Exploring the Effects of Bile Acid Inhibition

in

Cholestatic Pruritus

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ABSTRACT

Pruritus (itch) is an important symptom associated with cholestatic liver diseases. The aim of this work was to study cholestatic pruritus to further our understanding of the prevalence in primary biliary cholangitis (PBC) and explore the role of inhibiting circulating bile acids (BAs) in relieving cholestatic pruritus.

The cross-sectional study of pruritus from over 2800 patients from the UK-PBC research cohort showed that prevalence of pruritus in PBC is high (74%) with a significant proportion of patients reporting severe itch during the course of their disease. This study also highlighted the under-treatment of itch with inadequate use of guideline recommended drugs in the UK.

The impact of inhibiting circulating BAs on cholestatic pruritus was studied in two different ways- i) via nasobiliary drainage (NBD, i.e. external diversion of bile and BAs away from the ileum), and ii) via pharmacological inhibition of the ileal bile acid transporter (IBAT) that mediates enterohepatic circulation of BAs. The retrospective cohort study of NBD showed the intervention is a highly effective treatment, but only of short-term durability and associated with high complication rate. The phase 2 clinical trial of GSK2330672, a human IBAT inhibitor agent, showed that two-weeks of treatment significantly reduced pruritus severity compared to placebo.

The metabonomic and microbiome studies explored the serum metabonome and gut microbiota profile of pruritus in PBC. In addition, the effects of GSK2330672 on metabonome and gut microbiome were investigated. The study demonstrated that pruritus in PBC is associated with elevated serum total and glyco-conjugated BAs but no gut bacterial dysbiosis. Also, GSK2330672 was shown to reduce all taurine and glyco- conjugated serum BAs, increase faecal BAs and alter the gut-microbial composition.

Taken together, the research studies presented in this thesis suggest: i) high prevalence of pruritus and its under-treatment in PBC, ii) removal of BAs by NBD or inhibition by IBAT inhibitor drug improves cholestatic pruritus and, iii) serum BAs but not gut microbiome are altered in cholestatic pruritus and they can be modified by IBAT inhibitor treatment.

LIST OF PUBLICATIONS

Publications by the candidate relevant to the thesis:

Hegade VS, Pechlivanis A, McDonald JA, et al. Autotaxin, Bile Acid Profile and Effect of IBAT Inhibition in Primary Biliary Cholangitis Patients with Pruritus. *Liver Int*. 2019 Feb 8. doi: 10.1111/liv.14069. [Epub ahead of print]. **Chapter 6**

Hegade VS, Mells GF, Fisher H, et al. Pruritus is Common and Under-treated in Patients with Primary Biliary Cholangitis in the United Kingdom. *Clin Gastroenterol Hepatol*. 2018 Dec 14. [Epub ahead of print]. **Chapter 2**

Hegade VS, Kendrick SF, Dobbins RL, et al. Effect of ileal bile acid transporter inhibitor GSK2330672 on pruritus in primary biliary cholangitis: a double-blind, randomised, placebo-controlled, crossover, phase 2a study. *Lancet.* 2017 Mar 18; 389(10074):1114-1123. **Chapter 5**

Hegade VS, Kendrick SF, Dobbins RL, et al. BAT117213: Ileal bile acid transporter (IBAT) inhibition as a treatment for pruritus in primary biliary cirrhosis: study protocol for a randomised controlled trial. *BMC Gastroenterol*. 2016 Jul 19; 16(1):71. **Chapter 4**

Hegade VS, Krawczyk M, Kremer AE, et al. The safety and efficacy of nasobiliary drainage in the treatment of refractory cholestatic pruritus: a multicentre European study. *Aliment Pharmacol Ther*. 2016 Jan; 43(2):294-302. **Chapter 3**

Hegade VS, Jones DE, Hirschfield GM. Apical Sodium-Dependent Transporter Inhibitors in Primary Biliary Cholangitis and Primary Sclerosing Cholangitis. *Dig Dis*. 2017; 35(3):267-274.

Hegade VS, Bolier R, Oude Elferink RPJ, et al. A systematic approach to the management of cholestatic pruritus in primary biliary cirrhosis. *Frontline Gastroenterology* 2016; 7:158–166.

Hegade VS, Kendrick SF, Jones DE. Drug treatment of pruritus in liver diseases. *Clin Med (Lond)*. 2015 Aug; 15(4):351-7.

Hegade VS, Kendrick SF, Rehman J, et al. Itch and liver: management in primary care. *Br J Gen Pract.* 2015 Jun; 65(635):e418-20.

LIST OF ORAL AND POSTER PRESENTATIONS

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Poster presentations:

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Hegade VS, Mells GF, et al. A comparative study of pruritus in PBC cohorts from UK, USA and Italy. DDW annual meeting, 2015, Washington, USA

Hegade VS, Mells GF, et al. Patient experience and characteristics of cholestatic pruritus in the UK-PBC research cohort. EASL annual meeting, 2015, Vienna and AASLD annual meeting, 2014, Boston, USA

DEDICATION

My thesis is dedicated to my loving wife Meghna and our beautiful twin children Anvi and Adit. I would have been lost without them!

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LIST OF ABBREVIATIONS

AASLD	American Association for the Study of Liver Diseases
AE	Adverse event
AIH	Autoimmune hepatitis
ALD	Alcohol liver disease
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AMA	Anti-mitochondrial antibody
ASBT	Apical sodium-dependent bile acid transporter
AST	Aspartate aminotransferase
ATX	Autotaxin
BA	Bile acid
BAs	Bile acids
BSG	British Society of Gastroenterology
BRIC	Benign recurrent intrahepatic cholestasis
BS	Bile salt
BSEP	Bile salt export pump
C4	7-alpha hydroxy-4-cholestern-3-one
CA	Cholic acid
CDCA	Chenodeoxycholic acid
CDAD	*Clostridium difficile* associated diarrhoea
CNS	Central nervous system
CYP7A1	Cytochrome P450 7A1
DCA	Deoxycholic acid
DILI	Drug induced liver injury
DNA	Deoxyribonucleic acid
DRG	Dorsal root ganglion
EASL	European Association for the Study of Liver
ECG	Electrocardiogram
EDTA	Ethylenediaminetetraacetic acid
EHC	Enterohepatic circulation
ENBD	Endoscopic nasobiliary drainage
ENPP	Ecto-nucleotide pyrophosphatases/phosphodiesterases
ERC	Endoscopic retrograde cholangiography
ERCP	Endoscopic retrograde cholangiopancreatography
EST	Endoscopic sphincterotomy
FOB	Faecal occult blood
FGF15	Fibroblast growth factor 15
FGF19	Fibroblast growth factor 19
FDA	Food and Drug Administration
FDR	False discovery rate

FXR	Farnesoid -X receptor
g	Relative centrifugal force
GC	Gas chromatography
GCA	Glycocholic acid
GCDCA	Glycochenodeoxycholic acid
GCP	Good Medical Practice
GDCA	Glycodeoxycholic acid
GGT	Gamma glutamyl transferase
GI	Gastrointestinal
GLCA	Glycolithocholic acid
GLP	Glucagon like peptide
GRP	Gastrin releasing peptide
GSK	GlaxoSmithKline
GSRS	Gastrointestinal Symptom Rating Scale
GUDCA	Glycoursodeoxycholic acid
GWAS	Genome wide association Study
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HRQoL	Health related quality of life
HRT	Hormone Replacement Therapy
IARC	Interim Analysis Review Committee
IBAT	Ileal bile acid transporter
IBABP	Ileal bile acid binding protein
IBD	Inflammatory bowel disease
IBS	Irritable bowel disease
ICP	Intrahepatic cholestasis of pregnancy
IFSI	International forum for the study of itch
INR	International normalised ratio
IQR	Interquartile range
LC	Liquid chromatography
LCA	Lithocholic acid (Lithocholate)
Leu-ENK	leucine-enkephalin
LDL	Low-density lipoproteins
LFTs	Liver function tests
LPA	lysophosphatidic acid
LPC	lysophosphatidylcholine
LT	liver transplantation
MARS®	Molecular adsorbent recirculating system
mmol	milli mole
MS	Mass spectrometry
NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis

NBD	Nasobiliary drainage
NHS	National Health Service
NMR	Nuclear magnetic resonance
NPPB	Natriuretic Polypeptide B
NSAID	Nonsteroidal anti-inflammatory drug
NTCP	Sodium taurocholate co-transporting polypeptide
OATP	Organic anion transport polypeptide
OCA	Obeticholic acid
OH	hydroxyl group
OST	Organic solute transporter
OTU	operational taxonomic unit
PBC	Primary biliary cholangitis (previously cirrhosis)
PCR	Polymerase chain reaction
PD	Pancreatic duct
PEP	Post-ERCP pancreatitis
qPCR	quantitative PCR
PFIC1	Progressive familial intrahepatic cholestasis type 1
PFIC 2	Progressive familial intrahepatic cholestasis type 2
PK	Pharmacokinetics
PSC	Primary sclerosing cholangitis
PT	Prothrmobin time
PXR	pregnane X receptor
QoL	Quality of life
RDP	Ribosomal database project
RNA	Ribonucleic acid
SAE	Serious adverse event
SHP	Small heterodimer partner
SOP	Standard operating procedure
SSRI	Selective serotonin re-uptake inhibitor
T2DM	Type 2 Diabetes Mellitus
TBA	Total bile acid
TBS	Total bile salts
TCA	Taurocholic acid
TCDCA	Taurochenodeoxycholic acid
TDCA	Taurodeoxycholic acid
TGR5	Transmembrane G-protein coupled bile acid receptor
TLCA	Taurolithocholic acid
TRPA1	Transient receptor potential ankyrin 1
TUDCA	Tauroursodeoxycholic acid
UDCA	Ursodeoxycholic acid
UK	United Kingdom
UPLC-MS	Ultra-performance liquid chromatography-mass spectrometry

UV	Ultraviolet
VAS	Visual analogue scale
µm (µM)	micro mole

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1. CHAPTER 1: INTRODUCTION

1.1 Cholestatic pruritus

1.1.1 Definitions

Pruritus or itch can be defined as "an unpleasant sensation that leads to the desire to scratch" (Stander, Weisshaar et al., 2007). Although chronic pruritus (>6 weeks of duration) is most commonly seen in the setting of skin diseases it can occur as a consequence of systemic conditions [(**Figure 1-1**), adapted from (Yosipovitch and Bernhard, 2013)]. Cholestatic pruritus refers to pruritus caused by or associated with cholestatic diseases. In clinical practice, the most common cholestatic diseases associated with pruritus are primary biliary cholangitis (previously referred to as cirrhosis, PBC), primary sclerosing cholangitis (PSC), intrahepatic cholestasis of pregnancy (ICP) and benign recurrent intrahepatic cholestasis (BRIC).

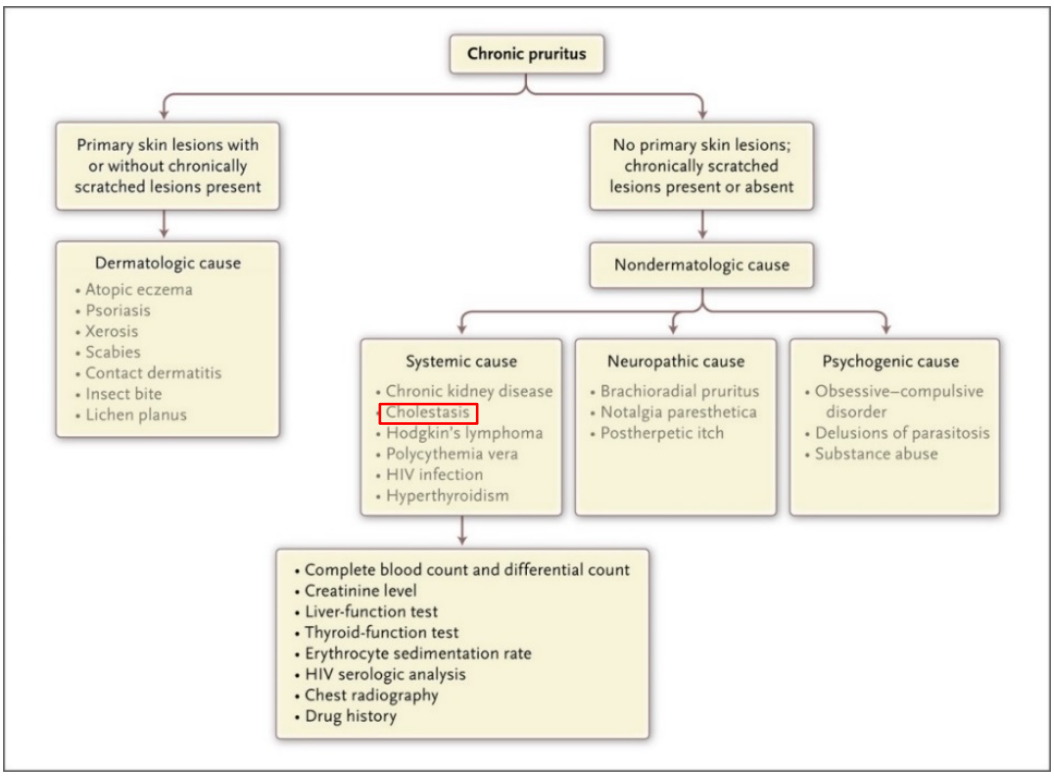


Figure 1-1 Classification of chronic pruritus

Cholestasis refers to impairment of bile formation or bile flow (Greek words 'chole' means bile and 'stasis' means standing still) and cholestatic diseases are specific group of conditions characterised by impairment of bile formation or bile flow, resulting in accumulation of bilirubin, cholesterol and their metabolites. The impairment could be within the liver (intrahepatic or non-obstructive cholestasis) or in the bile ducts draining the bile from the liver into the small intestine (extrahepatic or obstructive cholestasis). Intrahepatic cholestasis usually results either from immunologically mediated destruction of small bile ducts or defective bile acid transport proteins located within the hepatocytes or cholangiocytes. Extrahepatic cholestasis generally results from mechanical obstruction to the bile flow due to stones or strictures (inflammatory, benign or malignant).

Table 1-1 outlines the types of cholestatic conditions. This thesis mainly focusses on intrahepatic cholestasis.

Intrahepatic cholestasis	**Extrahepatic cholestasis**
• Primary biliary cholangitis (PBC) • Primary sclerosing cholangitis (PSC) • Alagille syndrome (paediatric) • Intrahepatic cholestasis of pregnancy (ICP) • Benign recurrent intrahepatic cholestasis (BRIC) • Progressive familial intrahepatic cholestasis types 1 and 2 (PFIC1, PFIC2) • Toxin or drug induced cholestasis • Chronic viral hepatitis C (HCV) infection • Sarcoidosis • Hepaticholithiasis • Intrahepatic cholangiocarcinoma • Intrahepatic biliary atresia	• PSC with strictures • Choledocholithiasis • Cholangiocarcinoma • IgG4-associated cholangitis • Pancreatic tumours • Ampullary tumours • Hilar lymphadenopathy • Bile duct adenoma • Biliary atresia • Benign biliary stricture • Biliary parasites (e.g. *Clonorchis sinensis*, *Fascioloa hepatica*)

Table 1-1 Types of cholestatic conditions

1.1.2 Incidence and prevalence

There is considerable variation in the incidence and prevalence of pruritus reported in different cholestatic conditions. Generally it is more common in intrahepatic than extrahepatic cholestasis. For example, while pruritus is the diagnostic symptom in ICP (i.e. pruritus defines ICP), only 5-15% of patients with chronic hepatitis C virus (HCV) infection experience pruritus during the course of their disease (Cacoub, Poynard et al., 1999, Chia, Bergasa et al., 1998, Cribier, Samain et al., 1998, Geenes and Williamson, 2009).

The epidemiology of pruritus in PBC and PSC is less clear due to scarcity of published literature. In general, it is suggested that up to 80% of PBC and PSC patients experience pruritus at some point in their illness (Bergasa, Mehlman et al., 2000, James, Macklon et al., 1981, Koulentaki, Ioannidou et al., 2006, Sherlock and Scheuer, 1973). However limited information exists about its natural history in affected individuals and the risk factors associated with its occurrence. One study reported that serum alkaline phosphatase (ALP) levels and Mayo risk score were independent risk factors for baseline pruritus (Talwalkar, Souto et al., 2003). It is known that in PBC pruritus has a fluctuating nature in untreated PBC patients, can develop at any stage of the disease and can often predate the diagnosis of the condition itself. The latter was suggested by an American study (n=238) in which 75% of PBC patients reported experience of pruritus preceding the formal diagnosis of PBC (Rishe, Azarm et al., 2008). Also, a significant number of initially asymptomatic PBC patients subsequently develop pruritus in the course of their illness. For example, in a large cohort study of 770 PBC patients from northeast England, the overall prevalence rate for pruritus was 33% and proportion of initially asymptomatic patients (n=422) developing pruritus was 15%, 31% and 47% at 1, 5 and 10 years of follow up, respectively (Prince, Chetwynd et al., 2002, Prince, Chetwynd et al., 2004). Similarly in a US study, among placebo treated patients (n=91), the annual risks for development or improvement/resolution of pruritus were 27% and 23%, respectively (Talwalkar, Souto et al., 2003).

1.1.3 Clinical assessment

A patient with known or suspected cholestatic disease presenting with pruritus needs a systematic clinical evaluation. Presence of pruritic skin lesions (other than scratch marks) should prompt referral to dermatology to rule out skin conditions contributing to pruritus (Bergasa, 2014). Intensity of pruritus should be assessed not only to allow objective

assessment of impact on patients' health and quality of life (QoL) but also to evaluate the effect of therapy. As is the case with pain, itch is a sensation and a multidimensional symptom, its assessment and quantification are inherently difficult. There is no gold standard and no clear recommendation of measurement tools for objective assessment of cholestatic pruritus. A few simple tools are currently available for use in clinical trials and practise.

1.1.3.1 Visual Analogue Scale (VAS)

VAS, the most commonly used tool for self-report of pruritus intensity is considered as the historical standard of pruritus assessment. It has been recently validated and shown to have high re-test reliability in a large (n=471) prospective study of patients with chronic pruritus (Phan, Blome et al., 2012). VAS provides an easy and rapid assessment of symptom severity and has been in use for many decades. VAS is a 10-cm long horizontal or vertical line, on which patients indicate the intensity of pruritus by marking the line at the point that corresponds to the severity of their pruritus (Furue, Ebata et al., 2013). The beginning of the scale (0 point) refers to no pruritus and the end of the scale (10 points) refers to most severe pruritus. Although easy-to use and reliable, VAS has methodological problems in the research and clinical settings. For example, various different expressions have been used for the 10-point end includes expressions such as "worst imaginable itch", "the most severe pruritus they can imagine", "most intense sensation imaginable", "maximal itch", "severe itching" and "unbearable pruritus". Therefore to establish uniformity, a recent consensus statement from the International Forum for the Study of Itch (IFSI) has proposed "worst imaginable itch" (**Figure 1-2**) as the most suitable and preferred definition of 10-point end of the VAS (Furue, Ebata et al., 2013).

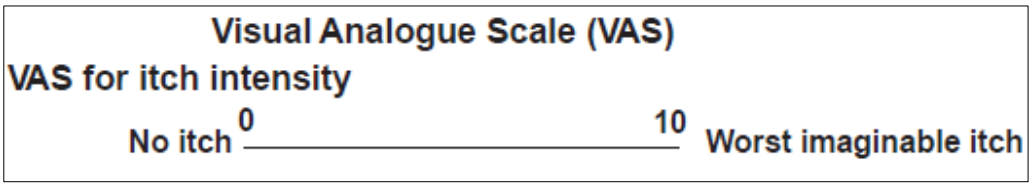


Figure 1-2 IFSI recommended visual analogue scale for itch

Although VAS is simple to use in clinical practise, it is not disease specific and has not been validated for use in PBC or other cholestatic diseases. In addition, VAS has certain drawbacks. VAS measures only the intensity of itch without impact on QoL and it has not been shown to detect changes over time. Completion of VAS requires the patient to use

thought processes to convert their itch severity to a mark on a continuum (Elman, Hynan et al., 2010). Despite these limitations, most clinical trials of drugs for cholestatic pruritus have utilised VAS as an outcome measure of pruritus severity in the study end-point (Bergasa, Schmitt et al., 1998, Ghent and Carruthers, 1988, Kuiper, van Erpecum et al., 2010, Mayo, Handem et al., 2007, Podesta, Lopez et al., 1991).

1.1.3.2 ***PBC-40 questionnaire***

PBC-40 is a disease specific QoL assessment tool developed and validated for self-completion by PBC patients (Jacoby, Rannard et al., 2005). It consists of 40 items grouped into six domains of typical PBC symptoms (fatigue, itch, cognition, emotional, social and other symptoms). The itch domain consists of three questions framed as statements. Each question is scored from 1 to 5 with higher scores representing more severe impairment and total domain scores are calculated by summing the individual item scores. Responses for these statements are on a standard five point Likert scale (score 1 for least burden or problem, score 5 for greatest burden or problem). The total score of the itch domain is obtained from summing individual question response scores (maximum score 15, minimum score 3). Based on the total score itch severity is classified as mild pruritus (score of 4-8), moderate pruritus (score of 9-11) and severe pruritus (score>12) (Newton, Hudson et al., 2007).

	Never	Rarely	Occasionally	Frequently	Always
i) Itching disturbed my sleep	1	2	3	4	5
ii) I scratched so much, I made my skin raw	1	2	3	4	5
iii) I have felt embarrassed because of the itching	1	2	3	4	5

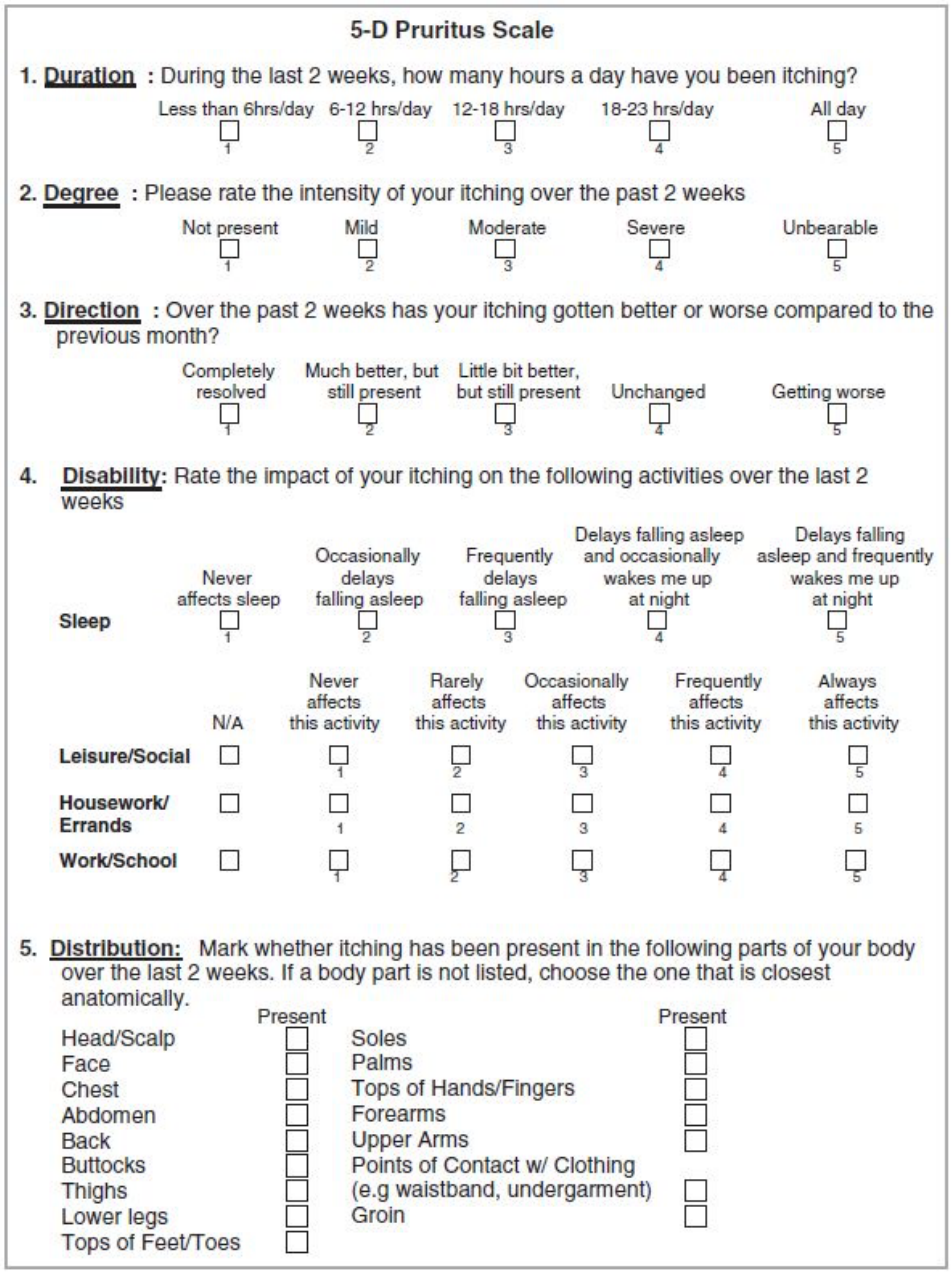
Figure 1-3 PBC-40 itch domain

1.1.3.3 ***5-D itch scale***

Recently, the 5-D itch scale has been designed to characterize the extent of itch and its impact by defining five dimensions of itch- degree, duration, direction, disability and distribution (**Figure 1-4**, adapted from (Elman, Hynan et al., 2010)). It is a brief (one page), easy to complete questionnaire with multiple choice or "check all boxes that apply" format and helps in both quantitative and qualitative assessment of pruritus. The total 5-D itch score (range 5-25) is achieved by summing together the scores of each of the five domains. For *duration, degree and direction* domains, the scores are equal to the value below the response choice (range 1-5). The score for the *disability* domain is achieved by taking the highest score on any of the four items. For the *distribution* domain, the number of affected body parts is tallied (potential sum 0–16) and the sum is sorted into five scoring bins: sum of 0–2 = score of 1, sum of 3–5 = score of 2, sum of 6–10 = score of 3, sum of 11–13 = score of 4, and sum of 14–16 = score of 5. The scores for all five dimensions are added and in total, the minimum score of 5 indicates no pruritus and a maximum score of 25 indicates most severe pruritus.

The 5-D itch scale was specifically designed to be useful as an outcome measure in clinical trials and has been validated and shown to be reliable measure of itch in patients with chronic pruritus of different aetiologies (Elman, Hynan et al., 2010). In this study, significant correlation between VAS and 5-D scale was demonstrated both at baseline and at 6-wek

follow up. Similarly, the disability domain of 5-D significantly correlated with the PBC-40 itch domain. It can be concluded that 5-D itch scale is a reliable, multidimensional measure of itching that has been validated in patients with chronic pruritus to able to detect changes over time. However, it is noteworthy that in this study only 27% (63/234) of patients had pruritus due to chronic liver disease.



5-D Pruritus Scale

1. **Duration** : During the last 2 weeks, how many hours a day have you been itching?

Less than 6hrs/day	6-12 hrs/day	12-18 hrs/day	18-23 hrs/day	All day
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

2. **Degree** : Please rate the intensity of your itching over the past 2 weeks

Not present	Mild	Moderate	Severe	Unbearable
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

3. **Direction** : Over the past 2 weeks has your itching gotten better or worse compared to the previous month?

Completely resolved	Much better, but still present	Little bit better, but still present	Unchanged	Getting worse
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

4. **Disability**: Rate the impact of your itching on the following activities over the last 2 weeks

	Never affects sleep	Occasionally delays falling asleep	Frequently delays falling asleep	Delays falling asleep and occasionally wakes me up at night	Delays falling asleep and frequently wakes me up at night
Sleep	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

	N/A	Never affects this activity	Rarely affects this activity	Occasionally affects this activity	Frequently affects this activity	Always affects this activity
Leisure/Social	☐	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
Housework/ Errands	☐	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
Work/School	☐	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

5. **Distribution:** Mark whether itching has been present in the following parts of your body over the last 2 weeks. If a body part is not listed, choose the one that is closest anatomically.

	Present		Present
Head/Scalp	☐	Soles	☐
Face	☐	Palms	☐
Chest	☐	Tops of Hands/Fingers	☐
Abdomen	☐	Forearms	☐
Back	☐	Upper Arms	☐
Buttocks	☐	Points of Contact w/ Clothing	
Thighs	☐	(e.g waistband, undergarment)	☐
Lower legs	☐	Groin	☐
Tops of Feet/Toes	☐		

Figure 1-4 5-D itch scale

1.1.4 Clinical features

Cholestatic pruritus has been mainly studied in PBC, an archetypal autoimmune cholestatic disease. Typically, cholestatic pruritus has predilection for limbs, soles of the feet and palms of the hands (palmoplantar distribution) but generalised itch may also occur (Bergasa, Mehlman et al., 2000). A vast majority of PBC patients report diurnal variation with worsening of itch in the late evening and early at night. This cortisol-like circadian rhythm of itch intensity has been convincingly shown by elegant experiments by Bergasa *et al.* using piezo film technology for the quantitative assessment of scratching (Talbot, Schmitt et al., 1991). Pruritus in PBC is often exacerbated by heat, psychological stress and contact with certain fabrics such as wool. Premenstrual period, late stages of pregnancy and hormone replacement therapy can also exacerbate the symptom. Most patients report their itch as a sensation of irritation deep under the skin and describe it as: "lying on a bed of cactus," "crawling", or "deep itch" "pins and needles" and those with severe itch report that the itch is "relentless" or so severe that it leads to wanting to "tear their skin off" or "scratching until bleeds" (Bergasa, 2003, Imam, Gossard et al., 2012, Rishe, Azarm et al., 2008).

Unlike other causes of pruritus, patients with cholestatic pruritus complain that scratching activity barely relieves their itch (Kremer, Oude Elferink et al., 2011). Patients' skin is generally devoid of primary skin lesions but longstanding intense scratching may result in secondary skin lesions such as excoriations, folliculitis (inflammation of hair follicles), prurigo nodularis (hard nodules on the skin), and lichenification (leathery hardening of the skin) (Swain, 1999).

Once pruritus develops its severity often fluctuates from day to day and it may diminish over time especially when the diseases becomes more advanced and liver synthetic function deteriorates (Lindor, Gershwin et al., 2009). However, in the majority of patients it is unlikely to completely resolve unless effective treatment is started (Mayo, 2008). Most patients suffer mild and tolerable symptoms but some patients may experience troublesome itch which may dramatically reduce their quality of life. Recently, health related quality of life (HRQoL) in chronic pruritus has been shown to be highly influenced by pruritus intensity irrespective of the underlying cause (Warlich, Fritz et al., 2015). In fact, patients with severe cholestatic pruritus may develop fatigue, cognitive symptoms, deranged sleep pattern, mood changes, anxiety, depression and sometimes suicidal ideations (Jones, 2012a).

For reasons that are unexplained the severity of cholestatic pruritus has no relationship with degree of severity of cholestasis i.e. patients with similar severities of cholestasis can have markedly different degrees of pruritus. In addition, cholestatic pruritus is independent of biochemical severity, duration of the disease and histological stage of PBC (Jones and Bergasa, 1999). For example, patients with a patient with early stage PBC and normal liver function tests (LFTs) may present with severe itch, whereas patients with advanced PBC and liver synthetic dysfunction might have no pruritus.

In a recent study of over 2300 PBC patients significantly higher pruritus severity was observed in patients who were unresponsive to ursodeoxycholic acid (UDCA) therapy (Carbone, Mells et al., 2013). The same study also suggested that intensity of pruritus may be associated with the age at disease presentation. The pruritus score measured on a visual analogue scale (VAS) was 64% higher in PBC patients who presented at younger than age 30 (n=24) in comparison to those presented at older than age 70 (n=178) suggesting that younger PBC patients are more likely to have severe pruritus.

1.2 Pathogenesis of cholestatic pruritus

In general, despite clinical and experimental research spanning over five decades the mechanism of development of pruritus in cholestasis is incompletely understood. A conventional hypothesis is that in cholestasis compounds (normally excreted in bile) are released into the systemic circulation and among these compounds one or more pruritogen(s) may diffuse from the plasma to the skin where they stimulate neural itch fibres. Subsequent transmission of nociceptive signal to the spinal cord and the brain then elicits a motor response of scratching. Over the past decades, many experimental and clinical studies have attempted to explore different putative pruritogens but to date no single substance has been conclusively shown to be the causative pruritogen in cholestasis. Most compelling evidence supports the direct or indirect roles of bile acids (bile salts), opioids and recently the 'autotaxin-lysophosphatidic acid (ATX-LPA)' axis.

1.2.1 Bile acids: an overview

Bile acids (BAs), along with phospholipids and cholesterol are major constituents of bile. They are amphipathic molecules (i.e. with both hydrophilic and hydrophobic regions) with detergent-like properties. Primary BAs are synthesized from enzymatic catabolism of cholesterol by the hepatocytes via either the classical pathway or the alternate pathway. Classical pathway results in the formation of cholic acid (CA) and accounts for 90% of BA synthesis, whereas the alternate pathway leads to the formation of chenodeoxycholic acid (CDCA). Cytochrome P450 7A1 (CYP7A1) is the gene encoding cholesterol 7α-hydroxylase, the rate-limiting enzyme in the classical pathway of BA synthesis.

After their synthesis, unconjugated CA and CDCA are targeted to the peroxisomes where they are conjugated (amidation) with glycine and taurine which renders them more hydrophilic and more readily secretable in the bile. In humans, predominant conjugated BAs are glyco-conjugates and under physiological PH conditions these conjugated BAs exist as anionic salts and are therefore called bile salts. Many use the terms "bile salts" and "bile acids" interchangeably, though in man, the bile salts are mainly conjugated and ionised (Kirby, Heaton et al., 1974).

The bile salts are stored in the gallbladder and upon ingestion of meal they are released into the intestinal lumen where they facilitate absorption of fat and fat soluble vitamins. CDCA is

partially epimerized into α3 β7-OH UDCA, the major tertiary bile acid (Humbert, Maubert et al., 2012). Besides amidation by glycine and taurine, BAs can also be conjugated as 3α sulphated BAs which are water-soluble and present abundantly in normal urine (Humbert, Maubert et al., 2012).

Conjugated primary BAs present in the intestinal lumen are modified by different bacterial phyla by deconjugation, oxidation and dehydroxylation to produce secondary BAs: lithocholic acid (LCA) and deoxycholic acid (DCA). These secondary BAs are subsequently reabsorbed through the ileal intestinal wall, into the portal circulation and reach liver.

Human bile predominantly contains primary CA and CDCA and a very small amount (about 1-3% of total BAs) of UDCA (Monte, Marin et al., 2009). The hydrophobicity of BAs (which determines their liver cytotoxicity) follows the order of UDCA<CA<CDCA<DCA<LCA (Benedetti, Alvaro et al., 1997, Heuman, 1989). The index of hydrophilicity depends on the number and position of hydroxyl (OH) groups, and whether amidation of the lateral chain is with glycine or taurine. BAs conjugated with taurine are more hydrophilic than those conjugated with glycine, and trihydroxylated BAs (CA, TCA, GCA) are more hydrophilic than dihydroxylated BAs (CDCA,GCDCA,TCDCA,DCA,GDCA). At concentrations ≥200micromoles/litre (μM/L) LCA, DCA and CDCA are toxic (Fiorucci, Distrutti et al., 2014).

Feeding status affects the serum BA concentration. After food ingestion serum BAs rise due to cholecystokinin mediated gallbladder contraction resulting in increasing bile flow into the intestine. Therefore in studies using serum BAs, feeding status should be controlled (Bathena, Thakare et al., 2015b). However, studies show that urinary BAs are affected to a lesser extent by food intake and therefore do not have to be obtained at fasting state (Bathena, Thakare et al., 2015a, Simko and Michael, 1998, Simko, Michael et al., 1987).

1.2.1.1 ***Enterohepatic circulation and Ileal bile acid transporter***

The average daily BA synthesis in healthy humans is about 1 milli mole (mmol) and daily BA secretion is 30-50 mmol (van Berge Henegouwen and Hofmann, 1978). The ability to secrete more bile than is synthesized is largely due to an efficient intestinal conservation mechanism mediated by the ileal bile acid transport system (Hofmann, 2007). This is briefly described below.

After their normal physiological function is completed in the intestine, BAs reach the ileum where most BAs are reabsorbed efficiently and returned to the liver via portal blood (enterohepatic circulation, (EHC)). The absorption of BAs in the terminal ileum is sodium dependent and saturable compared to that in jejunum where it is sodium independent and non saturable. Ileal bile acid transporter (IBAT or ASBT) expressed in the distal ileum is the predominant transporter mediating the ileal uptake of conjugated bile salts. IBAT belongs to family of apical sodium dependent bile acid transporter (ASBT, gene symbol *SLC10A2*). IBAT mediates active transfer of bile salts coupled to Na+ absorption across the luminal plasma membrane to an intracellular 14 kDa protein called ileal BA binding protein (IBABP).

Interestingly, IBAT mediated transport is not equal among BA species; conjugated (more hydrophilic) BAs are transported more efficiently than unconjugated forms. Also the affinity of IBAT is higher for dihydroxy BAs such as CDCA and DCA than for trihydroxy BAs such as CA, taurocholic acid (TCA) and glycocholic acid (GCA). BAs bind to IBABP which facilitates intracellular diffusion of BAs to the basolateral membrane. Finally, BAs exit the ileal enterocyte via the basolateral plasma membrane mediated by the organic solute transporter (OST), a heterodimeric bile salt transporter composed of two subunits (OSTα and OSTβ) and enter the portal bloodstream (Ballatori, Christian et al., 2005, Dawson, Hubbert et al., 2005) (**Figure 1-5**). In the ileal enterocytes, BAs also bind with farnesoid X receptor (FXR) which induces transcription of fibroblast growth factor 19 (FGF19 in humans, and FGF15 in rodents) (Holt, Luo et al., 2003, Inagaki, Choi et al., 2005). FGF19, an enterokine, is released into the portal circulation, and binds to its hepatocyte receptor FGF receptor 4 (FGFR4) and initiates a small heterodimer partner (SHP) independent downregulation of CYP7A1, resulting in inhibition of BA synthesis (Jones, 2012b, Kir, Zhang et al., 2012).

When BAs reach the liver via portal venous blood, they undergo efficient extraction (despite bound to albumin) and uptake at the basolateral membrane of hepatocytes mediated by transporters sodium taurocholate co-transporting polypeptide (NTCP, gene symbol *SLC10A1*) and organic anion transport polypeptides (OATPs). The hepatic re-uptake is greater for trihydroxy BAs than dihydroxy BAs and is greater for conjugated BAs than unconjugated BAs (Hofmann, 2007).

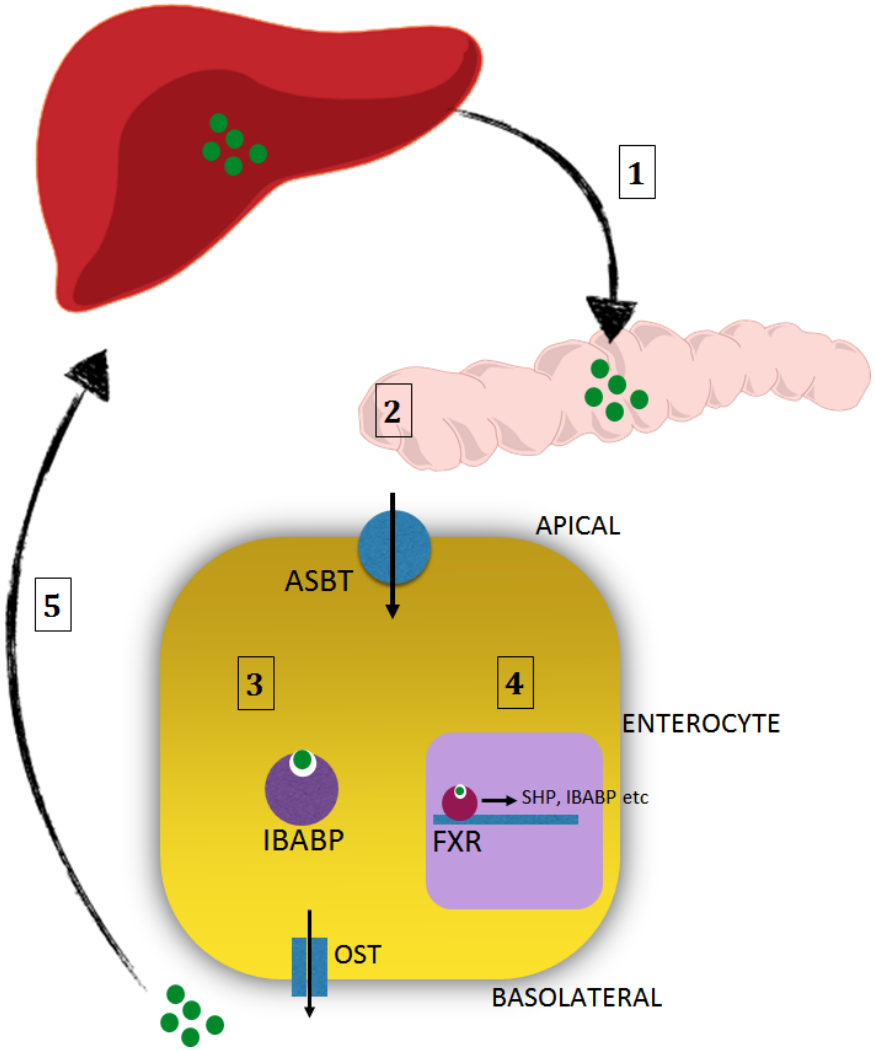


Figure 1-5 Enterohepatic circulation of bile acids via enterocyte in terminal ileum
1) Primary bile acids (BAs) synthesized in liver and excreted into duodenum as constituent of bile; 2) BAs avidly and actively reabsorbed in the terminal ileum via ASBT (also called IBAT); 3) BAs transported intracellularly by IBABP, 4) BAs free to bind with farnesoid X receptor (FXR), 5) BAs released into portal venous circulation via OST a/b and circulated back to liver. [Image courtesy: Richard A Speight, adapted from (Hegade, Speight et al., 2016b)]

Subsequently, BAs circulating in the portal circulation are transported across the basolateral membranes of the hepatocytes via NTCP. Finally, conjugated BAs are transported across the canalicular plasma membrane of the hepatocytes via the bile salt export pump (BSEP) and secreted into bile. This efficient cycle between the small intestine and the liver ensures 95% of BAs re-enter the liver, leaving only approximately 5% (or approximately 0.5 g/d) in the intestinal lumen. Also, due to their efficient uptake by the liver, BAs remain at a low concentration in the peripheral blood circulation (Monte, Marin et al., 2009).

1.2.2 Role of bile acids

Chronic cholestatic diseases such as PBC and PSC are characterised by elevated BAs in the circulation and tissues and accumulation of toxic BAs (Pusl and Beuers, 2006). At high hepatic concentrations BAs induce oxidative stress and apoptosis, resulting in damage to the liver parenchyma (Monte, Marin et al., 2009). In addition to their potential role in disease progression, BAs (or bile salts) have also been implicated as potential pruritogens in cholestatic liver diseases ('bile salt theory').

Many studies have evaluated serum levels of BAs in liver disease patients with pruritus. In an old study, patients with cholestatic pruritus were found to have higher mean values of serum total bile acid (TBA) compared to those without pruritus (126µM/l vs. 39.4µM/l; normal range is 3.11 ± 0.69 µM/l). Although the correlation between serum bile acid levels and pruritus was not distinct, patients with serum TBA concentration of more than 50µM/l were more likely to complain of pruritus. Also, those with pruritus tended to have markedly low ratio of glycine conjugated BAs to taurine conjugated BAs (Neale, Lewis et al., 1971).

It has also been shown that different bile salts differ in their ability to provoke pruritus. For example, in their bile salts study on healthy people Kirby *et al*, showed that dihydroxy bile salts (DCA, CDCA and their conjugates GDCA, TDCA and GCDCA, TCDCA respectively) were more effective in causing pruritus than trihydroxy bile salts (CA, GCA and TCA) (Kirby, Heaton et al., 1974).

Following body of evidence supports the hypothesis that BAs (or bile salts) cause or contribute to the development of pruritus in cholestasis:

- serum levels of bile salts are elevated in cholestasis (Carey, 1961);

- feeding cholylsarcosine (a synthetic bile acid) to cholestatic patients aggravates their pruritus (Ahrens, Payne et al., 1950, Ricci, Hofmann et al., 1998);
- bile salts have been recovered from the skin surface of jaundiced patients with pruritus and 85% of the recovered bile salts were in the unconjugated form (Schoenfield and Sjövall, 1967);
- intradermal application of bile salts induces pruritus in healthy volunteers (Kirby, Heaton et al., 1974, Varadi, 1974);
- dramatic reductions in pruritus seen in patients undergoing nasobiliary drainage (which removes bile salts from enterohepatic circulation) or extracorporeal albumin dialysis (which removes bile salts from systemic circulation) (Beuers, Gerken et al., 2006, Pares, Cisneros et al., 2004, Stapelbroek, van Erpecum et al., 2006);
- some antipruritic effect of cholestyramine/colesevelam (bile salt resins which bind to bile salts in the intestine and reduce serum levels of bile acids) (Carey and Williams, 1961, Datta and Sherlock, 1966, Oster, Rachmilewitz et al., 1965); and
- a positive linear relationship between itch and serum bile acids has been shown in one study (Di Padova, Tritapepe et al., 1984).
- Pruritus is a common adverse event seen in patients treated with Obeticholic acid (OCA), a semi-synthetic BA (see section **1.2.2.1**)

However, the 'bile salt theory' is not universally supported and the following arguments challenge the role of bile salts in cholestatic pruritus:

- no correlation has been shown between itch intensity and serum, urine or skin tissue concentrations of bile salts in cholestatic patients (Bartholomew, Summerfield et al., 1982, Carey, 1958, Datta and Sherlock, 1966, Freedman, Holzbach et al., 1981, Ghent, Bloomer et al., 1977, Neale, Lewis et al., 1971, Osborn, Wootton et al., 1959); One possible explanation for the discrepancy between total circulating bile salts concentration and pruritus may be due changes in the relative proportion of individual bile salts in cholestasis (e.g. lower levels of DCA and its conjugates but increased ratio of CDCA and CA) (Kirby, Heaton et al., 1974, Neale, Lewis et al., 1971).
- no correlation could be demonstrated between serum bile salt levels and itch relief after treatment with nasobiliary drainage (NBD) and extracorporeal albumin dialysis (Beuers, Gerken et al., 2006, Pusl, Denk et al., 2006);

- women with ICP, in which pruritus is the defining symptom (all suffer from pruritus) may have only mildly elevated serum bile salts (Geenes and Williamson, 2009);
- frequency and severity of itch do not correlate with degree of cholestasis (Bartholomew, Summerfield et al., 1982, Freedman, Holzbach et al., 1981, Ghent, Bloomer et al., 1977, Kremer, Martens et al., 2010);
- no association was found between the concentration of any particular conjugated or free BA and the presence or absence of pruritus; and pruritus can spontaneously ameliorate despite ongoing cholestasis (Murphy, Ross et al., 1972). The latter is exemplified by absence or disappearance of pruritus in advanced PBC patients with severe degree of cholestasis despite high levels of serum bile salts.
- a paradoxical observation that whilst pruritus can be seen in patients with normal serum levels of bile salts, patients with obstructive cholestasis who often have the highest bile salt levels do not always develop pruritus (Ghent and Bloomer, 1979, Murphy, Ross et al., 1972).
- patients with bile salt synthesis defects, while cholestatic, generally do not suffer from itch.

Also, the exact mechanism by which BAs (or bile salts) may cause or trigger pruritus has not been fully explained. An earlier suggestion that bile salts cause pruritus by mast cell activation and degranulation (shown in *in vitro* studies) has not been confirmed in *in vivo* studies (Quist, Ton-Nu et al., 1991).

1.2.2.1 ***Obeticholic acid and pruritus***

A recent proposal that bile salts probably modulate pruritus by activation of FXR is supported by observation of increased occurrence of pruritus as an adverse event (AE) in PBC patients treated with Obeticholic acid (OCA), a semisynthetic bile acid and a potent FXR agonist (Mason, Luketic et al., 2010). This section summarises the current evidence of pruritus as AE in OCA trial.

In the phase II double blind, placebo-controlled trial of PBC patients (n=165), OCA was given in 10 mg, 25 mg, or 50 mg doses once daily for 3 months. OCA resulted in significant reductions in total endogenous BAs (excluding UDCA) and OCA constituted less than 2% of total plasma BAs. In this study, pruritus was the principal AE and was shown to be dose related; pruritus incidence values in the OCA 10 mg, 25 mg, and 50 mg groups were 47% (not

significantly different), 87% (p<.0003), and 80% (p<.006), respectively vs 50% in the placebo group. Severe pruritus was reported in 16% (6/38) of the patients in the 10-mg group, 24% (9/37) 25-mg group and 37% (15/41) 50-mg group of patients, respectively. In the open-label extension phase of this study although 87% (68/78) of patients experienced some pruritus, only 13% (10/78) discontinued OCA treatment as a result (Hirschfield, Mason et al., 2015).

In the phase 3 trial of OCA (Nevens, Andreone et al., 2016), 217 patients with PBC were randomised to receive OCA at a dose of 10 mg (the 10-mg group, n=73), at a dose of 5 mg with adjustment to 10 mg if applicable (the 5–10-mg group, n=71), or placebo (n=73) over a 12 month period. A total of 63% of the patients had a history of disease related pruritus, and 59% reported pruritus at baseline. Similar to the phase 2 trial, and consistent with FXR activation, significant decreases from baseline in bile acid levels were seen in the OCA treated group. Pruritus was the most common AE that occurred during the double-blind phase across all groups, with higher incidence reported in the OCA group (56% in the 5–10-mg group and 68% in the 10-mg group) compared to 38% in the placebo group. Changes from baseline in the VAS score for pruritus and the 5-D questionnaire score were greater in the 10-mg group than in the placebo group (VAS: p<0.001 at week 2, p = 0.003 at month 3, and p = 0.03 at month 6; 5-D questionnaire: p<0.001 at week 2 and p = 0.005 at month 3). At month 12, the scores on the VAS and the 5-D questionnaire did not differ significantly between both OCA group and the placebo group. The percentage of patients who received an intervention (mostly bile acid sequestrants) was similar across groups (range, 50 to 62%). Discontinuation of treatment owing to pruritus occurred in 7 patients (10%) in the 10-mg group and in 1 (1%) in the 5–10-mg group. No patient in the placebo group discontinued the trial regimen owing to pruritus.

OCA has also been studies as a monotherapy in PBC patients intolerant to UDCA. In an international, randomized, double-blind, placebo-controlled phase 2 study PBC patients were randomized and dosed with placebo (n = 23), OCA 10 mg (n = 20), or OCA 50 mg (n = 16) given as monotherapy once daily for 3 months (Kowdley, Luketic et al., 2018). Pruritus was the most common adverse event; incidence was 35% in the placebo group, 70% in the OCA 10 mg and 94% in the OCA 50 mg groups. The median time to onset of pruritus was 33, 14, and 6 days in the placebo, OCA 10 mg, and OCA 50 mg groups, respectively. 15% of patients in the OCA 10 mg and 38% of patients in the OCA 50 mg discontinued due to pruritus. In the open label extension of this study twenty-eight patients continued OCA (18 patients

completed through 6 years) at a median weighted average daily dose of 14.0 mg. Again, pruritus was the most common AE reported with 89% of patients experiencing the symptom and 11% (3/12) discontinuing the drug. Twenty patients (71%) received concomitant medications for pruritus including antihistamines, bile acid sequestrants, and antibiotics (typically rifampicin).

Pruritus has also been reported with OCA use in non-PBC population. In the FLINT trial, of the 141 patients with non-cirrhotic, non-alcoholic steatohepatitis (NASH) treated with OCA 25mg once daily 72 weeks, 33 (23%) developed pruritus compared with nine (6%) of 142 in the placebo group (Neuschwander-Tetri, Loomba et al., 2015). Pruritus was also more severe in the OCA group and led to the use of antipruritic medications or short periods of withholding treatment in some patients, and treatment discontinuation in one patient.

Above data clearly suggest that pruritus related to OCA is dose-dependent pruritus and doses above 10mg/day are determined to be excessive. In these trial, management of pruritus caused by OCA included antipruritic agents (most commonly bile acid sequestrants) or temporary OCA interruption. Antihistamines and rifampicin have also been used (Kowdley, Luketic et al., 2018). Data from phase 3 trial suggested that initiation of therapy with OCA at a dose of 5 mg, with adjustment up to 10 mg if appropriate, was associated with a lower rate of discontinuation owing to pruritus and confirmed the benefit of dose titration in managing pruritus (Nevens, Andreone et al., 2016). Recently, an expert panel has provided guidance on the management of pruritus symptoms in patients receiving OCA as treatment for PBC (Pate, Gutierrez et al., 2019).

The mechanism of OCA induced pruritus is not entirely clear. Authors of the phase 2 trial speculated that OCA induced pruritus is not related TGR5 activation since OCA is a weak TGR5 agonist and OCA reduced levels of the endogenous human TGR5 agonist, DCA (Hirschfield, Mason et al., 2015). In the phase 3 trial, *post hoc* analysis showed no correlation between ATX activity and patient-reported measures of pruritus severity (according to the VAS, 5-D questionnaire, or PBC-40 itch scores), suggesting OCA induced pruritus is unlikely to be related to ATX (Nevens, Andreone et al., 2016). Clearly, more mechanistic studies are needed to understand OCA induced pruritus.

1.2.2.2 ***Bile acid- TGR5 axis***

More recently, two studies have attempted to provide novel insights on mechanism of BA induced pruritus. Alemi *et al*. suggest BAs induce itch by activating TGR5, a G protein–coupled plasma membrane receptor for BAs. In their elegant mouse experiments, they have shown that: i) TGR5 is expressed by spinal neurons and dermal macrophages, ii) BAs increase the intrinsic excitability of dorsal root ganglion (DRG) neurons by a TGR5-dependent mechanism, and iii) BAs stimulate release of gastrin releasing peptide (GRP) and leucine-enkephalin (Leu-ENK) which are neuropeptide transmitters of itch (Alemi, Kwon et al., 2013). These results have been supported by another mouse study suggesting BAs induce pruritus by co-activation of TGR5 and transient receptor potential ankyrin 1 (TRPA1) (Lieu, Jayaweera et al., 2014). However, it is noteworthy that in these mouse models very high concentrations of unconjugated deoxycholic acid (DCA), a BA that is not or barely found in cholestasis was investigated. The potential role of TGR5 in pruritus is further strengthened by recent evidence that pharmacological activation of TGR5 in mice provokes pruritus (Keitel, Reich et al., 2015). Therefore, it is possible that pruritus induced by OCA may be linked to its weak TGR5 agonism although the definite evidence is lacking (Hirschfield, Mason et al., 2015).

Figure 1-6 [adapted from (Lieu, Jayaweera et al., 2014)] shows a proposed model of Bile Acid-TGR5 induced pruritus in cholestasis.

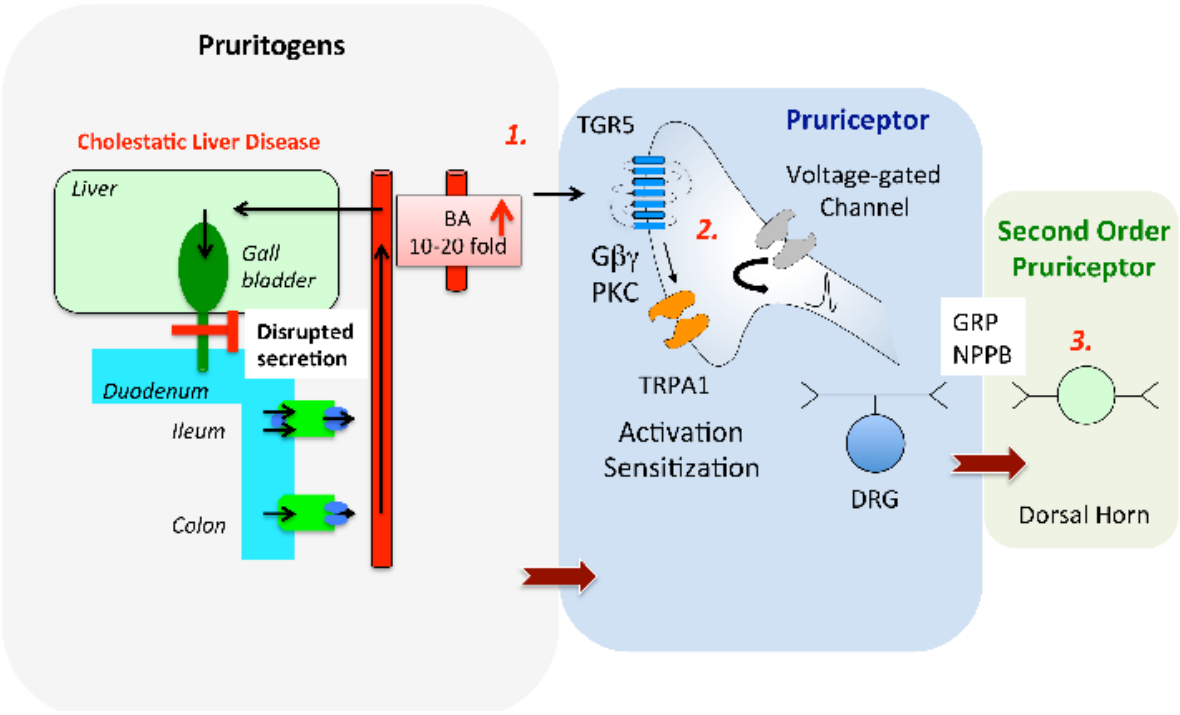


Figure 1-6 A proposed model of bile acid induced itch via TGR5 activation in cholestasis

The above two key observations that OCA, a semisynthetic BA causes itch and TGR5 mediates BA induced itch, have brought the focus back on BAs in the pathogenesis of cholestatic pruritus.

To some extent the BA-TGR5 axis can also explain why pruritus intensity has not been shown to correlate with serum or plasma BA concentrations. Fiorucci and colleagues argue that TGR5 (in addition to detecting BAs in systemic circulation) is also likely to be involved in detecting BAs that flow through the blood-brain barrier or are synthesized directly in the central nervous system (CNS). Alternatively, there may be distinct, yet unidentified pruritogens in the nervous system (such as neurosteroids) that are structurally related to bile acids and are potential agonists of TGR5 (Fiorucci, Distrutti et al., 2014).

1.2.3 Role of endogenous opioids

Another school of thought holds that cholestasis is associated with increased neurotransmission/neuromodulation mediated by endogenous opioids in the CNS. Interestingly, the beneficial effects of naloxone (an opiate antagonist) in relieving pruritus in PBC were first reported (Bernstein and Swift, 1979, Summerfield, 1980), a decade before it

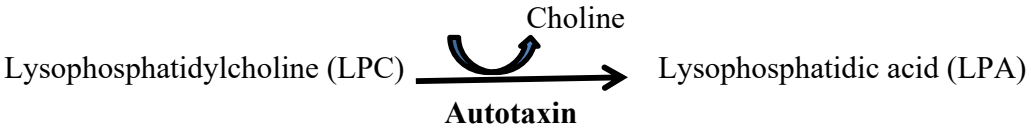
was shown that plasma and hepatic levels of endogenous opioids are increased in cholestatic patients and in animal models of cholestasis (Bergasa, Vergalla et al., 1996, Spivey, Jorgensen et al., 1994, Swain, Rothman et al., 1992). In addition, multiple lines of evidence support the 'opioid theory' of cholestatic pruritus. These include: i) cholestasis is associated with phenomena consistent with increased opioidergic tone, i.e. administration of opiate antagonists to patients with cholestasis is associated with an opiate withdrawal-like reaction and relief of pruritus (Thornton and Losowsky, 1988), ii) cholestatic pruritus can be ameliorated by parenteral administration naloxone (Bergasa, Alling et al., 1995, Bergasa, Talbot et al., 1992), and iii) administration of morphine or other opiate agonist drugs induces pruritus (Ballantyne, Loach et al., 1988, Koenigstein, 1948). More recently it has been shown that opioid induced itch is mediated both by an opioid receptor mechanism and through systemic and peripheral pathways (Greaves, 2010).

However, as is the case with the bile acids (or bile salts) theory, a number of observations are against the 'opioid theory'. No correlation has ever been found between plasma endogenous opioid levels and itch intensity in cholestatic patients and endogenous opioids are increased in advanced stages of PBC (Spivey, Jorgensen et al., 1994) whereas pruritus is typically seen in early stages. Also in the recent study of cholestatic patients by Kremer *et al*., serum μ-opioid activity was not increased in patients with ICP compared with regular pregnancies, only few pruritic PBC patients had increased μ-opioid levels and there was no correlation between serum μ-opioid activity and itch intensity (Kremer, Martens et al., 2010). These evidence dispute the major causative role of opioids in the pathogenesis of cholestatic pruritus.

1.2.4 Role of Autotaxin and Lysophosphatidic acid

1.2.4.1 ***Autotaxin***

Autotaxin (ATX), a potent human motility-stimulating protein, was first isolated from A2058 melanoma cell supernatants (Stracke, Krutzsch et al., 1992). It belongs to the family of ecto-nucleotide pyrophosphatases/phosphodiesterases (ENPP) and is also referred to as ENPP2 (Beuers, Kremer et al., 2014). ATX has extracellular lysophospholipase D activity which produces a bioactive phospholipid lysophosphatidic acid (LPA) by hydrolysing the choline group from lysophosphatidylcholine (LPC) (Tokumura, Majima et al., 2002, Umezu-Goto, Kishi et al., 2002).



ATX is present in the circulating blood and has number of physiological functions including angiogenesis, neuronal development and lymphocytic homing (Tanaka, Okudaira et al., 2006, van Meeteren, Ruurs et al., 2006). There is considerable interest in ATX in oncology as in addition to melanoma cells, it is overexpressed in several other tumour entities and has been linked to tumour cell proliferation, motility and metastases (Mills and Moolenaar, 2003). The effects of ATX are largely mediated by the enzymatic formation of LPA. The phosphodiesterase activity of ATX ('ATX activity assay') in the serum samples can be measured based on the amount of choline released with LPC as the substrate as detected by an enzymatic fluorimetric method (Nakamura, Ohkawa et al., 2007).

1.2.4.2 ***Lysophosphatidic acid***

LPA is a potent bioactive phospholipid that arises in blood as a consequence of enzymatic cleavage of choline from LPC by ATX. Therefore, circulating level of LPA primarily depends on ATX activity. LPA mediates multiple biological functions through activation of specific G protein-coupled receptors including cytokine production, platelet activation, cytoskeletal reorganization, chemotaxis, cell proliferation, cell migration and survival (Mills and Moolenaar, 2003, van Meeteren and Moolenaar, 2007). Animal studies have revealed that LPA is involved in both pathological and physiological states including brain development, neuropathy pain, lung fibrosis, renal fibrosis, protection against radiation-induced intestinal injury, implantation and hair growth (Aoki, Inoue et al., 2008). LPA is a highly unstable lipid derivative that undergoes rapid metabolism in the circulation. In addition, LPA can be formed during and after blood collection, and therefore levels depend on the procedure of processing and storage (Kremer, Martens et al., 2010).

1.2.4.3 ***Evidence for Autotaxin and Lysophosphatidic acid***

Recent experimental and clinical works by a group in Amsterdam provide new insights into cholestatic pruritus. They identified LPA as a potent neuronal activator and showed increased serum levels of LPA in patients with cholestasis (Kremer, Martens et al., 2010, Kremer, van Dijk et al., 2012). They observed significantly elevated concentrations of LPA in the sera of

pregnant patients with ICP compared to gestation matched non-cholestatic pregnant controls. They also confirmed the findings of a previous study showing that intradermal injection of LPA (but not the vehicle) initiated scratch response in mice (Hashimoto, Ohata et al., 2004). Kremer, *et al.* also showed that serum ATX activity was markedly increased in patients with ICP (versus pregnant controls, $p<0.0001$) and cholestatic patients with itch (versus those without itch, $p<0.001$).

Table 1-2 summarises current evidence of ATX and LPA in cholestatic pruritus.

- Serum LPA concentrations markedly increased in patients with ICP compared with gestation matched noncholestatic pregnant controls (n=13, $p<0.05$)
- LPA injected intradermally into mice induced dose dependent scratch response
- Irrespective of the cause of cholestasis serum ATX activity markedly elevated in patients with cholestatic pruritus (compared with cholestatic patients without pruritus and healthy controls)
- Increased serum ATX levels are specific for pruritus of cholestasis but not pruritus of other causes (uraemia, Hodgkin's disease or atopic dermatitis)
- Significant correlation between ATX activity and intensity of itch perception (measured by VAS) in patients with cholestatic itch ($r=0.77$, $p<0.0001$)
- ATX activity responds to and closely correlated with effectiveness of therapeutic interventions:
  - After 3 weeks of treatment Colesevelam marginally lowered ATX activity (n=17, $p<0.05$)
  - Two weeks of Rifampicin treatment (150 mg twice daily) was associated with significant decrease in serum ATX activity (n=6, $p<0.05$)
  - Significant reduction in ATX activity observed in patients with cholestatic pruritus who responded to MARS therapy (MARS responders, n=8) compared to MARS non responders (n=2) ($p<0.01$)
  - Nasobiliary drainage strongly reduced serum ATX levels (n=5, $p<0.01$)
  - Improvement of pruritus (measured in % of VAS after treatment) showed a linear correlation with reduction in serum ATX activity for all treatment groups (colesevelam, rifampicin, MARS and nasobiliary drainage; $r=0.62$, $p<0.0001$)

Table 1-2 Summary of evidence for lysophosphatidic acid and autotaxin in cholestatic pruritus
[References: (Kremer, Bolier et al., 2015, Kremer, Martens et al., 2010, Kremer, van Dijk et al., 2012)]

Although identification of ATX-LPA pathway is a key development in understanding cholestatic pruritus, a number of questions remain unanswered. First, cell (or source) of origin of ATX in cholestatic conditions is as yet unknown. ATX has been reported to be expressed in the liver (Giganti, Rodriguez et al., 2008), and released by adipocytes and endothelial cells (Ferry, Tellier et al., 2003, Kanda, Newton et al., 2008, Moulharat, Fould et al., 2008). It is proposed that either hepatocytes or biliary epithelial cells produce ATX under cholestatic conditions or cholestasis either increases ATX expression or reduces its clearance (Kremer, Martens et al., 2010). Also, evidence suggests liver sinusoidal endothelial cells play an important role in uptake and degradation of ATX (Jansen, Andries et al., 2009).

Second, an interesting observation in studies by Kremer *et al*., is that despite reduction in its serum activity, ATX could not be detected in the bile of patients who underwent nasobiliary drainage (NBD) or in the albumin dialysate of Molecular Adsorbent Recirculating System (MARS®) patients. This suggests that an as yet-unidentified factor ('Factor X') that drives ATX production is cleared from the bile (by NBD) and circulation (by MARS) (Jones, 2012a).

The third and possibly the most important as yet unexplored issue is the biological reason for elevation of ATX in cholestasis. It is plausible that elevation of ATX in cholestasis could be due to the up-regulation of body's homeostatic response to limit biliary epithelial cell injury (Jones, 2012a). The hypothesis that ATX may have a role in the regenerative capacity of biliary epithelial cells in cholestatic conditions has implications in that ATX antagonists may improve pruritus symptom but may be at the cost of worsening the disease progression in cholestasis (Jones, 2012a).

1.3 Current treatment of cholestatic pruritus

Ursodeoxycholic acid (UDCA) is the only FDA (Food and Drug Administration) approved drug licensed for treating PBC patients [Obeticholic acid (OCA), OCALIVA®, Intercept Pharmaceuticals, was conditionally approved as a second-line drug by the FDA in April 2016].

UDCA has been shown to be effective in improving liver biochemistry (Gong, Huang et al., 2007), reducing histological progression (Corpechot, Carrat et al., 2000) and need for liver transplantation and improving survival in PBC patients (Lammers, van Buuren et al., 2014). The mechanism of action of UDCA is described and comprehensively reviewed in a recent report (Beuers, Trauner et al., 2015). In brief, UDCA has potent anti-cholestatic, anti-apoptotic (cytoprotective) and anti-inflammatory effects in hepatocytes and cholangiocytes.

However, UDCA has not been convincingly shown to have any beneficial anti-pruritic effect in cholestatic conditions (except in ICP). An early study showed that at the end of two years of treatment with UDCA a significantly lower number of patients had pruritus (53% vs. 8%, $p<0.01$) (Poupon, Chretien et al., 1987). Combes *et al.,* noted a significant decrease in the likelihood of developing severe pruritus with UDCA, yet no significant improvement in mean pruritus scores compared to placebo was observed (Combes, Carithers et al., 1995).

A later study also observed a trend toward improvement in pruritus after 1 year of UDCA treatment compared to placebo (30% vs. 24%, $P = 0.08$) (Talwalkar, Souto et al., 2003). Despite these observations, there is no strong evidence proving the efficacy of UDCA therapy in reducing development or severity pruritus in PBC and therefore the current American Association for the Study of Liver Diseases (AASLD), European Association for the Study of Liver (EASL) and British Society of Gastroenterology (BSG) guidelines do not recommended UDCA as a specific anti-pruritic agent (European Association for the Study of the, 2017, Hirschfield, Dyson et al., 2018, Lindor, Gershwin et al., 2009). On the contrary, some anecdotal reports and an early study observed 'paradoxical itch' in patients taking UDCA, particularly at the beginning of therapy (Poupon, Chretien et al., 1987).

In the Talwalkar *et al*. study, ~8% of patients given UDCA developed pruritus and among patients treated with standard dose UDCA (13–15 mg/kg/day) reporting pruritus at study entry, there was no significant improvement in prevalence rate after 1 year (23% vs. 33%,

p=0.37). A recent Cochrane review concluded that UDCA did not influence the number of PBC patients with pruritus (168/321 (52.3%) versus 166/309 (53.7%); RR 0.96, 95% CI 0.84 to 1.09, I^2 = 0%; 6 trials) (Rudic, Poropat et al., 2012). Interestingly, more recent evidence suggests that severity of pruritus tends to be higher in those PBC patients who are unresponsive to UDCA (UDCA non-responders, defined using the Paris I criteria) (Carbone, Mells et al., 2013).

In PBC and other cholestatic diseases, symptomatic patients with itch need specific anti-pruritic interventions for effective symptom control and improve quality of life. There are, however, currently only limited treatment options available for patients suffering with cholestatic itch. Topical emollients plus coolants (such as 1% aqueous cream with menthol) can be used for mild or localised itch. For more severe and generalised itch associated with cholestasis AASLD and EASL recommend step-wise treatment using colestyramine, rifampicin, opioid antagonists and sertraline (Beuers, Boberg et al., 2009, Lindor, Gershwin et al., 2009) (**Figure 1-7**).

