further characterisation.

This data has been published in the World Journal of Surgery (appendix 10).

Chapter 4: Results – Improvement in GIQLI scores shown post-operatively despite no correlation to bowel habits.

Chapter summary

This was part of the prospective case control study. The patients identified for the study and control groups were administered the GIQLI questionnaire, and asked to identify number of stools per day as well as stool description using the Bristol stool chart, before and 3-4 months after surgery. The results were then compared using a Wilcoxon signed rank test. This showed that there was a significant improvement in quality of life after surgery in both the control groups and the study groups. Using a multivariate model adjusting for age and sex, there were no significant differences in GIQLI scores preoperatively and postoperatively between the two groups.

When it came to stool frequency and consistency, there were no significant differences in the study group when comparing pre- and post-operative results. In the control group there was a significant difference in stool frequency but not in stool consistency. The patients were also assessed for whether their symptoms would fit into the ROME IV criteria.

Symptom assessment: GIQLI and the Bristol stool chart.

The GIQLI questionnaire (Appendix 4) is a validated questionnaire developed to investigate the quality of life of patients with gastrointestinal symptoms. It consists of 36 items to which the patient has to select one of five answers. The answers are then scored, with ‘0’ points given to the least desirable answer and ‘4’ points given to the most desirable answer. A decision was made to use this questionnaire as it is a validated method of assessing gastrointestinal symptoms and thus a reliable method for internal comparison of patient symptoms pre- and post-operatively. It is also a reproducible method of investigating bowel symptoms such as diarrhoea.

The Bristol stool chart (figure 14) was also used as an investigative measure in this study (Lewis & Heaton, 1997). Patients were asked to quantify the number of times they opened their bowels daily and what their stool looked like as per the Bristol stool chart. This is another way of internally validating the patient’s responses and comparing pre- and post-operative stool numbers and consistency.

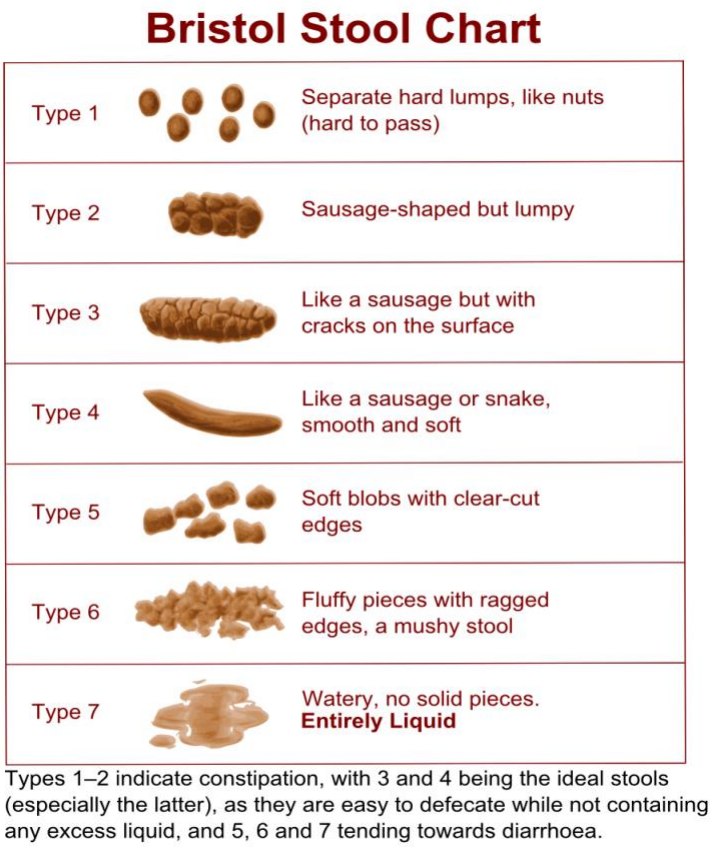

Figure 14: Bristol stool chart (Wikipedia commons/ CCBY-SA30)

ROME IV criteria

The ROME foundation created clear criteria for the diagnosis of irritable bowel syndrome (IBS). These are known as the Rome IV criteria. However it has been stated that 25 to 50% of patients with IBS-D (diarrhoea-predominant IBS) actually have bile acid diarrhoea (Camilleri, 2015). The criteria for diagnosis of IBS involve abdominal pain weekly for a minimum of 3 months, along with a minimum of two of the following criteria: increasing or improving pain related to defecation, associated with a change in stool frequency, or associated with a change in stool form. There are also subtypes associated with IBS, these being IBS -C (constipation type) where more than 25% of bowel movements are Bristol Stool scale types 1 or 2 and less than 25% types 6 or 7; IBS-D (diarrhoea predominant) where >25% of bowel movements are classified as Type 6 and 7 on the Bristol stool chart and <25% are type 1 and 2; IBS-M (mixed type) where >25% of bowel movements are classified as Type 6 and 7 on the Bristol stool

chart and >25% are type 1 and 2 and finally IBS-U (unclassified) where the diagnostic criteria for IBS are met however they do not fit any particular subtype (Drossman & Hasler, 2016)

Methods

The patients were identified from the theatre lists and approached for inclusion into the study as described in chapter 2. The GIQLI questionnaire and identification of number of stools per day and description as per the Bristol stool chart was performed on the day of operation, pre-operatively. A telephone number and/or email address, as per the patient's preference, were obtained for the follow up questionnaire which was administered 3-4 months post-operatively, either over the telephone with the questions being read out and the answers read out so that the participants could choose the most appropriate answer or sent to them by email where they could choose the most appropriate answer. The total scores for the questionnaire were added up and the scores pre- and post- operatively were compared for each group. The participants were also asked to provide number of stools per day and a description of the stool using the Bristol stool chart. These were also compared pre- and post-operatively.

The pre- and post-operative GIQLI scores were compared using a Wilcoxon signed rank test. This was chosen as the samples were paired since we were comparing the same group of patients before and after surgery, however the results were not normally distributed as seen in the charts in appendix 11. A multivariate model was also performed to adjust for age and sex. The average daily bowel frequency pre- and post-operatively was also compared using a Wilcoxon signed rank test as these were also not normally distributed.

GIQLI results

Study group

The maximum number of points available on the GIQLI questionnaire is 144 points, indicating a high subjective quality of life. For the study group, the pre-op questionnaire scores ranged from 51 to 135. The post-op questionnaire scores ranged from 57 to 140. The higher the score, the better the quality of life. These are shown in figure 15.

Of the 40 study patients, 36 were followed up. Four patients were lost to follow up either from inability to make contact or that declined to continue participation in the study. 33 patients showed an improvement in the post-operative quality of life score, with a median improvement of 19 points (range 1 to 63).

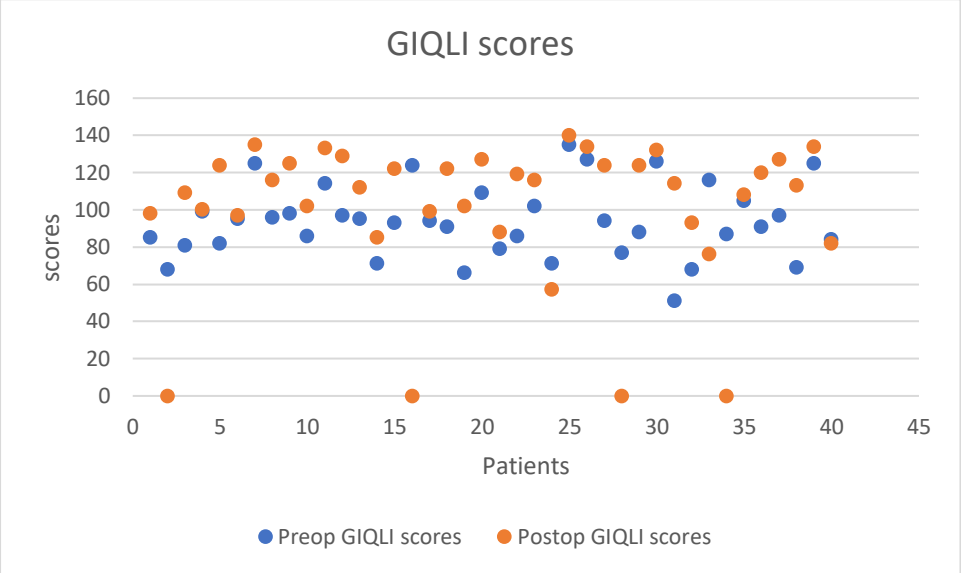

Figure 15: Pre- and post-operative GIQLI scores, study group. The patients who were not followed up in the post-operative setting are not included in the postoperative graph.

As the results were not normally distributed (appendix 11), a related samples Wilcoxon signed rank test was performed to analyse the pre- and post-operative results. The difference between the two groups was statistically significant (p=0.000) implying there is a statistically significant improvement in quality of life after laparoscopic cholecystectomy. This is shown in figure 16.

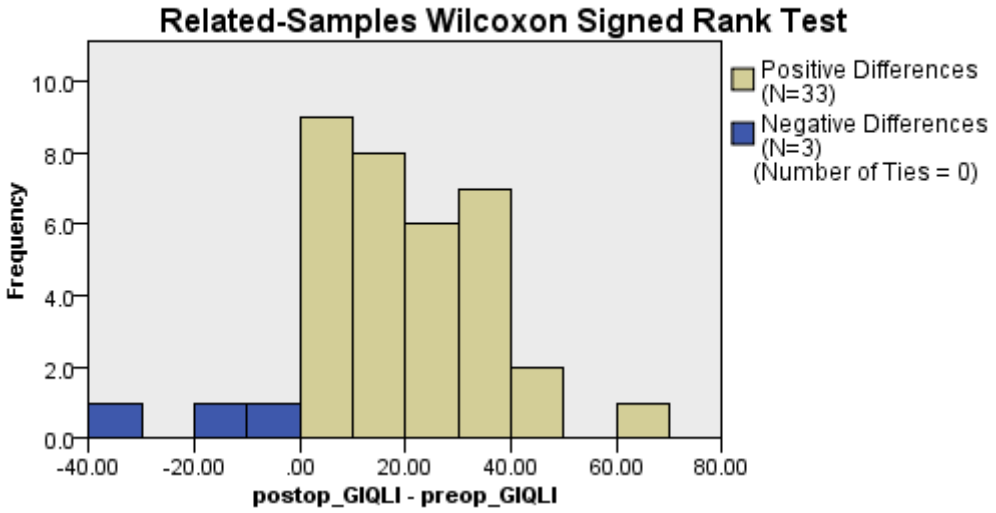

Figure 16: Related samples Wilcoxon signed rank test, study group

Symptom improvement as per the GIQLI questionnaire is shown in table 8 below.

Symptom	**Patients showing improvement (%)**
Abdominal pain	33 (91.7)
Upper abdominal distension	32 (88.9)
Bloating	30 (83.3)
Excessive passage of gas through the anus	31 (86.1)
Excessive burping or belching	34 (94.4)
Gurgling noises from the abdomen	31 (86.1)
Frequent bowel movements	27 (75)
Found eating to be a pleasure	30 (83.3)
Restricted foods to eat	34 (94.4)
Able to cope with everyday stresses	32 (88.9)
Sad about being ill	31 (86.1)
Nervous or anxious about illness	33 (91.7)
Happy with life in general	30 (83.3)

Frustration about illness	33 (91.7)
Feeling tired or fatigued	31 (86.1)
Feeling unwell	32 (88.9)
Waking up at night	30 (83.3)
Troubled by changes in appearance	28 (77.8)
Losing physical strength	29 (80.1)
Losing endurance	28 (77.8)
Feeling unfit	30 (83.3)
Complete normal daily activities	32 (88.9)
Complete normal patterns of leisure	29 (80.1)
Troubled by medical treatment	33 (91.7)
Personal relationships worsened	32 (88.9)
Sexual life impaired	31 (86.1)
Regurgitation	32 (88.9)
Slow speed of eating	34 (94.4)
Trouble swallowing	33 (91.7)
Urgent bowel movements	26 (72,2)
Diarrhoea	30 (83.3)
Constipation	32 (88.9)
Nausea	35 (97.2)
Blood in stool	35 (97.2)
Heartburn	36 (100)
Uncontrolled stools	26 (72,2)

Table 8: Symptom improvement, study group

There were no associations in GIQLI score differences between smokers, non-smokers and ex-smokers (p=0.10), alcohol excess and no alcohol excess (p=1.00)

using a Chi-squared test as this was comparing categorical with continuous variables. A Spearman's correlation coefficient was used to determine whether there was any correlation between BMI and change in GIQLI score postoperatively, and this was also not significant (p=0.757).

Control Group

20 patients were in the control group and 18 completed the questionnaire at 3 months post-operatively. 2 patients were lost to follow up. 15 patients showed score improvement postoperatively with a median improvement score of 15, range 2 to 46. The pre-operative and post-operative scores are shown in figure 17 below. While the pre-operative scores were normally distributed, the post-operative scores were not and therefore a Wilcoxon signed rank test was performed to assess whether the improvement in scores post-operatively was statistically significant. There was found to be a significant improvement in quality of life post-operatively, p=0.012. This is shown in figure 18.

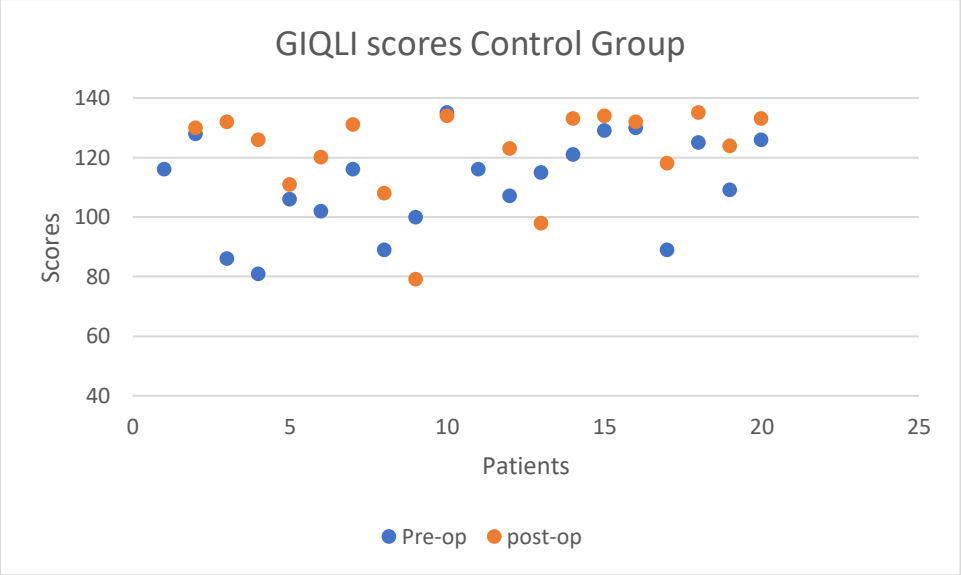

Figure 17: Pre- and post- operative GIQLI results, control group

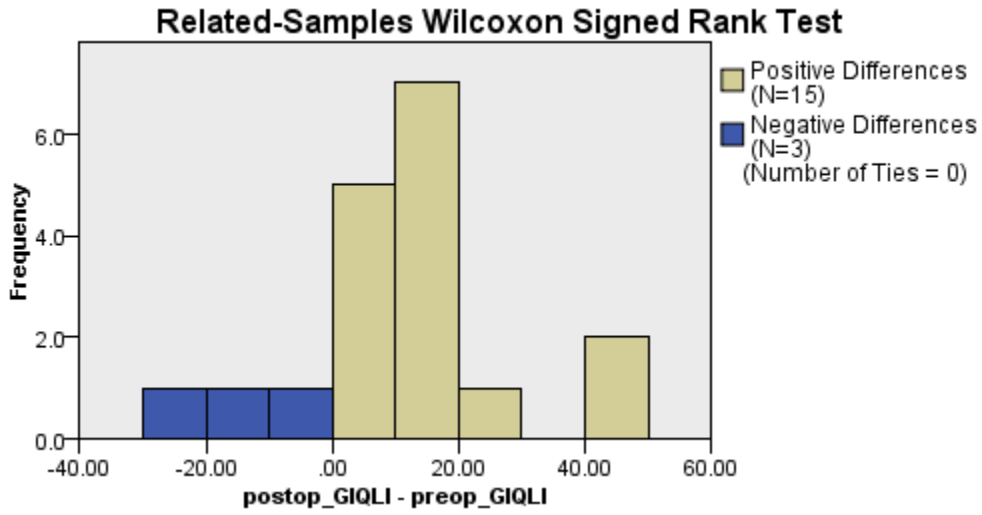

Figure 18:Related samples Wilcoxon signed rank test for control group GIQLI

Symptom improvement as per the GIQLI questionnaire is shown in table 9 below.

Symptom	**Patients showing improvement (%)**
Abdominal pain	18 (100)
Upper abdominal distension	16 (88.9)
Bloating	14 (77.8)
Excessive passage of gas through the anus	17 (94.4)
Excessive burping or belching	15 (83.3)
Gurgling noises from the abdomen	15 (83.3)
Frequent bowel movements	15 (83.3)
Found eating to be a pleasure	14 (77.8)
Restricted foods to eat	14 (77.8)
Able to cope with everyday stresses	17 (94.4)
Sad about being ill	17 (94.4)
Nervous or anxious about illness	17 (94.4)
Happy with life in general	16 (88.9)

Frustration about illness	14 (77.8)
Feeling tired or fatigued	15 (83.3)
Feeling unwell	16 (88.9)
Waking up at night	14 (77.8)
Troubled by changes in appearance	17 (94.4)
Losing physical strength	17 (94.4)
Losing endurance	17 (94.4)
Feeling unfit	18 (100)
Complete normal daily activities	16 (88.9)
Complete normal patterns of leisure	14 (77.8)
Troubled by medical treatment	17 (94.4)
Personal relationships worsened	16 (88.9)
Sexual life impaired	17 (94.4)
Regurgitation	15 (83.3)
Slow speed of eating	16 (88.9)
Trouble swallowing	15 (83.3)
Urgent bowel movements	17 (94.4)
Diarrhoea	17 (94.4)
Constipation	15 (83.3)
Nausea	17 (94.4)
Blood in stool	17 (94.4)
Heartburn	18 (100)
Uncontrolled stools	18 (100)

Table 9: Symptom improvement, control group

There was no correlation between change in GIQLI scores and smoking (p=1.00) and alcohol (p=0.97) in the control group.

Bowel habit results

Study group

The average daily bowel frequency preoperatively was 1.5. This increased to 1.66 postoperatively. The data was not normally distributed therefore a Wilcoxon signed rank test was used to compare the medians. This showed no significant difference (p=0.502). We can see that 13 patients had a greater daily stool frequency post operatively, while 8 had a lower frequency and 15 were the same both pre- and postoperatively. This is shown in figure 19.

In terms of stool consistency using the Bristol stool chart, the median both pre- and post-operatively was 4. We can see that 13 patients had looser stool while 10 had harder stool and 13 were the same. This is shown in figure 20.

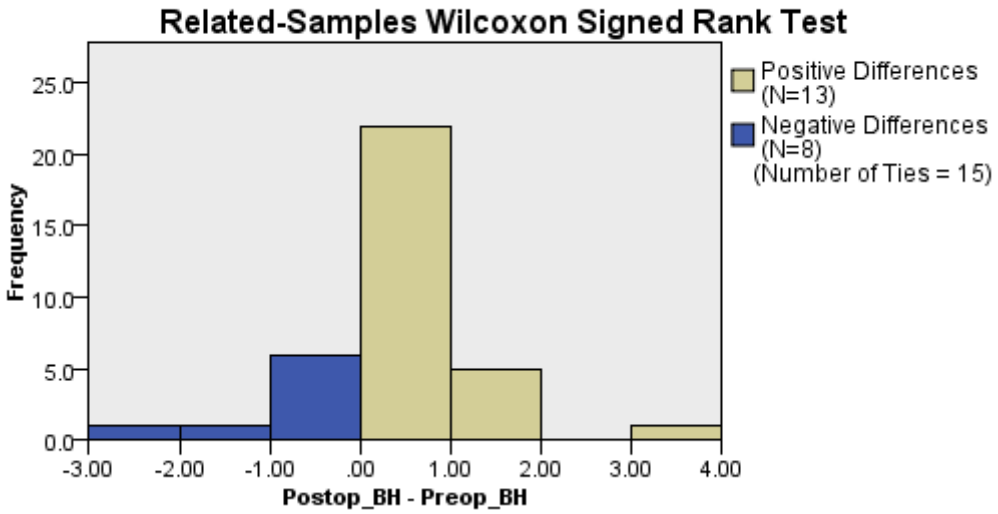

Figure 19: pre- and post- op number of stools per day, study group

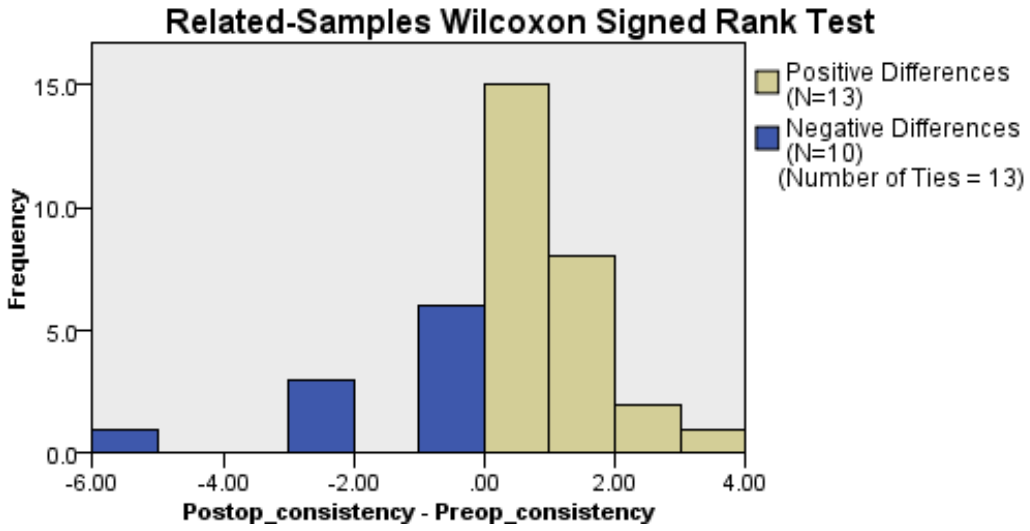

Figure 20: pre- and post- op stool consistency, study group

Control group

The median for pre- and post- op stool frequency was found to be statistically significant (p=0.042), with a higher stool frequency post-operatively. 7 patients remained the same while 9 patients had increased frequency and two patients had decreased frequency. This is shown in figure 21. However, there was no significant difference in stool consistency (p=0.739), with 12 patients remaining the same, three becoming looser and three describing firmer stools. This is seen in figure 22.

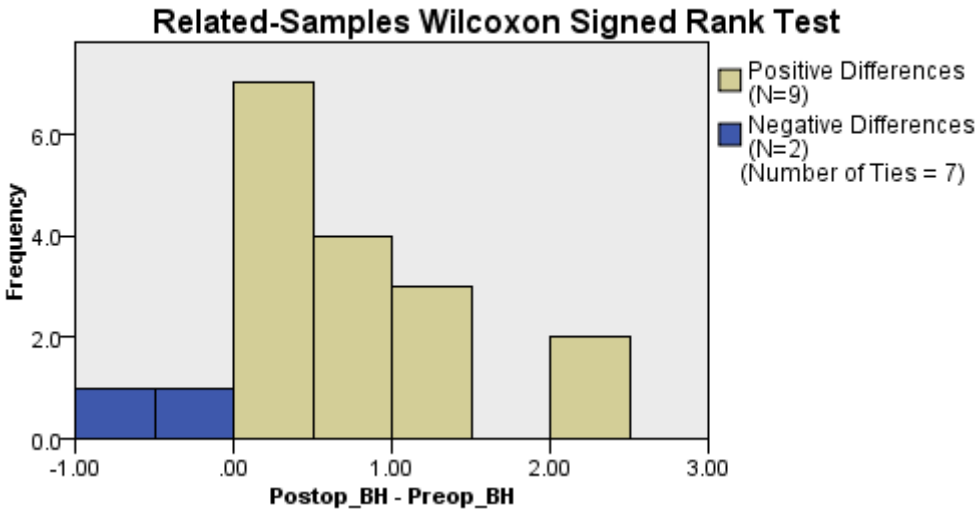

Figure 21: pre- and post- op stool frequency, control group

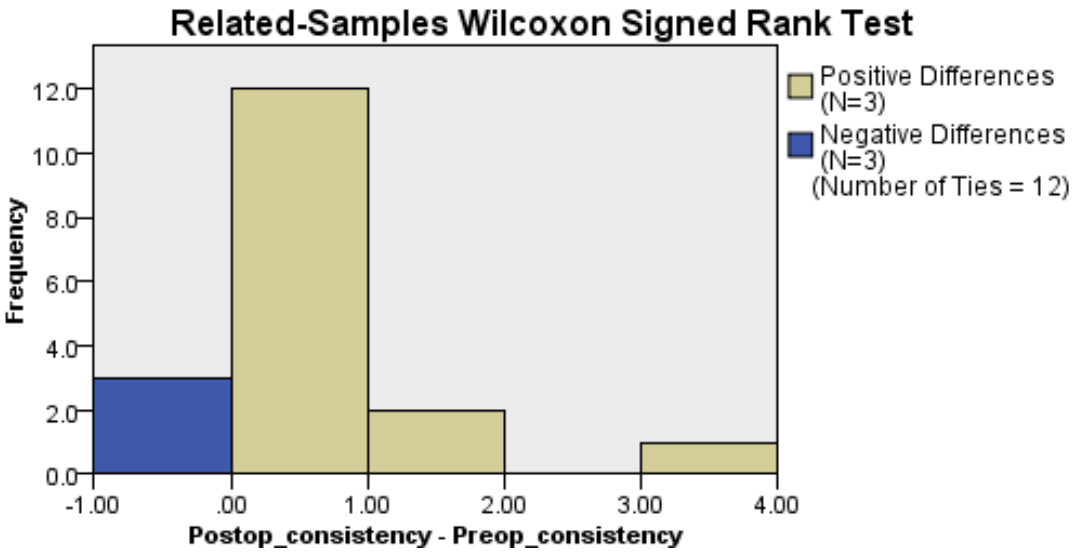

Figure 22: pre- and post-op stool consistency, control group

ROME IV criteria results: Study group

As per the ROME IV criteria, 18 patients would have fit the criteria for IBS pre-operatively. Three patients would have fit the criteria for IBS-D; eight for IBS-C, and seven for IBS-M. 12 patients fit the criteria for IBS in the post-operative period, of whom five were IBS-M, three were IBS-C and four were IBS-D. However, the patients who fit the IBS-M criteria were not eligible for further testing as stool consistency was not type-6 or 7 on the Bristol stool chart and having stool frequency of less than 3 per day.