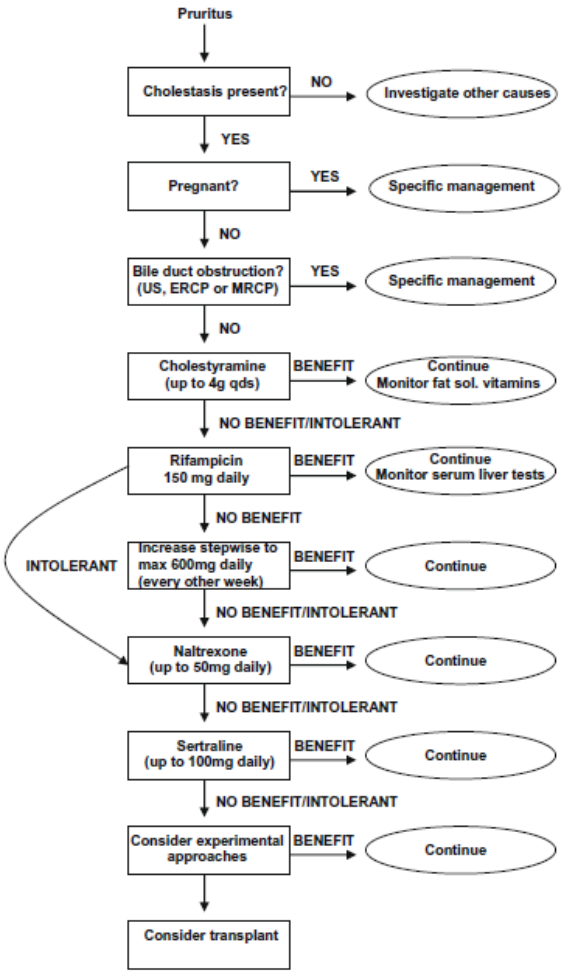


Figure 1-7 EASL recommendations on step-wise treatment of cholestatic pruritus

1.3.1 Cholestyramine

Cholestyramine (colestyramine) is an orally administered non-absorbable anion exchange resin which removes potential pruritogens (bile salts) from the enterohepatic circulation by enhancing their faecal excretion. Cholestyramine has been shown to preferentially bind dihydroxy bile salts (which are more effective in causing pruritus than trihydroxy bile salts) in the intestine (Johns and Bates, 1969, Johns and Bates, 1970, Kirby, Heaton et al., 1974) and produces 30-40% reduction in total serum bile salts levels with an increase in the ratio of trihydroxy to dihydroxy bile acids (Neale, Lewis et al., 1971).

Cholestyramine is the first line therapy recommended by the international guidelines for the amelioration of cholestatic pruritus (Beuers, Boberg et al., 2009, Lindor, Gershwin et al., 2009). However, the evidence basis for its recommendation is category II-2 (cohort or case control analytical studies), largely derived from uncontrolled studies performed in 1960s

(Carey and Williams, 1961, Datta and Sherlock, 1963, Datta and Sherlock, 1966, Oster, Rachmilewitz et al., 1965, Van Itallie, Hashim et al., 1961). In 1984, a single blind placebo controlled study of 7 PBC patients showed that compared to placebo, pruritus scores were significantly lower during two weeks of treatment with cholestyramine (Duncan, Kennedy et al., 1984). In the same year Di Padova *et al*. (the only double-blind, placebo controlled, randomized trial to date) reported a significant beneficial effect of cholestyramine (3g three times daily for 4 weeks) versus a placebo (p=0.01) in 10 patients (Di Padova, Tritapepe et al., 1984). A more recent meta-analysis also suggested that although colestyramine improves cholestatic pruritus, the scientific basis is weak (Tandon, Rowe et al., 2007).

The recommended initial dose of cholestyramine is 4g/day, preferably taken in the morning and gradually increased up to 16g/day based on clinical need. Cholestyramine should be taken at least 4 hours before or after any other medications (including UDCA) to avoid interference with their intestinal absorption. In clinical practice, the main limitation to the use of cholestyramine is its unpleasant taste which negatively affects the compliance. Other adverse effects such as anorexia, constipation, diarrhoea, abdominal discomfort or bloating and hypertriglyceridemia may also limit its regular use.

1.3.1.1 ***Colesevelam***

Colesevelam is an anion-exchange resin (similar to cholestyramine and colestipol). But, owing to the presence of abundant hydrophobic side chains on the hydrophilic polymer backbone, it has 7-fold higher BA–binding capacity than cholestyramine (Steinmetz, 2002). Also, it is better tolerated with a low incidence of gastrointestinal side effects (Davidson, Dillon et al., 1999).

As a treatment for cholestatic pruritus, colesevelam has been studied only once in a double bind randomised controlled trial of 35 patients with severe pruritus of different disease aetiology (Kuiper, van Erpecum et al., 2010). The trial showed that 3-week treatment with colesevelam (three 625mg tablets twice daily) significantly decreased serum BA levels but was no more effective than placebo in alleviating the severity of cholestatic pruritus. Although the mean morning and evening pruritus VAS scores decreased significantly during colesevelam treatment (p=0.01), the primary end point was not different between the colesevelam and placebo groups. The predefined primary endpoint (proportion of patients with at least a 40% reduction of pruritus based on VAS scores) was reached by 36% in the

colesevelam group [compared to 35% in the placebo group (p=1.0)] for the morning VAS scores and 40% for the evening VAS scores [compared to 50% in the placebo, (p=0.74)]. Also, at the end of 3-week treatment 76% of colesevelam-treated patients still reported severe pruritus. It is noteworthy that only 4 patients in the colesevelam group (n=17) had PBC. According to the authors the potential reasons for observed lack of efficacy of colesevelam were 1) small sample size or shorter duration of treatment, 2) intestinal BAs were not of key importance in the pathogenesis of pruritus, or 3) the observed decrease in serum BA levels was not enough to have an impact on the severity of pruritus.

Recently, colesevelam treatment has been shown to attenuate cholestatic liver and bile duct injury in Mdr2$^{-/-}$ mice by modulating composition, signalling and excretion of faecal bile acids. Colesevelam treatment for 8 weeks increased faecal BA excretion, enhanced BA conversion towards secondary BAs, thereby stimulating secretion of GLP-1 from enteroendocrine L-cells. In addition, faecal microbiota analysis showed increase of the phylum δ-Proteobacteria and a shift within the phyla Firmicutes from *Clostridiales* to *Lactobacillus* (Fuchs, Paumgartner et al., 2018).

1.3.2 Rifampicin

Rifampicin (or rifampin), an enzyme inducer and a commonly used anti-mycobacterial agent, is an approved and guideline recommended second line agent in the treatment of cholestatic pruritus (2009, Lindor, Gershwin et al., 2009). Studies on molecular mechanism of rifampicin in cholestasis have shown that it enhances BA detoxification as well as bilirubin conjugation and export systems by the induction of biotransformation of enzymes and transporters (LeCluyse, 2001, Marschall, Wagner et al., 2005). This would explain its anti-cholestatic mechanism in improving biochemical markers of liver injury but not its antipruritic effect in cholestatic liver disease. Induction of microsomal enzymes that metabolise systemic pruritogens and a reduction in bile salt-mediated disruption of hepatocyte membranes were the early hypothesized mechanism of therapeutic action (Galeazzi, Lorenzini et al., 1980, Miguet, Mavier et al., 1977). In addition, an unconfirmed suggestion was that rifampicin alters intestinal metabolism of potential pruritogens by its antibiotic effect on the intestinal flora. Recently, rifampicin was shown to significantly decrease serum ATX levels (compared to placebo) and reduce expression of ATX in HepG2 cells (*in vitro*) in pregnane X receptor (PXR)-dependent manner. As Rifampicin is a PXR agonist, these results suggest that anti-

pruritic effect of rifampicin may be via PXR agonism-mediated down regulation of autotaxin transcription (Kremer, van Dijk et al., 2012).

Following the first study (Hoensch, Balzer et al., 1985) that showed rifampicin ameliorates pruritus, four prospective randomised controlled clinical trials (Bachs, Pares et al., 1989, Bachs, Pares et al., 1992, Ghent and Carruthers, 1988, Podesta, Lopez et al., 1991) have confirmed its efficacy by using subjective end points including self-report of itch severity. Two meta-analyses (Khurana and Singh, 2006, Tandon, Rowe et al., 2007) further corroborate that treatment with rifampicin leads to complete or partial resolution of pruritus in up to 77% patients as compared with placebo or alternative. Dose of rifampicin in these studies was 300-600 mg/day or 10mg/kg/day. The old AASLD guideline recommendation was to use 150mg/day when serum bilirubin level is <3mg/dl (<51µmol/L) and 300mg/day (150mg twice daily) when serum bilirubin level is >3mg/dl (Lindor, Gershwin et al., 2009). However, according to the current AASLD guidelines rifampicin should not be used in patients with bilirubin levels >2.5 mg/dl (Lindor, Bowlus et al., 2019). Based on the clinical need, the dose can be titrated up to maximum of 600 mg/day with regular follow up of liver panel and blood counts.

Side effects associated with rifampicin use are nausea, vomiting, diarrhoea, decreased appetite, headaches, fever, rash and flushing (Khurana and Singh, 2006, Martinez, Collazos et al., 1999). Most of the side effects of rifampicin are transient and resolve on discontinuation of the drug. Side effects that are of serious concern include hepatitis, hepatic failure, haemolytic anaemia, thrombocytopenia, renal impairment and alteration in drug metabolism (Bachs, Pares et al., 1989, Khurana and Singh, 2006, Prince, Burt et al., 2002, Talwalkar, Souto et al., 2003). In addition, rifampicin and selective serotonin re-uptake inhibitor (SSRI) drugs should not be used together as rifampicin may obviate their anti-depressive effects (Markowitz and DeVane, 2000).

Although studies support both short and long-term use of rifampicin treatment as effective for relieving pruritus, hepatotoxicity remains a serious concern, especially with long term use. An early study reported 12.5% incidence of rifampicin-induced hepatitis (Bachs, Pares et al., 1992) and another study reported significant hepatitis in 7.3% of patients (necessitating liver transplantation in one case) treated with rifampicin for cholestatic liver disease (Prince, Burt et al., 2002). Therefore, in those taking rifampicin, close monitoring of blood counts and liver

function tests (serum transaminase levels) at regular intervals is strongly recommended. More recently, low risk of hepatotoxicity has been suggested by a retrospective study. In this study of 105 patients (with PBC or PSC) treated with rifampicin, drug induced hepatitis occurred in 4.8% cases at a median of 70 (range 27-130) days after drug initiation and all cases of hepatitis recovered after drug cessation (Webb, Rahman et al., 2018).

1.3.3 Opioid antagonists

Selective mu (μ) opioid receptor antagonist agents- naloxone and naltrexone are recommended as third line therapy for patients with cholestatic itch when the first and second line drugs are ineffective or not tolerated. Interestingly, the beneficial effect of naloxone in relieving pruritus in PBC was first reported in 1980 (Summerfield, 1980), a decade before it was shown that plasma and hepatic levels of endogenous opioids are increased in cholestatic patients and in animal models of cholestasis (Bergasa, Rothman et al., 1992, Bergasa, Vergalla et al., 1996, Swain, Rothman et al., 1992). More recent studies have shown that opioid induced itch is mediated both by an opioid receptor mechanism and by initiating itch through systemic and peripheral pathways (Greaves, 2010). Therefore, the likely mechanism of opiate antagonists in reducing itch is by blocking the opiate receptors and by modifying central and peripheral itch and/pain signalling by influencing the endogenous opioidergic system.

A number of prospective studies have shown that when administered either orally (naltrexone and nalmefene) (Bergasa, Alling et al., 1999, Bergasa, Schmitt et al., 1998, Carson, Tran et al., 1996, Mansour-Ghanaei, Taheri et al., 2006, Terg, Coronel et al., 2002, Thornton and Losowsky, 1988, Wolfhagen, Sternieri et al., 1997) or as intravenous infusion (naloxone) (Bergasa, Alling et al., 1995, Bergasa, Talbot et al., 1992) opioid antagonists are associated with amelioration of the perception of pruritus and reduction of scratching activity in cholestatic patients. Further support to their use comes from a recent meta-analysis of five studies that concluded that compared to the control intervention opiate antagonists are more likely to significantly reduce cholestasis associated pruritus (Tandon, Rowe et al., 2007).

A significant concern with the use of these agents is precipitation of 'opiate withdrawal like reaction' - a constellation of symptoms characterised by abdominal pain, tachycardia, high blood pressure, goose bumps, nightmares and depersonalisation (Bergasa, Alling et al., 1999, Bergasa, Schmitt et al., 1998, Thornton and Losowsky, 1988). This reaction can be

minimised by starting the opioid antagonists at a lower dose and gradually increasing the dose. The guidelines recommend starting naltrexone at 12.5mg/day and gradually increase by 12.5mg every 3-7 days until amelioration of pruritus (maximum daily dose 50mg) (Lindor, Gershwin et al., 2009). Another approach to minimise opioid withdrawal like reaction is by admitting patients to the hospital for 3-4 days to receive continuous daily intravenous infusion of naloxone (0.4mg intravenous bolus, then 0.2μg/kg/min continuous infusion) followed by introduction of oral naltrexone and discontinuation of the infusion. In those responding to this treatment, naltrexone can be continued as it is generally well tolerated during long-term treatment. Hepatotoxicity is uncommon but has been reported (Mitchell, 1986), therefore regular monitoring of liver biochemistry is recommended. Opioid antagonists are contraindicated in patients with acute hepatitis, liver failure, suppressed pulmonary function, drug addictions and in those receiving opioid containing medications (Imam, Gossard et al., 2012).

1.3.4 Sertraline

Sertraline, a selective serotonin re-uptake inhibitor (SSRI), commonly prescribed as an antidepressant, is the recommended fourth line therapy to alleviate cholestatic pruritus. The rationale for its use comes from evidence that serotonin system modulates nociception and perception of pruritus and sertraline can influence endogenous serotoninergic system and modify the central itch and/or pain signalling. Two studies showed sertraline was well tolerated and moderately effective in reducing the intensity of itch in cholestatic pruritus (Browning, Combes et al., 2003, Mayo, Handem et al., 2007). The recommended initial dose is 25mg/day, increased gradually by 25mg every 4-5 days to 75-100mg/day. Sertraline is usually well tolerated and uncommon adverse effects include nausea, dizziness, diarrhoea, visual hallucinations and increased fatigue (Mayo, Handem et al., 2007). As mentioned earlier, rifampicin and sertraline should not be used together as the later may avert the antidepressant effects of serotonin reuptake inhibition and induce SSRI withdrawal syndrome (Markowitz and DeVane, 2000).

1.3.5 Other drugs

Antihistamines are commonly prescribed and used by patients with cholestatic pruritus (Rishe, Azarm et al., 2008) even though there is no strong evidence to support their benefits. A single blind randomised crossover trial (n=8) of PBC and PSC patients with pruritus

showed that chlorpheniramine was ineffective and was associated with a high incidence of side effects and Terfenadine (non-sedative, selective H_1 specific antihistamine) had significant anti-pruritic effect (Duncan, Kennedy et al., 1984). Histamine does not play a major role in pathogenesis of cholestatic pruritus and classical histamine induced skin changes such as erythema, urticaria and flares seen in allergic reactions are not observed in patients with cholestatic pruritus. Nevertheless, due to their sedative properties antihistamines may help patients sleep (giving night time 'remission') and may dampen the itch severity during the day (Greaves, 2005). Side effects associated with their use such as dry mouth and dry eyes limit their use in PBC patients with sicca symptoms.

Other medications that have been studied in cholestatic pruritus include: stanozolol, phenobarbitone, propofol, flumenicol, ondansetron, dronabinol, butorphanol, lidocaine and gabapentin (Bachs, Pares et al., 1989, Bergasa, McGee et al., 2006, Borgeat, Wilder-Smith et al., 1993, Neff, O'Brien et al., 2002, Schworer, Hartmann et al., 1995, Turner, Rawlins et al., 1994, Villamil, Bandi et al., 2005, Walt, Daneshmend et al., 1988). These therapies are at best are considered experimental drug therapies and are not recommended by current guidelines for routine use.

1.3.5.1 ***Fibrates in cholestatic pruritus***

Fibrates (Bezafibrate, BZF and Fenofibrate, FF) are peroxisome proliferator activated receptor (PPAR) agonist agents and have long been in clinical use as effective treatment for hyperlipidaemia. FF is a selective PPAR-α agonist and BZF is a pan-PPAR (α, β/δ and γ) agonist. Their effect on lowering serum alkaline phosphatase was first reported in 1993 with a study reporting 25% reduction in serum ALP with six weeks of therapy with BZF for hyperlipidaemia (Day, Feher et al., 1993). Since then, many studies have reported fibrates improve liver biochemistry in patients with PBC who have suboptimal response to UDCA (summarised in the review article:(Ghonem, Assis et al., 2015)). Interestingly, some case reports and pilot studies have also reported beneficial effects of BZF and FF on pruritus (Han, Wang et al., 2012, Kanda, Yokosuka et al., 2003, Kita, Kita-Sasai et al., 2002, Kita, Takamatsu et al., 2006, Ohmoto, Mitsui et al., 2001, Ohmoto, Yoshioka et al., 2006). It is noteworthy that in these uncontrolled studies anti-pruritic effect of fibrates was not measured as a primary outcome and pruritus was not assessed objectively using validated tools. Therefore, no firm conclusion can be drawn on the anti-pruritic effect of fibrates.

Anecdotally, pruritus appears to occur or recur after stopping fibrates in PBC patients (personal communication: Prof. Albert Pares, Barcelona, Spain).

To date, there is no placebo-controlled RCT evaluating the role of fibrates as anti-pruritic drugs in cholestatic liver disease. However, the BEZURSO study (the only RCT to date of fibrates in PBC) has recently been published(Corpechot, Chazouilleres et al., 2018b). In this 24-month, double-blind, placebo-controlled, phase 3 trial, patients who had had an inadequate response to UDCA were randomised to receive BZF at a daily dose of 400 mg (50 patients), or placebo (50 patients), in addition to continued treatment with UDCA. At baseline, 32% in BZF group and 48% in the placebo group had clinically significant pruritus (defined as 0-10 VAS score of $\geq$3). The results on pruritus showed the reduction in the itch intensity score was greater in the bezafibrate group (47 patients) than in the placebo group (40 patients). The median percentage change in the VAS score was −100% (95% confidence interval [CI], −100 to −71) in the BZF group, as compared with 4% (95% CI, −40 to 47) in the placebo group. The median difference of changes from baseline to 24 months in itch intensity score between BZF and placebo groups was -95% [95%CI -241% to 50%]. However, we argued that since the pruritus intensity levels were low, particularly in the BZF group (median baseline itch intensity score of 1), no definitive conclusions can be drawn from this trial with regard to the effects of BZF on clinically significant itch in patients with PBC (Jones and Hegade, 2018). The authors of this study acknowledged that the trial was not specifically designed to assess the effect of BZF on pruritus and studies that are specifically designed to assess symptoms would be needed (Corpechot, Chazouilleres et al., 2018a).

The mechanism of potential anti-pruritic effect of fibrates is of importance but has not been explained. Researchers in the Academic Medical Centre (AMC, Amsterdam) have hypothesized that bezafibrate may improve pruritus by reducing serum ATX activity levels and/or other pruritogens. Their FITCH trial, designed to test this hypothesis, is currently recruiting patients(Bolier, de Vries et al., 2017). Metabonomic studies of BZF in PBC have shown BZF significantly decreases circulating metabolites such as phosphatidylcholines and some sterols(Reig, Pérez-Cormenzana et al., 2016).

It can be concluded that currently there is lack of strong evidence for anti-pruritic effect of fibrates but they would be an attractive therapeutic option for itch in PBC as they have proven anti-cholestatic effect and they appear to be safe for long-term administration.

1.3.6 Invasive/Experimental therapies

The anti-pruritic pharmacotherapies explained above provide relief only in a proportion of affected patients. As liver transplantation (LT) improves cholestatic pruritus, PBC patients with severe intractable itch should be referred for LT even in the absence of liver failure (Lindor, Gershwin et al., 2009). Invasive therapy may be offered to those with refractory cholestatic pruritus and to those who need relief of symptom (albeit temporary) while on the liver transplant waiting list. Currently available invasive therapies for patients with refractory cholestatic pruritus are:

- nasobiliary drainage (NBD)
- Plasmapheresis
- Albumin dialysis using molecular adsorbent recirculating system (MARS)

*1.3.6.1 **Nasobiliary drainage***

Nasobiliary drainage involves patient undergoing endoscopic retrograde cholangiography (ERC) and placing a fine tube (usually 7Fr size) in the extrahepatic bile duct which is brought out through the patient's nose. As bile is drained freely over a period of 1-7 days, it is likely that NBD removes the potential pruritogen(s) from the enterohepatic circulation. To date the evidence for using NBD in cholestatic pruritus has been mainly in the form of case series which report striking and rapid relief of pruritus after installing a NBD (Beuers, Gerken et al., 2006, Singh, Bhalla et al., 2009, Stapelbroek, van Erpecum et al., 2006). This thesis has explored the safety and efficacy of this intervention in more detail (see **Chapter 3**).

*1.3.6.2 **Plasmapheresis and MARS***

Small case reports and case series have shown both plasmapheresis and MARS® to be safe and effective therapeutic options for transiently relieving drug resistant cholestatic pruritus (Cisneros-Garza, Munoz-Ramirez Mdel et al., 2014, Pusl, Denk et al., 2006). Their therapeutic success is postulated secondary to removal of potential pruritogen(s) from the systemic circulation.

Interestingly, a recent study showed linear correlation between the effects of NBD and MARS in reducing the perception of itch with lowering of serum ATX levels (Kremer, van Dijk et al., 2012). However neither ATX protein nor its activity could be detected in the bile or in the

albumin dialysate suggesting 'factor X' that is capable of increasing ATX expression in cholestatic pruritus is removed by NBD and MARS (Jones, 2012a).

1.3.6.3 ***Phototherapy***

Patients with medically refractory cholestatic pruritus can also be treated with narrowband ultraviolet B (UV-B) phototherapy, an established treatment modality for pruritus of cutaneous conditions (e.g. psoriasis, atopic dermatitis). Narrowband UV-B, which has a focused and maximum emission at 311nm was first introduced following a study of the action spectrum of UV-B in psoriasis and appears relatively safe (Parrish and Jaenicke, 1981).

The evidence for UV light therapy in cholestatic pruritus comes mainly from case reports and case series (Cerio, Murphy et al., 1987, Decock, Roelandts et al., 2012, Hanid and Levi, 1980, Perlstein, 1981, Person, 1981, Pinheiro, Marinho et al., 2013, Rosenthal, Diamond et al., 1994). In the absence of randomised controlled trials, these studies as well as anecdotal evidence suggests UV light therapy provides only temporary relief from itch.

The exact mechanism of therapeutic effect is not clearly explained but UV-B light induced chemical modification of pruritogens in the skin or altered sensitivity to pruritogens have been postulated (Cerio, Murphy et al., 1987, Hanid and Levi, 1980).

1.4 Limitation of current anti-pruritic therapies

As described in previous sections, UDCA, the current mainstay treatment of PBC patients has no proven role in treating pruritus. The four main classes of drugs recommended by the American and European guidelines are limited by their lack of universal efficacy, poor compliance (especially cholestyramine) and the need for regular monitoring for liver toxicity (rifampicin). Of these, cholestyramine is the only licensed drug for treatment of cholestatic pruritus and use of other drugs is "off-label". Cholestyramine and rifampicin have good reports but clinical experience of both naltrexone and sertraline has been disappointing for many clinicians (Beuers, Boberg et al., 2009).

A critical review of published literature shows that the strength of evidence for available anti-pruritic drug therapy is poor. Cholestyramine, the current first-line therapy was last studied over five decades ago, and has evidence category II-2 (cohort or case control analytical studies) (Carey and Williams, 1961, Datta and Sherlock, 1963, Datta and Sherlock, 1966, Oster, Rachmilewitz et al., 1965, Van Itallie, Hashim et al., 1961). Only rifampicin and

naltrexone have been studied in controlled trials (both have evidence category I) (Ghent and Carruthers, 1988, Khurana and Singh, 2006, Tandon, Rowe et al., 2007, Terg, Coronel et al., 2002, Wolfhagen, Sternieri et al., 1997) and sertraline (evidence category II-2) was the last agent investigated with a positive clinical outcome on pruritus (Mayo, Handem et al., 2007). A number of other drugs have been investigated but with little success and more recently both gabapentin (2006) and colesevelam (2010) trials failed to show any therapeutic benefit in cholestatic pruritus (Bergasa, McGee et al., 2006, Kuiper, van Erpecum et al., 2010).

Invasive therapies such as NBD and MARS may be offered to patients with medically refractory pruritus but they have considerable limitations. NBD involves endoscopy and carries risks associated with ERCP (such as post-ERCP pancreatitis). MARS is only available in specialist centres and patients may have to travel long distances to access the treatment. Moreover, the duration of relief from pruritus induced by these invasive therapies is variable (few weeks at best) and many patients need repeated sessions of treatments to maintain ‘remission’ of itch symptom.

1.5 Metabonomics

Metabonomics is a part of the high-throughput, systems level 'omic' technologies (others include: genomics, proteomics, and transcriptomics) which have helped to significantly improve our understanding of the biology and development of a number of diseases. Although both use the same experimental tools, metabonomics (understanding the response of living systems to stimuli) and metabolomics (a comprehensive characterization of the metabolic complement of the cell) have been defined slightly differently (Nicholson, Lindon et al., 1999, Oliver, Winson et al., 1998).

Professor Jeremy Nicholson (of Imperial College London) first coined the term 'metabonomics' and defined it as the quantitative measurement of the multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modifications (Nicholson, Lindon et al., 1999). The similar term 'metabolomics' was defined later (Fiehn, 2002) and is now used interchangeably. Metabolomics was defined as 'a comprehensive analysis in which all the metabolites of a biological system are identified and quantified'. In contrast to the interventional definition of metabonomics, metabolomics has an observational definition which is difficult (if not impossible) to achieve (Dona, Kyriakides et al., 2016). Therefore *metabonome* or *metabolome* refers to a profile of chemicals in a sample (or a tissue) and the profile represents a snapshot in time of what chemicals are present in the sample (Marchesi, Adams et al., 2016). By measuring changes in metabolite concentrations the range of biochemical effects that are induced by a disease can be determined and such information can be complementary to genetic, epigenetic and proteomic knowledge. Metabonomic phenotyping corresponds to the use of analytical chemistry methods in metabolomics to generate high-resolution metabolic observations about various disease and treatment conditions (Dumas, Kinross et al., 2014).

The two main analytical platforms used in the metabonomic phenotyping studies are proton (^{1}H) nuclear magnetic resonance (NMR) spectroscopy, and Mass spectrometry (MS). The latter is usually coupled with a chromatographic technique such as Liquid Chromatography (LC) or Gas Chromatography (GC) to improve spectral resolution. At the instrumentation level, methods and principles of NMR and MS are different. NMR spectroscopy exploits the ability of spin active nuclei to absorb and re-emit pulsed electromagnetic radiation of a characteristic frequency pattern when placed in a magnetic field; interaction of nuclei with electromagnetic fields gives information about molecular structure, chemical environment and

molecular motion (Holmes, Wijeyesekera et al., 2015). In contrast, ultra-performance liquid chromatography-mass spectrometry (UPLC-MS) uses chromatographic columns packed with small particles (1.7μm) to allow the use of ultra-high pressure elution with improved chromatographic separation and reproducibility (Holmes, Wijeyesekera et al., 2015).

Both NMR and MS enable a fairly high-throughput generation of molecular fingerprints associated with health and disease (phenotype) of interest and information is usually complimentary. ^{1}H NMR is commonly used as a first stage metabolic screening tool as it enables the generation of untargeted metabolic profiles associated with disease phenotype of interest from study samples. This is then followed up using more sensitive MS methods- with liquid (LC-MS) or gas phase (GC-MS) chromatographic separation for targeted detection and quantification of biomarkers of interest. More recently ultra-performance liquid chromatography-mass spectrometry (UPLC) is used as a molecular separation phase before MS detection since it provides rapid analysis and delivers excellent chromatographic resolution (Plumb, Castro-Perez et al., 2004).

Human bio fluids that can be studied using NMR and/or MS include: blood (plasma/serum), faeces, cerebrospinal fluid, urine, sweat, tears, saliva, bile, milk, blister/cyst fluid, dialysis fluid and lavage fluid. There is a large and accumulating research evidence to show that using NMR and MS techniques metabonomic profile of wide range of pre-pathological and pathological conditions can be characterised [summarised in (Holmes, Wijeyesekera et al., 2015)].

Table 1-3 [adapted from (Holmes, Wijeyesekera et al., 2015)] outlines the relative strengths and limitations of NMR spectroscopy and ultra-performance liquid chromatography-mass spectrometry (UPLC-MS).

Platform	Relative strengths	Relative weaknesses
Nuclear magnetic resonance (NMR) spectroscopy	-Highly reproducible -Low cost per sample (mainly reagent free) -Exact quantification possible -Detailed SOPs and experimental parameters available -Minimal need for sample preparation, chemicals, reagents -Relatively high throughput (10–15 min per sample) -Good metabolite identification databases -2D methods applied to multiple samples informs statistical spectroscopic analysis (to aid in metabolite identification) -High linear dynamic range (~1 x 106) -Non-destructive -Analysis of wide range of chemical structures and molecular sizes	-Relatively insensitive -High capital cost of instrumentation -Overlap of metabolites in 1D spectra (mitigated by increased magnetic field strengths and ≥2D methods)
Ultra-performance liquid chromatography-mass spectrometry (UPLC-MS)	-Profiling or targeted quantitative modes depending on the MS detector -Sample handling simple -High throughput capability (typically 1–20 min per sample) -UPLC can be coupled to any type of MS -Any column chemistry possible, giving a wide range of detectable compounds	-Retention times are highly specific to exact chromatographic conditions -Databases only transferable when chromatographic conditions are identical -Batch effects can be introduced by mass detector drift of chromatography -Relatively young technology; metabolite databases are incomplete

Table 1-3 Strengths and weaknesses of NMR spectroscopy and UPLC-MS

1.5.1 Metabonomic studies of cholestasis

In health, due to the effective enterohepatic circulation only small quantities of BAs are found in the systemic (peripheral) circulation. However in cholestasis, due to the disturbance in bile flow or clearance, the concentration and profile of BAs in various pool compartments (serum, urine, and faeces) are likely to be affected (Yousef I. M., G. Bouchard et al., 1998). Therefore, using metabonomic studies to investigate BA profile in these bio fluids may be useful in patients with cholestatic pruritus.

To date only a few metabonomic studies have been piloted on serum or plasma from patients with PBC (Bell, Wulff et al., 2015, Masubuchi, Sugihara et al., 2015, Trottier, Białek et al., 2012).

Trottier *et al*., investigated the role of BAs as biomarkers in PBC (and PSC) by metabolomic profiling of 17 BAs in the serum from twelve (n=12) PBC patients (Trottier, Bialek et al., 2012). They showed that in cholestatic conditions (PBC and PSC) the serum concentration of total bile acids (TBA) and taurine conjugates of primary bile acids (such as taurocholic acid, TCA) was *elevated* (compared to non-cholestatic condition). In contrast to PSC the ratio of total glycine versus total taurine conjugates was *reduced* in patients with PBC (compared to PSC). However, in this study there was no additional information on the phenotype of PBC patients and no data on if the patients had pruritus.

In the global metabolic profile study by Bell *et al*., there was no information on pruritus in the study group (PBC, n=18) but 101 metabolites were found to be significantly ($p \leq 0.05$) different between PBC and healthy controls. The differential BA levels seen in patients with PBC were similar to the Trottier *et al*., study. They performed random forest analysis of low-molecular-weight metabolites and BAs. Compared to healthy control, *higher* levels of the conjugated primary bile acids glycocholic acid (GCA), taurocholic acid (TCA), glycochenodeoxycholic acid (GCDCA) and taurochenodeoxycholic acid (TCDCA) as well as hyocholic acid (HCA, an unusual trihydroxy BA) and its conjugates (glycohyocholic acid, GHCA and taurohyocholic acid, THCA) were seen in patients with PBC. On the contrary, secondary bile acid deoxycholic acid (DCA) and its glycine- and taurine-conjugated derivatives were not different. The authors further assessed the profiles and identified alterations in lipid metabolism, oxidative stress/lipid peroxidation, stress hormones and protein/amino acid metabolism in PBC (Bell, Wulff et al., 2015).

The focus of the study by Masubuchi *et al*. was to identify serum biomarkers to differentiate cholestatic injury from hepatocellular injury. Using LC-MS/MS based methods they showed *decrease* in the serum levels of lithocholic acid (LCA) and DCA to be significantly associated with cholestatic liver injury in contrast to increased levels of LCA and decreased UDCA level to be associated with hepatocellular injury (Masubuchi, Sugihara et al., 2015). This suggests secondary bile acids (LCA and DCA) have the potential to discriminate cholestatic liver injury from hepatocellular injury.

1.6 Microbiota and microbiome

Microbiota refers to the types of organisms that are present in an environmental habitat, whether they are bacteria, viruses or eukaryotes; and *microbiome* refers to collection of different microbes and their functions or genes found in an environmental habitat (Marchesi, Adams et al., 2016).

The microorganisms that reside in the human gut (i.e. gut microbiota) and their functions or genes (i.e. gut microbiome) have a profound influence on human physiology and nutrition, and are crucial for human life. Trillions of microbiota present in the human intestine, termed as "super-organism", consists of many hundreds of species of bacteria that play significant role in the life of host, affecting the balance between health and disease (Holmes, Li et al., 2011, Lederberg, 2000). One of the most exciting scientific advances in recent years has been the realization that bacteria in the human gut are not simple 'passengers' in our bodies, but instead have key roles in our physiology, including our immune responses and metabolism, as well as in disease (Blaser, Bork et al., 2013). Therefore, the last decade has seen rapid interest in this field and the gut microbiome (predominantly bacteria) is now increasingly being investigated in both health and disease.

The role of the gut microbiota in various gastrointestinal and liver diseases is being increasingly recognised. This evolving field has greatly benefited from recent developments in the high throughput sequencing technologies and bioinformatics which have finally reached a resolution needed for studying the ecosystem that is composed of 100 trillion cells. Culture independent quantitative PCR (qPCR) of the 16S rRNA gene is the primary tool used in these studies and it has been shown to be a powerful technique in studying the diverse and complex faecal microbiota (Mariat, Firmesse et al., 2009). The 16S rRNA gene is the most invariant gene in the bacterial genome and is considered the best phylogenetic maker for molecular taxonomy (Ridlon, Kang et al., 2014). Bacterial 16S rRNA genes sharing 97-99% identity is referred to as an operational taxonomic unit (OTU) and represents a "phylotype"(Ley, Peterson et al., 2006). The human gut microbiota contains more than 1000 phylotypes which are mainly divided into six phyla: *Firmicutes* (Gram-positive), *Bacteroidetes* (Gram-negative), *Actinobacteria* (Gram-positive), *Proteobacteria* (Gram-negative), *Fusobacteria* and *Verrucomicrobia* (Human Microbiome Project, 2012). The basic functions performed by the human gut microbiota include bile salt metabolism, vitamin synthesis, digestion and

fermentation of proteins and polysaccharides and stimulation of the immune function (Deda, Gika et al., 2015).

Dysbiosis refers to a disturbance or imbalance in a biological system and specific changes in the types and numbers of bacteria in the gut may lead to developing different diseases (Marchesi, Adams et al., 2016). Indeed, specific dysbiosis related to gut microbiota have been determined in various diseases.

Disease/Condition	**Reference**
Cirrhosis	(Bajaj, Ridlon et al., 2012)
Obesity	(Ley, 2010)
Type 2 Diabetes	(Larsen, Vogensen et al., 2010)
Irritable bowel syndrome (IBS)	(Rajilic-Stojanovic, Biagi et al., 2011, Saulnier, Riehle et al. 2011)
Inflammatory bowel disease (IBD)	(Frank, St Amand et al., 2007, Rajilic-Stojanovic, Shanahan 2013, Sokol, Seksik et al., 2009, Willing, Dicksved et al., 20
Colorectal cancer	(Wang, Cai et al., 2012)
Clostridium difficile associated diarrhoea (CDAD)	(Hopkins and Macfarlane, 2002)
Central nervous system (CNS) disorders	(Collins, Surette et al., 2012)

Table 1-4 Diseases associated with dysbiosis of the gut microbiota

1.6.1 Gut microbiota and PBC

To data, only few studies have addressed the role of microbes in PBC and shown interesting association of bacteria with the disease. For example, an early study showed that *E.coli* isolated from PBC patients' stools contained PBC-specific AMA-reactive proteins and proposed that antigens released from the bacterial cell wall contribute to the pathogenesis of the disease (Hopf, Moller et al., 1989). A later study confirmed that cross-reactivity to *E. coli* mimics was commonly seen in PBC (Bogdanos, Baum et al., 2004). Similarly, reactive serum against proteins of *Novosphingobium aromaticivorans* (a ubiquitous organism that metabolizes organic compounds and oestrogens) from stool specimens has been found in approximately 25% of PBC patients (Selmi, Balkwill et al., 2003) and IgG3 antibodies cross-reacting with β-galactosidase of *Lactobacillus delbrueckii* has been reported in 50% of PBC patients (Bogdanos, Baum et al., 2005).

Recently a Chinese study showed a different faecal microbiota composition in patients with early stage PBC (n=42) compared to healthy control. The gut of PBC patients was depleted of some potentially beneficial bacteria, but were enriched in some bacterial taxa containing opportunistic pathogens. They also showed association between altered microbiota and the immunity and metabolism of PBC patients, suggesting altered gut microbiome may be critical for the onset or development of PBC by interacting with metabolism and immunity (Lv, Fang et al., 2016).

1.6.2 Gut microbiota modulate bile acids

It is well-known that gut microbiota play a key role in the metabolic transformation of BAs by modifying primary BAs into secondary BAs, thus increasing chemical diversity of BA pool (Midtvedt, 1974, Payne, Bernstein et al., 2008). After their physiological role of fat and lipid digestion in the intestine, conjugated primary BAs (i.e. taurine and glycine conjugated CA and CDCA) undergo microbe-mediated enzymatic deconjugation, dehydrogenation and dehydroxylation in the terminal ileum or colon (Payne, Bernstein et al., 2008, Ridlon, Kang et al., 2006) (**Figure 1-8**, adapted from (Payne, Bernstein et al., 2008)).

Studies have shown that the deconjugation of BAs (from taurine and glycine) is primarily performed by intestinal *Bacteroides* species (sp.) (Narushima, Itoha et al., 2006) with *Bifidobaterium* sp. and *Lactobacillus* sp. identified as other key players (Gilliland and Speck, 1977). Further enzymatic transformation (mainly 7-alpha dehydroxylation) of a small portion of deconjugated BAs is carried out by *Clostridium* sp. to yield secondary bile acids –DCA and LCA (Hirano, Nakama et al., 1981, Narushima, Itoha et al., 2006).

The metabonomic studies comparing germ-free animals with conventionally housed animals have demonstrated the regulatory effects of the gut microbiota on the metabolism of BAs (Claus, Tsang et al., 2008, Swann, Want et al., 2011). Therefore, the regulatory effect of the gut microbiota on secondary BA metabolism means that the BA diversity depends on the gut microbial diversity and any shift in microbial diversity can influence BA diversity (Jones, Begley et al., 2008, Sayin, Wahlstrom et al., 2013).

In addition, novel insights from the recent study by Sayin *et al*. suggest role of FXR in BA regulation by gut microbiota. Their BA profile study of mice shows that the gut microbiota regulates expression of fibroblast growth factor 15 (FGF15) in the ileum and CYP7A1 in the liver by FXR-dependent mechanisms. They identified tauro-conjugated beta- and alpha-

muricholic (TβMC and TαMC) acids as FXR *antagonists* and suggested gut microbiota inhibit BA synthesis in the liver by alleviating FXR inhibition in the ileum (Sayin, Wahlstrom et al., 2013).

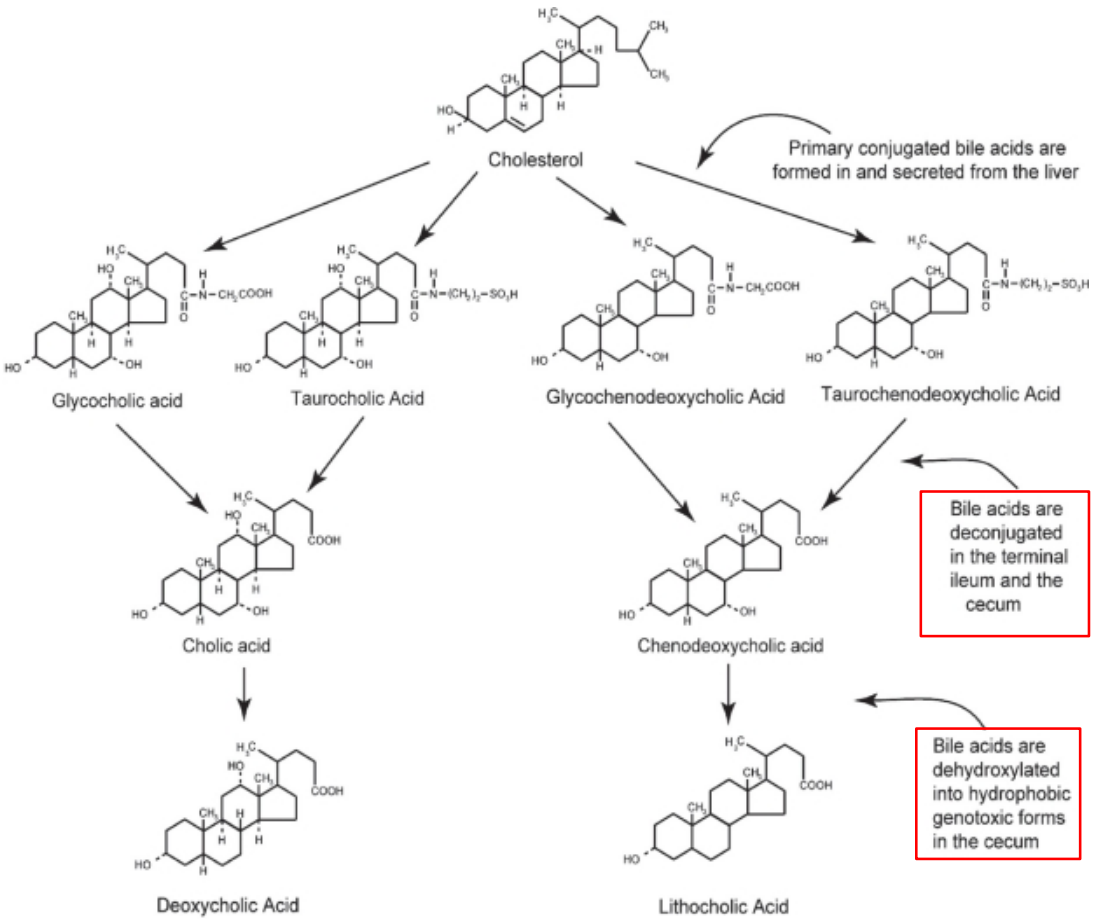


Figure 1-8 Modification of primary bile acids into secondary bile acids

1.6.3 Bile acids modulate gut microbiota

Evidence shows that BAs have both direct antimicrobial effects on gut microbes (Begley, Gahan et al., 2005), and indirect effects through FXR-induced antimicrobial peptides (Inagaki, Moschetta et al., 2006). Indeed, DCA exerts a strong detergent effect on bacterial membranes and has long been known to be a highly potent antimicrobial agent (Begley, Gahan et al., 2005). Recent experimental evidence suggests that increased levels of BAs reaching the large intestine (i.e. changing the faecal BA pool by feeding BA diet) can significantly alter the composition of the gut microbiome. In the Islam *et al*. study complex

and significant changes in the gut microbiome were observed by feeding rats with cholic acid (CA) diet. There was a 6-fold and a 20-fold increase in total faecal BAs after medium and high CA diet, respectively. The increased level of faecal BAs produced significant phylum-level alterations of the gut microbiome with Firmicutes expanding from 54% to 93-98% of the microbiome. In addition, at the class-level the Clostridia expanded from 39% to 70% and at the genus-level *Blautia* expanded from 8.3% to 55-62% (Islam, Fukiya et al., 2011). Another study observed feeding mice with CA diet resulted in 1000 fold increase in the levels of BA 7α-dehydroxylating bacteria which produce DCA (Ridlon, Alves et al., 2013).

The effect of modulation of faecal BAs on the gut-microbiome in patients has not been studied in detail. For example, a study of faecal BAs in liver cirrhosis patients and controls showed significantly lower proportion of cirrhotic patients had detectable secondary faecal BAs and higher Enterobacteriaceae (potentially pathogenic) abundance. This suggests cirrhosis, especially advanced disease, is associated with a decreased conversion of primary to secondary faecal BA in the gut which is likely to play an important role in allowing pro-inflammatory microbial taxa to expand (Kakiyama, Pandak et al., 2013).

In summary, the liver-bile acid-microbiome axis is emerging as an important research area to further our understanding of liver disease. Changes in the levels of faecal BAs has been found to be associated with dramatic shifts in gut microbiome. Decrease in BAs entering the intestines appears to favour overgrowth of pathogenic and pro-inflammatory members of the microbiome. In contrast, increasing levels of the primary BA cholic acid (CA) causes a dramatic shift toward the Firmicutes and increasing production of the harmful secondary bile acid deoxycholic acid (DCA).

This thesis has examined the effect of increasing faecal levels of BAs (by IBAT inhibition) on faecal bacterial diversity in patients with PBC and pruritus (see **Chapter 6**).

1.7 Ileal bile acid transporter

In health, BAs [cholic acid (CA) and chenodeoxycholic acid (CDCA)] are produced in the liver, secreted in the intestine and following their role in fat digestion most BAs (>95%) are reclaimed in the terminal ileum and returned to the liver via portal vein (Martinez-Augustin and Sanchez de Medina, 2008). This efficient process of recycling of BAs, referred to as the enterohepatic circulation (EHC), maintains the balance between hepatic synthesis and intestinal loss of BAs.

Section **1.2.1.1** above has outlined the physiological role of Ileal bile acid transporter (IBAT, also called apical sodium-dependent bile acid transporter, ASBT) in the enterohepatic circulation (EHC) of bile acids.

The physiological importance of the EHC has been well known for many decades (Hofmann, 1977) and the key proteins and sodium-dependent transporters involved in EHC have been characterised. It is now known that a number of specialized membrane transporters expressed on the apical (brush border) and basolateral membranes of the hepatocyte and ileal enterocytes mediate EHC of BAs (an in-depth review in (Alrefai and Gill, 2007)). Of these, ASBT (gene symbol *SLC10A2*) located on the apical membrane of ileal enterocytes was first cloned in humans in 1995 (Wong, Oelkers et al., 1995) and subsequently shown to function as a major gatekeeper for the intestinal compartment of EHC of BAs (Craddock, Love et al., 1998, Dawson, Haywood et al., 2003, Oelkers, Kirby et al., 1997). Indeed, by regulating the rate of biliary BA secretion ASBT is considered a major determinant of BA pool size in the human body and is an essential regulator of lipid and cholesterol homeostasis (Mosinska, Fichna et al., 2015). Interestingly, ASBT- mediated transport is not equal among BA species; conjugated (more hydrophilic) BAs being transported more efficiently than unconjugated forms. Also, the affinity of ASBT is higher for dihydroxy BAs such as CDCA than for trihydroxy BAs such as CA, taurocholic acid (TCA) and glycocholic acid (GCA) (Craddock, Love et al., 1998).

1.7.1 Effect of IBAT (ASBT) inhibition

In recent years, ASBT has gained more attention as a specific drug target to inhibit the EHC and alter the circulating pool of BAs. There are specific effects of pharmacological inhibition of ASBT which have therapeutic potential. First, inhibition of intestinal reabsorption of BAs leads to increased BA load in the colon (**Figure 1-9**) and causes BA-induced diarrhoea. The

latter effect is being utilised to treat constipation (Simren, Bajor et al., 2011). Second, decreased return of BAs to liver results in increased hepatic BA synthesis as a result of negative feedback regulation (inactivation of hepatic FXR) (Li, Xu et al., 2004). This lowers serum cholesterol level (due to increased conversion of cholesterol into BAs) which is an additional benefit in lipid metabolism and metabolic disorders and supports ASBT inhibitors as novel hypolipidaemic dugs (Kramer and Glombik, 2006). Third, ASBT inhibition may have anti-diabetic action mediated through BA-TGR5 axis. BAs in the colon activate TGR5 receptors (highly expressed in colon) resulting in stimulation of expression and secretion of the incretin glucagon like peptide-1 (GLP-1), a hormone that lowers plasma glucose (Harach, Pols et al., 2012). Finally, an as yet untested hypothesis suggests increased load of BAs in the colon may significantly impact the gut microbiome with potential secondary effects on cholestatic diseases (Wagner and Trauner, 2016).

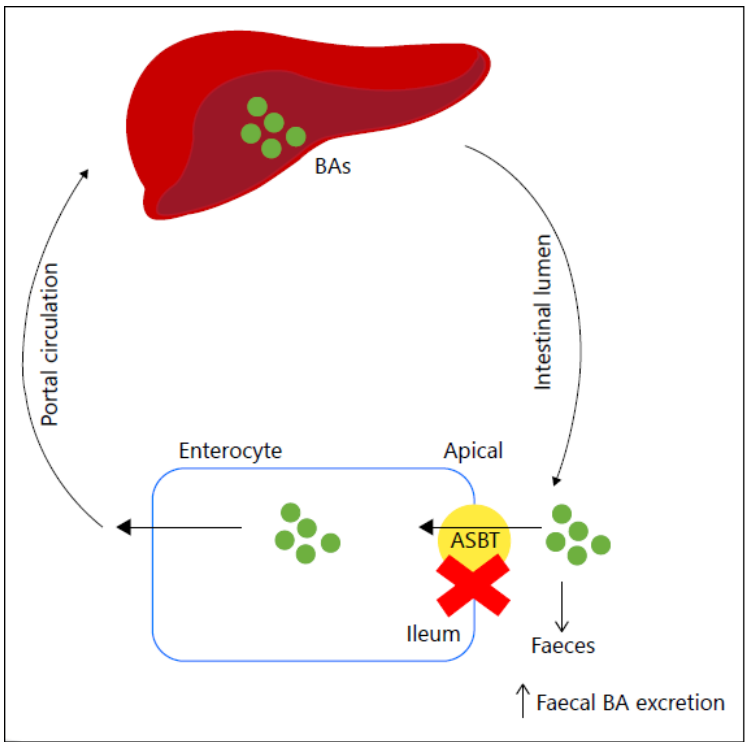


Figure 1-9 Proposed effect of IBAT inhibitor drug on bile acids

At the turn of the century, at least five classes of chemically divergent specific IBAT inhibitors were developed (**Table 1-5**) mostly for the treatment of hypercholesterolemia. The main IBAT inhibitors that have entered phase 2 trials are summarised in **Table 1-6**.

Class	Compound	Comments	Reference
Dimeric bile acid analogues	PB3, S0960		(Kramer, Stengelin et al., 1999, Kramer and Wess, 1996, Wess, Kramer et al., 1994)
Benzothiazepine derivatives	2164U90 264W94 GSK2330672	competitive inhibitor of murine ASBT ∼500-fold more potent inhibitor than 2164U90	(Lewis, Brieaddy et al., 1995, Root, Smith et al., 1995) (Root, Smith et al., 2002) (Wu, Aquino et al., 2013)
Benzothiepine derivatives	SC-435	-potent and non-absorbed; -increases faecal BA excretion; -decreases total and LDL-cholesterol plasma levels; -enhances expression of the hepatic LDL receptor	(West, McGrane et al., 2005, West, Ramjiganesh et al., 2002, West, Zern et al., 2003)
Naphthol derivatives	S8921	mixed competitive and non-competitive ASBT inhibitor	(Hara, Higaki et al., 1997, Tollefson, Vernier et al., 2000)
4-oxo-1-phenyl-1,4,-dihydroquinoline derivatives	/		(Kurata, Suzuki et al., 2004)

Table 1-5 Different classes of IBAT inhibitors

Compound	Sponsor	Clinical trial number	Phase	Disease
A4250	Albireo	NCT02360852	2	Cholestatic pruritus
		NCT02630875	2	Paediatric cholestasis
GSK2330672	GlaxoSmithKline	NCT01416324	1	Healthy subjects
		NCT01929863	2	T2DM
		NCT02202161	2	T2DM
LUM001 (SHP-625 or Lopixibat)	Shire	NCT01904058	2	PBC
		NCT02061540	2	PSC
		NCT01903460	2	Alagille Syndrome
		NCT02057692 NCT02117713		
		NCT02057718	2	PFIC
Volixibat (SHP-626)	Shire	NCT02787304	2	NASH

Table 1-6 IBAT inhibitors in clinical development

Following is a review of recent evidence to support therapeutic potential of IBAT inhibitors as novel therapy for cholestatic diseases.

1.7.2 IBAT (ASBT) inhibition in cholestasis

There are conflicting data on intestinal absorption of BAs during cholestasis. An adaptive regulation leading to downregulation of intestinal ASBT has been shown in both animal and human studies (Hruz, Zimmermann et al., 2006, Sauer, Stiehl et al., 2000). In contrast, increased absorption of BAs has been reported in PBC, thus contributing to cholestasis in this condition (Lanzini, De Tavonatti et al., 2003). Nevertheless, ASBT inhibition is an attractive therapeutic option in cholestatic conditions based on the hypothesis that interrupting the EHC of BAs may also reduce the circulating BA pool and hepatic levels of potentially cytotoxic BAs.

Early reports from non-cholestatic animal studies demonstrated that SC-435 (an IBAT inhibitor) leads to increased faecal BA (and diarrhoea) and reduced FXR stimulation, lower FGF19 synthesis, and consequently enhanced BA synthesis, expanding the BA pool and lowering plasma cholesterol (Li, Xu et al., 2004, West, McGrane et al., 2005, West, Zern et al., 2003). More recently, effects of IBAT inhibitors in animal models of cholestasis have been reported. Miethke *et al.* treated $mdr2^{-/-}$ mice (an established animal model for chronic cholestasis with some features of sclerosing cholangitis) with SC-435 for 14 days. They observed an 8-fold increase in faecal BA excretion associated with 65%, 98.9% and 98.8% decrease in hepatic, serum and biliary concentrations of BAs, respectively. The anti-cholestatic and anti-inflammatory effects of SC-435 was evidenced by decreased markers of liver injury; plasma ALT and bilirubin concentrations decreased by 86% and 93%, respectively and serum ALP by 55%. They also observed an improvement in liver histology of sclerosing cholangitis with decreased fibrosis and favourable alteration in the biliary phosphatidylcholine/BAs ratio indicating decreased bile toxicity. In addition, the livers from SC-435 treated mice showed reduction in the proinflammatory $Ly6C^{+}$ and increase in anti-inflammatory $Ly6C^{-}$ subset of monocytes accompanied by reduced levels of $F4/80^{+}$ $CD11b^{+}$ Kupffer cells and $Gr1^{+}$ $CD11b^{+}$ neutrophils. Taken together, the results from this study demonstrated the potential of IBAT inhibitors in halting progression of murine sclerosing cholangitis during the early phase of disease process (Miethke, Zhang et al., 2016).

Baghdasaryan *et al.* also examined the effects of a specific intestinal IBAT inhibitor (A4250, Albireo pharma, Sweden) in eight week old $Mdr^{-/-}$ ($Abcb4^{-/-}$) mice (model of cholestatic liver injury and sclerosing cholangitis). After four weeks of treatment with A4250 they observed reduced serum ALT, ALP and BA levels, decreased hepatic expression of proinflammatory (*Tnf-a, Vcam1, Mcp-1*) and pro-fibrogenic (*Col1a1,Col1a2*) genes and bile duct proliferation. Furthermore, A4250 was shown to significantly reduce bile flow and biliary BA output with preserved $HCO3^-$ and biliary phospholipid (PL) secretion resulting in an increased $HCO3^-$/BA and PL/BA ratio (Baghdasaryan, Fuchs et al., 2016).

The three main IBAT inhibitors currently being investigated in early phase trials are: A4250, GSK2330672 and Lopixibat chloride (Maralixibat, LUM001) (

Table 1-6). In non-cholestatic population ASBT inhibitors have been shown to be effective in changing circulating BA levels. For instance, in a randomised, double blind, placebo controlled study of 24 healthy subjects one week treatment with A4250 was found to be safe, well tolerated and produce significant decrease in plasma total BAs and FGF19 levels and increase plasma C4 (a marker of hepatic BA synthesis) and faecal BAs. The main adverse events were abdominal discomfort, nausea and mild diarrhoea which were dose-dependent (Graffner, Gillberg et al., 2016).

1.7.3 IBAT (ASBT) inhibition in cholestatic pruritus

Pruritus is specifically being targeted with novel IBAT inhibitors. The rationale for this is cholestatic pruritus is linked to circulating BAs and reducing their levels may improve the symptom. The results of CLARITY study funded by Lumena (part of the Shire group of companies) investigating use of oral Lopixibat chloride (Maralixibat chloride, SHP625, formerly LUM001) in PBC patients with pruritus was presented as an abstract at the International Liver Congress (EASL 2016) (M.J. Mayo, 2016). In this double blind, randomised placebo controlled trial PBC patients on stable doses of UDCA (or intolerant to UDCA) with baseline pruritus score >4 for each of two consecutive weeks in the screening period were randomised to daily Lopixibat 10 mg, 20 mg or placebo. The 13-week treatment period comprised dose-escalation (3–4 weeks) and stable-dosing periods (9–10 weeks). The primary endpoint was change in Adult Itch Reported Outcome (ItchRO™) weekly sum score at week 13 or early termination. The results from 66 enrolled patients (61 completed the study) showed significant decrease in ItchRO score from baseline in the within group

comparison (26% Lopixibat, $p<0.0001$ and 23% placebo, $p<0.0001$) but no significant difference between group comparison (Lopixibat vs. placebo, $p=0.47$). The changes in serum ALP from baseline were not significant for either group but reduction in mean serum BA levels (-14.23 vs.10.05) and increase in C4 levels (13.49 vs. -2.21) were greater for Lopixibat group compared to placebo. In this study the primary end point did not differ significantly between Lopixibat and placebo and authors concluded that a large placebo effect might have confounded assessment of pruritus. Full results of the study have been recently published (Mayo, Pockros et al., 2019a).

This thesis has investigated the efficacy of GSK2330672, a novel IBAT inhibitor in the treatment of pruritus in patients with PBC (see **Chapters 4 and 5**).

1.8 Scope of the thesis

My *thesis* is that pruritus is a key unmet need in cholestatic disease, is associated with perturbations of bile acid homeostasis, and is amenable to modulation by inhibition of the enterohepatic circulation. This thesis will be investigated by testing *hypotheses* to fill four important gaps in the current understanding of cholestatic pruritus in PBC.

First, the prevalence of pruritus in PBC in the United Kingdom (UK) is not known. It is important to assess the magnitude of this symptom burden to highlight the need for improved patient and clinician awareness about the condition. Also, there are no data from large studies on the utility of currently available medical therapies in treating pruritus in PBC patients. Using the large dataset from the UK-PBC Research Cohort Study, this work (**Chapter 2**) has attempted to understand the patient-reported characteristics of pruritus and its treatment in PBC.

After measuring the unmet need, the second element focused on investigating treatment interventions. Pruritus may be attenuated by interrupting enterohepatic circulation (EHC) of pruritogens either by oral administration of anion exchange resins such as cholestyramine or by diverting bile away from the ileum. The latter can be done by endoscopic nasobiliary drainage (NBD) as a definitive physical interruption of EHC. Although NBD has been available as a treatment modality, its role in clinical practice is less clearly defined. **Chapter 3** of this thesis attempts to describe the true role of NBD in refractory cholestatic pruritus and tries to understand the pros and cons of this treatment.

NBD is invasive. So, an alternative, less invasive approach would be using a pharmacological agent to achieve EHC interruption. In this regard, an ileal bile acid (BA) transporter (IBAT) inhibitor drug would be appealing. Therefore, **Chapter 5** explores the safety and efficacy of GSK2330672 (an experimental IBAT inhibitor drug) investigated in a randomised, double blind, placebo-controlled study in treating PBC patients with pruritus. The study protocol is described in **Chapter 4**.

Third, to date only a few metabonomic studies have been performed on bio fluids (serum/plasma, urine or faeces) from patients with PBC. It is noteworthy that none of the reported studies (see section **1.5.1**) focussed specifically on pruritus of PBC. Moreover, there are no published data on the global metabolic profile of PBC patients with pruritus and on

quantification of BAs in PBC patients with pruritus. Therefore, **Chapter 6** has attempted to explore the mechanism by which BA changes are related to pruritus (and intervention by IBAT inhibitor drug) by utilising metabolic profiling of serum and faeces.

Fourth, there are limited published studies on the composition of the gut microbiota in patients with PBC or in PBC patients with pruritus. Since BAs have been proposed to play either direct or indirect role in the pathogenesis of cholestatic pruritus, understanding the interaction between faecal BAs and the gut microbiome in PBC patients with itch may shed more light on the pathogenesis of cholestatic pruritus. To this end, **Chapter 6** of this thesis has attempted to investigate the gut microbiota composition of PBC patients with pruritus and evaluate if there is a shift in the representation of the dominant phyla of bacteria in the faeces of PBC patients with pruritus in comparison to control. In addition, this work examined the effect of increasing the faecal BA levels (by IBAT inhibitor drug) on the composition of gut-microbiota in PBC patients with pruritus (**Chapter 6)**.

1.9 Hypothesis and Aims

The broad aim of this thesis is to improve understanding of the characteristics of cholestatic pruritus in primary biliary cholangitis (PBC) and help fulfil the need for better, more effective drug treatment of pruritus in PBC and other related cholestatic conditions.

The main hypotheses are:

1. Pruritus is prevalent in patients with PBC.
2. Pruritus of cholestatic diseases can be treated by interrupting the enterohepatic circulation by using endoscopic nasobiliary drainage.
3. Pruritus in PBC can be treated by using a pharmacological agent to block the enterohepatic circulation of bile acids (BAs).
4. Pruritus in PBC is associated with distinct metabolic signature(s) which may be predominantly due to the metabolic perturbations associated with BA homeostasis.
5. Pruritus in PBC has distinct gut-microbiota profile (due to associated dysbiosis) which may be amenable to modulation by faecal BAs.

The aims of this thesis are:

1. To investigate the prevalence and treatment of pruritus in PBC patients using the UK-PBC Research cohort and other international PBC cohorts (**Chapter 2**).
2. To investigate the safety and efficacy of endoscopic nasobiliary drainage in treating patients with cholestatic pruritus (**Chapter 3**).
3. To investigate the safety and efficacy of ileal bile acid transporter (IBAT) inhibitor drug GSK2330672 in treating pruritus in PBC patients (**Chapter 4 and 5**).
4. To investigate the metabolic (mainly BAs) profile of PBC patients with pruritus and study the effect of IBAT inhibitor drug (**Chapter 6**).
5. To investigate the faecal microbial community ecology and diversity of PBC patients with pruritus and study the effect of changing faecal BA composition (using IBAT inhibitor) (**Chapter 6**).

An overview of the research projects is given in **Figure 1-10**.

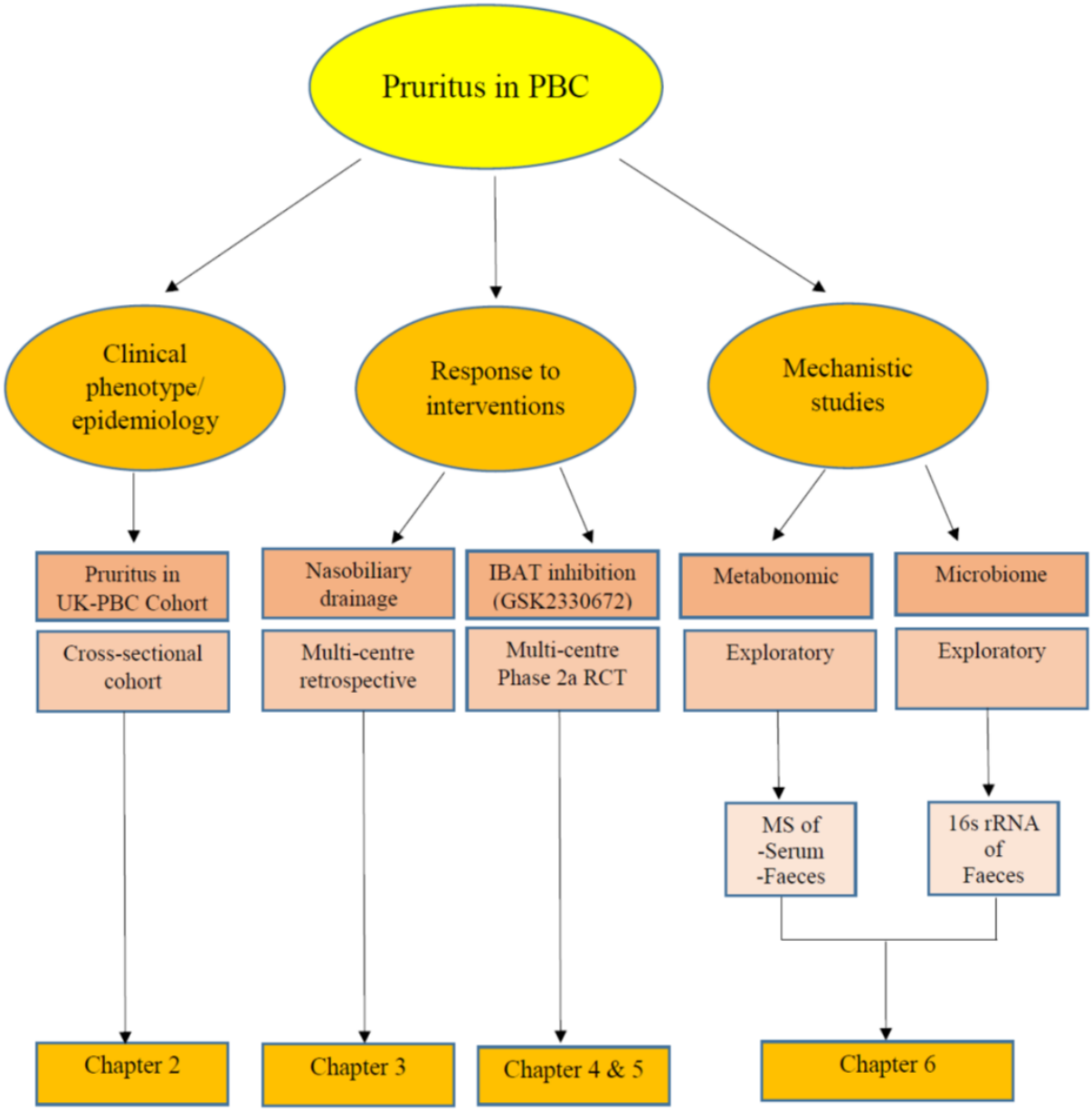


Figure 1-10 Overview of research projects undertaken in this thesis

2. CHAPTER 2: PREVALENCE OF PRURITUS IN PBC COHORTS

2.1 Introduction and Aims

The burden of pruritus (itch) in PBC has been less well studied but general clinical impression is that many patients with PBC are troubled by pruritus. Pruritus, recognised as a more specific symptom of PBC than fatigue (Lindor, Gershwin et al., 2009), can occur at any point in the disease course and in one study, pruritus was reported to precede the formal diagnosis of PBC in nearly 75% patients (Rishe, Azarm et al., 2008). Pruritus is reported not to respond to therapy with ursodeoxycholic acid (UDCA), the recognised first line, disease modifying therapy (Lindor, Gershwin et al., 2009, Rudic, Poropat et al., 2012). Scratching resulting from pruritus makes patients self-conscious, interferes with their body image and may lead to social isolation,(Fahey, 1999) while nocturnal pruritus affects sleep quality worsening fatigue. In addition, chronic pruritus associated with cholestatic liver diseases has been shown to produce substantial impairment of health-related quality of life (HRQoL) (Jin and Khan, 2016, Younossi, Kiwi et al., 2000).

Therefore, it is important that clinicians treating PBC patients recognise the symptom and initiate treatment with available therapies in line with current guidelines. Both the European Association for the Study of the Liver (EASL) and American Association for the Study of Liver Diseases (AASLD) recommend oral cholestyramine (colestyramine) as the first line therapy for pruritus in PBC followed by rifampicin (rifampin), opiate antagonists (naltrexone or naloxone) and sertraline as second, third and fourth line therapies respectively (European Association for the Study of the, 2009, Lindor, Gershwin et al., 2009).

The natural history of pruritus in PBC is less well understood and there is scarcity of data on prevalence of itch in PBC in the United Kingdom (UK) and its description from PBC patient's perspective. This may be partly due to the subjective nature of the symptom which some patients may find difficult to describe to the clinicians. In a US study, certain characteristics of pruritus in PBC were addressed by inviting 238 patients in an internet based on-line survey. Patients described their experience of the symptom in their own words; 35% patients described their itch as "bugs crawling" and 65% described worsening of itch at night and improvement with something cool (Rishe, Azarm et al., 2008). This study also explored different anti-pruritic treatments received by PBC patients and reported antihistamines and cholestyramine as the most commonly prescribed medications. Whilst this study was probably

the first published study to attempt to characterise itch from PBC patient's perspective, the authors admit their study had limitations as it was restricted to PBC patients with internet access and only those with itch were likely to have been motivated to respond to the survey. Literature also suggests that there is lack of appreciation of the patient-burden of pruritus from clinicians. Studies of chronic pruritus in non-PBC population indicate that patients often think that health professionals do not take their itch seriously (Bathe, Weisshaar et al., 2013) resulting in inadequate treatment and poor patient satisfaction.

Here, we report the characteristics of pruritus in a large, national cohort of patients with PBC. Our primary aim was to use the UK-PBC Research Cohort to study the prevalence of pruritus in PBC, investigate the associations between measures of itch intensity assessed using patient-reported outcomes and investigate the frequency of anti-pruritic treatments as reported by patients. Additionally, we conducted analyses using the Clinical Practice Research Datalink (CPRD) to explore how patient-reported information in the UK-PBC cohort compared to the actual recorded prescription for a cohort of PBC patients seeking anti-pruritic treatment from their primary care in the UK.

The main aim of this study was to use the UK-PBC Research Cohort, a unique, large cohort of PBC patients from across the UK to:

1. Explore the prevalence of pruritus;

2. To evaluate the co-relation between different measurements of itch score;

2. Assess the characteristics of pruritus treatment as reported by patients.

2.2 Patients and Methods

2.2.1 Study design and subjects

This was a cross-sectional observational study of patients with PBC recruited to the UK-PBC Research Cohort. Participants were recruited throughout the UK by the UK-PBC Consortium, a research network of 155 National Health Service (NHS) Trusts or Health Boards collaborating in the UK-PBC project (http://www.uk-pbc.com/). The UK-PBC project was approved by the Oxford C research ethics committee (REC reference: 07/H0606/96). The UK-PBC Research Cohort has been described in detail elsewhere (see http://www.uk-pbc.com/about/aboutuk-pbc/ws1/researchcohort). Clinical data regarding age at diagnosis, UDCA therapy and biochemical status of patients in the UK-PBC cohort have been previously published (Carbone, Mells et al., 2013, Dyson, Wilkinson et al., 2016, Mells, Pells et al., 2013).

The cohort included in the current study consisted of non-transplanted patients with PBC incident or prevalent between 2008 and 2012 (although the UK-PBC cohort includes transplanted patients, these were excluded from the current study). The diagnosis of PBC was based on established diagnostic criteria (two or more of the following criteria: cholestatic liver biochemistry, compatible or diagnostic liver histology, and antimitochondrial antibody [AMA] at a titre >1:40). In the UK-PBC cohort, symptoms and HRQoL have been thoroughly characterised using established and validated measures. Relevant to the current study, pruritus was assessed in detail using a standardised questionnaire (**Table 2-1**) that was mailed to the participants in February 2011 (first survey) and few years later in July 2014 (second survey). The rationale for two surveys from the same cohort was to evaluate the consistency of patient-reported information over time and to check if there was any recall bias.

1. Since you developed PBC, have you experienced itching caused by the PBC? *never, only rarely, occasionally, frequently, all the time*
Since you developed PBC, did you experience any of the following? 2. Itching has disturbed my sleep 3. I have scratched so much, I made my skin raw } PBC-40 Itch domain 4. I have felt embarrassed because of the itching For each question possible answers are *never, only rarely, occasionally, frequently, all the time*
Intensity of itching in the last seven days: 5. On a scale of 0 to 10 where 0 is no itch and 10 is unbearable itch, how would you rate the worst itching you have experienced in the last seven days? 6. Mark on the visual analogue scale below indicating the worst itch you have experienced in the last seven days
Intensity of itching since you first developed PBC: 7. On a scale of 0 to 10 where 0 is no itch and 10 is unbearable itch, how would you rate the worst itching you have experienced since you first developed PBC? 8. Mark on the visual analogue scale below indicating the worst itch you have experienced since you first developed PBC
9. Since you first developed PBC, have you ever received any of the following treatments for itching? *Colestyramine, rifampicin, naltrexone, phototherapy, admitted to hospital specifically for treatment of itching, other medications*

Table 2-1 Pruritus questionnaire used in the UK-PBC Cohort

2.2.2 Pruritus assessment measures

The core data for this study were obtained from the patient self-reported information using validated pruritus assessment tools.

2.2.2.1 ***PBC-40 Itch domain score***

PBC-40 is a validated, disease-specific QOL measure with robust psychometric properties and optimised for self-completion (Jacoby, Rannard et al., 2005). Itch domain forms one of the six domains within PBC-40 and consists of three questions framed as statements. Responses for these statements are on a standard five point Likert scale (score 1 for least burden or problem, score 5 for greatest burden or problem). The total score of the itch domain is obtained from summing individual question response scores (minimum score 3, maximum score 15). Empirical cut-offs for categorizing pruritus into 'no' (score <3), 'mild' (score 4-8), 'moderate' (score 9-11) and 'severe' (score >12) itch have been defined and validated (Newton, Hudson et al., 2007). The original PBC-40 itch domain refers to itch *in the last four weeks,* but the pruritus questionnaire in the UK-PBC cohort incorporated PBC-40 itch domain to refer to itch *since the development of PBC*. For each patient, the total score of the PBC-40 itch domain was obtained from summing individual response scores for questions 2, 3 and 4 in the questionnaire (**Table 2-1**).

2.2.2.2 ***Pruritus Visual Analogue Scale (VAS) and Numerical Rating Scale (NRS)***

VAS, first described may decades ago (Hayes and Patterson, 1921), remains a widely used tool to assess symptom severity. In the UK-PBC cohort, we used a 0-10 cm VAS that decodes pruritus into a point on a line (0=no itch, 10=worst itch possible) and patients were asked to mark their level of itch on the VAS to indicate the intensity of their pruritus *in the last seven days* (i.e. 'current' itch) and *since development of PBC* ('ever itch') (questions 6 and 8 in (**Table 2-1**)).

Pruritus Numerical Rating Scale (NRS): Patients were asked to rate their level of itch on a scale of 0 (no itch) to 10 (unbearable itch) *in the last seven days* (i.e. 'current' itch) and *since development of PBC* ('ever itch') (questions 5 and 7 in (**Table 2-1**)).

2.2.3 Definitions

In this study, 'ever itch' refers to itch at some point in their illness since development of PBC. We defined *persistent itch* as pruritus reported to be occurring 'frequently' or 'all the time' at some point in their illness since development of PBC. To assess the 'worst ever' itch, we used the validated cut-off scores of PBC-40 itch domain score and defined *severe itch* as PBC-40 itch domain score of ≥12. Pruritus VAS and NRS scores *in the last seven days* and *since development of PBC* were used to assess 'current' and 'ever' itch intensity, respectively.

2.2.4 Anti-pruritic therapy

In the UK-PBC pruritus questionnaire patients were asked to report if they had received specific treatment for itch at any point following PBC diagnosis and a list of guideline recommended treatments for patients with pruritus in PBC including colestyramine (Cholestyramine, Questran®), rifampicin, naltrexone and phototherapy was included. Patients were also asked to report if they were ever admitted to hospital specifically for treatment of itch and if they had taken any other medications including anti-histamines and natural or herbal remedies (question 9 of (**Table 2-1**)).

2.2.5 Primary care database

The Clinical Practice Research Datalink (CPRD; previously known as General Practice Research Database, GPRD) is a large longitudinal primary care database with information collected from a large number of general practices in the UK (Walley and Mantgani, 1997). The patient population captured in the database is broadly representative of the demographic breakdown of the UK population and of the activity of general practitioners (Hollowell, 1997). The data reflect the observations, diagnoses made by, and therapies prescribed by general practitioners (GPs), in addition to information communicated to them by hospitals. The CPRD is accepted as an excellent resource for conducting robust medical and epidemiological research (Walley and Mantgani, 1997) and has been extensively validated for a wide range of diagnoses and consistently found to be accurate (Jick, Jick et al., 1991, Lewis, Brensinger et al., 2002).

For the current study, the method used to identify pruritus data from the CPRD is given in **APPENDIX 1.** Briefly, CPRD database was searched for a recording of prescription for colestyramine, rifampicin, and naltrexone in PBC patients' entire medical history, including

time before PBC diagnosis until the end of available data (31 December 2014). The hypothesis was that the proportion of UK-PBC patients self-reporting use of anti-pruritic medications would be similar to the actual prescription record for anti-pruritic medications in the CPRD.

2.2.6 Statistical analysis

As part of quality control, the pruritus data set was checked for plausibility, accuracy, and completeness. Data analysis was performed using the statistical analysis software Prism 6.0 (GraphPad Prism, La Jolla,). Frequency data are presented as numbers (n) or percentages (%). Where data were distributed normally, they are presented as mean ± standard deviation (SD). Where data were distributed non-normally, they are presented as median and range. Correlation analyses of measures of itch intensity were determined using Spearman's rank correlation method to compute the correlation coefficient (r). Fisher's exact test was used to calculate difference between proportions. A p value of < 0.05 was considered statistically significant.

2.3 Results

The UK-PBC cohort symptom dataset formed the source data for the analyses. Pruritus data was available for 2975 unique, non-transplanted PBC patients. Of these, 250 (8.4%) patients were excluded owing to missing data (incomplete or partially completed information) and 541 (18.2%) were excluded because they reported having a skin disorder (eczema, psoriasis and/or urticaria) that might confound the analysis. A total of 2184 were therefore included in the final analysis. Main results from this cohort are given in **Table 2-2**.

2.3.1 Frequency of ever itch

560 (25.6%) patients reported that they had never experienced itch. 1624 (74.4%) patients reported that they had experienced itch at some point in their illness (i.e. any experience of itch). Of these, 749 (34.3%) patients reported experiencing itch 'frequently' or 'all the time' (i.e. persistent itch) at some point in their illness (**Figure 2-1**).

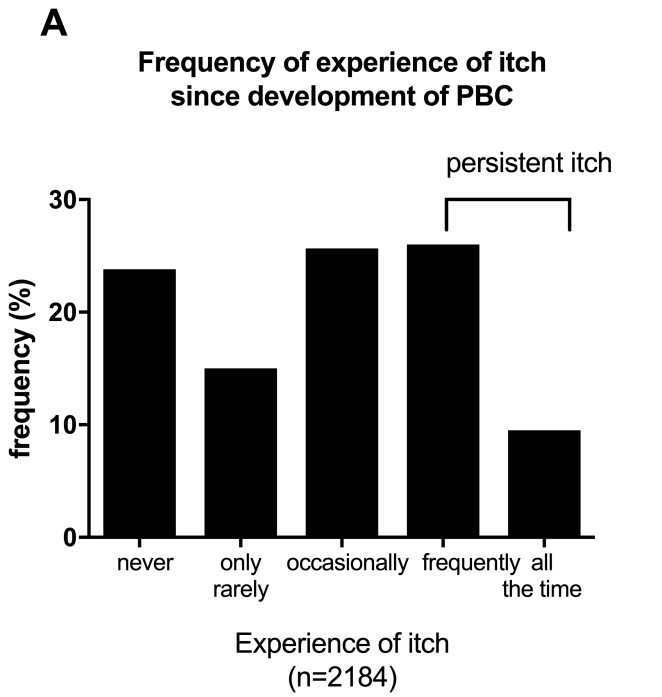
2.3.2 Severity of ever itch

For the whole study cohort the median 'ever' itch scores for PBC-40 itch domain score was 4 (IQR 0-9), for pruritus NRS was 6 (IQR 1-8) and for pruritus VAS was 5 (IQR 0.5-8).

We used PBC-40 itch domain score to define 'worst ever' itch. Based on the previously validated cut-off values for PBC-40 itch domain scores, 571 (26.1%), 347 (15.9%) and 249 (11.4%) patients met the criteria for mild, moderate and severe itch, respectively (**Figure 2-1**).

		n	%
1	Total number of patients - Females - Mean Age (yrs)	2975 2677 63.5	- 90
2	Excluded from analysis: -Incomplete data -Skin conditions	 250 541	 8.4 18.2
3	Patients included in final analysis	**2184**	-
4	**Frequency of itch**		
	Never experienced itch	560	25.6
	Any experience of itch	1624	74.4
	Persistent itch†	749	34.3
5	**Itch severity** (PBC 40 itch domain scores)		
	No itch (0-3)	1017	46.5
	Mild (4-8)	571	26.1
	Moderate (9-11)	347	15.9
	Severe (≥12)	249	11.4
6	**Treatment received**		
	A) Any experience of itch: n=1624		
	Colestyramine	394	24.2
	Rifampicin	93	5.7
	Naltrexone	35	2.1
	B) Persistent itch†: n=749		
	Colestyramine	284	37.9
	Rifampicin	84	11.2
	Naltrexone	34	4.5
	C) Severe itch‡: n=249		
	Colestyramine	129	51.8
	Rifampicin	59	25
	Naltrexone	23	9.7
	D) Pruritus VAS score >5 in last seven days:		
	Colestyramine	156	41.1
	Rifampicin	48	12.6
	Naltrexone	22	5.8

Table 2-2 Main results from the first survey of 2184 patients in the UK-PBC cohort
†*Persistent* itch= pruritus reported to be occurring 'frequently' or 'all the time' at some point in their illness since development of PBC.



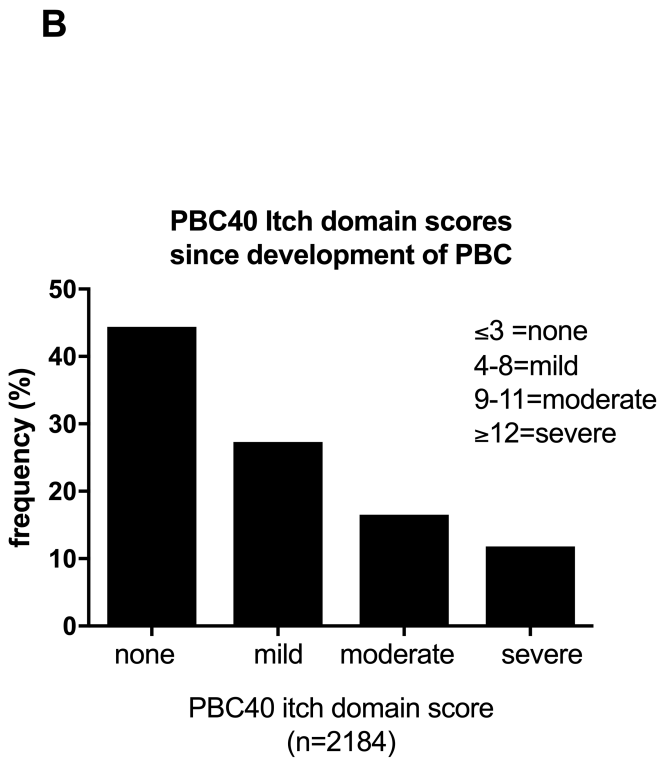


Figure 2-1 Frequency of experience of itch (A) and PBC-40 itch domain scores (B) since development of PBC for the entire UK-PBC cohort

In patients with *severe* itch (n=249), the median 'current' (i.e. in the last seven days) itch intensity score on NRS was 7 (IQR 4-8) and on VAS was 6 (IQR 3.5-8) with 145 (58.2%) patients reporting 'current' VAS score >5.

Persistent ('frequently' or 'always') sleep disturbance from itch was reported by 427 (19.5%) patients and 321 (14.7%) had persistently felt embarrassed because of the itching.

2.3.3 Correlation between measures of itch intensity

We observed statistically significant correlations between the pruritus NRS and the pruritus VAS *in the last seven days* (Spearman's rank correlation test, r=0.96, p<0.0001) (**Figure 2-2A**) as well as *since development of PBC* (r=0.96, p<0.0001) (**Figure 2-2B**), suggesting significant inter-changeability of the pruritus assessment tools. Also, the PBC-40 itch domain score and the pruritus VAS *since development of PBC* correlated significantly (r=0.80, p<0.0001) (**Figure 2-2C**).

2.3.4 Anti-pruritic therapy

Table 2-2 provides the key results. Patient-reported frequency of use of colestyramine (first-line treatment) in patients with any experience of itch, persistent itch and severe itch since development of PBC was 24.2%, 37.9% and 51.8%, respectively. The reported frequency of use of rifampicin (second-line treatment) for these three groups was 5.7%, 11.2% and 25%, respectively.

Of those reported to have some experience of itch (n=1624), only 77 (4.7%) reported to have received both colestyramine and rifampicin and only 21 (1.2%) patients reported to have received all three lines of treatment (colestyramine, rifampicin and naltrexone). 104 (6.4%) reported using anti-histamine drugs for their itch.

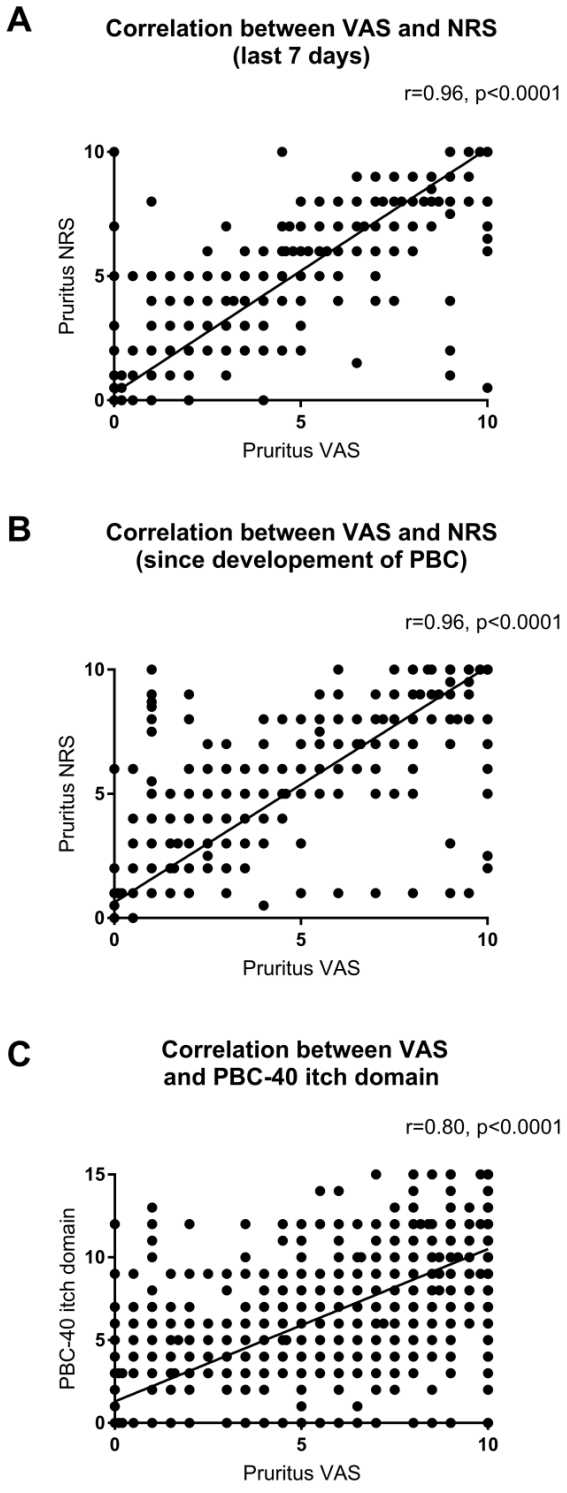


Figure 2-2 Correlations between measures of itch intensity in the UK-PBC cohort
A) VAS and NRS measured in the last seven days, **B**) since development of PBC; and **C**) correlation between the PBC-40 itch domain score and VAS since development of PBC.

2.3.5 Validation from CPRD database

In light of the low levels of reported anti-pruritic therapy in the UK-PBC cohort, we validated the findings using the CPRD, an entirely independent, primary care database. From CPRD database, 664 patients (89% females, mean age 64 years) were identified to have diagnosis of PBC. Of these, 181 (27.2%) patients had recorded diagnosis of pruritus related to PBC. For these patients the frequency of recorded prescription for colestyramine, rifampicin, and naltrexone was 30.4%, 3.3%, and 2.8%, respectively.

It is likely that these 181 patients approximate to the patients in the persistent itch category (34.3%) of the UK-PBC cohort. **Figure 2-3** shows the comparison of patient reported data (persistent itch category of UK-PBC cohort) with the recorded prescription data (in the CPRD) for anti-pruritic treatment.

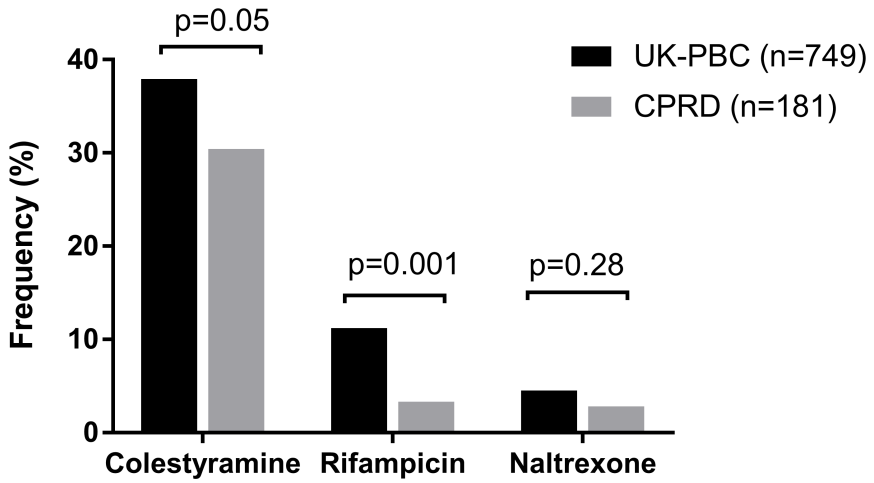


Figure 2-3 Comparison of anti-pruritic therapies in UK-PBC and CPRD datasets
Frequencies of patient-reported use of treatment in the persistent itch category in the UK-PBC cohort were compared with the actual recorded prescription in the CPRD cohort.

2.3.6 Longitudinal assessment of pruritus

A second survey of the UK-PBC cohort was performed in July 2014 to evaluate consistency in self-reporting pruritus (for prevalence and pruritus severity of ever itch) and to assess evolution of pruritus over time. Of 2184 patients in the first survey (February 2011), 1423 (65.1%) returned completed questionnaires in the second survey (July 2014). Comparison of individual data for 1423 patients from both surveys showed high consistency of self-reported data on prevalence and treatment of pruritus (**Table 2-3**).

Compared to the first survey, lower proportion of patients reported moderate (15.6% vs. 12.5%, p=0.018) and severe (9.7% vs. 7.5%, p=0.038) itch scores in the second survey. Overall, the reported frequency of use of anti-pruritic treatment was higher in the second survey across all categories of patients (**Table 2-3**). This was exemplified by patients with *severe itch* who reported higher use of colestyramine (70.1% vs. 50.7%) and rifampicin (31.8% vs 23.9%) in the second survey compared to the first survey.

The high consistency in patient reporting was supported by the observation that there were no significant differences in the reported frequencies of 'any experience' of itch (p=0.83) or 'persistent' itch (p=0.46) since development of PBC. In addition, a significant correlation ($r=0.72$, $p<0.0001$) was observed between the PBC-40 itch domain scores from both surveys.

		First Survey (Year 2011)		Second Survey (Year 2014)		
		n	%	n	%	p value
1	Frequency of Itch					
	Never experienced itch	389	27.3	384	27.0	0.83
	Any experience of itch	1034	72.7	1039	73.0	0.83
	Persistent itch†	463	32.5	445	31.3	0.46
2	Itch severity (PBC-40 itch domain scores)					
	Mild (4-8)	375	26.4	389	27.3	0.55
	Moderate (9-11)	222	15.6	178	12.5	**0.018**
	Severe (≥12)	138	9.7	107	7.5	**0.038**
3	Treatment received					
	A) Any experience of itch:					
	Colestyramine	242	23.4	278	26.8	0.078
	Rifampicin	54	5.2	73	7.0	0.087
	Naltrexone	19	1.8	28	2.7	0.190
	b) Persistent itch†:					
	Colestyramine	169	36.5	189	42.5	0.066
	Rifampicin	50	10.8	64	14.4	0.103
	Naltrexone	18	3.9	26	5.8	0.170
	C) Severe itch :					
	Colestyramine	70	50.7	75	70.1	**0.002**
	Rifampicin	33	23.9	34	31.8	0.171
	Naltrexone	11	8.0	14	13.1	0.190

Table 2-3 Comparison of patient-reported pruritus data from first and second surveys in the UK-PBC Cohort
n=1423. Significant p values are shown in bold.

2.4 PBC cohorts from US and Italy

Pruritus datasets were also available from PBC cohorts in USA and Italy. Unlike the UK-PBC, these datasets included patients from single centres, but similar to the UK-PBC cohort they included comprehensive patient-reported information on their pruritus and its treatment. Utilising these databases I intended to compare pruritus experienced by PBC patients from UK, USA and Italy to:

1. understand the prevalence of pruritus in PBC in each cohort,
2. study any differences in the frequency and intensity of pruritus between the cohorts,
3. assess the correlation between measures of itch intensity in each cohort and
4. report the frequency of anti-pruritic treatments received by PBC patients

The database from USA included 655 patients with PBC and that from Italy had 75 patients.

2.4.1 Frequency and severity of ever itch

- 445 (68%) USA and 45 (60%) Italy patients had experienced itch at some point in their illness ('ever itch').
- 221 (34%) in the USA cohort and 20 (27%) in the Italy cohort reported *persistent itch*.
- Severe itch (PBC-40 itch domain score ≥12) was reported by 69/655 (10.53%) in the USA cohort and 6 (8%) in the Italy cohort.

Table 2-4 shows comparison of prevalence of itch in UK, USA and Italy PBC cohorts.

	UK Cohort	**USA Cohort**	**p value[#]**	**Italy Cohort**	**p value[#]**
Any experience of itch	1624/2184 (74%)	445/655 (68%)	0.0025	45/75 (60%)	0.0069
Persistent itch	749/2184 (34%)	221/655 (34%)	1.0	20/75 (27%)	0.2076
Severe itch	249/2184 (11%)	69/655 (10.5%)	0.7349	6/75 (8%)	0.4125

Table 2-4 Patient-reported prevalence of pruritus in PBC cohorts from UK, USA and Italy
[#]proportions in USA and Italy cohorts compared to UK cohort; p values calculated from Chi-square test.

2.4.2 Correlation between measures of itch intensity

Significant correlations were seen between the PBC-40 itch domain score and the pruritus VAS *since development of PBC* in both cohorts [USA: r=0.77, p=0.01 and Italy: r=0.84, p=0.01].

2.4.3 Anti-pruritic therapy

Table 2-5 shows the proportion of patients in the USA and Italy PBC cohorts reported to have received anti-pruritic drugs during the course of their PBC.

Overall, the results from USA and Italy cohorts were similar to the UK-PBC cohort with large proportion of patients reporting no treatment with guideline-recommended drugs. The proportion of patients with *persistent* and *severe itch* reported to have received cholestyramine was 31% and 43% in the USA cohort and 30% and 50% in the Italy cohort, respectively.

	Treatment received, n (%)		
Category of patients	**Cholestyramine**	**Rifampicin**	**Naltrexone**
Any experience of itch			
USA (n=445)	90 (20.2)	37 (8.3)	11 (2.4)
Italy (n=45)	10 (22.2)	4 (8.8)	0
Persistent itch			
USA (n=221)	70 (31.6)	34 (15.3)	10 (4.5)
Italy (n=20)	6 (30)	3 (15)	0
Severe itch			
USA (n=69)	30 (43.4)	18 (26)	7 (10.1)
Italy (n=6)	3 (50)	3 (50)	0

Table 2-5 Patient-reported information on anti-pruritic therapy in PBC cohorts from USA and Italy

2.5 Discussion

This is the largest study to date recording the patient-reported characteristics of cholestatic pruritus. Our study provides important insights into pruritus in PBC by using the UK-PBC cohort, assessed using well-described and validated measures optimised for self-completion. Since the patients in the UK-PBC cohort have been recruited from every hospital in the UK, they robustly represent 'real world' patients without referral or treatment centre bias. The study therefore provides accurate assessment of the scale of unmet need in PBC.

In this study, we set out to assess the prevalence of pruritus in the UK-PBC cohort and explore different characteristics of pruritus and its treatment self-reported by patients.

The main finding of this study is that pruritus is a frequent ongoing symptom in patients with PBC, despite the availability of seemingly effective therapies. The overall prevalence of pruritus (74%) observed in this study highlights the significant symptom burden in PBC with more than one-third (34%) of patients experiencing persistent itch during their illness. This study is also the first to report the prevalence of pruritus severity in PBC by using the PBC-40 score, the only validated disease-specific score (Jacoby, Rannard et al., 2005). Using the previously validated cut-off scores for categorising itch severity, we observed that more than a quarter (27.3%) of patients experienced moderate to severe pruritus since their diagnosis of PBC.

A key finding of our study is that most patients with PBC and itch do not receive anti-pruritic treatment. The EASL and AASLD guidelines recommend a step-wise treatment of pruritus, starting with cholestyramine as the first-line drug followed by rifampicin and naltrexone. In our study, only 24% of patients who had experienced itch during their illness reported to have ever received treatment with cholestyramine. Although the reported frequency of use of cholestyramine was higher in those with persistent itch (37%) and severe itch (51%), it is noteworthy that approximately half of patients with severe itch reported no treatment with this medication. Similarly, the reported use of second-line (rifampicin) and third-line (naltrexone) drug therapies was also unsatisfactory with only 25% of patients with severe itch reported to have ever received rifampicin.

The longitudinal data from our study highlights the importance of treating itch in PBC. We observed that of the 60% of patients with persistent itch who did not receive any therapy, 68%

continued to experience persistent itch and 47% described current itch intensity score ≥5. These findings suggest that without adequate treatment pruritus is unlikely to improve in the majority of patients. Therefore, our observations from the UK-PBC cohort on the inadequate or inconsistent treatment of pruritus may have significant implications. It is possible that patients with pruritus did not seek medical intervention for their pruritus or patients' treating clinician (GPs or secondary care physicians) may not be familiar with the available guidelines for treating cholestatic pruritus and therefore did not initiate appropriate therapy to eligible patients. If the latter is true then it suggests that there is a real need for improvement in the awareness and management of cholestatic pruritus at the level of both GPs and gastroenterologists.

In the UK-PBC cohort, the data for pruritus prevalence and anti-pruritic therapy are provided by the patients and therefore may be prone to potential recall bias. We addressed this in two ways. Firstly, the pruritus survey was repeated after a gap of ~3.5 years and comparison of both surveys showed highly consistent findings on prevalence of itch, its severity and treatment. Overall, our results suggest the patient-reported information on their itch and medications was reliable and recall bias was unlikely. Furthermore, in the UK-PBC cohort we have previously published a high level of data accuracy on patient self-reported PBC therapy based on a cross validation of self-reported data of a subgroup of 1379 patients (~63% of the whole cohort) with the patients' hospital record data (Carbone, Mells et al., 2013). Secondly, we used the CPRD cohort to validate the findings from the UK-PBC cohort. The CPRD enabled to study the actual recorded pruritus prevalence and medication use in the primary care setting. The results showed important similarities between CPRD and UK-PBC cohort results. The recorded prevalence of pruritus in PBC patients in the CPRD cohort (27%) was similar to the prevalence of persistent itch (34%) in the UK-PBC cohort. This suggests that PBC patients who experience pruritus frequently or all the time (i.e. persistent itch) are more likely to present to their primary care physicians for treatment than those experiencing mild or infrequent itch. In addition, the percentage of persistent itch patients in the UK-PBC cohort self-reporting ever use of cholestyramine (37%) was similar to the actual recorded cholestyramine prescription data in the CPRD (30%). The recorded use of rifampicin in CPRD (3%) was lower than the self-reported use in UK-PBC cohort (11%), potentially because the latter included patients managed by secondary care physicians who are more likely to prescribe rifampicin than GPs. Overall, data recorded in the CPRD corroborate the

patient-reported data in the UK-PBC cohort and support that self-reported data in the UK-PBC cohort is reliable in accurately capturing anti-pruritic therapy.

In clinical practice, the assessment of itch intensity in PBC is difficult due to the lack of objective measures. The PBC-40 is a validated tool that provides valuable information but it is infrequently used in routine clinical practise as it is perceived as time consuming. The NRS and VAS are easy to use unidimensional scales and can be routinely used to assess pruritus intensity but they are not specific for PBC and have not been validated for use in PBC. In this study, we observed that pruritus NRS and VAS scores of PBC patients correlated significantly for both 'ever' itch and 'current' itch. We have also shown that the PBC-40 itch domain score correlates significantly with the pruritus VAS suggesting a strong correlation between the itch intensity and its functional consequences.

The main strengths of our study include the large sample size, cross sectional study of a national cohort, use of validated pruritus assessment tools, a follow-up study describing longitudinal pruritus data, and validation of patient-reported therapy data with an independent primary care database. The main limitation of the study is lack of a comparator group e.g. normal population or age/sex matched healthy volunteers. Therefore, it was not possible to directly compare differences in prevalence of pruritus between patients with PBC and in the general population. Nevertheless, our study provides further insights into important clinical issues related to pruritus in PBC.

2.6 Conclusion

In conclusion, in this UK-PBC cohort study we report that the prevalence of pruritus in PBC is high and a significant proportion of patients experience persistent and severe itch during the course of their disease. The patient-reported information on their pruritus and treatment is highly consistent over time and the data accuracy is validated by an independent primary care database. We observed under-treatment of pruritus in PBC with inadequate and unsatisfactory use of guideline recommended therapy. We suggest the need for improvement in the awareness and management of pruritus among both primary and secondary care physicians caring for patients with PBC in the UK.

After studying the unmet need in pruritus in this study, I subsequently investigated treatment of pruritus, with a focus on therapeutic role of interrupting the enterohepatic circulation of pruritogens.

2.7 Acknowledgements

The work presented in this chapter was a collaborative work between UK-PBC team, international collaborators from USA and Italy and the GSK personnel.

The UK-PBC pruritus data from 2011 and 2014 was collected by the UK-PBC team based in Cambridge. In particular, I am grateful to Geroge Mells and Steven Flack at Addenbrookes Hospital, Cambridge for their timely assistance with the datasets. My role included statistical analysis of these datasets and interpretation of results including creating the tables and figures.

The datasets from USA and Italy were provided by Dr Brian Juran and Dr Konstantinos Lazaridis (Mayo Clinic, Rochester, USA) and Dr Pietro Invernizzi (Humanitas Clinical and Research Center, Milan). I am very grateful to them. My role included statistical analysis of their datasets and interpretation of results including creating the tables and figures.

My special thanks to Julia DiBello of GSK for sharing her work on CPRD dataset. She did the data collection and analysis of CPRD dataset. I subsequently collaborated with her to utilise the main data to compare with the results from UK-PBC datasets. I created Figure 2-3.

I wrote the first manuscript of the study. After series of corrections the final manuscript was published in peer reviewed journal *Clinical Gastroenterology and Hepatology* in 2018.

3. CHAPTER 3: ENDOSCOPIC NASOBILIARY DRAINAGE AS A TREATMENT FOR REFRACTORY CHOLESTATIC PRURITUS

3.1 Introduction and Aims

The current medical treatment of cholestatic pruritus is described in **Chapter 1** of this thesis. These drugs are not universally effective and a significant number of patients remain refractory to medical therapy. In practise, treatment of patients with intractable or refractory cholestatic pruritus is highly challenging and frustrating for both clinicians and the patients. Very limited options exist for such patients who may be offered guideline recommended invasive therapies such as nasobiliary drainage (NBD), plasmapheresis or extracorporeal albumin dialysis (e.g. Molecular Adsorbent Recirculation System (MARS®) or Prometheus®) for symptom relief (Beuers, Boberg et al., 2009, Lindor, Gershwin et al., 2009). The evidence basis for these approaches is, in each case, relatively limited with little in the way of informed guidance as to how and when the approach should be considered.

Biliary drainage diverts the bile and bile salts (BS) away from the ileum where 90% of the BS are physiologically reabsorbed and returned to the liver (enterohepatic circulation), thus depleting the body of bile and potential pruritogenic substances (Hofmann and Huet, 2006). Biliary drainage can be achieved either surgically (such as partial external biliary diversion or ileal bypass) or endoscopically.

Endoscopic NBD (ENBD) was first developed by Cotton *et al*. as a technique for placing trans-nasal biliary catheterisation during ERCP (Cotton, Burney et al., 1979). Since then it has been successfully utilised for variety of applications such as treating patients with obstructive jaundice, cholangitis and post-operative bile leaks (Ishigaki, Sasaki et al., 2014). Compared to surgical biliary drainage, NBD is more appealing as it is less invasive, convenient, temporary, and can be used repeatedly. In brief, ENBD is carried out through the endoscopic placement of a 6Fr or a 7Fr nasobiliary catheter into the common bile duct during endoscopic retrograde cholangiopancreatography (ERCP). After ensuring free flow of bile from the external end of the catheter, the latter is re-routed through the nose and connected to a bag for continuous drainage (**Figure 3-1**). To maintain catheter patency whilst in use, the catheter is irrigated once daily with sterile normal saline.

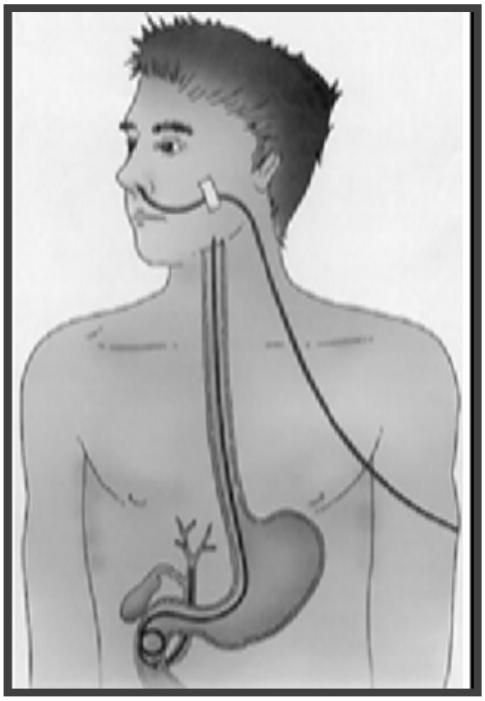


Figure 3-1 Nasobiliary drainage catheter
[Image courtesy: Andreas Kremer, Germany]

Data on the use of ENBD in treating cholestatic pruritus are limited to very few published studies. There are reports that NBD induces complete and long-lasting remission in BRIC [n=3](Stapelbroek, van Erpecum et al., 2006) and transiently relieves intractable pruritus in PBC [n=3] (Beuers, Gerken et al., 2006) and acute cholestatic viral hepatitis [n=6] (Singh, Bhalla et al., 2009). More recently long term NBD (i.e. continuous biliary drainage by leaving the NBD catheter *in situ* for few months) has also been suggested to be safe and effective (Appleby, Hutchinson et al.) These results are encouraging but inference is limited since they are single centre reports with small sample size and include patients with one specific disease aetiology.

The main aim of this study was to maximise our understanding of the potential benefits and optimal utility of ENBD in refractory cholestatic pruritus by systematically describing the cumulative experience of using NBD in this setting. To achieve this, we performed a multicentre retrospective study of NBD with a larger number of patients of different aetiologies of cholestasis. Specifically, we intended to study:

1) Efficacy of ENBD in cholestatic pruritus of different aetiologies.

2) Effect of ENBD on liver function tests (LFTs) and levels of total serum bile salts (TBS).

3) Any correlation between duration of treatment response and duration of drainage and volume of bile drained.

4) Safety of ENBD and adverse events (AEs) associated with the intervention.

3.2 Patients and Methods

3.2.1 Study design

This was a multi-centre retrospective study of patients treated with ENBD for medically refractory cholestatic pruritus. We retrospectively analysed data of patients treated with ENBD for cholestatic pruritus at five academic medical centres [Newcastle, United Kingdom (UK): Freeman Hospital; Paris (France): Hôpital Saint-Antoine; Erlangen (Germany): Friedrich-Alexander-University of Erlangen; Homburg (Germany): Saarland University Medical Center, and Rotterdam (Netherlands): Erasmus Medical Centre]. Due to the nature of the intervention it was not possible to do a placebo (sham) controlled or blinded study.

3.2.2 Data collection

Data were obtained from the medical records of patients with refractory cholestatic pruritus treated with ENBD between September 2006 and April 2015. Demographic, clinical, biochemical, radiological and endoscopic data were collected in a predesigned electronic case report form. A study investigator from each centre retrieved data after careful interrogation of patient medical records. The diagnosis of underlying cholestatic condition was based on appropriate clinical, laboratory, serological and genetic tests. All patients were informed and consented for ERCP to place a NBD catheter for biliary drainage. In patients who had repeated ENBD, outcome of each procedure was assessed as a unique case (i.e. number of cases>number of patients).

*3.2.2.1 **Evaluation of efficacy***

The effect of NBD on pruritus was evaluated using a 0-10 visual analogue scale (pruritus VAS). Patients had completed pruritus VAS before NBD (pre-NBD), repeatedly during the drainage period and at the end of drainage period or after the NBD catheter was removed (post-NBD). To assess the durability and treatment efficacy of NBD, we defined the duration of treatment response as the time (in days) taken to return to pre-treatment pruritus level after removal of the NBD catheter.

*3.2.2.2 **Safety and biochemical parameters***

We also collected data on duration of NBD, pre-and post-NBD laboratory parameters including serum bilirubin, alkaline phosphatase (ALP), alanine aminotransferase (ALT) and TBS. Endoscopy records were reviewed to collect procedure-related data such as the size of NBD catheter placed, use of endoscopic sphincterotomy (EST) and prophylactic temporary pancreatic duct (PD) stents. The volume of bile output from the catheter was monitored.

Any AEs associated with the NBD procedure were reviewed. Post ERCP pancreatitis (PEP) was defined as per the consensus definition (Elmunzer, Scheiman et al., 2012, Fazel, Quadri et al., 2003): 1) new or increased abdominal pain that was clinically consistent with a syndrome of acute pancreatitis, and 2) serum amylase or lipase ≥ 3x the upper limit of normal 24 hours after the procedure, and 3) prolongation of existing hospitalization for at least 2 days. Severe PEP was defined as that resulted in the development of pancreatic necrosis or pseudocyst, or required additional endoscopic, percutaneous, or surgical intervention. Cases that did not meet the definition of severe PEP were considered as mild PEP.

*3.2.2.3 **Statistical analysis***

Data were analysed with GraphPad Prism 6.0 (GraphPad Software, Inc. La Jolla, USA). Data were not normally distributed. Categorical variables were expressed as frequencies and percentages and continuous variables were expressed as median with interquartile range (IQR). The Wilcoxon signed-rank test (for paired samples) and Mann Whitney test (for unpaired samples) were used for the comparison of continuous data. Correlation between variables was evaluated using Spearman's rank correlation test to compute the correlation coefficient (r). Fisher's exact test was used to compare categorical variables. Statistical significance was set at $p < 0.05$.

3.3 Results

A total of 27 patients who underwent 29 NBD procedures (n=29 cases) were included in this study. Aetiologically, PBC (44%) was the commonest cause of pruritus followed by benign intrahepatic recurrent cholestasis (BRIC) (29%). **Table** 3-1 summarises the baseline demographic, clinical and biochemical characteristics of study patients.

Age†, years	41 (11)
Females, n (%)	16 (59.3%)
Diagnosis, n (%)	
PBC	12 (44.4)
BRIC	8 (29.6)
DILI	2 (7.4)
others	5 (18.5)
Previous medical treatments, n (%)	
Cholestyramine	21 (78)
Rifampicin	25 (93)
Opiate antagonists	18 (67)
Sertraline	8 (30)
Plasmapheresis/ extracorporeal albumin dialysis	4 (15)
ultraviolet phototherapy	4 (15)
Pre-ENBD serum biochemistry†	
ALP (IU/L)	367 (311)
ALT (IU/L)	61 (90.5)
Bilirubin (µMol/L)	203.5 (455.3)
TBS (µMol/L)	144 (225.5)
Duration of NBD†, days	
All patients	7 (9.5)
PBC patients	5.5 (3.7)
BRIC patients	9 (12.5)
Pruritus VAS (mean±SD)	
Pre-NBD	9.0 ±1.7
Post-NBD	2.0 ±2.9
Bile output (ml/day)†	
Minimum	150 (335)
Maximum	400 (412.5)
Duration of treatment response†, days	
All patients	50 (345)
PBC patients	13 (68.25)
BRIC patients	459.8 (720.8)

Table 3-1 Baseline clinical and biochemical characteristics of study patients
Data marked † are expressed as median and (IQR). DILI, drug induced liver injury.

3.3.1 Effect of NBD on pruritus

Of the 29 NBD cases, pre-and post-NBD pruritus VAS data was not available in two cases. At baseline the median VAS score was 10 (IQR 2) suggesting all patients had severe pruritus. The median duration of NBD was 7 days (mean 16; range 2-86 days).

Overall, a significant reduction in the median pruritus VAS was seen following NBD. Post-NBD pruritus VAS (median score 0.3) was significantly lower compared to pre-NBD pruritus VAS (median score 10) **(Figure 3-2**).

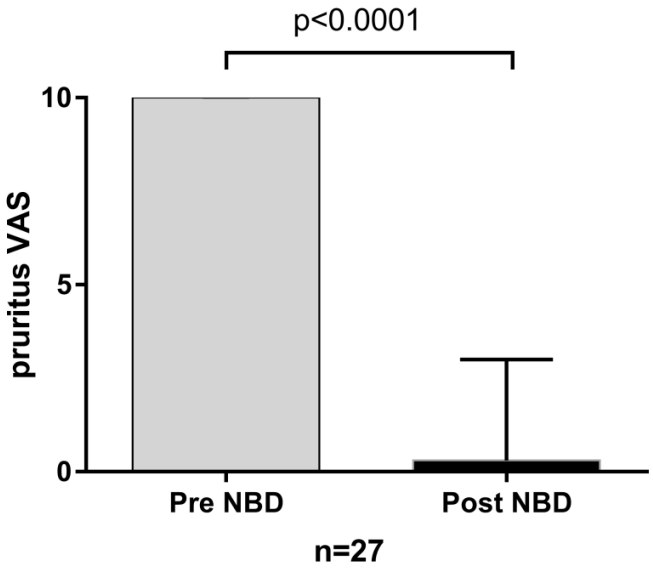


Figure 3-2 Effect of nasobiliary drainage on pruritus

Overall, improvement in pruritus VAS was seen in 26/29 (89.6%) cases with complete resolution of pruritus (zero on VAS) in 12 (41.3%) cases. Improvement in pruritus VAS was seen in all except one patient (**Figure 3-3**). The patient who did not benefit from NBD was a 69 yr. male with BRIC and had 21 days of NBD with an average bile output of 250ml/day but had no change in pruritus VAS following NBD.

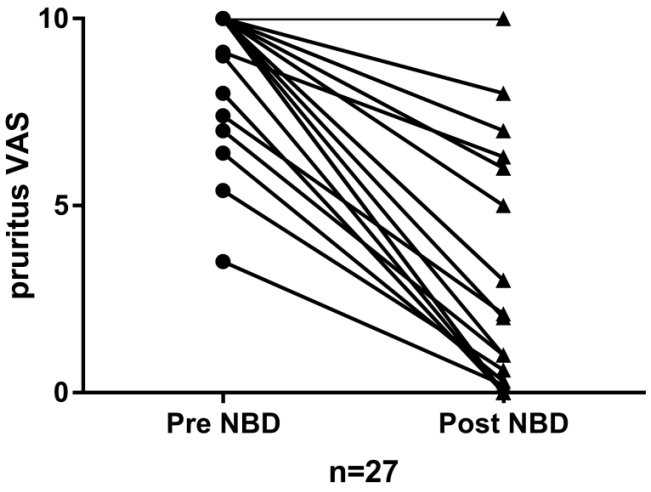


Figure 3-3 Changes in pruritus VAS after nasobiliary drainage

Overall, the median percentage decrease in pruritus VAS was 94% (mean 78.4%) (**Figure 3-4**). Also, NBD immediately resolved pruritus in nine (33%) patients who were free of pruritus within 24 hours of starting drainage.

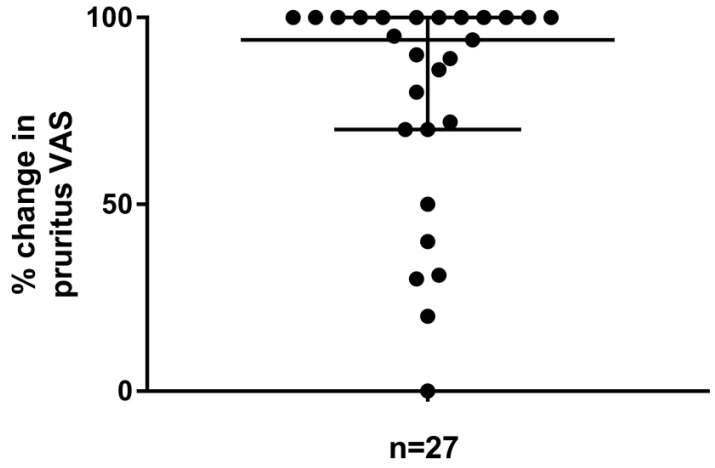


Figure 3-4 Percentage change from baseline in pruritus VAS after nasobiliary drainage

3.3.2 Duration of NBD and treatment response

Overall, the median duration of NBD was 7 days. As PBC (n=12) and BRIC (n=8) were the commonest causes of pruritus, we analysed data separately to compare these two groups of patients.

BRIC patients received longer duration of NBD compared to PBC patients (9 days vs. 5.5 days, p=0.04) (**Figure 3-5**).

Overall, the median duration of treatment response was 50 days (IQR 345 days).

The duration of treatment response was significantly longer for BRIC patients compared to PBC patients (median 459 days vs.13 days, p=0.02) (**Figure 3-6**).

When BRIC patients were excluded from the analysis, the overall median duration of treatment response was 14 days.

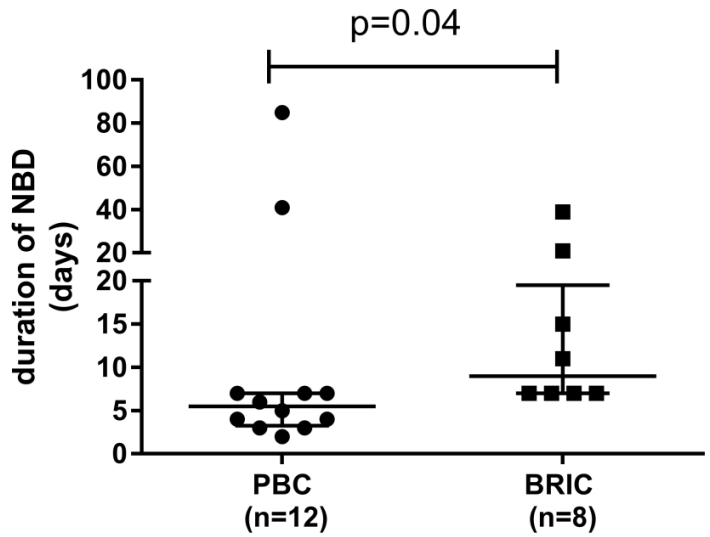


Figure 3-5 Duration of nasobiliary drainage treatment in study population

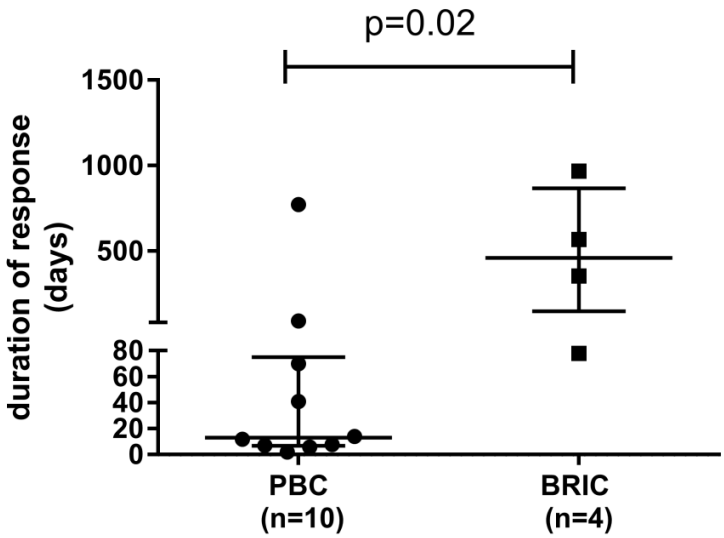


Figure 3-6 Duration of treatment response after nasobiliary drainage

3.3.3 Effect of NBD on serum biochemistry

NBD significantly decreased serum ALP [367 (IQR 311) vs. 288.5 (IQR 315.5), p=0.001] and serum bilirubin [203.5 (IQR 455.34) vs. 169.3 (IQR 285), p=0.03) but there was no significant change in the levels of serum ALT [61 (IQR 90.5) vs. 71 (IQR 105), p=0.37). Although a trend toward decline in the levels of TBS was observed, the change was not statistically significant [144 (IQR 225.5) vs.58.5 (IQR 150.3), p=0.07] (**Figure 3-7**)

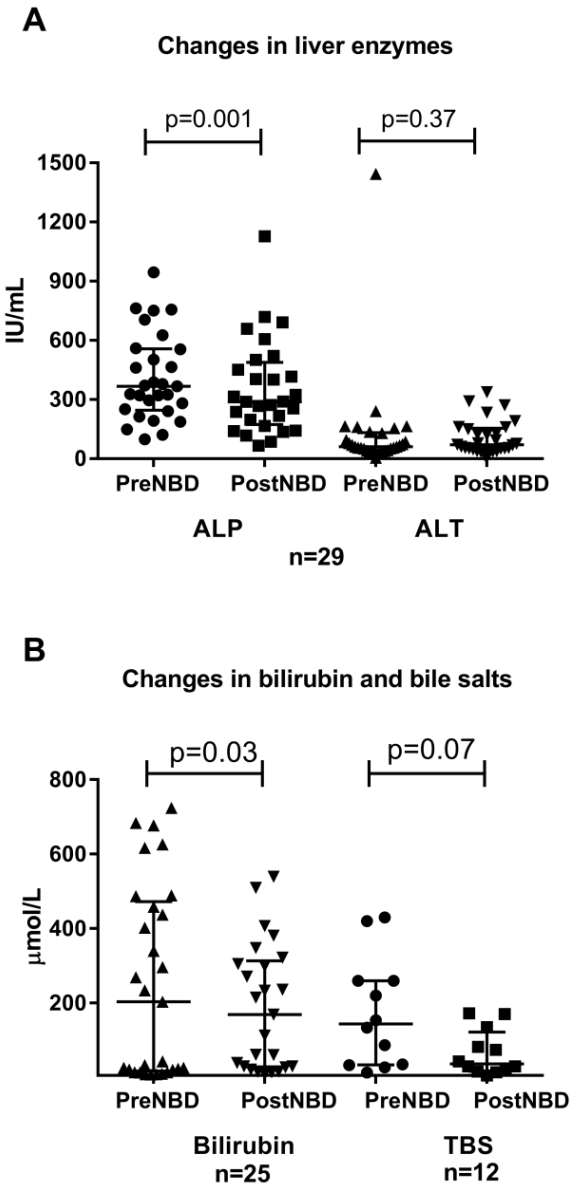


Figure 3-7 Effect of nasobiliary drainage on serum biochemistry

A sub-group analysis PBC patients (n=10) showed significant decrease in the mean (±SD) serum ALP following NBD (428±227 vs. 399±210 IU/L, p=0.0195, Wilcoxon matched-pairs signed rank test) (**Figure 3-8**).

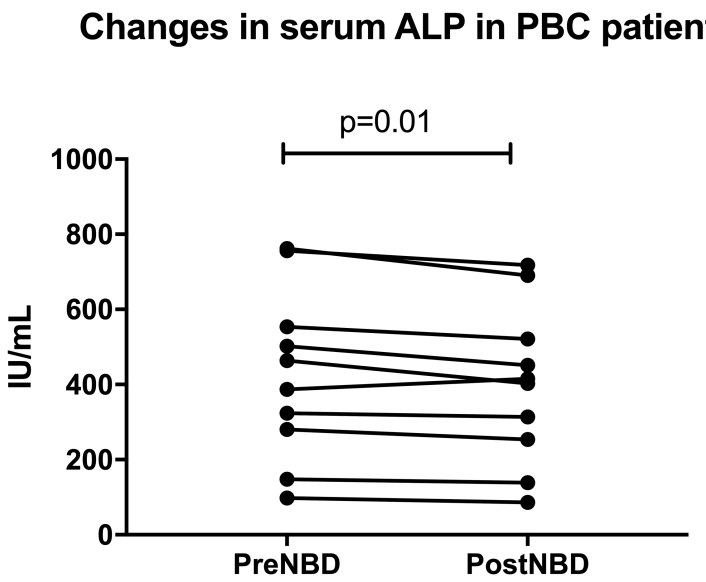


Figure 3-8 Changes in serum alkaline phosphatase after nasobiliary drainage in PBC patients

3.3.4 Treatment response associations

The hypotheses for these analyses were:

i) Longer duration of NBD results in longer duration of treatment response.

ii) Higher the daily volume of bile output, longer the duration of treatment response.

iii) Following NBD, there is a correlation between change in the total serum bile salt levels and change in the pruritus VAS.

However, the results showed:

i) No significant correlation between duration of NBD and duration of treatment response (r=0.36, p=0.12). This lack of correlation persisted even after excluding BRIC patients from analysis (r=0.20, p=0.49). (**Figure 3-9A&B**)

ii) Duration of treatment response did not correlate significantly with the daily volume of bile output (r=0.40, p=0.19 for minimum output and r=0.14, p=0.65 for maximum output) (**Figure 3-9 C&D**)

iii) There was no significant correlation between percentage (%) change in TBS levels and % change in pruritus VAS following NBD (r=-0.35, p=0.28) (**Figure 3-10**). Data was available in 11 patients. Reductions in both pruritus VAS and TBS levels were seen in six (54%) patients; decrease in pruritus VAS but increase in TBS levels was observed in four (36%) patients and in one patient there was no change in pruritus VAS although TBS levels had decreased.

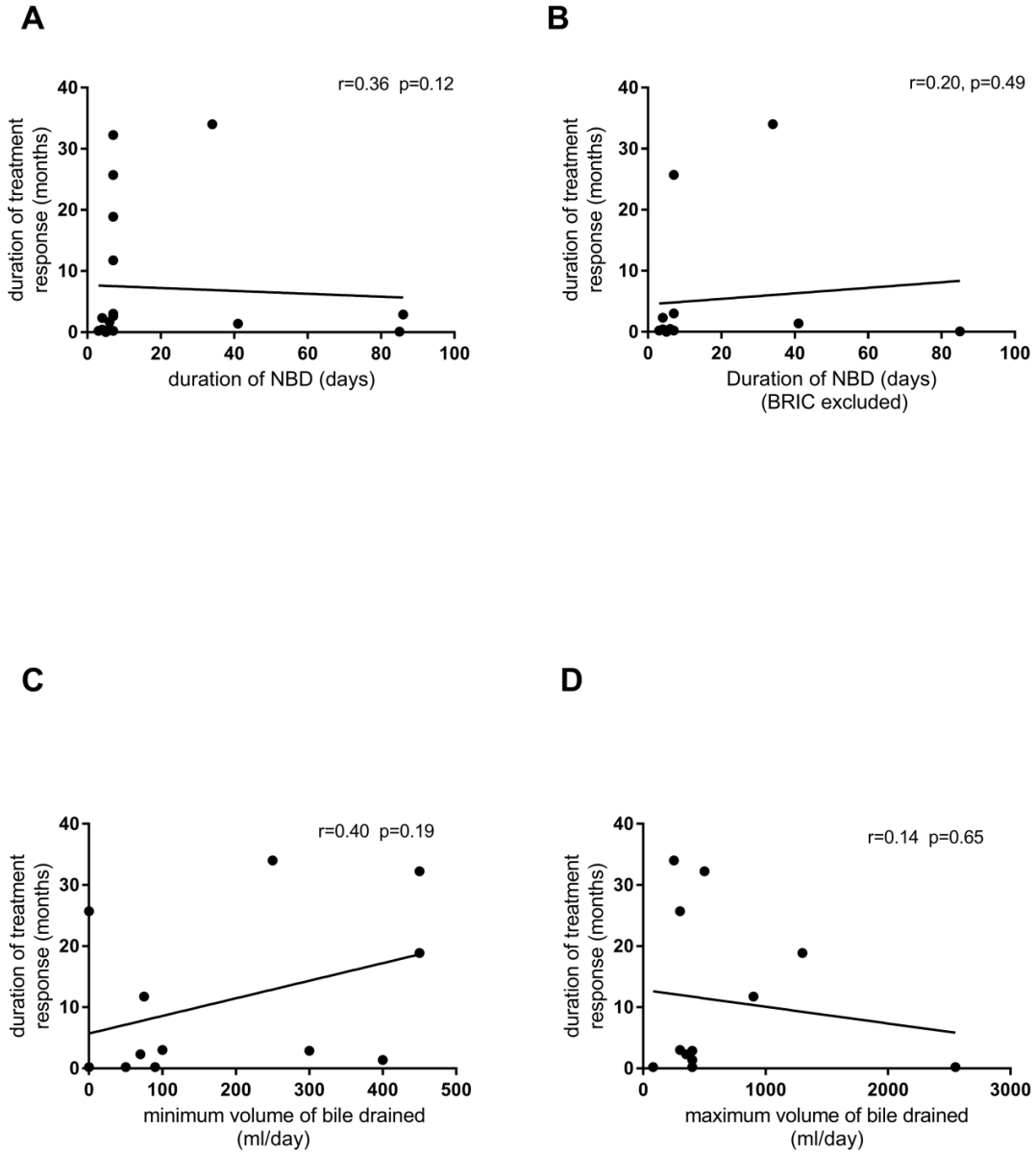


Figure 3-9 Associations between duration of treatment response and duration of nasobiliary drainage and volume of bile drained

The duration of treatment response did not signifianctly correlate with duration of NBD (**A&B**) or with the daily minimum or maximum volume of bile output (**C&D**).

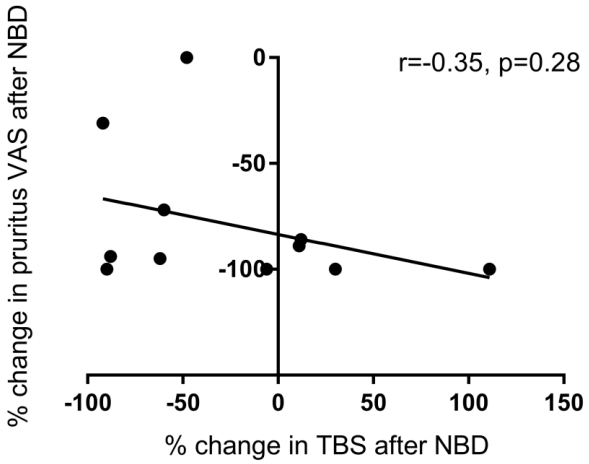


Figure 3-10 Correlation between changes in itch intensity and serum total bile salts after nasobiliary drainage

3.3.5 Adverse events

Adverse events (AEs) were observed in 10/29 (34%) of cases (**Table 3-2**). Of these 9 (31%) were diagnosed with mild PEP and one was post-ERCP acute cholangitis based on clinical, biochemical or radiological features. There were no cases of severe PEP. In total, two patients (6.9%) had received post-ERCP prophylactic single dose rectal indomethacin [non-steroidal anti-inflammatory drug (NSAID)] and four patients (13.8%) had prophylactic temporary PD stent placement. Of the 9 patients who developed PEP, 4 (44%) had EST and only one had prophylactic PD stent placement. There was no significant association between EST and pancreatitis [Fisher's exact test, p=0.40, relative risk 1.69 (95%CI 0.59-4.82)]. All AEs had resolved completely with appropriate medical management. There was no mortality associated with these AEs.

	Number of cases n (%)
Total procedures	29
Total adverse events (AEs)	10 (34.5)
Post ERCP Pancreatitis (PEP)	9 (31)
Acute cholangitis	1 (3.4)
Frequency of PEP according to disease aetiology	
PBC	5 (55.5)
BRIC	3 (33.3)
Acute Hepatitis A	1 (11.1)

Table 3-2 Summary of adverse events associated with endoscopic nasobiliary drainage

3.4 Discussion

Generally, treatment of cholestatic pruritus is targeted at reducing the hepatic and systemic concentration of BS or other putative pruritogens. Indeed, this is the rationale for using cholestyramine (bind to BS in the intestine and reduce their re-absorption) and opioid antagonists (reduce the pruritogenic effect of endogenous opioids) (Mela, Mancuso et al., 2003). However, in clinical practice the treatment of cholestatic pruritus remains a formidable challenge and may be frustrating as the drug therapy is limited and not universally effective. In those who do not respond to medications, invasive options such as ENBD are often explored.

Published literature on the use of endoscopic NBD in cholestatic pruritus is limited with only single centre case series. Stapelbroek *et al*. first showed quick (pruritus disappeared within 24 hours of NBD) and complete disappearance of pruritus and normalisation of TBS levels in three BRIC patients following 11-21 days of NBD and the duration of treatment response lasted for 8-12 months (Stapelbroek, van Erpecum et al., 2006). In the same year Beuers *et al.* reported that following a mean 4.1 days of NBD in three PBC patients, two were completely free of pruritus within 24hours (Beuers, Gerken et al., 2006). Subsequently, Singh *et al*. reported complete remission of pruritus following 7 days of NBD in six patients with intractable pruritus secondary to viral hepatitis A, B and E (Singh, Bhalla et al., 2009). More recently a UK single centre report of three patients (2 PBC, 1 BRIC) has suggested long term NBD is successful in maintaining remission of pruritus (Appleby, Hutchinson et al.).

In comparison to previous studies, the current study is unique since it is a multi-centre study and it is the largest retrospective study describing the utility of ENBD in the treatment of cholestatic pruritus. In addition to adding evidence to the reported advantages of ENBD, this study attempts to answer some of the previously unanswered questions and uncertainties about ENBD.

The key finding of the present study is that ENBD is an effective treatment option for refractory pruritus of different cholestatic aetiologies. Except one patient, all patients in this study benefitted from ENBD with significant improvement in pruritus. In particular, NBD effectively terminated pruritus attacks in all patients with BRIC. This observation is in line with previous reports and suggests that exacerbations of BRIC can be effectively treated with

NBD. The speed of induction of remission with NBD varies between patients with a third achieving immediate and dramatic remission of pruritus within 24hr of initiating drainage.

The present study also shows that after cessation of NBD patients achieve a short period of 'pruritus-free' remission. Patients usually wish to know the average duration they can expect to remain itch-free *after* stopping the drainage. Accordingly, we evaluated our data by defining the duration of treatment response as the time taken to return to pre-treatment pruritus level *after* the removal of NBD catheter. It seems that the duration of treatment response is variable and likely depends on the underlying disease aetiology. Overall, the median duration of treatment response was 50 days. Of note, the duration was shorter for PBC patients (median 13 days) in comparison to BRIC patients (median 459.8 days). This apparent difference in the beneficial effect of NBD between PBC and BRIC patients is likely due to the underlying pathophysiology of these conditions. Clinical observations suggest BRIC patients present in episodes and are known to remain in spontaneous remission for months to years in between attacks (Folvik, Hilde et al., 2012). On the contrary, pruritus in PBC usually recurs when treatment is stopped. The implication of this finding is that PBC patients undergoing NBD should be advised to expect only a couple of weeks of remission after NBD.

Another pertinent but previously unexplored question relates to the effect of duration of drainage on treatment response – i.e. "do patients need longer duration of drainage to achieve longer period of remission?" Our results suggest that duration of treatment response is essentially independent of duration of drainage and this lack of correlation was demonstrated even after removing BRIC patients from the analysis. Therefore, in clinical practise the duration of NBD should be guided by the patient tolerance of the catheter and benefit of drainage on their pruritus but in general BRIC patients usually do not need more than 7-10 days of drainage.

There are conflicting data on the effect of NBD on serum TBS with two studies showing significant reduction (Singh, Bhalla et al., 2009, Stapelbroek, van Erpecum et al., 2006) and one study showing only transient decrease (Beuers, Gerken et al., 2006) in serum TBS following NBD. We did not observe significant change in the levels of serum TBS and the percentage change in serum TBS levels did not correlate with the percentage change in pruritus VAS. Our results could be due to insufficient data as the pre-and post-NBD data on serum TBS was available in only 12/29 (41%) cases. But if our results are confirmed, they are

against the conventional hypothesis that NBD reduces the systemic levels of pruritogenic BS by interrupting their enterohepatic circulation (Hofmann and Huet, 2006). Therefore, more studies are needed to see any true effect of NBD on serum BS pattern and future studies should evaluate levels of sub-species of BS both in the serum and bile. Alternatively, the benefit of NBD on pruritus could be secondary to removal of other yet unidentified pruritogens from the enterohepatic circulation (Beuers, Kremer et al., 2014).

Treatment with ENBD procedure may be associated with AEs. In our study 34% had AEs; a majority were due to PEP. This high rate of AEs could be attributable to three main factors. Firstly, the NBD catheter is a transpapillary endoprosthesis that obstructs the pancreatic orifice and inhibits the flow of pancreatic fluids, thus increasing the risk of PEP (Huibregtse and Tytgat, 1982). The gauge of the NBD catheters may be another factor contributing to the high risk of PEP. Conventionally a 6Fr or a 7Fr NBD catheter is used and all patients in our study received 7Fr catheter. However, a recent study (n=165) proposed that compared to a 6Fr catheter, using a 4Fr catheter for NBD significantly reduces the incidence of PEP (15.7% vs. 3.7%, p=0.02) without any significant difference in the biliary output (Ishigaki, Sasaki et al., 2014). Secondly, 44% of patients who developed PEP had endoscopic sphincterotomy (EST) during placement of NBD catheter. Since EST is a known independent risk factor for PEP (Cotton, Garrow et al., 2009) we advocate caution against routine use of EST while placing NBD catheter. Finally, recently published meta-analyses strongly support the use of rectal NSAIDs high risk patients to prevent PEP (Akshintala, Hutfless et al., 2013, Elmunzer, Scheiman et al., 2012, Yaghoobi, Rolland et al., 2013). But majority of NBD procedures in our study were performed prior to these publications and only a small number of patients received prophylactic rectal NSAIDs. This may also have contributed to the high rate of AEs. Overall, the results of our study show that ENBD is an effective salvage therapy but it carries high risk of AEs. Therefore based on current evidence in high risk ERCP (Akshintala, Hutfless et al., 2013, Elmunzer, Scheiman et al., 2012, Yaghoobi, Rolland et al., 2013) we recommend routine use of prophylactic rectal NSAIDs (indomethacin or diclofenac) in all patients undergoing ENBD.

The main strength of the current study is the real life data and large sample size of different aetiologies of cholestasis collected from multiple centres. But the observed results may be limited due the retrospective analysis of the data. Lack of a placebo (or sham) control is

another weakness of this study. Nevertheless, our study demonstrates that ENBD is an important and an effective rescue treatment of refractory cholestatic pruritus.

There are no guidelines on how to use ENBD in cholestatic pruritus and little information is available for clinicians to deliver this treatment effectively in routine clinical practise. Therefore, based on the study results and the cumulative experience of using ENBD, we propose few recommendations (**Table 3-3**) to optimise its safety and effectiveness in treating cholestatic pruritus.

- NBD should be offered to patients with severe pruritus who have failed to respond to conventional drug therapy recommended by current guidelines.
- NBD is a high risk procedure, therefore should be used selectively and cautiously and ideally performed by experienced endoscopists in high volume centres with specialist input from hepatologists.
- When consenting patients for NBD:
  i. Give a realistic estimation of expected benefit;
  ii. Reassure that majority of patients stop itching within few days of drainage;
  iii. Warn that benefit is temporary and itch might recur after removal of the NBD catheter;
  iv. Inform PBC patients to expect shorter duration (only couple of weeks) of remission;
  v. Explain the potential risks of complications including post-ERCP pancreatitis (PEP).
- For BRIC patients limit the duration of NBD to 7-10 days.
- For PBC patients, the duration of NBD should be guided by the patient tolerance of the catheter and benefit of drainage on their pruritus.

Table 3-3 A suggested approach to the use of endoscopic nasobiliary drainage in treating patients with cholestatic pruritus

3.5 Conclusion

In conclusion, in this relatively large retrospective study of ENBD in treating cholestatic patients with refractory pruritus we provide further evidence that ENBD is effective in inducing remission of pruritus of different aetiologies. In addition, ENBD has favourable effect on serum alkaline phosphatase (especially in PBC patients) and bilirubin levels. The duration of response to NBD is independent of the duration of drainage and the daily bile output.

Unfortunately, the effect of ENBD is usually temporary and the procedure is invasive and frequently associated with complications. All patients undergoing ENBD should receive prophylaxis for post-ERCP pancreatitis. We outline our proposals to optimise the use of ENBD in clinical practise. We urge the need for prospective studies to confirm our findings, assess the effect of ENBD on levels of bile salts in the serum and bile and evaluate the role of long term ENBD.