ROME IV criteria results: Control group

Three patients fit the criteria for IBS in the control group, two for IBS-C and one for IBS-U. In the postoperative period, only one patient fit the criteria and that was for IBS-U (the same patient as the preoperative period).

Group differences

Using a multivariate model adjusting for age and sex, there were no significant differences in GIQLI scores preoperatively and postoperatively between the two groups (p=0.570 and p=0.505 respectively).

Discussion

There is a demonstrable improvement in the quality of life of patients following laparoscopic cholecystectomy in our study, with 33 out of 36 patients (92%) showing an improvement in their subjective quality of life. This is consistent with previous literature that has confirmed that in most cases, gallstone-related symptoms of abdominal pain, bloating and heartburn improved post-operatively (Gui *et al.*, 1998; Topcu *et al.*, 2003) are improved. General quality of life parameters such as tiredness, anxiety, feeling unfit and ability to perform daily tasks related to work and recreation are also improved after surgery. Most patients commented on the general quality of life questions that other factors impacted on their questionnaire responses. These include unrelated medical conditions (joint pains, heart conditions, mental health issues) as well as social factors also impacting their quality of life, as some patients were scheduled for their follow up appointments during the government-imposed lockdown for the COVID-19 pandemic.

Despite 10 patients stating that they had uncontrolled stools more often and more frequent bowel motions, analysis of pre- and post-op bowel habits using daily bowel motion as recorded by the same patients and the Bristol stool chart did not confirm these changes in 6 of 10 patients. Further analysis showed that those patients who did not show improvement went from ‘never’ to ‘a little of the time’ on the questionnaire. This could possibly be the reason why they were not eligible for further testing as per the British Society of Gastroenterology guidelines(Arasaradnam *et al.*, 2018). Four were sent for ^{75}SeHCAT and colonoscopy, however one patient did not attend for either test. As these were self-reported questionnaires, an element of bias and problems with recall may be present in these results. Two of the patients who were sent for further testing had ^{75}SeHCAT scan results of <15%,thus being diagnosed with BAD after their cholecystectomy, with normal colonoscopy results. The other patient did not have bile acid diarrhoea and had a normal colonoscopy.

We can see that when comparing stool frequency and stool consistency there were no significant differences in the pre-and post-op groups. However, when the data is more closely examined, one can see that 13 patients had a greater stool frequency and 13 patients had looser stools postoperatively. However, this did not necessarily mean that they had enough stool episodes per day or loose enough stools that meant

further investigations were warranted. Also, only 10 patients reported more frequent bowel movement on their GIQLI questionnaire, however when asked specifically regarding their bowel habit and the number of times they passed stool per day there were 13 patients who stated number of stools per day postoperatively had increased . Also, they were not the same patients, as only 4 patients stated increased bowel movements on both the GIQLI and the Bristol stool chart. Conversely, eight patients reported less frequent stool and 10 reported harder stool post-cholecystectomy. While this has been documented previously(Wilson & Macintyre, 1993), it is interesting to observe such differences in bowel habit changes between patients.

In the control group a number of patients reported that they were restricting their diet in the post-operative period and had less pleasure in eating than they did pre-operatively. This could possibly have been the subset of patients who had laparoscopic sleeve gastrectomy which is surgery performed for weight loss and which requires a careful postoperative diet to ensure its success. This is in contrast to those patients who underwent laparoscopic hernia repair, which requires no dietary modifications, and laparoscopic Nissens fundoplication which requires a liquid/blended diet for a few days post operatively but then allows a return to normal diet. There were no particular changes in bowel habit identified in the questionnaire in this group. Despite this, the daily stool frequency was significantly higher in this group in the postoperative period, although none of the patients individually required further investigations as the highest stool frequency was two per day. In terms of stool consistency there were no significant changes, which was as expected in this group. None of the patients in the control group required further investigations.

Chapter 5: Results – Higher triglyceride levels in the post-cholecystectomy study group with no correlation to bowel habit or GIQLI scores.

Chapter summary

11 patients in the study group and 9 patients in the control group had pre- and postoperative lipid levels measured. These consisted of HDL, LDL, triglycerides and cholesterol. 7 patients in the study group also had pre- and post-operative FGF19 plasma levels measured.

In the study group there were no significant differences between pre- and post-op level of cholesterol (p=0.812), HDL (p=0.944), and LDL (p=0.082). There was a significant difference in pre- and post- operative triglyceride levels (p=0.021). There was no significant correlation between the change in pre- and post- op GIQLI scores and triglyceride level changes (p=0.422). There was also no significant correlation between triglyceride level changes and changes in pre- and post- op bowel habit (p=0.890) or triglyceride level changes and stool consistency as per the Bristol stool chart (p=0.887). There were no significant differences in cholesterol, HDL, triglyceride or LDL levels in the pre- and post-operative period for the control group.

Methods

Patients in the case-control study were offered blood tests pre- and post-operatively. While 11 patients in the study group had pre- and post-op lipid levels, only 7 patients had pre- and post-op FGF19 levels. This discrepancy is due to 4 patients being unable to come into hospital to have FGF19 levels taken however went to their GP to have lipid levels repeated post-operatively. Patients were fasted for a minimum of 6 hours prior to testing. Lipid samples were analysed at the University Hospitals Coventry and Warwickshire pathology laboratories, and results obtained consisted of HDL, LDL, triglyceride and cholesterol levels, these all being different types of lipids. FGF19 samples were sent to Affinity Biomarker labs at Imperial College, London, due to their previous experience in analysing FGF19 plasma samples.

A Wilcoxon signed rank test was used to analyse differences between lipid levels pre- and post-operatively. A Spearman's correlation coefficient test was then used to analyse whether there was any relationship between changes in lipid levels and bowel habit, stool consistency and GIQLI results. These tests were used due to non-parametric distribution of results.

A Wilcoxon signed rank test was also used to analyse differences in plasma FGF19 levels pre- and post-operatively. A Spearman's correlation coefficient test was then used to check for any relationship between change in plasma FGF19 levels and stool consistency, bowel habit and GIQLI results.

Study group – lipid levels

16 patients agreed to have lipid levels taken before and after surgery. 11 patients had blood tests post operatively, as 3 patients were lost to follow up (one patient agreed to undertake the questionnaire by telephone but was unable to attend for blood tests) and 2 patients were unable to come into hospital for repeat blood tests due to COVID19. The Wilcoxon signed rank test was used to assess for change in lipid level as data was not normally distributed. This is presented visually as raw linked data.

There were no significant differences between pre- and post-op level of cholesterol ($p=0.812$) HDL ($p=0.944$), and LDL ($p=0.082$). These are shown in figures 23, 24 and 25. There was found to be a significant difference between the pre- and post-operative fasting plasma FGF19 levels ($p=0.018$). There was no correlation between the change that occurred in plasma FGF19 levels and change in stool consistency ($p=0.67$) or change in GIQLI scores ($p=0.43$).

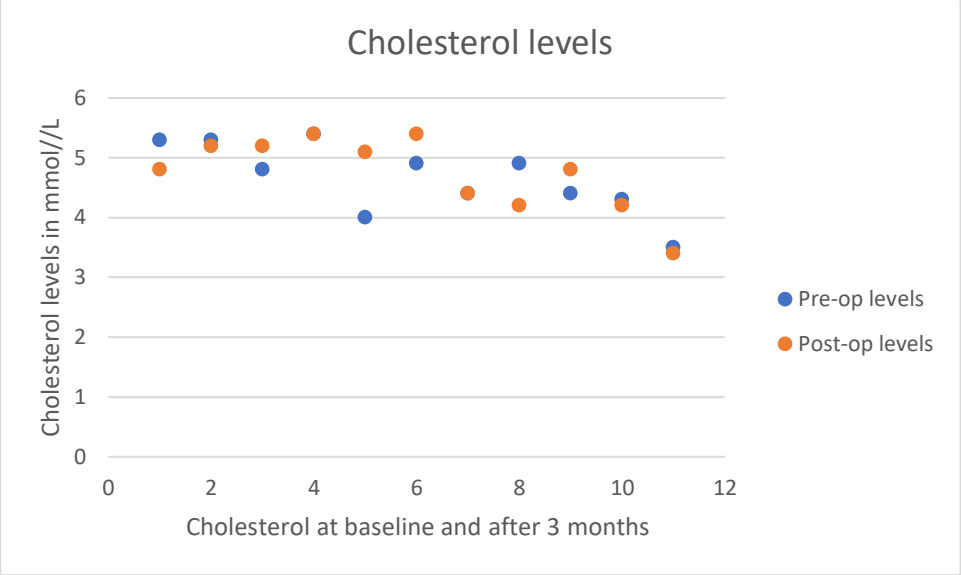

Figure 23: Pre- and post-operative cholesterol levels, study group

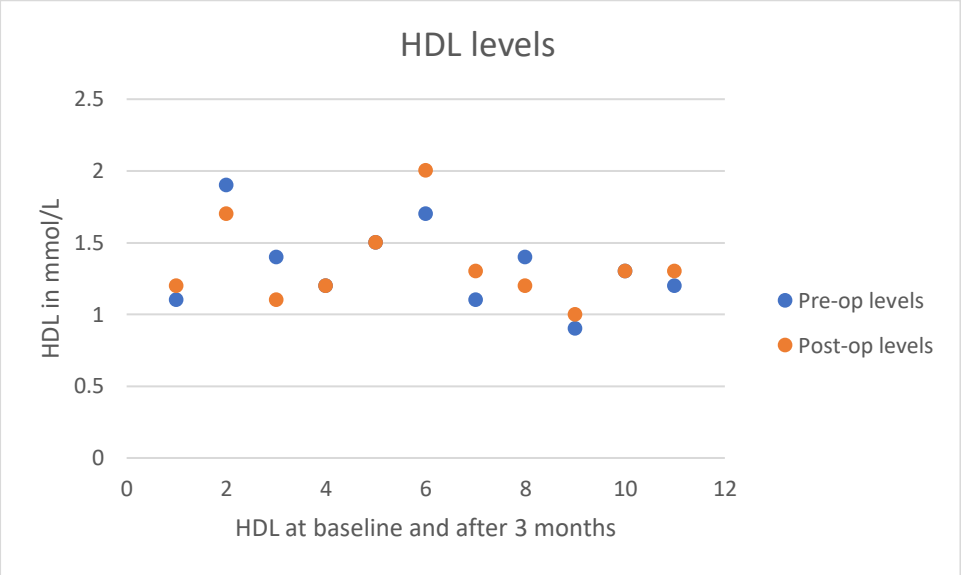

Figure 24: Pre- and post-operative HDL levels, study group

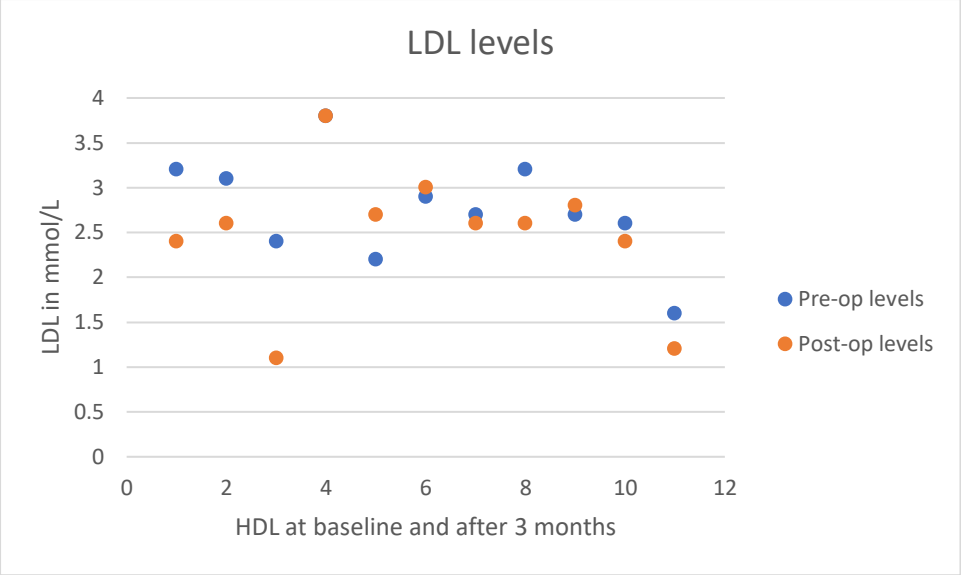

Figure 25: Pre- and post-operative LDL levels, study group

There was a significant difference in pre- and post- operative triglyceride levels (p=0.021). This is shown in figure 26. Triglyceride levels were significantly increased post operatively, as the mean pre-op was 1.25 and the average post-op was 2.07.

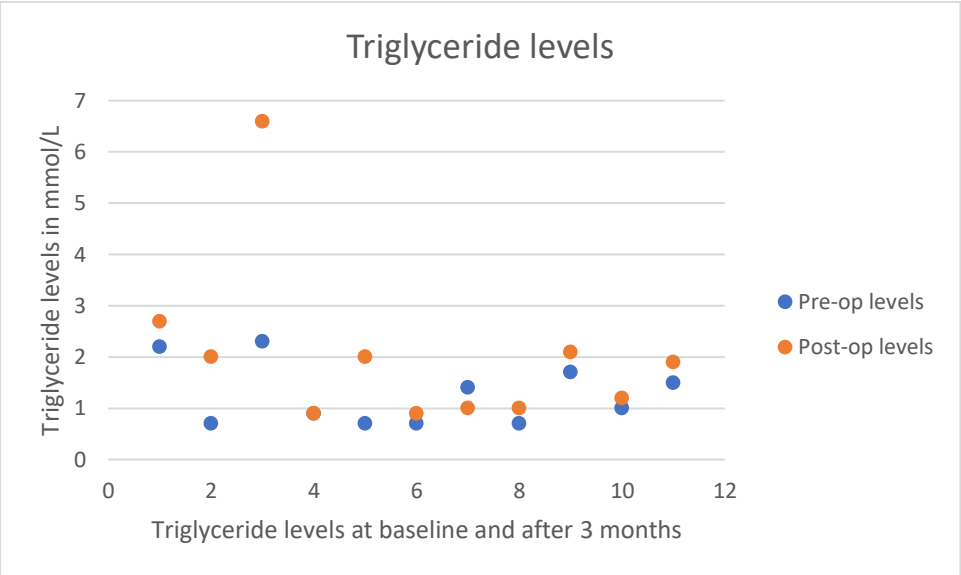

Figure 26: Pre- and post-operative triglyceride levels, study group

Control group

12 patients agreed to have lipid levels measured, however only 9 could be followed up because of the COVID19 pandemic. Wilcoxon signed rank test was used to assess for change in lipid levels as data was not normally distributed. Once again, this is presented visually as a histogram. There were no significant differences in cholesterol, HDL, triglyceride or LDL levels in the pre- and post-operative period for the control group. These are shown in figures 27, 28, 29 and 30.

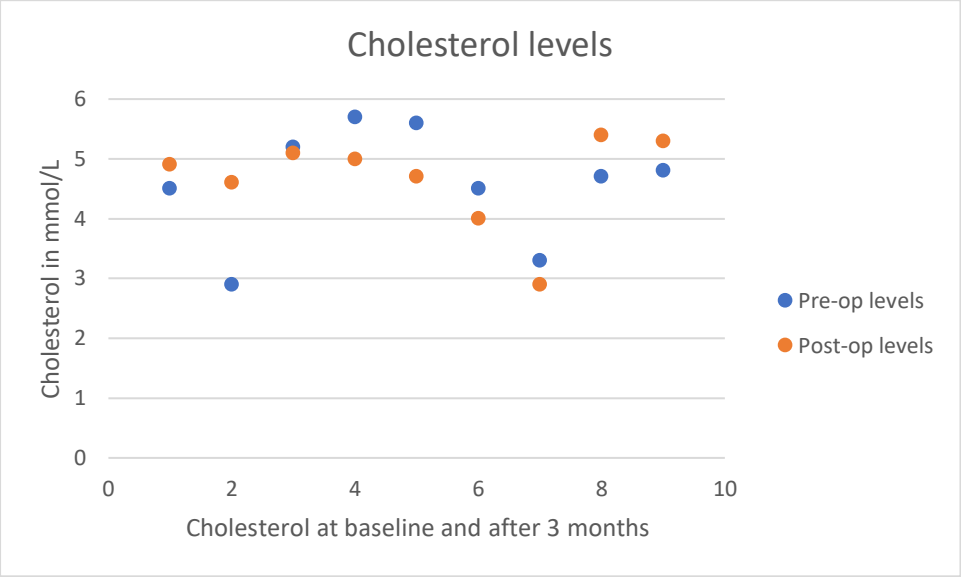

Figure 27: Pre- and post-operative cholesterol levels, control group

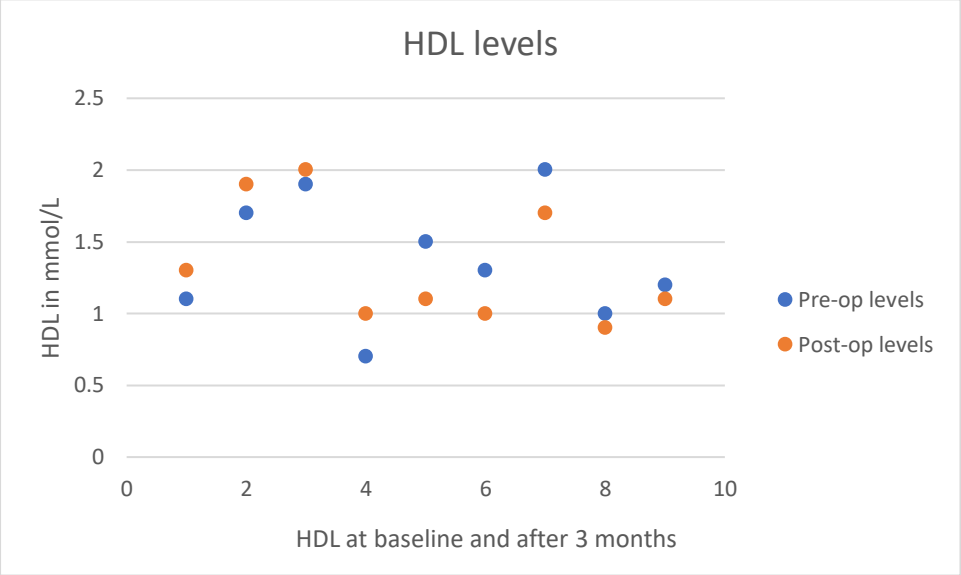

Figure 28: Pre- and post-operative HDL levels, control group

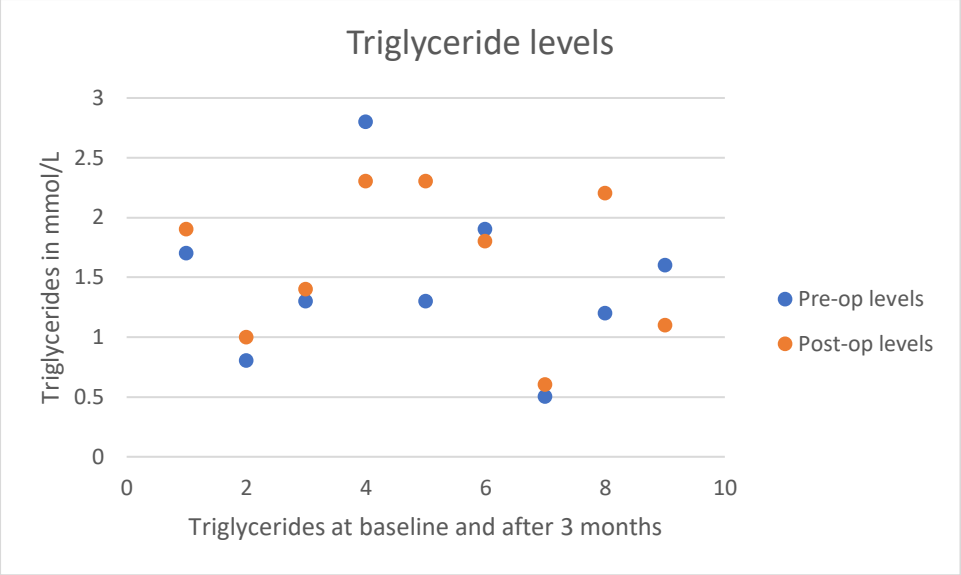

Figure 29: Pre- and post-operative triglyceride levels, control group, with 95% confidence intervals

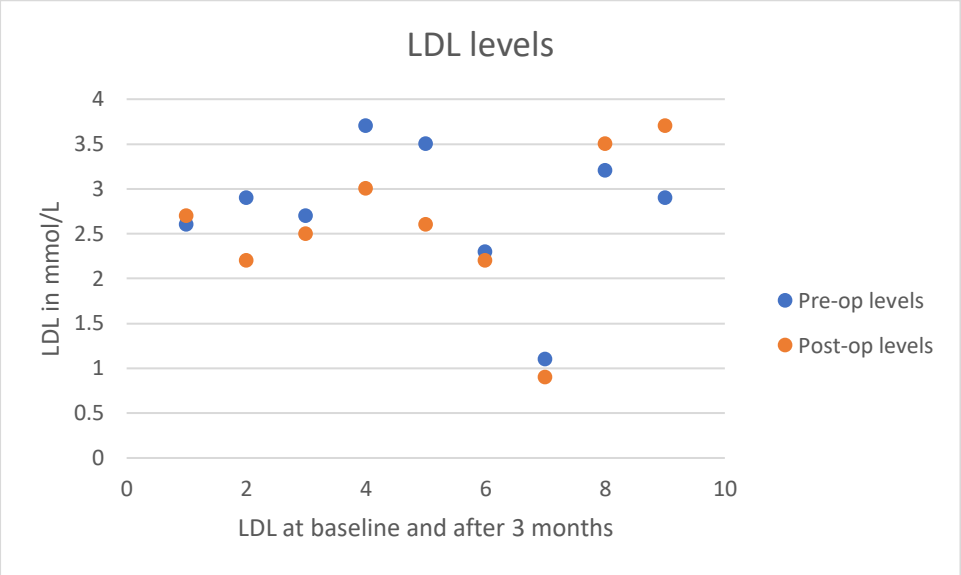

Figure 30: Pre- and post-operative LDL levels, control group

Serum results

ELISA testing for FGF19 was undertaken for serum samples. Five random samples were tested at 1:1, 1:10, 1:25, 1:50, 1:100 and 1:250 dilutions. ELISA testing was performed as described above for the gallbladder samples. However, despite repeat experiments, FGF19 was not detected in any of the samples at any concentration. Therefore plasma FGF19 levels were tested for.

Plasma FGF19 results

Due to no FGF19 being found in serum, plasma FGF19 tests were conducted at Affinity Biomarker labs in London. ELISA testing was used to analyse the samples. The results can be seen in figure 31 below.

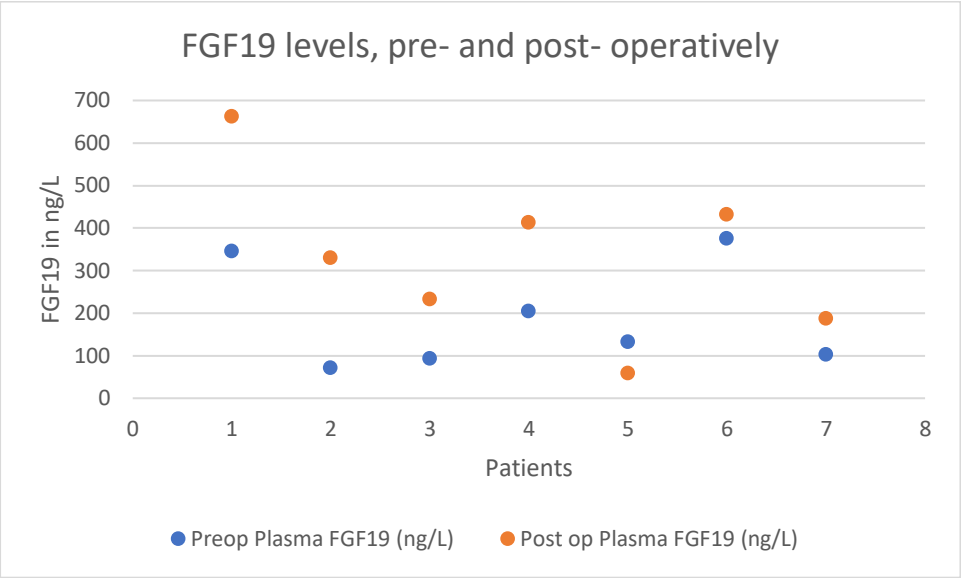

Figure 31: FGF19 plasma levels pre- and post-operatively.

There is a general trend in increasing FGF19 plasma concentration levels post-operatively. There was found to be a significant difference between the pre- and post-operative fasting plasma FGF19 levels ($p=0.043$ using a Wilcoxon signed rank test as the values were not normally distributed), with the levels being significantly higher post op. There was no correlation between the change that occurred in plasma FGF19 levels and change in stool consistency ($p=0.40$), change in bowel habit ($p=0.99$) or change in GIQLI scores ($p=0.1$)

C4 results

C4 levels were also analysed at Affinity biomarker labs using mass spectrometry. Figure 32 shows pre- and post-operative plasma C4 levels.

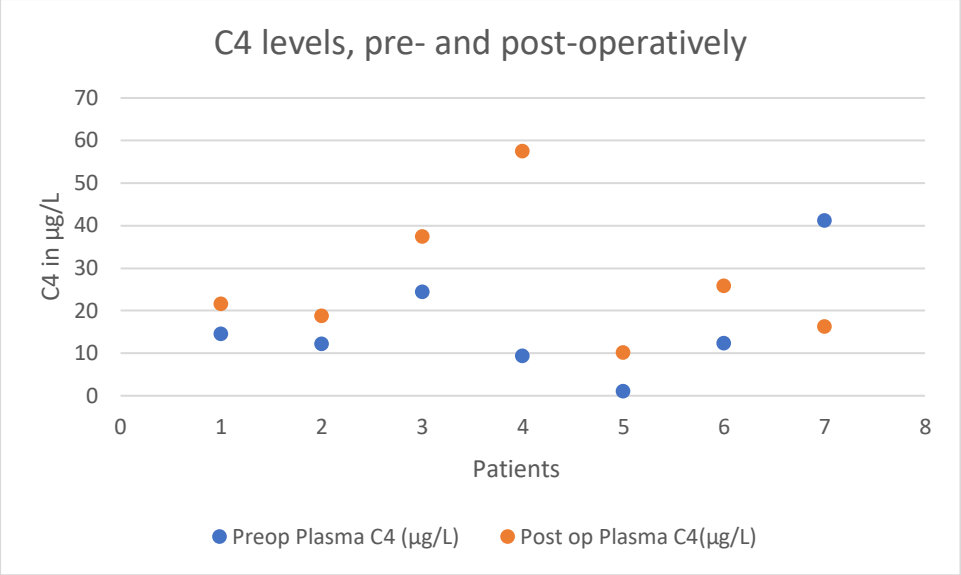

Figure 32: Pre- and post-op C4 levels in µg/L

There were no significant differences in the pre- and post-op C4 levels (p=0.18). There was also no correlation between change in C4 levels and change in bowel habit, stool consistency and GIQLI results (p=0.72, p=0.23, and p=0.071 respectively).

Relationship between change in triglyceride levels and bowel habits.

Spearman's correlation coefficient was used to examine the relationship between the triglyceride level changes and bowel habit, stool consistency and GIQLI scores of those patients in the study group who had lipid function tests. This test was chosen as the data was non-parametric.

There was no significant correlation between the change in pre- and post- op GIQLI scores and triglyceride level changes (p=0.422). This is shown in figure 33.

There was also no significant correlation between triglyceride level changes and changes in pre- and post- op bowel habit (p=0.890) (figure 34) or triglyceride level changes and stool consistency as per the Bristol stool chart (p=0.887) (figure 35).

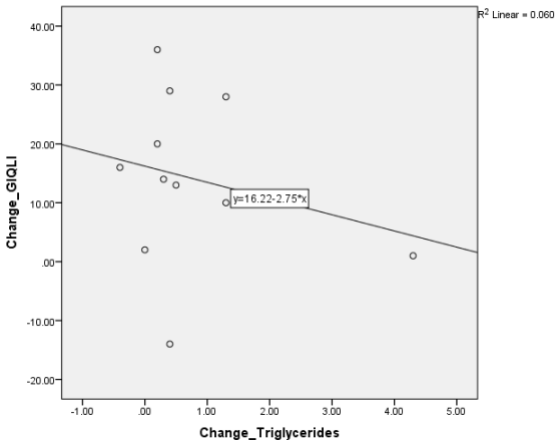

Figure 33: correlation between change in Triglyceride levels and change in GIQLI scores, study group

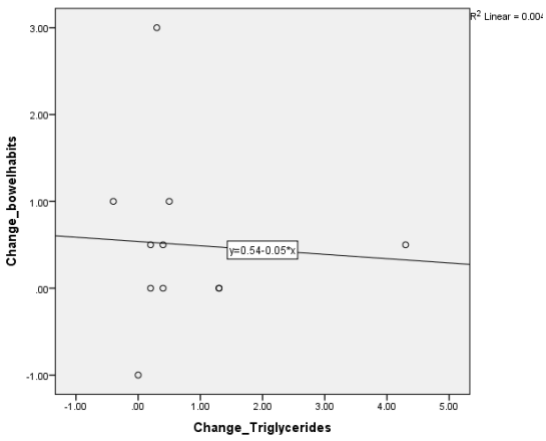

Figure 34: correlation between change in Triglyceride levels and change in bowel habits, study group

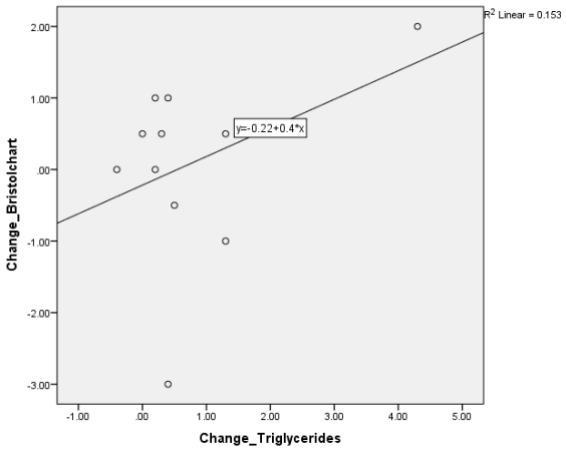

Figure 35: correlation between change in Triglyceride levels and change in stool consistency as per Bristol stool chart, study group

Discussion

It is interesting to note that triglyceride levels are higher in post-cholecystectomy patients but there is no significant difference in cholesterol, HDL and LDL levels. This is not congruent with some previous studies, which may however be explained by our

small numbers (Gill & Gupta, 2017; Malik *et al.*, 2011), though Sauter et al. also found no difference in pre- and post- cholecystectomy lipid levels (Sauter *et al.*, 2002b). Lower triglyceride levels have been described previously in post-cholecystectomy patients, again contrary to our patient cohort (Amigo *et al.*, 2011).

Primary bile acid diarrhoea has been associated with higher triglyceride levels (Appleby *et al.*, 2017). Post-cholecystectomy bile acid diarrhoea may have a similar physiology. It may be that even though these patients have not developed bile acid diarrhoea, there may have been enough of an increase in their bile acid synthesis rate that it had an effect on the serum triglyceride levels. The fact that there were no differences in triglyceride levels in the control group, who did not have their gallbladder removed, may indicate that this is happening, however the numbers in the control group were also small.

We looked into the correlation between change in triglyceride levels and change in bowel habit as well as FGF19 concentration. None of these showed any correlation. A previous study has found that up to 30% of patients with primary bile acid diarrhoea had hypertriglyceridaemia and higher FGF 19 concentrations, however no correlation was found here (Johnston IM, 2016), contrary to normal physiology where hypertriglyceridaemia leads to reduced FGF19 levels due to inhibition of ASBT expression in the terminal ileum, which therefore leads to reduced FXR activation (Renner *et al.*, 2008). This may be as despite a small increase in number stools per day after cholecystectomy none of these patients required further investigation for bile acid diarrhoea as they did not have more than three stools per day with a stool consistency of 6 or 7 on the Bristol stool chart. Our small numbers may also account for this disparity from previous studies. This may also be because the changes seen in primary bile acid diarrhoea are not necessarily seen in the post-cholecystectomy bile acid diarrhoea.

In terms of correlating lipid levels to bowel habit, some studies have demonstrated that long-chain fatty acid bacteria within the bowel contribute to increased colonic motility in rats, these being bacteria from the genii Prevotella, Lactobacillus, and Alistipes (Zhao *et al.*, 2018). These genii have already been shown to be more highly prevalent in patients with bile acid diarrhoea (Sagar *et al.*, 2018; Sagar *et al.*, 2016). Therefore,

a larger group analysis especially of patients who actually develop bile acid diarrhoea rather than a mild change in bowel habit may show some correlation.

Another interesting point to note was that the FGF19 levels were significantly higher postoperatively and there is a generally increasing trend of FGF19 levels in plasma postoperatively. The increased concentration of FGF19 could be due to some negative feedback post-gallbladder removal, as there is no longer a 'storage system' for bile acids. Thus, these are continuously being released into the small bowel and triggering more FGF19 transcription in the terminal ileum. It would be interesting to see this with larger numbers and assess whether the results obtained in this pilot project are still reflected in a larger cohort. There has been a similar study with 18 patients which did not find any difference in the FGF19 levels post-cholecystectomy however once patients were given a meal, there was a significant difference. This is interesting to note, as we found a significant difference post-operatively without the patients being given a meal (Borup *et al*. 2021).

When analysing C4 levels, there were no significant correlations to bowel habit, stool consistency and GIQLI results. There were also no significant differences in C4 levels pre- and post-op. This is interesting as C4 levels would be expected to be higher post-operatively to reflect increased bile acid production, as we have shown in figure 32, as has been previously reported (Sauter *et al.*, 2002b), though as the increase in levels was not statistically significant it is difficult to assess However they also did not report any changes associated with bowel habits. Potentially, a larger sample size could help to eliminate confounding in this regard. The study by Borup et al. also did not find any significant differences in pre- and post- operative C4 levels (Borup *et al*. 2021).

The overall increased FGF19 levels would imply a reduction in bile acid synthesis (as seen in figure 1, chapter 1) via a negative feedback loop, with FGF19 acting on FXR within the liver (Keely & Walters, 2016). However, the increased C4 levels would actually indicate an increase in bile acid synthesis(Barrera *et al.*, 2015). As there were no significant differences in stool frequency and consistency within the study group it may be that the increase in FGF19 was not enough to effect a change in the bowel habit of this population. The increase in C4 level may be explained by the increase in enterohepatic cycles post-removal of the gallbladder.

Chapter 6: No significant correlation between gallbladder FGF19 concentration and bowel habit or GIQLI.

Chapter summary

Gallbladder tissue was retrieved from the operating theatre and analysed for FGF19, SHP and PPARα. The results obtained were then correlated with the bowel habit, blood and plasma results to assess for any significant correlation.

FGF19 concentration in gallbladder tissue was assessed and then correlated with change in bowel habit, stool consistency, BMI and smoking. There were no significant results. FGF19 concentration was also correlated with triglyceride levels and there was no significant relationship found. SHP was not found in the gallbladder tissue despite attempting ELISA three times and therefore a conclusion was made that SHP is not found within gallbladder tissue.

PPARα concentration was found to have a significant relationship with stool consistency, however not with change in bowel habit or change in triglyceride levels.

Methodology

ELISA for FGF 19, SHP and PPARα

Gallbladder tissue samples were homogenized using homogenization buffer and lysis buffer and protease inhibitors.

R and D Duoset ® ELISA kits were used to detect FGF19 and SHP levels in gallbladder samples. ABCAM ELISA kit was used to detect PPARα in gallbladder samples.

Homogenisation of gallbladder samples

A solution of 2mls RIPA buffer and 18 mls of milliq H_2O was made up with two tabs of protease inhibitor. They were mixed with the help of a vortex machine and a sonicator bath and chilled on ice. A 2-4mm^2 sample of gallbladder tissue was taken from each sample and 0.6mls of the buffer solution was added to each sample. Each sample was

homogenized using a Polytron PT1200E homogenizer. The samples were centrifuged for 10 minutes at 4 degrees at 10000rpm. The supernatant was aliquoted into sample bottle and the debris discarded.

Setting up the ELISA plates - FGF 19

The capture antibody was diluted with PBS to 4micrograms/ml as per the catalogue. The capture antibody vial had 720 micrograms therefore it was calculated that the dilution concentration was 1:180. 11mls of PBS (11000 micrograms) were calculated to require 61.1micrograms of the reconstituted capture antibody. This was then pipetted into microplates and left to incubate overnight. They were then washed three times with 350microlitres of PBTS per well and blocked with 300micrograms of 1% BSA in PBS per well. This was left for one hour then washed again with PBTS. The plates were then filled with 100 microlitres of the standard and the samples as required. Two of each sample and the standards at each concentration were incubated for 2 hours. They were then washed with PBTS again. The Detection antibody was diluted to 20ng/ml as per the catalogue which made up a concentration of 1:180. Thus, once again a 61.1micrograms of the detection antibody solution were required in 11 mls of 1% BSA in PBS. The solution was made up and 100micrograms of the detection antibody were pipetted into each well with a multichannel pipette. This was left to incubate for two hours. PBTS wash was performed again. Streptavidin-HRP was diluted to 1:200 in 11mls of 1%BSA in PBS (55 microlitres in 11mls). 100microlitres were pipetted into each well and it was incubated for 20 minutes. The substrate reagent was mixed using 5.5mls of substrate reagent A and 5.5 mls of substrate reagent B. 100micrograms were pipetted per well. After 20 minutes 50micrograms of the stop solution was added to each well. The plates were read using a PheraStar plate reader immediately.

Making up the standard for ELISA – FGF 19

The FGF19 standard in the kit consisted of 27.5ng. This was made up with 0.5ml of 1% BSA in PBS to 55ng/ml. Thus, the concentration was 1:55. The working concentrations started at 1000pg/ml. Thus 1000micrograms/55 - 18.2mls of standard to acquire working concentration of 1000pg/ml. Sequential dilutions were done to get

concentrations of 500pg/ml, 250pg/ml, 125 pg/ml, 62.5pg/ml, 31.25 pg/ml and 15.7pg/ml.

Finding the concentration for gallbladder samples – FGF 19

Five random homogenised gallbladder samples were diluted to 1:10, 1:25, 1:50, 1:100 and 1:250 to assess which concentration would be best to use for the ELISA tests. When comparing against the standard, 1:25 was determined to be the ideal concentration. Thus, all the gallbladder tissue samples were subsequently diluted to 1:25.

Setting up the ELISA plates - SHP

The capture antibody was diluted with PBS to 2 micrograms/ml as per the catalogue. The capture antibody vial had 360 micrograms therefore it was calculated that the dilution concentration was 1:180. 11mls of PBS (110000micrograms) were calculated to require 61.1micrograms of the reconstituted capture antibody. This was then pipetted into microplates and left to incubate overnight. They were then washed three times with 350 microlitres of PBTS per well and blocked with 300 micrograms of 1% BSA in PBS per well. This was left for one hour then washed again with PBTS. The plates were then filled with 100 microlitres of the standard and the samples as required. Two of each sample and the standards at each concentration were incubated for 2 hours. They were then washed with PBTS again. The detection antibody was diluted to 400 ng/ml as per the catalogue which made up a concentration of 1:36. Thus, 305 micrograms of the detection antibody solution were required in 11 mls of 1% BSA in PBS. The solution was made up and 100 micrograms of the detection antibody were pipetted into each well with a multichannel pipette. This was left to incubate for two hours. PBTS wash was performed again. Streptavidin-HRP was diluted to 1:200 in 11mls of 1%BSA in PBS (55 microlitres in 11mls). 100microlitres were pipetted into each well and it was incubated for 20 minutes. The substrate reagent was mixed using 5.5mls of substrate reagent A and 5.5 mls of substrate reagent B. 100micrograms were pipetted per well. After 20 minutes 50 micrograms of the stop solution was added to each well. The plates were read immediately using a PheraStar plate reader.

Making up the standard for ELISA – SHP

The FGF19 standard in the kit consisted of 27.5ng. This was made up with 0.5ml of a diluent (IC diluent #10) made up of 50mM HEPES, 0.1mM EDTA, 0,1 mM EGTA, 0.5% NP-40 alternative and 120 mM NaCl at a pH of 7.5 as per the product catalogue to create a standard of 240 ng/ml. Thus, the concentration was 1:240. The working concentrations started at 10000pg/ml. Thus 10000micrograms/240 – 41.6mls of standard to acquire working concentration of 1000pg/ml. Sequential dilutions were made to achieve concentrations of 5000pg/ml, 2500pg/ml, 1250 pg/ml, 625pg/ml, 312.5 pg/ml and 156.25pg/ml.

Finding the concentration for gallbladder samples – SHP

Five random homogenised gallbladder samples were diluted to 1:1, 1:10, 1:25, 1:50, 1:100 and 1:250 in IC diluent #10 to assess which concentration would be best to use for the ELISA tests. This was repeated twice with no success, despite the standards working well. A final test was performed using all undiluted gallbladder samples and once again was unsuccessful despite the standards working well.

ELISA plates – PPARα

The complete transcription factor binding (CTFB) assay was prepared as per the protocol. For the 96-well plate, this consisted of 7008µlitres of water, 2400µlitres of transcription factor binding assay buffer, 96µl of reagent A and 96µl DTT. 90µl of this was placed in the sample wells along with 10µl of sample. The non-specific binding wells contained 100µl of CTFB, as were the blank wells. The positive control wells contained 90µl of CTFB with 10µl of positive control in the kit. The specific competitor dsDNA cells contained 80µl of CTFB as well as 10µl of PPAR alpha and 10µl of control. They were incubated overnight. They were washed 5 times with 200µl of wash buffer.

96µl of the primary antibody was diluted in 9504µl of antibody binding buffer and 100µl was added to each well except the blank. It was then incubated for one hour at room temperature and washed 5 times with 200µl of wash buffer. 96µl of the Goat anti-rabbit

HRP conjugate was diluted in 9504µl of antibody binding buffer and 100µl was added to each well except the blank wells. It was incubated for one hour at room temperature and washed 5 times with 200µl of wash buffer again. 100µl of developing solution was added to each well and incubated at 15-45 minutes with gentle agitation. 100µl of stop solution was added to each well being used. It was read at 450nm using the PheraStar plate reader.

Protein assays

Protein assays were performed to standardise the ELISA samples. The samples were diluted to 1:25 in 0.1M NaOH. Standards were prepared using 4mg/ml BSA. 1ml of 4mg/ml BSA was serially diluted seven times in 0.5mls of 0.1M NaOH to get concentrations of 2mg/ml, 1mg/ml, 0.5mg/ml, 0.25mg/ml, 0.125mg/ml, 62.5µg/ml and 31.25µg/ml. One additional 0.5mls 0.1M NaOH was also prepared and left blank (no BSA). Two of each standard (4 microlitres) was pipetted into wells, as well as 4 microlitres of 0.1M NaOH. 4 microlitres of two of each sample were also pipetted into wells. 200 microlitres of Bradford reagent was pipetted into each well. After 10 minutes the plates were read using PheraStar Reader, using the protocol Bradford at wavelength of 595.

Statistics

A Spearman's correlation coefficient test was then used to check for any relationship between change in plasma FGF19 levels and gallbladder FGF19 levels. This was also used to assess for any relationship between FGF19 and stool consistency, bowel habit, BMI, smoking and triglyceride levels, as well as for relationships between PPARα and bowel habit, stool consistency and triglycerides. The Spearman's correlation coefficient was used as the data was non-parametric and the variables were all continuous.

Results: Gallbladder tissue – FGF19

ELISA tests and protein assays for FGF 19 were conducted as above. The ELISA results were analysed using a software program from elisaanalysis.com. The results obtained from the program were multiplied by the dilution factor (25). The results from the protein assays were also multiplied by the dilution factor (25). Thus, FGF19

concentration (picograms per millilitre) of protein was calculated. The raw data from the ELISA tests is shown in Appendix 12.

Results: Gallbladder tissue – SHP

As described above, gallbladder tissue samples were also tested for SHP at multiple dilutions (1:1, 1:10, 1:25, 1:50, 1:100 and 1:250). The experiment was repeated twice with different gallbladder samples at these concentrations. Despite the standards working correctly each time, the gallbladder samples did not reveal a signal for SHP. The experiment was repeated using all gallbladder samples with no dilution (1:1). Once again, despite the standards showing good signal, no signal was obtained from the gallbladder samples. This implies that up to a concentration of 156.25pg/ml (the lowest concentration standard), there was no SHP within the gallbladder. The raw data is shown in Appendix 12.

Results: Gallbladder tissue – PPAR alpha

ELISA tests and protein assays for PPAR alpha were conducted as above. The ELISA results were analysed using a software program from elisaanalysis.com. The raw data from the ELISA is shown in Appendix 12.

The results obtained from the ELISA were used to determine exact concentrations of each protein using elisaanalysis.com and the final protein concentrations were used to perform statistical analysis.

Gallbladder FGF19 and bowel habit

Spearman's correlation coefficient was used to test for correlation between FGF19 concentration in gallbladder tissue and change in bowel habit and this revealed no significant correlation (p=0.124). This is illustrated in figure 36 below. Change in stool consistency was also tested for correlation with FGF19 concentration and once again this was not statistically significant (p=0.173, figure 37). Correlation with BMI and smoking status (non-smoker, ex-smoker and smoker) was also examined and these were not statistically significant, p=0.424 and p=0.523 respectively.

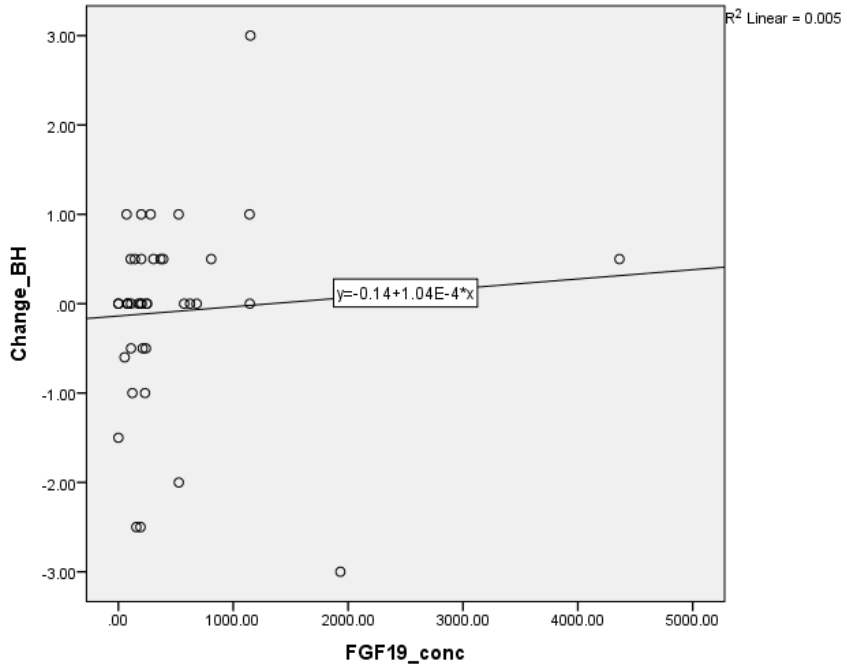

Figure 36: FGF19 correlation to change in bowel habit

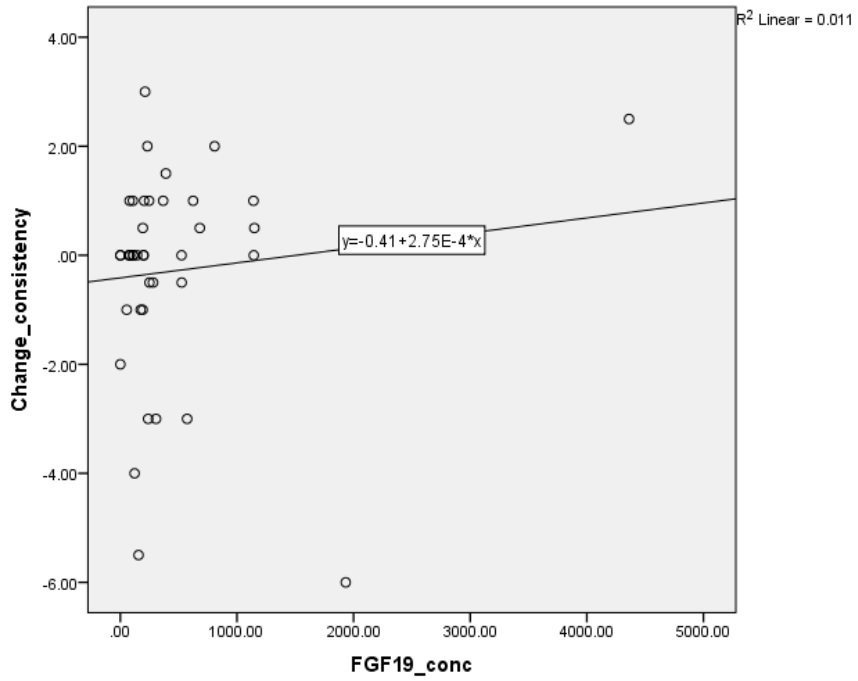

Figure 37: FGF19 correlation to change in stool consistency

Relationship between change in triglyceride levels and FGF19 levels.

The mean concentration of FGF 19 in pg/ml was also correlated to the change in triglyceride levels using Spearman's correlation coefficient. There was no significant correlation between the two variables (p=0.581). This is shown in figure 38 below.

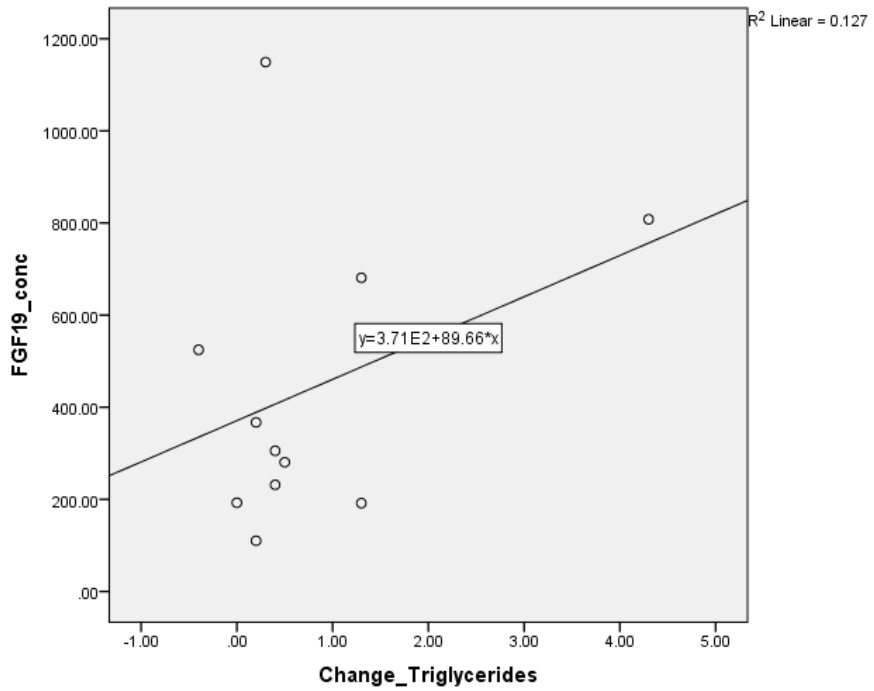

Figure 38: Correlation between change in triglyceride levels and FGF19 concentration

PPAR alpha concentration and bowel habits

Spearman's correlation coefficient was used to test for correlation between PPARα concentration and change in bowel habit, and there was no significant correlation (p=0.12). This is shown in Figure 39. However, there was a significant correlation with change in stool consistency (p=0.003), shown in figure 40, showing a lower concentration with higher Bristol stool chart value (looser stool).