This study confirmed the beneficial role of physical interruption of enterohepatic circulation in improving cholestatic pruritus. However, the invasive nature of the intervention is less appealing for routine use in clinical practice. Therefore, the role of achieving interruption of enterohepatic circulation using pharmacological approaches will be explored in the next chapter.

3.6 Acknowledgements

This study was planned by me and my supervisor (DEJ) in March 2014. We contacted all the major liver centres in the UK to find out if ENBD service was provided in their units. It became clear that Newcastle and Leeds were the only two major units where ENBD was used as a treatment for cholestatic pruritus patients. The clinicians in Leeds had already published their early results and did not have all the data needed for this study. Therefore, I approached centres in other European countries. Favourable replies were received from four large academic centres who agreed to collaborate and share their unpublished data.

The work presented in this chapter would not have been possible without the successful collaboration with other European centres who regularly perform nasobiliary drainage for treating patients with refractory cholestatic pruritus. I am grateful to following people for sharing their patient data: M. Krawczyk and F. Lammert (Saarland University Medical Center, Homburg, Germany); A. Kramer and J. Kuczka (Friedrich-Alexander-University of Erlangen, Erlangen, Germany); F. Gaouar and C. Corpechot (Centre de reference des Maladies Inflammatoires des voies biliaires, Hopital Saint-Antoine, Paris, France); E. Kuiper and H. van Buuren (Erasmus MC, Rotterdam, The Netherlands).

After collecting data from the above centres, I did all the data analysis including statistics, interpreted the results, created figures and tables, and wrote the first manuscript. Other co-authors provided intellectual input in improving the manuscript which was subsequently published as an original article in peer-reviewed journal *Alimentary Pharmacology and Therapeutics* in 2016.

4. CHAPTER 4: STUDY DESIGN FOR A RANDOMISED CONTROLLED TRIAL OF GSK2330672 IN THE TREATMENT OF PRURITUS IN PBC

4.1 Introduction

Chapter 2 studied the scale of the pruritus symptom within the United Kingdom (UK)-PBC cohort, a national cohort of ~3000 PBC patients recruited from every hospital in the UK. In this cohort 74% of PBC patients reported experience of pruritus at some point in the course of the disease. Also, 34% reported persistent pruritus and 11% reported severe pruritus since the diagnosis of PBC. A similar scale of symptom burden was also observed in PBC cohorts from USA and Italy (**Chapter 2**).

4.1.1 Need for novel anti-pruritic drugs in PBC

Pruritus has a negative impact on perceived quality of life in PBC patients and has been associated with sleep deprivation, worsened day time fatigue and when severe, may lead to depression and suicidal tendencies (Mells, Pells et al., 2013). Ursodeoxycholic acid (UDCA), the current standard of care for PBC patients and the only licenced therapy for PBC has no role in treating pruritus (Beuers, Boberg et al., 2009). Current treatment of pruritus in PBC involves step-wise use of specific anti-pruritic agents in line with current international guidelines (Beuers, Boberg et al., 2009, Lindor, Gershwin et al., 2009). These drugs include bile acid sequestrants (cholestyramine), enzyme inducers (rifampicin), opioid antagonists (naltrexone) and selective serotonin re-uptake inhibitors (sertraline).

The current drug therapy in cholestatic pruritus is limited by their lack of universal efficacy, poor compliance (especially cholestyramine) and the need for regular monitoring for liver toxicity (rifampicin). Cholestyramine and rifampicin have good reported efficacy but clinical experience of both naltrexone and sertraline has been disappointing for many clinicians (Beuers, Boberg et al., 2009).

A critical review of literature shows that the strength of evidence for current anti-pruritic drug therapy is poor. Cholestyramine, the current first-line therapy was last studied over five decades ago but has never been subjected to randomised placebo-controlled trials and has evidence category II-2 (cohort or case control analytical studies) (Carey and Williams, 1961, Datta and Sherlock, 1963, Datta and Sherlock, 1966, Oster, Rachmilewitz et al., 1965, Van Itallie, Hashim et al., 1961). Only rifampicin and naltrexone have been studied in controlled

trials (Ghent and Carruthers, 1988, Khurana and Singh, 2006, Tandon, Rowe et al., 2007, Terg, Coronel et al., 2002, Wolfhagen, Sternieri et al., 1997) and sertraline (evidence category II-2) is the last agent investigated with a positive outcome on pruritus (Mayo, Handem et al., 2007). A number of other drugs have been investigated but with little success and more recently both gabapentin (2006) and colesevelam (2010) trials failed to show any therapeutic benefit in cholestatic pruritus (Bergasa, McGee et al., 2006, Kuiper, van Erpecum et al., 2010). Therefore, development of better drug therapies with fewer side effects is an unmet clinical need for PBC patients (Dyson, Webb et al., 2015).

In pruritus trials, placebo response may be a confounder, as seen in at least three recent trials which failed to meet their primary end points due to significant placebo response (Bergasa, McGee et al., 2006, Kuiper, van Erpecum et al., 2010, M.J. Mayo, 2016). Therefore, careful consideration should be given regarding the choice of trial design to adequately compare and demonstrate therapeutic advantage over the placebo.

4.2 Ileal bile acid transporter

Primary BAs are synthesized in the liver from an enzymatic catabolism of cholesterol, a process regulated by enzyme cytochrome P450 (CYP) 7A1. Unconjugated BAs are conjugated in hepatocytes with glycine and taurine, secreted into the bile and stored in the gallbladder. Upon ingestion of a meal, conjugated BAs ("bile salts") are released into the intestinal lumen where they facilitate absorption of fat and fat soluble vitamins. After their normal physiological function is completed in the intestine, BAs reach the ileum where they are reabsorbed. The ileal bile acid transporter [(IBAT), also called apical sodium dependent bile acid transporter (ASBT)], is a protein predominantly located in the terminal ileum and serves as the main transporter mediating the ileal uptake of conjugated BAs and their return to the liver via the portal circulation (enterohepatic circulation) (Dawson, Haywood et al., 2003).

Bile salts (and their protonated form, BAs) have been suggested to play role in the pathogenesis of pruritus in cholestatic conditions. In cholestasis, the ileal uptake of BAs has been shown to be upregulated (Lanzini, De Tavonatti et al., 2003). Also, the evidence that pruritus dramatically improves in patients undergoing nasobiliary drainage (**Chapter 3**) (Hegade, Krawczyk et al., 2016) and is effectively cured by LT (Neuberger and Jones, 2001) suggests a direct or indirect role for BAs in mediating cholestatic pruritus. Therefore a

pharmaceutical agent that can interrupt their enterohepatic circulation and reduce their levels in the systemic circulation may be predicted to improve pruritus.

In two animal model cholestatis studies treatment with IBAT inhibitors SC-435 and A4250 produced BA malabsorption and attenuated BA-mediated cholestatic liver injury by reducing biliary BA output (Baghdasaryan, Fuchs et al., 2016, Wong, Oelkers et al., 1995). In humans, use of IBAT inhibitor A4250 has been shown to decrease the serum BAs and increase faecal BAs by highly efficient interruption of their enterohepatic circulation with no serious adverse events (Graffner, Gillberg et al., 2016).

4.2.1 GSK2330672

GSK2330672 is a selective inhibitor of human IBAT and it is designed to be a non-absorbable agent restricted to the gastrointestinal (GI) tract. GSK2330672 is expected to block the uptake of BAs in the terminal ileum, increase their excretion in the faeces and decrease the amount of BAs returning to the liver via enterohepatic circulation (**Figure 4-1**). Therefore, treatment of PBC patients with oral GSK2330672 is postulated to reduce concentrations of BAs in the systemic circulation and in turn improve pruritus.

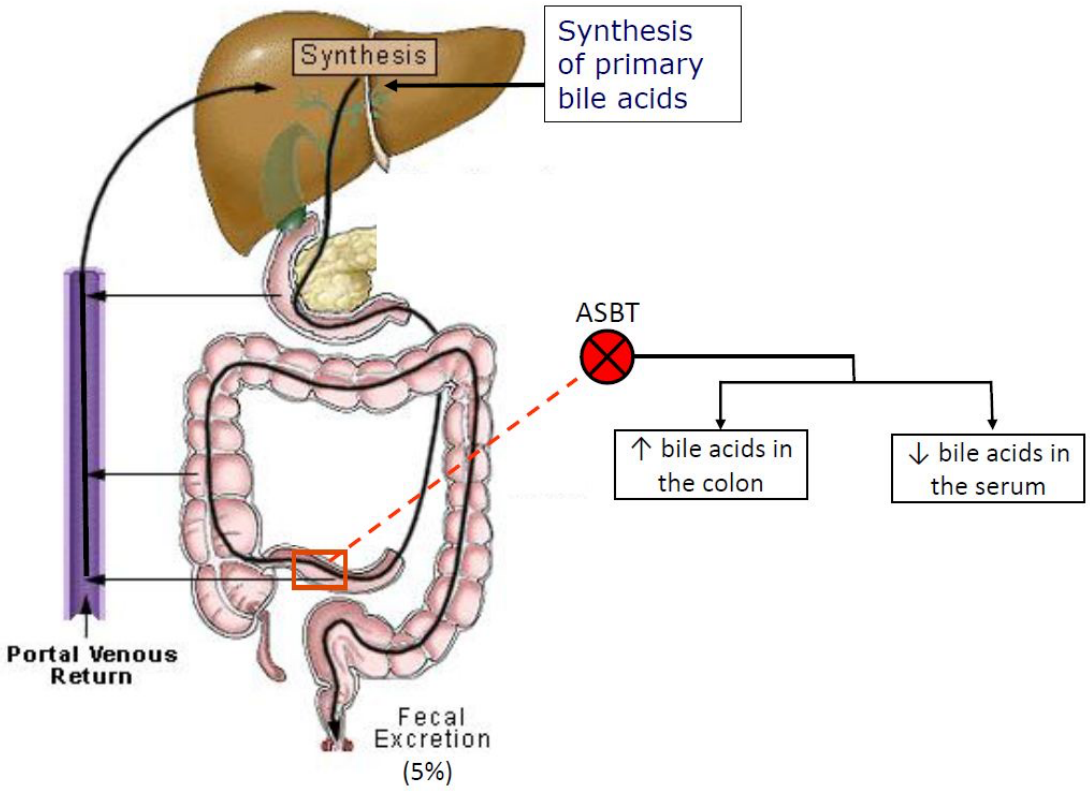


Figure 4-1 Postulated effects of GSK2330672, an IBAT inhibitor drug

In two phase 1 studies (59 healthy volunteers) it was well tolerated with a good safety profile at a dose range of 0.1 to 90 mg (unpublished data from clinical trial NCT01416324 and NCT01607385). GI symptoms were the most common reported drug-related adverse events (AEs). These included diarrhoea, abdominal pain, bowel movement irregularity and positive faecal occult blood tests. All AEs were considered mild or moderate in severity

4.3 Study Design and Patients

The BAT117213 study was a phase 2a multicentre, randomized, double-blind, placebo-controlled, two-period crossover trial, designed to investigate treatment with repeated doses of GSK2330672 in PBC patients with pruritus (ClinicalTrials.gov Identifier: NCT01899703). The crossover design was determined to be the most appropriate and efficient to study pruritus outcome measure in a proof-of-concept study in a rare disease with a rapid efficacy readout. In addition to studying the safety and efficacy of the drug the study was designed to provide an opportunity to conduct explorative studies (including metabonomic and microbiomic studies) to develop novel mechanistic insights into cholestatic pruritus.

The National Research Ethics Service Committee North East and Sunderland (REC reference 13/NE/0290) and the Medicine and Healthcare products Regulatory Agency approved all versions of the study protocol. All recruitment sites obtained approval from their respective hospital Research and Development (R&D) departments before screening patients. All participants provided written informed consent before enrolment. The trial was done in accordance with the International Conference on Harmonisation Good Clinical Practice guidelines and the Declaration of Helsinki.

Following written informed consent, patients with PBC and pruritus were screened to establish study eligibility. Eligible subjects participated in a two-week placebo run-in period followed by randomization in a crossover fashion to receive placebo or GSK2330672 treatment during two consecutive two-week study periods (Sequence 1 / Sequence 2) (**Figure 4-2**). Subjects then participated in a two-week follow up period of placebo dosing. There was no washout period between two treatment periods. Patients then entered 14 days of follow-up during which they received blinded placebo treatment.

Total duration of the study was 56 days from the first day of dosing. When taking GSK2330672 (or matching placebo), participants received 45 mg twice per day on days 1–3, and were then asked to increase the dose to 90 mg twice daily on days 4–14. The dose titration was mainly to reduce development of diarrhoea which was an anticipated adverse event (AE) with this drug.

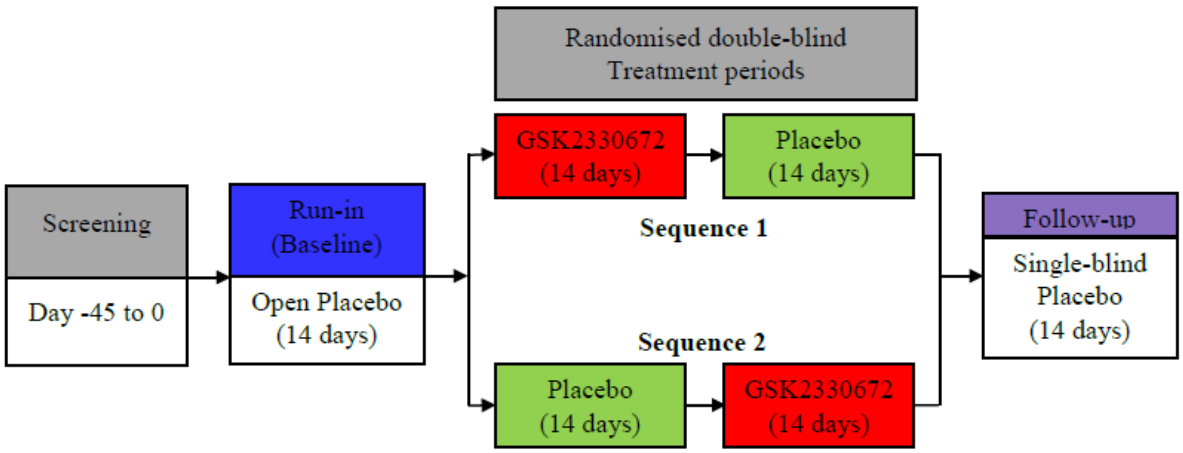


Figure 4-2 Trial design

The study population consisted of PBC patients with ongoing pruritus. Patients were eligible for inclusion in the trial if they were aged 18–75 years, had proven or likely PBC (established according to recognised criteria (Beuers, Boberg et al., 2009, Lindor, Gershwin et al., 2009) with ongoing pruritus, were on stable doses of UDCA for more than 8 weeks at the time of screening, and had serum alkaline phosphatase (ALP) value no more than 10 times the upper limit of normal.

The trial entry criteria for ongoing pruritus was defined as: i) severe pruritus significantly impacting daily life and proven refractory to medical therapy, or ii) pruritus that is newly diagnosed or untreated, or iii) pruritus that is unresolved with the use of a single antipruritic agent. To determine subject eligibility for study enrolment outpatient screening was performed within 45 days before the first dose administration. Subjects meeting all the inclusion criteria and no exclusion criteria were enrolled by a designated investigator from the centre.

Key inclusion and exclusion criteria for study eligibility are detailed in **Table 4-1** and **Table 4-2**

1. Male or female aged between 18 and 75years of age inclusive, at the time of signing the informed consent.
2. Proven or likely PBC, as demonstrated by the patient presenting with at least 2 of the following:
   i) History of sustained increased ALP levels first recognized at least 6 months prior to Day 1,
   ii) Positive AMA titre (>1:40 titre on immunofluorescence or M2 positive by ELISA) or PBC-specific antinuclear antibodies (antinuclear dot and nuclear rim positive);
   iii) Liver biopsy consistent with PBC.
3. Screening ALP value < 10×ULN.
4. Subjects should be on stable doses of UDCA for >8 weeks at time of screening.
5. Symptoms of pruritus as follows (one of the following): i) PBC patients with severe symptoms of pruritus that significantly impact daily life and have proven refractory after at least one previous therapy has been discontinued due to inadequate clinical response, poor tolerability or adverse events. Temporary response to cooling, 1% menthol in aqueous cream, nasobiliary drainage or MARS therapy is still compatible with refractory itch. ii) PBC patients with unresolved symptoms with use of a single antipruritic agent who can tolerate washout of current therapy for the duration of the trial. iii) PBC patients seeking treatment for pruritus that is newly diagnosed or previously untreated.
6. A female subject is eligible to participate if she is not pregnant, as confirmed by a negative serum human chorionic gonadotropin (hCG) test or at least one of the following conditions applies: i) Non-reproductive potential defined as pre-menopausal females with a documented tubal ligation or hysterectomy; or postmenopausal defined as 12 months of spontaneous amenorrhea. ii) Females on hormone replacement therapy (HRT) and whose menopausal status is in doubt will be required to use one of the highly effective contraception methods along with either a second form of highly effective contraception or barrier protection (condoms with spermicide) if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of post-menopausal status prior to study enrolment. iii) Reproductive potential and agrees to follow one of the specified contraception options for the specified duration of time.
7. Capable of giving written informed consent, which includes compliance with the requirements and restrictions listed in the consent form.

Table 4-1 Inclusion criteria

1. Screening total bilirubin >1.5x ULN. Isolated bilirubin >1.5xULN is acceptable if bilirubin is fractionated and direct bilirubin <35%.
2. Screening ALT or AST >4x ULN.
3. Screening serum creatinine >2.5 mg/dL (221 umol/L).
4. History or presence of hepatic decompensation (e.g., variceal bleeds, encephalopathy, or poorly controlled ascites).
5. History or presence of other concomitant liver diseases including hepatitis due to hepatitis B or C virus (HCV, HBV) infection, primary sclerosing cholangitis (PSC), alcoholic liver disease, definite autoimmune hepatitis or biopsy proven non-alcoholic steatohepatitis (NASH).
6. Administration of the following drugs at any time during the 3 months prior to screening for the study: colchicine, methotrexate, azathioprine, or systemic corticosteroids.
7. Current or chronic history of inflammatory bowel disease, chronic diarrhoea, Crohn's disease or diarrhoea related to malabsorption syndromes.
8. Faecal occult blood (FOB) positive test at screening.
9. Based on averaged QTc values of triplicate ECGs obtained at least 5 minutes apart:
    a. QTc ≥ 450 msec; or
    b. QTc ≥ 480 msec in subjects with Bundle Branch Block.
10. History of sensitivity to heparin or heparin-induced thrombocytopenia.
11. History of sensitivity to any of the study medications, or components thereof or a history of drug or other allergy that, in the opinion of the investigator or GSK Medical Monitor, contraindicates their participation.
12. History of regular alcohol consumption within 6 months of the study defined as an average weekly intake of >21 units for males or >14 units for females.
13. A positive pre-study drug/alcohol screen. A minimum list of drugs that will be screened for include amphetamines, barbiturates, cocaine, opiates, cannabinoids and benzodiazepines.
14. Where participation in the study would result in donation of blood or blood products in excess of 500 mL within a 56 day period.
15. The subject has participated in a clinical trial and has received an investigational product within the following time period prior to the first dosing day in the current study: 30 days, 5 half-lives or twice the duration of the biological effect of the investigational product (whichever is longer).
16. Exposure to more than four new chemical entities within 12 months prior to the first dosing day

Table 4-2 Exclusion criteria

4.3.1 Study objectives and outcomes

The primary objective of the study was to investigate the safety and tolerability of oral GSK2330672 when administered for 14 days to patients with primary biliary cholangitis with pruritus. Details of the primary, secondary and exploratory objectives and outcome measures (endpoints) are given in **Table 4-3**.

4.3.2 Recruitment

The study was a UK multicentre study and recruitment was originally planned in three large, tertiary referral National Health Service (NHS) hospitals based in Newcastle, Birmingham and Cambridge. One centre (Cambridge) did not enrol any participants and the study enrolment was done at two centres in the UK: Newcastle Upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, and University Hospitals Birmingham NHS Foundation Trust, Birmingham, in collaboration with the UK-PBC Consortium. Patients were recruited from the out-patient department cohorts of these hospitals and in addition, trial information was published in newsletters and magazines from the UK-PBC research group and patient support groups (LIVErNORTH and PBC Foundation).

Any PBC patient interested in participating in the study could contact the study team at the centre nearest to their location either directly or via referral from local primary or secondary care physicians. The UK-PBC platform was utilised for recruitment using a similar approach to the to the RIT-PBC trial reported recently by our group (Jopson, Newton et al., 2015). The established UK-PBC database was screened for patients with PBC-40 itch domain scores meeting the definitions of persistent and/or severe pruritus. The clinicians looking after these patients were contacted to approach the patients and interested patients were referred to their local recruiting centre. All participants gave their written consent to participation before screening investigations were performed. Participants completed the consent process with study investigators trained in Good Clinical Practice (GCP) and assessment of capacity.

Objectives	Endpoints
Primary	
To investigate the safety and tolerability of oral GSK2330672 compared with placebo when administered for 14 days to patients with primary biliary cholangitis treated with ursodeoxycholic acid (UDCA).	-Safety and tolerability parameters following repeat doses of GSK2330672 administered twice daily (BID), including adverse events, and assessments of clinical laboratory, ECGs and vital signs. -Tolerability as rated by the Gastrointestinal Symptom Rating Scale (GSRS). -Faecal occult blood (FOB) testing.
Secondary	
To demonstrate the lack of effect of oral GSK2330672 on steady-state pharmacokinetics of UDCA when UDCA is administered alone or in combination with GSK2330672.	Steady-state pharmacokinetic parameters of UDCA and its taurine and glycine conjugates tauroursodeoxycholic acid (TUDCA) and glycoursodeoxcholic acid (GUDCA) will be calculated: Cmax, tmax , AUC (0-24hours), and elimination $t_{1/2}$.
To investigate the steady state pharmacokinetics of oral GSK2330672 when administered for 14 days to patients with primary biliary cholangitis treated with UDCA.	Plasma samples will be collected for measurement of GSK2330672 and pharmacokinetic parameters will be reported.
To evaluate the effects of oral GSK2330672 administered for 14 days to patients with primary biliary cholangitis treated with UDCA, on total serum bile acid concentrations and serum markers of bile acid synthesis (C4).	Measurement of serum profiles of total bile acid concentrations and 7-alpha hydroxy-4-cholesten- 3-one (C4), the first committed step of bile acid synthesis from cholesterol.
To evaluate the effects of oral GSK2330672 administered for 14 days to patients with PBC treated with UDCA on subjects' experience of pruritus and its impact.	Patient reported outcomes – daily pruritus 0 to 10 point scale, 5D-itch scale, PBC-40.
Exploratory	
-Markers of disease progression -Experience of pruritus and its impact on the patient and subject's experience of benefits and disadvantages with GSK2330672 -Metabonomics & microbiomics and Pharmacogenomics	-ALT/AST, ALP, GGT, bilirubin, albumin, PT/INR -Responses to exit interview conducted at end of follow-up phase. -Metabonomics – bile acid species, autotaxin, FGF-19. Stool bacterial species; Pharmacogenomics for genes related to itching and IBAT response

Table 4-3 Study objectives and endpoints

4.3.3 Randomisation

A single randomisation schedule for all sites was generated using a dedicated randomization creation and publishing tool for GSK studies (Randall) by the GSK statistician. Randomisation numbers were allocated in a 1:1 ratio to sequence 1 (GSK2330672 followed by placebo) or Sequence 2 (placebo followed by GSK2330672) with a block size of 4. Until the study was unblinded Randall limits access only to pharmacy personal involved in drug preparation (i.e. the statistician and site staff were fully blinded).

Randomisation numbers were allocated to participants by site staff. At the time of randomisation sites obtained the next available randomisation number via a dedicated electronic system (RAMOS: Randomisation and Medication Ordering System).

4.3.4 Sample size

The initial sample size of approximately 40 subjects was decided based on feasibility and consideration of desired precision for estimating treatment effects for both efficacy and pharmacokinetic (PK) endpoints. Further details are given in **Appendix 1**.

4.4 Study treatment

The investigational medicinal product used in this study was GSK2330672. The control intervention was placebo. Both GSK233072 and placebo were manufactured at a dedicated manufacturing unit in London (UK) and dispensed as 30g aliquots of oral solution into amber glass bottles for distribution to participating study centres. The study centres supplied solutions to subjects in accordance with the randomization schedule. Subjects consumed the entire quantity of one or two bottles of study drug twice daily followed by two 50mL rinses of water. All patients started the study with 14-days placebo run in period followed by 14-days treatment with GSK2330672 or placebo in a cross over fashion. The initial dose of GSK2330672 was 45mg BD and all patients were asked to increase the dose to 90mg BD on day 4. If this was not tolerated, they were asked to continue at 45mg BD and attempt a dose increase again two days later. If 90mg BD could not be tolerated by the end of day 7, subjects were asked to continue only 45mg BD.

4.4.1 Concomitant medications

Before starting the study, all patients were advised to stop using their usual anti-pruritic agents including cholestyramine, colesevelam, rifampicin, naltrexone, sertraline, gabapentin and anti-histamines. The use of these medications was prohibited during the study period until

the final follow-up period when rescue medications were permitted. Application of topical agents used to relieve pruritus was permitted during the study only if agents did not contain active ingredients in the list of prohibited agents and with prior agreement of the clinical investigator. Subjects were asked to abstain from taking new prescription or new non-prescription drugs (including vitamins and dietary or herbal supplements), from the start of the placebo run-in period until completion of the follow-up visit. The use of UDCA was permitted and patients who were on UDCA were standardised to receive Ursofalk®(Dr. Falk Pharma UK Ltd) once daily preparation at dose 13-15mg/kg/day and instructed to take it at bed time.

4.5 Study conduct

The conduct of the trial followed the principles outlined in the NHS research governance framework for health and social care, GCP and the guiding principles of the 2008 Declaration of Helsinki. The trial involved the participant visiting the study centre a total of six times including screening visit, day 1 visit, three consecutive fortnightly in-patient stays (each up to 36 hours) and a follow up visit. The schedule of study procedures during these visits and data collection is summarised in **Table 4-4**

Protocol deviation or exemptions were not allowed with the exception of immediate safety concerns. All Investigators at recruiting sites followed standard operative procedures for collection, handling, processing and storage of samples (blood, urine and stool) collected at study visits. All clinical and non-clinical subject data including medical history (to capture co-morbidities and concomitant medications) and physical examinations were entered into electronic case report forms (eCRFs). No patient identifiable information was entered in the eCRFs. All participants were allocated a unique study identifier which was used on eCRFs transmitted electronically to the sponsor and combined with data provided from other sources in a validated data system.

AEs and serious adverse events (SAEs) were collected from the start of the placebo run-in period (day 1) until the follow-up contact (day 56). The investigator and site staff were responsible for detecting, documenting and reporting events that meet the definition of an AE or SAE. All SAEs were recorded and reported to the study sponsor within 24 hours. Periodic reviews of the safety data were performed and presented during interim analysis to both the sponsor and the study investigators.

Period description	**Screening**	**Placebo Run-in**				**Treatment Period 1**				**Treatment Period 2**				**Follow-up‡‡**			
Day (relative to Day 1)	**-45 to -1 days**	**1**	**2-12**	**13**	**14**	**15**	**16-26**	**27**	**28**	**29**	**30-40**	**41**	**42**	**43**	**44**	**45-55**	**56**
Admission to Unit				X				X				X					
Discharge						X				X				X			
Outpatient visit	X	X															X
Screening assessments†	X																
Brief Physical		X															X
12-lead ECG‡	X	X			X				X				X				X
Vital signs	X	X			X				X				X				X
Urine drug/alcohol screen	X	X		X				X				X					X
β-hcg (women)	X	X		X				X				X					X
standard blood tests and urinalysis	X				X				X				X				X
Randomisation	X																
Study treatment dosing		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Concomitant medication review		X		X				X				X					X
Meal served				X	X	X		X	X	X		X	X	X			
Blood samples*					X				X				X				
Metabonomics (Stool and urine)				<---------->				<---------->				<---------->					
Microbiomics (Stool)				<---------->				<---------->				<---------->					

PBC-40 questionnaire††		X		X				X				X					X
GSRS††		X		X				X				X					X
5-D Itch scale††		X		X				X				X					X
Faecal occult blood	X				X				X				X				X
Pruritus 0-10 point scale (electronic diary)#		<---------->															
AE assessment		<---------->															
PGx	For subjects who consent only. Collect one PGx sample after the start of dosing, preferably on day 1																
Exit interview																	X

Table 4-4 Study procedures

†Screening assessments: informed consent for the study and PGX; demographics; complete physical; medical/medication/drug/alcohol history; Hepatitis B and Hepatitis C screen

‡ Single ECG to be performed, with the exception of screening and day 14 when this will be in triplicate

*Blood samples for GSK2330672, UDCA, bile acids, bile acid species, C4, Autotaxin, FGF19 and metabonomics

††patient reported outcome (PRO) assessments. On days in which PRO assessments are administered at study visits they should be administered before any other study procedure

#symptoms recorded twice daily (pre-dose of study drug)

‡‡Rescue treatment with antipruritic agents can be instituted in subjects with severe itching during the placebo follow up period

4.6 Sample collection and Assays

Blood count, liver enzymes [serum ALT, ALP and gamma glutamyl transferase (GGT)], bilirubin and albumin were measured on days 1, 14, 28, 42 and 56. Tolerability was assessed using the gastrointestinal symptom rating scale (GSRS) on days 1, 13, 27, 41 and 56. This scale included 15 questions and was used to assess symptoms experienced over the preceding 5 to 7 days (Svedlund, Sjodin et al., 1988).

Blood samples for measuring serum total bile acids (TBA), individual BA species, autotaxin (ATX) activity, fibroblast growth factor 19 (FGF19) and 7 alpha hydroxy-4-cholesten-3-one (C4) were collected in fasting state, before dosing with study drug on the mornings of day 14 (baseline/end of placebo run-in), day 28 and day 42. Stool samples for FOB tests and faecal BA analysis were also collected on the same time points. All samples were stored at -20 to -80°C until analysed. Samples for clinical chemistry and haematology safety endpoints were processed by the local certified laboratory.

Serum BA analysis to identify and quantify individual BA species was conducted by High Performance Liquid Chromatography (HPLC) coupled with Charged Aerosol Detector (CAD) as published before (Bolier, Tolenaars et al., 2016, Kunne, Acco et al., 2013). Serum autotaxin (ATX) assay was analysed as recently described (Kremer, Martens et al., 2010). Serum FGF19 was measured by a quantitative sandwich enzyme immunoassay technique according to the manufacturer's instructions (Human FGF19, Quantikine® ELISA, R&D Systems, Oxford, UK). These assays were conducted in the Academic Medical Centre, Amsterdam. Serum C4 and TBA were measured by Covance BioAnalysis (Madison, WI, USA) with validated HPLC / Mass Spectrometry (MS) methods.

4.7 Patient reported outcomes

Existing patient reported outcome (PRO) measures to assess the impact of PBC symptoms include the PBC-40, a widely acceptable, validated, disease-specific questionnaire and the 5-D Itch scale (Elman, Hynan et al., 2010, Jacoby, Rannard et al., 2005). However, for this study a more specific PRO measure was needed that could detect the severity and variability of pruritus and other PBC symptoms and potential treatment effects on a daily basis with a short recall period. The development of such a measure began with interviews with PBC patients to identify additional characteristics of pruritus and other symptoms and their impact

on sleep and daily activities. With input from PBC patients and PRO experts a new electronic patient reported outcome (ePRO) diary (**Figure 4-3** and **Table 4-5**) was developed to assess the severity of the pruritus and other PBC symptoms. Subjects completed the ePRO diary every morning and evening before dosing the study drug. In the ePRO diary pruritus severity was rated using a 0-10 numerical rating scale (NRS).

Figure 4-3 Electronic diary used by the study participants
This diary contained the morning and evening symptom questionnaires. Pre-set alarm sounds prompted patients to enter in the information. Data was transformed to the sponsor's central database via phone network or Wi-Fi.

Evening (PM) questions:

1. Rate the worst itching that you experienced between waking this morning and now.
2. Rate the overall intensity of your itching between waking this morning and now.
3. How much time did you experience any itching between waking this morning and now?
4. Rate how bothersome your itching was between waking this morning and now.
5. How much did your itching interfere with your daily activities today?
6. Rate your tiredness or weariness at its worst today.
7. How much of the time were you tired or weary today?
8. How much did tiredness or weariness interfere with your daily activities today?
9. Rate your ability to concentrate today.
10. Rate your ability to remember things today.

Morning (AM) questions:

11. Rate the worst itching that you experienced between bedtime last night and now.
12. Rate the overall intensity of your itching between bedtime last night and now.
13. Rate how bothersome your itching was between bedtime last night and now.
14. How much did your itching interfere with your sleep last night?

Questions 2 and 12 were combined to derive an overall daily *itch intensity* score.

Questions 1 and 11 were combined to derive a daily *worst itch* score.

Questions 4 and 13 were combined to derive a daily *bothersome itch* score.

Question 14 was used to assess *sleep interference*.

Table 4-5 Questions included in the electronic diary for patient reported outcome

4.8 Discussion

The apparent lack of novel drug development in cholestatic pruritus can be attributed partly to incomplete understanding of the complex pathophysiology of the disease. More recent advances in molecular research have identified novel targets for drug development in cholestasis. IBAT inhibitors are novel class of drugs with therapeutic potential in cholestasis. They have been shown be beneficial in cholestasis by the experimental studies and their desired effects on serum and faecal bile acid profile has been proven in healthy people (Baghdasaryan, Fuchs et al., 2016, Graffner, Gillberg et al., 2016).

The BAT117213 study was designed to be the first phase 2 multicentre, double-blinded, placebo-controlled crossover trial to investigate the safety and efficacy of IBAT inhibitor in PBC patients with pruritus. Unlike the only other phase 2 trial of an IBAT inhibitor drug (LUM001) in PBC (CLARITY study, NCT01904058), the main strength of the BAT117213 study is its crossover design which allowed estimating the treatment effect in a smaller number of patients and reduced the between-patient variability and yields a more efficient comparison of treatments than a similar sized parallel group trial. In the BAT117213 study every patient received both the study drug and the placebo; therefore each patient served as his/her own matched control.

An additional strength of this trial is the utility of patient reported outcomes to measure the treatment response objectively using existing validated tools including the PBC-40 questionnaire and 5-D itch scale as well as a novel, easy-to-use electronic symptom diary. The latter has been specifically developed for this study and it contains morning and evening diaries with questions on itch, fatigue and concentration to comprehensively capture the severity of the symptoms over the preceding 12 hours. In addition, the exit interviews conducted at the end of the study provide the opportunity for patients to express their experiences in the study in a semi-structured method that may not have been detected with the more structured patient reported outcomes measures.

The BAT117213 study also provided a unique opportunity to conduct novel, explorative, mechanistic research in patients with cholestatic pruritus. Serum and urine samples obtained during the study will be used to study the metabolic phenotype (metabonomics) of pruritus in PBC by using ^{1}H (proton)-nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS). Similarly, using the faecal samples from study patients gut-microbiome

studies will be undertaken to study the association between gut microbiota composition and pruritus in PBC. Results of these metabonomic and microbiomic studies are likely to provide more mechanistic insight into cholestatic pruritus (This is covered in **Chapter 6**).

The main drawback of this trial is the potential carryover effect (i.e. effect of the treatment from the previous time period may "carry over" on the response to subsequent period) and lack of "washout period" between treatment periods. Carryover effect is a common problem inherent to the crossover study design and may potentially confound direct estimates of treatment effect. Therefore the statistical analysis the data will be assessed for any evidence of carry over and appropriate sensitivity analyses will be performed. To mitigate against the lack of "washout period" the outcome measurements will be restricted to the latter part of each treatment period.

In summary, BAT117213 study is a phase 2 study to evaluate the safety and tolerability of a unique class of drug in treating pruritus in PBC patients and provide novel information about bile acids and metabolic changes and gut microbiome profile in cholestatic pruritus. The results from this trial will inform the trial design of future development phase of the IBAT inhibitor drug.

4.9 Acknowledgements

GlaxoSmithKline (GSK) was the sponsor of this drug trial and following people from GSK made significant contributions in writing the study protocol. I am grateful to Robert L. Dobbins, Duncan Richards, James Storey, George Dukes, Sam Miller, Kim Gilchrist, and Susan Vallow. My role in the study protocol included suggesting and implementing major changes to the study inclusion and exclusion criteria (which subsequently helped in increasing recruitment numbers) and changing the exploratory aims and objectives of the study. I also attended the Research Ethics Committee meeting for study approval and completed all regulatory requirements for study initiation.

5. CHAPTER 5: SAFETY AND EFFICACY OF GSK2330672 IN THE TREATMENT OF CHOLESTATIC PRURITUS

5.1 Introduction

As described in previous sections, pharmacotherapy of cholestatic pruritus is limited and challenging. Bile acid (BA) sequestrants cholestyramine and colesevelam are often given to treat pruritus with variable success. Cholestyramine remains the only FDA approved therapy for cholestatic pruritus and despite its poor tolerability profile and the lack of well-conducted, randomized, controlled trials (RCTs) it is recommended by both the American and European practice guidelines as the current first line agent (Beuers, Boberg et al., 2009, Lindor, Gershwin et al., 2009). Colesevelam is a better tolerated BA sequestrant but was found to be ineffective in the only RCT reported so far (Kuiper, van Erpecum et al., 2010). Other drug therapies (rifampicin, naltrexone and sertraline), although recommended by the scientific guidelines, are not licensed for treating cholestatic pruritus (Beuers, Boberg et al., 2009, Lindor, Gershwin et al., 2009). Moreover, they have the disadvantage of needing regular monitoring due to the risk of liver injury and other limiting adverse effects.

In clinical practice, response rates below 50% are common for most of the guideline recommended drugs (Levy, 2011) and despite their step-wise use, many patients report refractory itch; these cases may need referral for invasive (usually temporary) treatments or liver transplantation (the only definitive cure). The current lack of effective anti-pruritic therapies in PBC will likely be compounded by the fact that the key emerging second line disease modifying agent, Obeticholic acid (OCA) which has recently been licensed by the FDA, is associated with an increased frequency and severity of pruritus (Hirschfield, Mason et al., 2015, Nevens, Andreone et al., 2016). Many other BA based therapies in PBC that are currently in development (Hegade, Speight et al., 2016a) may also be associated with pruritus. Therefore, effective pruritus management in PBC is likely to become increasingly important and challenging and new approaches are needed.

Ileal bile acid transporter [IBAT, also called apical sodium-dependent bile acid transporter (ASBT); gene symbol *SLC10A2*], is an integral brush border membrane glycoprotein mainly expressed in the distal ileum (Dawson, Haywood et al., 2003, Dawson, Lan et al., 2009). The main physiological function of IBAT is reabsorption of BAs and maintenance of their enterohepatic circulation. In cholestatic liver disease ileal BA absorption has been shown to be increased (Hofmann, 2003, Lanzini, De Tavonatti et al., 2003) and inhibiting ileal BA

transport was proposed to prevent inappropriate conservation of BAs (Hofmann, 2009). Using an IBAT inhibitor to reduce BA reabsorption and modulate the BA pool in the systemic circulation is an interesting, yet unexplored therapeutic strategy in PBC.

To date, published reports of IBAT inhibitor drugs include a study in healthy people (A4250) (Graffner, Gillberg et al., 2016), two reports in animal models of cholestasis (A4250 and SC435) (Baghdasaryan, Fuchs et al., 2016, Miethke, Zhang et al., 2016) and a more recent abstract report of Lopixibat chloride (formerly LUM001) in patients with PBC and pruritus (M.J. Mayo, 2016). GSK2330672 is a highly potent, soluble, minimally absorbed, selective inhibitor of the human IBAT. It has been successfully evaluated in both animal models of type 2 diabetes mellitus (T2DM) and an early phase trial of T2DM patients (Nunez, Yao et al., 2016, Wu, Aquino et al., 2013). In two phase I studies (59 healthy volunteers) it was found to be well tolerated with a good safety profile at a dose range of 0.1 to 90 mg (unpublished data from clinical trial NCT01416324).

We designed and conducted a phase IIa study of GSK2330672 and here we report the first randomised, placebo-controlled, double blind, cross over trial of an IBAT inhibitor in subjects with PBC and pruritus. We postulated that GSK2330672 would interrupt enterohepatic circulation of BAs and exert therapeutic benefit on pruritus associated with PBC.

5.2 Methods

As described in the study protocol- **Chapter 4**

5.3 Data analysis

The study was designed to estimate the effect of GSK2330672 (when co-administered with UDCA) relative to placebo on pruritus and the pharmacokinetics (PK) of UDCA. Due to the early clinical and exploratory nature of the study no formal hypothesis testing was planned and the sample size was based on feasibility with consideration of efficacy (using pruritus 0-10 NRS) and potential PK interaction between GSK2330672 and UDCA. An initial sample size of 40 subjects was estimated to be sufficient for both efficacy and PK based on the assumption that GSK2330672 was at least as effective as rifampicin and the standard deviation (SD) was similar to the reported SDs in trials of other anti-pruritic drugs (Khurana and Singh, 2006, Tandon, Rowe et al., 2007).

Given the uncertainties associated with sample size assumptions two interim analyses were performed to assess for futility and possible sample size re-estimation. Data from the pruritus 0 to 10 NRS were reviewed by an unblinded Interim Review Committee (composed of personnel not directly involved in study conduct). The first interim analysis was undertaken after 11 subjects and a second interim analysis after 19 patients completed the treatment period at which point the final target sample size was reduced. The study was closed for recruitment after 22 subjects were randomised.

To summarise the daily pruritus 0-10 point NRS during the placebo run-in period and each treatment period for each individual patient, we calculated trimmed means of weekly itch scores. Trimmed means removed the highest and lowest daily score (an average of the morning and evening scores) to provide a more robust summary not influenced by potential data-entry errors. For statistical analysis we used the second week of each period to provide an ‘analytical washout’ (i.e. seven days between the analysed periods to allow treatment effects to stabilise). The efficacy end-point analysis used a mixed effects model with fixed effect terms for treatment period, and sequence with subjects treated as a random effect in the model. Baseline results were included within the model as an additional period. Point estimates and their associated 95% confidence interval (CI) and corresponding p value were constructed for the mean differences of interest in pruritus scores [i.e. changes from baseline on each treatment and between double-blind GSK2330672 and placebo]. Data on PK

parameters, bile acids and biomarkers were log-transformed for analysis, and results are therefore reported as percentage changes or ratios. In tables, summary statistics for continuous variables are shown as mean ± SD and categorical variables are shown in numbers (or percentages, %) unless otherwise stated. Although not formally a hypothesis-testing study, two-sided p values <0·05 were considered statistically significant. All analyses were performed using SAS, version 9·2 or greater (SAS Institute, Cary, NC, USA).

5.3.1 Analysis of the 5-D Itch scale

The scores of each of the five domains were achieved separately and then summed together to obtain a total 5-D itch score. 5-D itch scores could potentially range between 5 (no pruritus) and 25 (most severe pruritus). Single-item domain scores (duration, degree and direction) were equal to the value indicated below the response choice (range 1–5). The disability domain included four items that assessed the impact of itching on daily activities: sleep, leisure/social activities, housework/errands and work/school. The score for the disability domain was achieved by taking the highest score on any of the four items. Taking an average score across all four items might underestimate the impact of itching on daily activities due to the lower impact of itching on other activity items compared with the impact on sleep. For the distribution domain, the number of affected body parts was tallied (potential sum 0– 16) and the sum was sorted into five scoring bins: sum of 0–2 = score of 1, sum of 3–5 = score of 2, sum of 6–10 = score of 3, sum of 11–13 = score of 4, and sum of 14–16 = score of 5. Descriptive analysis of the range of actual domain scores, mean domain scores where applicable, and standard deviations were evaluated between treatment groups with GSK2330672 and without GSK2330672.

5.3.2 Analysis of the PBC-40 domains

The PBC-40 has six domains; Cognitive, Itch, Fatigue, Social, Emotional and (other) Symptoms with individual questions scored in the range 1–5 (with high scores denoting greater symptom impact and worse QOL). Descriptive analysis of the range of actual domain scores, mean domain scores, and standard deviations were evaluated between treatment groups with GSK2330672 and without GSK2330672.

5.4 Results

5.4.1 Patients

Between March 10, 2014, and Oct 7, 2015, we enrolled and randomised 22 patients. 21 patients completed all the planned study procedures as per protocol. One patient was withdrawn from the study due to withdrawal of consent in the placebo run-in period (**Figure 5-1**). The safety population therefore comprised a total of the 22 randomly assigned patients, while the analysis population comprised 21 patients who completed all the planned study procedures as per protocol (although one patient did not attend the full follow-up period). 19 of 21 patients were taking UDCA during the study period at the guideline recommended dose.

The baseline demographic and clinical characteristics of the participants are shown in **Table 5-1**. A summary of the frequency of use of anti-pruritic treatments prior to the start of the study is provided in **Table 5-2**. As per the study protocol, use of these drugs was stopped at the study entry.

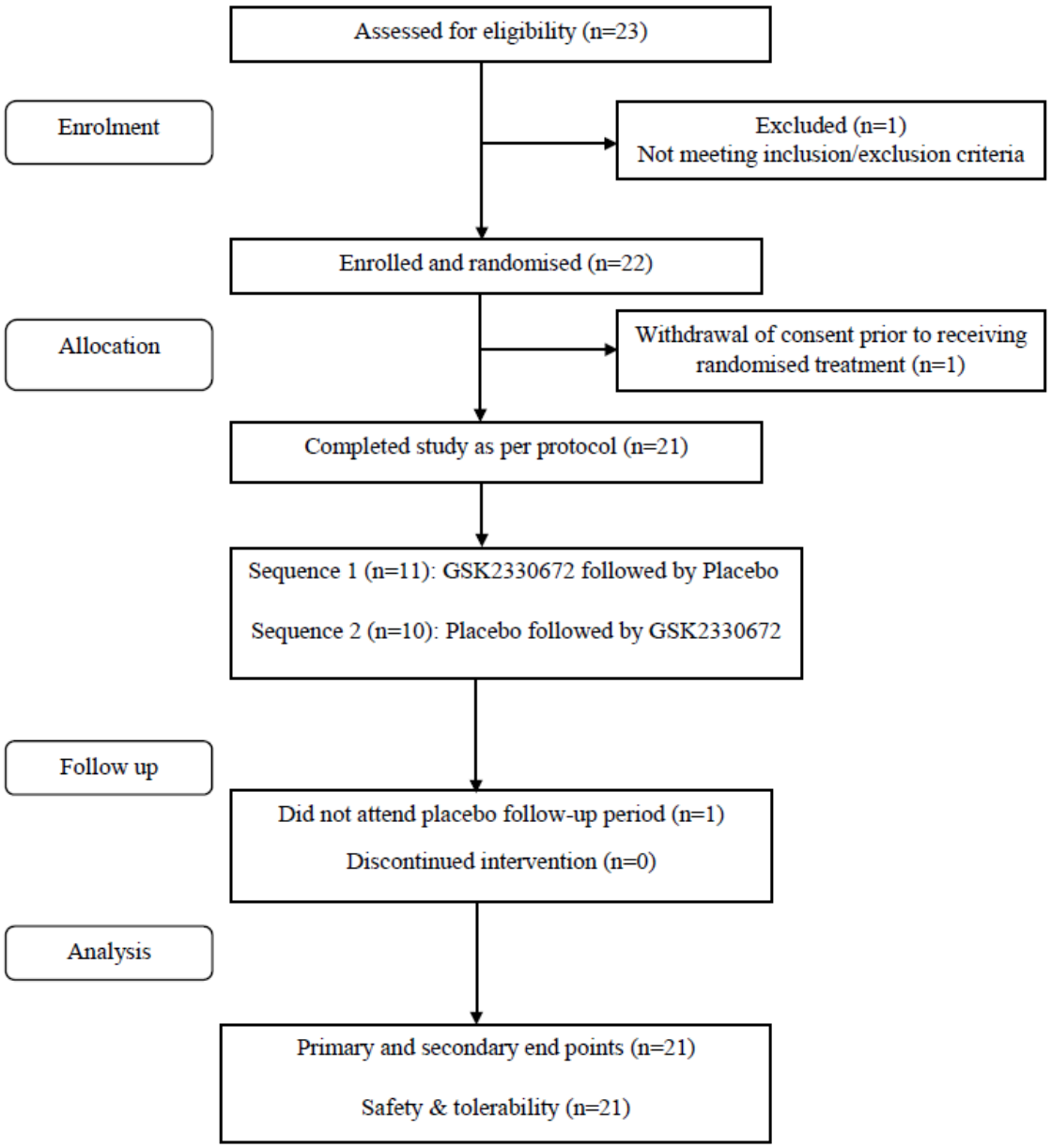


Figure 5-1 Study flow chart

The safety population comprised a total of the 22 randomly assigned patients. Analysis population comprised 21 patients who completed all the planned study procedures as per protocol.

Age (years)	52.9 (10.6)
Female	19 (86)
Body Mass Index (kg/m^2)	27.2 (4.9)
Body Weight (kg)	72.8 (13.5)
Duration of PBC, years	5 (4.8)
Ethnicity, n (%)	
Hispanic/Latino	0
Not Hispanic/Latino	22 (100)
Race, n (%)	
White	21 (95)
Black	0
Asian: Central/South Asian Heritage	1 (5)
UDCA use, n (%)	15 (68)
Total UDCA daily dose at study entry (mg/day)	883 (208.5)
Total UDCA daily dose during study period (mg/day)	967 (185.8)
Pruritus Scores*	
Itch intensity on NRS (min 0, max 10), trimmed mean	5.33 (2.16)
PBC-40 Itch Domain Score (min 3, max 15)	10.5 (3.3)
5-D Itch scale (min 5, max 25)	18.7 (3.6)
Laboratory markers*	
Alkaline phosphatase (IU/L)	264 (174.1)
Gamma glutamyl transferase (IU/L)	211 (172.6)
Alanine amino transaminase (IU/L)	59.3 (44.8)
Aspartate amino transaminase (IU/L)	60.8 (35.8)
Total bilirubin (µMol/L)	12.2 (5.49)
Total protein (g/L)	73.32 (5.9)
Albumin (g/L)	41.9 (4.2)
Creatinine (µMol/L)	65.8 (9.1)
Autotaxin activity (nMol/ml/min)	8.2 (4.1)
FGF19 (pg/mL)	162.9 (107.5)
C4 (ng/ml)	13.1 (10.0)
Serum total bile acids (µM)	48.6 (68.7)

Table 5-1 Baseline characteristics of trial population
Data are shown in mean (SD) unless otherwise stated.*Baseline data at the end of placebo run-in period.

Treatment	N	%
Anti-histamines	8	38.1
Colestyramine (e.g. Questran)	4	19.0
Naltrexone	2	9.5
Sertraline	2	9.5
Rifampicin	1	4.8
Gabapentin	1	4.8
Phototherapy	1	4.8
None	2	9.5
Total	**21**	

Table 5-2 Frequency of use of anti-pruritic treatments prior to the start of the study

5.4.2 Safety and tolerability

All subjects started with GSK2330672 dose of 45mg twice daily for three days and successfully increased to 90mg twice daily on days 4-14. During the study there were no reports of serious adverse events (SAEs). There were no clinically significant changes in vital signs, laboratory values or ECG parameters, and no positive FOB tests were reported. There were no reports of liver toxicity and no significant changes were seen in serum total bilirubin, ALP, GGT, ALT, AST or albumin during the study period (**Table 5-3**).

	Run-in (Baseline)		Sequence 1				Sequence 2			
	Post Placebo		Post GSK2330672		Post Placebo		Post Placebo		Post GSK2330672	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
ALP (IU/L)	264	174.1	229.4	114.5	247.1	126.3	291.8	202.4	318.1	252.3
GGT (IU/L)	211	172.6	197.8	152.8	182.9	129.8	312.3	447.5	349.3	534.5
ALT (IU/L)	59.3	44.8	57.2	30.79	50.4	17.1	62	41.8	72.5	69.5
AST (IU/L)	60.8	35.8	43.4	18.9	45.6	25.2	52.3	37.9	57.3	50
Total bilirubin (µmol/L)	12.2	5.49	10.9	4	12.6	6.1	15.7	8.8	14.4	9.54
Albumin (g/L)	41.9	4.2	40.7	3.9	39.5	3.6	40.9	3.1	41.6	2.01
Creatinine (µmol/L)	65.8	9.18	63.3	9.79	65.5	9.1	67.1	11.8	66.5	10.8
Urea (mmol/L)	4.7	1.17	4.6	0.94	4.5	0.9	4.6	1	4.1	0.8

Table 5-3 Changes in clinical biochemistry during study period
None of the parameters changed significantly ($p>0.05$) after treatment with GSK2330672.

Overall, GSK2330672 was well tolerated. A summary of all adverse events (AEs) reported for more than one subject (>5%) during any treatment period is given in (**Table 5-4**).

The frequency of any AEs was similar (81%) in both treatment periods. The most common AE observed during the study was headache, reported by 14 (64%) subjects.

	Placebo Run-in (N=22) **n (%)**	**GSK2330672 (N=21)** **n (%)**	**Placebo (N=21)** **n (%)**
Subjects with any AE	15 (68)	17 (81)	17 (81)
Gastrointestinal system			
Diarrhoea	1 (5)	7 (33)	1 (5)
Upper abdominal pain	0	3 (14)	1 (5)
Abdominal distension	0	3 (14)	1 (5)
Abdominal pain	0	3 (14)	0
Vomiting	0	1 (5)	2 (10)
Nausea	0	2 (10)	0
Nervous system			
Headache	7 (32)	6 (29)	7 (33)
Dizziness	1 (5)	1 (5)	2 (10)
Paraesthesia	0	0	2 (10)
Infections			
Nasopharyngitis	0	1 (5)	2 (10)
General			
Fatigue	0	0	2 (10)

Table 5-4 Summary of adverse events
Adverse event (AEs) were monitored from day 1 to 56 of the study including follow-up period. The listed AEs (any severity) have an incidence >5% in any treatment group.

A total of 16 (73%) subjects reported AEs related to GI system. The most common GSK2330672 related AE was diarrhoea (i.e. too frequent emptying of the bowels) reported by 7 (33%) subjects with majority (n=5, 71%) reporting it with mild severity (lasting up to 4 days and no or minimal impact on daily life). The frequency of diarrhoea reported during GSK2330672 treatment was significantly higher compared to placebo treatment (33% vs. 5%, p=0.0406, Chi-square test with Yates' correction). No subject discontinued the drug or had their dose decreased secondary to diarrhoea. Two AEs (diarrhoea, abdominal distension)

reported in the GSK2330672 arm and one AE (upper abdominal pain) reported in placebo arm were considered to be of severe intensity.

5.4.3 Effect on pruritus

After GSK2330672 treatment, percentage (%) changes from baseline itch scores were **(Figure 5-2**):

- NRS -57% (95% CI -73 to -42), p<0.0001
- PBC-40 itch domain -30% (-42 to -20), p<0.0001
- 5-D itch scale -35% (-45 to -25), p<0.0001

GSK2330672 reduced itch intensity significantly more than the double-blind placebo in all three scales:

- NRS -23% (95% CI -1 to -45), p=0.037
- PBC-40 itch domain -14% (95% CI -1 to -26), p=0.034
- 5-D itch -20% (95% CI -7 to -34); p=0.0045

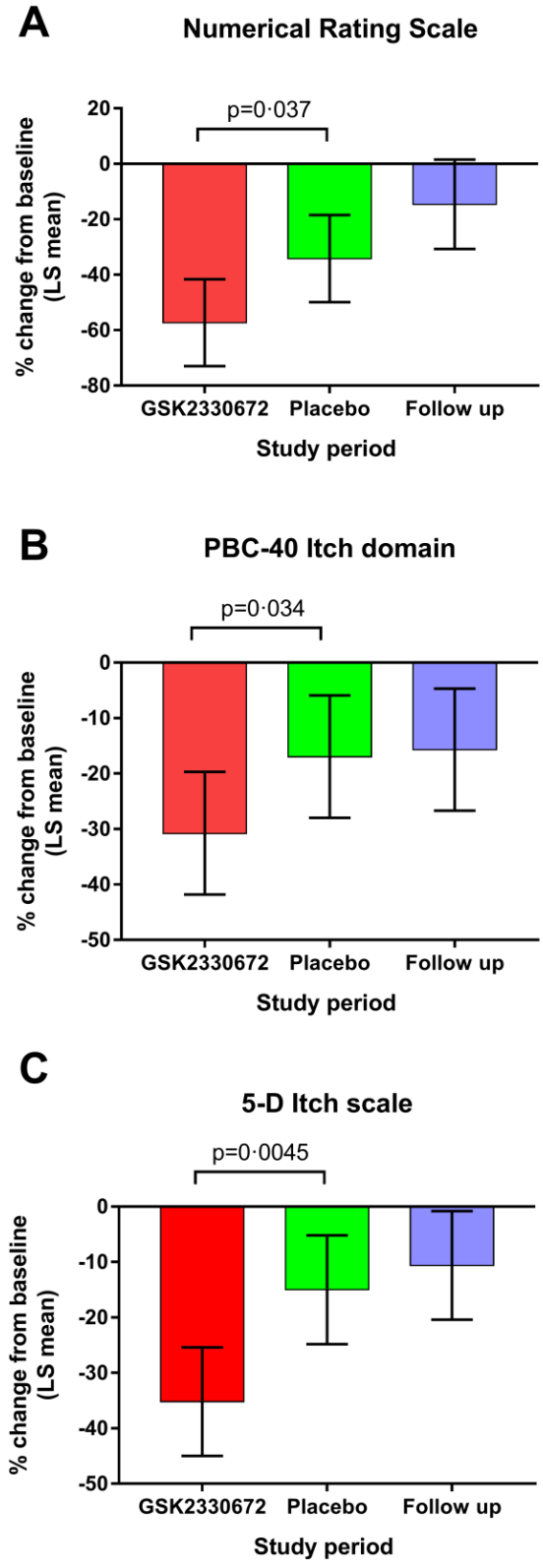


Figure 5-2 Changes from baseline in itch intensity scores according to treatment period
A) 0- 10 numerical rating scale (NRS), **B**) PBC-40 itch domain score, and **C**) 5-D itch scale. Data are shown as least squares mean percentage (%) changes. Error bars show 95% CI.

For individual patients, changes in the weekly trimmed mean of their itch intensity score are shown in the "spaghetti plot" (**Figure 5-3**).

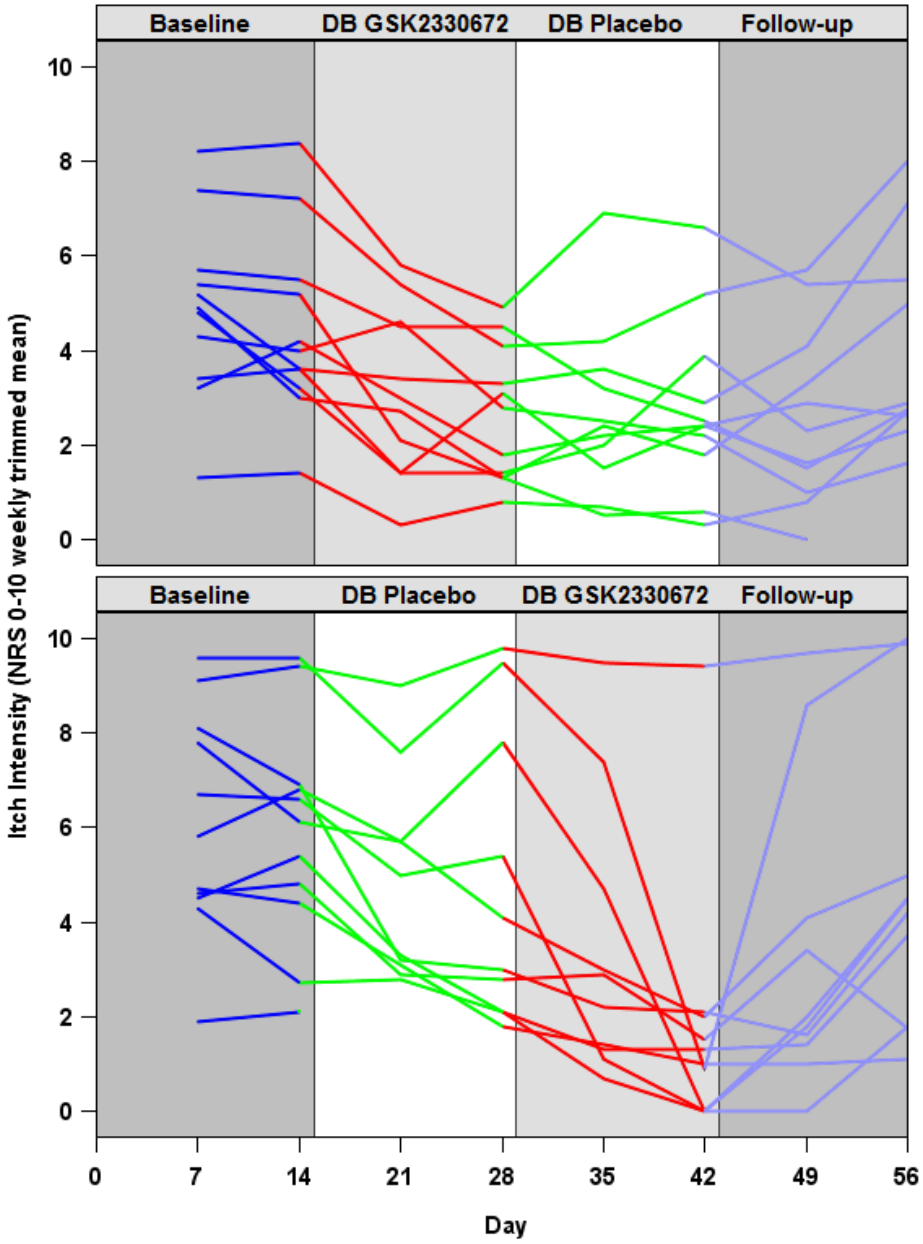


Figure 5-3 Changes in individual itch intensity scores measured by 0-10 numerical rating scale during the entire study period

Sequence 1 (top panel, n=11) and Sequence 2 (bottom panel, n=10). Data shown are weekly trimmed mean of NRS. Baseline=Run-in/open placebo, DB=double blind, Follow up=single blind placebo.

Overall, the mean NRS itch intensity score significantly reduced from baseline after GSK2330672 treatment period (**Figure 5-4 A**) and the reduction was significant in both the sequences of treatment (**Figure 5-4 B&C**).

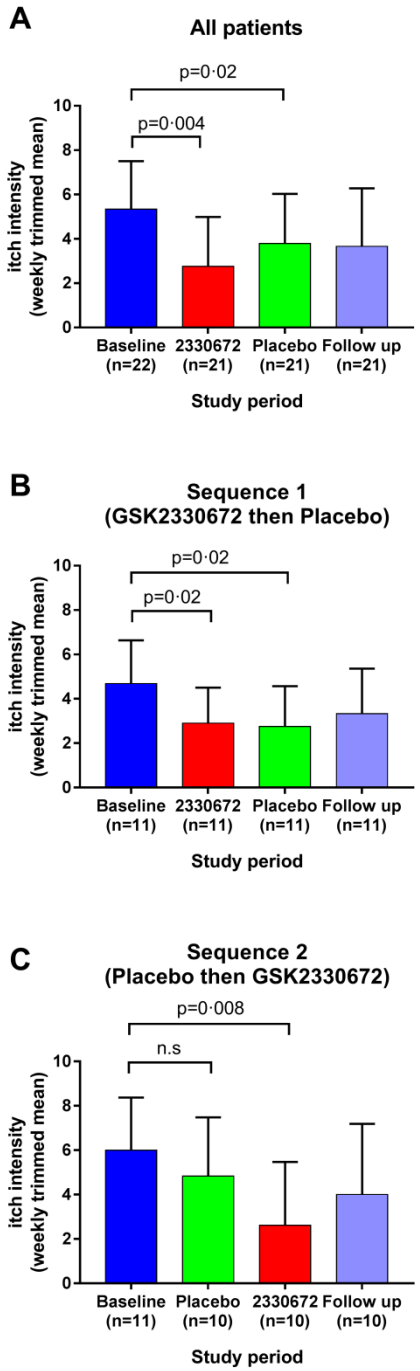


Figure 5-4 Changes in itch intensity scores measured by 0-10 numerical rating scale
Results according to the treatment period (**A**) and according to sequence of treatment (**B &C**). Data shown are group means of individual subject trimmed mean for the second week of each period. Error bars show SD.

In the NRS, itch was also evaluated for worst itch, bothersome itch and sleep interference. Significant reductions were seen in these scores following GSK2330672 treatment (**Table 5-5**).

Itch type	Treatment sequence / Comparison	Visit	n	Absolute score (0-10 NRS)		Change from Baseline (%)	
				LS Mean (SE)	**95% CI**	**LS Mean (SE)**	**95% CI**
Worst itch	Sequence 1	Baseline	11	4.78 (0.733)	3.29, 6.27		
		GSK2330672	11	2.91 (0.733)	1.42, 4.40	-36 (11)	-58, -14
		Placebo	11	3.00 (0.733)	1.51, 4.49	-42 (11)	-63, -20
		Follow-up	10	4.07 (0.752)	2.55, 5.59	-2 (11)	-25, 21
	Sequence 2	Baseline	11	6.13 (0.733)	4.64, 7.62		
		Placebo	10	4.86 (0.757)	3.33, 6.39	-25 (11)	-48, -3
		GSK2330672	10	1.52 (0.757)	-0.01, 3.05	-76 (11)	-98, -53
		Follow-up	10	4.54 (0.757)	3.01, 6.07	-28 (11)	-51, -6
	GSK2330672 v Placebo		**21**	**-1.72 (0.473)**	**-2.66, -0.77**	**-22 (11)**	**-44, -1**
	GSK2330672 (sequences combined)		21			-56 (8)	-72, -40
	Placebo (sequences combined)		21			-34 (8)	-49, -18
Bothersome itch	Sequence 1	Baseline	11	4.28 (0.804)	2.65, 5.92		
		GSK2330672	11	2.45 (0.804)	0.82, 4.09	-43 (13)	-70, -16
		Placebo	11	2.63 (0.804)	0.99, 4.26	-44 (13)	-71, -17
		Follow-up	10	3.44 (0.822)	1.77, 5.11	-3 (14)	-32, 25
	Sequence 2	Baseline	11	5.54 (0.804)	3.90, 7.17		
		Placebo	10	4.42 (0.828)	2.74, 6.10	-28 (14)	-56, 1
		GSK2330672	10	1.27 (0.828)	-0.41, 2.95	-79 (14)	-107, -51
		Follow-up	10	4.07 (0.828)	2.39, 5.75	-27 (14)	-55, 2
	GSK2330672 v Placebo		**21**	**-1.66 (0.484)**	**-2.63, -0.69**	**-25 (14)**	**-53, 3**
	GSK2330672 (sequences combined)		21			-61 (10)	-81, -42
	Placebo (sequences combined)		21			-36 (10)	-55, -16
Sleep interference*	Sequence 1	Baseline	11	4.10 (0.870)	2.34, 5.87		
		GSK2330672	11	2.52 (0.870)	0.75, 4.29		
		Placebo	11	2.53 (0.870)	0.76, 4.29		
		Follow-up	10	3.09 (0.890)	1.28, 4.89		
	Sequence 2	Baseline	11	4.94 (0.870)	3.17, 6.71		
		Placebo	10	3.53 (0.897)	1.72, 5.35		
		GSK2330672	10	0.52 (0.897)	-1.29, 2.34		
		Follow-up	10	2.86 (0.897)	1.04, 4.67		
	GSK2330672 v Randomised Placebo		**21**	**-1.51 (0.541)**	**-2.59, -0.43**		

Table 5-5 Changes in Numerical Rating Scales scores for worst itch, bothersome itch and sleep interference during the study

*Baseline sleep interference scores included values at/close to zero, and therefore percent change from baseline analyses were not robust and are not reported.

GSK2330672 treatment was associated with significant reductions in the mean PBC-40 itch domain and 5-D itch score (**Figure 5-5 A&D**). The mean decrease from baseline in 5-D itch score after GSK2330672 treatment was significant in both sequences of treatment (**Figure 5-5 E&F**), whereas the decrease in PBC-40 itch domain score was only significant in sequence 2 (**Figure 5-5 B&C**).

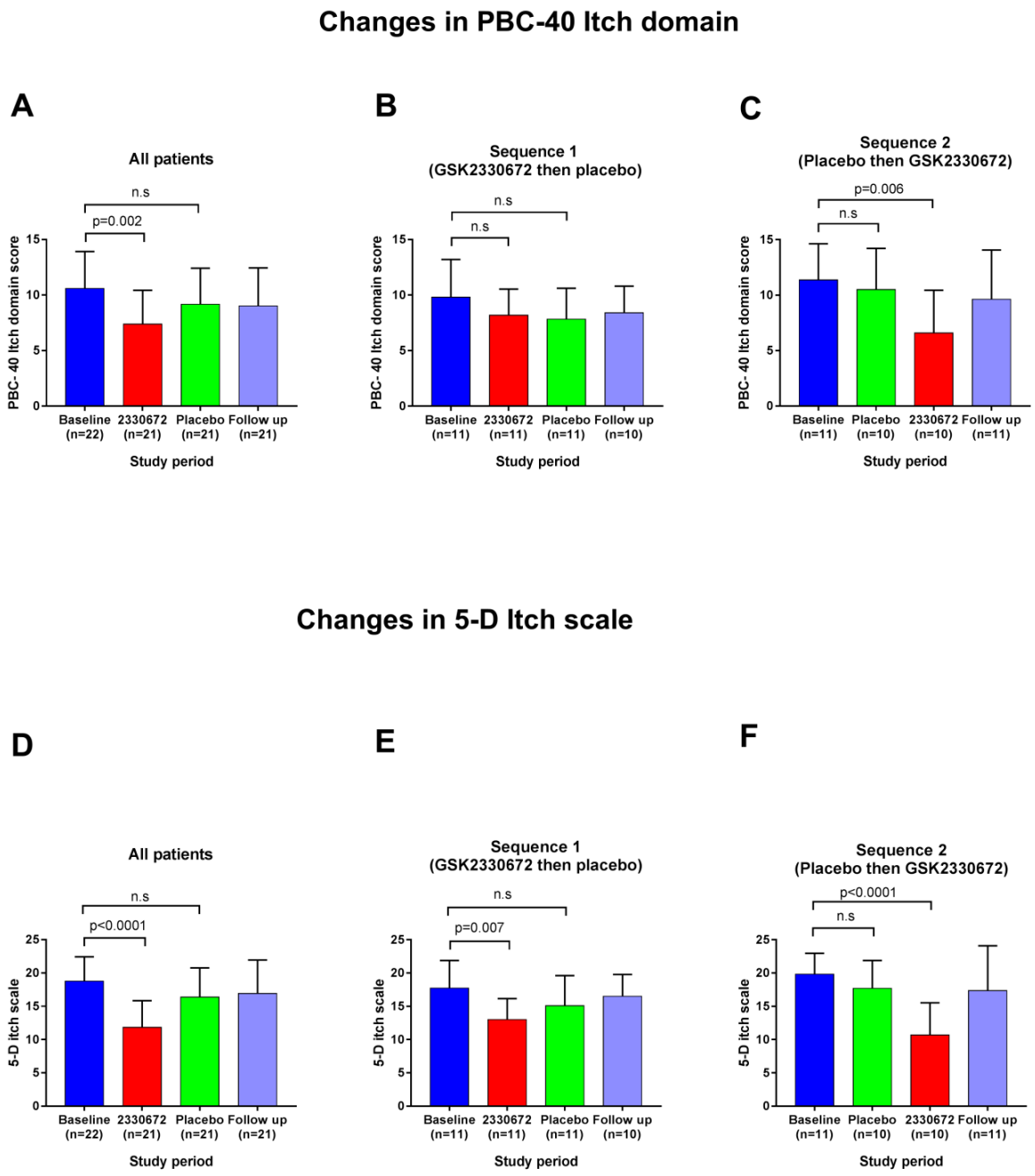


Figure 5-5 Changes in PBC-40 itch domain and 5-D itch scale
Data shown are for all patients according to the treatment period (**A &D**) and according to sequence of treatment (**B-C** and **E-F**). Data shown are mean scores and error bars show SD.