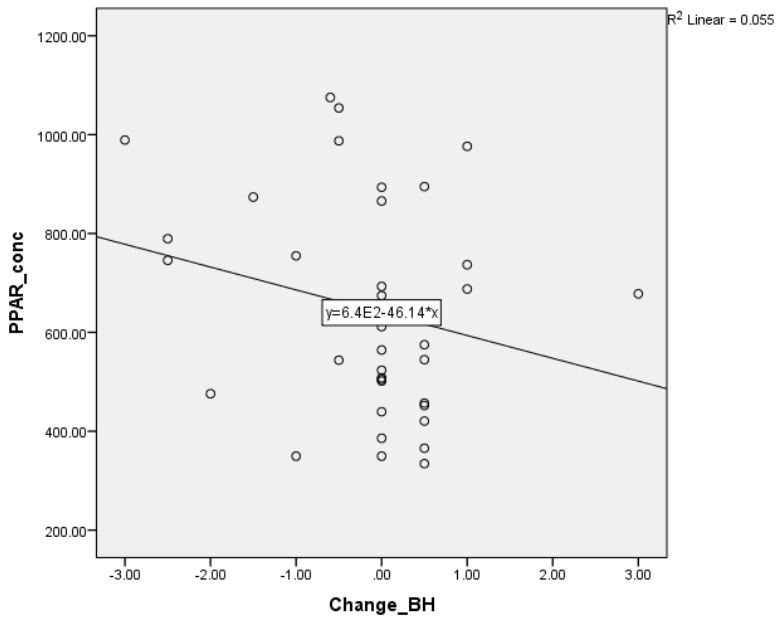

Figure 39: Correlation of PPARα concentration and change in bowel habit.

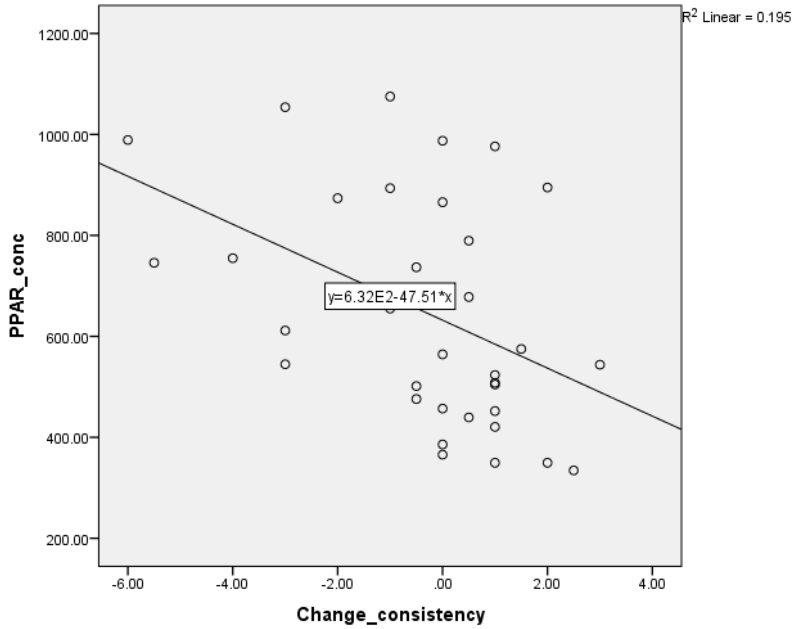

Figure 40: Correlation of PPARα concentration and change in stool consistency.

PPAR alpha concentration was also correlated to change in triglyceride levels pre- and post-operatively, again using Spearman's correlation coefficient. Once again there was no significant correlation between PPAR alpha concentration and change in triglyceride concentration (p=0.748). This is shown in figure 41 below.

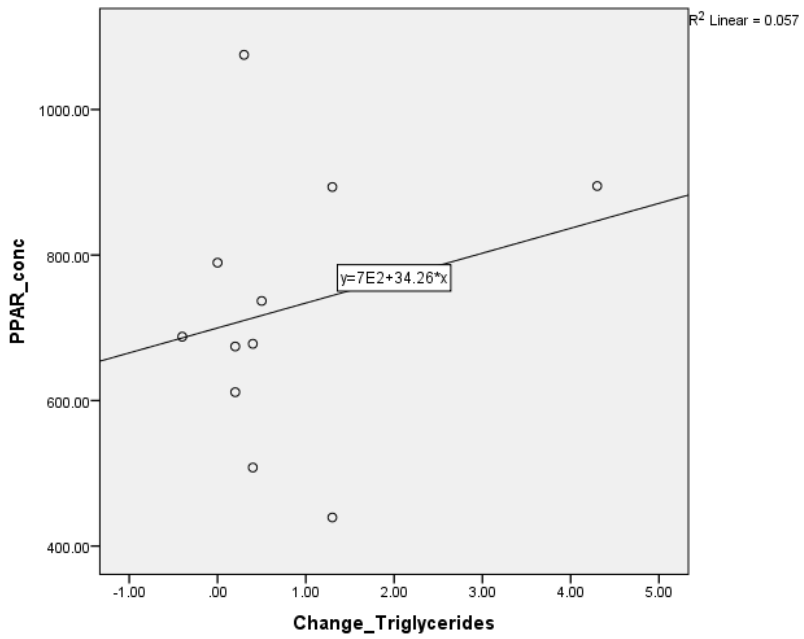

Figure 41: Correlation of PPARα concentration with change in triglyceride levels

Correlation between Plasma FGF19 concentration and gallbladder FGF19 concentration

A Spearman's correlation coefficient was used to analyse whether there was a relationship between the change in plasma FGF19 levels and the gallbladder FGF19 concentration. This was found to be negative (p=0.65). The values are shown in table 10.

Patient code	Preop Plasma FGF19 in ng/L	Post op plasma FGF19 in ng/L	Change in plasma FGF19 in ng/L	Gallbladder FGF19 in pg/mg
BCLC001	344.26	661.97	317.71	280.49
BCLC003	69.79	329.53	259.74	681
BCLC004	92.44	231.40	138.96	808.02
BCLC006	204.10	412.15	208.05	192.73
BCLC007	57.36	131.43	74.07	191.65
BCLC010	374.09	430.84	56.75	524.73
BCLC015	101.22	186.90	85.68	305.53

Table 10: Plasma FGF19 pre- and post-op; and gallbladder tissue FGF19 concentration

Discussion

FGF19

FGF19 is synthesized in the terminal ileum due to absorption of bile acids, and through the portal circulation acts on hepatic FXR to decrease bile acid synthesis as part of a negative feedback loop (Walters & Appleby, 2015). While FGF19 is present within the gallbladder, its function within this organ is unknown. We postulated that since FGF19 is also secreted by the gallbladder, a higher FGF19 concentration in the gallbladder may result in the development of bile acid diarrhoea once it is removed due to a potential role in this negative feedback loop (Zweers *et al.*, 2012). However, there were no significant correlations between FGF19 concentration within the gallbladder and the changes in bowel habits exhibited by the patients. This may imply either that the FGF19 level secreted by the gallbladder are not high enough to be an effective part of the negative feedback loop, or that the feedback loop is interrupted at a level downstream from FGF19 when bile acid diarrhoea develops. It may also imply that the FGF 19 from the gallbladder is not related to the negative feedback loop at all. This may explain also why there is no correlation between the plasma FGF19 concentration levels and gallbladder FGF19 concentration levels.

Unfortunately, no patients consented for liver biopsy as it would have been possible to measure FXR levels and differences between patients who develop BAD and those who don't as well as correlate and changes in bowel habit or stool consistency.

Obesity has been shown to increase the risk of bile acid diarrhoea, however there was no correlation in our study (Camilleri *et al.*, 2017; Sadik *et al.*, 2004). We did not find any significant differences in FGF19 concentration with change in bowel habit, change in stool consistency, smoking status or BMI.

SHP

The role of SHP within the bile acid synthesis loop is to act with FXR on CYP7A1 to decrease bile acid synthesis (Zhou & Hylemon, 2014). It also acts on LXR to decrease SREBP-1 stimulated lipogenesis therefore decreasing triglyceride concentration. Thus, we wanted to investigate whether this is present within the gallbladder as there is no published literature on this subject, though we know it is present in the liver

(Zhang *et al.*, 2018), and if it is found within the gallbladder, then does it have a role in the development of post-cholecystectomy diarrhoea. ELISA tests for SHP were performed on multiple gallbladder samples at different concentrations and no SHP was found within the gallbladder tissue. This implies that SHP has no direct role in the development of post-cholecystectomy bile acid diarrhoea. It would have been interesting to see whether there was a difference in SHP concentrations in the liver of patients who developed bile acid diarrhoea and those who did not. However unfortunately of all the patients approached, none consented for a liver biopsy during cholecystectomy. Other patients were not approached for this as it could only be performed in the main operating theatre of the hospital. Laparoscopic cholecystectomy is frequently performed in the day surgery unit and this is an area that is not equipped for liver surgery should there be complications arising from taking the liver biopsy. Other patients were not approached as liver biopsy could only be undertaken by a consultant hepatobiliary surgeon as per the ethical approval. However, many of these operative procedures were performed by upper gastrointestinal surgeons who do not routinely operate on the liver. With the liver biopsy it would also have been possible to correlate SHP level with the FGF19 and FXR levels as we believe these to be inter-related and involved in the bile acid synthesis cycle.

PPARα

PPARα is involved in the regulation of lipid levels by regulating fatty acid metabolism once it is activated by FXR. It decreases hepatic apo C-III production and increases LPL-mediated lipolysis which then increases triglyceride metabolism and decreases LDL secretion. This causes increased free fatty acid oxidation and decreasing serum triglyceride levels (Amigo *et al.*, 2011; Ferrebee & Dawson, 2015). Thus, we investigated the effect of gallbladder PPARα on bowel habits as well as on triglyceride levels. We have shown that there was no correlation of PPARα concentration within the gallbladder with the change in triglyceride levels post-operatively. However, when taking the whole group into account, there was a significant correlation between PPARα concentration levels and change in stool consistency postoperatively, though this was not reflected in the change in bowel habits. This correlation with change in stool consistency may be a reflection of the interruption of the bile acid synthesis loop where there are higher FXR levels leading to more PPARα activation, with the

interruption of the negative feedback loop coming later in the pathway thus leading to higher bile acid synthesis rates (rather than lower synthesis rates as it should be with higher FXR concentrations).

Chapter 7: Discussion and Conclusion

We started this project with a systematic review of the literature pertaining to post-cholecystectomy diarrhoea as well as a review of the literature available regarding the pathophysiology of BAD. Our systematic review has shown that there is a 13.3% rate of post-cholecystectomy diarrhoea, however we were not able to elicit any concrete conclusions regarding possible predictive factors as the evidence was conflicting.

We then proceeded to perform a local audit regarding post-cholecystectomy diarrhoea which found that only 1.4% of the population was investigated for BAD and of these 59% were positive. We also found a long time from surgery to investigation with women being tested significantly later than men (^{75}SeHCAT, p=0.006). We then extended this audit nationally and five centres were involved in the final analysis. We found that 2.1% of patients post-cholecystectomy were investigated for diarrhoea, much less than the numbers that our systematic review suggested. Despite a long time from surgery to investigation, there were no significant differences between investigation times for men and women within this part of the study.

Recruitment and follow up for our case-control study was highly impacted by COVID-19. However, we managed to recruit 40 patients for the study group and 20 for the control group. Both groups showed a significant improvement in quality of life, via the GIQLI questionnaire, in the post-operative period. In the study group there were no significant changes in the stool frequency and consistency when comparing and the pre- and post-operative period, however the control group showed an increased stool frequency. Plasma FGF19 levels and C4 levels both increased post-operatively in the study group.

There were no significant results when assessing the effect of gallbladder FGF19 concentration on bowel habit, stool consistency, lipid levels, BMI or smoking. Gallbladder PPAR α was found to have a significant correlation with stool consistency, with the lower the PPARα concentration the higher the Bristol stool chart number (i.e. looser stool).

The study group showed a significant increase in triglycerides post-operatively, however there were no changes in cholesterol, HDL and LDL levels. Correlation of

these increased triglyceride levels and GIQLI, stool consistency and bowel habits showed no significant results.

In our review of the literature available regarding post-cholecystectomy diarrhoea in Chapter one, we found an overall rate of 13.3% of patients who are likely to suffer from PCD. The rest of the literature is highly variable, with rates reported ranging from 2.1% to 57.2% (Fisher *et al.*, 2008; Fort *et al.*, 1996; Lamberts *et al.*, 2013b; Luman *et al.*, 1996). The number of patients investigated for BAD after cholecystectomy as seen by our multicentre audit, is 2.14%, much lower than our review of the literature would suggest. This implies that though not all these patients may have BAD specifically, as we know that approximately two thirds of patients with post cholecystectomy diarrhoea have BAD, there are a large number of patients who may be suffering from a potentially controllable condition which could be significantly affecting their quality of life(Walters *et al.*, 2017). There is also a potential lack of awareness regarding PCD and a number of patients are not consented for this possibility pre-operatively(Hussain *et al.*, 2016), implying that patients may not correlate the onset of this symptom with their surgery.

We have also shown a delay between surgery and investigation, which may mean that patients are finding it difficult to access care for this condition. Another possible issue is that despite the BSG guidelines advising endoscopy (colonoscopy) and ^{75}SeHCAT for the investigation of chronic diarrhoea, a large number of these patients were also investigated via a CT scan which is not usually the best investigation to visualise bowel(Arasaradnam *et al.*, 2018). Another potential confounding factor is that all these patients should have endoscopy, again as per BSG guidelines, to exclude inflammatory bowel disease (IBD, such as Crohn's disease or Ulcerative colitis). However, only 65.8% of patients had endoscopy thus IBD was not fully excluded in all patients.

The GIQLI results in the case control study are encouraging in that there is a general tend in improvement in overall quality of life post-operatively in all patients, both study group and control group. This is shown in both gastrointestinal symptom-specific part of the questionnaire as well as the general quality of life part of the questionnaire. 10 patients related that in the questionnaire that they had more frequent bowel motions and uncontrolled stools after surgery, however when comparing their own reported

bowel habits (number of bowel motions per day and description of bowel motion as per the Bristol stool chart) there were 6 patients in whom this increase was not confirmed. The other four were the patients who were referred on to further testing. Thus, as in all patient-reported questionnaires, there may be an element of recall bias in these results. This is a limitation in this study and potentially could be avoided by asking patients to keep accurate prospective diaries of their bowel habit.

When comparing stool frequency and consistency pre-and post-operatively in the study group, despite 13 patients having looser stools and 13 (different) patients having more frequent stools not all these patients had enough to warrant further testing for bile acid diarrhoea. The control group patients also reported higher stool frequency postoperatively but once again this did not meet the criteria of three or more bowel motions per day and therefore did not require further investigations.

We did not find any evidence that gallbladder FGF19 has a direct role in the development of bile acid diarrhoea after cholecystectomy, as there were no significant differences in gallbladder FGF19 concentrations when compared with bowel habit changes (frequency and consistency), BMI, smoking status, GIQLI results or plasma FGF19 levels. SHP was not found within the gallbladder tissue on ELISA testing and therefore we concluded that it has no role in the development of BAD after cholecystectomy.

We found a significant difference in postoperative triglyceride levels in the study group and we postulated that this could be due to involvement of PPAR alpha which is involved in the regulation of lipids. As PPARα is found within the gallbladder we decided to investigate whether there was a relationship between this and the change in triglyceride levels (van Raalte *et al.*, 2004). It is also known that patients with primary BAD are more likely to have hypertriglyceridaemia (Sagar NM, 2016). However, we did not find a significant relationship between the increase in triglyceride levels postoperatively and PPARα concentration levels within the gallbladder. One significant limitation is that we had a very small number of participants who contributed with lipid levels and therefore this is an experiment that may be worth repeating in a larger cohort.

When it comes to blood results, despite the increase in triglyceride levels post-cholecystectomy, there were no other significant increases in the other parts of the lipid profile (HDL, LDL and cholesterol). This could once again be due to our small numbers as there have been some studies which show significant differences postoperatively (Gill & Gupta, 2017; Malik *et al.*, 2011; Johnston *et al* 2016). This is interesting as idiopathic BAD has been associated with hypertriglyceridaemia, and we postulated that this might also be the case in patients where BAD develops post-cholecystectomy. It may be that even though these patients did not all develop bile acid diarrhoea, there may have been enough of an increase in their bile acid synthesis rate that it had an effect on the serum triglyceride levels. We did not find any correlation between triglyceride levels and gallbladder FGF19 concentration. However, we did find that plasma FGF19 levels are significantly higher post-operatively and are associated with stool frequency, though not consistency.

This study has shown that post-cholecystectomy diarrhoea is more common than suspected, however investigations for the condition are often delayed. We now know that FGF19 concentration within the gallbladder is unlikely to have an effect on the development of post-cholecystectomy bile acid diarrhoea, or any other bowel habit changes. Plasma FGF19 levels are higher and associated with increased stool frequency, thus may represent the increase in enterohepatic cycling and negative feedback on the bile acid production loop. We know that triglyceride levels do increase post-operatively in cholecystectomy patients and we also know that triglyceride levels in patients with BAD are higher so this is still an avenue to explore despite not finding any correlation with PPARα within the gallbladder, as PPARα in the liver could still be involved.

Some limitations of this work include the subjective nature of some of the data collection, especially when concerning bowel habits and other symptoms. One mitigating factor could have been to ask patient to record every single bowel movement for a period of time pre- and post-surgery and the consistency as per the Bristol stool chart to ensure accurate data rather than that based on recollection. Another limitation is the fact that the blood samples were collected at different times of day depending on what was convenient for the patients. However, the diurnal variation in plasma FGF19 and C4 levels could mean that this introduces a

confounding factor in our study. This could be mitigated by ensuring all blood tests are collected at the same time.

Further work needs to be done especially concerning the gut microbiome and changes post-cholecystectomy. There is convincing preliminary evidence that it may be involved in the development of bile acid diarrhoea. While the initial plan for this study was to also collect stool samples, this was quickly abandoned as patients were reluctant to take part in this part of the study. Most patients undergoing laparoscopic cholecystectomy are young and they stated work and childcare commitments as being the reasons why they were unwilling to return to hospital for blood tests and stool samples, however they were happy to take part in the post-operative questionnaire as this could be done over the phone. Thus, comparing the gut microbiome pre- and post- laparoscopic cholecystectomy with further comparison to bowel habits and stool consistency could yield some answers regarding whether there are changes in the microbiome of post-cholecystectomy patients developing BAD. This could be performed as a cohort study comparing post-cholecystectomy microbiome to pre-cholecystectomy as well as a case-control study comparing the microbiome of patients having had cholecystectomy to those who did not. Differences in the microbiome could lead to further conclusions regarding the possible aetiology of BAD.

The subset of patients who were able to return for blood tests was also small. Unfortunately, there were those who were willing to return for blood tests who were unable to do so as the COVID19 pandemic occurred around this time and therefore hospital visits which were not vital for care (such as research visits) were cancelled to avoid exposure. Thus, these patients did fill in the questionnaire over the telephone but were told not to come in for blood tests for their safety. Another sequelae of COVID was that it affected the recruitment of both study and control groups, as well as closure of the university laboratories thus delaying testing of samples. Another possibility to improve the data obtained would be to repeat the experiments with larger cohorts. As a pilot study this study gave some indications that there are changes in triglyceride levels after surgery, as well and general increased of plasma FGF19 levels, however the significance of these results can be further elaborated in larger cohorts using this study as a basis. Again, further studies such as case-controls could be used to investigate the role of plasma FGF19 further, with all patients pre- and post-

cholecystectomy having FGF19 plasma levels taken to compare with bowel habits. A ^{75}SeHCAT test should also be performed on all these patients to assess their degree of bile acid retention.

Further work can also be done on biomarkers in the liver which may affect the development of bile acid diarrhoea, such as SHP which was eventually not found to be present in the gallbladder. Thus, experiments of SHP levels within the liver could be useful in assessing whether the FXR-FGF19 feedback loops are in play in the development of post-cholecystectomy BAD since SHP is involved in the inhibition of CYP7A1 when it is activated by FXR. Other liver biomarkers that could be looked into would be liver FGF19 levels, as this could be correlated with plasma FGF-19 and levels could be used to investigate whether they are involved in BAD development. As we found an increase in triglyceride levels but no real correlation with gallbladder PPARα, another possible experiment would be to assess whether liver PPARα could be involved in the changes in triglyceride level changes postoperatively, and once again assess whether these are related to the development of BAD. In this case a liver biopsy would need to be taken in such a study and could be done intra-operatively, and once again a cohort of patients having cholecystectomy could be compared to a cohort not having cholecystectomy. In this case due to the need to assess bowel habit it is important that the patients not having cholecystectomy are not a bowel resection group as this would also impact the bowel habit.

There is an upcoming NICE guideline due to be published in November 2021 which states that there is not enough evidence to recommend routine use of ^{75}SeHCAT to diagnose BAD in patients with chronic diarrhoea or diarrhoea-predominant IBS, and that this should be only used in trial settings (NICE, 2021). This is in direct contrast with the guidance provided by the British Society of Gastroenterology which recommends ^{75}SeHCAT as a first-line test in secondary care in patients with chronic diarrhoea and grades the recommendation as ‘strong’ (grade 1) evidence (Arasaradnam, 2018). The decision is based on four papers published between 1985 and 1987 investigating the dose-response relationship between ^{75}SeHCAT results of patients taking bile acid sequestrants. There is also a systematic review which pools 15 studies and concludes that there is a dose-response relationship between ^{75}SeHCAT retention and response to colestyramine, which is one of the bile acid

sequestrants used, which has not been quoted as evidence in the upcoming guidance (Wedlake *et al.*, 2009). Also, the sensitivity of ^{75}SeHCAT testing is 96% with a specificity of 100% at 7 days (Sciarretta *et al.*, 1986) which is higher than C4 which has a sensitivity of 90% and specificity of 79% (Sauter *et al.*, 2002). As a comparator to ^{75}SeHCAT the authors chose to use trial of bile acid sequestrants which is not a recommended course of action (Orekoya *et al.*, 2015; Farrugia and Arasaradnam, 2020). The decision to exclude ^{75}SeHCAT from the investigation of chronic diarrhoea from these guidelines does not seem based on full facts.

We have performed a pilot, human study investigating the pathophysiology of bile acid diarrhoea after cholecystectomy which may form the basis of multiple larger studies in the future, including those investigating the gut microbiome and relationship with cholecystectomy and the role of biomarkers such as FGF19 or PPARα in the development of BAD.

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Appendices

Appendix 1: Publication Bile acid diarrhoea: pathophysiology, diagnosis and management

REVIEW

Bile acid diarrhoea: pathophysiology, diagnosis and management

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ABSTRACT

The actual incidence of bile acid diarrhoea (BAD) is unknown, however, there is increasing evidence that it is misdiagnosed in up to 30% with diarrhoea-predominant patients with irritable bowel syndrome. Besides this, it may also occur following cholecystectomy, infectious diarrhoea and pelvic chemoradiotherapy.

BAD may result from either hepatic overproduction of bile acids or their malabsorption in the terminal ileum. It can result in symptoms such as bowel frequency, urgency, nocturnal defecation, excessive flatulence, abdominal pain and incontinence of stool.

Bile acid synthesis is regulated by negative feedback loops related to the enterohepatic circulation, which are dependent on the farnesoid X receptor and fibroblast growth factor 19. Interruption of these feedback loops is thought to cause bile acid overproduction leading to BAD. This process may occur idiopathically or following a specific trigger such as cholecystectomy. There may also be an interplay with the gut microbiota, which has been reported to be significantly different in patients with severe BAD.

Patients with suspected BAD are investigated in various ways including radionucleotide imaging such as SeHCAT scans (though this is not available worldwide) and blood tests. However, other methods such as bile acid measurement in stool (either spot test or 48 hours samples) and urine tests have been explored. Importantly, delay in diagnosis and treatment of BAD greatly affects patient's quality of life and may double the overall cost of diagnosis.

Key points

- Idiopathic bile acid diarrhoea (BAD) is due to overproduction of bile acids (rather than malabsorption).
- The negative feedback loops involved in bile acid synthesis are interrupted in BAD but there is lack of data regarding what causes the interruption.
- There is increasing evidence of an interplay between the gut microbiota, farnesoid X receptor and fibroblast growth factor 19 in BAD.
- Tests for BAD such as SeHCAT are not available worldwide but alternatives include plasma C4 testing and possibly faecal bile acid measurement.
- Idiopathic BAD is due to overproduction of bile acids (rather than malabsorption).

INTRODUCTION

Bile acid diarrhoea (BAD), sometimes also known as bile acid malabsorption or bile salt malabsorption (though this is not always the correct terminology), can result in symptoms such as bowel frequency, urgency, nocturnal defecation, excessive flatulence, abdominal pain and incontinence of stool.[1] This phenomenon was first described in 1967 and was initially known as choleric enteropathy.[2] It has since been classified primary BAD which is idiopathic and is usually secondary to hepatic overproduction of bile acids due to interruption of the negative feedback loop rather than malabsorption in the ileum.[3] Secondary BAD is secondary to a terminal ileum resection or occurs in conditions such as Crohn's disease or postradiation where the terminal ileum reabsorbs bile acids as part of the enterohepatic circulation, thus being a true malabsorption syndrome.