Analysis of other domains of PBC-40 showed significantly greater reduction in the fatigue domain score after GSK2330672 treatment compared to the placebo [-9% (95% CI -3 to -16); p=0·0033]. No significant changes were apparent for other domains of PBC-40 (**APPENDIX 1**).

5.4.4 Changes in serum bile acids

Serum total bile acid (TBA) levels changed from baseline with a 50% decrease (95% CI -37 to -61; p<0.0001) after GSK2330672 treatment compared to a 12% increase (95% CI -12 to +42; p=0.35) after placebo.

The changes in serum TBA levels following GSK2330672 were significant when compared to baseline and placebo (**Figure 5-6**) and the changes were reversed within two weeks of stopping GSK2330672.

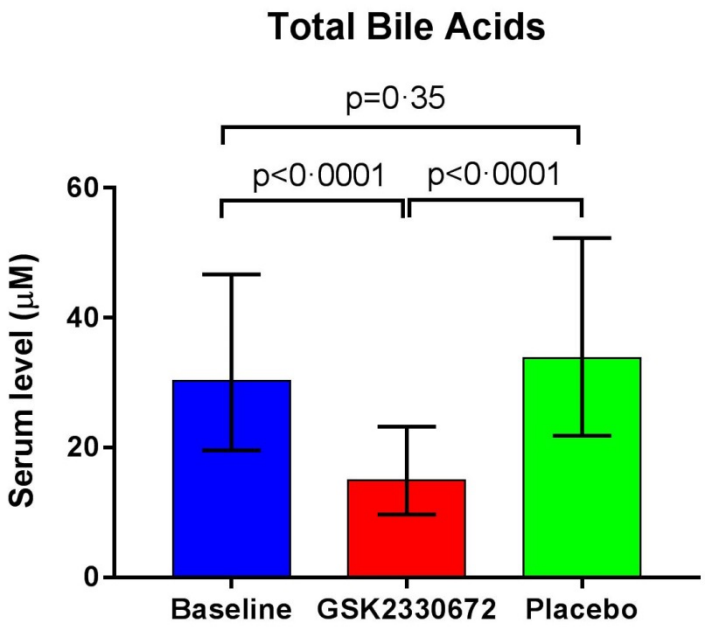


Figure 5-6 Changes in serum total bile acids according to treatment period
Data shown are geometric mean values and error bars show 95% CI.

Serum levels of conjugated BAs significantly decreased after GSK2330672 compared to baseline (**Figure 5-7**), with the largest percentage reductions observed in taurocholate [(TCA) -74% (95% CI -53 to -86); p<0·0001], glycocholate [(GCA) -64% (95% CI -23 to -83); p=0·0099] and taurochenodeoxycholate [(TCDCA) -58% (95% CI -32 to -74); p=0·0007].

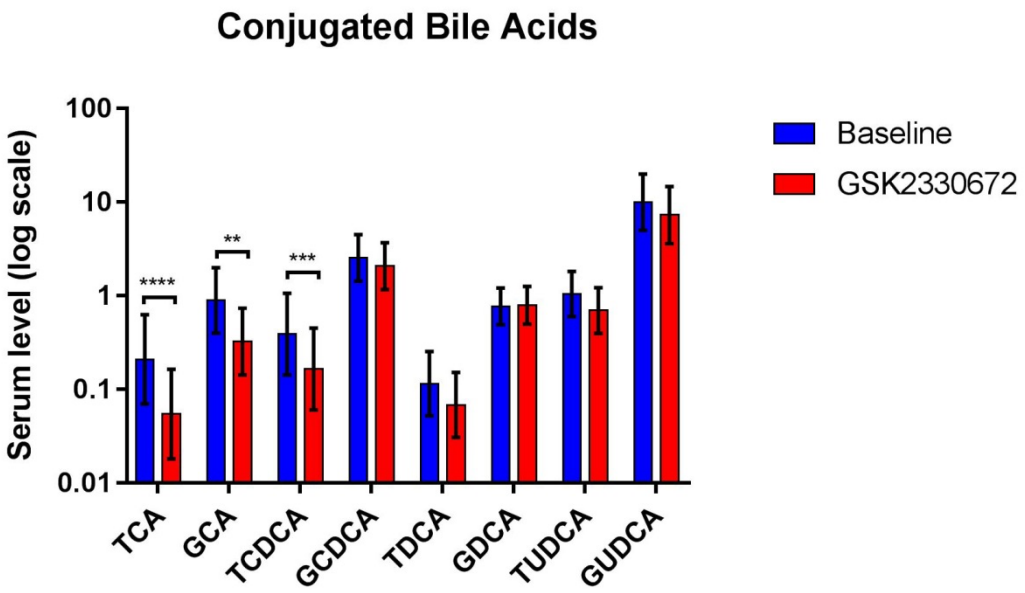


Figure 5-7 Changes in serum levels of conjugated bile acids
Data shown are log scaled mean levels with error bars showing upper and lower limits. **p<0.01, ***p<0.001 and ****p<0.0001

In contrast, unconjugated primary BAs cholate [(CA) -13% (95% CI -60 to +86); p=0·70] and chenodeoxycholate [(CDCA) -4% (95% CI -34 to +38); p=0·80] did not change significantly after GSK2330672 (**Figure 5-8**).

A significant increase in serum ursodeoxycholic acid [(UDCA) +57% (95% CI +15 to +116); p=0·0062] was observed after GSK2330672 (**Figure 5-8**). No significant changes from baseline were seen in any BA species after placebo treatment.

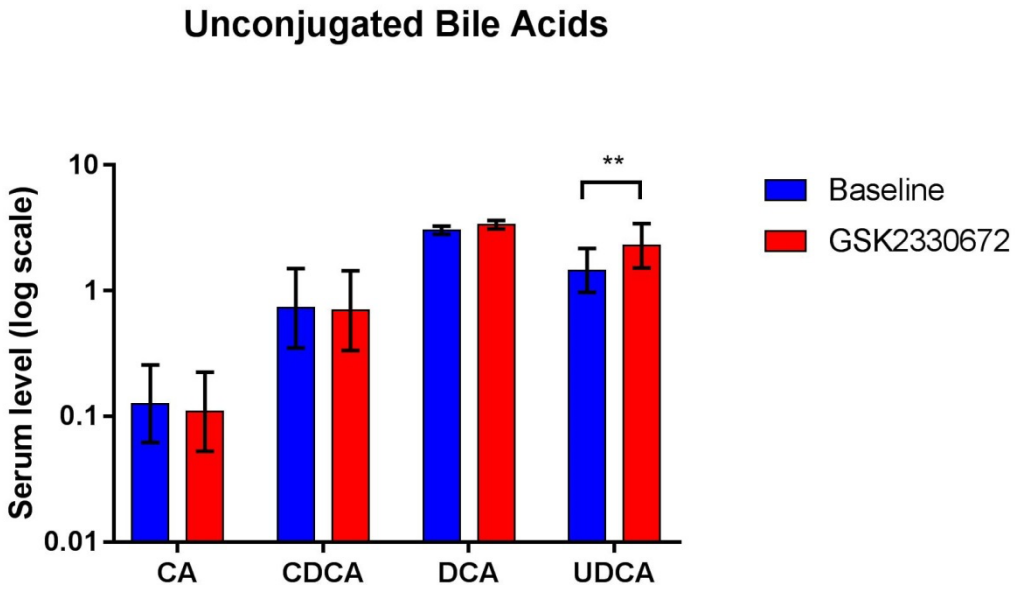


Figure 5-8 Changes in serum levels of unconjugated bile acids
Data shown are log scaled mean levels with error bars showing upper and lower limits. **p<0.01

5.4.5 Changes in faecal bile acids

Faecal TBA showed a mean 36% increase after GSK2330672 (95% CI -1 to +85) compared to 16% decrease after placebo (95% CI -40 to +15).

5.4.6 Changes in serum C4, autotaxin activity and FGF-19

There was a significant 3·1-fold (95% CI 2·4 to 4·0, p<0.0001) increase in serum C4 levels from baseline after GSK2330672 treatment (**Figure 5-9**) and the increase was seen in both sequences of treatment. No significant changes in C4 were seen after placebo treatment.

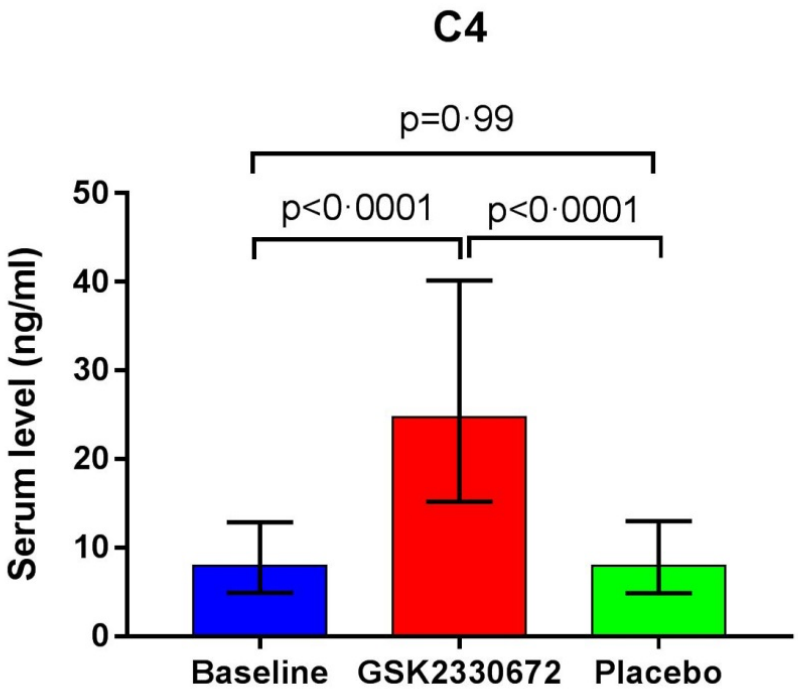


Figure 5-9 Changes in serum C4 levels according to treatment period
Data shown are geometric mean values and error bars show 95% CI.

Serum ATX activity [-11% (95% CI -3 to -19); p=0.0070] and FGF19 levels [-78% (95% CI -60 to -88); p<0.0001] decreased significantly compared to the baseline following GSK2330672 treatment (**Figure 5-10**) but not after placebo.

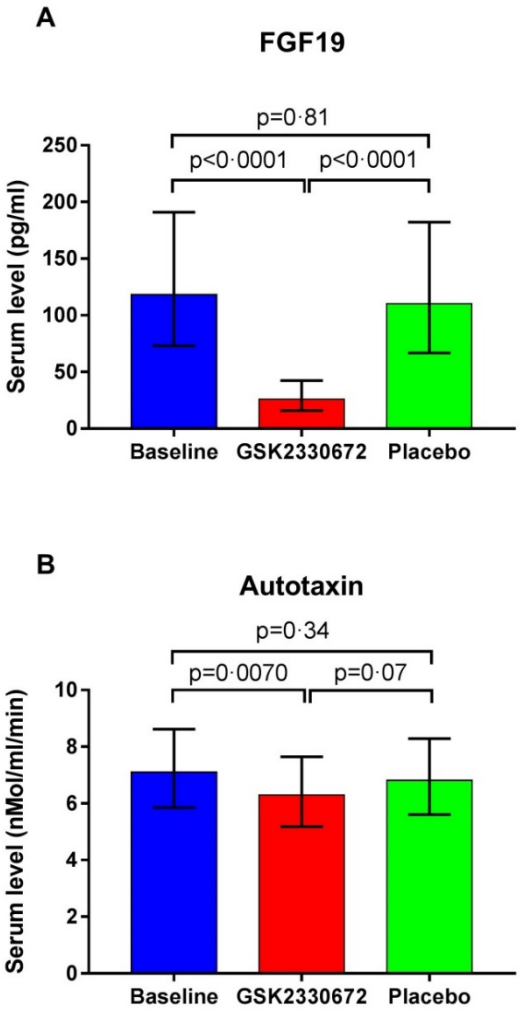


Figure 5-10 Changes in serum fibroblast growth factor-19 and serum autotaxin activity levels
Data shown are geometric mean and error bars show 95% CI.

5.4.7 Pharmacokinetics

GSK2330672 is designed as a non-absorbable agent restricted to the gastrointestinal (GI) tract. Eight out of 22 subjects (36%) had measurable plasma concentrations of GSK2330672. However, the peak concentration (5.33ng/mL achieved at 2hr post-dose in one subject) suggests minimal absorption. All 14 subjects who provided faecal samples for drug analysis had detectable levels of GSK2330672 in the faeces. No GSK2330672-related metabolites were detected in plasma or urine.

5.5 Discussion

This trial of GSK2330672 shows safety and efficacy of a novel agent in treating pruritus associated with PBC. Despite the significant symptom burden and need for better anti-pruritic drugs, there has been little recent progress in developing new treatments for pruritus in PBC. We have attempted to fill the treatment gap by conducting the first randomised, placebo-controlled, crossover trial of an IBAT inhibitor drug in patients with PBC and significant pruritus. In this trial we have demonstrated that interrupting enterohepatic circulation of BAs by inhibiting IBAT with GSK2330672 improves pruritus in patients with PBC.

We found that GSK2330672 at 45-90mg dose, given twice daily for two weeks in patients with PBC is safe and generally well tolerated. No SAEs and no clinically significant abnormality related to haematology, clinical chemistry or ECG was reported following treatment with GSK2330672.

Diarrhoea (33%) was the most frequent AE associated with GSK2330672 and this finding is in concordance with previous reports of IBAT inhibition in healthy volunteers (Graffner, Gillberg et al., 2016) and in patients with T2DM (Nunez, Yao et al., 2016). Increased BA load in the colon increases colonic motility and reduces colonic transit time, causing diarrhoea (Alrefai, Saksena et al., 2007, Raimondi, Santoro et al., 2008, Rao, Wong et al., 2010). In our study, the severity of diarrhoea was mild to moderate and no subject discontinued GSK2330672 or had their dose decreased. Taken together, the safety and tolerability profile of GSK2330672 in patients seen in our study would not preclude further clinical investigation of the drug to treat patients with PBC.

The main finding of the study is that compared to placebo, GSK2330672 was significantly more effective in improving itch intensity. This was evidenced by decreases in pruritus scores measured by three different tools of itch measurement. GSK2330672 treatment was clearly associated with improvement in pruritus and the changes from baseline were significant regardless of the dosing sequence. Notably, pruritus scores improved within the first week of GSK2330672; continued to decrease through two weeks of treatment and returned towards baseline upon switch to blinded placebo (as shown in **Figure 5-3**).

Despite the differences in the results between active (GSK2330672) and randomised placebo treatment the magnitudes of the effect may, in fact, be underestimated because the crossover

study design lacked a washout between treatment periods. Incorporation of analytical washout mitigates this somewhat, but placebo responses and carry-over effects still resulted in a sequence effect that influenced the magnitude of response depending on the order of treatment. Comparing the GSK2330672 period with the run-in open placebo period avoids the sequence effect and gives an alternative estimate of the magnitude of effect, although conversely this may be an overestimate due to the unblinded placebo run-in being used to form this comparison. Nevertheless, individual subject responses (**Figure 5-3**) clearly demonstrated rapid improvement of pruritus during GSK2330672 treatment and greater response was observed in patients with higher baseline itch intensity than those with lower itch intensity. GSK2330672 also decreased sleep interference score, disability (5-D itch scale) and fatigue (PBC-40 scale) domain scores confirming the treatment had meaningful impact on the symptom complex associated with PBC. Despite these encouraging results, our study had small number of patients, treatment duration was short and the study was not designed to make definitive conclusions on superiority of the study drug over placebo treatment. Therefore, the efficacy of GSK2330672 on pruritus needs to be confirmed in larger studies of longer duration.

Thus far, the evidence for the role of BAs in the development of pruritus in PBC is equivocal and the topic has been controversial (Kremer, Feramisco et al., 2014). A strong correlation between plasma BA levels and the severity of itch has never been demonstrated in cholestatic patients (Kremer, Feramisco et al., 2014) but more recent evidence has linked BA-mediated cholestatic pruritus via TGR5 receptors (Alemi, Kwon et al., 2013, Lieu, Jayaweera et al., 2014). In this study, GSK2330672 treatment had substantial effect on the circulating BA pool as demonstrated by 50% decrease in serum TBA and decreased serum levels of all taurine and glycine conjugated primary BAs. These findings are consistent with a preferential effect of GSK2330672 on ileal reuptake of conjugated BAs. However, we cannot exclude the possibility that pruritogens other than BAs are also transported via IBAT.

Cholesterol 7α-hydroxylase (CYP7A1), the rate limiting enzyme in hepatic BA synthesis is regulated by farnesoid X receptor (FXR) and FGF19. Following GSK2330672 treatment we observed a significant 3-fold increase in serum C4, a surrogate marker for hepatic CYP7A1 enzymatic activity (Sauter, Berr et al., 1996). Fasting C4 has been shown to provide a good measure of the overall flux through the BA synthetic pathway and a reliable assessment of the

degree of IBAT inhibition (Nunez, Yao et al., 2016). Taken together, these findings suggest significant target (i.e. IBAT) engagement and inhibition by GSK2330672.

The significant decrease in serum FGF19 levels observed in our study would be consistent with decreased ileal FXR activation following IBAT inhibition. Decreased circulating levels of FGF19 result in decreased inhibition of hepatic BA synthesis as reflected by increased serum C4 levels. In this regard, the effect of GSK2330672 on FGF19 is in contrast to other agents proposed for PBC treatment. Obeticholic acid, recently approved for PBC, is a strong ileal FXR activator that increases FGF19 levels and reduces BA synthesis while fibrates reduce BA synthesis despite reduction in FGF19. The long-term use of an IBAT inhibitor is likely to maintain suppressed levels of FGF19 and therefore, the effect of resulting upregulated BA synthesis on cholestasis needs careful evaluation. However, the circulating BA pool is unlikely to expand as the loss of BAs (in faeces) may exceed increased BA synthesis. The apparent paradox of contrasting actions of different agents in PBC on FGF19 reflects the fact that this factor is only an intermediate step in one pathway reducing BA levels. If reduction in BA levels is the critical pathway then direct reduction through IBAT inhibition could be as valuable a mechanism as suppression of synthesis despite contrasting actions on FGF19.

Recently, autotaxin (ATX) has been proposed as a key potential factor in the pruritogenic pathway in cholestasis (Beuers, Kremer et al., 2014). Interestingly, we observed significantly reduced level of serum ATX activity after treatment with GSK2330672 but not after placebo. It is possible that IBAT inhibition with GSK2330672 also interrupts the ileal reabsorption of 'Factor X' (an as yet unidentified molecule proposed to upregulate ATX activity) (Jones, 2012a) which in turn decreases the expression or synthesis of ATX and reduces serum ATX levels. Further work is required to probe the mechanisms involved in ATX effects of IBAT inhibitors.

(The differential effect of BAs, their correlations with pruritus scores and BA-ATX link is further explored in **Chapter 6**).

The ability of GSK2330672 to remain in the GI tract reduces concerns of systemic toxicity and drug interactions. Notably, GSK2330672 did not have any significant interaction with UDCA absorption or recycling. UDCA is not transported via IBAT and there was a significant increase in serum UDCA levels after GSK2330672 treatment. However, the glyco

(GUDCA) and tauro (TUDCA) conjugates of UDCA are transported by IBAT and we observed a 3-4 fold decrease in their serum levels. The clinical relevance of this effect is not known. Reassuringly, we did not observe any adverse effect of GSK2330672 on the therapeutic efficacy of UDCA since the serum liver biochemistry (mainly ALP) did not adversely increase during the study treatment. In the recently published trial of Maralixibat (an IBAT inhibitor) in PBC patients with pruritus, investigators did not quantify the effect of the drug on serum UDCA levels (Mayo, Pockros et al., 2019b). As UDCA is the mainstay of treatment in PBC the clinical implication of inhibitory effect of GSK2330672 on UDCA conjugates merits further investigation.

Overall, there were no significant changes in the liver enzymes following GSK2330672 treatment. The lack of significance may be due to the short duration of treatment used in this study and longer treatment with GSK2330672 may be required to study the effect of IBAT inhibition on ALP (biochemical marker of cholestasis) and other liver enzymes.

5.5.1 Strengths and Limitations

This study has a number of strengths. Firstly, this is the first ever crossover RCT of an IBAT inhibitor drug to treat pruritus in patients with PBC. The crossover design of the study is unique as it allowed estimation of the treatment effect in a smaller number of patients and provided a more efficient comparison of treatments than a similar sized parallel group trial. Secondly, we used patient reported outcomes to measure the treatment response objectively by employing the existing validated tools (PBC-40 questionnaire and 5-D itch scale) as well as a novel, easy-to-use electronic symptom diary.

The main limitations of our study are the relatively small sample size, short duration (two weeks) of treatment and the lack of washout discussed above. We also acknowledge that maintaining blinding is difficult in the crossover design and due to the prominent side effect (i.e. diarrhoea) of the active durg, inadvertent unblinding may have occurred. It is likely that patients receiving a medication with a characteristic side effect may readily distinguish between active treatment and placebo.

5.6 Conclusion

In conclusion, this phase IIa randomised controlled trial showed that two weeks of treatment with an oral IBAT inhibitor GSK2330672 in patients with PBC and symptoms of pruritus was safe, well tolerated and was significantly more effective than placebo in reducing the severity of pruritus. There was a significant reduction in serum total and conjugated BAs, FGF19 and serum autotaxin activity. Our results suggest that GSK2330672 may be a significant and novel advance for the treatment of pruritus in PBC. Diarrhoea, the most common adverse event associated with GSK2330672 may limit the long-term use of this drug.

5.7 Acknowledgements

This early phase clinical trial forms the backbone of my PhD and I am proud of the achievement. This trial could not have been possible without the participation of patients and I am extremely grateful to their involvement. My role in this study started in Septmeber 2013 and concluded in March 2017 when the results were published in the *Lancet*.

I recruited all thirteen patients in Newcastle with the help of the team at the clinical research facility at Royal Victoria Infirmary. I am grateful to the research team at Birmingham who recruited nine patients. I am grateful to Ruth Bolier and Jacqueline Langedijk at Academic Medical Centre (Amsterdam) for their valuable assistance with analysis of serum bile acids, ATX and FGF19.

Following the completion of recruitment, my role extended to data analysis which I did in close collaboration with the GSK statistician Mr Sam Miller. His contribution has been immense, particulary with the analysis of complex data generated from the electronic itch diary and pharmacokinetics. I did the statistical analysis of serum and faecal biomarkers and created tables and figures (Sam Miller created Figure 5-3).

Following the data analysis and interpretation, I wrote the first manuscript. Other co-authors provided intellectual input in improving the manuscript which was subsequently published as an original article in the *Lancet*.

6. CHAPTER 6: BILE ACID AND GUT BACTERIAL PROFILE IN PBC PATIENTS WITH PRURITUS AND EFFECT OF IBAT INHIBITION

6.1 Introduction

This chapter reports the works undertaken to explore the metabonomic and the gut microbiome characteristics of pruritus in PBC and investigate the effect of IBAT inhibition.

The role of BAs in the pathophysiology of pruritus in PBC has been debated but the exact mechanism remains elusive (Herndon, 1972). **Section 1.2.2** above describes the current evidence for the role of BAs in cholestatic pruritus. In addition to the ambiguity in the role of BAs, it is currently not known if any other serum metabolites are associated with cholestatic pruritus.

Recently, metabonomic studies have helped to identify specific metabolic profile in other liver diseases such as alcoholic liver disease (ALD), acute alcoholic hepatitis, non-alcoholic fatty liver disease (NAFLD), autoimmune hepatitis (AIH) and hepatocellular carcinoma (HCC) (Holmes, Wijeyesekera et al., 2015). However, in the current literature, there are limited studies in cholestatic liver diseases with metabonomic profiling of serum/plasma and urine from patients with PBC and PSC (Bell, Wulff et al., 2015, Masubuchi, Sugihara et al., 2015, Tang, Wang et al., 2015, Trottier, Bialek et al., 2012) Importantly, none of these studies specifically investigated pruritus associated with cholestasis.

Also, effect of anti-pruritic therapy on metabolites associated cholestatic pruritus is less well explored with only a few abstract reports on the effect of bezafibrate and albumin dialysis (Pares, Perez-Cormenzana et al., 2014, Reig, Perez-Cormenzana et al., 2016). More recently, IBAT inhibitor agents are emerging as potential novel treatment for pruritus in PBC (**Chapter 5**), but their effect on the metabolites associated with pruritus is currently unknown.

Emerging evidence shows altered gut microbiota (compared to healthy individuals) is associated with NAFLD, ALD, PSC and hepatic encephalopathy (Bajaj, Ridlon et al., 2012, Kakiyama, Pandak et al., 2013, Marchesi, Adams et al., 2016). A recent study of patients with early-stage PBC reported alterations of the gut microbiome (Lv, Fang et al., 2016) and another study showed a distinct microbial diversity in UDCA- treatment naïve PBC patients (Tang, Wei et al., 2017). BAs modulate the gut microbiota with changes in intestinal BAs

shown to significantly alter the composition of the gut microbiome in animal studies (Islam, Fukiya et al., 2011). Also, the gut microbiota modulate the BA pool by metabolic transformation of primary BAs into secondary BAs (Midtvedt, 1974). Therefore, it is conceivable that in cholestatic pruritus, changes in BAs or microbiota or in the interaction of the two may have a role in the aetiology of the symptom, and may be modified by IBAT inhibition. However, to date, there are no studies reporting gut microbiota composition in patients with PBC and pruritus. Also, the effect of increased BA load in the human colon following treatment with IBAT inhibitor drug on the faecal microbiota has not been previously investigated.

The overarching goal of this study was to characterise, for the first time, the serum metabolite profile and the faecal microbial composition in PBC patients with pruritus. We set out to perform a comprehensive research study with following hypothesis:

1) PBC patients with pruritus have a distinct serum metabonomic signature and gut microbiome composition, compared to PBC patients without pruritus and/or healthy people; and

2) Pharmacological inhibition of enterohepatic circulation of BAs with an IBAT inhibitor can alter the serum and faecal BA profile, as well as change the faecal microbial composition in PBC patients with pruritus.

6.2 Materials and Methods

6.2.1 Participants

This prospective case-control study was carried out in two parts. In the first part, patients with PBC with pruritus were recruited to the BAT117213 study, a phase 2a, RCT of IBAT inhibitor drug GSK2330672. This clinical trial was sponsored by GlaxoSmithKline (GSK) and registered with EudraCT (2012-005531-84) and ClinicalTrials.gov (Identifier: NCT01899703). Ethical approval for BAT117213 study was given by the Research Ethics Committee NRES Committee North East – Sunderland (13/NE/0290). We recruited 22 PBC patients with pruritus between March 10, 2014, and Oct 7, 2015. The trial protocol is described in **Chapter 4** and safety and efficacy data of GSK2330672 are detailed in **Chapter 5**. Itch severity was assessed using the PBC-40 itch domain score and 5-D itch scale (Elman, Hynan et al., 2010, Jacoby, Rannard et al., 2005).

In the second part, we set up the metabonomic and microbiota profile of pruritus in PBC (MetaMic) study and recruited asymptomatic PBC patients (PBC-control) and healthy volunteers (HC). Participants in the PBC-control group were recruited only if they did not have any itch (assessed using PBC-40 itch domain score ≤3) and were not taking any anti-pruritic medications at the time of the study enrolment. Healthy volunteers who self-reported good health, could enter the study when no known liver diseases were documented in their medical history. This study was sponsored by NIHR Newcastle BRC and approved by NRES Committee North East - Newcastle & North Tyneside 2 (14/NE/1036). PBC-control and HC were non-related but were age (±2 years), gender and ethnicity- matched to the PBC patients with pruritus of BAT117213 study.

The recruitment of participants in both studies occurred at two centres in the UK: Newcastle Upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, and University Hospitals Birmingham NHS Foundation Trust, Birmingham.

6.2.2 Sample collection

In the BAT117213 study, PBC patients with pruritus provided fasting peripheral blood samples and stool samples at baseline and after two weeks of treatment with GSK2330672 as per the study protocol. All samples were collected while the participants were at the study site. Blood samples were placed on ice with light excluded (or placed in a +4°C fridge) for a minimum of 30 minutes before processing. All samples were processed within a maximum of

2.5 hours from collection. Each blood sample was centrifuged at $+4^0$C at 1000g for 10 minutes after which 200µL of the supernatant was aliquoted into 2mL cryovials for immediate storage at -80˚C. The study staff weighed and transferred at least 200mg of stool in two 2mL cryovials for immediate storage at -80˚C.

All participants in the MetaMic study provided one sample of fasting peripheral venous blood as per the study protocol. Using instructions and stool collection kit provided by the study staff participants also provided a stool sample at a single time point by collecting stool at their homes and immediately refrigerated the sample in their home freezers. A research nurse visited all participants to collect the stool samples shortly after production (usually within 72 hours) and transported the samples on ice to the research laboratory. Upon arrival at the laboratory, the samples were divided into aliquots and immediately stored at -80˚C until processing for DNA isolation.

6.2.3 Metabonome analysis

The BA profiling analysis in serum and faecal samples was performed using a 'semi-targeted' profiling method, utilizing an ultraperformance liquid chromatography coupled to a quadrupole time-of-flight mass spectrometry (UPLC-QToF-MS) assay at Imperial College London as previously reported (Sarafian, Lewis et al., 2015). In addition, quantitative measurements of up to 16 BAs in human serum was performed using Biocrates® Bile Acids Kit (BIOCRATES Life Sciences AG, Innsbruck, Austria). The assay was used with Waters Xevo® TQ MS triple quadrupole mass spectrometer (Waters Inc., Milford, Massachusetts, USA). We also used Biocrates Absolute*IDQ*® p150 kit (BIOCRATES Life Sciences AG, Innsbruck, Austria) with Waters TQ-MS to quantify metabolites from four analyte groups: acylcarnitines, amino acids, glycerophospho-and sphingolipids, and one hexose. Using this platform, serum samples were submitted to the MS by a flow injection analysis method and all metabolites were measured using a single experimental setup. The sample preparation, assay, data calibration and analysis were conducted as per the manufacturer's instruction manual and software (Met*IDQ*®).

Serum ATX assay was quantified as recently described (Nakamura, Ohkawa et al., 2007). Serum fibroblast growth factor 19 (FGF19) was measured by a quantitative sandwich enzyme immunoassay technique according to the manufacturer's instructions (Human FGF19,

Quantikine® ELISA, R&D Systems, Oxford, UK). These assays were conducted in the Academic Medical Centre, Amsterdam.

6.2.4 Metataxonomic analysis

We sequenced the V3-V4 region of the 16S rRNA gene to study the faecal bacterial composition in the study population. Frozen stool samples were thawed and DNA was extracted from approximately 200mg of stool using the PowerLyzer® PowerSoil® DNA Isolation Kit (MO BIO Laboratories, Inc., San Diego, CA, USA) as per the manufacturer's instructions and bead beating samples using the Bullet Blender® Storm instrument (Next Advance, Inc. NY, USA). Following extraction, DNA concentration was quantified using the Qubit® dsDNA BR Assay Kit (ThermoFisher Scientific Inc., Waltham, Massachusetts, USA) and the Qubit® Fluorometer. We followed Illumina's 16S Metagenomic Sequencing Library Preparation Protocol to prepare sample libraries with the following modifications. We used the SequalPrep Normalization Plate Kit (Life Technologies Ltd, Paisley, UK) to clean up and normalize the index PCR reactions. In addition, we used the NEBNext Library Quant Kit for Illumina (New England Biolabs Ltd, Hitchin, UK) to quantify the sample libraries prior to denaturing and diluting the pooled libraries to load onto the flowcell. Sequencing was performed on the Illumina MiSeq platform (Illumina Inc., Saffron Walden, UK) using the MiSeq Reagent Kit v3 (Illumina) using paired-end 300bp chemistry.

6.2.5 Data processing and statistical analysis

Demographic and biochemical data for study groups (PBC patients with pruritus, PBC-control and HC) were analysed using unpaired t tests.

6.2.5.1 Metabonomic data analysis

All metabonomic data were analysed using non parametric tests. Serum BA data at baseline and post-GSK2330672 (or post-placebo) were analysed using Wilcoxon matched-pairs signed rank test and data for disease (PBC with pruritus) and control groups were analysed using Mann-Whitney test, comparing ranks. Given the number of parallel tests conducted for a typical spectral dataset, all resultant *p* values for metabolites were subsequently adjusted to account for multiple testing by false discovery rate (FDR) method. For instance, *p* values for faecal BA profile data analysis were adjusted for multiple comparisons with FDR correction using Benjamini, Krieger and Yekutieli method. Correlations were computed using Spearman

correlation coefficient (r). Statistical analysis were performed using GraphPad Prism 7.01 (GraphPad Software, Inc. La Jolla, USA). We regarded *p* values of <0.05 as significant.

6.2.5.2 ***Microbiome data analysis***

Quality filtering and analysis of the 16S rRNA gene sequence data was performed using the Mothur package following the MiSeq SOP pipeline (Kozich, Westcott et al., 2013). Sequence alignments were performed using the SILVA 16S rRNA gene bacterial database (www.arb-silva.de/) and classification of sequences was performed using the ribosomal database project (RDP) reference sequence files and a previously described method (Wang, Garrity et al., 2007). Analysis of the study groups at the individual operational taxonomic unit (OTU) level for different phylogenetic levels was undertaken in Statistical Analysis of Metagenomic Profiles (STAMP®) (Parks and Beiko, 2010) software package.

We compared samples from different time points using the Kruskal–Wallis H test and Tukey-Kramer multiple comparisons test. Comparison between two groups were done using White's non-parametric t-test and corrected for multiple testing using the Benjamini-Hochberg FDR correction implemented in STAMP®. Microbial richness was calculated based on the Chao1 index and within-samples (alpha) diversity was calculated using Shannon index. These measures were analysed using Mann-Whitney test for comparison between two study groups and Student t-tests were used for comparison between baseline and post GSK233672 (or post placebo) samples.

We used unweighted UniFrac distance metrics as measures of between-sample (beta) diversity and applied non-metric multidimensional scaling (NMDS) in R (https://www.r-project.org/) to evaluate ordination patterns. Using distance matrices, we performed permutational multivariate analysis of variance (PERMANOVA), a non-parametric, multivariate statistical test to test for differences in microbial community composition.

6.3 Results

6.3.1 Study population characteristics

The present study included 22 PBC patients with pruritus and control group of 31 asymptomatic PBC (PBC-control) patients and 18 healthy volunteers (HC). None of the participants had received any antibiotics in the preceding three months prior to study participation. The baseline characteristics of the study groups are summarised in **Table 6-1**. The demographics, serum levels of FGF-19 and dose of UDCA were not significantly different in PBC patients with pruritus and the control groups. Serum levels of alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT) and bilirubin and PBC-40 itch domain scores were significantly higher in PBC patients with pruritus compared to PBC-control.

	PBC with pruritus (n=22)	**PBC control (n=31)**		**Healthy control (n=18)**	
	Mean ± SD	Mean± SD	p value	Mean± SD	p value
Age (yr)	52.9± 10.5	58.1± 9.1	0.0603	53.0 ± 9.5	0.9607
Gender (M:F), n	3:19	All Females	0.1574	3:15	0.7894
BMI (kg/m^2)	27.2± 4.9	27.6± 5.4	0.7917	26.3 ± 5.4	0.6164
Body weight (kg)	72.81± 13.55	71.93± 14.81	0.8262	70.2 ± 13.4	0.5589
PBC-40 itch domain score	10.5± 3.3	2± 1.5	**<0.00001**		
ALP, (IU/L)	264± 174.13	176.8± 132.7	**0.044**		
GGT, (IU/L)	211± 172.6	84.3± 112.5	**0.002**		
ALT, (IU/L)	59.3± 44.8	39.93± 31.71	0.071		
Total bilirubin (µmol/L)	12.2± 5.4	8.2± 4.3	**0.004**		
Serum albumin (g/L)	41.9± 4.2	44.7± 2.7	0.006		
Serum FGF-19 (pg/mL)	162.9± 107.5	127.8±102.9	0.245	111.2± 53.8	0.09
UDCA dose (mg/day)	967± 185.8	836.6± 375.0	0.139		

Table 6-1 Baseline characteristics of the study groups
Significant p values are given in bold.

6.3.2 PBC patients with pruritus have altered serum metabolic profile

Data from serum BA quantification showed significantly elevated total BA level in patients with PBC patients with pruritus compared to patients with PBC-control and HC (**Table 6-2**). Glyco-cholic acid (GCA) and glyco-chenodeoxycholic acid (GCDCA) levels were significantly higher in patients with PBC patients with pruritus compared to PBC-control. There were no significant differences between study groups in any other conjugated or unconjugated serum BAs.

	PBC with pruritus	**PBC control**	**p value***	**Healthy control**	**p value***
Total BA	48.9±56.1	17.3±24	**0.0190**	6.13± 5.93	**0.0003**
Cholic acid (CA)	0.29±0.38	0.22±0.31	0.8893	0.25± 0.39	0.6222
Glyco- CA (GCA)	8.95±16.1	0.93±1.51	**0.0134**	0.67± 0.52	0.0800
Tauro- CA (TCA)	4.04±9.21	0.32±0.64	0.0837	0.19± 0.33	0.0965
Chenodeoxycholic acid (CDCA)	0.22±0.23	0.32±0.36	0.6561	0.32± 0.37	0.7376
Glyco- CDCA (GCDCA)	13.4±25.6	1.89±2.94	**0.0285**	2.16± 1.6	0.5042
Tauro- CDCA (TCDCA)	3.83±8.85	0.33±0.47	0.1712	0.42± 0.70	0.4141
Deoxycholic acid (DCA)	0.33±0.29	0.41±0.43	0.5736	0.41± 0.47	0.8723
Tauro- DCA (TDCA)	0.33±0.60	0.17±0.38	0.2035	0.30± 0.66	0.5651
Glyco- DCA (GDCA)	1.97±2.08	0.75±0.84	0.0599	1.1± 1.69	0.2979

Table 6-2 Serum bile acid levels in PBC patients with pruritus and control group
BA levels in μM (mean ±SD). Significant p values are given in bold. *compared to PBC patients with pruritus. Statistical significance was determined by unpaired non-parametric test with Mann-Whitney test, comparing ranks.

In PBC patients with pruritus we observed significant correlations between baseline 5-D itch score and serum glycocholic acid (GCA) and taurocholic acid (TCA) (**Table 6-3**). However, there were no significant correlations between serum BAs and baseline PBC-40 itch domain score or NRS (**Table 6-4** and **Table 6-5**).

Metabolite	r	95%CI	p value
Glycocholic acid	0.47	0.05283 to 0.7525	**0.0257**
Taurocholic acid	0.45	0.02374 to 0.7396	**0.0349**
Autotaxin	0.20	-0.253 to 0.5831	0.3686
Tauroursodeoxycholic acid	0.07	-0.3661 to 0.4945	0.7271
Glycoursodeoxycholic acid	0.20	-0.2519 to 0.5839	0.3658
Taurochenodeoxycholic acid	0.38	-0.05935 to 0.6996	0.0786
Taurodeoxycholic acid	0.17	-0.2788 to 0.5645	0.4368
Glycochenodeoxycholic acid	0.40	-0.02985 to 0.7144	0.0595
Glycodeoxycholic acid	0.18	-0.2728 to 0.569	0.4201
Cholic acid	0.01	-0.4222 to 0.4427	0.9555
Ursodeoxycholic acid	-0.34	-0.6766 to 0.1027	0.1158
Chenodeoxycholic acid	-0.19	-0.5814 to 0.2555	0.3747
Deoxycholic acid	0.005	-0.4283 to 0.4366	0.982
Total bile acids	-0.013	-0.4435 to 0.4213	0.95

Table 6-3 Correlation between 5-D itch scores and serum bile acids and autotaxin activity in PBC patients with pruritus at baseline
Significant p values are given in bold.

Metabolite	r	95% CI	p value
Autotaxin	0.2453	-0.2094 to 0.6128	0.2712
Tauroursodeoxycholic acid	0.2048	-0.2498 to 0.5854	0.3606
Taurocholic acid	0.313	-0.1381 to 0.6566	0.156
Glycoursodeoxycholic acid	0.1677	-0.2855 to 0.5596	0.4556
Glycocholic acid	0.283	-0.1704 to 0.6374	0.202
Taurochenodeoxycholic acid	0.2484	-0.2062 to 0.6149	0.2649
Taurodeoxycholic acid	0.099	-0.3484 to 0.5097	0.6612
Glycochenodeoxycholic acid	0.3167	-0.1341 to 0.6589	0.151
Glycodeoxycholic acid	0.1586	-0.2941 to 0.5531	0.4809
Cholic acid	-0.1818	-0.5695 to 0.2721	0.4182
Ursodeoxycholic acid	-0.2185	-0.5948 to 0.2363	0.3286
Chenodeoxycholic acid	-0.1004	-0.5107 to 0.3471	0.6565
Deoxycholic acid	0.07816	-0.3667 to 0.4939	0.7296
Total bile acids	-0.2219	-0.5971 to 0.2329	0.3209

Table 6-4 At baseline no significant correlations between PBC-40 itch domain scores and serum bile acids and autotaxin activity in PBC patients with pruritus

	r	95% CI	p
Autotaxin	0.1113	-0.3374 to 0.5188	0.622
Tauroursodeoxycholic acid	0.1604	-0.2924 to 0.5544	0.4758
Taurocholic acid	0.151	-0.3012 to 0.5477	0.5025
Glycoursodeoxycholic acid	0.1689	-0.2844 to 0.5604	0.4525
Glycocholic acid	0.2576	-0.1968 to 0.6209	0.2472
Taurochenodeoxycholic acid	0.1634	-0.2895 to 0.5566	0.4674
Taurodeoxycholic acid	-0.0608	-0.4806 to 0.3817	0.7881
Glycochenodeoxycholic acid	0.2305	-0.2243 to 0.6029	0.302
Glycodeoxycholic acid	-0.02598	-0.4534 to 0.4111	0.9086
Cholic acid	0.1417	-0.3098 to 0.541	0.5294
Ursodeoxycholic acid	-0.02768	-0.4547 to 0.4097	0.9027
Chenodeoxycholic acid	-0.00961	-0.4403 to 0.4246	0.9662
Deoxycholic acid	-0.05422	-0.4755 to 0.3873	0.8106
Total bile acids	0.02485	-0.4121 to 0.4525	0.9126

Table 6-5 At baseline no significant correlations between itch intensity scores measured by numerical rating scale and serum bile acids and autotaxin activity in PBC patients with pruritus.

Analysis of other quantified serum metabolites showed significant differences in 43 metabolites between PBC patients with pruritus and HC (**Table 6-6**). **Table 6-7** shows the comparision of serum metabolites in PBC patients with prurtus compared to the control group (PBC-control and HC). Only one metabolite (C10:2, decadienylcarnitine) was significantly higher in PBC patients with pruritus (0.084±0.026 μM) compared to PBC-control (0.055±0.01 μM, p=0.013; Mann-Whitney test with FDR).

No	Short name	Biochemical name	PBC with pruritus n=22		Healthy control n=8		p value*
			mean	SD	mean	SD	
	Acylcarnitines						
1	C2	Acetylcarnitine	6.47	2.49	4.1	1.31	0.0391
2	C3:1	Propenoylcarnitine	0.06	0.013	0.05	0.004	0.0249
3	C3-OH	Hydroxypropionylcarnitine	0.057	0.013	0.046	0.004	0.0230
4	C5	Valerylcarnitine	0.141	0.032	0.108	0.021	0.0330
5	C5:1	Tiglylcarnitine	0.057	0.012	0.048	0.004	0.0350
6	C5:1-DC	Glutaconylcarnitine	0.049	0.012	0.038	0.003	0.0173
7	C5-DC (C6-OH)	Glutarylcarnitine	0.056	0.012	0.044	0.004	0.0249
8	C5-OH (C3-DC-M)	Hydroxyvalerylcarnitine	0.071	0.014	0.058	0.004	0.0249
9	C7-DC	Pimelylcarnitine	0.056	0.018	0.036	0.01	0.0225
10	C8	Octanoylcarnitine	0.244	0.09	0.134	0.026	0.0082
11	C8:1	Octenolycarnitine	0.169	0.061	0.11	0.027	0.0202
12	C9	Nonalycarnitine	0.051	0.019	0.037	0.009	0.0426
13	C10	Decanoylcarnitine	0.298	0.134	0.129	0.043	0.0082
14	C10:2	Decadienylcarnitine	0.084	0.026	0.046	0.009	0.0105
15	C12	Dodecanoylcarnitine	0.134	0.048	0.075	0.02	0.0091
16	C12-DC	Dodecanedioylcarnitine	0.062	0.004	0.058	0.003	0.0350
17	C14	Tetradecanoylcarnitine	0.048	0.013	0.034	0.006	0.0137
18	C14:1	Tetradecenoylcarnitine	0.092	0.023	0.056	0.009	0.0082
19	C14:1-OH	Hydroxytetradecenoylcarnitine	0.023	0.006	0.016	0.002	0.0156
20	C14:2	Tetradecadienylcarnitine	0.036	0.013	0.019	0.004	0.0082
21	C14:2-OH	Hydroxyetetradecadienylcarnitine	0.017	0.004	0.012	0.001	0.0114
22	C16:1	Hexadecenoylcarnitine	0.04	0.012	0.023	0.004	0.0098
23	C16:1-OH	Hydroxyhexadecenoylcarnitine	0.034	0.02	0.015	0.002	0.0082
24	C16:2	Hexadecadienylcarnitine	0.018	0.004	0.013	0.001	0.0082
25	C16:2-OH	Hydroxyhexadecadienylcarnitine	0.023	0.006	0.018	0.002	0.0249
26	C16-OH	Hydroxyhexadecanoylcarnitine	0.016	0.005	0.012	0.002	0.0230
27	C18:1	Octadecenoylcarnitine	0.123	0.042	0.077	0.023	0.0249
28	C18:1-OH	Hydroxyoctadecenoylcarnitine	0.016	0.004	0.012	0.001	0.0156
	Amino acids						
1	Phe	Phenylalanine	95.8	17.9	69.9	9.76	0.0091
	Glycerophospholipids						
1	lysoPC a C18:1	lysophosphatidylcholine acyl C18:1	23.7	4.67	29.4	6.86	0.0279
2	lysoPC a C18:2	lysophosphatidylcholine acyl C18:2	19.2	5.46	41.4	10.7	0.0082
3	PC aa C30:0	phosphatidylcholine diacyl C30:0	6.07	2.32	3.95	0.887	0.0350
4	PC aa C32:1	phosphatidylcholine diacyl C32:1	32.6	14.1	16.7	6.55	0.0182
5	PC aa C32:2	phosphatidylcholine diacyl C32:2	5.73	2.18	3.42	0.809	0.0249
6	PC aa C34:2	phosphatidylcholine diacyl C34:2	79.5	3.64	10.4	1.33	0.0426
7	PC aa C34:3	phosphatidylcholine diacyl C34:3	27.2	9.62	17.3	2.91	0.0182
8	PC aa C36:1	phosphatidylcholine diacyl C36:1	77.9	11.5	72.4	5.62	0.0303

No	Short name	Biochemical name	PBC with pruritus n=22		Healthy Control n=8		p value
			mean	SD	mean	SD	
9	PC aa C38:3	phosphatidylcholine diacyl C38:3	71.2	11.1	54	8.31	0.0125
10	PC aa C38:4	phosphatidylcholine diacyl C38:4	77.3	4.17	72.8	2.5	0.0303
11	PC aa C40:4	phosphatidylcholine diacyl C40:4	4.55	1.24	3.27	0.547	0.0230
12	PC aa C40:5	phosphatidylcholine diacyl C40:5	13.4	4.34	9.69	2.39	0.0330
13	PC ae C34:3	phosphatidylcholine acyl-alkyl C34:3	5.81	1.45	8.23	1.68	0.0156
14	PC ae C42:3	phosphatidylcholine acyl-alkyl C42:3	0.756	0.127	0.959	0.173	0.0475

Table 6-6 List of significantly altered serum metabolites in PBC patients with pruritus compared to healthy control

Metabolite concentrations (μM) are shown in mean ±SD. Values in red font are higher compared to the other group. *compared to PBC with pruritus; statistical significance was determined by Mann-Whitney test with FDR

No	Short name	Biochemical name	PBC with itch (n=22)		All control (n=28)		p value[#]
			mean	SD	mean	SD	
	Acylcarnitines						
2	C3:1	Propenoylcarnitine	0.06	0.013	0.052	0.008	0.04
3	C3-OH	Hydroxypropionylcarnitine	0.057	0.013	0.048	0.008	0.02
4	C4:1	Butenylcarnitine	0.052	0.018	0.054	0.074	0.02
5	C5	Valerylcarnitine	0.141	0.032	0.117	0.037	0.02
6	C5:1	Tiglylcarnitine	0.057	0.012	0.05	0.007	0.04
7	C5:1-DC	Glutaconylcarnitine	0.049	0.012	0.041	0.007	0.02
15	C10:2	Decadienylcarnitine	0.084	0.026	0.052	0.011	0.001
16	C12	Dodecanoylcarnitine	0.134	0.048	0.102	0.035	0.04
17	C12-DC	Dodecanedioylcarnitine	0.062	0.004	0.059	0.004	0.02
18	C14	Tetradecanoylcarnitine	0.048	0.013	0.04	0.009	0.02
19	C14:1	Tetradecenoylcarnitine	0.092	0.023	0.073	0.019	0.02
21	C14:2	Tetradecadienylcarnitine	0.036	0.013	0.027	0.009	0.04
22	C14:2-OH	Hydroxyetetradecadienylcarnitine	0.017	0.004	0.014	0.002	0.02
24	C16:1-OH	Hydroxyhexadecenoylcarnitine	0.034	0.02	0.018	0.004	0.007
26	C16:2-OH	Hydroxyhexadecadienylcarnitine	0.023	0.006	0.019	0.003	0.02
	Amino acids						
1	Phe	Phenylalanine	95.8	17.9	81.4	20.6	0.03
2	Pro	Proline	155	45	197	59.6	0.04
	Glycerophospholipids						
1	lysoPC a C14:0	lysophosphatidylcholine acyl C14:0	4.89	0.978	4.19	0.608	0.04
2	lysoPC a C16:1	lysophosphatidylcholine acyl C16:1	5.35	1.56	4.17	1.38	0.03
4	lysoPC a C18:2	lysophosphatidylcholine acyl C18:2	19.2	5.46	30.5	12.4	0.02
5	PC aa C30:0	phosphatidylcholine diacyl C30:0	6.07	2.32	4.44	1.45	0.02
6	PC aa C32:1	phosphatidylcholine diacyl C32:1	32.6	14.1	20.7	9.88	0.02
7	PC aa C32:2	phosphatidylcholine diacyl C32:2	5.73	2.18	3.67	1.18	0.01
8	PC aa C34:1	phosphatidylcholine diacyl C34:1	86.6	3.75	83.8	2.11	0.03
9	PC aa C34:2	phosphatidylcholine diacyl C34:2	79.5	3.64	76.8	1.94	0.04
10	PC aa C34:3	phosphatidylcholine diacyl C34:3	27.2	9.62	19.2	5.78	0.01
12	PC aa C36:2	phosphatidylcholine diacyl C36:2	75.7	3.48	72.5	2.05	0.01
13	PC aa C36:4	phosphatidylcholine diacyl C36:4	91.2	4.27	87.3	3.07	0.02
15	PC aa C38:4	phosphatidylcholine diacyl C38:4	77.3	4.17	74.1	3.29	0.03
19	PC ae C34:3	phosphatidylcholine acyl-alkyl C34:3	5.81	1.45	7.34	2.15	0.03

Table 6-7 List of serum metabolites in PBC patients with pruritus compared to the control group (PBC and HC)

Metabolite concentrations (μM) are shown in mean ±SD. Values in red font are higher compared to the other group. [#]statistical significance was determined by Mann-Whitney test with FDR.

6.3.3 Serum autotaxin correlates with bile acids

In PBC patients with pruritus significant correlations were observed between conjugated primary and secondary BA levels and serum ATX activity at baseline (**Table 6-8**). Following GSK2330672 treatment, percentage (%) changes (Δ) in serum BA levels from baseline correlated significantly with %Δ in serum ATX activity from baseline (**Table 6-8**).

A) At baseline			
	r	**95% CI**	**p value**
Glycochenodeoxycholic acid (GCDCA)	0.80	0.56 to 0.91	**<0.0001**
Taurochenodeoxycholic acid (TCDCA)	0.74	0.45 to 0.88	**<0.0001**
Glycodeoxycholic acid (GDCA)	0.71	0.41 to 0.87	**0.0002**
Glycocholic acid (GCA)	0.69	0.37 to 0.86	**0.0003**
Taurocholic acid (TCA)	0.68	0.35 to 0.86	**0.0005**
Taurodeoxycholic acid (TDCA)	0.68	0.36 to 0.86	**0.0004**
Tauroursodeoxycholic acid (TUDCA)	0.51	0.10 to 0.77	**0.0148**
Cholic acid (CA)	0.01	-0.42 to 0.44	0.9578
Chenodeoxycholic acid (CDCA)	0.01	-0.41 to 0.44	0.9364
B) Post GSK2330672 treatment			
	r	**95% CI**	**p value**
%Δ Taurocholic acid (TCA)	0.60	0.22 to 0.8	**0.0034**
%Δ Taurochenodeoxycholic acid (TCDCA)	0.56	0.16 to 0.80	**0.0079**
%Δ Glycochenodeoxycholic acid (GCDCA)	0.55	0.15 to 0.80	**0.0084**
%Δ Glycocholic acid (GCA)	0.48	0.05 to 0.76	**0.0268**
%Δ Taurodeoxycholic acid (TDCA)	0.39	-0.05 to 0.71	0.0754
%Δ Glycodeoxycholic acid (GDCA)	0.42	-0.02 to 0.72	0.0563
%Δ Cholic acid (CA)	0.15	-0.31 to 0.55	0.5058
%Δ Chenodeoxycholic acid (CDCA)	0.15	-0.30 to 0.55	0.5045

Table 6-8 Correlations between serum autotaxin activity and serum bile acid levels in PBC patients with pruritus at baseline

(**A**) At baseline and (**B**) between percentage (%) change (Δ) in serum autotaxin activity and bile acid levels after GSK2330672 treatment. Significant p values are given in bold.

However, %Δ in serum BAs (total or individual) or ATX activity after GSK2330672 did not significantly correlate with %Δ in 5-D itch, PBC-40 itch domain or NRS cores (**Table 6-9**, **Table 6-10** and **Table 6-11**).

Serum bile acid	r	95%CI	p value
% Δ GCA	0.07	-0.3834 to 0.4984	0.7582
% Δ TCA	0.12	-0.3404 to 0.5347	0.603
% Δ TCDCA	-0.05	-0.4838 to 0.3997	0.8218
% Δ GCDCA	0.006	-0.4378 to 0.4477	0.9788
% Δ CA	0.29	-0.1724 to 0.651	0.198
% Δ CDCA	0.36	-0.09633 to 0.6935	0.107
% Δ DCA	0.031	-0.4173 to 0.4675	0.893
% Δ GDCA	0.049	-0.4021 to 0.4816	0.8317
% Δ TDCA	-0.17	-0.5702 to 0.2944	0.4597
% Δ UDCA	0.03	-0.41 to 0.47	0.8700
% Δ TUDCA	0.22	-0.24 to 0.60	0.3363
% Δ GUDCA	0.14	-0.32 to 0.54	0.5401
% Δ ATX	0.22	-0.2388 to 0.6093	0.3201

Table 6-9 Following GSK2330672 treatment no correlations were seen between percentages (%) change (Δ) from baseline in 5-D itch scores and %Δ in serum bile acids or autotaxin activity in PBC patients with pruritus

Serum bile acid	r	95%CI	p value
% Δ GCA	0.01	-0.4311 to 0.4542	0.9508
% Δ TCA	0.07	-0.381 to 0.5005	0.7492
% Δ TCDCA	-0.11	-0.5301 to 0.3461	0.6223
% Δ GCDCA	-0.05	-0.4827 to 0.4009	0.8267
% Δ CA	0.28	-0.1855 to 0.6432	0.2186
% Δ CDCA	0.22	-0.2421 to 0.6071	0.3274
% Δ DCA	0.05	-0.3975 to 0.4858	0.8133
% Δ GDCA	-0.06	-0.4966 to 0.3854	0.766
% Δ TDCA	-0.24	-0.6199 to 0.2227	0.2862
% Δ UDCA	-0.05	-0.48 to 0.40	0.8256
% Δ TUDCA	0.21	-0.25 to 0.59	0.3593
% Δ GUDCA	0.06	-0.38 to 0.49	0.7832
% Δ ATX	0.19	-0.2757 to 0.5838	0.4087

Table 6-10 Following GSK2330672 treatment no correlations seen between percentages (%) change (Δ) from baseline in PBC-40 itch domain scores and % Δ in serum bile acids or autotaxin activity in PBC patients with pruritus

Serum bile acid	r	95%CI	p value
% Δ GCA	0.01	-0.4343 to 0.4511	0.9643
% Δ TCA	0.02	-0.4219 to 0.4631	0.9124
% Δ TCDCA	0.01	-0.4285 to 0.4568	0.9397
% Δ GCDCA	-0.10	-0.5249 to 0.3524	0.6441
% Δ CA	0.27	-0.1861 to 0.6428	0.2195
% Δ CDCA	0.08	-0.3706 to 0.5095	0.71
% Δ DCA	0.11	-0.3441 to 0.5317	0.6155
% Δ GDCA	0.007	-0.4364 to 0.449	0.9732
% Δ TDCA	-0.047	-0.48 to 0.4038	0.8385
% Δ UDCA	0.09	-0.36 to 0.51	0.6810
% Δ TUDCA	0.14	-0.31 to 0.55	0.5180
% Δ GUDCA	0.09	-0.36 to 0.51	0.6945
% Δ ATX	0.14	-0.3224 to 0.549	0.5439

Table 6-11 Following GSK2330672 treatment no correlations between percentages (%) change (Δ) from baseline in itch intensity score measured using numerical rating scale (NRS) and % Δ in serum bile acids or autotaxin activity in PBC patients with pruritus

In PBC patients with pruritus baseline serum ATX activity was significantly higher and the mean serum level of total lysophosphatidylcholine (LPC) was significantly lower (221±35.3 µM) compared to HC (259±47.3 µM, p=0.04) but not PBC-control (231±57.2 µM, p=0.72) (**Figure 6-1**).

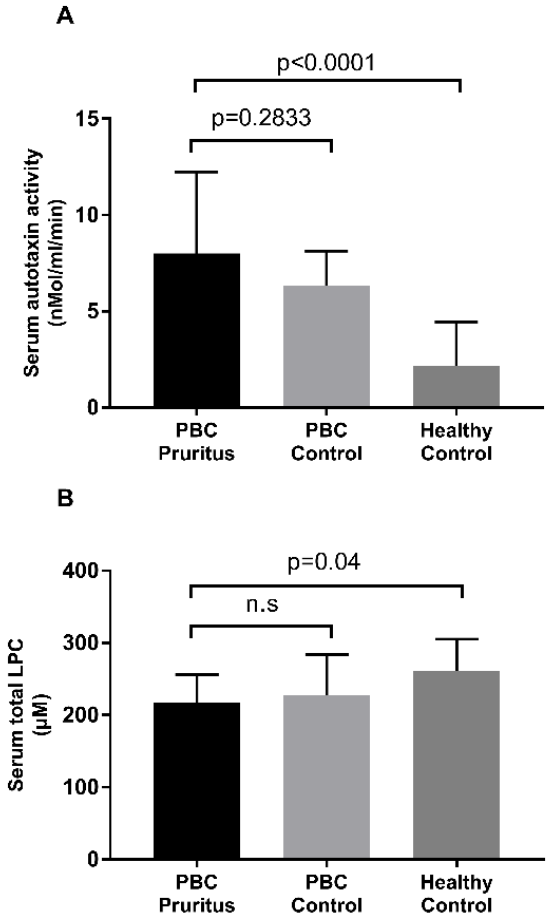


Figure 6-1 Serum autotaxin activity and total lysophosphatidylcholine levels in the study cohorts

A significant negative correlation between serum total LPC and serum ATX activity was observed in the entire study cohort (n=71, r= -0.28, p=0.0097), but this correlation was not significant in the PBC-pruritus group (n=22, r=-0.09, p=0.6764).

6.3.4 IBAT inhibition alters serum and faecal bile acid profile

Serum and faecal BA profile data for pre- and post-GSK2330672 were available for 16 PBC patients with pruritus (samples from six patients were insufficient for analysis). Compared to the baseline, two weeks of treatment with GSK2330672 significantly reduced serum levels of all tauro- and glyco-conjugated BAs (**Table 6-12**). Serum total BA level decreased, but did not reach statistical significance (p=0.0577). Serum levels of chenodeoxycholic acid (CDCA, a primary BA) significantly increased (p=0.029) but serum levels of cholic acid (CA, another primary BA) did not change significantly (p=0.782) and deoxycholic acid (DCA) significantly increased (p=0.011).

	PBC patients with pruritus n=16		
Serum BA levels in µM (mean ±SD)	**At baseline**	**After GSK2330672**	**p value***
Total BA	50.8±51.3	32.1±39.2	0.0577
Cholic acid (CA)	0.29±0.38	0.10±0.09	0.7820
Glyco- CA (GCA)	9.56±16.7	1.72±2.0	**<0.0001**
Tauro- CA (TCA)	4.46±10.1	0.43±0.88	**0.0002**
Chenodeoxycholic acid (CDCA)	0.21± 0.24	0.41±0.40	**0.0290**
Glyco- CDCA (GCDCA)	11.7± 19.5	4.15±4.99	**0.0131**
Tauro- CDCA (TCDCA)	3.68±8.83	0.60±1.06	**0.0021**
Deoxycholic acid (DCA)	0.35±0.31	0.65±0.65	**0.0110**
Tauro- DCA (TDCA)	0.40±0.69	0.16±0.19	**0.0125**
Glyco- DCA (GDCA)	2.26±2.25	1.64±1.93	**0.0214**
Lithocholic acid (LCA)	0.04±0.03	0.03±0.03	0.3755
Taurolithocholic acid (TLCA)	0.05±0.07	0.01±0.01	**0.0004**
Glycolithocholic acid (GLCA)	0.17±0.20	0.06±0.05	**0.0052**

Table 6-12 Changes in serum BA levels after treatment with GSK2330672 in PBC patients with pruritus
Significant p values are given in bold.

Compared to the baseline, no significant changes were seen in serum acylcarnitines, glycerophospholipids or sphingolipids following GSK2330672.

Faecal BA profiling showed significantly increased levels of total BA, CA, CDCA and DCA following GSK2330672, compared to the baseline (**Figure 6-2**).

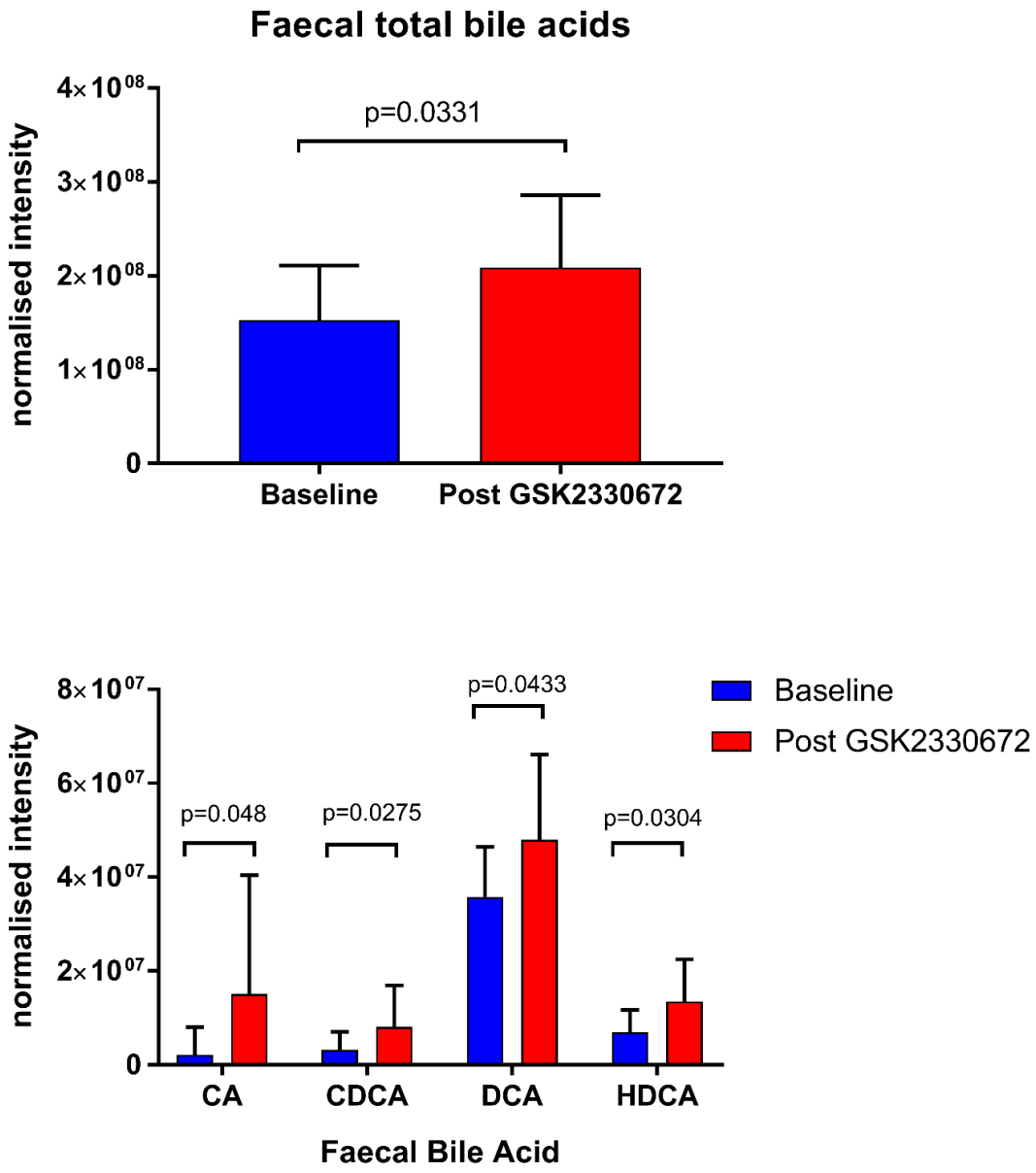


Figure 6-2 Changes in total and individual faecal bile acid profile after treatment with GSK2330672 in PBC patients with pruritus
Data in mean±SD. *p* values adjusted with FDR correction as described in method section

6.3.5 PBC patients with pruritus have no specific gut bacterial profile

The faecal bacterial composition of PBC patients with pruritus was not significantly different from the two control cohorts. Analyses performed on phylum, class and order levels showed relative abundance of faecal bacteria from PBC patients with pruritus was not significantly different from those of PBC-control or HC ($p>0.05$ for all comparisons, ANOVA with Benjamini Hochberg FDR).

Comparison of alpha diversity indices showed no significant differences in the Chao1 index ($p=0.051$, Kruskal-Wallis test) or Shannon index ($p=0.923$, Kruskal-Wallis test) between PBC patients with pruritus and PBC-control or HC (**Figure 6-3**).

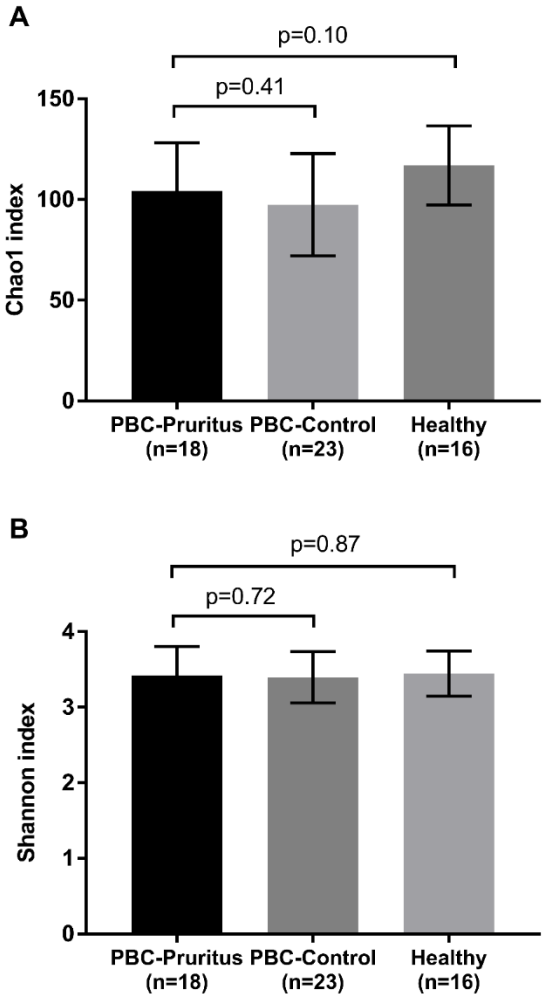


Figure 6-3 Bacterial diversity indices in the study cohorts
No significant differences were observed in the Chao1 index (**A**) or Shannon index (**B**) in PBC patients with pruritus, PBC-control and HC individuals.

6.3.6 IBAT inhibition alters gut bacterial profile

Gut bacterial composition of PBC patients with pruritus was compared at baseline and after 14 days of treatment with GSK2330672. Analysis of samples for estimation of depth showed 4100 reads/sample (with >99% coverage in all samples) in the baseline samples and 4500 reads/sample in the post-GSK2330672 samples.

For each subject, relative abundance of bacterial species (determined at the phylum level) at baseline, after 14 days of GSK2330672 and after 14 days of placebo treatment is shown in **Figure 6-4**.