Emerging evidence over the last decade has shown that BAD is not as uncommon as previously perceived. Up to 30% of patients with diarrhoea-predominant irritable bowel syndrome (IBS) have evidence of BAD as determined by ^{75}SeHCAT testing.[4] Compared with controls, patients with IBS had lower ^{75}SeHCAT values and higher C4 levels but similar fibroblast growth factor 19 (FGF-19) levels and more than 50% responded to bile acid sequestrant (colestipol).[5] In addition to patients with ileal disease (eg, Crohn's disease and right hemicolectomy

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Table 1 Causes of bile acid diarrhoea

Hepatic overproduction	True malabsorption
Idiopathic	Crohn's disease
Postcholecystectomy	Right hemicolectomy
Irritable bowel syndrome-diarrhoea predominant type	Enteropathy (such as HIV (human immunodeficiency syndrome)
Pancreatic insufficiency	Pelvic radiation
	Bariatric surgery
	Microscopic colitis
	Small bowel bacterial overgrowth

where the terminal ileum is resected or HIV causing enteropathy),[6 7] BAD has also been reported in those following cholecystectomy,[8] and those with postinfectious diarrhoea,[9] as well as patients having metformin[10] and those with pancreatic insufficiency[11] (table 1). For those not responding to treatment, other additional causes should be sought, for example, bacterial overgrowth, pancreatic insufficiency or microscopic colitis,[12] even if ^{75}SeHCAT testing has been abnormal. Another under recognised group are those with cancer especially those receiving pelvic chemoradiotherapy as >50% have BAD.[13]

Bile acids are vital in the digestion and absorption of fat. They are synthesised from cholesterol in the liver and excreted in bile as primary bile acids (PBA) (cholic and chenodeoxycholic acids). Dehydroxylation then occurs to form lithocolic and deoxycholic acid and these are reabsorbed via the enterohepatic circulation. There is a negative feedback process regulating this, working through the nuclear farnesoid X receptor (FXR) and FGF19. Disruption of this process results in excessive bile acid loss to the colon which, among other factors are contributory to symptoms of diarrhoea.

PATHOPHYSIOLOGY

Bile acid synthesis and enterohepatic circulation

Bile acids are synthesised in the liver from cholesterol into PBA (cholic acid (CA) and chenodeoxycholic acid (CDA)) by means of CYP7A1 (cholesterol 7α hydroxylase) enzyme. Some are converted to lithocholic and deoxycholic acid by means of 7α hydroxylase produced by intestinal bacteria and are then expressed into the duodenum to aid in digestion of fatty acids.[14] The latter is achieved by emulsification and formation of micelles. The diameter of the micelles is 4–5 nm which allows them pass into the intermicrovillous spaces and reach the epithleial cells to allow for absorption.[15] Approximately 95% of bile acids are reabsorbed from the ileum per cycle and the amount of cycles per day tends to vary between individuals, though it is estimated that bile acids actually undergo enterohepatic cycling about five times per day.[16 17] The cumulative daily loss of bile acids in faeces daily is 200–400 mg, however, the total bile acid pool is between 3 and 4 g.[18] The enterohepatic cycling process ensures that there are sufficient quantities of bile acids to handle dietary fat. Remarkably this process still remains efficient in most individuals even with the onslaught of high fat within the modern diet. Bile acid production may also be stimulated by dietary intake, such as ingestion of long-chain triglycerides possibly through FXR receptor.[19 20]

Negative feedback loops

Bile acids in the terminal ileum are absorbed via apical sodium-dependent bile acid transporter to activate ileal FXR, which induces transcription of FGF19. This is then released into the portal circulation and travels to the liver to activate hepatic FXR which acts on CYP7A1 via short heterodimer primer, thus decreasing bile acid synthesis.[21] FGF 19 also binds to FGF receptor 4 (FGFR4) in the hepatocytes, which interacts with β-klotho (KLB) to inhibit CYP7A1 leading to a further decrease in bile acid synthesis via the classical pathway and activating hepatocyte FXR.[22 23] Production of FGF19, therefore, inhibits BA synthesis by these two negative feedback loops.[24] These processes are shown in figure 1.

Bile acid diarrhoea

The mechanism behind primary BAD relates to the negative feedback mechanism in the rate-limiting step catalysed by CYP7A1. When the negative feedback mechanism is disrupted (potentially due to impaired FGF19 signalling), as occurs in BAD, the activity of CYP7A1 is increased with resultant sixfold to sevenfold increase in the synthesis of bile acids.[25] Bile acids have been shown to induce fluid secretion and increase mucosal permeability in the colon, cause high amplitude propagated contractions.[26–28]

Putative mechanisms of symptoms in BAD

Patients with BAD usually present with diarrhoea, which may be persistent or intermittent, frequency, urgency, flatulence, abdominal pain, octurnal defecation and even faecal incontinence.[1] There is no actual malabsorption in primary BAD, unlike that occurring due to terminal ileal disease such as Crohn's. Rather, there is hepatic overproduction of bile acids due to interruption of the negative feedback loop regulating bile acid synthesis, resulting in a larger than normal proportion of bile acids entering the colon and exerting its effects.[3] Beyond this the reason behind the development of BAD has not yet been determined, such as where the disruption of this negative feedback loop occurs. There has been a suggestion that there are different phenotypes of BAD, including possible associations in patients with familial hypertriglyceridaemia as well as potential functional genetic variation in the receptors such as FGFR4 and β-klotho.[29]

It is known that the symptoms of BAD occur mainly due to the entry of a higher concentration of bile acids into the colon. Mekhjian *et al* proposed the theory of

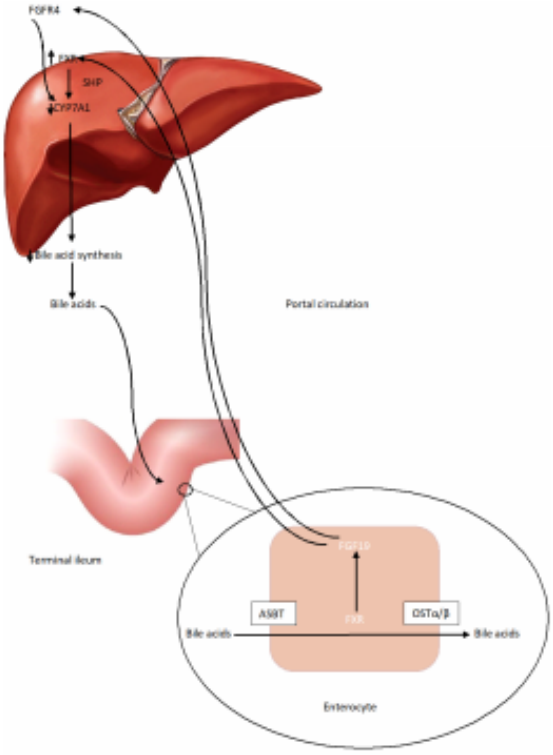

Figure 1 Enterohepatic circulation: Bile acids activate transcription of FGF19 which then acts on hepatic FXR to inhibit bile acid synthesis via CYP7A1. Bile acids are also released into the portal circulation via organic solute transporter (OST)α/β. They are transported back to the liver where they bind with FGFR4 to interact with β-klotho to decrease bile acid synthesis via CYP7A1. ASBT, apical sodium-dependent bile acid transporter; FGF19, fibroblast growth factor 19; FGFR4, FGF receptor 4; FXR, farnesoid X receptor; SHP, short heterodimer primer.

induction of intra-luminal secretion of sodium and water.[27] Others have proposed bile acids stimulating the colonic motility and defecatory reflex, and resultant mucosal damage leading to increased permeability[30]

There have been studies investigating bowel transit and its association with BAD. Some have suggested an accelerated transit both in the small bowel and in the distal colon.[31] However, other studies suggest no association.[32] Overall there is little evidence to support claims that symptoms of diarrhoea in BAD are due to changes in intestinal transit, except perhaps for a genetic variation associated with TGR5, a g-protien coupled receptor, (acting on intestinal motility) which increases transit times.[33]

Gut microbiome

Gut microbiota affect bile acids by causing deconjugation, dehydrogenation and dihydroxylation of PBA in the distal small intestine and colon. This process causes a change in the bile acid pool composition therefore activating FXR and thus inhibiting bile acid synthesis. However, it is not known whether a change in the gut microbiota has any effect on symptoms of diarrhoea. The amount of secondary bile acids (mainly DCA) in the bile acid pool depends on the rate of formation and absorption via the colon, the colonic transit time and colonic pH. There has been a correlation between high DCA levels and gallstones.[34]

Gut microbiome is involved in the digestion of complex carbohydrates to form short chain fatty acids (SCFA), such as acetate, butyrate and proprionate.[35] As both the levels of faecal bile acids in the colon and levels of SCFA are dependent on gut microbiota, any changes in faecal bile acids may affect SCFA and in turn effect the presence of diarrhoea. Patients with BAD also have a higher proportion of primary faecal bile acids, potentially due to decreased *Bifidobacteria* and *Leptum* species as well as an increased *Escherichia coli* in their gut microbiota. This may change the affinity of BAs to FXR and TGR5, thus leading to decreased FXR activation and to increased delivery of bile acids to the colon.[36] Wang *et al*,[37] have shown an increase in *Bifidobacteria* abundence with concurrent decrease in secondary faecal bile acids and resultant increase in acetate and proprionate levels. The relationship between the increase of SCFA with the decrease in total faecal bile acids has not been explored. The gut microbiome is also heavily involved in the lipid metabolism and absorption, and thus, dysbiosis may affect the interplay between lipids and bile acid synthesis.[38]

There have also been studies investigating the differences in faecal microbiota of different gut conditions. The faecal microbiota of patients with severe BAD (<5%) is significantly different to that of patients with diarrhoea secondary to IBS and that of patients with less severe forms of BAD.[39–41]

DIAGNOSTIC METHODS

There are several methods by which BAD can be diagnosed, all of varying reliability outlined in table 2.

Nuclear medicine

The BSG guidelines state that patients with chronic diarrhoea should all be investigated to exclude BAD either with a ^{75}SeHCAT scan where available or C4 given current evidence base.[42] ^{75}SeHCAT (Selenium-75 homocholic acid taurine test), first described in 1982, is used to determine the amount of bile acids retained after 7 days.[43 44] The National Institute for Health and Care Excellence diagnostic guidance report on ^{75}SeHCAT in 2012 stated that given the prevalence of undiagnosed BAD, there is potential for patient and system benefits associated with ^{75}SeHCAT investigations.[45] The report also suggested that insufficient evidence exists to determine its cost-effectiveness and recommended further research to evaluate this technology and effects of treatment[46] Its 2016 review, made no changes in light of lack of new evidence on ^{75}SeHCATs comparative diagnostic accuracy and

Table 2 Comparison of diagnostic methods of bile acid diarrhoea (BAD)

Diagnostic method	Favourable points	Limitations
^{75}SeHCAT	Well established Predicts response to treatment	Involves radiation Limited availability in certain countries for example, unavailable in USA
C4	No radiation Simple	Diurnal variation Fasting sample Not widely available (in the UK as a research tool)
FGF19	No radiation Simple Commercial assay available	Diurnal variation Requires further validation
Faecal bile acids/faecal metabolomics	No radiation	Cumbersome 48 hours sample collection versus spot test Not widely available Poor patient compliance
Urine	Easy collection	Experimental Not widely available
Therapeutic trial	Easily available	Unreliable Poor response does not exclude diagnosis of BAD Not cost-effective Delays diagnosis and affects patients quality of life.

FGF19, fibroblast growth factor 19 .

2020 review is awaited. A systematic review and meta-analysis comprising 36 studies and 5028 patients on BAD biomarkers concluded that ^{75}SeHCAT had a highest diagnostic yield to date (limited by study heterogeneity) with 25% previously diagnosed as having functional bowel disorders actually had primary BAD.[47]

Selenium-75 homocholic acid taurine is a synthetic analogue of taurocholic acid, which is a natural conjugated bile acid, and behaves in the same way as bile acids except that it is resistant to deconjugation by intestinal bacteria.[48] It is ingested in the form of a capsule after an overnight fast, and a standard gamma camera is used to detect the baseline level 3 hours after ingestion. The scan is repeated after 7 days and the overall retention in the abdomen is measured. Retention values of 10%–15% are considered mild bile acid malabsorption, while 5%–10% is moderate bile acid malabsorption and less than 5% is severe bile acid malabsorption.[45 49] However, its use is not widespread despite the ability to be used in any nuclear medicine department supporting a gamma camera and it is not licensed for use in the USA.[50] Sensitivity of ^{75}SeHCAT testing is 96% with a specificity of 100% at 7 days.[51]

^{75}SeHCAT may also predict response to therapy. Pooled data from 15 studies shows a dose–response relationship between the severity of malabsorption and the effect of treatment with a bile acid sequestrant: clinical response to colestyramine occurred in 96% of patients with <5% retention of SeHCAT, 80% at <10% retention and 70% at <15% retention.[52] In general, the lower the SeHCAT retention value the greater the likelihood of response to sequestrants.

Blood

C4 (7α-hydroxy-4-cholesten-3-one) levels may also be used to diagnose BAD, especially in the absence of ^{75}SeHCAT. This is a marker of bile acid synthesis via CYP7A1, and baseline C4 levels are raised in patients with BAD secondary to impaired feedback of FGF19.[3] This indicates increased bile acid synthesis and thus increased levels of bile acids in the colonic lumen.[48] Patients with terminal ileal disease may also have increased C4 levels, as decreased reabsorption may increase synthesis.[53] When compared with ^{75}SeHCAT testing, C4 levels have a negative predictive value of 98%, making it an attractive test to exclude BAD.[48] When comparing ^{75}SeHCAT values <10%, fasting C4 levels >48.4 ng/mL has a sensitivity of 90% and a specificity of 79%.[54] Timing of specimen collection is critical due to the diurnal variation of C4 levels as well as variation with hypertriglyceridaemia and ethanol levels (both of which are associated with higher bile acid synthesis, therefore, higher C4 levels).[53 55–58] C4 levels correlate negatively with faecal bile acid excretion[59] and inversely correlated with FGF19 levels.[60]

Fasting serum FGF19 levels are inversely correlated with C4 levels. C4 levels are usually higher in patients with BAD. Since FGF19 inhibits bile acid synthesis, decreased FGF19 levels may indicate BAD. FGF19 levels correlate well with ^{75}SeHCAT results, with a negative predictive value of 82% for ^{75}SeHCAT of <10%, sensitivity of 58% and specificity of 84% for a serum FGF19 level of <145 pg/mL. Its negative predictive value rises to 94% for ^{75}SeHCAT <5%,[60] FGF19 levels change rapidly after meals, and there is also a natural diurnal variation. Thus like C4, standardisation would be required if FGF19 levels is to be used for

diagnosis.[61] FGF19 levels are not yet routinely used in the diagnosis of BAD.

Stool

Measurement of faecal bile acids may also be another diagnostic test for BAD if ^{75}SeHCAT is unavailable.[62] This is a measure of the total excess bile acids exiting the colon. Within the colon, a proportion of bile acids are absorbed and conjugated into secondary bile acids. The amount of primary faecal bile acids (CDA and CA) are found to be higher in patients with BAD and even correlate with frequency and consistency of stool.[59 63] Patients with BAD have a higher stool weight. Total faecal bile acids of more than 2337 µmol/48 hours are diagnostic for BAD, however, elevated primary faecal bile acids may also be used as a diagnostic test, as >4% PBA are indicative of BAD since healthy volunteers usually only have about 0.02% primary faecal bile acids. A 4% cut-off may be used even when total faecal bile acids measure 1000 µmol/48 hours.[17] However, this requires a 48-hour faecal collection taken during the last 2 days of a 4-day 100 g fat diet, due to variation in dietary fat intake and consequently bile acid levels. Faeces needs to be homogenised, deconjugated and separated before performing either gas chromatography-mass spectrometry, liquid chromatography-tandem mass spectrometry and HPLC-mass spectrometry.[64 65] This poses difficulty in patient compliance, labour-intensive analytical process and further complicated by the fact that there will also be diurnal variation in bile acid secretion. A pilot study has shown feasibility with a single spot faecal sample but requires further validation, as >10% PBA in a faecal spot sample had a 45% sensitivity and an 63% to detect ^{75}SEHCAT value of <15%.[66] Given that stool uptake in the UK bowel cancer screening programme is 50%–58%.[67] It is hard to conceive this will be used routinely for diagnosis in the UK due to poor patient compliance coupled with dietary restriction.

Urine

There has also been a study to detect volatile organic compounds in urine of patients with BAD. This revealed detection of 2-propanol and acetamide as markers of BAD, compared with healthy controls and patients with inflammatory bowel disease.[68] This may prove a novel way to diagnose BAD but further validation studies are required.

There has also been some experimental work using an electronic nose to detect volatile organic compounds. This is still being investigated, however, the chemical signature profile of patients with BAD were different to those with ulcerative colitis and healthy controls. Its purported mechanisms include gut dysbiosis or dysfermention in response to disease.[69] The main gases identified in patients with BAD were 2-propanolol and acetamide.[68]

Therapeutic trials

Bile acid sequestrants are sometimes used in a therapeutic trial if no other diagnostic methods are available. In a series of 264 patients where 53% had BAD, 44% failed to respond to cholestyramine alone. Half of these non-responders derived benefit from Colesevelam (unlicensed but used with extended indication). Thus, lack of response to cholestyramine does not constitute exclusion of BAD hence therapeutic trials of bile acid sequestrants (cholestyramine or colesevelam) are not recommended.[70] This has been affirmed also by the BSG chronic diarrhoea guidelines.[42] A patient-reported outcome study has shown that if the diagnosis of BAD is delayed, often by more than 5 years, due to poor recognition of the disease patients quality of life is affected negatively.[71] Delayed investigation and treatment of BAD has been shown to almost double the diagnostic care-of-package cost.[72]

MANAGEMENT

Bile acid sequestrants are the first-line management of BAD. Colestyramine has long been used as a first line and response has been associated with ^{75}SeHCAT value. Ninety-six per cent of patients with <5% retention responded to cholestyramine, 80% of patients responded at <10% retention and 70% at <15% retention.[52] Colestipol has similarly been shown to improve symptoms of diarrhoea in patients with low ^{75}SEHCAT.[5]

Unfortunately, colestryamine and colestipol are often discontinued by patients as they are poorly tolerated due to the taste and texteure of the resin powder. Another issue arises as patients find that while diarrhoea settles, they often have constipation, bloating, nausea and abdominal cramps.[73] Colesevelam is another bile acid sequestrant which is often better tolerated than colestyramine and has also been shown to create a firmer consistency of stool in such patients, however, its use is unlicenced.[74–76]

Some other medications have been tried, such as hydroxypropyl cellulose which was compared with colestryamine. There was no difference between patients on colestyramine and on hydroxypropyl cellulose who achieved clinical remission in 8 weeks, however, it was found that colestyramine was superior in decreasing the number of watery stools.[77] Obeticholic acid has been shown to stimulate FGF19, thus reducing bile acid synthesis and causing symptom improvement related to stool frequency and stool form. It was also effective in patients with ileal resections, improving abdominal pain and urgency, though more so in patients with a shorter resected length.[78]

Dietary interventions may be used to improve symptoms. A low-fat diet has been shown to improve gastrointestinal symptoms in patients, with improvements shown in urgency, bloating, lack of control, bowel frequency, abdominal pain and nocturnal defecation.[79 80] A combined approach using both

colesevelam and low fat approach has also been shown to be helpful.[81]

Specific conditions causing BAD secondary to malabsorption will need treatment, such as antibiotics for small bowel overgrowth,[82] or steroids (mainly budesonide due to lack of systemic side effects) for microscopic colitis.[83]

CONCLUSION

While part of the pathophysiology behind BAD has been elicited, there are gaps in knowledge as to what causes the disruption of the feedback loop in the case of idiopathic BAD. This may be limiting advances in diagnosis and treatment of the disease. ^{75}SeHCAT or C4 measuement is still the most commonly used methods for diagnosis though novel technologies such as e-nose are emerging. Spot faecal bile acid measurement may hold some potential but requires further validation and may prove difficult to use routinely in clinic. BAD is a treatable disease, however, the delay in diagnosis causes a significant increase in the diagnostic care cost as well as affecting the quality of life of patients. Further avenues for research should look into further defining the reasons behind the disruption of the negative feedback loops to target treatment.

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Appendix 2: Ethical approval

NHS
Health Research Authority

East Midlands - Leicester Central Research Ethics Committee
The Old Chapel
Royal Standard Place
Nottingham
NG1 6FS

25 January 2019

Miss Alexia Farrugia
Clinical Research Fellow in Specialist Surgery
University Hospitals Coventry and Warwickshire NHS Trust
Walsgrave General Hospital
Clifford Bridge Road
Coventry West Midlands
CV2 2DX

Dear Miss Farrugia

Study title:	**Bile acid diarrhoea after laparoscopic cholecystectomy**
REC reference:	**18/EM/0395**
Protocol number:	**N/A**
IRAS project ID:	**249381**

Thank you for your response of 23rd January 2019, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to make a request to postpone publication, please contact hra.studyregistration@nhs.net outlining the reasons for your request.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation

as revised, subject to the conditions specified below.

Conditions of the favourable opinion

The REC favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements. Each NHS organisation must confirm through the signing of agreements and/or other documents that it has given permission for the research to proceed (except where explicitly specified otherwise).

Guidance on applying for HRA and HCRW Approval (England and Wales)/ NHS permission for research is available in the Integrated Research Application System, at www.hra.nhs.uk or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of management permissions from host organisations

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database within 6 weeks of recruitment of the first participant (for medical device studies, within the timeline determined by the current registration and publication trees).

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g. when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non-clinical trials this is not currently mandatory.

If a sponsor wishes to request a deferral for study registration within the required timeframe, they should contact hra.studyregistration@nhs.net. The expectation is that all clinical trials will be registered, however, in exceptional circumstances non registration may be permissible with prior agreement from the HRA. Guidance on where to register is provided on the HRA website.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
IRAS Application Form [IRAS_Form_26112018]		26 November 2018
Letter from sponsor [Trust Sponsorship Letter]		
Letters of invitation to participant [Invitation letter]	1.1	17 January 2019
Non-validated questionnaire [Data collection form]	1	23 November 2018
Other [Response]	1	07 January 2019
Participant consent form [Consent form control group]	1.3	20 December 2018
Participant consent form [Consent form study group]	1.3	20 December 2018
Participant information sheet (PIS) [PIS control group]	1.3	17 January 2019
Participant information sheet (PIS) [PIS study group]	1.3	17 January 2019
Referee's report or other scientific critique report [Peer review]	1	12 September 2018
Research protocol or project proposal [Protocol]	1.1	22 January 2019
Summary CV for Chief Investigator (CI) [CV - CI]	1	28 September 2018
Summary CV for student [Alexia Farrugia CV]	1	26 June 2018
Summary CV for supervisor (student research) [CV - supervisor]	1	28 September 2018
Validated questionnaire [GIQLI - supplementary information]	1	05 October 2018
Validated questionnaire [GIQLI questionnaire]	1	23 November 2018

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document *"After ethical review – guidance for researchers"* gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports

- Notifying the end of the study

The HRA website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

User Feedback

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application procedure. If you wish to make your views known please use the feedback form available on the HRA website:
http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance/

HRA Training

We are pleased to welcome researchers and R&D staff at our training days – see details at http://www.hra.nhs.uk/hra-training/

18/EM/0395	**Please quote this number on all correspondence**

With the Committee's best wishes for the success of this project.

Yours sincerely

[redacted]

Mrs Rita Patel
Chair

Email:nrescommittee.eastmidlands-leicestercentral@nhs.net

Enclosures: "After ethical review – guidance for researchers"

Copy to: *Mrs Ceri Jones*
Prof Ramesh Arasaradnam
Miss Sonia Kandola , University Hospitals Coventry and Warwickshire NHS Trust

Appendix 3: HRA approval

Ymchwil Iechyd a Gofal Cymru
Health and Care Research Wales

NHS
Health Research Authority

Prof Ramesh Arasaradnam
University Hospitals Coventry and Warwickshire NHS Trust
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25 January 2019

Dear Prof Arasaradnam

HRA and Health and Care Research Wales (HCRW) Approval Letter

Study title:	**Bile acid diarrhoea after laparoscopic cholecystectomy**
IRAS project ID:	**249381**
Protocol number:	**N/A**
REC reference:	**18/EM/0395**
Sponsor	**Organization not set**

I am pleased to confirm that HRA and Health and Care Research Wales (HCRW) Approval has been given for the above referenced study, on the basis described in the application form, protocol, supporting documentation and any clarifications received. You should not expect to receive anything further relating to this application.

How should I continue to work with participating NHS organisations in England and Wales?
You should now provide a copy of this letter to all participating NHS organisations in England and Wales, as well as any documentation that has been updated as a result of the assessment.

This is a single site study sponsored by the site. The sponsor R&D office will confirm to you when the study can start following issue of HRA and HCRW Approval.

It is important that you involve both the research management function (e.g. R&D office) supporting each organisation and the local research team (where there is one) in setting up your study. Contact details of the research management function for each organisation can be accessed here.

How should I work with participating NHS/HSC organisations in Northern Ireland and Scotland?
HRA and HCRW Approval does not apply to NHS/HSC organisations within the devolved administrations of Northern Ireland and Scotland.

If you indicated in your IRAS form that you do have participating organisations in either of these devolved administrations, the final document set and the study wide governance report (including this

letter) has been sent to the coordinating centre of each participating nation. You should work with the relevant national coordinating functions to ensure any nation specific checks are complete, and with each site so that they are able to give management permission for the study to begin.

Please see IRAS Help for information on working with NHS/HSC organisations in Northern Ireland and Scotland.

How should I work with participating non-NHS organisations?
HRA and HCRW Approval does not apply to non-NHS organisations. You should work with your non-NHS organisations to obtain local agreement in accordance with their procedures.

What are my notification responsibilities during the study?
The document "*After Ethical Review – guidance for sponsors and investigators*", issued with your REC favourable opinion, gives detailed guidance on reporting expectations for studies, including:

- Registration of research
- Notifying amendments
- Notifying the end of the study

The HRA website also provides guidance on these topics, and is updated in the light of changes in reporting expectations or procedures.

I am a participating NHS organisation in England or Wales. What should I do once I receive this letter?
You should work with the applicant and sponsor to complete any outstanding arrangements so you are able to confirm capacity and capability in line with the information provided in this letter.

The sponsor contact for this application is as follows:

Mrs Ceri Jones
E-mail R&DSponsorship@uhcw.nhs.uk
Telephone 02476965031

Who should I contact for further information?
Please do not hesitate to contact me for assistance with this application. My contact details are below.

Your IRAS project ID is **249381**. Please quote this on all correspondence.

Yours sincerely

Catherine Adams
Senior Assessor

Email: hra.approval@nhs.net

Copy to: *Mrs Ceri Jones, Sponsor's Representative*
Miss Sonia Kandola , University Hospitals Coventry and Warwickshire NHS Trust
Miss Alexia Farrugia, Student

IRAS project ID	249381

List of Documents

The final document set assessed and approved by HRA and HCRW Approval is listed below.