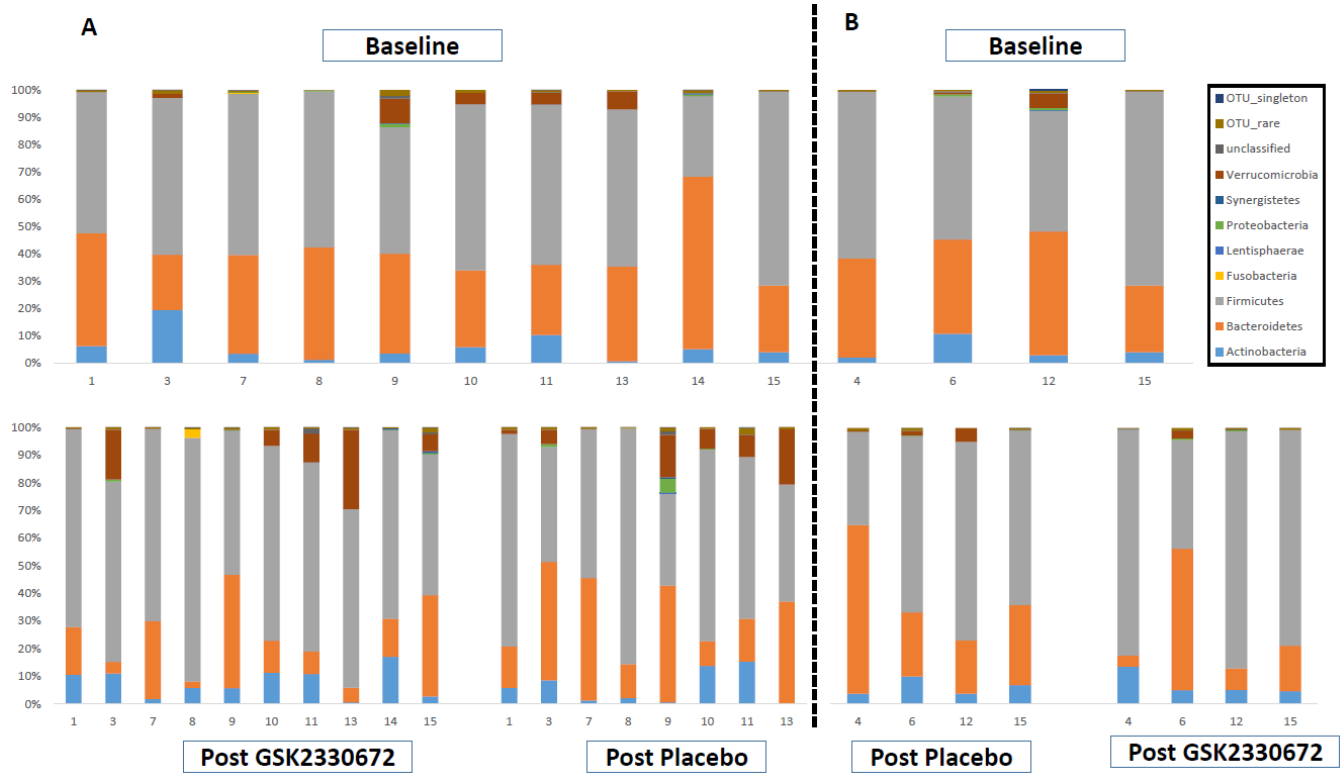


Figure 6-4 Relative abundance of phylum level bacterial species in PBC patients with pruritus and effect of IBAT inhibition
For each subject (shown with a number under the bar chart) bacterial abundances are shown at three time points (baseline, post-GSK2330672 and post-placebo). Subjects shown in panel (**A**) received GSK2330672 for two weeks followed by placebo for two weeks. Subjects in panel (**B**) received placebo for two weeks followed by GSK2330672 for two weeks.

A non-metric multidimensional scaling (NMDS) plot showed clear separation of bacterial composition after GSK2330672 treatment **(Figure 6-5**). Overall, GSK2330672 significantly changed the bacterial community composition at the phylum level (PERMANOVA p=0.027), (**Figure 6-6 A**) with a significant decrease in *Bacteroidetes* (p=0.033) and increase in *Firmicutes* (p=0.033). Further analysis showed significant changes at the class and order levels with decrease in *Bacteroidia* (p=0.040) and *Bacteroidales* (p=0.011) and increase in Clostridia (p=0.040) and *Clostridiales* (p=0.044), respectively (**Figure 6-6 B&C**). No significant changes were seen at other taxonomic levels.

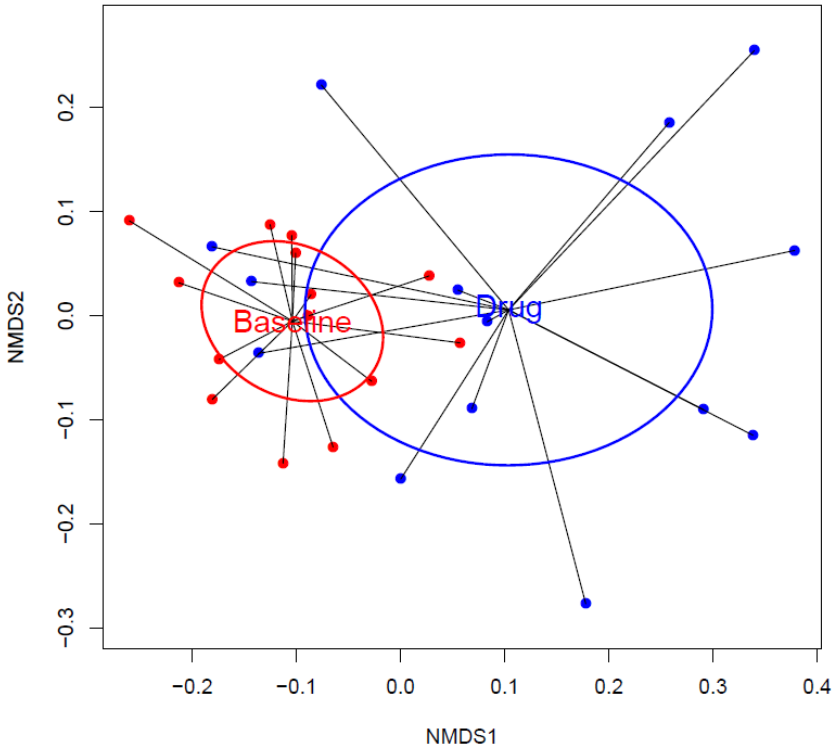
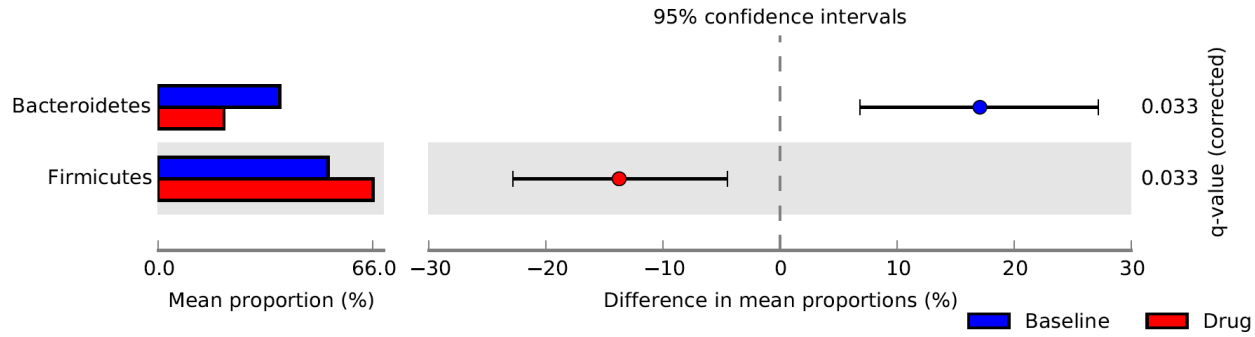
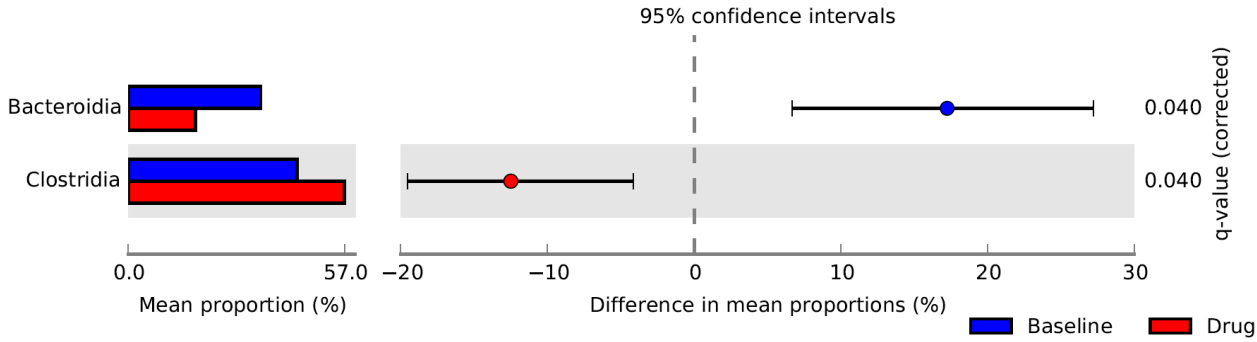


Figure 6-5 NMDS plot showing changes in faecal bacterial community composition following treatment with GSK2330672 in PBC patients with pruritus

B



C



D

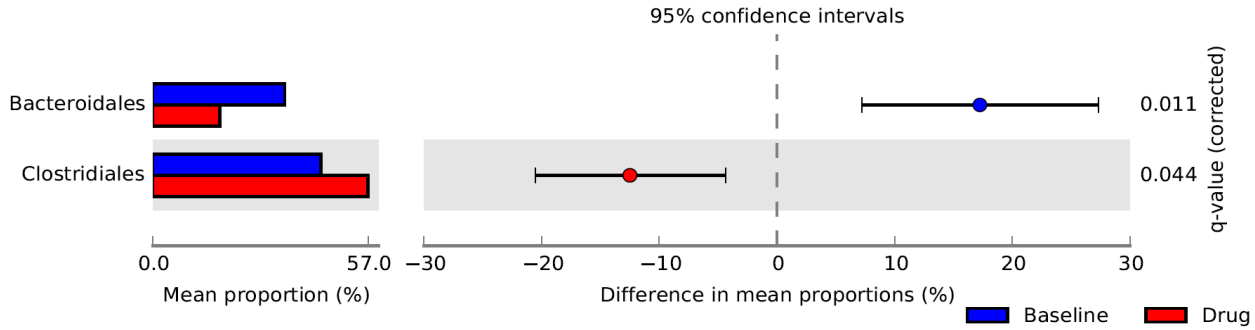


Figure 6-6 Changes in faecal bacterial community composition following treatment with GSK2330672 in PBC patients with pruritus

Extended error bar plots showing significant taxonomic changes in phylum Bacteroidetes and Firmicutes (panel **B**), class Bacteroidia and Clostridia (panel **C**) and order *Bacteroidales and Clostridiales* (panel **D**)

Changes in microbiome and faecal BA levels following GSK2330672 correlated with strongly positive correlation seen between phylum *Firmicutes* and CA (r=0.99) and CDCA (r=0.95) and negative correlation between phylum *Bacteroidetes* and CA (r=-0.74) and CDCA (r=-0.68) (**Figure 6-7**).

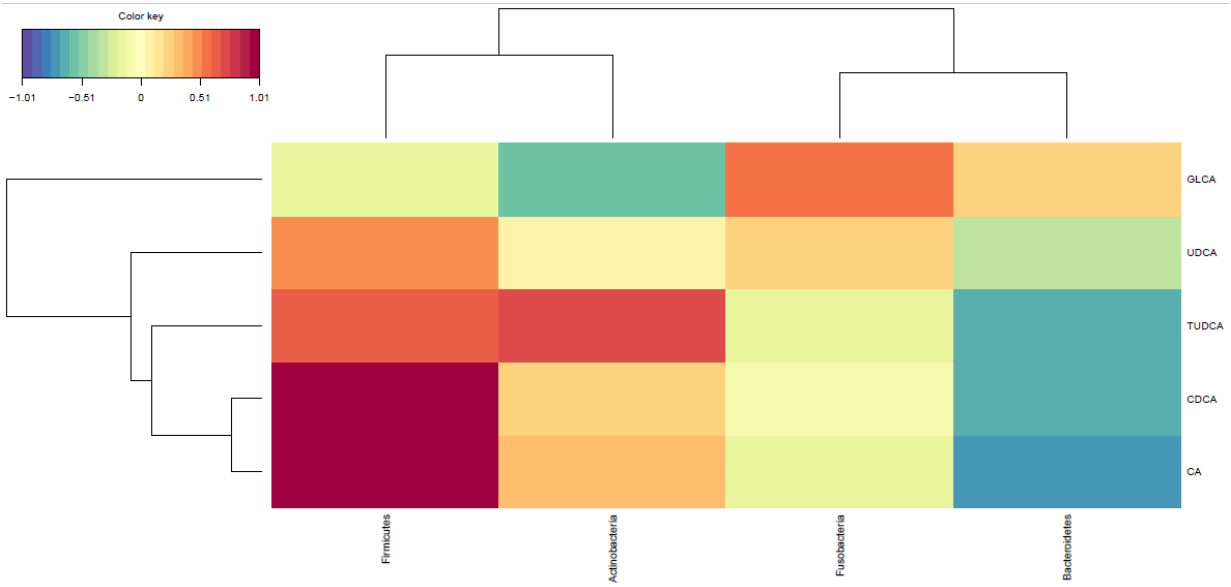


Figure 6-7 Heat map showing correlations between changes in faecal bile acid levels and the gut microbiome (at phylum level) after GSK2330672 treatment in PBC patients with pruritus
Strongly positive correlations were observed between faecal CA and CDCA levels and Firmicutes and strongly negative correlations were seen between faecal CA and CDCA levels and Bacteroidetes.

6.4 Discussion

This is the first study to report the serum metabonomic profile and gut bacterial composition in PBC patients with pruritus. In addition, we describe the effects of IBAT inhibition on serum and faecal BAs and compositional alterations in faecal bacteria in this patient group.

The relationship between serum BAs and cholestatic pruritus is complex. In a historic study of BAs in patients with liver diseases, fasting total BA levels were found to be higher in patients with pruritus compared to those without pruritus (Neale, Lewis et al., 1971). A positive relationship between pruritus and serum BAs has been shown (Di Padova, Tritapepe et al., 1984) and improvement in pruritus with BA binding resin cholestyramine further supports their role (Tandon, Rowe et al., 2007). Despite these, the pruritogenic role of BAs in cholestatic conditions is not unequivocal, with lack of correlation between BA levels and itch scores often quoted as a counter-argument (Beuers, Kremer et al., 2014). Recent studies on metabolic profiling of BAs in PBC and PSC did not include patients with pruritus (Bell, Wulff et al., 2015, Trottier, Bialek et al., 2012).

In this study, we found that PBC patients with pruritus have altered serum BA profile compared to PBC patients without pruritus. In addition to significantly higher levels of total BA, GCA and GCDCA in PBC patients with pruritus, we observed GCA and TCA correlated with 5-D itch scores. Furthermore, serum total and conjugated BAs significantly decreased following pharmacological IBAT inhibition with GSK2330672. However, reductions in serum BAs did not correlate with reductions in pruritus scores. Nevertheless, we have recently reported that GSK2330672 treatment in PBC patients with pruritus significantly improved pruritus scores (Hegade, Kendrick et al., 2017). Therefore, the anti-pruritic effect of an IBAT inhibitor agent could be mediated by reduction in circulating BAs.

Taken together, our findings on differential BAs in PBC patients with pruritus and changes after IBAT therapy may suggest that serum (total or individual) BAs may have a pathogenetic role in cholestatic pruritus. In our study cohort, the baseline characteristics of participants in the PBC-pruritus and PBC-control groups were comparable with respect to age, BMI and use of UDCA. Higher levels of ALP, GGT and bilirubin in the PBC-pruritus group may suggest they had more severe cholestasis compared to the PBC-control. However, it is noteworthy that baseline serum FGF19 levels were not significantly different between the study groups. A recent study has shown strong link between serum FGF19 levels and severity of cholestasis in

PBC patients (Li, Lin et al., 2017). Therefore, the severity of cholestasis in our PBC-pruritus group is unlikely to have biased the serum BA results.

We also studied serum ATX, a novel proposed pruritogen in cholestatic diseases (Kremer, van Dijk et al., 2012). Recently, we reported significant reduction in serum ATX activity after treatment with GSK2330672 (8.25±4.18 vs. 6.95±2.62, p=0.007) in PBC patients with pruritus (**Chapter 5**) (Hegade, Kendrick et al., 2017). Similar to previous studies, we found elevated serum ATX activity in PBC patients with pruritus. Since serum ATX drives enzymatic conversion of LPC into lysophosphatidic acid (LPA) (Kremer, Martens et al., 2010), it may be anticipated that sera of PBC patients with pruritus have lower LPC levels. Our results are consistent with this prediction since we observed significantly lower serum levels of total LPC in PBC patients with pruritus and a significant negative correlation between LPC and ATX activity.

Interestingly, we observed correlations between serum BAs and ATX activity at baseline, with a strong correlation between GCDCA and ATX ($r=0.80$, $p<0.0001$). Also, reductions in tauro- and glyco-conjugated primary BAs and ATX levels after GSK2330672 treatment correlated significantly. Our observations on association between serum BAs and ATX are novel. This, in addition to the recent intriguing finding of the inhibitory effect of GCDCA on ATX activity (Keune, Hausmann et al., 2016) merits further investigation into the complex interplay between BAs and ATX in cholestatic pruritus.

In the current literature there are only two reports on intestinal microbiota composition in PBC. In their study Lv *et al*. observed early stage PBC patients (n=42) had reductions of several potentially beneficial gut microbiota (such as Acidobacteria, *Lachnobacterium* sp., etc.), and the enrichment of some opportunistic pathogens (such as γ-Proteobacteria, Enterobacteriaceae, etc.) (Lv, Fang et al., 2016). Tang and co-workers observed reduced species richness and a lower level of microbial diversity in patients with PBC and partial restoration of these changes after UDCA treatment (Tang, Wei et al., 2017). However, these investigators did not report gut microbiota in relation to pruritus associated with PBC. We hypothesized that pruritus in PBC is associated with specific gut bacterial dysbiosis. But our results did not show any significant difference in faecal bacterial composition or diversity between PBC patients with pruritus compared to the control group. This lack of difference may suggest that cholestatic pruritus may not be associated with a specific gut bacterial

composition. However, since our cohort size was small and we did not study functional alterations in the gut microbiota, we cannot exclude the possibility of microbial metabolites contributing to cholestatic pruritus. Therefore, our negative findings on gut microbiota need to be confirmed in larger studies and additional studies are needed to investigate the role of gut microbial metabolites in cholestatic pruritus.

Evidence suggest that BAs are important in regulating gut microbial community structure (Li, Tang et al., 2017, Ridlon, Kang et al., 2014) and animal data show regulatory effects of gut microbiota on BA homeostasis (Claus, Tsang et al., 2008, Swann, Want et al., 2011). Although effects of IBAT inhibitor agents on serum and faecal BA levels have been studies in animal models of cholestasis (Baghdasaryan, Fuchs et al., 2016, Miethke, Zhang et al., 2016), to date, there are no human studies on the effect of IBAT inhibition on the gut microbiota. We observed that in PBC patients with pruritus treated with an IBAT inhibitor faecal BA levels increased and faecal bacterial composition significantly changed from baseline. Increased faecal DCA levels could indicate increased conversion of CA to DCA by gut microbiota derived 7-α-dehydroxylase enzymes. Major taxonomic alterations were seen at the phylum, class and order-levels respectively, with significant decreases in *Bacteroidetes*, *Bacteroidia* and *Bacteroidales* and increases in *Firmicutes*, *Clostridia* and *Clostridiales*. We hypothesize that these changes are at least in part due to the direct effect of increased BA load in the colon resulting from IBAT inhibition. This idea is supported by increased faecal CA and CDCA levels after GSK2330672 and their strong correlations with *Firmicutes* and *Bacteroidetes*. Interestingly, our findings are similar to Islam and colleagues study, where rats fed with high CA diet showed significant expansions in *Firmicutes* (from 54% to 93-98%) and *Clostridia* (from 39% to 70%) and significant inhibition of the *Bacteroidetes* (Islam, Fukiya et al., 2011). However, an important question that remains unanswered by our study, but that merits further investigation is, whether the changes in the gut microbiome produced by the IBAT inhibitor contribute to its anti-pruritic effect in PBC via changes in faecal microbial metabolites.

Although we have attempted to provide a comprehensive insight into the serum metabonome and gut microbiota in cholestatic pruritus, our study has limitations to be addressed in future studies. First, our relatively small cohort may have resulted in insufficient statistical power to unravel all metabolic perturbations. To determine the complete metabonome profile and microbial diversities, a large cohort of PBC patients with pruritus is required. Ongoing clinical development of GSK2330672 (NCT02966834) may present the opportunity for

further study of metabonomic and microbiomic profile in cholestatic pruritus. Second, we did not investigate the metagenome (functional composition profile) of microbiota which may help in analysis of pathway(s) associated with cholestatic pruritus. We only studied relative abdundance of bacteria. We need to focus on what the bacteria are doing rather than how their levels are changing. Third, instead of mucosal microbiota we opted to study stool samples, but it is known that faecal bacterial profiles do not fully replicate mucosa associated profiles (Sartor, 2015). Finally, although our cohort was matched for age, BMI and ethnicity, results could be influenced by other confounding effects such as environment and dietary factors.

In summary, our results show that pruritus in PBC is associated with an altered serum metabolome with elevated total and glyco-conjugated bile acids which may be contributing to the symptom. Serum bile acids and autotaxin correlated both at baseline and post IBAT inhibition. No differences were identified in the gut microbiota of PBC patients with pruritus compared to control. In PBC patients with pruritus, IBAT inhibition reduced serum bile acids and autotaxin, increased faecal bile acids, and altered the gut microbiome. Our findings need to be confirmed in subsequent studies which should focus on further dissecting the underlying molecular mechanism of cholestatic pruritus and clarifying the mechanisms of the anti-pruritic effect of IBAT inhibitor agents.

6.5 Acknowledgements

Metabonomics (UPLC-MS) and metagenomics (16S sequencing) are complex science with a steep learning curve. The studies presented in this chapter would not have been possible without the help of many people at Imperial College London who taught me basics and fundamental science behind these 'omic' studies. They also helped me in analysing and interpreting the complex data. I am grateful to Alexandros Pechlivanis, Douglas Rees, Julie McDonald, Professor Simon Taylor-Robinson, Professor Elaine Holmes and Professor Julian Marchesi for their invaluable contribution with the work presented in this chapter.

Between October 2015 and July 2016, I regularly travelled from Newcastle to London to analyse study samples at Imperial College London laboratories. I stayed on site for 2-3 days at a time and was closely involved with sample handling and understanding basic workflow involved in metabomic and microbiome studies. All works presented in this chapter were done under direct supervision of Alex and Julie.

With faecal microbiota analysis, I was involved with harvesting bacterial DNA, amplication and sequencing of 16S RNA, data processing and analysis and interpretation of results. With serum and faecal metabonimic studies, I prepared samples, observed UPLC-MS methods, performed data pre-processing and analysed and interpreted results. I did not get involved with equipment setup and instead focussed on application of MS to my studies.

I wrote the first draft of the manuscript of the study results. Other co-authors provided intellectual input in improving the manuscript which was subsequently published as an original article in *Liver International* in 2019.

7. CHAPTER 7: SUMMARISING DISCUSSION

This chapter discusses the main conclusions drawn from the research studies presented in the earlier chapters of this thesis.

Overall, the work carried out during this research project aimed to study cholestatic pruritus with main focus on understanding the prevalence and unmet need, investigate therapeutic role of interrupting enterohepatic circulation (invasive as well as pharmacological), explore role of bile acids and faecal microbiome and study the effect of inhibiting intestinal bile acids.

Investigation was developed around three principle hypotheses.

- That pruritus is a prevalent symptom in patients with primary biliary cholangitis.

Chapter 1 explored the burden of pruritus in patients with PBC. We noted that there are limited studies of 'real-world' experience of pruritus and its treatment in international PBC cohorts. By studying the UK-PBC research cohort, a nationally representative data from over 2500 patients in the UK, we have shown that 74% of patients with PBC experience pruritus at some point during their disease. We also evaluated pruritus in large PBC cohorts from USA and Italy and observed similar results. The comparative study of these three independent international PBC cohorts suggested prevalence of pruritus in PBC is 60%- 74%. Persistent itch (i.e. itch occurring frequently or all the time since development of PBC) was reported by 34.3% (UK), 34% (USA) and 27% (Italy) patients and severe itch (PBC-40 itch domain score ≥12) by 11.4% of UK, 6% of USA and 8% of Italy patients. We also observed significant correlations between VAS scores and PBC-40 itch scores in all three cohorts.

An important observation in the study was under-treatment of pruritus in all three cohorts. The patient-reported frequencies of cholestyramine treatment in those with persistent itch were 37.9% (UK), 31.6% (USA) and 30% (Italy). In those with severe itch the treatment also appeared unsatisfactory in all three cohorts since only 51.8% (UK), 43.4% (USA) and 50% (Italy) patients reported receiving colestyramine. Overall, this study suggested high prevalence of pruritus in PBC and under-treatment with guideline recommended drugs and highlighted the need for improvement in the knowledge among clinicians caring for patients with PBC.

- That cholestatic pruritus can be treated by interrupting or inhibiting enterohepatic circulation of bile acids.

The core part of the thesis is aimed to address the impact of inhibiting or interrupting enterohepatic circulation (EHC) of bile acids on cholestatic pruritus. This was carried out in two ways. First, we studied the nasobiliary drainage (NBD) that diverts bile and bile acids away from ileum and second, we investigated a novel pharmacological agent called Ileal Bile Acid Transporter (IBAT) inhibitor that blocks EHC of bile acids.

In **Chapter 3** we showed via a well-designed, multi-centre, retrospective European cohort study that nasobiliary drainage is a highly effective treatment for medically refractory pruritus in cholestatic liver diseases. We also observed favourable effect of NBD on serum alkaline phosphatase and serum bilirubin levels. The majority of patients undergoing NBD showed rapid and significant improvement in their itch scores. Interestingly, the duration of response to NBD was independent of the duration of drainage and the daily bile output. But rather disappointingly, the pruritus remission was noted to be temporary with itch returning after the cessation of biliary drainage. In addition, insertion of NBD is invasive and the procedure is associated with adverse events. We noted 31% of patients in our study developed mild post-ERCP pancreatitis (PEP). Therefore, we have outlined proposals to optimise the use of NBD in clinical practice including routine use of rectal NSAIDs in all patients undergoing NBD to reduce risk of PEP. Our study results need to be confirmed by prospective studies which should also focus on studying the effect of NBD on the levels of serum and biliary bile acids and other potential pruritogens in cholestasis. There is also a need for studying the role of long-term NBD where patients with severe cholestatic pruritus can be discharged home with a NBD catheter *in situ* and managed as out-patients. This may be an attractive option for some patients (e.g. patients on liver transplantation waiting list) and merits further investigation.

The above observation that diverting bile away from the ileum (via NBD) improves pruritus in cholestasis raised an interesting question on the role of GSK2330672, a novel, human IBAT inhibitor in the treatment of cholestatic pruritus. Therefore, we developed a study protocol for a multi-centre, phase 2a, randomised controlled trial (RCT) to investigate the safety and efficacy of 14-day treatment with oral GSK2330672 in patients with PBC with pruritus (**Chapter 4**).

In this cross-over RCT we studied the effect of GSK2330672 in 22 PBC patients with pruritus and showed that compared to placebo, GSK2330672 produced a significant improvement in the patient-reported pruritus scores that was assessed using three different assessment tools. We have also shown that treatment with IBAT inhibitor drug significantly reduces serum total and conjugated bile acid levels, increases hepatic bile acid synthesis (as shown by rise in serum C4) and decreases serum FGF19 and significantly elevated faecal bile acid levels. These results were consistent with the postulated mechanism of action of IBAT inhibitor drug. We found that GSK2330672 at 45-90mg dose, given twice daily for two weeks was safe and well tolerated with no significant adverse events. Diarrhoea (33%) was the most frequent AE associated with the drug but the severity of diarrhoea was mild to moderate and no subject discontinued GSK2330672 or had their dose decreased (**Chapter 5**).

- That cholestatic pruritus is associated with distinct bile acid metabolic signature and gut microbial dysbiosis and these may be modified by inhibiting intestinal bile acids.

Chapter 6 focussed on studying the serum and faecal metabolites and the gut-microbiota associated with pruritus in PBC. Using the metabonomic techniques developed at the Imperial College London, we studied the serum and faeces of PBC patients with pruritus at baseline and after treatment with GSK2330672 and compared with control group of healthy people and PBC patients without pruritus. We have shown that PBC patients with pruritus have altered serum metabolic profile and we have identified a specific serum metabonomic signature associated with pruritus in PBC. The glyco-conjugated bile acids were found to be significantly higher in the sera of PBC patients with itch compared to the control and IBAT inhibitor treatment significantly reduced all serum taurine and glyco- conjugated bile acids. In addition, we have identified other metabolites (28 acylcarnitines and 14 glycerophospholipids) significantly altered in PBC patients with itch compared to healthy control.

The study on faecal microbial composition suggested that pruritus in PBC is not associated with gut-microbial dysbiosis. The microbial diversity in the stool from PBC patients with pruritus was not significantly different from the control groups. However, 14-days of treatment with GSK2330672 was shown to significantly alter the microbial community at the phylum level with significant decrease in Bacteroidetes and increase in Firmicutes. This shift

is most likely secondary to the increased faecal bile acid levels resulting after GSK2330672 treatment and the effect of high faecal bile acids on the faecal microbiota.

In summary, this thesis describes important aspects of cholestatic pruritus and includes:

- large international cohort studies to understand the epidemiology of pruritus in PBC and its treatment, as reported by patients;
- a multi-centre, international cohort study to establish the role of nasobiliary drainage in the treatment algorithm of cholestatic pruritus;
- first evidence of safety and efficacy for a new class of drugs-IBAT inhibitors in the treatment of cholestatic pruritus and
- experimental metabolomic and microbiome studies that provide important mechanistic insights into pathogenesis of pruritus in PBC.

7.1 Implications of findings

Following table summarises main findings of the research studies and their implications.

Chapter	Main finding(s)	Implication(s)
Chapter 2	Prevalence of pruritus in PBC cohorts from UK, USA and Italy was 60%- 74% and in all three cohorts pruritus was undertreated with guideline recommended drugs.	Among clinicians caring for patients with PBC there is a need to understand the high prevalence of pruritus and improve their knowledge for better utility of available drugs.
Chapter 3	Nasobiliary drainage is a highly effective treatment for medically refractory pruritus in cholestatic liver diseases with favourable effects on serum ALP. Pruritus remission with NBD is temporary with itch returning after the cessation of biliary drainage.	NBD should be offered to patients with refractory cholestatic pruritus with a realistic estimation of expected benefit. In PBC the duration of drainage should be guided by the patient tolerance of the nasobiliary catheter and benefit of drainage on their pruritus.
Chapter 5	GSK2330672, an ileal bile acid transporter inhibitor agent, produced significant improvement (compared to placebo) in the patient-reported pruritus. The drug at 45-90mg dose, given twice daily for two weeks was safe and well tolerated with diarrhoea (33%) as the most frequent AE.	IBAT inhibitor agent may become a novel anti-pruritic drug in clinical use. The optimum dose of this agent needs further evaluation in a larger study of longer duration to limit the AEs.
Chapter 6	PBC patients with pruritus have altered serum metabolic profile with significantly higher levels of glyco-conjugated bile acids. Microbial diversity in the stool from PBC patients with pruritus was not significantly different from control group. Serum BAs and ATX activity showed significant correlations in PBC patients with pruritus, both at baseline and after treatment with IBAT inhibitor agent.	Role of BAs and ATX and their relationship with microbiome in patients with cholestatic pruritus merits further investigation as it may have therapeutic implications. The mechanism of anti-pruritic effect of IBAT inhibitior agent needs better understanding, as it may be related to attenuating complex interplay between BAs and ATX.

7.2 Future directions

I have attempted to comprehensively explore cholestatic pruritus by utilising the available methods during my research period (2013-2017). I believe, the work presented in this thesis provides novel insights in this disease area. However, I could not explore a number of facets of cholestatic pruritus which clearly need further attention. I have highlighted few.

- Bile samples taken during nasobiliary drainage were not utilised for metabonomic analysis. The available sample size (n=3) was too small to undertake such analysis. It is possible that the metabolite(s) causing pruritus is present in the bile and not in the blood. We need a large prospective study to compare bile samples from PBC (or PSC) patients with pruritus and control group to analyse the metabolites and identify potential pruritogen(s). Of course, the invasive nature of intervention to obtain bile (ERCP) may pose a potential issue with recruitment of patients.
- In the future faecal microbiota study, the focus probably should shift from measuring the bacterial abundance to investigating the functional metagenomics. We need to investigate what the bacteria are doing rather than how their levels are changing. The relationship between bile acids (in serum/stool/skin) and microbiota (skin/faeces) needs to be explored in greater detail.
- Genetics of pruritus in PBC is less explored and candidate genes associated with pruritus are yet to be identified. Large number of PBC patients have been recruited in the UK-PBC Research Cohort, which provides great opportunity to conduct a GWAS of cholestatic pruritus.

  Also, the relationship between itch and response to IBAT inhibitor agent needs to be explored. In the GSK2330672 trial (presented in chapter 4), patients provided samples for pharmacogenetics/pharmacogenomics but these are yet to be analysed. Data from such study may help in better understanding of the genetic factors associated with itch and treatment response.

- Currently, BAs and ATX are considered to be potential mediators of cholestatic pruritus and we found novel association between ATX and serum BAs (Chapter 6). This merits further investigation as the understanding of their relationship and interaction may be crucial for developing better therapies.
- We clearly need a better tool to objectively assess pruritus, particularly for clinical trials. Currently available patient reported outcome measures (VAS, 5-D itch and PBC-40) may be inadequate to detect changes in itch intensity. Also, these measures may be not overcome the bias introduced by placebo effect. Accelerometer (wrist actigraphy) may overcome this issue. Future drug trials of anti-pruritic therapy should utilise this method to measure scratching activity and inform response to intervention.
- Pruritus is a well-documented adverse event with Obeticholic acid (OCA) treatment in PBC patients, but the mechanism of OCA induced itch remains unclear. Bile acid-TGR5 axis is a proposed pathway in cholestatic pruritus in animal models. OCA, a semi-synthetic BA, may mediate itch via TGR5 agonism but this is yet to be proven. We need trials of TGR5 agonist agents (e.g. INT-777) and if itch is found to be associated with these agents, it may support the argument that TGR5 is involved in pruritus.
- UDCA will remain the mainstay of treatment in PBC and all future therapies will be adjuvant to UDCA. As seen in Chapter 5, GSK2330672 treatment significantly *increased* serum levels of UDCA (but decreased all other conjugated BAs). This effect of IBAT inhibitor drug on UDCA level merits explanation. One hypothesis may be that UDCA competes with other BAs for ileal absorption and IBAT inhibitor agent blocks other BAs but not UDCA; therefore, increasing serum UDCA levels.
- Perhaps, we will never find a cure for cholestatic pruritus until we find the causative pruritogen. Prospective large scale studies of patients with pruritus will be needed for detailed metabonomic and microbiome analysis. Such studies should incorporate existing and/or new anti-pruritic intervention(s) to identify pruritogenic metabolites and assess novel pathways to unravel the complex pathogenesis of cholestatic pruritus.

8. APPENDIX 1

8.1 Identification of PBC patients in the CPRD database

Patients ≥18 year of age, diagnosed with PBC between 2000 and 2008 with ≥1 year of medical history available (before the start of the study period) were selected from CPRD. This definition of PBC was used by Jackson *et al*., in a study of mortality and malignancy in PBC patients conducted in CPRD (Jackson, Solaymani-Dodaran et al., 2007). A validation study in CPRD has demonstrated high accuracy for the diagnosis of inflammatory bowel disease (92%) (Lewis, Brensinger et al., 2002) and therefore any error in the recorded diagnosis of PBC in CPRD is likely to be small as this is a diagnosis acquired almost exclusively in secondary care in the United Kingdom, much like inflammatory bowel disease (Jackson, Solaymani-Dodaran et al., 2007).

Within the CPRD based cohort of PBC patients, pruritus was identified by searching for codes for generalized pruritus. There is no validated method for selecting patients with pruritus from the CRPD data. According to Stander *et al*., patients with chronic pruritus can be classified clinically by the presence or absence of skin disease into the following three groups (Stander, Weisshaar et al., 2007):

Group 1: Patients with cutaneous diseases, such as eczema or psoriasis, as the etiology of their pruritus

Group 2: Patients with normal skin and pruritus

Group 3: Patients with chronic scratch lesions and pruritus.

Those who make up groups 2 and 3 are thought to have systemic, neurologic, or psychogenic aetiologies for their itch (Stander, Weisshaar et al., 2007). Patients in group 2 and 3 were of interest for our study. They were identified using the following algorithm adapted from Fett *et al* (Fett, Haynes et al., 2014).

- At least 2 READ codes for generalized pruritus separated by at least 6 weeks. The date of diagnosis for pruritus was considered the first date on which a READ code for generalized

pruritus (that was separated by 6 weeks or more from a prior READ code for generalized pruritus) occurred.

- To ensure that patients had chronic pruritus with normal skin, patients were excluded if they had any READ codes for dermatologic disorders associated with pruritus (e.g. rash, psoriasis, eczema, xerosis) or secondary scratch lesions (e.g. prurigo) prior to the diagnosis date.
- Patients with secondary scratch lesions were not excluded. Additionally, based on initial analyses, the requirement for 2 codes for pruritus with no dermatological codes prior to the pruritus codes, yielded only approximately 8% of the PBC sample with a pruritus diagnosis. Given that PBC patients are more likely to have pruritus than the general population, the study team decided to use a more sensitive definition for pruritus requiring that patients have only 1 code for pruritus with no codes for dermatologic conditions within 6 months of a pruritus code.

In the CPRD PBC cohort, patients' entire medical history, including time before PBC diagnosis until the end of available data (31 December 2014), was searched for a recording of prescription for cholestyramine (colestyramine, Questran), rifampicin, and naltrexone. "Ever use" of medication was defined as anytime in a patients' medical history including time before PBC diagnosis (on or prior to 31 Dec 2014).

8.2 Sample size estimation in GSK2330672 clinical trial

The initial sample size of approximately 40 subjects was decided based on feasibility and consideration of desired precision for estimating treatment effects for both efficacy and pharmacokinetic (PK) endpoints.

For the estimation of the effect of GSK2330672 versus placebo on pruritus, sample-size was based on literature results from placebo-controlled trials using a 0-10 point scale to assess pruritus. Tandon *et al.,* (2007) reported pooled between-subject standard deviation (SDb) for Rifampin of 3.84 pts, with SDb for other anti-pruritic drugs (e.g. opioid antagonists) being smaller, ranging from 1.26 to 2.43 pts. Assuming a within-subject correlation of 0.5 (i.e. within-subject SD = SDb/√2) and a sample-size of 40 subjects the expected half-width of the 95% CI for GSK2330672 versus placebo was calculated for a range of values of SDb. Given that a 2 point difference from placebo was considered clinically significant, a half-width of the 95% CI of below 1.25 was considered adequate to meet the estimation objective of the trial, and so 40 subjects was considered likely to be sufficient.

Given the uncertainties associated with sample size assumptions this study was designed to include prespecified interim analyses for futility or sample-size re-estimation, scheduled to be begin once at least 10 subjects had been recruited into the study and completed the run-in and two cross-over periods. Subsequent interims were to be conducted based on convenience and accrual rates thereafter. Results were reviewed by an unblinded Interim Analysis Review Committee (IARC, composed of GSK personnel not directly involved in study conduct). An Interim Analysis Charter described in advance the procedures that the IARC would follow during its review of data.

A predetermined stopping rule for futility was agreed by the study team. This futility rule was based on a responder analysis where a patient was defined a responder provided they achieved at least a 2 point reduction in itch or more on drug versus preceding placebo as assessed on the NRS. Given the natural floor effect in the 0-10 scale, only those patients with a mean itch-intensity score of more than 3 points during run-in were included in the interim analysis. Operating characteristics for various target response rates and posterior probability cut-offs were evaluated by simulation prior to the first interim analysis and the following rule was considered acceptable to the team and specified in the interim analysis charter: the study

would stop for futility if the posterior probability that the responder rate was greater than 60% was less than 5%, using a neutral non-informative prior, Beta (1/3, 1/3).

At the first interim the number of eligible patients for review was 10/11, of which 6 were responders and 4 were non-responders, yielding a posterior probability that the true response rate was > 60% of 50%. The study therefore continued as planned.

At the second interim the number of eligible patients for review was 17/19, of which 9 were responders and 8 were non-responders, yielding a posterior probability that the true response rate was > 60% of 28%. The study therefore did not stop for futility. Three further results reported at the second interim were:

1. Mixed effects model results estimated a half-width for the 95% CI for the mean reduction in itch with GSK2330672 compared to placebo of approximately 1 (initial sample-size had targeted achieving below 1.25), i.e. the within-subject SD was lower than had been assumed for initial sample-size calculations.

2. The same model estimated a high posterior probability (>90% with an uninformative prior) that the mean reduction in itch with GSK2330672 was 2 or more points compared to preceding placebo.

3. Although no PK samples had been analysed a high proportion of subjects were taking UDCA (almost 90%), considerably higher than had been assumed for initial sample-size calculations for PK objectives.

These three factors, in combination with consideration of the recruitment rate at the time, supported the decision to continue enrolment for approximately one month with a reduced target sample size of 20-25 patients. A total of 22 patients were ultimately enrolled onto the study.

8.3 Effect of GSK2330672 on PBC-40 domains

Domain	Treatment sequence / Comparison	Visit	n	Mean domain score (1-5)		Change from Baseline (%)	
				LS Mean (SE)	95% CI	LS Mean (SE)	95% CI
Cognitive	Sequence 1	Baseline	11	2.82 (0.348)	2.10, 3.54		
		GSK2330672	11	2.83 (0.348)	2.12, 3.55	2 (5)	-8, 12
		Placebo	11	2.77 (0.348)	2.05, 3.49	0 (5)	-10, 10
		Follow-up	10	2.71 (0.350)	1.99, 3.44	-3 (5)	-13, 8
	Sequence 2	Baseline	11	2.89 (0.348)	2.18, 3.61		
		Placebo	10	2.70 (0.350)	1.97, 3.42	-5 (5)	-16, 5
		GSK2330672	10	2.28 (0.350)	1.56, 3.00	-19 (5)	-29, -8
		Follow-up	11	2.58 (0.348)	1.86, 3.29	-10 (5)	-20, 1
	GSK2330672 v Placebo		**21**	**-0.18 (0.117)**	**-0.41, 0.05**	**-6 (3)**	**-13, 1**
	GSK2330672 (sequences combined)		21			-8 (4)	-16, -1
	Placebo (sequences combined)		21			-3 (4)	-10, 5
Emotional	Sequence 1	Baseline	11	2.85 (0.375)	2.08, 3.62		
		GSK2330672	11	3.06 (0.375)	2.29, 3.83	12 (9)	-5, 30
		Placebo	11	2.91 (0.375)	2.14, 3.68	7 (9)	-10, 25
		Follow-up	10	3.00 (0.379)	2.22, 3.78	10 (9)	-8, 28
	Sequence 2	Baseline	11	3.42 (0.375)	2.65, 4.20		
		Placebo	10	3.31 (0.379)	2.53, 4.09	3 (9)	-15, 21
		GSK2330672	10	3.08 (0.379)	2.30, 3.86	-8 (9)	-26, 9
		Follow-up	11	3.48 (0.375)	2.71, 4.26	4 (9)	-14, 21
	GSK2330672 v Placebo		**21**	**-0.04 (0.151)**	**-0.34, 0.26**	**-3 (4)**	**-12, 5**
	GSK2330672 (sequences combined)		21			2 (6)	-11, 14
	Placebo (sequences combined)		21			5 (6)	-7, 18
Fatigue	Sequence 1	Baseline	11	3.25 (0.329)	2.57, 3.93		
		GSK2330672	11	3.06 (0.329)	2.38, 3.74	-7 (5)	-17, 2
		Placebo	11	3.17 (0.329)	2.49, 3.84	-4 (5)	-14, 5
		Follow-up	10	3.03 (0.331)	2.34, 3.71	-8 (5)	-18, 2
	Sequence 2	Baseline	11	3.34 (0.329)	2.66, 4.02		
		Placebo	10	3.04 (0.331)	2.36, 3.72	-10 (5)	-20, 0
		GSK2330672	10	2.46 (0.331)	1.78, 3.14	-26 (5)	-36, -16
		Follow-up	11	2.98 (0.329)	2.30, 3.66	-12 (5)	-22, -3
	GSK2330672 v Placebo		**21**	**-0.34 (0.111)**	**-0.56, -0.12**	**-9 (3)**	**-16, -3**
	GSK2330672 (sequences combined)		21			-17 (3)	-24, -10
	Placebo (sequences combined)		21			-7 (3)	-14, -0
Social	Sequence 1	Baseline	11	2.76 (0.310)	2.13, 3.40		
		GSK2330672	11	2.65 (0.310)	2.02, 3.29	-5 (7)	-19, 8
		Placebo	11	2.66 (0.310)	2.03, 3.30	-4 (7)	-18, 10
		Follow-up	10	2.76 (0.312)	2.11, 3.40	-1 (7)	-15, 13
	Sequence 2	Baseline	11	3.15 (0.310)	2.51, 3.78		
		Placebo	10	3.24 (0.312)	2.60, 3.88	3 (7)	-11, 17
		GSK2330672	10	3.21 (0.312)	2.57, 3.85	2 (7)	-12, 16
		Follow-up	11	3.15 (0.310)	2.51, 3.78	0 (7)	-14, 14
	GSK2330672 v Placebo		**21**	**-0.02 (0.112)**	**-0.24, 0.20**	**-1 (3)**	**-7, 5**
	GSK2330672 (sequences combined)		21			-2 (5)	-12, 8
	Placebo (sequences combined)		21			-0 (5)	-10, 9
Symptoms	Sequence 1	Baseline	11	2.56 (0.213)	2.12, 3.00		
		GSK2330672	11	2.39 (0.213)	1.95, 2.83	-6 (5)	-16, 4
		Placebo	11	2.42 (0.213)	1.98, 2.85	-5 (5)	-15, 5
		Follow-up	10	2.14 (0.215)	1.70, 2.58	-16 (5)	-27, -6
	Sequence 2	Baseline	11	2.30 (0.213)	1.86, 2.74		
		Placebo	10	2.06 (0.215)	1.62, 2.50	-7 (5)	-18, 3
		GSK2330672	10	1.99 (0.215)	1.55, 2.43	-10 (5)	-21, 0
		Follow-up	11	2.13 (0.213)	1.69, 2.57	-7 (5)	-17, 3
	GSK2330672 v Randomised Placebo		**21**	**-0.05 (0.090)**	**-0.23, 0.13**	**-2 (3)**	**-8, 5**
	GSK2330672 (sequences combined)		21			-8 (4)	-15, -1
	Placebo (sequences combined)		21			-6 (4)	-13, 1

Table 8-1 Changes in PBC-40 domain scores during the study period

9. APPENDIX 2 PUBLICATIONS RELATED TO THIS THESIS

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Pruritus Is Common and Undertreated in Patients With Primary Biliary Cholangitis in the United Kingdom

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BACKGROUND AND AIMS: **Little is known about the prevalence or treatment of pruritus associated with primary biliary cholangitis (PBC). We analyzed data from patients with PBC recruited from all clinical centers in the United Kingdom (UK) to characterize the prevalence, severity, progression, and treatment of pruritus.**

METHODS: **We performed cross-sectional and longitudinal studies of patients in the UK-PBC cohort to assess trajectories of pruritus. Data on pruritus frequency, severity, and therapy were collected via paper questionnaires completed by 2194 patients at their initial assessment in 2011 and then again in 2014 and 2017. Self-reported treatment data were validated against the prescription record of PBC cohort in the Clinical Practice Research Datalink, a primary care database. We defined persistent pruritus as itch that occurs frequently or all the time and severe pruritus as PBC-40 pruritus domain scores of 12 or more, throughout their disease course. Latent class mixed models were used to study pruritus trajectories and identify factors associated with high pruritus.**

RESULTS: **At initial assessment, 1613 (73.5%) patients had experienced pruritus at some point since their development of PBC—persistent pruritus was reported by 34.5% of the patients and severe pruritus by 11.7%. Only 37.4% of patients with persistent pruritus and 50% with severe pruritus reported ever receiving cholestyramine. Frequencies of rifampicin use were 11% in patients with persistent pruritus and 23% in patients with severe pruritus. Comparison of 2011 and 2014 surveys (comprising 1423 patients) showed consistent self-reported data on pruritus. Proportions of patients in the UK-PBC cohort treated with cholestyramine or naltrexone (37.4% and 4.4%) did not differ significantly from proportions treated in the Clinical Practice Research Datalink cohort (30.4% and 4.4%) ($P = .07$ for cholestyramine and $P = .32$ for naltrexone). Latent class mixed models (n = 1753) identified 3 different groups of pruritus. Multivariable analysis identified younger age at diagnosis and higher level of alkaline phosphatase at 12 months after diagnosis as factors significantly associated with persistent high pruritus.**

CONCLUSIONS: **In a large national cohort study of patients with PBC, we found a high prevalence of pruritus and inadequate guideline-recommended therapy. Patient-reported data used to determine pruritus prevalence and treatment are reliable. Younger age and levels of higher alkaline phosphatase were associated with persistent pruritus. We need to increase awareness and management of pruritus in PBC in the UK.**

Keywords: CPRD; LCMM; cholestatic; itching.

Abbreviations used in this paper: CPRD, Clinical Practice Research Datalink; IQR, interquartile range; LCMM, latent class mixed model; NRS, numerical rating scale; PBC, primary biliary cholangitis; UDCA, ursodeoxycholic acid; VAS, visual analog scale.

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Most current article

Primary biliary cholangitis (PBC) (previously primary biliary cirrhosis)[1] is a chronic cholestatic liver disease of autoimmune origin in which cholestasis, if not treated effectively, results in liver fibrosis and cirrhosis. Pruritus (itch) is a characteristic symptom of PBC that may occur at any point in the disease course and is reported not to respond to ursodeoxycholic acid (UDCA), the first-line disease-modifying therapy.[2–4] Scratching resulting from chronic pruritus makes patients self-conscious, interferes with their body image, and may lead to social isolation,[5] while nocturnal pruritus may affect sleep quality, worsening fatigue. The diverse impacts of pruritus in cholestatic liver disease contribute to the substantial impairment of health-related quality of life.[6,7] Both the European Association for the Study of the Liver and American Association for the Study of Liver Diseases recommend oral cholestyramine (colestyramine) as the first-line therapy for pruritus in PBC followed by rifampicin (rifampin), opiate antagonists (naltrexone or naloxone), and sertraline as second-, third-, and fourth-line therapies, respectively.[3,4]

Data on the natural history of pruritus in PBC are limited, with few studies describing the epidemiology of the symptom. An early study of PBC clinical trial participants observed a 55% prevalence rate for pruritus with 27% annual risk for development of pruritus in placebo-treated patients.[8] More recently, a study from the UK-PBC Research Cohort (see the following) reported that pruritus is less severe in male PBC patients and more severe in patients with younger age at presentation or inadequate response to UDCA.[9] There is a paucity of studies describing pruritus in PBC from the patient perspective and there are no large PBC cohort studies on patient-reported experience on frequency, severity, and treatment characteristics of pruritus. Previously, an American study evaluated the perception of pruritus in PBC via an Internet-based online survey of 238 patients with PBC.[10]

Here, we report the characteristics of pruritus in a large, national cohort of patients with PBC. Our primary aim was to use the UK-PBC Research Cohort to study the prevalence of pruritus in PBC, investigate the associations between measures of pruritus intensity assessed using patient-reported outcomes, evaluate the frequency of antipruritic treatments as reported by patients, and explore the trajectory of pruritus over time. Additionally, we conducted analyses using the Clinical Practice Research Datalink (CPRD) to verify patient-reported information in the UK-PBC cohort against the actual recorded prescription for a cohort of PBC patients seeking antipruritic treatment from their primary care physician in the United Kingdom to confirm key findings.

What You Need to Know

Background

We analyzed data from patients with primary biliary cholangitis (PBC) recruited from clinical centers throughout the United Kingdom to characterize the prevalence, severity, progression, and treatment of pruritus.

Findings

In a cross-sectional study of questionnaires completed by 2194 patients at their initial assessment and then 3 and 5 years later, we found that most patients (73.5%) had experienced pruritus at some point since the development of PBC. Persistent pruritus was reported by 34.5% of the patients and severe pruritus by 11.7%, although most patients did not receive treatment for this symptom. Younger age at diagnosis and higher level of alkaline phosphatase at 12 months after diagnosis were significantly associated with persistent high pruritus.

Implications for patient care

In patients with PBC, pruritus is highly prevalent and undertreated. We need to increase awareness and management of pruritus in PBC in the United Kingdom.

Patients and Methods

Study Design and Subjects

This was a cross-sectional observational study of patients with PBC recruited to the UK-PBC Research Cohort with a longitudinal follow-up element to explore pruritus trajectory over time. The UK-PBC project and UK-PBC Research Cohort have been described in detail elsewhere (see http://www.uk-pbc.com/about/aboutuk-pbc/ws1/researchcohort). Briefly, all participants in the cohort have PBC defined by 2 or more of the following criteria: cholestatic liver biochemistry, compatible liver histology, and antimitochondrial antibody at a titer >1:40). Participants are recruited throughout the United Kingdom by the UK-PBC Consortium, a research network of 155 National Health Service Trusts or Health Boards collaborating in the UK-PBC project. The symptoms and health-related quality of life of all participants are thoroughly characterized using established and validated measures. Clinical data regarding age at diagnosis, UDCA therapy, and biochemical status of patients in the UK-PBC cohort have been previously published.[9,11,12] The UK-PBC project was approved by the Oxford C research ethics committee (REC reference: 07/H0606/96).

Participants included in the current study were patients with prevalent or incident PBC between 2008 and 2011, who had never undergone liver transplantation. Pruritus in these participants was assessed in detail using a standardized, self-completed questionnaire (Supplementary Table 1) that was mailed to the participants in February 2011 (first survey), July 2014 (second survey), and again in March 2017 (third survey). The rationale for multiple surveys from the same cohort was to evaluate the consistency of patient-reported

information (regarding historic pruritus prevalence) and to explore the evolution of pruritus over time. Using the date of the first mail shot (February 25, 2011) as an approximation for questionnaire completion date, pruritus was deemed prevalent if the recorded date of PBC diagnosis was more than 6 months before the date of the first mail shot. Otherwise, pruritus was deemed incident. None of the patients were receiving obeticholic acid and patients participating in clinical trials of investigational medicinal products were not included in the analysis.

Pruritus Assessment Measures

The core data for this study were obtained from the patient self-reported information using validated pruritus assessment tools. The tools were used to assess pruritus severity and pruritus episode frequency at the time of assessment and at its worst in the past, reflecting the complexity of pruritus patterns reported by patients.

PBC-40 Pruritus Domain Score. The PBC-40 is a validated, disease-specific quality-of-life measure with robust psychometric properties and optimized for self-completion.[13] Pruritus domain forms 1 of the 6 domains within PBC-40 and consists of 3 questions framed as statements. Responses for these statements are on a standard 5-point Likert-type scale with responses ranging from 0 (least burden or problem to 5 (greatest burden or problem). The total score of the pruritus domain is obtained from summing individual question response scores. Empirical cutoffs for categorizing pruritus into no (score <3), mild (score 4–8), moderate (score 9–11) and severe (score ≥ 12) pruritus have been defined and validated.[14] The original PBC-40 pruritus domain refers to pruritus in the last 4 weeks, but the pruritus questionnaire in the UK-PBC cohort incorporated PBC-40 pruritus domain to refer to pruritus since the development of PBC. For each patient, the total score of the PBC-40 pruritus domain was obtained from summing individual response scores for questions 2, 3, and 4 in the questionnaire (Supplementary Table 1).

Pruritus Visual Analog Scale. The visual analog scale (VAS), first described many decades ago,[15] remains a widely used tool to assess symptom severity. In the UK-PBC cohort, we used a 0- to 10-cm VAS that decodes pruritus into a point on a line (0 = no itch, 10 = worst itch possible) and patients were asked to mark their level of pruritus on the VAS to indicate the intensity of their pruritus in the last 7 days (current pruritus VAS) and since development of PBC (ever pruritus VAS) (questions 6 and 8 in Supplementary Table 1).

Pruritus Numerical Rating Scale. Patients were asked to rate their level of pruritus on a scale of 0 to 10 in the last 7 days (current pruritus numerical rating scale [NRS]) and since development of PBC (ever pruritus NRS) (questions 5 and 7 in Supplementary Table 1).

Definitions. In this study, ever pruritus refers to pruritus at any point in their illness since development of PBC and persistent pruritus refers to experiencing pruritus frequently or all the time since development of PBC. We defined severe pruritus as PBC-40 pruritus domain score ≥ 12.

Antipruritic Therapy

In the UK-PBC pruritus questionnaire, patients were asked to report if they had received specific treatment for pruritus at any point after PBC diagnosis. A list of drug treatments (and their trade names) including cholestyramine (cholestyramine, Questran), rifampicin, and naltrexone was included. Patients were also asked to report if they ever received phototherapy treatment, were admitted to hospital specifically for treatment of itch, and had taken any other medications including antihistamines and natural or herbal remedies.

Clinical Practice Research Datalink

The CPRD, previously known as General Practice Research Database, is a large longitudinal primary care database with information collected from a large number of general practices in the United Kingdom.[16] The patient population captured in the database is broadly representative of the demographic breakdown of the UK population and of the activity of general practitioners.[17] The data reflect the observations of, diagnoses made by, and therapies prescribed by general practitioners, in addition to information communicated to them by hospitals. The CPRD is a well-recognized resource for conducting robust medical and epidemiological research[16] and has been extensively validated for a wide range of diagnoses.[18,19] The methodology to identify pruritus data from the CPRD is given in the Supplementary Material. We hypothesized that the proportion of UK-PBC patients self-reporting use of antipruritic medications would be similar to the actual prescription record for antipruritic medications in the CPRD.

Statistical Analysis

Quantitative variables are gives as mean $\pm$ SD and descriptive statistics are presented as frequencies, n (%). Non-normally distributed data are presented as median (interquartile range [IQR]). Correlation coefficients (r) were determined using Spearman's rank correlation test. Chi-square test or Fisher's exact test were used to analyze the difference between proportions. To explore latent groupings within longitudinal trajectories of pruritus, linear mixed-effects models was developed using the latent class mixed model (LCMM) package in R software.[20] The dependent variable was total PBC-40 pruritus domain score. Patients were included in this longitudinal analysis if they returned at least 2 of the 3 surveys from 2011, 2014, or 2017. The correlation between repeated measures of the dependent variables within participants was accounted for in the mixed effects model structure. Goodness of fit statistics were used to determine the optimal number of latent

Table 1. Main Results From the First Survey (Year 2011) in the UK-PBC Cohort (n = 2194)

Sex	
Female	1988 (90.61)
Male	206 (9.38)
Age (n = 2193), *y*	63.5 ± 11.0
Time since diagnosis (n = 2127), *y*	6.4 (3.1–11.0)
Serum alkaline phosphatase 12 mo postdiagnosis (n = 1689), *IU/L*	163 (115–289)
Serum transaminase 12 mo postdiagnosis (n = 1686), *IU/L*	32 (23–49)
Serum bilirubin 12 mo postdiagnosis (n = 1673), *μmol/L*	9 (7–12)
Platelet count at diagnosis (n = 1988), × 10^9/*L*	272 (225.5–324)
Serum albumin at diagnosis (n = 2051), *g/L*	41 (38–44)
Frequency of pruritus	
Any experience of pruritus (ever pruritus)	1613 (73.51)
Persistent pruritus[a]	759 (34.59)
Never experienced pruritus	581 (26.48)
Pruritus severity (PBC-40 pruritus domain scores)	
No (<3)	992 (45.21)
Mild (4–8)	577 (26.29)
Moderate (9–11)	368 (16.77)
Severe (≥12)	257 (11.71)
Treatment received	
Persistent pruritus (n = 759)[a]	
Cholestyramine	284 (37.41)
Rifampicin	84 (11.06)
Naltrexone	34 (4.47)
Severe pruritus (n = 257)[b]	
Cholestyramine	129 (50.19)
Rifampicin	59 (22.95)
Naltrexone	23 (8.94)

Values are n (%), mean ± SD, or median (interquartile range).
[a]Patients with experience of pruritus occurring "frequently" or "all the time" since development of primary biliary cholangitis.
[b]Severe pruritus patients with PBC-40 itch domain score ≥12 since development of primary biliary cholangitis.

groups within the data. For each participant, a posterior probability associated with each latent group was calculated. In subsequent analyses, participants were assigned to the latent group with the highest posterior probability. Multinomial logistic regression was used to assess the association between various clinical and demographic covariates and class membership. The biochemical measures included as covariates were those previously demonstrated to associate with disease activity in the UK-PBC cohort (serum alkaline phosphatase, transaminase and bilirubin levels after 12 months of treatment, and platelet count and serum albumin level at the time of diagnosis). Odds ratios and 95% confidence intervals are presented for comparison of latent groups. All analysis were performed using the statistical software GraphPad Prism 6.0 (GraphPad Software, San Diego, CA) and R version 3.3.0 (R Foundation for Statistical Computing, Vienna, Austria).

Results

The UK-PBC cohort symptom dataset formed the primary source data. In the initial assessment (year 2011) pruritus data were available for 2975 unique PBC patients who had not undergone liver transplantation. Of these, 250 (8.4%) patients were excluded owing to missing data (incomplete or partially completed information) and 531 (17.8%) were excluded because they reported having a skin disorder (eczema, psoriasis, or urticaria) that might confound the analysis. A total of 2194 records were, therefore, included in the final analysis. The main results of are listed in Table 1. Pruritus was deemed to be prevalent in 2033 (93%) cases and incident in 161 (7%) cases and their characteristics are reported in Supplementary Table 2.

Frequency of Pruritus

A total of 1613 (73.5%) patients reported that they had experienced pruritus at some point in their illness (ever pruritus). A total of 759 (34.5%) patients reported experiencing pruritus frequently or all the time (persistent pruritus*)* since their diagnosis of PBC. A total of 581 (26.4%) patients reported that they had never experienced pruritus due to their PBC (Figure 1*A*).

Severity of Pruritus

The median ever pruritus scores for PBC-40 pruritus domain was 4 (IQR, 0–9), for pruritus NRS was 6 (IQR, 1–8) and for pruritus VAS was 5 (IQR, 0.5–8).

We used the PBC-40 pruritus domain score to define severity of pruritus. Based on the previously validated cutoff values, 577 (26.2%), 368 (16.7%), and 257 (11.7%) patients met the criteria for mild, moderate, and severe pruritus, respectively (Figure 1*B*). In patients with severe pruritus (n = 257), the median current pruritus (ie, in the last 7 days) score on NRS was 7 (IQR, 4–8) and on the VAS was 6 (IQR, 3.5–8).

Persistent (frequently or always) sleep disturbance from pruritus was reported by 427 (19.5%) patients and 321 (14.7%) had persistently felt embarrassed because of the itching.

Anti-pruritic therapy

We analyzed the patient-reported data for cholestyramine, rifampicin, and naltrexone by dividing the cohort into 3 categories of patients based on their reported previous pruritus history: ever pruritus, persistent pruritus, and severe pruritus. Of patients with any experience of pruritus since their PBC diagnosis (ever pruritus, n = 1613), 24.2% reported to have ever received cholestyramine and 5.7% received rifampicin before the 2011 study point. Only 37.4% of patients with persistent pruritus and 50% with severe pruritus reported to have ever received cholestyramine. The reported frequency of ever use of rifampicin in patients with persistent pruritus was 11% and 23% in those with severe pruritus (Table 1). Overall, 104 (6.4%) patients reported ever

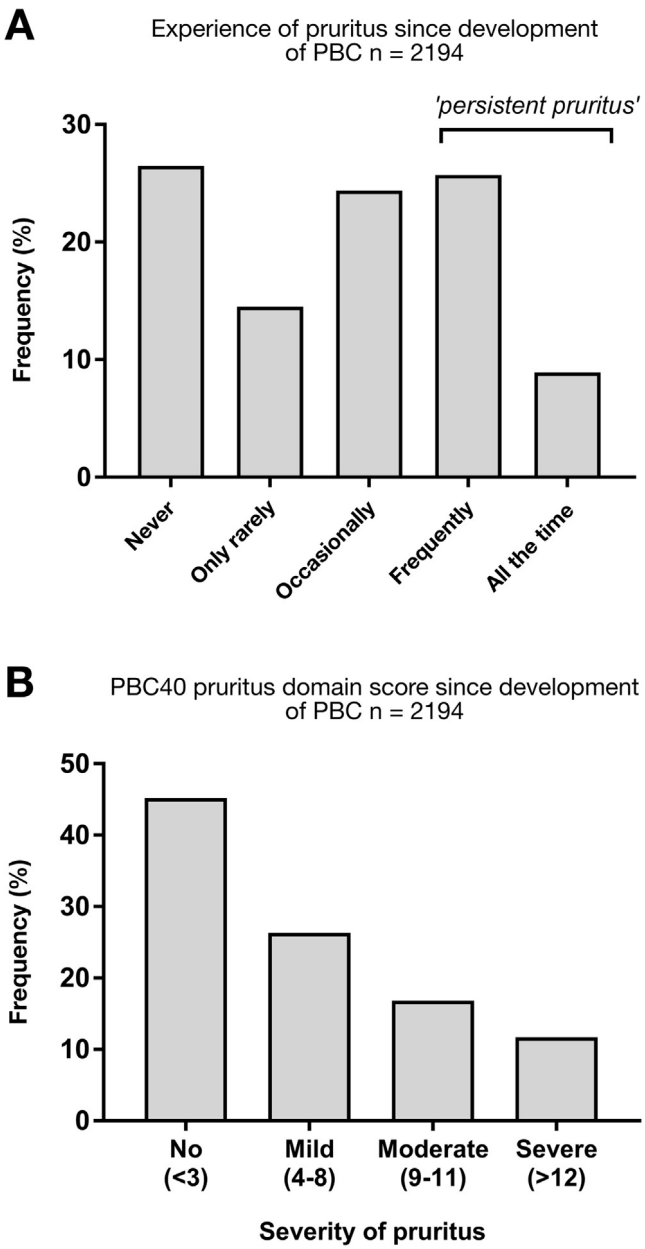

Figure 1. Pruritus in the UK-PBC cohort (n = 2194). (*A*) Frequency of experience of itch and (*B*) PBC-40 itch domain scores since development of primary biliary cholangitis (PBC).

using antihistamine drugs and 25 (1.5%) patients had received phototherapy.

In light of the low levels of reported antipruritic therapy in the UK-PBC cohort, we validated the findings using the CPRD, an entirely independent, primary care database. From the CPRD database, 664 patients (89% women, mean age 64 years) were identified as having a diagnosis of PBC. Of these, 181 (27.2%) patients had a recorded diagnosis of pruritus related to PBC. For these patients the frequency of actual recorded prescription for cholestyramine, rifampicin, and naltrexone was 30.4%, 3.3%, and 2.8%, respectively. Comparison of CPRD data with the self-reported treatment data for persistent pruritus (n = 759) patients of the UK-PBC cohort showed no significant difference for cholestyramine (37.4% vs 30.4%; $P = .07$) or naltrexone (4.4% vs 2.8%; $P = .32$) use (Figure 2).

Longitudinal Assessment of Pruritus

In the UK-PBC cohort, of 2194 patients in the first survey (year 2011), 1423 (64%) returned completed questionnaires in the second survey (year 2014), providing comparative and longitudinal data. Reporting of previous pruritus history was consistent between 2011 and 2014 (Supplementary Table 3), with 27.3% and 27%, respectively, stating that they had never experienced pruritus ($P = .83$). Also, we observed no significant differences in the reported frequencies of ever pruritus ($P = .83$) or persistent pruritus ($P = .46$). Overall, the reported frequency of use of antipruritic treatment was higher in the second survey across all categories of patients (Supplementary Table 3). This was exemplified by significantly lower proportion of patients reporting severe pruritus (7.5% vs 9.7%; $P = .038$) and higher use cholestyramine in patients with severe pruritus (70.1% vs 50.7%; $P = .002$) in the second survey compared with the first survey. Increased treatment use may account for the presence of an improved pruritus group in this cohort.

In the UK-PBC cohort, pruritus trajectory was assessed using the LCMM analysis utilizing the baseline data set (2011) and the 2 follow-up points (2014 and 2017). A total of 1753 patients participated in at least 2 of the 3 surveys and analysis of their individual pruritus severity scores (using the PBC-40 pruritus domain) identified 3 latent groups (archetypal disease patterns) (Figure 3). We have termed these groups as persistent high pruritus group (18% of the cohort), persistent moderate pruritus group (28% of the cohort), and persistent low pruritus group (54% of the cohort).

Univariable multinomial regression analysis identified higher levels of alkaline phosphatase, higher transaminase levels (at 12 months postdiagnosis), higher platelet count, lower serum albumin, and lower age (at diagnosis) in patients in the persistent high pruritus group compared with the persistent low pruritus and persistent moderate pruritus groups (Table 2). Multivariable analysis identified higher alkaline phosphatase and lower age as significant factors associated with the persistent high pruritus group (Table 3).

Correlation Between Measures of Itch Intensity

Using data from all 2194 patients in the first survey, we observed significant correlations between the current pruritus NRS and VAS (Spearman's rank correlation test, $r = 0.96$, $P < .0001$) as well as ever pruritus NRS and VAS ($r = 0.96$, $P < .0001$). Also, the PBC-40 pruritus domain score correlated significantly with ever pruritus NRS ($r = 0.81$, $P < .0001$) and ever pruritus VAS ($r = 0.80$, $P < .0001$).

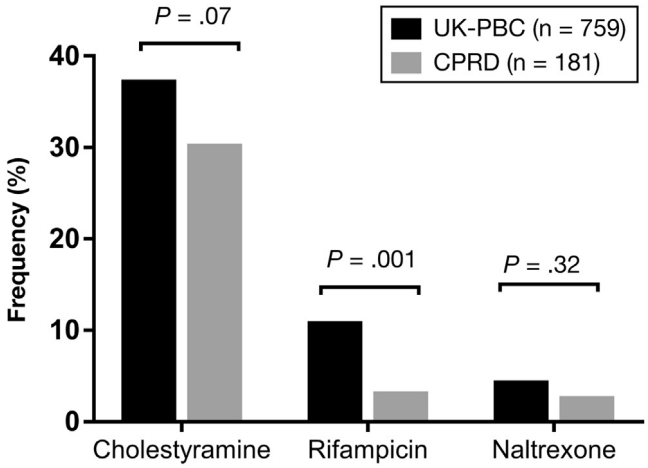

Figure 2. Comparison of patient-reported treatment data in the persistent pruritus category of the UK-PBC cohort with the actual recorded prescription data in the Clinical Practice Research Datalink (CPRD).

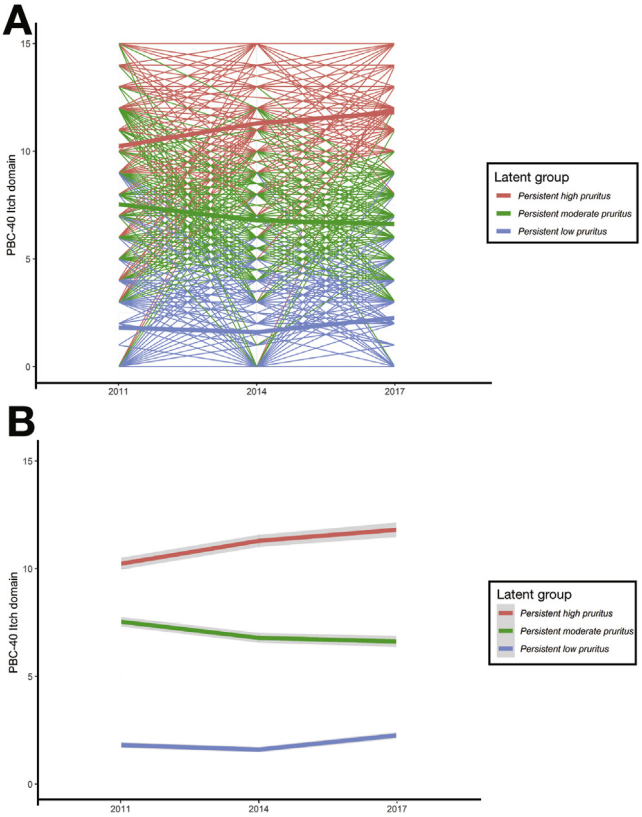

Figure 3. Latent class mixed model analysis of pruritus trajectory from 2011 to 2017 in the UK-PBC cohort (n = 1753). (*A*) Based on the individual total PBC-40 pruritus domain scores, 3 latent groups identified: persistent high pruritus (red), persistent moderate pruritus (green), and persistent low pruritus (blue). (*B*) Mean trajectories for each of the 3 latent groups with associated confidence intervals (gray shadows).

Discussion

This is the largest study to date recording the patient-reported characteristics of cholestatic pruritus. Our study provides important insights into pruritus in PBC by using the UK-PBC cohort, assessed using well-described and validated measures optimized for self-completion. As the patients in the UK-PBC cohort have been recruited from every hospital in the United Kingdom, they robustly represent real-world patients without referral or treatment center bias. The study therefore provides accurate assessment of the scale of unmet need in PBC.

In this study, we set out to assess the prevalence of pruritus in the UK-PBC cohort and explore different characteristics of pruritus and its treatment self-reported by patients.

The main finding of this study is that pruritus is a frequent ongoing symptom in patients with PBC, despite the availability of seemingly effective therapies. The overall prevalence of a history of pruritus at some point in the disease course (73%) observed in this study highlights the significant symptom burden in PBC with more than one-third (34%) of patients experiencing persistent pruritus during their illness. The patient-reported prevalence of pruritus in our cohort is higher than that reported in previous smaller studies. In the historic UDCA-placebo trial, pruritus was reported by 55% of patients at study entry.[8] More recently, in the phase 3 trial of obeticholic acid 59% of patients and in the bezafibrate (Bezafibrate in combination with Ursodeoxycholic Acid in Primary Biliary Cholangitis trial) trial 40% of patients reported pruritus at baseline.[21,22] These clinical trial data, however, relate to symptom frequency at a single time point in the disease (enrolment) and therefore underestimate the full impact of the symptom over the whole course of the disease.

This study is also the first to report the prevalence of pruritus severity in PBC by using the PBC-40 score, the only validated disease-specific score.[13] Using the previously validated cut-off scores for categorizing pruritus severity, we observed that more than a quarter (28%) of patients had experienced moderate to severe pruritus since their diagnosis of PBC.

A key finding of our study is that most PBC patients with pruritus do not receive antipruritic treatment. The European Association for the Study of the Liver and American Association for the Study of Liver Diseases guidelines recommend a step-wise treatment of pruritus, starting with cholestyramine as the first-line drug followed by rifampicin (second line) and naltrexone (third line). In our study, only 24% of patients who had experienced pruritus during their illness reported to have ever received treatment with cholestyramine. Although the reported frequency of use of cholestyramine was higher in those with persistent pruritus (37%), it is noteworthy that ~50% of patients with severe pruritus reported no treatment with this medication. Similarly, use of rifampicin and naltrexone was also unsatisfactory, with only 23% and 9% of patients with severe pruritus reported to have ever received rifampicin and naltrexone, respectively.

In the UK-PBC cohort, the data for pruritus prevalence and antipruritic therapy are provided by the patients and therefore may be prone to potential recall bias.

Table 2. Univariable Multinomial Regression Analysis Comparing Persistent Low Pruritus and Persistent Moderate Pruritus Groups to Persistent High Pruritus (Reference Group) Identified Using LCMM

Covariate	Subjects	$OR_{(Low\ vs\ High)}$	$OR_{(Moderate\ vs\ High)}$	*P* value
Age (in 2011)	1753	1.05 (1.04–1.07)	1.03 (1.02–1.05)	<.001
Alkaline phosphatase level (at 12 months postdiagnosis)[a]	1369	0.6 (0.53–0.68)	0.74 (0.65–0.83)	<.001
Transaminase level (at 12 months postdiagnosis)[a]	1235	0.58 (0.49–0.7)	0.81 (0.69–0.94)	<.001
Platelet count[b]	1577	0.78 (0.64–0.96)	0.8 (0.64–1.01)	.031
Albumin level[b]	1629	4.68 (1.64–13.35)	1.66 (0.53–5.21)	.005
Bilirubin level[a]	1346	0.91 (0.67–1.25)	0.96 (0.69–1.34)	.834

On average, compared with the persistent high pruritus group, patients in the persistent low pruritus and persistent moderate pruritus groups were significantly older and had lower alkaline phosphatase and transaminase levels at 12 months postdiagnosis.
LCMM, latent class mixed model; OR, odds ratio.
[a]Ratio with upper limit of normal (log transformed).
[b]Ratio with lower limit of normal (log transformed).

We addressed this in 2 ways. First, the pruritus survey was first repeated after a gap of ~3.5 years and comparison of both surveys showed highly consistent findings on historic prevalence of pruritus, its severity and treatment. Overall, our results suggest the patient-reported information on their pruritus and medications was reliable and recall bias was unlikely. Furthermore, in the UK-PBC cohort we have previously published a high level of data accuracy on patient self-reported PBC therapy based on a cross validation of self-reported data of a subgroup of 1379 patients (~63% of the whole cohort) with the patients' hospital record data.[9] Second, we used the CPRD cohort to determine the actual prescription rate for antipruritic therapy for PBC in primary care. Recorded pruritus rate was similar in the CPRD and UK-PBC cohorts with the slightly higher rate in UK-PBC cohort likely to reflect either the interest of patients with more symptoms in joining a research program or the use of objective quantification tools in the UK-PBC setting, which are not used in primary care. Our observation that recorded prescriptions rates for antipruritic treatment were in fact lower in CPRD than in the UK-PBC cohort suggests that the apparently low treatment rate in the UK-PBC was not simply a reporting issue. There would, therefore, appear to be a genuine issue with treatment reach in the United Kingdom.

Therefore, our observations from the national PBC cohort on the inadequate or inconsistent treatment of pruritus may have significant implications. It is possible that patients with pruritus did not seek medical intervention for their pruritus or patients' treating clinician (general practitioners or secondary care physicians) may not be familiar with the available guidelines for treating cholestatic pruritus and therefore did not initiate appropriate therapy to eligible patients. If the latter is true, then we propose there is a strong need for improvement in the awareness and management of cholestatic pruritus at the level of both general practitioners and gastroenterologists. A recent study on educational awareness has shown significant knowledge gaps in clinicians managing PBC and experts have urged for improving access to better patient care in this rare disease.[23,24]

Our exploratory analysis of pruritus trajectory over a period of 6 years using the LCMM method shows novel findings. We identified 3 different pruritus archetypes in PBC, with nearly half of patients experiencing persistent moderate or high pruritus in their disease course. Again, this highlights the symptom burden in PBC patients who, despite the availability of guideline-recommended therapy, have significant ongoing pruritus. In line with our previous report,[9] we observed that persistent high pruritus was significantly associated with younger age at diagnosis and higher levels of alkaline phosphatase at 12 months postdiagnosis. Overall, our study highlights the challenge of delivering better care for PBC patients with pruritus as well as the need for newer antipruritic therapy.

In clinical practice, the assessment of pruritus intensity in PBC is difficult due to the lack of objective measures. The PBC-40 is a validated tool that provides valuable information but it is infrequently used in

Table 3. Multivariable Multinomial Regression Analysis Comparing Persistent Low Pruritus and Persistent Moderate Pruritus Groups to Persistent High Pruritus (Reference Group) Identified Using LCMM

Covariate	$OR_{(Low\ vs\ High)}$	$OR_{(Moderate\ vs\ High)}$	*P* Value
Age (in 2011)	1.04 (1.02–1.06)	1.02 (1.01 1.04)	<.001
Alkaline phosphatase level (at 12 months postdiagnosis)[a]	0.68 (0.58- 0.8)	0.7 (0.6 0.83)	<.001
Transaminase level (at 12 months postdiagnosis)[a]	0.86 (0.7–1.06)	1.07 (0.89 1.28)	.08
Platelet count[b]	0.9 (0.73–1.1)	0.89 (0.69 1.14)	.582
Albumin level[b]	3.72 (1.06–13.14)	1.35 (0.35 5.26)	.061

Multinomial regression analysis included a total of 1116 patients with complete data. Compared with persistent high pruritus group, patients in the persistent low pruritus and persistent moderate pruritus groups were older and had lower alkaline phosphatase levels at 12 months postdiagnosis.
LCMM, latent class mixed model; OR, odds ratio.
[a]Ratio with upper limit of normal (log transformed).
[b]Ratio with lower limit of normal (log transformed).

routine clinical practice, as it is perceived as time consuming. The NRS and VAS are easy to use unidimensional scales and can be routinely used to assess pruritus intensity but they are not specific for PBC and have not been validated for use in PBC. In this study, we observed that pruritus NRS and VAS scores correlated significantly for both ever pruritus and current pruritus. We have also shown that the PBC-40 itch domain score correlates significantly with the pruritus VAS suggesting a strong link between the pruritus intensity and its functional consequences.

The main strengths of our study include the large sample size, cross sectional study of a national cohort, use of validated pruritus assessment tools, 2 follow-up studies describing longitudinal pruritus data, and validation of patient-reported therapy data with an independent primary care database. The main limitation of the study is lack of a comparator group (eg, normal population or age- or sex-matched healthy volunteers). Therefore, it was not possible to directly compare differences in prevalence of pruritus between patients with PBC and in the general population. Nevertheless, our study provides further insights into important clinical and treatment issues related to pruritus in PBC.

Conclusion

In conclusion, in this UK-PBC cohort study we report that the prevalence of pruritus in PBC is high and a significant proportion of patients experience persistent and severe pruritus during the course of their disease. The patient-reported information on their pruritus and treatment is highly consistent over time and the data accuracy is validated by an independent primary care database. We observed undertreatment of pruritus in PBC with inadequate and unsatisfactory use of guideline-recommended therapy. We suggest the need for improvement in the awareness and management of pruritus among both primary and secondary care physicians caring for patients with PBC in the United Kingdom. In PBC, younger age at diagnosis and higher serum alkaline phosphatase levels at 12 months post-diagnosis appear to be associated with persistent high pruritus.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Clinical Gastroenterology and Hepatology* at www.cghjournal.org, and at https://doi.org/10.1016/j.cgh.2018.12.007.

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Conflicts of interest
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AP&T Alimentary Pharmacology and Therapeutics

The safety and efficacy of nasobiliary drainage in the treatment of refractory cholestatic pruritus: a multicentre European study

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SUMMARY

Background
Pruritus is a common symptom associated with cholestatic liver diseases. To date only small single centre case series have suggested efficacy of nasobiliary drainage in relieving cholestatic pruritus.

Aim
To perform a multicentre study to evaluate the safety and efficacy of nasobiliary drainage in cholestatic pruritus.

Methods
This was a retrospective study of all patients treated with nasobiliary drainage for refractory cholestatic pruritus between 2006 and 2015 at five European centres. Pruritus was quantified using a visual analogue scale (VAS) and liver enzymes, serum bilirubin and total serum bile salts (TBS) were measured before (pre-NBD) and after nasobiliary drainage (post-NBD). We analysed the duration of treatment response and associated complications.

Results
In total, 27 patients (59% females) underwent 29 nasobiliary drainage procedures. The median duration of NBD was 7 days. NBD decreased pruritus in 89.6% of cases (VAS from 10.0 to 0.3, $P < 0.0001$). The median percentage decline in pruritus VAS was 94% and 33% of patients were free of pruritus within 24 h of starting drainage. The duration of treatment response was independent of duration of drainage ($P = 0.12$) and bile output. Significant improvements were seen in the median levels of serum alkaline phosphatase ($P = 0.001$) and serum bilirubin ($P = 0.03$) but not in serum TBS ($P = 0.07$). Mild post-endoscopic retrograde cholangiopancreatography pancreatitis (31%) was the most frequent complication.

Conclusions
Nasobiliary drainage is effective in relieving cholestatic pruritus in most patients and has favourable effect on biomarkers of cholestasis. Nasobiliary drainage may be associated with high risk of adverse events, especially pancreatitis. Prospective studies are needed to confirm our findings.

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INTRODUCTION

Pruritus (itch) is a common and often a prominent symptom of cholestatic diseases such as primary biliary cholangitis (PBC) and primary sclerosing cholangitis. It is also a frequent symptom in other less common cholestatic conditions such as benign recurrent intrahepatic cholestasis (BRIC), progressive familial intrahepatic cholestasis and drug-induced liver injury (DILI). The current standard of medical therapy of cholestatic pruritus includes four classes of anti-pruritic drugs: anion exchange resins (cholestyramine), enzyme inducers (rifampicin), opioid antagonists (naltrexone) and sertraline. Step-wise use of these drugs is recommended by both the American Association for the Study of Liver Diseases and the European Association for the Study of the Liver (EASL) guidelines.[1, 2] However, these drugs are not universally effective and a significant number of patients remain symptomatic despite medical therapy. Patients with pruritus not responding to drugs may be offered other therapeutic approaches such as nasobiliary drainage (NBD), ultraviolet phototherapy, plasmapheresis or extracorporeal albumin dialysis (e.g. Molecular Adsorbent Recirculation System, MARS) for symptom relief.[1, 2] The evidence for efficacy of these approaches is, in each case, relatively limited with little in the way of informed guidance as to how and when the approach should be considered.

Biliary drainage diverts the bile and bile salts (BS) away from the ileum, where 90% of the BS are physiologically reabsorbed and returned to the liver (enterohepatic circulation), thus depleting the body of BS and other potential pruritogenic substances.[3] Evidence suggests that surgical biliary drainage and partial external biliary diversion are effective in treating pruritus in patients with intrahepatic cholestasis.[4–8] Biliary drainage can also be achieved endoscopically by placing a NBD catheter, a procedure first developed by Cotton *et al.* as a technique for transnasal biliary catheterisation during endoscopic retrograde cholangiopancreatography (ERCP).[9] Since then, NBD has been successfully utilised for variety of applications such as treating patients with obstructive jaundice, cholangitis and post-operative bile leaks.[10] Compared to surgical biliary drainage, NBD is more appealing as it is less invasive, convenient, temporary and can be used repeatedly. In brief, NBD is carried out through the endoscopic placement of a 6Fr or a 7Fr nasobiliary catheter into the common bile duct during ERCP. After ensuring free flow of bile from the external end of the catheter, the latter is re-routed through the nose and connected to a bag for continuous drainage. To maintain catheter patency while in use, the catheter is irrigated once daily with sterile normal saline.

Data on the use of NBD in cholestatic pruritus are limited to very few published studies. There are reports that NBD induces complete and long-lasting remission in BRIC ($n = 3$)[11] and transiently relieves intractable pruritus in PBC ($n = 3$)[12] and acute cholestatic viral hepatitis ($n = 6$).[13] More recently long-term NBD (i.e. continuous biliary drainage by leaving the NBD catheter *in situ* for few months) has also been suggested to be safe and effective.[14] These results are encouraging but inference is limited since they are single centre reports with small sample size and include patients with one specific disease aetiology. Therefore to maximise our understanding of the potential benefits and optimal utility of NBD in refractory pruritus, we performed a multicentre retrospective study of NBD with a larger number of patients with different aetiologies of cholestasis.

PATIENTS AND METHODS

Study design

This was a retrospective, multicentre study of patients treated with NBD for cholestatic pruritus (Figure 1). We retrospectively analysed data of patients from five academic medical centres [Newcastle, United Kingdom (UK): Freeman Hospital; Paris (France): Hôpital Saint-Antoine; Erlangen (Germany): Friedrich-Alexander-University of Erlangen; Homburg (Germany): Saarland University Medical Center, and Rotterdam (the Netherlands): Erasmus Medical Centre]. We defined refractory pruritus as persistent pruritus despite treatment with at least two anti-pruritic drugs recommended by current guidelines on cholestatic pruritus. These include cholestyramine, rifampicin, naltrexone and sertraline. To assess the effect of NBD on pruritus, we defined the duration of treatment response as the time taken to return to pre-treatment pruritus level after removal of the NBD catheter.

Our primary aim was to systematically describe our experience of using NBD in treating patients with cholestatic pruritus. Specifically, we intended to study: (i) efficacy of NBD in cholestatic pruritus of different aetiologies, (ii) effect of NBD on the levels of serum bilirubin, liver enzymes [alkaline phosphatase (ALP) and alanine aminotransferase (ALT)] and total serum bile salts (TBS), (iii) any correlation between duration of treatment response and duration of drainage and volume

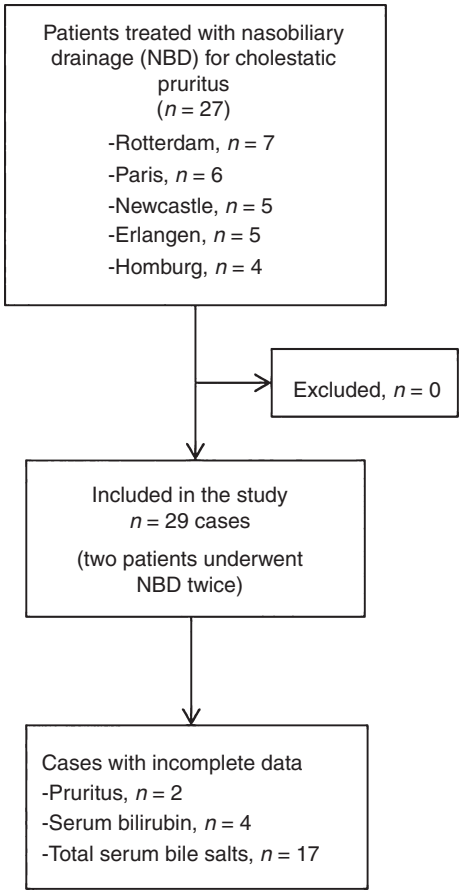

Figure 1 | Study flow chart.

of bile drained and (iv) safety of NBD and adverse events (AEs) associated with NBD.

Data collection

Data were obtained from the medical records of patients with refractory cholestatic pruritus treated with NBD between September 2006 and April 2015. Demographical, clinical, biochemical, radiological and endoscopic data were collected in a pre-designed electronic case report form. A study investigator from each centre retrieved data after careful interrogation of patient medical records. The diagnosis of underlying cholestatic condition was based on appropriate clinical, laboratory, serological and genetic tests. All patients were informed and consented for ERCP to place a NBD catheter for biliary drainage. In patients who had repeated NBD, outcome of each procedure was assessed as a unique case (i.e. number of cases > number of patients).

In four study centres, intensity of pruritus was evaluated using a 0–10 visual analogue scale (pruritus VAS). Patients had completed pruritus VAS before NBD (pre-NBD), repeatedly during the drainage and at the end of drainage period (post-NBD). In the Paris centre, intensity of pruritus was assessed as: 'none', 'mild', 'moderate' and 'severe'. To have comparable units, the data in the six patients obtained from this centre were converted to VAS as follows: none = 0, mild = 3, moderate = 6 and severe = 10. This approach of recalculation of pruritus severity was based on a method used in a previous study.[15] The pruritus VAS measured on the last day of drainage or on the day after removal of the NBD catheter was considered as post-NBD VAS. Following the removal of NBD catheter and patient discharge, pruritus VAS was measured during out-patient follow-up visits. The follow-up interval for individual patients varied across the study centres and was determined according to the clinical need.

We also collected data on duration of NBD and pre- and post-NBD laboratory parameters including serum bilirubin, ALP, ALT and TBS. Post-NBD measurements of all laboratory parameters were performed on the day of NBD catheter removal. The methods used to measure TBS were: high performance liquid chromatography (HPLC) coupled with tandem-mass spectrometry (HPLC-MS/MS) in the Paris centre, an enzymatic-fluorimetric method in the Rotterdam centre, and Diazyme total bile salts kits (Diazyme Laboratories, Poway, CA, USA) in the Erlangen centre.

Endoscopy records were reviewed to collect procedure-related data such as the size of NBD catheter placed, use of endoscopic sphincterotomy [(EST) i.e. cutting the biliary sphincter during ERCP], insertion of prophylactic temporary pancreatic duct (PD) stents and use of prophylactic rectal nonsteroidal anti-inflammatory drugs (NSAIDs). The volume of bile output from the catheter was recorded. Any AEs associated with the NBD procedure were reviewed. Post-ERCP pancreatitis (PEP) was defined as per consensus definition[16, 17]: (i) new or increased abdominal pain that was clinically consistent with a syndrome of acute pancreatitis and (ii) serum amylase or lipase ≥3× the upper limit of normal 24 h after the procedure, and (iii) prolongation of existing hospitalisation for at least 2 days. Severe PEP was defined as that resulted in the development of pancreatic necrosis or pseudocyst, or required additional endoscopic, percutaneous or surgical intervention. Cases that did not meet the definition of severe PEP were considered as mild PEP.

Statistical analysis

Data were analysed with GraphPad Prism 6.0 (GraphPad Software, Inc. La Jolla, USA). Data were not normally

distributed. Categorical variables were expressed as frequencies and percentages and continuous variables were expressed as median with interquartile range (IQR). The Wilcoxon signed-rank test (for paired samples) and Mann–Whitney test (for unpaired samples) were used for the comparison of continuous data. Correlation between variables was evaluated using Spearman's rank correlation test to compute the correlation coefficient (r). Fisher's exact test was used to compare categorical variables. Statistical significance was set at $P < 0.05$.

RESULTS

A total of 27 patients who underwent 29 NBD procedures ($n = 29$ cases) were included in this study. Table 1 summarises the baseline demographical, clinical and biochemical characteristics of study patients. Aetiologically, PBC (44%) was the commonest cause of pruritus followed by BRIC (29%). Before undergoing NBD, the proportion of patients treated with one, two, three and all four guideline recommended anti-pruritic drugs were 92.5%, 85.2%, 62.9% and 29.6% respectively. Twenty-three (85.2%) patients met the definition of refractory pruritus. Of the remaining four (14.8%) patients, two patients (both BRIC) had received only rifampicin and two patients (DILI and acute severe alcoholic hepatitis) had contraindications to receive anti-pruritic drug therapy.

Table 1 | Baseline clinical and biochemical characteristics of study patients

Age (years)*	41 (11)
Females, *n* (%)	16 (59.3)
Diagnosis, *n* (%)	
PBC	12 (44.4)
BRIC	8 (29.6)
DILI	2 (7.4)
Others†	5 (18.5)
Previous medical treatments, *n* (%)	
Rifampicin	25 (93)
Cholestyramine	21 (78)
Opiate antagonists	18 (67)
Sertraline	8 (30)
Plasmapheresis/ extracorporeal albumin dialysis	4 (15)
Ultraviolet phototherapy	4 (15)
Pre-NBD serum biochemistry*	
ALP (IU/L)	367 (311)
ALT (IU/L)	61 (90.5)
Bilirubin (μmol/L)	203.5 (455.3)
TBS (μmol/L)	144 (225.5)
Duration of NBD, days*	
All patients	7 (9.5)
PBC patients	5.5 (3.7)
BRIC patients	9 (12.5)
Pruritus VAS*	
Pre-NBD	10 (2)
Post-NBD	0.3 (3)
Bile output (mL/day)*	
Minimum	150 (335)
Maximum	400 (412.5)
Duration of treatment response, days*	
All patients	50 (345)
PBC patients	13 (68.25)
BRIC patients	459.8 (720.8)

PBC, primary biliary cholangitis; BRIC, benign recurrent intrahepatic cholestasis; DILI, drug-induced liver injury; ALP, alkaline phosphatase; ALT, alanine aminotransferase; TBS, total serum bile salts; NBD, nasobiliary drainage.

* Data expressed as median and (IQR).

† Other diagnoses included one case each of acute severe alcoholic hepatitis, acute viral hepatitis A, primary sclerosing cholangitis, progressive familial intrahepatic cholestasis and post-liver transplant biliary anastomotic stricture.

Effect of NBD on pruritus

Of the 29 NBD cases, pre- and post-NBD pruritus VAS data were not available in two cases. At baseline the median VAS score was 10 (IQR 2) suggesting all patients had severe pruritus. The median duration of NBD was 7 days (mean 16; range 2–86 days). Following NBD significant reduction was seen in the median pruritus VAS (from pre-NBD VAS 10.0 to post-NBD VAS 0.3, $P < 0.0001$) (Figure 2a). Overall, improvement in pruritus VAS was seen in 26/29 (89.6%) cases with complete resolution of pruritus (zero on VAS) in 12 (41.3%) cases (Figure 2b). Overall the median percentage decrease in pruritus VAS was 94% (Figure 2c). Also, NBD immediately resolved pruritus in nine (33%) patients who were free of pruritus within 24 h of starting drainage. One patient (3.7%) did not benefit from NBD (69 years male with BRIC; 21 days of NBD; average bile output 250 mL/day but post-NBD no change in pruritus VAS; no AEs).

Overall the median duration of treatment response was 50 days (IQR 345 days). When BRIC patients were excluded from the analysis, the overall median duration of treatment response was 14 days. As PBC ($n = 12$) and BRIC ($n = 8$) were the commonest causes of pruritus, we analysed data to compare these two groups of patients. The median duration of NBD was longer for BRIC patients compared to PBC patients (9 days vs. 5.5 days, $P = 0.04$) (Figure 3a). Similarly, the duration of treatment response was significantly longer for BRIC

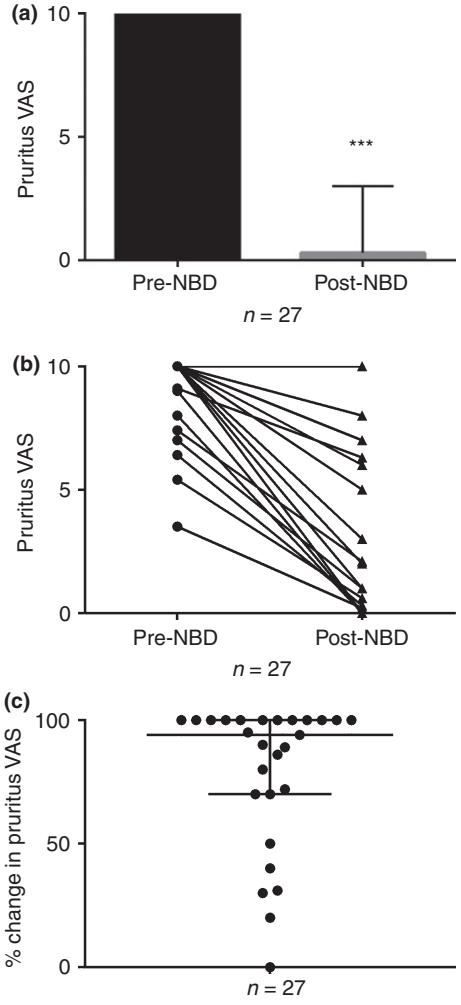

Figure 2 | Intensity of pruritus as measured by visual analogue scale (VAS) before (pre-NBD) and after (post-NBD) treatment with NBD. Significant decrease in pruritus VAS was seen post-NBD (a); pruritus VAS decreased in all but one patient (b) and the median percentage decline in pruritus VAS was 94% (c). ***$P < 0.0001$.

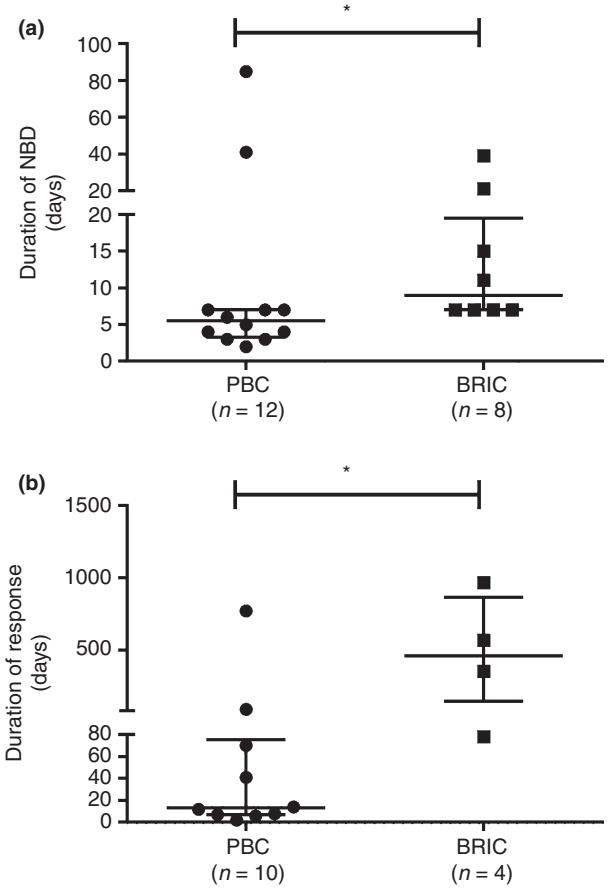

Figure 3 | Comparison of nasobiliary drainage (NBD) in PBC and BRIC patients. BRIC patients had significantly longer duration of drainage (a), and longer duration of treatment response (b). PBC, primary biliary cholangitis; BRIC, benign recurrent intrahepatic cholestasis. *$P < 0.05$.

patients compared to PBC patients (median 459 days vs. 13 days, $P = 0.02$) (Figure 3b).

Effect of NBD on serum biochemistry

Nasobiliary drainage significantly decreased serum ALP [367 (IQR 311) vs. 288.5 (IQR 315.5), $P = 0.001$] and serum bilirubin [203.5 (IQR 455.34) vs. 169.3 (IQR 285), $P = 0.03$], but there was no significant change in the levels of serum ALT [61 (IQR 90.5) vs. 71 (IQR 105), $P = 0.37$]. Only 12 (44.4%) of patients had TBS measured pre- and post-NBD and although a trend towards decline in the levels of TBS was observed, the change was not statistically significant [144 (IQR 225.5) vs. 58.5 (IQR 150.3), $P = 0.07$] (Figure 4).

Correlation analysis

No correlation was observed between total duration of drainage and duration of treatment response ($r = 0.36$, $P = 0.12$) (Figure S1a). This lack of correlation persisted even after excluding BRIC patients from analysis ($r = 0.20$, $P = 0.49$). Also, duration of treatment response did not correlate with daily volume of bile output ($r = 0.40$, $P = 0.19$ for minimum output and $r = 0.14$, $P = 0.65$ for maximum output) (Figure S1b, c). In addition, there was no correlation between percentage change in TBS levels and percentage change in pruritus VAS post-NBD ($r = -0.31$, $P = 0.30$).

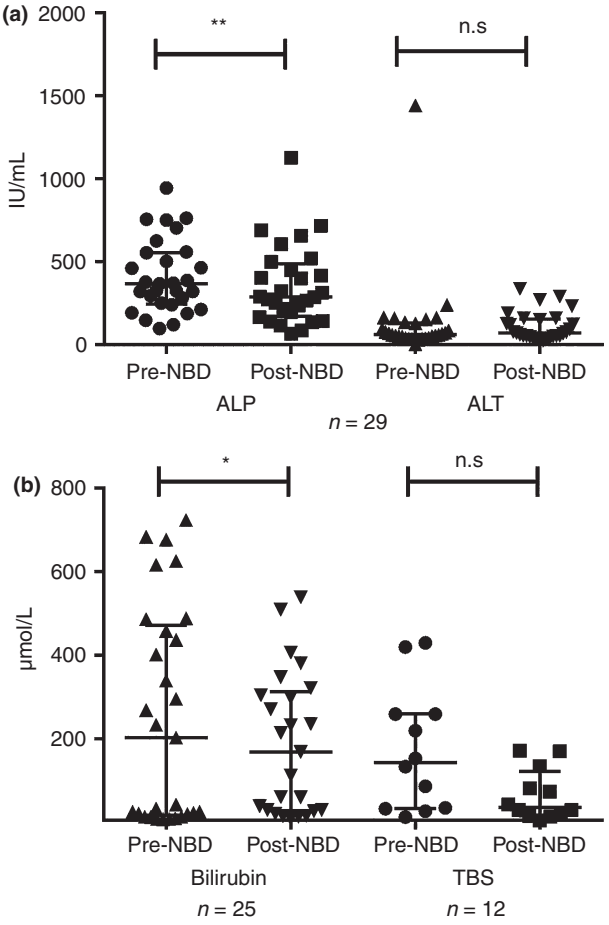

Figure 4 | Effects of NBD on laboratory parameters. Significant reductions were seen in serum ALP (a) and bilirubin (b) levels but not in serum ALT (a) or TBS (b). ALP, alkaline phosphatase; ALT, alanine aminotransferase; TBS, total serum bile salts. *$P < 0.05$, **$P = 0.001$, n.s, not significant.

Table 2 | Summary of adverse events associated with nasobiliary drainage

	Number of cases, *n* (%)
Total NBD procedures	29
Total AEs	10 (34.5)
PEP	9 (31)
Acute cholangitis	1 (3.4)
Frequency of PEP according to disease aetiology	
PBC	5 (55.5)
BRIC	3 (33.3)
Acute hepatitis A	1 (11.1)

NBD, nasobiliary drainage; AEs, adverse events; PEP, post-ERCP pancreatitis; PBC, primary biliary cholangitis; BRIC, benign recurrent intrahepatic cholestasis.

Adverse events

Adverse events were observed in 10/29 (34%) of cases (Table 2). Of these, nine (31%) were diagnosed with mild PEP and one was post-ERCP acute cholangitis based on clinical, biochemical or radiological features. There were no cases of severe PEP. Of the nine patients who developed PEP, four (44%) had EST at the time of placement of NBD catheter. There was no significant association between EST and pancreatitis [Fisher's exact test, $P = 0.40$, relative risk 1.69 (95% CI 0.59–4.82)]. Two (6.9%) patients had received prophylactic single-dose rectal indomethacin and none developed PEP. Of the four (13.8%) patients who had prophylactic temporary PD stent placement, one developed mild PEP. All AEs had resolved completely with appropriate medical management. There was no mortality associated with these AEs.

DISCUSSION

Pruritus is a frequent symptom reported by patients with cholestatic diseases. Recently, we reported the burden of pruritus in PBC patients in a comparative study of three large independent PBC cohorts (over 3500 patients in total) from UK, USA and Italy. The prevalence of pruritus in PBC was 60–70% and of these, nearly one-third of patients reported to suffer with persistent pruritus and up to 15% experienced severe pruritus since their diagnosis of PBC.[18] Generally, treatment of cholestatic pruritus is targeted at reducing the hepatic and systemic concentration of BS or other putative pruritogens. Indeed, this is the rationale for using cholestyramine (bind to BS in the intestine and reduce their re-absorption) and opioid antagonists (reduce the pruritogenic effect of endogenous opioids).[19] However, the treatment of cholestatic pruritus remains a formidable challenge and may be frustrating as the drug therapy is limited and not universally effective. In those who do not respond to medications, invasive options such as NBD are often explored.

Published literature on the use of endoscopic NBD in cholestatic pruritus is limited with only single centre case series. Stapelbroek *et al.* first showed quick (pruritus disappeared within 24 h of NBD) and complete disappearance of pruritus and normalisation of TBS levels in three BRIC patients following 11–21 days of NBD and the duration of treatment response lasted for 8–12 months.[11] Similarly, Beuers *et al.* reported that following a mean 4.1 days of NBD in three PBC patients, two were completely free of pruritus within 24 h.[12] Subsequently, Singh *et al.* reported complete remission of pruritus

following 7 days of NBD in six patients with intractable pruritus secondary to viral hepatitis A, B and E.[13] More recently, a UK single centre report of three patients (2 PBC, 1 BRIC) has suggested long-term NBD is successful in maintaining remission of pruritus.[14]

In comparison to previous studies, our study is unique since it is a multicentre study and to the best of our knowledge, it is the largest retrospective study describing the utility of NBD in cholestatic pruritus. In addition to adding evidence to the reported advantages of NBD, our study attempts to answer some of the previously unanswered questions and uncertainties about NBD.

This study shows that NBD is an effective treatment option for refractory pruritus of different cholestatic aetiologies. Except for one patient, all patients in our study benefitted from NBD with significant improvement in pruritus. In particular, NBD effectively terminated pruritus attacks in all patients with BRIC. This observation is in line with previous reports and suggests that exacerbations of BRIC can be effectively treated with NBD. The speed of induction of remission with NBD varies between patients with a third achieving immediate and dramatic remission of pruritus within 24 h of initiating drainage.

This study also shows that after cessation of NBD patients achieve a short period of 'pruritus-free' remission. Patients usually wish to know the average duration they can expect to remain itch-free *after* stopping the drainage. Accordingly, we evaluated our data by defining the duration of treatment response as the time taken to return to pre-treatment pruritus level after the removal of NBD catheter. It seems that the duration of treatment response is variable and likely depends on the underlying disease aetiology. Overall, the median duration of treatment response was 50 days. Of note, the duration was shorter for PBC patients (median 13 days) in comparison to BRIC patients (median 459.8 days). This apparent difference in the beneficial effect of NBD between PBC and BRIC patients is likely due to the underlying pathophysiology of these conditions. It is a known clinical fact that while pruritus in PBC usually recurs when treatment is stopped, BRIC patients present in episodes and are known to remain in spontaneous remission for months to years in between attacks.[20] The implication of this finding is that PBC patients undergoing NBD should be advised to expect only a couple of weeks of remission after NBD.

Another pertinent but previously unexplored question relates to the effect of duration of drainage on treatment response – i.e. 'do patients need longer duration of drainage to achieve longer period of remission?' Our results suggest that duration of treatment response is essentially independent of duration of drainage and this lack of correlation was demonstrated even after removing BRIC patients from the analysis. Therefore, the duration of NBD should be guided by the patient tolerance of the catheter and benefit of drainage on their pruritus but in general BRIC patients usually do not need more than 7–10 days of drainage.

We observed favourable effect of NBD on liver biochemical parameters with significant improvement in the levels of serum ALP (a biomarker of cholestasis) and bilirubin following NBD. The positive impact of NBD on liver biochemistry may have implications on patients with chronic cholestasis. In this regard, it has been suggested that long-term NBD in PBC patients achieves sustained remission from pruritus and improvement in liver biochemistry.[14]

There are conflicting data on the effect of NBD on TBS with two studies showing significant reduction[11, 13] and one study showing only transient decrease[12] in TBS following NBD. We did not observe significant change in the levels of TBS and the percentage change in TBS levels did not correlate with the percentage change in pruritus VAS. Our results could be due to insufficient data as the pre- and post-NBD data on TBS were available in only 12/29 (41%) cases. Also, different centres used different methods to measure TBS and the variation in the assays may have affected the TBS measurements and contributed to our negative results. But if our results are confirmed, they are against the conventional hypothesis that NBD reduces the systemic levels of pruritogenic BS by interrupting their enterohepatic circulation.[3] Therefore, more studies are needed to see any true effect of NBD on BS pattern and future studies should evaluate levels of sub-species of BS both in the serum and bile. Alternatively, the benefit of NBD on pruritus could be secondary to removal of other yet unidentified pruritogens from the enterohepatic circulation.[21]

Treatment with NBD procedure may be associated with AEs. In our study, 34% had AEs; a majority was due to PEP. This high rate of AEs could be attributable to three main factors. First, the NBD catheter is a transpapillary endoprosthesis that obstructs the pancreatic orifice and inhibits the flow of pancreatic fluids, thus increasing the risk of PEP.[22] The gauge of the NBD catheters may be another factor contributing to the high risk of PEP. Conventionally, a 6Fr or a 7Fr NBD catheter is used and all patients in our study received 7Fr catheter. However, a recent study (n = 165) proposed that compared to a 6Fr catheter, using a 4Fr catheter for NBD reduces the incidence of PEP (15.7% vs. 3.7%,

$P = 0.02$) without any significant difference in the biliary output.[10] Second, 44% of patients who developed PEP had EST during placement of NBD catheter. Since, EST is a known independent risk factor for PEP[23] we advocate caution against routine use of EST while placing NBD catheter. Finally, recently published meta-analyses strongly support the use of rectal NSAIDs in high-risk patients to prevent PEP.[17, 24, 25] But majority of NBD procedures in our series (started in 2006) were performed prior to these publications and only a small number of patients received prophylactic rectal NSAIDs. This may also have contributed to the high rate of AEs.

Overall, the results of our study show that NBD is an effective salvage therapy but it carries high risk of AEs. Therefore based on current evidence in high-risk ERCP[17, 24, 25], we recommend routine use of prophylactic rectal NSAIDs (indomethacin or diclofenac) in all patients undergoing NBD.

The main strength of our study is the real life data and large sample size of different aetiologies of cholestasis collected from multiple centres. But the obtained results may be limited due the retrospective analysis of the data. Nevertheless, our study demonstrates that NBD is an important and an effective rescue treatment of refractory cholestatic pruritus. There are no guidelines on how to use NBD in cholestatic pruritus and little information is available for clinicians to deliver this treatment effectively in routine clinical practise. Therefore, based on our study results and our cumulative experience of using NBD, we propose recommendations (Box 1) to optimise its safety and effectiveness in treating cholestatic pruritus.

CONCLUSIONS

In this large retrospective study of NBD in treating cholestatic patients with refractory pruritus, we provide further evidence that NBD is effective in inducing remission of pruritus of different aetiologies. In addition, NBD has favourable effect on serum alkaline phosphatase and bilirubin levels. The duration of response to NBD is independent of the duration of drainage and the daily bile output. Unfortunately, the effect of NBD is usually temporary and the procedure is invasive and frequently associated with complications. All patients undergoing NBD should receive prophylaxis for post-ERCP pancreatitis. We outline our proposals to optimise the use of NBD in clinical practise. We urge the need for prospective studies to confirm our findings, assess the effect of NBD on levels of BSs in the serum and bile and evaluate the role of long-term NBD.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Duration of treatment response did not correlate with total duration of nasobiliary drainage (a)

Box 1 A suggested approach to the use of nasobiliary drainage (NBD) in treating patients with cholestatic pruritus in clinical practise: 'Dos and Don'ts'.

- Nasobiliary drainage can be considered in patients with severe pruritus who have failed to respond to conventional drug therapy recommended by current guidelines.
- Nasobiliary drainage is a high-risk procedure, therefore, should be used selectively and cautiously and ideally performed by experienced endoscopists in high-volume centres with specialist input from hepatologists.
- When obtaining informed consent for NBD:
  - i Give a realistic estimation of expected benefit;
  - ii reassure that majority of patients stop itching within few days of drainage;
  - iii warn that benefit is temporary and itch is likely to recur after removal of the NBD catheter.
  - iv Inform PBC patients to expect shorter duration (only couple of weeks) of remission
  - v Explain the potential risks of complications including post-ERCP pancreatitis (PEP).
- To reduce the incidence and severity of PEP:
  - i Avoid routine use of endoscopic sphincterotomy;
  - ii give a single-dose rectal indomethacin or diclofenac immediately before or after ERCP.
- For BRIC patients limit the duration of NBD to 7–10 days.
- For PBC patients, the duration of NBD should be guided by the patient tolerance of the catheter and benefit of drainage on their pruritus.

or with daily minimum or maximum volume of bile output (b, c).

AUTHORSHIP

Guarantor of the article: Vinod S. Hegade.
Author contributions: VSH and DEJ were involved in study concept and design. VSH served as the co-ordinating investigator, conducted the statistical analysis and wrote the first draft of the manuscript. All authors were involved in the critical revision of the manuscript. All authors approved the final version of the manuscript, including the authorship list.

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STUDY PROTOCOL Open Access

BAT117213: Ileal bile acid transporter (IBAT) inhibition as a treatment for pruritus in primary biliary cirrhosis: study protocol for a randomised controlled trial

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Abstract

Background: Pruritus (itch) is a symptom commonly experienced by patients with cholestatic liver diseases such as primary biliary cholangitis (PBC, previously referred to as primary biliary cirrhosis). Bile acids (BAs) have been proposed as potential pruritogens in PBC. The ileal bile acid transporter (IBAT) protein expressed in the distal ileum plays a key role in the enterohepatic circulation of BAs. Pharmacological inhibition of IBAT with GSK2330672 may reduce BA levels in the systemic circulation and improve pruritus.

Methods: This clinical study (BAT117213 study) is sponsored by GlaxoSmithKline (GSK) with associated exploratory studies supported by the National Institute for Health Research (NIHR). It is a phase 2a, multi-centre, randomised, double bind, placebo controlled, cross-over trial for PBC patients with pruritus. The primary objective is to investigate the safety and tolerability of repeat doses of GSK2330672, and explore whether GSK2330672 administration for 14 days improves pruritus compared with placebo. The key outcomes include improvement in pruritus scores evaluated on a numerical rating scale and other PBC symptoms in an electronic diary completed twice daily by the patients. The secondary outcomes include the evaluation of the effect of GSK2330672 on total serum bile acid (BA) concentrations, serum markers of BA synthesis and steady-state pharmacokinetics of ursodeoxycholic acid (UDCA).

Discussion: BAT117213 study is the first randomised controlled crossover trial of ileal bile acid transporter inhibitor, a novel class of drug to treat pruritus in PBC. The main strengths of the trial are utility of a novel, study specific, electronic symptom diary as patient reported outcome to measure the treatment response objectively and the crossover design that allows estimating the treatment effect in a smaller number of patients. The outcome of this trial will inform the trial design of future development phase of the IBAT inhibitor drug. The trial will also provide opportunity to conduct metabonomic and gut microbiome studies as explorative and mechanistic research in patients with cholestatic pruritus.

Trial registration: EudraCT number: 2012-005531-84, ClinicalTrials.gov Identifier: NCT01899703, registered on 3rd July 2013

Keywords: Pruritus, Primary biliary cholangitis, PBC, Ileal bile acid transporter, IBAT

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Background

Primary biliary cholangitis (cirrhosis) (PBC) is an autoimmune chronic cholestatic liver disease with a prevalence of 30/100,000, typically affecting middle aged women (female: male ratio 10:1) [1]. In untreated cases immunologically mediated chronic cholestasis ultimately results in liver cirrhosis with associated complications such as portal hypertension, varices, ascites, hepatocellular carcinoma and death. The precise aetiology of PBC is unclear, although genetic and environmental factors are thought to play a key role.

Pruritus (itch) is one of the characteristic symptoms of PBC and can affect patients at any stage of the disease [2]. Recently, we studied the scale of the pruritus symptom within the United Kingdom (UK)-PBC cohort, a national cohort of over 3000 PBC patients recruited from every hospital in the UK. In this cohort 60–70 % of PBC patients reported experience of pruritus at some point in the course of the disease, 30 % had persistent pruritus and 15 % suffered with severe pruritus since the diagnosis of PBC [3]. A similar scale of symptom burden has also been reported in PBC cohorts from USA and Italy [4]. Pruritus has a negative impact on perceived quality of life in PBC patients and has been associated with sleep deprivation, worsened day time fatigue and when severe, may lead to depression and suicidal tendencies [5].

Ursodeoxycholic acid (UDCA), the current standard of care for PBC patients and the only licenced therapy for PBC has no role in treating pruritus [2]. Current treatment of pruritus in PBC involves step-wise use of specific anti-pruritic agents in line with current international guidelines [2, 6]. These drugs include cholestyramine, rifampicin, naltrexone and sertraline. Of these, cholestyramine is the only licensed drug for treatment of cholestatic pruritus and use of other drugs is "off-label". The limitations of these drugs are that their efficacy is not universal, treatment is often associated with side effects and there is a need for regular monitoring for liver toxicity. Patients with medically refractory pruritus may either need to undergo phototherapy, invasive interventions such as nasobiliary drainage or extracorporeal albumin dialysis for temporary relief of pruritus, or may be considered for liver transplantation (LT) which is typically curative. Therefore, development of better drug therapies with fewer side effects is an unmet clinical need for PBC patients [7].

Ileal bile acid transporter (IBAT)

Primary BAs are synthesized in the liver from an enzymatic catabolism of cholesterol, a process regulated by enzyme cytochrome P450 (CYP) 7A1. Unconjugated BAs are conjugated in hepatocytes with glycine and taurine, secreted into the bile and stored in the gallbladder. Upon ingestion of a meal, conjugated BAs ("bile salts") are released into the intestinal lumen where they facilitate absorption of fat and fat soluble vitamins. After their normal physiological function is completed in the intestine, BAs reach the ileum where they are reabsorbed. The ileal bile acid transporter [(IBAT), also called apical sodium dependent bile acid transporter (ASBT)], is a protein predominantly located in the terminal ileum and serves as the main transporter mediating the ileal uptake of conjugated BAs and their return to the liver via the portal circulation (enterohepatic circulation) [8].

Bile salts (and their protonated form, BAs) have been suggested to play role in the pathogenesis of pruritus in cholestatic conditions. In cholestasis, the ileal uptake of BAs has been shown to be upregulated [9]. Also, the evidence that pruritus dramatically improves in patients undergoing nasobiliary drainage [10] and is effectively cured by LT [11] suggests a direct or indirect role for BAs in mediating cholestatic pruritus. Therefore a pharmaceutical agent that can reduce their levels in the enterohepatic and systemic circulations may be predicted to improve pruritus. In two animal studies treatment with IBAT inhibitors SC-435 and A4250 produced BA malabsorption and attenuated BA-mediated cholestatic liver injury by reducing biliary BA output [12, 13]. In humans, use of IBAT inhibitor A4250 has been shown to decrease the serum BAs and increase faecal BAs by highly efficient interruption of their enterohepatic circulation with no serious adverse events [14].

GSK2330672

GSK2330672 is a selective inhibitor of human IBAT and it is designed to be a non-absorbable agent restricted to the gastrointestinal (GI) tract. GSK2330672 is expected to block the uptake of BAs in the terminal ileum, increase their excretion in the faeces and decrease the amount of BAs returning to the liver via enterohepatic circulation (Fig. 1). Therefore treatment of PBC patients with oral GSK2330672 is postulated to reduce concentrations of BAs in the systemic circulation and in turn improve pruritus.

In phase I studies involving 42 healthy volunteers single and repeat doses of GSK2330672 for 12 days were shown to be safe and tolerable (ClinicalTrials.gov Identifier: NCT01416324). GI symptoms were the most common reported drug-related adverse events (AEs). These included diarrhoea, abdominal pain, bowel movement irregularity and positive faecal occult blood tests. All AEs were considered mild or moderate in severity.

Methods

Study design and overview

The BAT117213 study is a Phase 2a trial, designed to investigate treatment with GSK2330672 in PBC patients with

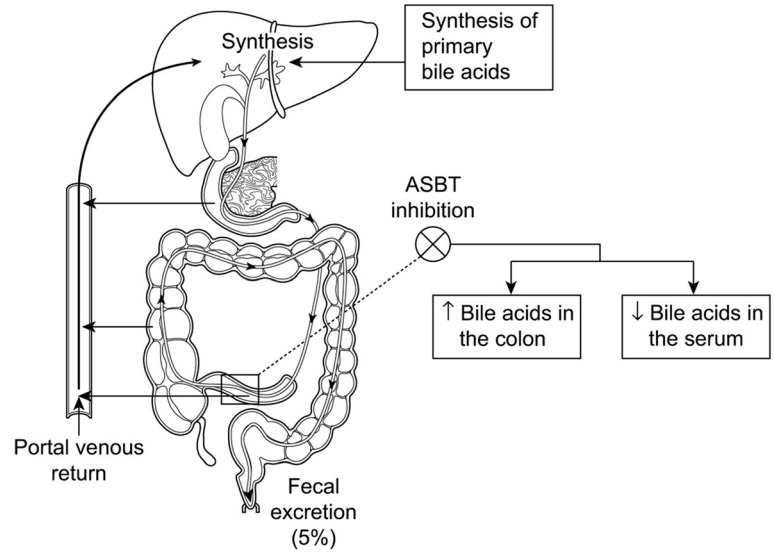

Fig. 1 Mechanism of IBAT (ASBT) inhibitor drug. GSK2330672 interrupts the enterohepatic circulation of bile acids by selectively inhibiting the IBAT protein located in the terminal ileum, thereby reducing the levels of bile acids in the systemic circulation. (Image reproduced with permission from [31]). IBAT, ileal bile acid transporter; ASBT, apical sodium-dependent bile acid transporter

pruritus (ClinicalTrials.gov Identifier: NCT01899703). This is a multicentre, randomized, double-blind, placebo-controlled, two-period cross-over trial which in addition to studying the safety and efficacy of the drug will provide an opportunity to conduct explorative studies (including metabonomic and microbiomic studies) to develop novel mechanistic insights into cholestatic pruritus.

Following written informed consent, patients with PBC and pruritus were screened to establish study eligibility. Eligible subjects participated in a two-week placebo run-in period followed by randomization in a crossover fashion to receive placebo or GSK2330672 treatment during two consecutive two-week study periods (Sequence 1/Sequence 2) (Fig. 2). Subjects then participated in a two-week follow up period of placebo dosing. Total duration of the study was 56 days from the first day of dosing.

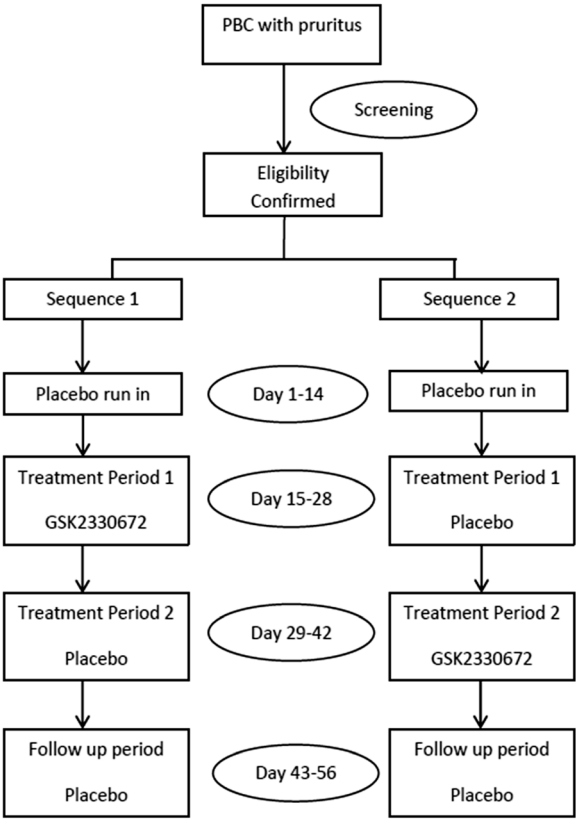

Fig. 2 Flowchart of BAT117213 study design

Study population

The study population consisted of PBC patients with ongoing pruritus. All participants had a diagnosis of definite or probable PBC established according to recognised criteria [2, 6]. Key inclusion and exclusion criteria for study eligibility are detailed in Table 1. The trial entry criteria for ongoing pruritus was defined as: i) severe pruritus significantly impacting daily life and proven refractory to medical therapy, or ii) pruritus that is newly diagnosed or untreated, or iii) pruritus that is unresolved with the use of a single antipruritic agent. To determine subject eligibility for study enrolment outpatient screening was performed within 45 days before the first dose administration. Subjects meeting all the inclusion criteria

Table 1 Eligibility criteria

A subject will be eligible for inclusion in this study only if all of the following criteria apply:
1. Male or female aged between 18 and 75 years of age inclusive, at the time of signing the informed consent.
2. Proven or likely PBC, as demonstrated by the patient presenting with at least 2 of the following:
• History of sustained increased AP levels first recognized at least 6 months prior to Day 1
• Positive AMA titer (>1:40 titer on immunofluorescence or M2 positive by ELISA) or PBC-specific antinuclear antibodies (antinuclear dot and nuclear rim positive)
• Liver biopsy consistent with PBC.
3. Screening AP value < 10 × ULN.
4. Subjects should be on stable doses of UDCA for >8 weeks at time of screening. Subjects not taking UDCA due to intolerance may be enrolled into this study following agreement by the GSK medical monitor.
5. Symptoms of pruritus as follows (one of the following):
• PBC patients with severe symptoms of pruritus that significantly impact daily life and have proven refractory after at least one previous therapy has been discontinued due to inadequate clinical response, poor tolerability or adverse events. Temporary response to cooling, 1 % menthol in aqueous cream, nasobiliary drainage or MARS therapy is still compatible with refractory itch.
• PBC patients with unresolved symptoms with use of a single antipruritic agent who can tolerate washout of current therapy for the duration of the trial.
• PBC patients seeking treatment for pruritus that is newly diagnosed or previously untreated.
6. A female subject is eligible to participate if she is not pregnant, as confirmed by a negative serum human chorionic gonadotropin (hCG) test or at least one of the following conditions applies:
• Non-reproductive potential defined as pre-menopausal females with a documented tubal ligation or hysterectomy; or postmenopausal defined as 12 months of spontaneous amenorrhea
• Females on hormone replacement therapy (HRT) and whose menopausal status is in doubt will be required to use one of the highly effective contraception methods along with either a second form of highly effective contraception or barrier protection (condoms with spermicide) if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of post-menopausal status prior to study enrolment.
• Reproductive potential and agrees to follow one of the specified contraception options for the specified duration of time.
7. Capable of giving written informed consent, which includes compliance with the requirements and restrictions listed in the consent form.
Main exclusion criteria:
1. Screening total bilirubin >1.5x ULN. Isolated bilirubin >1.5xULN is acceptable if bilirubin is fractionated and direct bilirubin <35 %.
2. Screening ALT or AST >4x ULN.
3. Screening serum creatinine >2.5 mg/dL (221 umol/L).
4. History or presence of hepatic decompensation (e.g., variceal bleeds, encephalopathy, or poorly controlled ascites).
5. History or presence of other concomitant liver diseases including hepatitis due to hepatitis B or C virus (HCV, HBV) infection, primary sclerosing cholangitis (PSC), alcoholic liver disease, definite autoimmune hepatitis or biopsy proven non-alcoholic steatohepatitis (NASH).
6. Administration of the following drugs at any time during the 3 months prior to screening for the study: colchicine, methotrexate, azathioprine, or systemic corticosteroids.
7. Current or chronic history of inflammatory bowel disease, chronic diarrhoea, Crohn's disease or diarrhoea related to malabsorption syndromes.
8. Faecal occult blood positive test at screening.
9. Based on averaged QTc values of triplicate ECGs obtained at least 5 min apart:
• QTc ≥ 450 msec; or
• QTc ≥ 480 msec in subjects with Bundle Branch Block.
10. History of sensitivity to heparin or heparin-induced thrombocytopenia.
11. History of sensitivity to any of the study medications, or components thereof or a history of drug or other allergy that, in the opinion of the investigator or GSK Medical Monitor, contraindicates their participation.
12. History of regular alcohol consumption within 6 months of the study defined as an average weekly intake of >21 units for males or >14 units for females.
13. A positive pre-study drug/alcohol screen. A minimum list of drugs that will be screened for include amphetamines, barbiturates, cocaine, opiates, cannabinoids and benzodiazepines.
14. Where participation in the study would result in donation of blood or blood products in excess of 500 mL within a 56 day period.
15. The subject has participated in a clinical trial and has received an investigational product within the following time period prior to the first dosing day in the current study: 30 days, 5 half-lives or twice the duration of the biological effect of the investigational product (whichever is longer).
16. Exposure to more than four new chemical entities within 12 months prior to the first dosing day.

and no exclusion criteria were enrolled by a designated investigator from the centre.

Study objectives and outcomes

The primary objective of this trial is to investigate the safety and tolerability of oral GSK2330672 compared with placebo when administered for 14 days to PBC patients treated with UDCA. The secondary objectives are: 1) to evaluate the effects of oral GSK2330672 on subjects' experience of pruritus and its impact; 2) to demonstrate the lack of effect of oral GSK2330672 on steady-state pharmacokinetics (PK) of UDCA when UDCA is administered alone or in combination with GSK2330672; 3) to investigate the steady state PK of oral GSK2330672; 4) to evaluate the effects of oral GSK2330672 on total serum BA concentrations and serum markers of BA synthesis [7-alpha-hydroxy-4-cholesten-3-one (C4)]. Exploratory objectives of the study include investigating effects of 14-day oral administration of GSK2330672 on markers of disease progression, subject's experience of benefits and disadvantages with GSK2330672, metabonomics, microbiomics and pharmacogenomics. The primary, secondary and exploratory outcome measures are given in Table 2.

Recruitment and consent

The study is a UK multicentre study and recruitment was planned in three large, tertiary referral National Health Service (NHS) hospitals based in Newcastle, Birmingham and Cambridge. Patients were recruited from the out-patient department cohorts of these hospitals and in addition, trial information was published in newsletters and magazines from the UK-PBC research group and patient support groups (LIVErNORTH and PBC Foundation). Any PBC patient interested in participating

Table 2 Primary, secondary and exploratory outcome measures of the BAT117213 study

1. Primary outcome measures:
• Safety assessment following repeat doses of oral GSK2330672
Safety will be assessed using clinical haematology, clinical chemistry, urinalysis, single 12-lead electrocardiograms (ECGs), vital sign measurements including systolic and diastolic blood pressure (BP) and pulse rate.
• Tolerability assessment using Gastrointestinal Symptom Rating Scale (GSRS)
Subjects will be asked to complete GSRS, a validated scale and the scale will be used to assess symptoms experienced by subject over the preceding 5 to 7 days
• Faecal occult blood (FOB) testing
FOB monitoring for symptomatic or visible gastrointestinal bleeding or asymptomatic occult bleeding
2. Secondary outcome measures:
• Subject reported outcomes-daily pruritus 0 to 10 point scale
This scale will be implemented to measure symptoms of itching as well as other associated symptoms twice daily in the morning and evening (approximately the time of drug dosing). The severity of itching symptoms from "0" (no itching) to "10" (worst possible itching) will be recorded
• Subject reported outcomes-5D-itch scale
The 5-D itch scale covers five dimensions of itching experienced by subjects including duration, degree, direction, disability and distribution
• Subject reported outcomes-PBC-40 quality of life (QoL) scale
The PBC-40 QoL scale has six domains; cognitive, itch, fatigue, social, emotional and (other) symptoms
• Measurement of serum profiles of total bile acid concentrations and 7-alpha hydroxy-4-cholesten-3-one (C4). C4 is the first committed step of bile acid synthesis from cholesterol
• Steady-state pharmacokinetics (PK) assessment of UDCA and its taurine and glycine conjugates taurodeoxycholic acid (TUDCA) and glycoursodeoxcholic acid (GUDCA).
Blood sample will be collected for measurements of steady state PK parameters of UDCA and its metabolites including maximum observed plasma concentration (C_{max}), time to C_{max} (t_{max}) and terminal phase half-life (t1/2).
3. Exploratory outcome measures:
• Markers of disease progression: ALT/AST, AP, GGT, bilirubin, albumin, PT/INR
• An exit interview conducted at end of follow-up phase to assess subject's experience of benefits and disadvantages with GSK2330672
• Pharmacogenomics for genes related to pruritus and GSK2330672 response
• Metabonomics to study serum bile acid species, serum autotaxin and FGF-19 before and after treatment with GSK2330672
• Microbiomics to study gut microbiota in PBC patients with pruritus

in the study could contact the study team at the centre nearest to their location either directly or via referral from local primary or secondary care physicians. The UK-PBC platform was utilised for recruitment using a similar approach to the to the RIT-PBC trial reported recently by our group [15]. The established UK-PBC database was screened for patients with PBC-40 itch domain scores meeting the definitions of persistent and/or severe pruritus. The clinicians looking after these patients were contacted to approach the patients and interested patients were referred to their local recruiting centre. All participants gave their written consent to participation before screening investigations were performed. Participants completed the consent process with study investigators trained in Good Clinical Practice (GCP) and assessment of capacity.

Randomisation

All eligible subjects enrolled in the study were randomised to either Sequence 1 or Sequence 2 to receive oral placebo or GSK2330672 for a 14-day period in a cross-over fashion (Fig. 2). Sequence 1 was GSK2330672 for 14 days followed by placebo for 14 days and Sequence 2 was placebo for 14 days followed by GSK2330672 for 14 days. Randomisation was carried out via a dedicated electronic system for randomisation-RAMOS (Randomisation and Medication Ordering System) by generating a unique randomisation number for each participant that linked to the corresponding allocated sequence of study drug.

Study treatment

The investigational medicinal product used in this study was GSK2330672. The control intervention was placebo. Both GSK233072 and placebo were manufactured at a dedicated manufacturing unit in London (UK) and dispensed as 30 g aliquots of oral solution into amber glass bottles for distribution to participating study centres. The study centres supplied solutions to subjects in accordance with the randomization schedule. Subjects consumed the entire quantity of one or two bottles of study drug twice daily followed by two 50 mL rinses of water. All patients started the study with 14-days placebo run in period followed by 14-days treatment with GSK2330672 or placebo in a cross over fashion.

Dose escalation and stopping criteria

The initial dose of GSK2330672 was 45 mg and all patients were asked to increase the dose to 90 mg on day 4. If this was not tolerated, they were asked to continue at 45 mg and attempt a dose increase again two days later. If 90 mg could not be tolerated by the end of day 7, subjects were asked to continue only 45 mg.

Following stopping criteria were in place to assure subject safety: 1) to stop the study treatment if the stopping criteria for liver chemistry were met [ALT >5-8 x upper limit of normal (ULN), bilirubin > 1.5-2 x ULN], and 2) to withdraw the subject from the study if corrected QT (QTc) interval withdrawal criteria were met based on their average values on triplicate ECGs separated by five minutes. These were QTc > 500 msec, or uncorrected QT >600 msec, or QTc >60 msec change from baseline). If a subject met the stopping criteria, appropriate safety follow-up assessments and procedures were completed.

Concomitant medications

Before starting the study, all patients were advised to stop using their usual anti-pruritic agents including cholestyramine, colesevelam, rifampicin, naltrexone, sertraline, gabapentin and anti-histamines. The use of these medications was prohibited during the study period until the final follow-up period when rescue medications were permitted. Application of topical agents used to relieve pruritus was permitted during the study only if agents did not contain active ingredients in the list of prohibited agents and with prior agreement of the clinical investigator. Subjects were asked to abstain from taking new prescription or new non-prescription drugs (including vitamins and dietary or herbal supplements), from the start of the placebo run-in period until completion of the follow-up visit. The use of UDCA was permitted and patients who were on UDCA were standardised to receive Ursofalk®(Dr. Falk Pharma UK Ltd) once daily preparation at dose 13–15 mg/kg/day and instructed to take it at bed time.

Patient reported outcomes

Existing patient reported outcome (PRO) measures to assess the impact of PBC symptoms include the PBC-40, a widely acceptable, validated, disease-specific questionnaire and the 5-D Itch scale [16, 17]. However, for this study a more specific PRO measure was needed that could detect the severity and variability of pruritus and other PBC symptoms and potential treatment effects on a daily basis with a short recall period. The development of such a measure began with interviews with PBC patients to identify additional characteristics of pruritus and other symptoms and their impact on sleep and daily activities. With input from PBC patients and PRO experts a new electronic patient reported outcome (ePRO) diary was developed to assess the severity of the pruritus and other PBC symptoms. Subjects completed the ePRO diary every morning and evening before dosing the study drug. In the ePRO diary pruritus severity was rated using a numerical rating scale (NRS). Psychometric testing to support the validity and reliability of the ePRO will be evaluated with data from the current clinical study.

Data analysis

Statistical analysis

This trial is designed to estimate the effect of study drug GSK2330672 relative to placebo when co-administered with UDCA on pruritus symptom, markers of efficacy and disease progression and the PK of UDCA. No formal hypothesis will be tested.

The efficacy endpoint in this study is the patient reported rating of pruritus severity scores. Pruritus will be measured in three different PROs: pruritus NRS using the ePRO, the 5-D itch scale and the PBC-40 questionnaire [16, 17]. Changes in pruritus NRS will be used as the key measure of the efficacy endpoint and will be analysed using a mixed effects model with fixed effect terms for treatment period and sequence to examine differences between GSK2330672 and placebo. Subject will be treated as a random effect in the model. Point estimates and their associated 95 % confidence interval (CI) will be constructed for the mean differences in pruritus severity scores.

Data from subjects that are co-administered UDCA as part of their standard care will be analysed similarly for PK endpoints. Following log-transformation, maximum observed plasma concentration (C_{max}), AUC (0–12 h) and AUC (12–24 h) of UDCA and glycine and taurine conjugated metabolites of UDCA (TUDCA and GUDCA) will be separately analysed. This will be done using a mixed effects model with fixed effect terms for treatment period and sequence to examine differences between UDCA administration with and without GSK2330672. Point estimates and corresponding 90 % CI will also be constructed for the difference and/or ratio between the mean of the test treatment (UDCA plus GSK2330672) and the mean of the reference treatment (UDCA alone).

Sample size

The efficacy endpoint in this study is pruritus score and the sample size for efficacy endpoint is based on the pruritus 0 to 10 points scale. On this scale the average effect of rifampicin is 1.62 points and the reported pooled total standard deviations of various anti-pruritic drugs ranges from 1.22 to 3.84 points [18, 19]. Assuming that GSK2330672 is at least as effective as rifampicin, a sample size of 40 will result in a reasonable power (>90 %) if the standard deviation (SD) is 3.1 points or less. For estimation of relative bioavailability 20 subjects taking UDCA are required to ensure that the resultant 90 % CI of the ratio will be within 0.8 and 1.25 assuming that the true ratio is 1 and the SD on the log10 scale is less than 0.25.

An initial sample-size of 40 subjects was selected based on considerations of both efficacy and PK endpoints. However, due to the uncertainty around sample-size assumptions a series of interim analyses for futility and possible sample-size re-estimation were carried out at regular intervals. Data from completed patients were reviewed by an unblinded review committee (composed of GSK personnel not directly involved in study conduct). As the probability of demonstrating sufficient difference was high, the sponsor revised the sample-size from 40 to 22. No other changes to study conduct were planned as a result of the interim analyses.