Document	*Version*	*Date*
IRAS Application Form [IRAS_Form_26112018]		26 November 2018
IRAS Application Form XML file [IRAS_Form_26112018]		26 November 2018
Letter from sponsor [Trust Sponsorship Letter]		
Letters of invitation to participant [Invitation letter]	1.1	17 January 2019
Non-validated questionnaire [Data collection form]	1	23 November 2018
Other [Response]	1	07 January 2019
Participant consent form [Consent form study group]	1.3	20 December 2018
Participant consent form [Consent form control group]	1.3	20 December 2018
Participant information sheet (PIS) [PIS control group]	1.3	17 January 2019
Participant information sheet (PIS) [PIS study group]	1.3	17 January 2019
Referee's report or other scientific critique report [Peer review]	1	12 September 2018
Research protocol or project proposal [Protocol]	1.1	22 January 2019
Summary CV for Chief Investigator (CI) [CV - CI]	1	28 September 2018
Summary CV for student [Alexia Farrugia CV]	1	26 June 2018
Summary CV for supervisor (student research) [CV - supervisor]	1	28 September 2018
Validated questionnaire [GIQLI questionnaire]	1	23 November 2018
Validated questionnaire [GIQLI - supplementary information]	1	05 October 2018

IRAS project ID	249381

Summary of assessment

The following information provides assurance to you, the sponsor and the NHS in England and Wales that the study, as assessed for HRA and HCRW Approval, is compliant with relevant standards. It also provides information and clarification, where appropriate, to participating NHS organisations in England and Wales to assist in assessing, arranging and confirming capacity and capability.

Assessment criteria

Section	Assessment Criteria	Compliant with Standards	Comments
1.1	IRAS application completed correctly	Yes	No comments
2.1	Participant information/consent documents and consent process	Yes	No comments
3.1	Protocol assessment	Yes	No comments
4.1	Allocation of responsibilities and rights are agreed and documented	Yes	This is a single site study, sponsored by the same organisation. No agreement is expected or required. Although formal confirmation of capacity and capability is not expected of all or some organisations participating in this study, and such organisations would therefore be assumed to have confirmed their capacity and capability should they not respond to the contrary, we would ask that these organisations pro-actively engage with the sponsor in order to confirm at as early a date as possible. Confirmation in such cases should be by email to the CI and Sponsor confirming participation based on the relevant Statement of Activities and information within this letter.
4.2	Insurance/indemnity arrangements assessed	Yes	No comments
4.3	Financial arrangements assessed	Yes	No comments

IRAS project ID	249381

Section	Assessment Criteria	Compliant with Standards	Comments
5.1	Compliance with the Data Protection Act and data security issues assessed	Yes	No comments
5.2	CTIMPS – Arrangements for compliance with the Clinical Trials Regulations assessed	Not Applicable	No comments
5.3	Compliance with any applicable laws or regulations	Yes	No comments
6.1	NHS Research Ethics Committee favourable opinion received for applicable studies	Yes	No comments
6.2	CTIMPS – Clinical Trials Authorisation (CTA) letter received	Not Applicable	No comments
6.3	Devices – MHRA notice of no objection received	Not Applicable	No comments
6.4	Other regulatory approvals and authorisations received	Not Applicable	No comments

Participating NHS Organisations in England and Wales

This provides detail on the types of participating NHS organisations in the study and a statement as to whether the activities at all organisations are the same or different.
This is a single site study and therefore there is only one 'site-type' undertaking activities as detailed in the protocol and supporting documentation. If this study is subsequently extended to other NHS organisation(s) in England or Wales, an amendment should be submitted, with a Statement of Activities and Schedule of Events for the newly participating NHS organisation(s) in England or Wales.. If chief investigators, sponsors or principal investigators are asked to complete site level forms for participating NHS organisations in England and Wales which are not provided in IRAS, the HRA or HCRW websites, the chief investigator, sponsor or principal investigator should notify the HRA immediately at hra.approval@nhs.net or HCRW at Research-permissions@wales.nhs.uk. We will work with these organisations to achieve a consistent approach to information provision.

Principal Investigator Suitability

This confirms whether the sponsor position on whether a PI, LC or neither should be in place is correct for each type of participating NHS organisation in England and Wales, and the minimum expectations for education, training and experience that PIs should meet (where applicable).
The Chief Investigator is responsible for study activity at the participating organisation. GCP training is not a generic training expectation, in line with the HRA/HCRW/MHRA statement on training expectations.

HR Good Practice Resource Pack Expectations

This confirms the HR Good Practice Resource Pack expectations for the study and the pre-engagement checks that should and should not be undertaken
No Honorary Research Contracts, Letters of Access or pre-engagement checks are expected for local staff employed by the participating NHS organisations. Where arrangements are not already in place, research staff not employed by the NHS host organisation undertaking any of the research activities listed in the research application would be expected to obtain an honorary research contract. This would be on the basis of a Research Passport (if university employed) or an NHS to NHS confirmation of pre-engagement checks letter (if NHS employed). These should confirm enhanced DBS checks, including appropriate barred list checks, and occupational health clearance.

Other Information to Aid Study Set-up

This details any other information that may be helpful to sponsors and participating NHS organisations in England and Wales to aid study set-up.
The applicant has indicated that they do not intend to apply for inclusion on the NIHR CRN Portfolio.

Appendix 4: GIQLI questionnaire

How often during the past 2 weeks have you had pain in the abdomen?

all of the time, most of the time, some of the time, a little of the time, never

How often during the past 2 weeks have you had a feeling of fullness in the upper abdomen?

all of the time, most of the time, some of the time, a little of the time, never

How often during the past 2 weeks have you had bloating (sensation of too much gas in the abdomen)?

all of the time, most of the time, some of the time, a little of the time, never

How often during the past 2 weeks have you been troubled by excessive passage of gas through the anus?

all of the time, most of the time, some of the time, a little of the time, never

How often during the past 2 weeks have you been troubled by strong burping or belching?

all of the time, most of the time, some of the time, a little of the time, never

How often during the past 2 weeks have you been troubled by gurgling noises from the abdomen?

all of the time, most of the time, some of the time, a little of the time, never

How often during the past 2 weeks have you been troubled by frequent bowel movements?

all of the time, most of the time, some of the time, a little of the time, never

How often during the past 2 weeks have you found eating to be a pleasure?

all of the time, most of the time, some of the time, a little of the time, never

Because of your illness, to what extent have you restricted the kinds of food you eat?

very much, much, somewhat, a little, not at all

During the past 2 weeks, how well have you been able to cope with everyday stresses?

extremely poorly, poorly, moderately, well, extremely well

How often during the past 2 weeks have you been sad about being ill?
all of the time, most of the time, some of the time, a little of the time, never

How often during the past 2 weeks have you been nervous or anxious about your illness?
all of the time, most of the time, some of the time, a little of thetime, never

How often during the past *2* weeks have you been happy with life in general?
never, a little of the time, some of the time, most of the time, all of the time

How often during the past 2 weeks have you been frustrated about your illness?
all of the time, most of the time, some of the time, a little of the time, never

How often during the past 2 weeks have you been tired or fatigued?
all of the time, most of the time, some of the time, a little of the time, never

How often during the past 2 weeks have you felt unwell?
all of the time, most of the time, some of the time, a little of the time, never

Over the past week, have you woken up in the night?
every night, 5-6 nights, 3-4 nights, 1-2 nights, never

Since becoming ill, have you been troubled by changes in your appearance?
a great deal, a moderate amount, somewhat, a little bit, not at all

Because of your illness, how much physical strength have you lost?
a great deal, a moderate amount, some, a little bit, none

Because of your illness, to what extent have you lost your endurance?
a great deal, a moderate amount, somewhat, a little bit, not at all

Because of your illness, to what extent do you feel unfit?

extremely unfit, moderately unfit, somewhat unfit, a little unfit, fit

During the past 2 weeks, how often have you been able to complete your normal daily activities (school, work,household)?

all of the time, most of the time, some of the time, a little of the time, never

During the past 2 weeks, how often have you been able to take part in your usual patterns of leisure or recreational activities?

all of the time, most of the time, some of the time, a little of the time, never

During the past 2 weeks, how much have you been troubled by the medical treatment of your illness?

very much, much, somewhat, a little, not at all

To what extent have your personal relations with people close to you (family or friends) worsened because of your illness?

very much, much, somewhat, a little, not at all

To what extent has your sexual life been impaired (harmed) because of your illness?

very much, much, somewhat, a little, not at all

How often during the past 2 week, have you been troubled by fluid or food coming up into your mouth (regurgitation)?

all of the time, most of the time, some of the time, a little of the time, never

How often during the past *2* weeks have you felt uncomfortable because of your slow speed of eating?

all of the time, most of the time, some of the time, a little of the time, never

How often during the past 2 weeks have you had trouble swallowing your food?

all of the time, most of the time, some of the time, a little of the time, never

How often during the past 2 weeks have you been troubled by urgent bowel movements?

all of the time, most of the time, some of the time, a little of the time, never

How often during the past 2 weeks have you been troubled by diarrhoea?

all of the time, most of the time, some of the time, a little of the time, never

How often during the past 2 weeks have you been troubled by constipation?
all of the time, most of the time, some of the time, a little of the time, never

How often during the past 2 weeks have you been troubled by nausea?
all of the time, most of the time, some of the time, a little of the time, never

How often during the past 2 weeks have you been troubled by blood in the stool?
all of the time, most of the time, some of the time, a little of the time, never

How often during the past 2 weeks have you been troubled by heartburn?
all of the time, most of the time, some of the time, a little of the time, never

How often during the past 2 weeks have you been troubled by uncontrolled stools?
all of the time, most of the time, some of the time, a little of the time, never

Calculation of score: most desirable option 4 points, least desirable option 0 points.

Appendix 5: Patient group support letter

BAD UK
3 New Street
Higham
Derbyshire
DE55 6BP

31st January 2019

Re: BADCaP Bile Acid Diarrhoea in Post-Cholecystectomy Patients Study IRAS number: 24938

BAD UK have been involved in advising the Chief Investigator and Principal Investigator in writing up the protocol and patient information for the above-named study.

From the patients perspective this is a very much needed area of research and we welcome the study being undertaken. A commonly asked question from patients whom attend our patient support groups and connect with us on our Facebook private support forums are:

- Why has having a cholecystectomy resulted in such debilitating diarrhoea that has a significant long term impact on quality of life?
- Can it be prevented?
- Are there any better treatment options / cures?

From our own review of the research literature available to us, and through our discussions with medical professionals about why people develop BAD post cholecystectomy, it is apparent to us that this is a little known entity amongst the medical community despite the possibility of this happening in 1-35% of patients after cholecystectomy, an extremely common procedure.

Our view is that this study does address where patients want further research into the condition to be focussed on as it is looking to determine why this disease happens, how to predict it pre-operatively and identify possible ways to improve future management of the condition.

Best wishes,

Michelle O'Connor

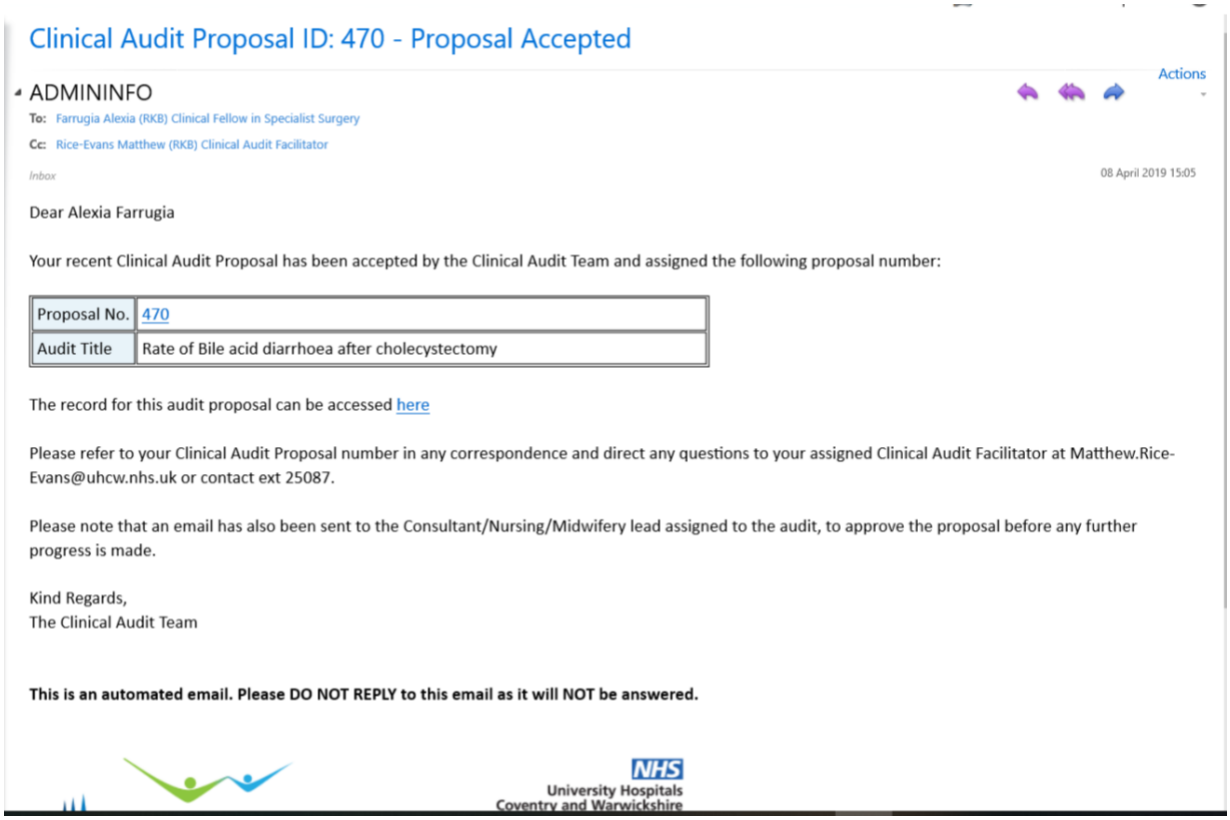
Chairman

BAD UK: EW40884

www.bad-uk.org

www.abadstory.com

Appendix 6- audit approval (local)



Clinical Audit Proposal ID: 470 - Proposal Accepted

ADMININFO

Actions

To: Farrugia Alexia (RKB) Clinical Fellow in Specialist Surgery

Cc: Rice-Evans Matthew (RKB) Clinical Audit Facilitator

Inbox

08 April 2019 15:05

Dear Alexia Farrugia

Your recent Clinical Audit Proposal has been accepted by the Clinical Audit Team and assigned the following proposal number:

Proposal No.	470
Audit Title	Rate of Bile acid diarrhoea after cholecystectomy

The record for this audit proposal can be accessed here

Please refer to your Clinical Audit Proposal number in any correspondence and direct any questions to your assigned Clinical Audit Facilitator at Matthew.Rice-Evans@uhcw.nhs.uk or contact ext 25087.

Please note that an email has also been sent to the Consultant/Nursing/Midwifery lead assigned to the audit, to approve the proposal before any further progress is made.

Kind Regards,
The Clinical Audit Team

This is an automated email. Please DO NOT REPLY to this email as it will NOT be answered.

NHS
University Hospitals
Coventry and Warwickshire

Appendix 7: Poster

Rates of bile acid diarrhoea diagnosis in patients following cholecystectomy

Farrugia A, Khan S, Williams N, Arasaradnam R.
University Hospitals Coventry and Warwickshire NHS Trust

Introduction

Bile acid diarrhoea (**BAD**) is known to happen after laparoscopic cholecystectomy (**LC**). Changes in the enterohepatic circulation (Figure 1) are thought to be involved. The rate of post-cholecystectomy diarrhoea is unknown though rates vary from 0.9% to 35% in the literature. The time from surgery to diagnosis of **BAD** is also unknown.

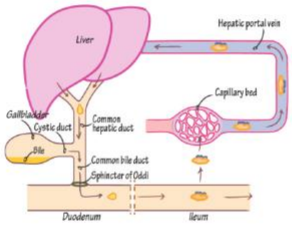


Figure 1: Enterohepatic circulation

Methodology

A prospectively maintained list of **LC** patients between 2013 and 2017 was cross-referenced with list of patients who had ^{75}**SeHCAT** during the same time frame. Patient demographics were collected and compared for significance using T-test ($p<0.05$). ^{75}**SeHCAT** <15% was considered positive.

Results

- 2371 patients had cholecystectomy between 2013 and 2017 at UHCW.
- 33 patients also had ^{75}**SeHCAT** testing (1.4%).
- Indication for ^{75}**SeHCAT** was diarrhoea (31), change in bowel habit (1) and diarrhoea alternating with constipation (1).
- 20 (60.1%) had ^{75}**SeHCAT** retention <15%, as shown in figure 2.

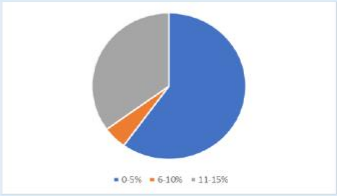


Figure 2—^{75}SeHCAT retention results

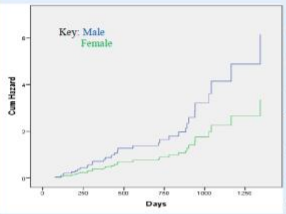


Figure 3: Cox regression analysis

- Mean time from surgery to ^{75}**SeHCAT** was 564 days (SD 371).
- Women were tested significantly later than men (660 vs 287 days, p=0.006). This is shown in the Cox regression analysis (Figure 3).
- 29 patients who had ^{75}**SeHCAT** were also investigated via endoscopy while 10 were also investigated via CT scan.

Conclusions

There is a significant delay in testing for **BAD** and only a small proportion of patients are sent for investigation. 59% of patients with diarrhoea post-**LC** may develop **BAD**. The true prevalence is unknown. **BAD** requires higher profile as it is an easily treatable condition which is known to happen after **LC** and can impact quality of life.

References

- Lamberts MP, Lugtenberg M, Rovers MM, Roukema AJ, Drenth JP, Westert GP, et al. Persistent and de novo symptoms after cholecystectomy: a systematic review of cholecystectomy effectiveness. Surgical endoscopy. 2013;27(3):709-18.
- Fort JM, Azpiroz F, Casellas F, Andreu J, Malagelada JR. Bowel habit after cholecystectomy: physiological changes and clinical implications. Gastroenterology. 1996;111(3):617-22.
- Sauter GH, Moussavian AC, Meyer G, Steitz HO, Parhofer KG, Jüngst D. Bowel habits and bile acid malabsorption in the months after cholecystectomy. American Journal Of Gastroenterology. 2002;97:1732

Appendix 8 – Multicentre audit protocol

Multicentre retrospective audit on the investigation of bile acid diarrhoea after laparoscopic cholecystectomy.

Steering Committee:

Alexia Farrugia

Clinical Research Fellow

University Hospitals Coventry and Warwickshire NHS Trust

Warwick Medical School, University of Warwick

Siobhan C McKay

ST7 General Surgery and HPB

University Hospitals Coventry and Warwickshire NHS Trust

Joseph Anthony Attard

Clinical Research Fellow

Queen Elizabeth Hospital, University Hospitals, Birmingham

Centre for Liver and Gastrointestinal Research, Institute of Immunology and Immunotherapy, University of Birmingham, Birmingham

Stacey Coleman

Foundation Doctor

University Hospitals Coventry and Warwickshire NHS Trust

Stuart Hanmer

Foundation Doctor

University Hospitals Coventry and Warwickshire NHS Trust

Stuart Bullock

Foundation Doctor

University Hospitals Coventry and Warwickshire NHS Trust

Saboor Khan

Consultant HPB Surgeon

University Hospitals Coventry and Warwickshire NHS Trust

Nigel Williams

Consultant Colorectal Surgeon

University Hospitals Coventry and Warwickshire NHS Trust

Ramesh Arasaradnam

Consultant Gastroenterologist

University Hospitals Coventry and Warwickshire NHS Trust

Sponsoring site: University Hospitals Coventry and Warwickshire

Supported by the Roux group

Introduction

This audit aims to determine the number of patients investigated for chronic diarrhoea with ^{75}SeHCAT after cholecystectomy.

While up to 35% of patients can have diarrhoea after cholecystectomy, it is not known what proportion of these are due to bile acid diarrhoea (1). Furthermore, in those patients diagnosed with bile acid diarrhoea up to 27.4% have had a previous cholecystectomy (2). One of the ways in which post-cholecystectomy diarrhoea develops is interruption in the enterohepatic circulation (3, 4). Normally, bile acids are stored in the gallbladder and secreted into the duodenum when stimulated by food intake. Following their release, they travel down the small bowel to be absorbed in the ileus. Bile acid synthesis is controlled by a finely tuned negative feedback mechanism via the FXR-FGF19 pathway. Upon bile acid production, FXR (a central transcriptional sensor of BA metabolic cascades) leads to up regulation of the enterokine FGF 19 which is secreted into the portal blood. FGF19 reaches the liver where it activates the duo FGF receptor 4 (FGFR4)/beta KLOTHO on the hepatocyte basolateral membrane triggering intracellular pathways that lead to repression of cholesterol 7-α-hydroxylase (CYP7A1), the rate limiting enzyme in BA synthesis (5). In bile acid diarrhoea this negative feedback loop via the FXR-FGF19 pathway is interrupted, leading to the overproduction of bile acids (Figure 1).

Diarrhoea affects patients' quality of life, leading to a myriad of issues including social isolation and economic issues due to problems in the workplace(6).^{75}SeHCAT is a nuclear medicine test that is used to diagnose bile acid malabsorption using Selenium-75, a gamma emitter. SeHCAT undergoes secretion into the biliary tree, gallbladder and intestine in response to food, and is reabsorbed efficiently in the ileum similar to natural bile acids (7). In order to diagnose bile acid malabsorption, the percent retention of SeHCAT at 7 days is calculated. A 7-day SeHCAT retention >15% is considered to be normal, with values less than 15% signifying excessive bile acid loss.

The BSG guidelines state that investigation using ^{75}SeHCAT and/or serum 7-alpha-hydroxy-cholesten-3-one (C4) are required in the clinical investigation of persistent (>4

weeks), undiagnosed diarrhoea (8). Bile acid diarrhoea can effectively be treated by bile acid sequestrants.

We aim to determine the number of patients undergoing ^{75}SeHCAT testing post-cholecystectomy and how many of these were eventually diagnosed with bile acid diarrhoea. If an association is found, then this will impact the consent process for laparoscopic cholecystectomy. This audit will also identify the frequency of this disease, the time from surgery to diagnosis, and may identify a real-world problem suggesting the need for further investigation and clarification of referral pathways.

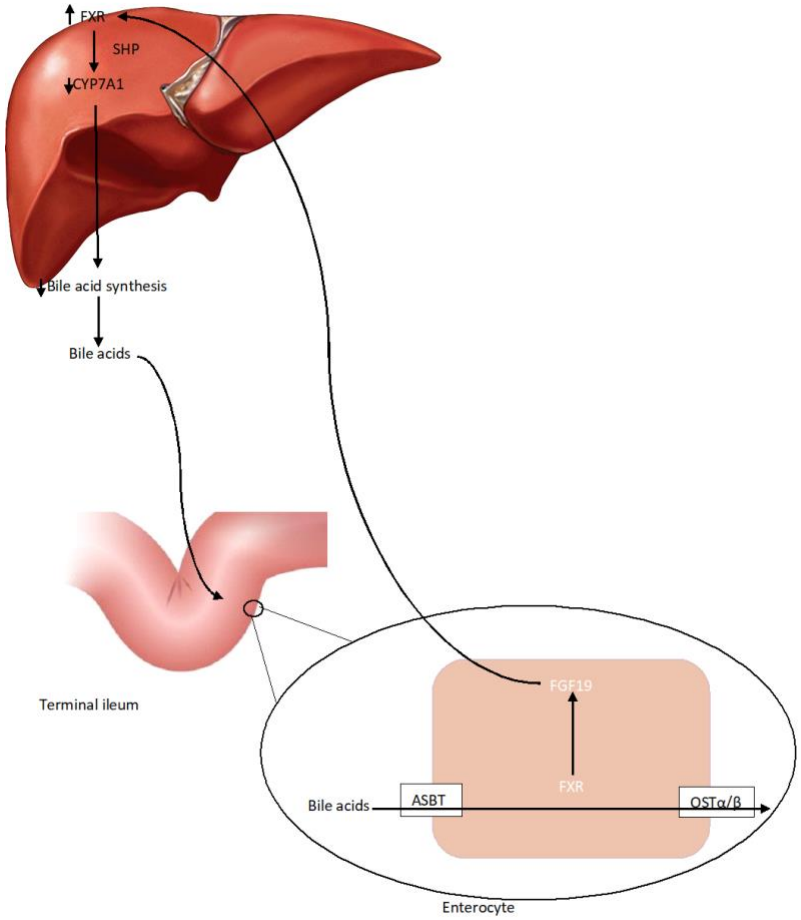


Figure 1: Bile acid production is controlled through negative feedback mechanisms. Bile acids production activates transcription of FGF19 via the transcriptional regulator FXR. FG19 expression ultimately leads to inhibition of bile acid synthesis by repressing CYP7A1, the rate limiting enzyme in bile acid synthesis.

Hypothesis:

A significant proportion of patients develop diarrhoea due to bile acid malabsorption following a cholecystectomy

Primary endpoints

- Percentage of patients investigated for BAD after cholecystectomy
- Percentage of patients diagnosed with BAD after cholecystectomy

Secondary endpoints

- Time from cholecystectomy to ^{75}SeHCAT
- Gender differences in referral
- Are BSG criteria for referral being followed
- Other diagnoses

Methods

A multicentre, retrospective audit of patients who underwent cholecystectomy and ^{75}SeHCAT

Governance

Ethics not required as this is a clinical audit. Each participating centre will be required to obtain the necessary governance approvals to conduct this audit.

Inclusion criteria

- Patients >16 years old at time of cholecystectomy (laparoscopic or open)

- AND patients who were investigated with ^{75}SeHCAT after cholecystectomy/OR coded as having bile acid diarrhoea/bile acid malabsorption

Exclusion criteria

- Patients diagnosed with bile acid diarrhoea prior to cholecystectomy

Study Period

Cholecystectomy performed between 1st January 2013 – 31st December 2017

Case identification

Each centre should contact the hospital coding team to aid in the identification of patients who have had both cholecystectomy and ^{75}SeHCAT test or diagnosed with bile acid diarrhoea/bile acid malabsorption.

Data Collection and Storage

The following data points will be extracted for each patient who had both cholecystectomy and ^{75}SeHCAT:

- Non-identifiable patient demographics,
- Relevant PMH
- Investigations
- Diagnosis

A CRF has been created to simplify data collection. Please refer to CRF (appendix 1).

In the case of patients having more than one ^{75}SeHCAT, CT scan or endoscopic investigation, the investigation that must be taken into account should be the one that occurred after the cholecystectomy.

Data will be entered into the spreadsheet which has locked cells for most fields which will act as a form of data validation. All data will be anonymised and sent back to the steering committee via hospital email accounts. Data will be stored on one hospital password-protected computer. No patient identifiable data will be shared outside the individual trusts. Local sites will be required to keep patient NHS number and/or local hospital number on one hospital password-protected computer until all the data is analysed.

Statistical analysis

A 7-day ^{75}SeHCAT retention time of <15% will be deemed positive. Patient demographics are to be collected and compared for significance ($p<0.05$) using non-parametric t-Test. The data will be collected on an excel sheet using locked cells for internal validation.

Data analysis will be conducted using IBM SPSS Statistics Version 23.

Data collection will occur in teams consisting of one consultant and up to three data collectors, one of whom will be the trainee lead. Data collectors can be a doctor, CNS, ACP or medical student. Centre recruitment will open in March 2019. Data collection will start in spring/summer, with analysis in autumn and dissemination in winter. Individual site data will not be identifiable, but sites will be expected to present their local data in an appropriate local forum.

Authorship

A collaborative authorship model will be used, under the name the 'BADCAP Study Group'. All contributors shall be acknowledged with Pubmed citation identified by role (steering committee, lead consultant, lead trainee, data collector).

Contact:

badcapstudy@gmail.com

References

1. Lamberts MP, Lugtenberg M, Rovers MM, Roukema AJ, Drenth JP, Westert GP, et al. Persistent and de novo symptoms after cholecystectomy: a systematic review of cholecystectomy effectiveness. Surgical endoscopy. 2013;27(3):709-18.

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5. Inagaki T, Choi M, Moschetta A, Peng L, Cummins CL, McDonald JG, et al. Fibroblast growth factor 15 functions as an enterohepatic signal to regulate bile acid homeostasis. Cell metabolism. 2005;2(4):217-25.

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7. Boyd GS, Merrick MV, Monks R, Thomas IL. Se-75-labeled bile acid analogs, new radiopharmaceuticals for investigating the enterohepatic circulation. Journal of nuclear medicine : official publication, Society of Nuclear Medicine. 1981;22(8):720-5.

8. Arasaradnam RP, Brown S, Forbes A, Fox MR, Hungin P, Kelman L, et al. Guidelines for the investigation of chronic diarrhoea in adults: British Society of Gastroenterology, 3rd edition. Gut. 2018.

Appendix 9 – CRF

Hospital number:_____

NHS number_____

Age 20-25 ☐ 26-30 ☐ 31-35 ☐

36-40 ☐ 41-45 ☐ 46-50 ☐

51-55 ☐ 56-60 ☐ 61-65 ☐

66-70 ☐ 71-75 ☐ 76-80 ☐

81-85 ☐ 86-90 ☐ 91-95 ☐

96-100 ☐

Gender Male ☐ Female ☐

History of IBD Ulcerative colitis ☐ Crohn's ☐

Indeterminate ☐ None ☐

Previous bowel resection involving terminal ileum Yes ☐ No ☐

Indication for ^{75}SeHCAT _____

Date of ^{75}SeHCAT test _____

^{75}SeHCAT result 0-5% ☐ 6-10% ☐ 11-15% ☐

>15% ☐

Indication for cholecystectomy Gallstones ☐ Acalculous cholecystitis ☐

Gallbladder polyps ☐ Gallstones and polyps ☐

Other ☐

Date of cholecystectomy _____

Colonoscopy/flex sig Yes ☐ No ☐

Date of colonoscopy_____

Endoscopy result Normal ☐ IBD ☐ polyps ☐

Cancer ☐ Diverticular disease ☐ Other ☐

CT scan Yes ☐ No ☐

Date of CT _____

CT result Normal ☐ IBD ☐ bowel tumour ☐

Non-bowel pathology ☐ Diverticular disease ☐ Other ☐

Episodes of Diarrhoea/day 1-5 ☐ 6-10 ☐ 11-15 ☐ >15 ☐

Duration of diarrhoea >4 weeks ☐ <4 weeks ☐

Final diagnosis Bile Acid malabsorption ☐ IBD ☐

Dumping syndrome ☐ Bowel cancer ☐

Infectious ☐ IBS ☐

Unknown ☐

Appendix 10: Publication: Rates of Bile Acid Diarrhoea after Cholecystectomy: A Multicentre audit

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ORIGINAL SCIENTIFIC REPORT

Rates of Bile Acid Diarrhoea After Cholecystectomy: A Multicentre Audit

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Abstract

Introduction Bile acid diarrhoea (BAD) can occur due to disruption to the enterohepatic circulation, e.g. following cholecystectomy. Post-cholecystectomy diarrhoea has been reported in 2.1–57.2% of patients; however, this is not necessarily due to BAD. The aim of this study was to determine the rates of bile acid diarrhoea diagnosis after cholecystectomy and to consider investigation practices.

Methods A retrospective analysis of electronic databases from five large centres detailing patients who underwent laparoscopic cholecystectomy between 2013 and 2017 was cross-referenced with a list of patients who underwent ^{75}SeHCAT testing. A 7-day retention time of <15% was deemed to be positive. Patient demographics and time from surgery to investigation were collected and compared for significance ($p < 0.05$).

Results A total of 9439 patients underwent a laparoscopic cholecystectomy between 1 January 2013 and 31 December 2017 in the five centres. In total, 202 patients (2.1%) underwent investigation for diarrhoea via ^{75}SeHCAT, of which 64 patients (31.6%) had a ^{75}SeHCAT test result of >15%, while 62.8% of those investigated were diagnosed with bile acid diarrhoea (BAD). In total, 133 (65.8%) patients also underwent endoscopy and 74 (36.6%) patients had a CT scan. Median time from surgery to ^{75}SeHCAT test was 672 days (SD ± 482 days).

Discussion/Conclusion Only a small proportion of patients, post-cholecystectomy, were investigated for diarrhoea with significant time delay to diagnosis. The true prevalence of BAD after cholecystectomy may be much higher, and clinicians need to have an increased awareness of this condition due to its amenability to treatment. ^{75}SeHCAT is a useful tool for diagnosis of bile acid diarrhoea.

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Introduction

Cholecystectomy is a common surgical procedure performed for diseases of the gallbladder, commonly offered for the treatment of symptomatic gallstones [1]. However, post-operatively some patients may develop symptoms which can cause discomfort and disruption to their quality of life, one of which is diarrhoea. The frequency of diarrhoea in the post-operative period is highly variable with previous studies identifying prevalence of up to 57.2% [2–6]. The high variability within the literature is the result of most studies not being specifically powered to investigate post-cholecystectomy diarrhoea. One of the causes of post-cholecystectomy diarrhoea is bile acid diarrhoea (BAD) [7].

The British Society of Gastroenterology (BSG) guidelines for investigation of chronic diarrhoea suggest endoscopic examination and a ^{75}SeHCAT scan as first-line investigations[8]. ^{75}SeHCAT testing is useful to determine bile acid diarrhoea where patients who have a less than 15% retention of gamma-emitting Selenium-75-homocholic acid taurine are diagnosed with bile acid diarrhoea. This is divided into three groups, with 11–15% retention classified as mild, while 6–10% retention is moderate and less than 5% is severe. The cut-off value of 15% demonstrated a 100% sensitivity and 91% specificity [9]. While there are other ways of diagnosing BAD such as using serum C4 and faecal bile acid levels, the ^{75}SeHCAT scan is more commonly used in the UK [10]. It is a condition which is amenable to treatment with bile acid sequestrants; however, it is often overlooked [10].

In this study we aimed to accurately determine the incidence of post-cholecystectomy diarrhoea across a number of hospital sites, how many patients are investigated, and how much of this is bile acid diarrhoea.

Methods

This project was a multicentre retrospective study. Local approval was sought from the Research and Development unit of each centre separately for retrospective review of data.

An electronic retrospective database of patients undergoing laparoscopic cholecystectomy between January 2013 and December 2017 was cross-referenced with all the patients who underwent ^{75}SeHCAT testing during the same time period at these centres. A 7-day^{75}SeHCAT retention of less than 15% was deemed to be positive. Patient demographics were collected and compared for significance ($p < 0.05$) Mann–Whitney U test. Time from surgery to investigation was also noted, and any differences between men and women were compared using a Mann–Whitney *U* test. To further investigate this, a log of the time from cholecystectomy to investigation was taken and a Student T test was used to determine whether there were still differences in investigation times. To further quantify this difference, a regression model of time to investigation adjusted for sex was also performed. Statistical advice was sought in the data analysis.

Results

A total of 9439 patients underwent a laparoscopic cholecystectomy between 1 January 2013 and 31 December 2017 in five UK centres: Oxford University Hospitals, North Bristol NHS Trust; Royal United Hospitals Bath NHS Trust, Queen Elizabeth University Hospital Glasgow and University Hospitals Coventry, and Warwickshire NHS Trust. Of these, 202 patients (2.1%) were investigated for BAD via ^{75}SeHCAT.

Demographic data

The sampled population consisted of 160 female patients (80%) and 42 male patients (20%). The age range of patients was from 20 to 90 with the highest number of patients diagnosed with BAD between the ages of 46 and 50. All patients younger than 35 were females, and the proportion of male patients increased after the age of 51. This is shown in Table 1, and the proportion of diagnosis is shown in Fig. 1.

Of patients included in the study, 10 patients had known inflammatory bowel disease (IBD) prior to laparoscopic cholecystectomy, this being Crohn's disease (six patients), ulcerative colitis (one patient), or indeterminate colitis (three patients). Five patients had had terminal ileal resection, only one of which had Crohn's disease.

In total, 127 patients were diagnosed with bile acid diarrhoea (62.8% of those investigated), and four patients were newly diagnosed with IBD. Nine patients were diagnosed with IBS, and two were diagnosed as chronic pancreatic insufficiency and four as chronic cholecystitis. One patient was diagnosed with an insulinoma of the pancreas, another with Sphincter of Oddi dysfunction, one with dumping syndrome, and another with functional bowel disorder. However, 38 patients (18.8%) had a diagnosis of 'unknown' at the end. This is also seen in Table 1.

Indications for ^{75}SeHCAT testing

Indications for ^{75}SeHCAT referral were mainly due to diarrhoea, chronic diarrhoea, loose stool, or watery stool

Table 1 Demographics

Age	Number of patients (male:female)	Endoscopy *n* (%)	CT *n* (%)	Final diagnosis of BAD *n* (%)	Final diagnosis of IBD *n* (%)	Final diagnosis of IBS *n* (%)	Final diagnosis unknown *n* (%)
20–25	9 (0:9)	6 (2.9%)	1 (0.5%)	7 (3.5%)	0	1 (0.5%)	0
26–30	13 (0:12)	8 (3.9%)	4 (1.9%)	11 (5.4%)	0	0	2 (0.9%)
31–35	11 (0:11)	5 (2.5%)	1 (0.5%)	7 (3.5%)	1 (0.5%)	0	2 (0.9%)
36–40	17 (2:13)	9 (4.5%)	5 (2.5%)	10 (4.9%)	0	1 (0.5%)	2 (0.9%)
41–45	27 (4:23)	17 (8.4%)	8 (3.9%)	14 (6.9%)	0	1 (0.5%)	8 (3.9%)
46–50	25 (3:22)	15 (7.4%)	6 (2.9%)	18 (8.9%)	1 (0.5%)	1 (0.5%)	1 (0.5%)
51–55	26 (7:19)	16 (7.9%)	6 (2.9%)	15 (7.4%)	0	3 (1.5%)	4 (1.9%)
56–60	19 (4:15)	13 (6.4%)	6 (2.9%)	12 (5.9%)	0	1 (0.5%)	4 (1.9%)
61–65	21 (7:14)	14 (6.9%)	12 (5.9%)	9 (4.5%)	1 (0.5%)	0	8 (3.9%)
66–70	11 (4:7)	11 (5.4%)	6 (2.9%)	8 (3.9%)	0	1 (0.5%)	1 (0.5%)
71–75	15 (5:10)	11 (5.4%)	7 (3.5%)	10 (4.9%)	1 (0.5%)	0	3 (1.5%)
76–80	7 (4:3)	2 (0.9%)	5 (2.5%)	4 (1.9%)	0	0	1 (0.5%)
81–85	2 (0:2)	2 (0.9%)	2 (0.9%)	1(0.5%)	0	0	1 (0.5%)
86–90	2 (2:0)	1 (0.5%)	0	1 (0.5%)	0	0	1 (0.5%)

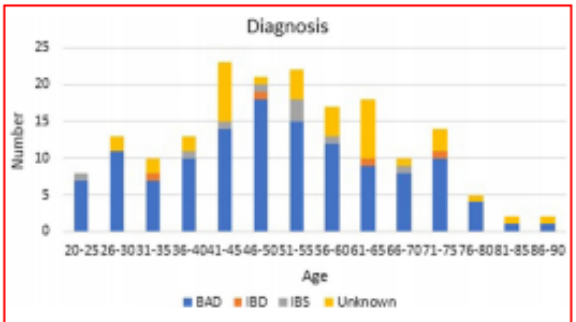

Fig. 1 Diagnosis (*BAD* bile acid diarrhoea; *IBD* inflammatory bowel disease; *IBS* irritable bowel syndrome)

(137 patients). In total, 21 patients were simply referred as "query of bile acid diarrhoea" or "bile acid malabsorption". Seven patients were listed as having a change in bowel habit, and a further 17 patients reported abdominal pain, often accompanied by diarrhoea. Other reasons for referral included steatorrheoa and bloating.

Other investigations

In total, 133 (65.8%) patients also underwent endoscopic examination (colonoscopy or flexible sigmoidoscopy) of which 86 were normal, 29 showed diverticular disease, 16 showed polyps (tubular adenomas), and two showed mild inflammation. Of those with a normal endoscopy, 43 were eventually diagnosed as having BAD.

In total, 74 (36.6%) patients had a CT scan of the abdomen and pelvis. Of these, 45 were normal, 11 showed diverticular disease, 2 demonstrated inflammatory bowel disease, and 15 showed non-bowel-related pathology.

^{75}SeHCAT results and correlation with symptoms

The distribution of patients and their ^{75}SeHCAT results is shown in Table 2. All patients had diarrhoea duration of >4 weeks. In total, 104 patients had one to five episodes per day, 34 had six to ten episodes a day, 10 patients had eleven to fifteen episodes per day, and 3 patients had more than fifteen episodes per day. For the remainder, bowel frequency was not recorded by the assessing clinician. There was no significant correlation between the ^{75}SeHCAT result and the number of episodes of diarrhoea per day ($p = 0.382$, using Chi-squared test). This is also seen in Table 2.

Table 2 ^{75}SeHCAT results and correlation with bowel habits

^{75}SeHCAT results	<5%	6–10%	11–15%	>15%
Total	72	40	26	64
Male	17	11	4	10
Female	55	29	22	54
1–5 episodes/day	28	19	16	41
6–10 episodes/day	20	6	2	6
11–15 episodes/day	5	3	1	1
>15 episodes/day	2	1	0	0

Time to investigation

There was no significant difference between men and women in time from laparoscopic cholecystectomy to referral for ^{75} SeHCAT scan or endoscopy. There was a significant difference between referral time for men and women for CT scan ($p = 0.022$); however, this does not hold up on taking a log and performing a Students' t test, or on performing a regression analysis adjusting for sex. This is shown in Table 3 and Figs. 2, 3, and 4

Discussion

One reason for the development of post-cholecystectomy diarrhoea is from disruption to the enterohepatic circulation, causing hepatic overproduction of bile acids. This is known as bile acid diarrhoea (BAD) of which there are three types: type one occurs secondary to ileal inflammation, thus interfering with bile acid absorption; type two is primary or idiopathic; and type three occurs secondary to other conditions where the ileum appears normal. In the latter, one of these conditions is following cholecystectomy [11, 12].

The mechanism of action to balance bile acid secretion is a negative feedback loop. Bile acid reabsorption in the ileum leads to activation of ileal FXR (farnesoid × receptor), thus inducing transcription of FGF19 (fibroblast growth factor 19) which then activates hepatic FXR. This inhibits CYP7A1 (cholesterol 7-αhydroxylase), which is the rate-limiting enzyme in bile acid synthesis, thus decreasing bile acid formation. When this is disrupted, as in BAD, there is overproduction of FGF19 leading to higher concentrations of bile acids which, in turn, leads to diarrhoea [12, 13].

In this study involving collaboration from five tertiary centres, only a small number of patients (2.1%) were investigated for diarrhoea following laparoscopic cholecystectomy. This may imply either that the rest of the patients did not require any investigation as they did not develop diarrhoea, or that their symptoms were short term and settled spontaneously without warranting medical investigation. The published literature reveals a large

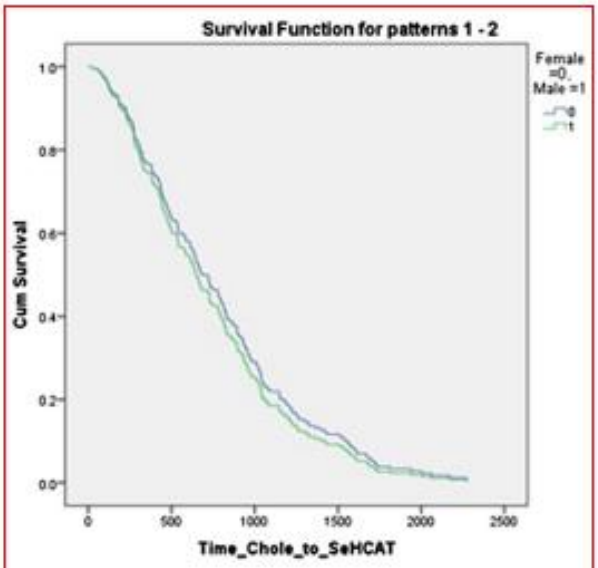

Fig. 2 Regression analysis for time to 75 SEHCAT, adjusted for sex

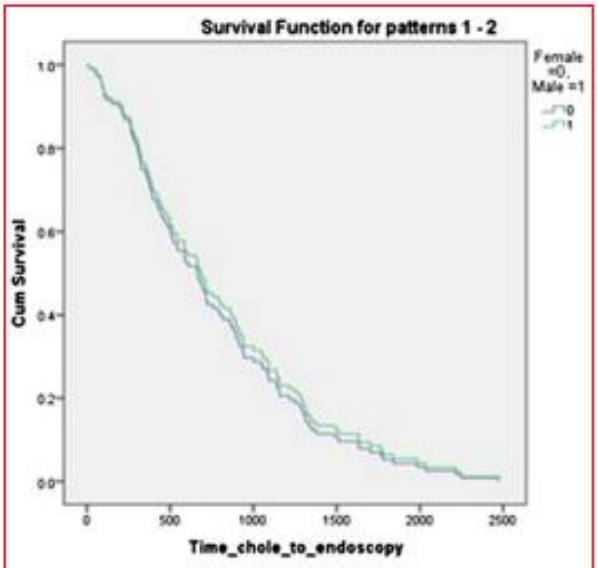

Fig. 3 Regression analysis for time to endoscopy, adjusted for sex

Table 3 Comparison of male and female median time from cholecystectomy to investigation

	Total/days (SD)	Female/days (SD)	Male/days (SD)	p value (Mann–Whitney U test)	p value(log and T test)	Regression analysis p value (hazard ratio with 95%CI)
^{75}SeHCAT	672 (482)	726 (461)	539 (548)	0.139	0.212	0.55 (0.901; 0.63.–1.277)
Endoscopy	696 (545)	723 (517)	545 (623)	0.290	0.66	0.739 (1.078; 0.691–1.682)
CT	778 (595)	938 (531)	388 (709)	0.022	0.41	0.323 (1.39; 0.723–2.674)

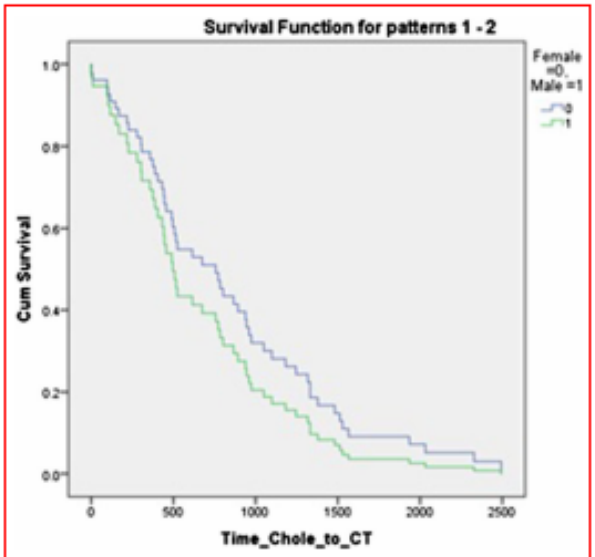

Fig. 4 Regression analysis for time to CT, adjusted for sex

variation in the quoted incidence of post-cholecystectomy diarrhoea. This ranges from 2.1 to 57.2% [2–6, 14]. Our own review of the literature showed a post-cholecystectomy diarrhoea rate of 13% (Farrugia et al., Post-Cholecystectomy diarrhoea rate and predictive factors—a systematic review of the literature). Despite this, the true rate of post-cholecystectomy diarrhoea due to altered bile acid physiology has not been determined. C4 (7α-hydroxy-4-cholesten-3-one) levels, which directly correlate with bile acid synthesis, have been shown to increase following cholecystectomy, while FGF19 levels decrease [5, 15]. Despite this, the increase in C4 levels has not been shown to be related to increased frequency of bowel movements or type of stool [5].

Thus, the number of patients being investigated does not necessarily correlate with the presumed rate of post-cholecystectomy diarrhoea that is reported in the literature. This may be due to a lack of awareness that diarrhoea may develop after cholecystectomy due to faults in the pre-operative consent process. Indeed, up to 70.3% of patients are not being consented for the possibility of developing diarrhoea after laparoscopic cholecystectomy [16].

There is a clear delay in initiating investigations, with a median of 672 days between surgery and ^{75}SeHCAT testing found in this study, implying that there is poor awareness within the medical community of the possibility of developing BAD after cholecystectomy. There was a difference in time to investigation between women and men, with median time to testing for female patients being 726 days while median time to testing for male patients, 539 days. While not statistically significant ($p = 0.139$), there is a median difference of 187 days. This may imply that complaints are not well regarded and in indeed one study suggests that there is a perceived reduction in constipation in women after cholecystectomy, but no real diarrhoea [17]. However, we can see from our results that it is not simply perception as patients have had positive ^{75}SeHCAT tests after developing diarrhoea post-cholecystectomy.

Furthermore, we have noted that not all patients underwent endoscopic investigation in addition to ^{75}SeHCAT testing, as is recommended by the British Society of Gastroenterology guidelines [8]. This could also imply that inflammatory bowel disease (IBD) was not excluded in all patients. As IBD (ileal Crohn's) can be a cause of BAD, this is a confounding factor in our study. Another confounding factor is that some patients were known to have Crohn's disease prior to laparoscopic cholecystectomy and others had had a previous right hemicolectomy for other conditions. As both of these factors affect the terminal ileum and may lead to bile acid malabsorption, it is unclear, for these patients, whether the BAD that developed was a consequence of malabsorption from the terminal ileum, or from bile acid overproduction following cholecystectomy, or perhaps a mixture of both. With endoscopic investigations there was an added delay of 178 days between women and men (median of 723 days for women and 545 days for men). Whilst failing to reach statistical significance ($p = 0.29$), it does represent an extra period of time with a reduced quality of life [18].

Despite CT scan being more useful in the investigation of structural rather than functional disorders, a large number of patients still had a CT scan as part of their initial investigation. In this there was a significant difference between referral time for women and men ($p = 0.022$), 938 days for women and 388 days for men. For all investigations, the median time to investigation of female patients was longer. This is a pattern that has been previously reported in other aspects of healthcare, resulting in higher morbidity and mortality for female patients [19, 20]. It is also interesting as CT scan is not recommended by the BSG guidelines for the investigation of chronic diarrhoea. However, there may have been other aspect if the clinical history led to a referral for CT scan.

Despite men being investigated (^{75}SeHCAT, endoscopy and CT scan) more rapidly from initial presentation compared to women, we can still see that there is a significant delay in initiating investigations after laparoscopic cholecystectomy with a median time to investigation longer than 18 months for each investigation. Symptoms tend to develop within the first 3 months after cholecystectomy, and it is therefore apparent that these patients are not being investigated in a timely manner [21] and to the detriment of their quality of life [18]. However, there may be other

issues at play such as social factors preventing some patients from seeking help or attending for tests, delays resulting from local processes such as referral practices and waiting list times for tests such as ^{75}SeHCAT (which is not found in all centres) and endoscopy waiting times. As such, it is difficult to say what effect this has on time from cholecystectomy to testing. As this is a multicentre study there may also be differences in practice between regions to take into account.

This study has confirmed that the degree of BAD, as seen on the ^{75}SeHCAT result, does not necessarily correlate with patient symptoms ($p = 0.382$), which is in keeping with previous work on the subject [22]. However, all patients were investigated after having diarrhoea for 4 weeks and the majority had a up to 10 episodes per day, which is congruent with the BSG guidelines for the investigation of chronic diarrhoea [8]. It is also interesting to note that whilst 62.8% of the cohort was diagnosed with BAD and 18.4% had another diagnosis, in 18.8% of patients a definitive diagnosis was not secured. This highlights that further work is required in this area to benefit this large group of patients with clinical symptoms.

We found that patients younger than 35 years of age were all females and there are generally fewer males in each age group under the age of 50. This seems to imply that younger women are at higher risk of developing PCD in our dataset. This correlates with some studies [23] but not with others that suggest younger males to be more at risk [4, 24, 25].

This study is based upon real-time linked clinical data, thus showing the true perspective of patients who were investigated post-laparoscopic cholecystectomy for diarrhoea. Patients who were empirically started on bile acid sequestrants rather than being investigated via ^{75}SeHCAT would not have been captured in the present study. Another possible limitation is that not all patients who develop diarrhoea are investigated via ^{75}SeHCAT; thus, the true numerator remains unknown. BAD is not a well-known condition, and therefore, the only patients who were referred for ^{75}SeHCAT testing were those seen by GPs, physicians, and surgeons who are aware of the condition. We also have no data regarding response to treatment in these patients identified here who were diagnosed with BAD. We have identified a large discrepancy between the number of male and female patients within our dataset, as such there may be an element of selection bias. However, the advantage of this study is that it is a multicentre study using ^{75}SeCHAT as the investigation of choice with defined cut-off values for diagnosis of BAD. It also benchmarks the current clinical scenario when it comes to the investigation of chronic diarrhoea after cholecystectomy. While this is the largest study of its kind to date, further studies involving direct comparison between those patients investigated, and those who are not, for diarrhoea following cholecystectomy would present a more comprehensive picture of this difficult condition and would not only improve our understanding but allow for improved patient care.

Conclusion

A small proportion of post-cholecystectomy patients were investigated for BAD (2.1%), and in those that were investigated 62.8% were positive for BAD as indicated by ^{75}SeHCAT testing (^{75}SeHCAT results <15%). There was a significant time delay to diagnosis following the onset of symptoms. This may in part be because cholecystectomy is mostly undertaken as a day case procedure and routine follow-up is rarely required. The true prevalence of BAD post-cholecystectomy may be much higher, and clinicians in both primary and secondary care need to have an increased awareness of this condition due to its amenability to treatment. Other options including serum C4 and faecal measurements of bile acid remain alternatives where ^{75}SeHCAT is unavailable.

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Author Contributions AF, JAA, SM, SK, NW, RPA were involved in setting up the protocol. AF and SM contacted other centres for inclusion. AF, SH and SB were involved in communicating with other centres during the study period. AF, JAA, SH, SB and SM were involved in the creation of the data collection sheet. AF, JAA, SH and SB analysed the data. AF, JAA, SH and SB wrote the manuscript. RPA, NW, SK and SM reviewed the manuscript. RPA, NW and SK supervised the whole project. MAA, RA, GBS, BC, SD, BM, MA, AO, RP, MS, ES, SS, WS, FT, JW and RW were involved in setting up local audit approvals and collecting data in the various centres and reviewed the final manuscript. NP provided statistical advice.

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Compliance with ethical standards

Competing interests None declared.

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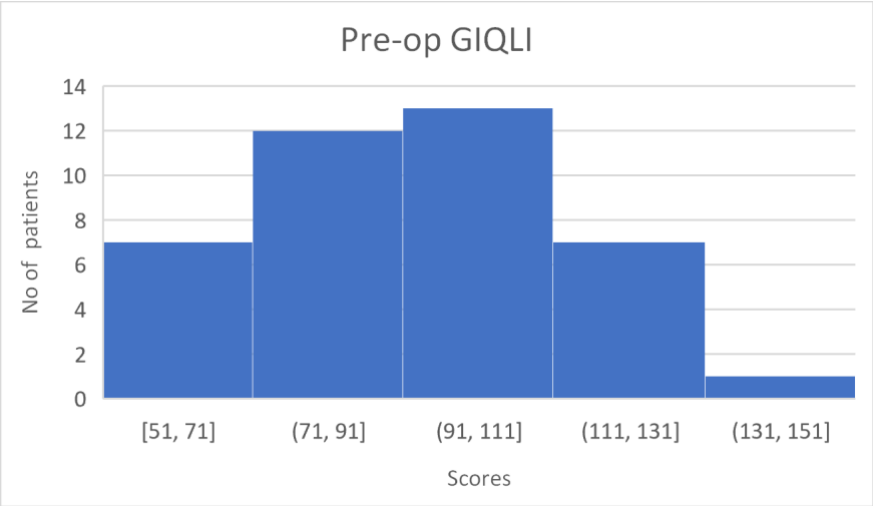
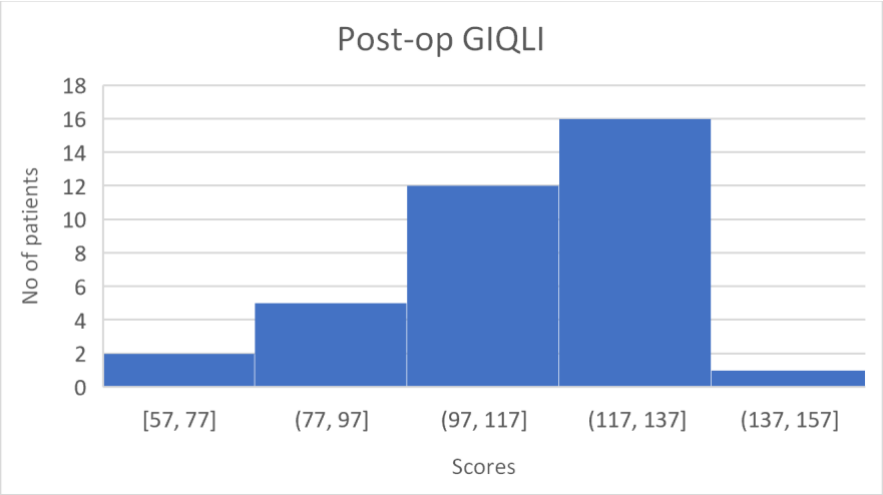
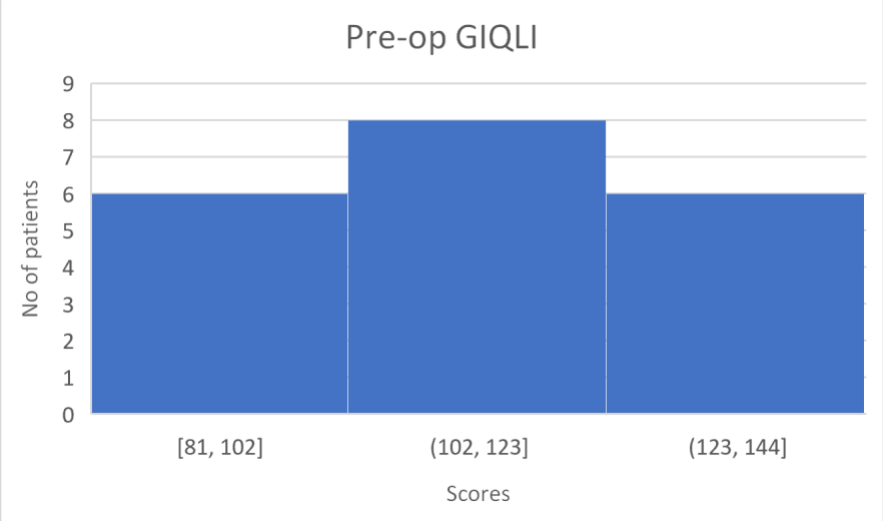
Appendix 11 – distribution graphs for GIQLI

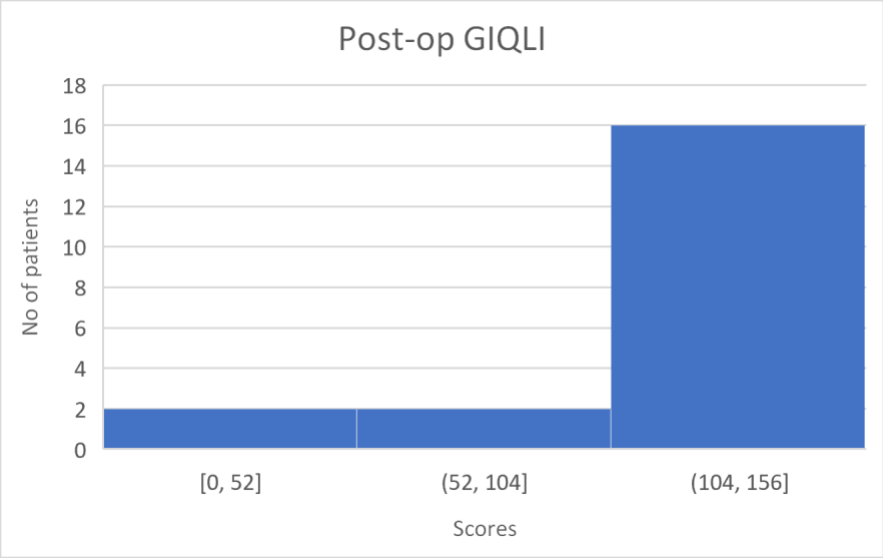
Study group





Control group





Appendix 12: ELISA raw data

	1	2	3	4	5	6	7	8	9	10	11	12
A	2.617	2.312	1.583	1.385	1.119	0.999	1.235	1.62	1.137	1.597	1.293	2.554
B	2.647	2.137	1.638	1.329	1.218	1.013	1.3	1.751	1.127	1.455	1.365	2.439
C	2.648	2.281	1.68	1.453	0.999	0.997	1.21	1.63	1.158	1.754	1.619	2.578
D	2.537	1.606	1.543	1.368	0.818	1.227	1.41	1.779	1.205	1.421	1.252	2.652
E	2.178	0.777	1.089	1.035	1	1.26	0.893	1.651	1.154	1.477	1.202	2.008
F	1.269	1.269	1.034	1.159	1.615	0.996	1.292	1.859	1.426	2.202	1.38	2.436
G	2.351	0.897	0.962	1.19	0.847	1.363	0.915	1.664	1.195	1.244	1.065	2.3
H	1.72	0.721	0.95	1.567	0.831	0.755	1.237	1.722	1.073	1.396	1.195	2.604

Test 1 FGF19 gallbladder samples BCLC001 - BCLC031 05/12/2019

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1	BCLC001	BCLC001	BCLC010	BCLC010	BCLC018	BCLC018	BCLC026	BCLC026	BLANK	BLANK
B	S2	S2	BCLC002	BCLC002	BCLC011	BCLC011	BBLC019	BBLC019	BCLC027	BCLC027	BLANK	BLANK
C	S3	S3	BCLC003	BCLC003	BCLC012	BCLC012	BCLC020	BCLC020	BCLC028	BCLC028	BLANK	BLANK
D	S4	S4	BCLC004	BCLC004	BCLC013	BCLC013	BCLC021	BCLC021	BCLC029	BCLC029	BLANK	BLANK
E	S5	S5	BCLC005	BCLC005	BCLC014	BCLC014	BCLC022	BCLC022	BCLC030	BCLC030	BLANK	BLANK
F	S6	S6	BCLC006	BCLC006	BCLC015	BCLC015	BCLC023	BCLC023	BCLC031	BCLC031	BLANK	BLANK
G	S7	S7	BCLC007	BCLC007	BCLC016	BCLC016	BCLC024	BCLC024	BLANK	BLANK	BLANK	BLANK
H	BLANK	BLANK	BCLC008	BCLC008	BCLC017	BCLC017	BCLC025	BCLC025	BLANK	BLANK	BLANK	BLANK

Test 1 FGF19 gallbladder samples BCLC001 - BCLC031 05/12/2019 Legend. S = standard, BCLC*** - sample, BLANK- no sample*

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.651	0.841	0.217	0.207	0.387	0.468	0.511	0.452	1.327	1.286	0.063	0.065
B	0.358	0.464	1.275	1.498	0.161	0.222	0.236	0.368	0.315	0.297	0.061	0.065
C	0.206	0.218	0.57	0.572	0.763	0.725	0.105	0.106	0.132	0.167	0.069	0.066
D	0.126	0.12	0.576	0.692	0.104	0.101	0.185	0.204	0.507	0.438	0.064	0.063
E	0.11	0.089	0.764	0.886	0.751	0.898	0.16	0.262	0.071	0.073	0.063	0.064
F	0.093	0.076	0.173	0.167	0.281	0.261	0.124	0.092	0.377	0.466	0.064	0.063
G	0.073	0.082	0.163	0.171	0.096	0.161	0.269	0.137	0.066	0.063	0.062	0.063
H	0.072	0.081	0.117	0.107	0.149	0.18	0.227	0.21	0.063	0.063	0.065	0.062

Test 2 FGF19 gallbladder samples BCLC001 - BCLC031 14/01/2020

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1	BCLC001	BCLC001	BCLC010	BCLC010	BCLC018	BCLC018	BCLC026	BCLC026	BLANK	BLANK
B	S2	S2	BCLC002	BCLC002	BCLC011	BCLC011	BBLC019	BBLC019	BCLC027	BCLC027	BLANK	BLANK
C	S3	S3	BCLC003	BCLC003	BCLC012	BCLC012	BCLC020	BCLC020	BCLC028	BCLC028	BLANK	BLANK
D	S4	S4	BCLC004	BCLC004	BCLC013	BCLC013	BCLC021	BCLC021	BCLC029	BCLC029	BLANK	BLANK
E	S5	S5	BCLC005	BCLC005	BCLC014	BCLC014	BCLC022	BCLC022	BCLC030	BCLC030	BLANK	BLANK
F	S6	S6	BCLC006	BCLC006	BCLC015	BCLC015	BCLC023	BCLC023	BCLC031	BCLC031	BLANK	BLANK
G	S7	S7	BCLC007	BCLC007	BCLC016	BCLC016	BCLC024	BCLC024	BLANK	BLANK	BLANK	BLANK
H	BLANK	BLANK	BCLC008	BCLC008	BCLC017	BCLC017	BCLC025	BCLC025	BLANK	BLANK	BLANK	BLANK

Test 2 FGF19 gallbladder samples BCLC001 - BCLC031 14/01/2020 figure legend S = standard, BCLC*** - sample, BLANK- no sample*

	1	2	3	4	5	6	7	8	9	10	11	12
A	1.999	1.394	0.468	0.511	0.025	0.044	0.035	0.044	1.678	0.028	0.151	0.035
B	1.537	1.535	0.572	0.763	0.046	0.028	0.095	0.049	0.047	0.031	0.047	0.032
C	1.034	0.977	0.507	0.438	0.041	0.029	0.037	0.041	0.035	0.037	0.042	0.029
D	0.523	0.589	0.764	0.886	0.048	0.042	0.05	2.678	2.683	0.05	0.129	0.038
E	0.303	0.323	0.927	0.897	0.064	0.043	0.038	0.111	0.052	0.042	0.03	0.056
F	0.175	0.198	0.255	0.347	0.037	1.987	0.038	0.048	0.044	0.045	0.04	0.026
G	0.124	0.133	0.555	0.249	0.045	2.476	0.061	0.038	0.113	0.076	0.036	0.039
H	0.082	0.081	0.098	0.097	0.045	0.042	0.049	0.048	0.043	0.089	0.184	0.048

Test 1 FGF19 gallbladder samples BCLC032 - BCLC040 16/07/2020

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1	BCLC032	BCLC032	BLANK	BLANK	BLANK	BLANK	BLANK	BLANK	BLANK	BLANK
B	S2	S2	BCLC033	BCLC033	BLANK	BLANK	BLANK	BLANK	BLANK	BLANK	BLANK	BLANK
C	S3	S3	BCLC035	BCLC035	BLANK	BLANK	BLANK	BLANK	BLANK	BLANK	BLANK	BLANK
D	S4	S4	BCLC036	BCLC036	BLANK	BLANK	BLANK	BLANK	BLANK	BLANK	BLANK	BLANK
E	S5	S5	BCLC037	BCLC037	BLANK	BLANK	BLANK	BLANK	BLANK	BLANK	BLANK	BLANK
F	S6	S6	BCLC038	BCLC038	BLANK	BLANK	BLANK	BLANK	BLANK	BLANK	BLANK	BLANK
G	S7	S7	BCLC039	BCLC039	BLANK	BLANK	BLANK	BLANK	BLANK	BLANK	BLANK	BLANK
H	BLANK	BLANK	BCLC040	BCLC040	BLANK	BLANK	BLANK	BLANK	BLANK	BLANK	BLANK	BLANK

Test 1 FGF19 gallbladder samples BCLC032 - BCLC040 16/07/2020 figure legend. S = standard, BCLC*** - sample, BLANK- no sample*

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	1.892	1.712	0.133	0.204	0.162	0.165	0.199	0.218	0.231	0.26	0.258	0.26
B	1.082	1.148	0.19	0.169	0.169	0.155	0.199	0.158	0.205	0.174	0.187	0.21
C	0.622	0.645	0.2	0.166	0.168	0.167	0.194	0.158	0.172	0.163	0.175	0.18
D	0.413	0.421	0.164	0.162	0.09	0.135	0.158	0.156	0.169	0.154	0.176	0.15
E	0.248	0.237	0.17	0.136	0.142	0.151	0.158	0.151	0.169	0.153	0.149	0.139
F	0.203	0.203	0.174	0.166	0.164	0.166	0.16	0.158	0.163	0.16	0.178	0.144
G	0.179	0.185	0.165	0.141	0.123	0.137	0.149	0.152	0.153	0.156	0.177	0.162
H	0.167	0.173	0.079	0.148	0.074	0.075	0.077	0.051	0.052	0.048	0.051	0.051

Test 1 SHP gallbladder samples BCLC001-BCLC040 06/08/2020

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	S1	S1	BCLC001	BCLC001	BCLC010	BCLC010	BCLC018	BCLC018	BCLC027	BCLC027	BCLC036	BCLC036
B	S2	S2	BCLC002	BCLC002	BCLC011	BCLC011	BBLC019	BBLC019	BCLC028	BCLC028	BCLC037	BCLC037
C	S3	S3	BCLC003	BCLC003	BCLC012	BCLC012	BCLC020	BCLC020	BCLC029	BCLC029	BCLC038	BCLC038
D	S4	S4	BCLC004	BCLC004	BCLC013	BCLC013	BCLC021	BCLC021	BCLC030	BCLC030	BCLC039	BCLC039
E	S5	S5	BCLC005	BCLC005	BCLC014	BCLC014	BCLC022	BCLC022	BCLC031	BCLC031	BCLC040	BCLC040
F	S6	S6	BCLC006	BCLC006	BCLC015	BCLC015	BCLC023	BCLC023	BCLC032	BCLC032	BLANK	BLANK
G	S7	S7	BCLC007	BCLC007	BCLC016	BCLC016	BCLC024	BCLC024	BCLC033	BCLC033	BLANK	BLANK
H	BLANK	BLANK	BCLC008	BCLC008	BCLC017	BCLC017	BCLC026	BCLC026	BCLC035	BCLC035	BLANK	BLANK

Test 1 SHP gallbladder samples BCLC001-BCLC040 06/08/2020 figure legend. S = standard, BCLC*** - sample, BLANK- no sample*

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.062	0.062	0.067	0.065	0.06	0.054	0.065	0.053	0.051	0.061	0.057	0.067
B	0.061	0.054	0.06	0.057	0.05	0.049	0.055	0.059	0.08	0.071	0.063	0.071
C	0.1	0.053	0.062	0.056	0.054	0.052	0.058	0.054	0.056	0.068	0.07	0.066
D	0.06	0.064	0.062	0.058	0.057	0.075	0.067	0.061	0.068	0.072	0.054	0.077
E	0.055	0.067	0.069	0.064	0.062	0.076	0.065	0.071	0.077	0.067	0.066	0.079
F	0.072	0.074	0.064	0.067	0.064	0.063	0.075	0.074	0.069	0.064	0.065	0.08
G	0.068	0.066	0.061	0.062	0.059	0.068	0.07	0.059	0.071	0.064	0.092	0.063
H	0.079	0.059	0.068	0.067	0.069	0.074	0.073	0.064	0.074	0.11	0.06	0.063

Test 2 SHP gallbladder samples BCLC001-BCLC040 13/08/2020

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1	BCLC001	BCLC001	BCLC010	BCLC010	BCLC018	BCLC018	BCLC027	BCLC027	BCLC036	BCLC036
B	S2	S2	BCLC002	BCLC002	BCLC011	BCLC011	BBLC019	BBLC019	BCLC028	BCLC028	BCLC037	BCLC037
C	S3	S3	BCLC003	BCLC003	BCLC012	BCLC012	BCLC020	BCLC020	BCLC029	BCLC029	BCLC038	BCLC038
D	S4	S4	BCLC004	BCLC004	BCLC013	BCLC013	BCLC021	BCLC021	BCLC030	BCLC030	BCLC039	BCLC039
E	S5	S5	BCLC005	BCLC005	BCLC014	BCLC014	BCLC022	BCLC022	BCLC031	BCLC031	BCLC040	BCLC040
F	S6	S6	BCLC006	BCLC006	BCLC015	BCLC015	BCLC023	BCLC023	BCLC032	BCLC032	BLANK	BLANK
G	S7	S7	BCLC007	BCLC007	BCLC016	BCLC016	BCLC024	BCLC024	BCLC033	BCLC033	BLANK	BLANK
H	BLANK	BLANK	BCLC008	BCLC008	BCLC017	BCLC017	BCLC026	BCLC026	BCLC035	BCLC035	BLANK	BLANK

Test 2 SHP gallbladder samples BCLC001-BCLC040 13/08/2020 legend S = standard, BCLC*** - sample, BLANK- no sample*

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.731	0.751	0.068	0.056	0.065	0.069	0.056	0.069	0.072	0.067	0.084	0.087
B	0.541	0.513	0.088	0.059	0.067	0.061	0.068	0.07	0.07	0.061	0.074	0.094
C	0.32	0.31	0.075	0.073	0.067	0.073	0.067	0.077	0.071	0.061	0.078	0.081
D	0.191	0.205	0.054	0.07	0.056	0.07	0.068	0.073	0.065	0.068	0.075	0.07
E	0.121	0.136	0.07	0.057	0.069	0.067	0.088	0.072	0.069	0.071	0.07	0.071
F	0.101	0.101	0.063	0.063	0.059	0.064	0.067	0.069	0.067	0.088	0.082	0.065
G	0.08	0.081	0.067	0.06	0.057	0.059	0.063	0.063	0.07	0.068	0.064	0.064
H	0.08	0.067	0.064	0.056	0.064	0.066	0.06	0.063	0.07	0.069	0.067	0.075

Test 3 SHP gallbladder samples BCLC001-BCLC040 14/08/2020

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1	BCLC001	BCLC001	BCLC010	BCLC010	BCLC018	BCLC018	BCLC027	BCLC027	BCLC036	BCLC036
B	S2	S2	BCLC002	BCLC002	BCLC011	BCLC011	BBLC019	BBLC019	BCLC028	BCLC028	BCLC037	BCLC037
C	S3	S3	BCLC003	BCLC003	BCLC012	BCLC012	BCLC020	BCLC020	BCLC029	BCLC029	BCLC038	BCLC038
D	S4	S4	BCLC004	BCLC004	BCLC013	BCLC013	BCLC021	BCLC021	BCLC030	BCLC030	BCLC039	BCLC039
E	S5	S5	BCLC005	BCLC005	BCLC014	BCLC014	BCLC022	BCLC022	BCLC031	BCLC031	BCLC040	BCLC040
F	S6	S6	BCLC006	BCLC006	BCLC015	BCLC015	BCLC023	BCLC023	BCLC032	BCLC032	BLANK	BLANK
G	S7	S7	BCLC007	BCLC007	BCLC016	BCLC016	BCLC024	BCLC024	BCLC033	BCLC033	BLANK	BLANK
H	BLANK	BLANK	BCLC008	BCLC008	BCLC017	BCLC017	BCLC026	BCLC026	BCLC035	BCLC035	BLANK	BLANK

Test 3 SHP gallbladder samples BCLC001-BCLC040 14/08/2020 figure legend. S = standard, BCLC*** - sample, BLANK- no sample*

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.352	0.338	0.341	0.363	0.352	0.337	0.304	0.341	0.34	0.384	0.558	0.387
B	0.496	0.463	0.453	0.432	0.415	0.456	0.409	0.557	0.484	0.439	0.178	0.171
C	0.503	0.45	0.449	0.738	0.495	0.387	0.422	0.445	0.374	0.414	0.16	0.179
D	0.418	0.38	0.362	0.452	0.372	0.449	0.388	0.404	0.385	0.447	0.405	0.274
E	0.412	0.454	0.474	0.458	0.501	0.456	0.446	0.471	0.466	0.437	0.408	0.426
F	0.424	0.329	0.375	0.355	0.472	0.365	0.342	0.316	0.426	0.381		
G	0.432	0.452	0.462	0.404	0.47	0.428	0.371	0.363	0.456	0.466		
H	0.408	0.373	0.363	0.349	0.41	0.37	0.399	0.34	0.533	0.595		

Test 1 PPAR alpha gallbladder BCLC001-BCLC040 29/09/2020

	1	2	3	4	5	6	7	8	9	10	11	12
A	BCLC001	BCLC001	BCLC010	BCLC010	BCLC018	BCLC018	BCLC027	BCLC027	BCLC036	BCLC036	PC	PC
B	BCLC002	BCLC002	BCLC011	BCLC011	BBLC019	BBLC019	BCLC028	BCLC028	BCLC037	BCLC037	BLANK	BLANK
C	BCLC003	BCLC003	BCLC012	BCLC012	BCLC020	BCLC020	BCLC029	BCLC029	BCLC038	BCLC038	BLANK	BLANK
D	BCLC004	BCLC004	BCLC013	BCLC013	BCLC021	BCLC021	BCLC030	BCLC030	BCLC039	BCLC039	C1	C1
E	BCLC005	BCLC005	BCLC014	BCLC014	BCLC022	BCLC022	BCLC031	BCLC031	BCLC040	BCLC040	C1	C1
F	BCLC006	BCLC006	BCLC015	BCLC015	BCLC023	BCLC023	BCLC032	BCLC032	NSB	NSB		
G	BCLC007	BCLC007	BCLC016	BCLC016	BCLC024	BCLC024	BCLC033	BCLC033	NSB	NSB		
H	BCLC008	BCLC008	BCLC017	BCLC017	BCLC026	BCLC026	BCLC035	BCLC035	PC	PC		

*Test 1 PPAR alpha gallbladder BCLC001-BCLC040 29/09/2020 figure legend. BCLC*** - sample; NSB = non-specific binding wells, PC – positive control wells; BLANK – blank, C1 – specific competitor dsDNA wells*