Conduct of the trial

The conduct of the trial followed the principles outlined in the NHS research governance framework for health and social care, GCP and the guiding principles of the 2008 Declaration of Helsinki. The trial involved the participant visiting the study centre a total of six times including screening visit, day 1 visit, three consecutive fortnightly in-patient stays (each up to 36 h) and a follow up visit. The schedule of study procedures during these visits and data collection is summarised in Table 3.

Protocol deviation or exemptions were not allowed with the exception of immediate safety concerns. All Investigators at recruiting sites followed standard operative procedures for collection, handling, processing and storage of samples (blood, urine and stool) collected at study visits. All clinical and non-clinical subject data including medical history (to capture co-morbidities and concomitant medications) and physical examinations were entered into electronic case report forms (eCRFs). No patient identifiable information was entered in the eCRFs. All participants were allocated a unique study identifier which was used on eCRFs transmitted electronically to the sponsor and combined with data provided from other sources in a validated data system.

Study monitoring

The study sponsor performed periodic monitoring at each study centre to monitor the study conduct and site activity. The monitor had direct access to all relevant documents to verify the data for completeness, accuracy and authenticity and the site's compliance with study protocol. All monitoring findings were reported and followed up in a timely manner. Periodic interim analysis of the trial were undertaken to determine as to whether the study should be modified, continued or terminated.

Adverse events

AEs and serious adverse events (SAEs) were collected from the start of the placebo run-in period (day 1) until the follow-up contact (day 56). The investigator and site staff were responsible for detecting, documenting and reporting events that meet the definition of an AE or SAE. All SAEs were recorded and reported to the study sponsor within 24 h. Periodic reviews of the safety data were performed and presented during interim analysis to both the sponsor and the study investigators.

Sponsorship, insurance and indemnity

In accordance with the Association of the British Pharmaceutical Industry (ABPI) guidance, the trial sponsor had policies in place regarding compensation for any trial related harm due to negligence or otherwise. The trial sponsor had insurance to cover indemnity in respect of potential liability arising from negligent harm related to study design. Due to the commercial nature of the study there were also arrangements for non-negligent compensation. The participating study centres were NHS hospitals and the NHS indemnity covered NHS staff and medical academic staff with honorary NHS contracts conducting the study for potential liability in respect of negligent harm arising from the conduct of the study.

Trial status

The BAT117213 study was opened for recruitment in January 2014 with first patient recruited in March 2014. The initial recruitment target was 40 subjects. Following review of safety and efficacy of data from 11 patients at the first interim analysis in March 2015, the sponsor decided to continue the study recruitment. A second interim analysis of the data from 19 patients was performed in July 2015 and the sponsor decided to reduce the total sample size to 22 patients. The recruitment ended in October 2015 with all 22 patients randomised from two trial sites (Newcastle 13; Birmingham 9). The treatment follow-up of participants was completed in December 2015. The analysis of study data is currently ongoing and results are scheduled to be available in November 2016.

Discussion

Need for novel anti-pruritic drugs in PBC

Pruritus is a complex symptom and the drug treatment of pruritus in PBC patients remains a challenge in clinical practice. The four main classes of drugs that are recommended by current guidelines [2, 6] include bile acid sequestrants (cholestyramine), enzyme inducers (rifampicin), opioid antagonists (naltrexone) and selective serotonin re-uptake inhibitors (sertraline). These drugs are limited by their lack of universal efficacy, poor compliance (especially cholestyramine) and the need for regular monitoring for liver toxicity (rifampicin). Cholestyramine and rifampicin have good reports but clinical experience of both naltrexone and sertraline has been disappointing for many clinicians [2].

A critical review of literature shows that the strength of evidence for current anti-pruritic drug therapy is poor. Cholestyramine, the current first-line therapy was last studied over five decades ago but has never been subjected to randomised placebo-controlled trials and has evidence category II-2 (cohort or case control analytical studies) [20–24]. Only rifampicin and naltrexone have been studied in controlled trials [18, 19, 25–27] and sertraline (evidence category II-2) is the last agent investigated with a positive outcome on pruritus [28]. A number of other drugs have been investigated but with little success and more recently both gabapentin (2006)

Table 3 BAT117213 study: schedule of procedures and data collection

Period description	Screening	Placebo run-in					Treatment period 1					Treatment period 2					Follow-up[f]				
Day (relative to Day 1)	-45 to-1 days	1	2–12	13	14	15	16–26	27	28	29	30–40	41	42	43	44	45–55	56
Admission to Unit				X				X				X					
Discharge						X				X				X			
Outpatient visit	X	X															X
Screening assessments[a]	X																
Brief Physical		X															X
12-lead ECG[b]	X	X			X				X				X				X
Vital signs	X	X			X				X				X				X
Urine drug/ alcohol screen	X	X		X				X				X					X
β-hcg (women)	X	X		X				X				X					X
standard blood tests and urinalysis	X				X				X				X				X
Randomisation	X																
Study treatment dosing		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Concomitant medication review		X		X				X				X					X
Meal served				X	X	X		X	X	X		X	X	X			
Blood samples[c]					X				X				X				
Metabonomics (Stool and urine)				<————	————	————	———>	<————	————	————	———>	<————	————	————	———>		
Microbiomics (Stool)				<————	————	————	———>	<————	————	————	———>	<————	————	————	———>		
PBC-40 questionnaire[d]		X		X				X				X					X
GSRS[d]		X		X				X				X					X
5-D Itch scale[d]		X		X				X				X					X
Faecal Occult blood	X				X				X				X				X

Table 3 BAT117213 study: schedule of procedures and data collection *(Continued)*

Pruritus 0–10 point scale (electronic diary)[e]	<——————————————————————————————————————>	
AE assessment	<——————————————————————————————————————>	
PGx	For subjects who consent only. Collect one PGx sample after the start of dosing, preferably on day 1	
Exit interview		X

[a]Screening assessments: informed consent for the study and PGX; demographics; complete physical; medical/medication/drug/alcohol history; Hepatitis B and Hepatitis C screen
[b]Single ECG to be performed, with the exception of screening and day 14 when this will be in triplicate
[c]Blood samples for GSK2330672, UDCA, bile acids, bile acid species, C4, Autotaxin, FGF19 and metabonomics
[d]patient reported outcome (PRO) assessments. On days in which PRO assessments are administered at study visits they should be administered before any other study procedure
[e]symptoms recorded twice daily (pre-dose of study drug)
[f]Rescue treatment with antipruritic agents can be instituted in subjects with severe itching during the placebo follow up period

and colesevelam (2010) trials failed to show any therapeutic benefit in cholestatic pruritus [29, 30].

IBAT2330672 trial

The apparent lack of novel drug development in cholestatic pruritus can be attributed partly to incomplete understanding of the complex pathophysiology of the disease. More recent advances in molecular research have identified novel targets for drug development in cholestasis. IBAT inhibitors are novel class of drugs with therapeutic potential in cholestasis. They have been shown be beneficial in cholestasis by the experimental studies and their desired effects on serum and faecal bile acid profile has been proven in healthy people [13, 14].

The BAT117213 study is the first phase 2 multicentre, double-blinded, placebo-controlled crossover trial designed to investigate the safety and efficacy of IBAT inhibitor in PBC patients with pruritus. Unlike the only other phase 2 trial of an IBAT inhibitor drug (LUM001) in PBC (CLARITY study, NCT01904058), the main strength of the BAT117213 study is its crossover design which allows estimating the treatment effect in a smaller number of patients and reduces the between-patient variability and yields a more efficient comparison of treatments than a similar sized parallel group trial. In the BAT117213 study every patient will receive both the study drug and the placebo; therefore each patient will serve as his/her own matched control.

An additional strength of this trial is the utility of patient reported outcomes to measure the treatment response objectively using existing validated tools including the PBC-40 questionnaire and 5-D itch scale as well as a novel, easy-to-use electronic symptom diary. The latter has been specifically developed for this study and it contains morning and evening diaries with questions on itch, fatigue and concentration to comprehensively capture the severity of the symptoms over the preceding 12 h. In addition, the exit interviews conducted at the end of the study provide the opportunity for patients to express their experiences in the study in a semi-structured method that may not have been detected with the more structured patient reported outcomes measures.

The BAT117213 study also provides a unique opportunity to conduct novel, explorative, mechanistic research in patients with cholestatic pruritus. Serum and urine samples obtained during the study will be used to study the metabolic phenotype (metabonomics) of pruritus in PBC by using ^{1}H (proton)-nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS). Similarly, using the faecal samples from study patients gut-microbiome studies will be undertaken to study the association between gut microbiota composition and pruritus in PBC. Results of these metabonomic and microbiomic studies are likely to provide more insight into the biology of pruritus in PBC and may identify potential biomarkers for cholestatic pruritus.

The main drawback of this trial is the potential carryover effect (i.e. effect of the treatment from the previous time period may "carry over" on the response to subsequent period) and lack of "washout period" between treatment periods. Carryover effect is a common problem inherent to the cross over study design and may potentially confound direct estimates of treatment effect. Therefore the statistical analysis the data will be assessed for any evidence of carry over and appropriate sensitivity analyses will be performed. To mitigate against the lack of "washout period" the outcome measurements will be restricted to the latter part of each treatment period.

In summary, BAT117213 study is a phase 2 study to evaluate the safety and tolerability of a unique class of drug in treating pruritus in PBC patients and provide novel information about bile acids and metabolic changes and gut microbiome profile in cholestatic pruritus. The results from this trial will inform the trial design of future development phase of the IBAT inhibitor drug.

Abbreviations

ABPI, association of the British Pharmaceutical Industry; AE, adverse event; ALT: alanine transaminase; ASBT, apical sodium dependent bile acid transporter; AUC, area under the curve; BA, bile acid; C4, 7-alpha-hydroxy-4-cholesten-3-one; CI, confidence interval; CYP, cytochrome P450; ECG, electrocardiogram; eCRF, electronic case report form; ePRO, electronic patient reported outcome; GCP, good clinical practice; GI, gastrointestinal; GSK, GlaxoSmithKline; GUDCA, glyco ursodeoxycholic acid; IBAT, Ileal bile acid transporter; LT, liver transplantation; MS, mass spectrometry; NHS, National Health Service (of the UK); NMR, nuclear magnetic resonance; NRS, numerical rating scale; PBC, primary biliary cholangitis (previously cirrhosis); PGX, pharmacogenetics; PK, pharmacokinetics; PRO, patient reported outcome; RAMOS, randomisation and medication ordering system; RCT, randomised controlled trial; SAE, serious adverse event; SD, standard deviation; TUDCA, tauro ursodeoxycholic acid; UDCA, ursodeoxycholic acid; ULN, upper limit of normal.

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Availability of data and materials
Not applicable.

Authors' contributions
SFWK, RLD, DR and GD designed the study and developed the trial protocol. DEJJ, GMH and GA are principal investigators (PIs) of this study and advised on the study design and protocol. JS was involved in trial coordination. SRM as the trial statistician did the sample size calculation and analysis plan. KG and SV designed and developed the electronic diary. VSH and MC managed the trial. VSH was the sub-investigator of the participating centre and wrote the manuscript for this publication. All authors read and approved the final manuscript.

Competing interests
SFWK, RLD, SRM, DR, JS, GD, KG and SV are GlaxoSmithKline (GSK) employees. GMH and DEJJ are investigators on the UK-PBC consortium which has received research funding from GSK. VSH and MC have no competing interest to declare.

Consent for publication
Not applicable.

Ethics approval and consent to participate
The National Research Ethics Service (NRES) Committee North East and Sunderland (REC reference 13/NE/0290) and the Medicine and Healthcare products Regulatory Agency (MHRA) approved all versions of the study protocol. In addition, all recruitment sites obtained approval from their respective hospital Research and Development (R&D) departments before starting screening patients. This study was conducted according to the ethical principles that have their origin in the Declaration of Helsinki and the Good Clinical Practice Guidelines (ICH-GCP).

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Effect of ileal bile acid transporter inhibitor GSK2330672 on pruritus in primary biliary cholangitis: a double-blind, randomised, placebo-controlled, crossover, phase 2a study

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Summary

Background Up to 70% of patients with primary biliary cholangitis develop pruritus (itch) during the course of their disease. Treatment of pruritus in primary biliary cholangitis is challenging and novel therapies are needed. Ursodeoxycholic acid, the standard first-line treatment for primary biliary cholangitis, is largely ineffective for pruritus. We investigated the efficacy and safety of GSK2330672, a selective inhibitor of human ileal bile acid transporter (IBAT), in patients with primary biliary cholangitis with pruritus.

Methods We conducted this phase 2a, double-blind, randomised, placebo-controlled, crossover trial in two UK medical centres. Following 2 weeks of open placebo run-in, patients were randomly assigned in a 1:1 ratio with a block size of 4 to receive GSK2330672 or placebo twice daily during two consecutive 14-day treatment periods in a crossover sequence. The treatment periods were followed by a 14-day single-blinded placebo follow-up period. The primary endpoints were safety of GSK2330672, assessed using clinical and laboratory parameters, and tolerability as rated by the Gastrointestinal Symptom Rating Scale. The secondary endpoints were changes in pruritus scores measured using the 0 to 10 numerical rating scale (NRS), primary biliary cholangitis-40 (PBC-40) itch domain score and 5-D itch scale, changes in serum total bile acids and 7 alpha hydroxy-4-cholesten-3-one (C4), and changes in the pharmacokinetic parameters of ursodeoxycholic acid and its conjugates. The trial was registered with ClinicalTrials.gov, number NCT01899703.

Findings Between March 10, 2014, and Oct 7, 2015, we enrolled 22 patients. 11 patients were assigned to receive intervention followed by placebo (sequence 1), and 11 patients were assigned to receive placebo followed by intervention (sequence 2). One patient assigned to sequence 2 withdrew consent prior to receiving randomised therapy. One patient did not attend the placebo follow-up period, but was included in the final analysis. GSK2330672 treatment for 14 days was safe with no serious adverse events reported. Diarrhoea was the most frequent adverse event during treatment with GSK2330672 (seven with GSK2330672 *vs* one with placebo) and headache was the most frequent adverse event during treatment with placebo (seven with placebo *vs* six with GSK2330672). After GSK2330672 treatment, the percentage changes from baseline itch scores were –57% (95% CI –73 to –42, p<0·0001) in the NRS, –31% (–42 to –20, p<0·0001) in the PBC-40 itch domain and –35% (–45 to –25, p<0·0001) in the 5-D itch scale. GSK2330672 produced significantly greater reduction from baseline than the double-blind placebo in the NRS (–23%, 95% CI –45 to –1; p=0·037), PBC-40 itch domain, (–14%, –26 to –1; p=0·034), and 5-D itch scale (–20%, –34 to –7; p=0·0045). After GSK2330672 treatment, serum total bile acid concentrations declined by 50% (95% CI –37 to –61, p<0·0001) from 30 to 15 µM, with a significant 3·1-times increase (95% CI 2·4 to 4·0, p<0·0001) in serum C4 concentrations from 7·9 to 24·7ng/mL.

Interpretation In patients with primary biliary cholangitis with pruritus, 14 days of ileal bile acid transporter inhibition by GSK2330672 was generally well tolerated without serious adverse events, and demonstrated efficacy in reducing pruritus severity. GSK2330672 has the potential to be a significant and novel advance for the treatment of pruritus in primary biliary cholangitis. Diarrhoea, the most common adverse event associated with GSK2330672 treatment, might limit the long-term use of this drug.

Funding GlaxoSmithKline and National Institute for Health Research.

Introduction

Primary biliary cholangitis (previously called primary biliary cirrhosis)[1] is a chronic autoimmune liver disease characterised by progressive cholestasis. Pruritus (itch) is a frequent and troublesome symptom, seen in 60–70% of patients at some point during the disease process.[2–4] The pathogenesis of cholestatic pruritus is complex and several putative pruritogens have been proposed.[5] The use of ursodeoxycholic acid (UDCA), the standard of care in primary biliary cholangitis, has improved outcomes in primary biliary cholangitis but has not been shown to improve pruritus.[6]

The bile acid sequestrant cholestyramine is often given to treat pruritus, but its effectiveness in practice is

Research in context

Evidence before this study

Chronic pruritus is a common symptom associated with cholestatic liver diseases and, along with fatigue, accounts for the greatest burden of symptoms in patients with primary biliary cholangitis. Up to 70% of patients develop pruritus at some point during the course of their disease and many patients describe persistent or severe pruritus which can be debilitating. This form of pruritus goes beyond cutaneous irritation, with secondary effects through sleep deprivation, daytime somnolence, fatigue, depression, and even on occasions, suicidal ideation. Overall, patients with primary biliary cholangitis and pruritus have a poor quality of life reflecting the limitations of current approaches to treatment. We searched PubMed for clinical studies published in English between Jan 1,1950, and Sept 1, 2016, with terms "PBC", "cholestasis", "pruritus", or "itch" and "bile acids", "bile salts", "IBAT", or "ASBT". Ursodeoxycholic acid, the standard of care for primary biliary cholangitis, has no substantial effect on pruritus and obeticholic acid, a second-line therapy, can actually worsen it. The four classes of available, guideline recommended drugs for pruritus include bile acid sequestrants (cholestyramine), pregnane X receptor agonists (rifampicin), opioid antagonists (naltrexone), and selective serotonin reuptake inhibitors (sertraline). However, the strength of evidence for these drugs is poor to moderate with only rifampicin and naltrexone ever studied in methodologically robust randomised controlled trials. Furthermore, rifampicin is associated with a risk of liver injury necessitating regular monitoring of liver enzymes, and naltrexone is associated with unpleasant symptoms of the opioid withdrawal syndrome. Cholestyramine remains the only licensed treatment but is poorly tolerated by many patients due to its unpleasant taste and texture, resulting in poor therapy adherence. Another bile acid sequestrant colesevelam has better tolerability but the only randomised controlled trial so far did not show that it was more effective than placebo in treating pruritus in primary biliary cholangitis.

Added value of this study

This study is a first-in-class, randomised, placebo-controlled trial of an ileal bile acid transporter (IBAT) inhibitor to treat pruritus in patients with primary biliary cholangitis. Using three different complementary patient-reported outcome measurements, GSK2330672 showed greater effects than placebo in reducing itch intensity, as well as night-time sleep interference and daytime fatigue. GSK2330672 significantly decreased serum activity of autotaxin, which forms lysophosphatidic acid, a novel proposed pruritogen in cholestasis shedding further light on the potential mechanisms for cholestatic pruritus and the actions of the drug. In addition to improving symptoms of primary biliary cholangitis, this study has shown that pharmacological inhibition of IBAT can be used as a therapeutic strategy to decrease the circulating bile acid pool in cholestatic patients. GSK2330672 decreased serum conjugated bile acids and resulted in ~50% decrease in total bile acid concentrations.

Implication of all the available evidence

Because of the great burden of pruritus in patients and the limitations of treatment options, there is a real unmet need for effective anti-pruritic therapies in primary biliary cholangitis. IBAT inhibitors are a novel class of drugs that have shown therapeutic potential in cholestasis. The results of our early phase randomised controlled trial in conjunction with previous experimental evidence and healthy volunteer trials provide support to further investigate anti-pruritic effect of IBAT inhibitor drugs in larger and longer-term studies of patients with primary biliary cholangitis.

limited. Despite its modest evidence base, and poor tolerability profile, it is the only US Food and drug Administration (FDA)-approved therapy for cholestatic pruritus and is recommended by both the American and European practice guidelines as the first-line agent.[7,8] Colesevelam is a better tolerated bile acid sequestrant, but was not efficacious in the only randomised controlled trial reported so far.[9] Other drug therapies (rifampicin, naltrexone, and sertraline), although recommended by the scientific guidelines,[7,8] are not actually licensed for treating cholestatic pruritus. Moreover, they have the disadvantage of needing regular monitoring due to risk of liver injury and other limiting adverse effects. This is particularly an issue with rifampicin, the most widely used second-line therapy, which has well reported hepatotoxicity.[10] In clinical practice, response rates less than 50% are common for most of the guideline recommended drugs[11] and despite their step-wise use, many patients report refractory itch, which can only be treated by invasive (usually temporary) treatments such as nasobiliary drainage[12] or liver transplantation (the only definitive cure). Therefore, effective anti-pruritic drug therapy is an unmet clinical need in primary biliary cholangitis and other cholestatic liver diseases.[13]

The paucity of effective anti-pruritic therapies in primary biliary cholangitis is probably compounded by the fact that the key emerging second-line disease modifying agent, obeticholic acid, which has been licensed by the FDA and European Medicines Agency, is associated with an increased frequency and severity of pruritus.[14,15] Many other bile acid-based therapies in primary biliary cholangitis that are in development[16] might also be associated with pruritus. Therefore, effective pruritus management in primary biliary cholangitis is likely to become increasingly important and challenging and new approaches are needed.

Ileal bile acid transporter (IBAT, also called apical sodium-dependent bile acid transporter [ASBT]) is an integral brush border membrane glycoprotein mainly expressed in the distal ileum.[17,18] The main physiological

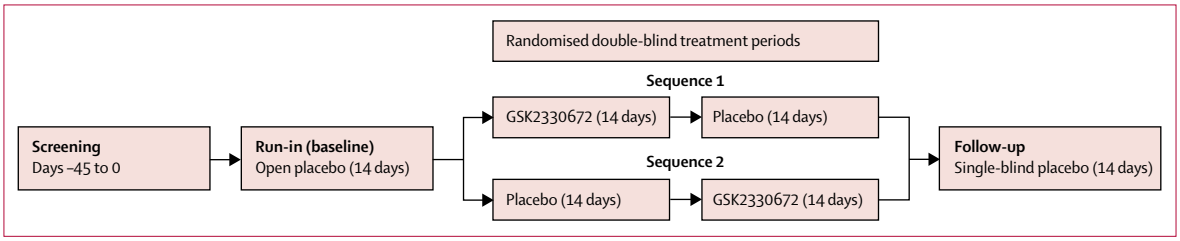

Figure 1: **Trial design**

function of IBAT is reabsorption of bile acids and maintenance of their enterohepatic circulation. In cholestatic liver disease, ileal bile acid absorption is increased[19,20] and inhibiting ileal bile acid transport was proposed to prevent inappropriate conservation of bile acids.[21] Using an IBAT inhibitor to reduce bile acid reabsorption and modulate the bile acid pool in the systemic circulation is an unexplored therapeutic strategy in primary biliary cholangitis, a condition in which retention of toxic hydrophobic bile acids is postulated to play a key pathogenetic role.

To date, published reports of IBAT inhibitor drugs include a study in healthy people (A4250),[22] two reports in animal models of cholestasis (A4250 and SC435)[23,24] and a report of lopixibat chloride (formerly LUM001) in patients with primary biliary cholangitis and pruritus.[25] GSK2330672 is a highly potent, soluble, minimally absorbed, selective inhibitor of the human IBAT. It has been assessed in animal models of type 2 diabetes mellitus (T2DM) and an early phase trial of T2DM patients.[26,27] In two phase 1 studies (59 healthy volunteers) it was well tolerated with a good safety profile at a dose range of 0·1 to 90 mg (unpublished data from clinical trial NCT01416324 and NCT01607385).

See Online for appendix

Here we report the first randomised, placebo-controlled, double-blind, crossover, phase 2 trial of an IBAT inhibitor in people with primary biliary cholangitis and pruritus. We postulated that GSK2330672 would interrupt enterohepatic circulation of bile acids and exert therapeutic benefit on pruritus associated with primary biliary cholangitis.

Methods

Study design and patients

BAT117213 was a phase 2a randomised, double-blind, placebo-controlled study to assess the safety, tolerability, pharmacokinetics, and pharmacodynamics of repeat doses of GSK2330672 in patients with primary biliary cholangitis and symptoms of pruritus (figure 1). This study was done at two centres in the UK: Newcastle Upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, and University Hospitals Birmingham NHS Foundation Trust, Birmingham, in collaboration with the UK-PBC Consortium.[28] The National Research Ethics Service Committee North East and Sunderland (REC reference 13/NE/0290) and the Medicine and Healthcare products Regulatory Agency approved all versions of the study protocol which is available online.[28] All recruitment sites obtained approval from their respective hospital Research and Development (R&D) departments before screening patients. All participants provided written informed consent before enrolment. The trial was done in accordance with the International Conference on Harmonisation Good Clinical Practice guidelines and the Declaration of Helsinki.[29] The trial protocol is available online.[28]

For more on the **UK-PBC Consortium** see http://www.uk-pbc.com

Patients were eligible for inclusion in the trial if they were aged 18–75 years, had proven or likely primary biliary cholangitis with ongoing pruritus, were on stable doses of UDCA for more than 8 weeks at the time of screening, and had serum alkaline phosphatase value no more than 10 times the upper limit of normal. Exclusion criteria are shown in the appendix.

Eligible volunteers entered a 2 week open placebo run-in period followed by randomised, blinded, two-period crossover treatment periods (sequence 1 and sequence 2) of 14 days each during which patients received either GSK2330672 or placebo twice daily. Sequence 1 was GSK2330672 followed by placebo and sequence 2 was placebo followed by GSK2330672. There was no washout period between two treatment periods. Patients then entered 14 days of follow-up during which they received blinded placebo treatment. When taking GSK2330672 (or matching placebo), participants received 45 mg twice per day on days 1–3, and were then asked to increase the dose to 90 mg twice daily on days 4–14.

Outcomes

The primary objective of the study was to investigate the safety and tolerability of oral GSK2330672 when administered for 14 days to patients with primary biliary cholangitis with pruritus. The secondary objectives were to investigate the effects of oral GSK2330672 on participants' experience of pruritus, study the effect of the drug on serum total bile acids and 7 alpha hydroxy-4-cholesten-3-one (C4), a serum marker of bile acid synthesis, and investigate the steady-state pharmacokinetic parameters of UDCA and its taurine and glycine conjugates. Exploratory endpoints included changes in the concentrations of serum liver biochemistry, bile acid species, autotaxin (ATX) activity, and fibroblast growth factor (FGF19; appendix).

Randomisation and masking

A single randomisation schedule for all sites was generated using a dedicated randomisation creation and publishing tool for GlaxoSmithkline (GSK) studies (RandAll) by the GSK statistician. Randomisation numbers were allocated in a 1:1 ratio to sequence 1 or sequence 2 with a block size of four. Randomisation numbers were allocated to participants by site staff. Patients, investigators, clinical trial site staff, and sponsor staff directly involved with the study were masked to treatment sequence assignment throughout the study.

Statistical analysis

The study was designed to estimate the effect of GSK2330672 (when co-administered with UDCA) relative to placebo on pruritus and the pharmacokinetics of UDCA. Due to the early clinical and exploratory nature of the study, no formal hypothesis testing was planned and the sample size was based on feasibility with consideration of efficacy (using pruritus 0–10 numerical rating scale [NRS]) and potential pharmacokinetics interaction between GSK2330672 and UDCA. An initial sample size of 40 participants was estimated to be sufficient for both efficacy and pharmacokinetics based on the assumption that GSK2330672 was at least as efficacious as rifampicin and the SD was similar to that reported in trials of other anti-pruritic drugs.[30,31] Given the uncertainties associated with sample size assumptions, two interim analyses were done to assess futility and determine if sample size re-estimation was necessary. Data from the pruritus 0–10 NRS were reviewed by a non-blinded Interim Review Committee (composed of people not directly involved in study conduct). The first interim analysis was done after 11 patients had completed the treatment period, and a second interim analysis was done after 19 patients had completed the treatment period, at which point the final target sample size was reduced (appendix). The study was closed for recruitment after 22 people were randomly assigned.

To summarise the daily pruritus 0–10 point NRS during the placebo run-in period and each treatment period for each individual patient, we calculated trimmed means of weekly itch scores. Trimmed means removed the highest and lowest daily score (an average of the morning and evening scores) to provide a more robust summary not influenced by potential outlying values. For statistical analysis we used the second week of each period to provide an analytical washout (ie, 7 days between the analysed periods to allow treatment effects to stabilise). The efficacy endpoint analysis used a mixed effects model with fixed effect terms for treatment period and sequence, with participants treated as a random effect in the model. Baseline (placebo run-in) results were included in the model as an additional period. Point estimates and their associated 95% CI and p value were constructed for the mean differences of interest in pruritus scores (ie, changes from baseline on each treatment and between double-blind GSK2330672 and placebo). The statistical analysis of pruritus scores in PBC-40 and 5-D itch was similarly done (appendix). Data on pharmacokinetics parameters, bile acids, and biomarkers were log-transformed for analysis, and results are reported as percentage changes or ratios. Although not formally a hypothesis-testing study, two-sided p values less than 0·05 were considered statistically significant. All analyses were done using SAS, version 9.2 or greater (SAS Institute, Cary, NC, USA). This trial is registered on ClinicalTrials.gov, number NCT01899703.

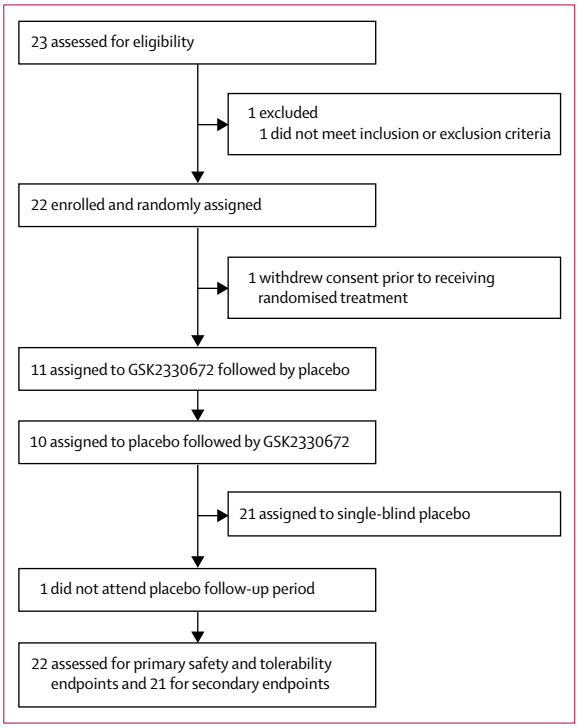

Figure 2: **Trial profile**

Role of the funding source

The sponsor and main study funder (GlaxoSmithKline; GSK) was involved in the study design, data analysis, and data interpretation with inputs from VSH, DEJ, and GMH. The NIHR Newcastle Biomedical Research Centre provided additional funding but had no role in study design, data collection, analysis, or interpretation. The trial was supervised by the research and development department of the Newcastle Upon Tyne Hospitals NHS Foundation Trust and data collection occurred at the clinical trial units of the study centres. The corresponding author (VSH) had access to the raw data, did analysis of primary and secondary outcomes with SRM and DT (GSK statisticians) and wrote the study report. All authors had access to the data, reviewed the manuscript, and share final responsibility for the decision to submit for publication.

	Measurement at baseline
Age (years)	52·9 (10·6)
Female (n)	19 (86%)
Body-mass index (kg/m^2)	27·2 (4·9)
Bodyweight (kg)	72·8 (13·5)
Duration of primary biliary cholangitis (years)	5 (4·8)
Race	
White (n)	21 (95%)
Asian: Central/South Asian (n)	1 (5%)
Ursodeoxycholic acid (UDCA)	
People taking UDCA during study period (n)	19 (90·4%)
Total daily dose during study period (mg/day)	967 (185·8)
Bodyweight adjusted daily dose during study period (mg/kg/day)	14 (1·7)
Pruritus scores*	
Itch intensity on numerical rating scale (min 0, max 10), trimmed mean	5·33 (2·1)
Primary biliary cholangitis-40 itch domain score (min 3, max 15)	10·5 (3·3)
5-D itch scale (min 5, max 25)	18·7 (3·6)
Laboratory markers*	
Alkaline phosphatase (IU/L)	264 (174·1)
Gamma glutamyl transferase (IU/L)	211 (172·6)
Alanine aminotransferase (IU/L)	59·3 (44·8)
Aspartate aminotransferase (IU/L)	60·8 (35·8)
Total bilirubin (μmol/L)	12·2 (5·5)
Total protein (g/L)	73·32 (5·9)
Albumin (g/L)	41·9 (4·2)
Creatinine (μmol/L)	65·8 (9·1)
Autotaxin activity (nmol/ml per min)	8·2 (4·1)
FGF19 (pg/mL)	162·9 (107·5)
C4 (ng/mL)	13·1 (10·0)
Total bile acids (μM)	48·6 (68·7)

Data are shown in mean (SD) unless otherwise stated. *Baseline data at the end of placebo run-in period.

***Table 1*: Baseline characteristics of volunteers**

Results

Between March 10, 2014, and Oct 7, 2015, we enrolled and randomised 22 patients. 21 patients completed all the planned study procedures as per protocol. One patient was withdrawn from the study due to withdrawal of consent in the placebo run-in period (figure 2). The safety population therefore comprised a total of the 22 randomly assigned patients, while the analysis population comprised 21 patients who completed all the planned study procedures as per protocol (although one patient did not attend the full follow-up period). 19 of 21 patients were taking UDCA during the study period at the guideline recommended dose. The baseline demographic and clinical characteristics of the participants are shown in table 1. A summary of the frequency of use of anti-pruritic treatments prior to the start of the study is provided in the appendix (p 15). As per the study protocol, use of these drugs was stopped at the study entry.

All patients in the active treatment period started with GSK2330672 at a dose of 45 mg twice per day for 3 days and successfully increased to 90 mg twice per day on days 4–14. During the study there were no reports of serious adverse events. There were no clinically significant changes in vital signs, laboratory values or ECG parameters, and no positive faecal occult blood tests were reported. There were no reports of liver toxicity and no significant changes were seen in serum total bilirubin, alkaline phosphatase, or other liver enzymes during the study period (appendix p 16).

Overall, GSK2330672 was well tolerated. A summary of all adverse events reported for more than one participant during any treatment period (irrespective of sequence) is given in table 2. The frequency of any adverse events was similar in active drug and placebo treatment periods (81% *vs* 81%). The most common adverse event observed during the study was headache, reported by 14 of 22 participants. 16 patients reported adverse events related to the gastrointestinal system. The most common GSK2330672-related adverse event was diarrhoea, reported by seven participants, with five rating the severity as mild (lasting up to 4 days with no or minimal effect on daily life). The frequency of diarrhoea reported during GSK2330672 treatment was significantly higher compared with placebo (seven patients during GSK2330672 treatment *vs* one patient during placebo treatment, p=0·0391, post-hoc mid-p McNemar test). No patient discontinued the drug or had their dose decreased secondary to diarrhoea. Two adverse events (diarrhoea, abdominal distension) reported during GSK2330672 treatment and one adverse event of upper abdominal pain reported during placebo treatment were considered to be severe.

After GSK2330672 treatment, changes from baseline itch scores were: –57% (95% CI –73 to –42, p<0·0001) for NRS, –30% (–42 to –20, p<0·0001) for PBC-40 itch domain, and –35% (–45 to –25, p<0·0001) for 5-D itch scale (figure 3). GSK2330672 reduced itch intensity significantly more than the double-blind placebo in all three scales: –23% (–1 to –45, p=0·0374) for NRS, –14% (–1 to –26, p=0·0335) for PBC-40 itch domain, and –20% (–7 to –34, p=0·0045) for 5-D itch.

For individual patients, changes in the weekly trimmed mean of their itch intensity score are shown in the appendix. Overall, the mean NRS itch intensity score decreased significantly from baseline after GSK2330672 treatment period (appendix p 17) and the reduction was significant in both the sequences of treatment (appendix p 18). In the NRS, itch was also assessed for worst itch, bothersome itch, and sleep interference. Significant reductions were seen in these scores following GSK2330672 (appendix p 19).

GSK2330672 treatment was also associated with significant reductions in the mean PBC-40 itch domain

and 5-D itch score (appendix p 20). The mean decrease from baseline in 5-D itch score after GSK2330672 treatment was significant in both sequences of treatment, whereas the decrease in PBC-40 itch domain score was only significant in sequence 2. Adjusting for sequence and period, the mean PBC-40 itch domain and 5-D itch scores following GSK2330672 treatment were significantly lower than placebo.

Analysis of other domains of PBC-40 showed a significantly greater reduction in the fatigue domain score after GSK2330672 treatment compared with placebo (–9%, 95% CI –3 to –16; p=0·0033). No significant changes were apparent for other domains of PBC-40 (appendix pp 21–22). All five domains of the 5-D itch scale, including the disability domain, showed significantly lower scores after GSK2330672 compared with placebo (appendix pp 23–24).

Serum total bile acid concentrations changed from baseline with a 50% decrease (95% CI –37 to –61, p<0·0001) after GSK2330672 treatment compared with a 12% increase (–12 to 42, p=0·3540) after placebo. The changes in serum total bile acid concentrations following GSK2330672 were significant when compared with baseline and placebo (figure 4A) and the changes were reversed within 2 weeks of stopping GSK2330672. Conversely, faecal total bile acid showed a mean 36% increase after GSK2330672 (95% CI –1 to 85) compared with 16% decrease after placebo (–40 to 15).

Serum concentrations of conjugated bile acids decreased after GSK2330672 compared with baseline (appendix p 25), with the largest percentage reductions observed in taurocholate (–74%; 95% CI –53 to –86, p<0·0001), glycocholate (–64%; –23 to –83, p=0·0099), and taurochenodeoxycholate (–58%; –32 to –74, p=0·0007). In contrast, unconjugated primary bile acids cholate (–13%; –60 to 86, p=0·7040) and chenodeoxycholate (–4%; –34 to 38, p=0·8049) did not change significantly after GSK2330672 (appendix p 25). A significant increase in serum UDCA (57%; 15 to 116, p=0·0062) and a marginal increase in serum deoxycholate (11%; –1 to 23%, p=0·0619) were observed after GSK2330672 (appendix p 25). No significant changes from baseline were seen in any bile acid species after placebo treatment (appendix pp 27–29).

There was a significant 3·1-times increase in serum C4 concentration from baseline after GSK2330672 treatment (95% CI 2·4 to 4·0, p<0·0001; figure 4B) and the increase was seen in both sequences of treatment. No significant changes in C4 were seen after placebo treatment. Significant decreases compared with baseline were seen in serum ATX activity (– 11%, 95% CI –3 to –19; p=0·0070) and FGF19 concentration (–78%, –60 to –88; p<0·0001) following GSK2330672 treatment, but not after placebo (appendix).

GSK2330672 is designed as a minimally absorbable agent with minimal systemic exposure. Eight of 22 participants had measurable plasma concentrations of GSK2330672 and the peak concentration (5·33 ng/mL, achieved at 2 h post-dose in one patient) is consistent with minimal systemic exposure. All 14 patients who provided faecal samples for drug analysis had detectable GSK2330672 in the faeces. No GSK2330672-related metabolites were detected in plasma or urine. Comparing co-administration of UDCA with GSK2330672 or with placebo showed 90% CIs for UDCA maximum concentration (Cmax) and area under curve (AUC) ratios within conventional bioequivalence limits of 0·8–1·25 but reduced exposure in the metabolites tauro ursodeoxycholic acid (TUDCA) and glyco ursodeoxycholic acid (GUDCA; appendix).

	Placebo run-in (n=22), n (%)	GSK2330672 (n=21), n (%)	Placebo (n=21), n (%)
Participants with any adverse event	15 (68)	17 (81)	17 (81)
Gastrointestinal system			
Diarrhoea	1 (5)	7 (33)	1 (5)
Upper abdominal pain	0	3 (14)	1 (5)
Abdominal distension	0	3 (14)	1 (5)
Abdominal pain	0	3 (14)	0
Vomiting	0	1 (5)	2 (10)
Nausea	0	2 (10)	0
Nervous system			
Headache	7 (32)	6 (29)	7 (33)
Dizziness	1 (5)	1 (5)	2 (10)
Paraesthesia	0	0	2 (10)
Infections			
Nasopharyngitis	0	1 (5)	2 (10)
General			
Fatigue	0	0	2 (10)

Adverse events were monitored from day 1 to 56 of the study including follow-up period. Data are in n (%). The listed adverse events (any severity) have an incidence greater than one patient (5%) in any treatment period.

Table 2: **Summary of adverse events**

Discussion

This trial of GSK2330672 shows safety and efficacy of a first-in-class, novel agent in treating pruritus associated with primary biliary cholangitis. Despite the substantial symptom burden and need for better anti-pruritic drugs, little progress in developing new treatments for pruritus in primary biliary cholangitis has been made. We have attempted to fill this treatment gap with the first randomised, placebo-controlled, crossover trial of an IBAT inhibitor drug in patients with primary biliary cholangitis and clinically significant pruritus. Our results suggest that interrupting enterohepatic circulation of bile acids by inhibiting IBAT with GSK2330672 improves pruritus in patients with primary biliary cholangitis.

We found that GSK2330672 at 45–90 mg dose, given twice per day for 2 weeks in patients with primary biliary cholangitis, was safe and generally well tolerated. No serious adverse events and no clinically significant abnormality related to haematology, clinical chemistry, or ECG was reported following GSK2330672 treatment.

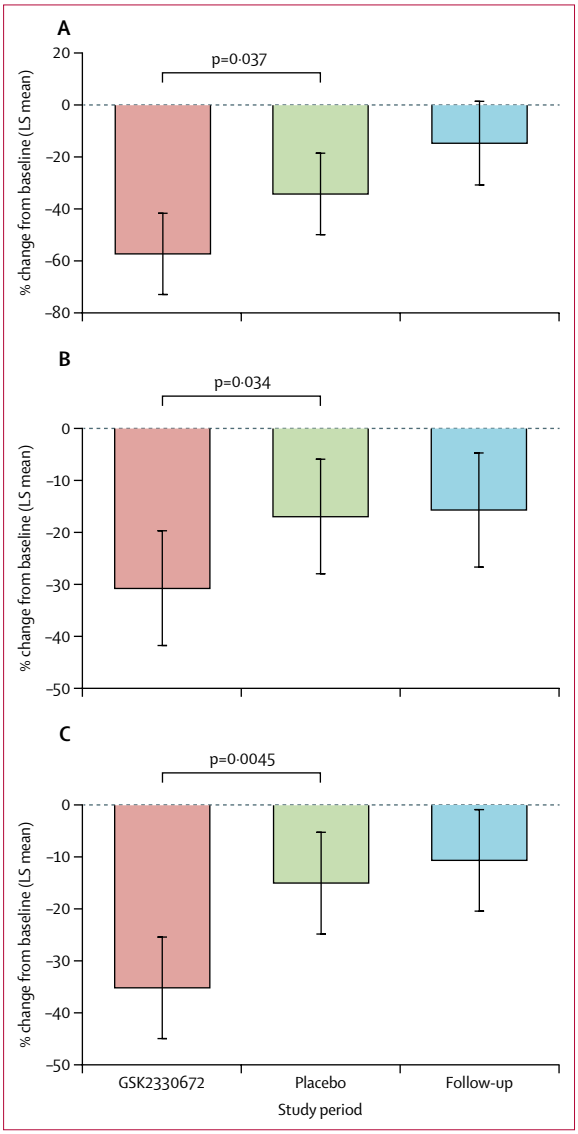

***Figure 3*: Changes from baseline in itch intensity scores according to treatment period**

(A) 0–10 numerical rating scale. (B) Primary biliary cholangitis-40 itch domain score. (C) 5-D itch scale. Error bars are 95% CI. LS=least squares.

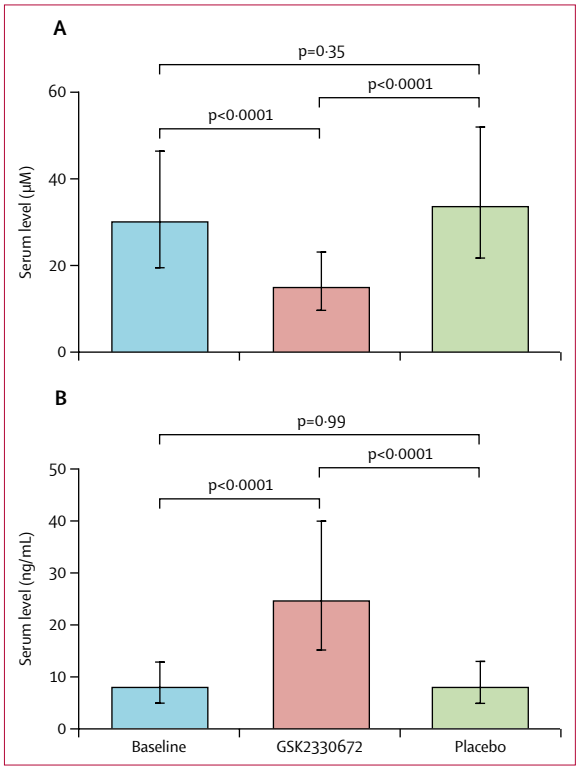

***Figure 4*: Changes in serum total bile acids and C4 according to treatment period**

Following treatment with GSK2330672 a significant decrease in total bile acid (A) and significant increase in C4 (B) was seen. Data shown are geometric mean values and error bars show 95% CI.

Diarrhoea was the most frequent adverse event associated with GSK2330672, which is in concordance with previous reports of IBAT inhibition in healthy volunteers[22] and in patients with T2DM.[27] Increased bile acid load in the colon increases colonic motility and reduces colonic transit time causing diarrhoea.[32–34] In our study, severity of diarrhoea was mild to moderate and no patients discontinued GSK2330672 or had their dose decreased. Taken together, the safety and tolerability profile of GSK2330672 in patients seen in our study does not preclude further clinical investigation of the drug to treat patients with primary biliary cholangitis; however, gastrointestinal disturbance might ultimately limit the usefulness of this agent in practice.

The key finding of this study is that GSK2330672 was significantly more efficacious than placebo in improving itch intensity. This was substantiated by decreases in pruritus scores measured by three different tools of itch measurement. GSK2330672 treatment was associated with improvement in pruritus and the changes from baseline were significant regardless of the dosing sequence. Notably, pruritus scores improved within the first week of GSK2330672, continued to decrease through 2 weeks of treatment, and returned towards baseline on switch to blinded placebo. The improvement in pruritus severity seen in the placebo phases was in keeping with other trials of anti-pruritic agents in primary biliary cholangitis and was significantly exceeded by that seen in the active drug phases.

Despite the differences in the results between intervention and placebo treatment, the magnitudes of the effect could be underestimated because the crossover study design lacked a washout between treatment periods. Incorporation of the analytical washout mitigates this somewhat, but placebo responses and carry-over effects still resulted in a sequence effect that influenced the magnitude of response depending on the order of treatment. Comparing the GSK2330672 period with the run-in open placebo period avoids the sequence effect

and gives an alternative estimate of the magnitude of effect, although this might be an overestimation due to the unblinded placebo run-in being used to form this comparison. Nevertheless, individual patient responses (appendix) clearly showed rapid improvement of pruritus during GSK2330672 treatment and greater response was observed in patients with higher baseline itch intensity than in those with lower itch intensity. GSK2330672 also decreased sleep interference score, disability (5-D itch scale), and fatigue (PBC-40 scale) domain scores agreeing that the treatment had clinically meaningful effect on the symptom complex associated with primary biliary cholangitis. The study provides early evidence of effect in this debilitating symptomatic condition, but treatment duration was not sufficient to assess durability of benefit. This study had few patients and was not designed to deliver definitive conclusions on superiority of study drug over placebo. The interim analyses might have introduced some statistical bias in the estimated effect sizes, though as the interim analyses primarily assessed lack of effect we do not anticipate that this bias would greatly affect the results. Therefore, the efficacy of GSK2330672 on pruritus needs to be substantiated in larger studies of longer duration.

The evidence for the role of bile acids in the development of pruritus in primary biliary cholangitis has been equivocal, and the topic has been controversial.[35] A strong association between plasma bile acid concentration and the severity of itch has never been recorded in cholestatic patients[35] but other evidence has linked bile acid-mediated cholestatic pruritus via TGR5 receptors.[36,37] In this study, GSK2330672 treatment had substantial effect on the circulating bile acid pool, as shown by 50% decrease in serum total bile acid and decreased serum concentration of all taurine and glycine conjugated primary bile acids. These findings are consistent with a preferential effect of GSK2330672 on ileal reuptake of conjugated bile acids. However, we cannot exclude the possibility that pruritogens other than bile acids are also transported via IBAT. Cholesterol 7α-hydroxylase (CYP7A1), the rate limiting enzyme in hepatic bile acid synthesis, is regulated by farnesoid X receptor (FXR) and FGF19. Following GSK2330672 treatment we observed a significant 3-times increase in serum C4, a surrogate marker for hepatic CYP7A1 enzymatic activity.[38] Fasting C4 has been shown to provide a good measure of the overall flux through the bile acid synthetic pathway and a reliable assessment of the degree of IBAT inhibition.[27] Taken together, these findings suggest substantial target (ie, IBAT) engagement and inhibition by GSK2330672. However, post-hoc correlation analyses estimated correlation coefficients between circulating biomarkers and pruritus scores to be less than 0·4, indicating that these markers are not highly associated with efficacy at an individual patient level after a short period of treatment.

Lopixibat chloride (maralixibat or LUM001), an IBAT inhibitor, has been studied in a phase 2 RCT as a novel treatment of pruritus in patients with primary biliary cholangitis (CLARITY study).[25] Although the itch scores decreased from baseline in both lopixibat and placebo groups, lopixibat was not shown to be more efficacious than placebo in reducing pruritus. The negative result is likely to be, in part, due to a lower bile acid depleting effect of lopixibat. In our study, the effects of GSK2330672 on both total bile acid and C4 were more marked than those reported with lopixibat; a finding which might explain the difference in clinical efficacy of the two agents.

The significant decrease in serum FGF19 concentration observed in our study is consistent with decreased ileal FXR activation following IBAT inhibition. Decreased circulating concentrations of FGF19 result in decreased inhibition of hepatic bile acid synthesis, as reflected by increased serum C4 concentration. In this regard, the effect of GSK2330672 on FGF19 is different to that of other agents proposed for primary biliary cholangitis treatment. Obeticholic acid is a strong ileal FXR activator that increases FGF19 concentration and reduces bile acid synthesis, and fibrates reduce bile acid synthesis despite reduction in FGF19. The long-term use of an IBAT inhibitor is likely to maintain suppressed FGF19 and therefore, the effect of resulting upregulated bile acid synthesis on cholestasis needs careful investigation. However, the circulating bile acid pool is unlikely to expand as the loss of bile acids in faeces might exceed increased bile acid synthesis. The apparent paradox of contrasting actions of different agents in primary biliary cholangitis on FGF19 reflects that this is only an intermediate step in one pathway reducing bile acid concentration. If reduction in bile acid is the crucial pathway, then direct reduction through IBAT inhibition could be as valuable a mechanism as suppression of synthesis despite contrasting actions on FGF19.

Autotaxin (ATX) has been proposed as a key potential factor in the pruritogenic pathway in cholestasis.[39,40] We observed significantly reduced serum ATX activity after treatment with GSK2330672 but not after placebo. It is possible that IBAT inhibition with GSK2330672 also interrupts the ileal reabsorption of an as yet unidentified molecule that might upregulate ATX activity,[41] which in turn decreases the expression or synthesis of ATX and reduces serum ATX concentration. Further work is required to probe the mechanisms involved in ATX effects of IBAT inhibitors.

The ability of GSK2330672 to remain in the gastrointestinal tract reduces concerns of systemic toxicity and drug interactions. Notably, GSK2330672 did not have any significant interaction with UDCA absorption or recycling. UDCA is not transported via IBAT and there was a significant increase in serum UDCA concentration after GSK2330672 treatment. However, the glyco (GUDCA) and tauro (TUDCA) conjugates of UDCA are transported by IBAT and we observed a 3–4-times decrease in their serum concentration. The clinical relevance of this effect is not known. Reassuringly, we did not observe any

adverse effect of GSK2330672 on the therapeutic efficacy of UDCA since the serum liver biochemistry (mainly alkaline phosphatase) did not adversely increase during the study treatment. As UDCA is the mainstay of treatment in primary biliary cholangitis the clinical implication of inhibitory effect of GSK2330672 on UDCA conjugates merits further investigation.

Overall, there were no significant changes in the liver enzymes following GSK2330672 treatment. The absence of significance might be due to the short duration of treatment used in this study. Longer treatment with GSK2330672 could be required to study the effect of IBAT inhibition on alkaline phosphatase (biochemical marker of cholestasis) and other liver enzymes.

This study was designed to efficiently gather information about potential effects of GSK2330672, which if sufficiently encouraging could be used to subsequently design a definitive trial. In this context, our study has several strengths. Firstly, this is the first crossover RCT of an IBAT inhibitor drug to treat pruritus in patients with primary biliary cholangitis. The crossover design of the study allowed estimation of the treatment effect in a few patients and provided a more efficient comparison of treatments than a similar sized parallel group trial. Secondly, we used patient reported outcomes to measure the treatment response objectively by using the existing validated tools (PBC-40 questionnaire and 5-D itch scale) and a novel, easy-to-use electronic symptom diary.

The study was not designed to make definitive conclusions on superiority of the study drug over placebo treatment and it is limited by the small sample size, short duration (2 weeks) of treatment, and the absence of a washout period, as discussed above. Therefore the efficacy of GSK2330672 on pruritus needs to be substantiated by larger studies of longer duration.

In conclusion, this phase 2a randomised controlled trial showed that 2 weeks of treatment with an oral IBAT inhibitor GSK2330672 in patients with primary biliary cholangitis and symptoms of pruritus was safe, well tolerated, and was more efficacious than placebo in reducing the severity of pruritus. There was a significant reduction in serum total and conjugated bile acids, consistent with the postulated mechanism of efficacy, interruption of enterohepatic circulation of biliary pruritogens. Our results suggest that GSK2330672 could be a substantial advance for the treatment of pruritus in primary biliary cholangitis. Depending on its occurrence, duration, and effect in further studies, diarrhoea, the most common adverse event associated with GSK2330672, might limit the long-term use of this drug.

Contributors

SFWK, RLD, DR, and DEJ had the original concept of this trial and developed the trial protocol. SFWK, RLD, SRM, DR, JS, GMH, and DEJ wrote or reviewed all protocol versions. DEJ and GMH were principal investigators of this study. SFWK, JS, and VSH submitted all research ethics committee applications, local research and development applications and coordinated the trial sites. VSH, SFWK, MC, GMH, and DEJ recruited the participants and were responsible for data collection. VSH, SFWK, RLD, SRM, DT, JS, GED, GMH, and DEJ participated in data analysis and interpretation. SRM and DT did the sample size calculation and analysis plan. VSH, SRM, and DT did all the statistical analysis. RPJOE and UB contributed in analysis and interpretation of bile acids, FGF19, and autotaxin data. VSH was the sub-investigator of the participating centre and wrote the manuscript for this publication. SRM, DT, and VSH were responsible for preparation of the tables and figures. All authors participated in the Article review and approved the final manuscript. VSH, GED, GMH, and DEJ are guarantors.

Declaration of interests

SFWK, RLD, SRM, DT, DR, JS, and GED are or were GlaxoSmithKline (GSK) employees. SFWK reports grants and other from GSK during the conduct of the study and outside the submitted work. RLD reports personal fees from GSK during the conduct of the study. SRM reports personal fees and other from GSK during the conduct of the study. DR reports other from GSK during the conduct of the study. RPJOE received reimbursement of costs of autotaxin, bile salts, and FGF19 measurement from Newcastle University. GMH and DEJ are investigators on the UK-PBC consortium which has received research funding from GSK. GMH attended an advisory board on primary biliary cholangitis for GSK and received travel expenses for attending data analysis meeting. VSH reports personal fees and non-financial support from GSK during the conduct of the study. MC and UB declare no competing interests.

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ORIGINAL ARTICLE

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Autotaxin, bile acid profile and effect of ileal bile acid transporter inhibition in primary biliary cholangitis patients with pruritus

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Abstract

Background and Aims: Pruritus is a common symptom in patients with primary biliary cholangitis (PBC) for which ileal bile acid transporter (IBAT) inhibition is emerging as a potential therapy. We explored the serum metabonome and gut microbiota profile in PBC patients with pruritus and investigated the effect of GSK2330672, an IBAT inhibitor.

Methods: We studied fasting serum bile acids (BAs), autotaxin and faecal microbiota in 22 PBC patients with pruritus at baseline and after 2 weeks of GSK2330672 treatment. Control group included 31 asymptomatic PBC patients and 18 healthy volunteers. BA profiling was done by ultra performance liquid chromatography coupled to a mass spectrometry (UPLC-MS). Faecal microbiomes were analysed by 16S ribosomal RNA gene sequencing.

Results: In PBC patients with pruritus, serum levels of total and glyco-conjugated primary BAs and autotaxin were significantly elevated. Autotaxin activity correlated significantly with tauro- and glyco-conjugated cholic acid (CA) and chenodeoxycholic acid (CDCA), both at baseline and after GSK2330672. GSK2330672 significantly reduced autotaxin and all tauro- and glyco- conjugated BAs and increased faecal levels of CA ($P = 0.048$) and CDCA ($P = 0.027$). Gut microbiota of PBC patients with pruritus was similar to control groups. GSK2330672 increased the relative abundance of Firmicutes ($P = 0.033$) and Clostridia ($P = 0.04$) and decreased Bacteroidetes ($P = 0.033$) and Bacteroidia ($P = 0.04$).

Conclusions: Pruritus in PBC does not show a distinct gut bacterial profile but is associated with elevated serum bile acid and autotaxin levels which decrease after IBAT inhibition. In cholestatic pruritus, a complex interplay between BAs and autotaxin is likely and may be modified by IBAT inhibition.

Abbreviations: ALP, alkaline phosphatase; ALT, alanine transaminase; ANOVA, analysis of variance; ATX, autotaxin; BA, bile acid; BMI, body mass index; CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; FDR, false discovery rate; FGF19, fibroblast growth factor 19; GCA, glycocholic acid; GCDCA, glycochenodeoxycholic acid; GDCA, glycodeoxycholic acid; GGT, gamma-glutamyl transferase; GUDCA, glycoursodeoxycholic acid; HC, healthy control; IBAT, ileal bile acid transporter; LPA, lysophosphatidic acid; LPC, lysophosphatidylcholine; NMDS, non-metric multidimensional scaling; NRS, numerical rating scale; OTU, operational taxonomic unit; PBC, primary biliary cholangitis; PERMANOVA, permutational multivariate analysis of variance; PSC, primary sclerosing cholangitis; RCT, randomised controlled trial; rRNA, ribosomal RNA; TCA, taurocholic acid; TCDCA, taurochenodeoxycholic acid; TDCA, taurodeoxycholic acid; UDCA, ursodeoxycholic acid; UPLC-QToF-MS, ultra performance liquid chromatography coupled to a quadrupole time-of-flight mass spectrometry.

KEYWORDS
metabonome, microbiota, PBC, pruritus

1 | INTRODUCTION

Primary biliary cholangitis (PBC) is a cholestatic liver disease, characterised by chronic inflammation and fibrotic destruction of interlobular bile ducts. If untreated, PBC may lead to biliary cirrhosis and need for liver transplantation.[1] Pruritus (itch) is a common and often a disabling symptom affecting up to 75% of patients at some point in their disease course.[2] It causes significant symptom burden and can produce a negative impact on health-related quality of life.[2,3] The antipruritic actions of bile acid (BA) sequestrants (eg cholestyramine) point to a potential role of BAs in the pathophysiology of cholestatic pruritus but the exact mechanism remains elusive.[4] Recent evidence shows serum autotaxin (ATX) activity is associated with cholestatic pruritus and its product lysophosphatidic acid (LPA) has been proposed as a candidate pruritogen in cholestasis.[5,6] However, the relative contributions of ATX and total and individual BA species, and their mechanistic interactions in cholestatic pruritus remain obscure.

The treatment of pruritus in PBC is challenging because of the limited efficacy and poor tolerability of currently available drugs and lack of effective new therapies. Ileal bile acid transporter (IBAT) inhibitor agents are emerging as potential novel therapy for pruritus in PBC.[7-10] Recently, we investigated GSK2330672, a novel, selective human IBAT inhibitor in a phase 2a, randomised controlled trial (RCT) and showed that PBC patients with pruritus receiving 2 weeks of oral treatment with GSK2330672 had significant improvement in their pruritus compared to placebo.[11]

Over the years, metabonomics has been applied to study the metabolic signatures in a variety of liver diseases.[12] There are limited studies in cholestatic liver diseases with metabonomic profiling of serum/plasma and urine from patients with PBC and primary sclerosing cholangitis (PSC) and none of these studies specifically investigated pruritus associated with cholestasis.[13-16] Also, the effect of antipruritic therapy on metabolites-associated cholestatic pruritus has had only preliminary exploration in published abstract reports on the effect of bezafibrate and albumin dialysis.[17,18] Moreover, the effect of IBAT inhibitor on the metabolites associated with pruritus is currently unknown.

The role of gut microbiota in PBC is not clear. A recent study of patients with early stage PBC reported alterations of the gut microbiome[19] and another study showed a distinct microbial diversity in ursodeoxycholic acid (UDCA)-treatment naïve PBC patients.[20] BAs modulate the gut microbiota and changes in intestinal BAs have been shown to significantly alter the composition of the gut microbiome in animal studies.[21] Also, the gut microbiota modulate the BA pool by metabolic deconjugation and transformation of primary BAs into secondary BAs.[22] Therefore, it is conceivable that in cholestatic pruritus, changes in BAs or microbiota or in the interaction of the two may have a role in the aetiology of the symptom, and may be modified by IBAT inhibition. However, to date, there are no studies reporting gut microbiota composition in patients with PBC and pruritus.

Key Points

- We compared serum bile acid, autotaxin and stool bacterial profile in PBC patients with and without itch and studied the effect of GSK2330672, a novel antipruritic drug.
- In PBC patients with itch, elevated levels of bile acids and autotaxin were found without any significant difference in the gut bacterial composition.
- In PBC patients with itch, GSK2330672 treatment decreased autotaxin and all major serum bile acids, increased faecal bile acids and changed gut bacterial composition.
- Bile acids and/or autotaxin may have role in itch associated with PBC and they may be modified by GSK2330672 treatment to improve itch.

The main aim of this study was to characterise the serum metabolite profile and the faecal microbial composition in PBC patients with pruritus. We set out to test the following hypotheses:

1. PBC patients with pruritus have a distinct serum metabonomic signature and gut microbiome composition, compared to PBC patients without pruritus and/or healthy people; and
2. Pharmacological inhibition of enterohepatic circulation of BAs with an IBAT inhibitor can alter the serum and faecal BA profile, as well as change the faecal microbial composition in PBC patients with pruritus.

2 | MATERIALS AND METHODS

2.1 | Participants

This prospective case-control study was carried out in two parts. In the first part, patients with PBC with pruritus were recruited to the BAT117213 study, a phase 2a, RCT of IBAT inhibitor GSK2330672. This RCT was sponsored by GlaxoSmithKline (GSK) and registered with EudraCT (2012-005531-84) and ClinicalTrials.gov (Identifier: NCT01899703). Ethical approval was given by the Research Ethics Committee NRES Committee North East—Sunderland (13/NE/0290). We recruited 22 PBC patients with pruritus between 10 March 2014, and 7 October 2015. Itch severity was assessed using a 0-10 numerical rating scale (NRS), PBC-40 itch domain score and

5-D itch scale.[23,24] The trial protocol is available online[7] and we have recently published the safety and efficacy data of GSK2330672 in PBC patients with pruritus.[11]

In the second part, we recruited asymptomatic PBC patients (PBC-control) and healthy volunteers (HC). Participants in the PBC-control group were recruited only if they did not have any itch (assessed using PBC-40 itch domain score ≤3) and were not taking any antipruritic medications at the time of the study enrolment. Healthy volunteers who self-reported good health could enter the study when no known liver diseases were documented in their medical history. This study was sponsored by the NIHR Newcastle BRC and approved by NRES Committee North East - Newcastle & North Tyneside 2 (14/NE/1036). PBC-control and HC were non-related, but were age (±2 years), gender and ethnicity matched to the PBC patients with pruritus of BAT117213 study.

The recruitment of participants in both studies occurred at two centres in the UK: Newcastle Upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, and University Hospitals Birmingham NHS Foundation Trust, Birmingham. Informed consent was obtained from each patient included in the study and the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki.

2.2 | Metabonomic analysis

The BA profiling analysis in faecal samples was performed using a 'semi-targeted' profiling method, utilising an ultra performance liquid chromatography coupled to a quadrupole time-of-flight mass spectrometry (UPLC-QToF-MS) assay at Imperial College London as previously reported.[25] In addition, quantitative measurements of up to 15 BAs in human serum were performed using Biocrates® Bile Acids Kit (BIOCRATES Life Sciences AG, Innsbruck, Austria). The assay was used with Waters Xevo® TQ MS triple quadrupole mass spectrometer (Waters Inc, Milford, MA). Total bile acid (TBA) level was calculated by summation of 15 conjugated and unconjugated primary and secondary BA levels. We also used Biocrates Absolute*IDQ*® p150 kit (BIOCRATES Life Sciences AG, Innsbruck, Austria) with Waters TQ MS to quantify acylcarnitines, amino acids, glycerophospholipids and sphingolipids. Serum ATX assay was quantified as recently described.[26] Serum fibroblast growth factor 19 (FGF19) was measured by a quantitative sandwich enzyme immunoassay technique according to the manufacturer's instructions (Human FGF19, Quantikine® ELISA, R&D Systems, Oxford, UK). ATX and FGF19 assays were conducted in the Academic Medical Centre, Amsterdam.

2.3 | Metataxonomic analysis

We sequenced the V3-V4 region of the bacterial 16S ribosomal RNA (rRNA) gene to study the faecal bacterial composition in the study population. Sequencing was performed on the Illumina MiSeq platform (Illumina Inc, Saffron Walden, UK) using the MiSeq Reagent Kit v3 (Illumina) using paired-end 300bp chemistry. Further details of sample collection, preparation and statistical analysis of metabonomic and metataxonomic data sets are given in the supplementary information Data S1.

3 | RESULTS

We studied data from 22 PBC patients with pruritus, 31 PBC patients without pruritus (PBC-control) and 18 healthy volunteers (HC). None of the participants had taken any antibiotics for at least 3 months prior to study entry. The baseline demographic and clinical characteristics of the study groups are summarised in Table 1. The demographics and UDCA dose were comparable between PBC

TABLE 1 Demographic and biochemical characteristics of study cohorts

	PBC pruritus (n = 22)	PBC-control (n = 31)		Healthy control (n = 18)	
	Mean ± SD	Mean ± SD	*P* value*	Mean ± SD	*P* value*
Age (years)	52.9 ± 10.5	58.1 ± 9.1	0.0603	53.0 ± 9.5	0.9607
Gender (M:F), n	3:19	All Females	0.1574	3:15	0.7894
BMI (kg/m^2)	27.2 ± 4.9	27.6 ± 5.4	0.7917	26.3 ± 5.4	0.6164
Body weight (kg)	72.81 ± 13.55	71.93 ± 14.81	0.8262	70.2 ± 13.4	0.5589
PBC-40 itch domain score	10.5 ± 3.3	2 ± 1.5	**<0.00001**		
Serum ALP (IU/L)	264 ± 174.13	176.8 ± 132.7	**0.044**		
Serum GGT (IU/L)	211 ± 172.6	84.3 ± 112.5	**0.002**		
Serum ALT (IU/L)	59.3 ± 44.8	39.93 ± 31.71	0.071		
Total serum bilirubin (μmol/L)	12.2 ± 5.5	8.2 ± 4.3	**0.004**		
Serum albumin (g/L)	41.9 ± 4.2	44.7 ± 2.7	0.006		
Serum FGF19 (pg/mL)	162.9 ± 107.5	127.8 ± 102.9	0.245	111.2 ± 53.8	0.09
UDCA dose (mg/day)	967 ± 185.8	836.6 ± 375.0	0.139		

*Fisher's exact test was used to compare gender distribution between PBC with pruritus and controls. Unpaired *t* test was used to compare all other parameters between PBC with pruritus and controls. Significant differences shown in bold.

patients with pruritus and PBC-control. Serum levels of alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT) and bilirubin and PBC-40 itch domain scores were significantly higher in PBC patients with pruritus compared to PBC-control.

3.1 | Bile acids and autotaxin in PBC with pruritus

PBC patients with pruritus had significantly elevated the total BA level compared to PBC-control and HC with glycocholic acid (GCA) and glycochenodeoxycholic acid (GCDCA) levels were significantly higher than PBC-control (Table 2). There was no difference in the levels of UDCA or its conjugates between PBC patients with pruritus and PBC-control.

In PBC patients with pruritus, baseline serum ATX activity was significantly higher (Figure 1A) with a mean serum level of total lysophosphatidylcholine (LPC) significantly lower (221 ± 35.3 μM) compared to HC (259 ± 47.3 μM, $P = 0.04$) but not PBC-control (231 ± 57.2 μM, $P = 0.72$).

At baseline, 5-D itch scores significantly correlated with serum GCA ($r = 0.47$, $P = 0.0257$) and taurocholic acid ([TCA], $r = 0.45$, $P = 0.0349$] levels in PBC patients with pruritus (Table S1). No significant correlations were seen between serum BAs, autotaxin and baseline PBC-40 itch domain score or NRS (Tables S2 and S3).

Analysis of other quantified serum metabolites showed significant differences in 43 metabolites between PBC patients with pruritus and HC (Table S4). However, only one metabolite (C10:2, decadienylcarnitine) was significantly higher in PBC patients with pruritus (0.084 ± 0.026 μM) compared to PBC-control (0.055 ± 0.01 μM, $P = 0.013$; Mann-Whitney test with FDR).

3.2 | IBAT inhibition reduces bile acids and autotaxin

Serum and faecal BA profile data for pre- and post-GSK2330672 were available for 16 patients (samples from six patients were insufficient for analysis). Compared to the baseline, 2 weeks of GSK2330672 treatment significantly reduced the serum levels of all tauro- and glyco-conjugated BAs (Table 3). Total BA level also decreased, but did not reach statistical significance ($P = 0.057$). Serum levels of chenodeoxycholic acid (CDCA) and deoxycholic acid (DCA) significantly increased but cholic acid (CA) did not change significantly ($P = 0.78$). GSK2330672 treatment significantly decreased the serum ATX activity levels (Figure 1B).

Faecal BA profiling (n = 14) showed significantly increased levels of total BA, CA, CDCA and DCA following GSK2330672 treatment, compared to the baseline (Figure 2). No significant differences were seen in other conjugated primary or secondary BAs (Figure S1).

Compared to the baseline, no significant changes were seen in other measured serum metabolites including acylcarnitines, glycerophospholipids or sphingolipids following GSK2330672 (data not shown).

TABLE 2 Serum total and individual bile acid levels in PBC patients with pruritus and control groups

	PBC pruritus	PBC-control	*P* value*	Healthy control	*P* value*
Total bile acids	48.9 ± 56.1	17.3 ± 24	**0.0190**	6.13 ± 5.93	**0.0003**
Cholic acid (CA)	0.29 ± 0.38	0.22 ± 0.31	0.8893	0.25 ± 0.39	0.6222
Taurocholic acid (TCA)	4.04 ± 9.21	0.32 ± 0.64	0.0837	0.19 ± 0.33	0.0965
Glycocholic acid (GCA)	8.95 ± 16.1	0.93 ± 1.51	**0.0134**	0.67 ± 0.52	0.0800
Chenodeoxycholic acid (CDCA)	0.22 ± 0.23	0.32 ± 0.36	0.6561	0.32 ± 0.37	0.7376
Taurochenodeoxycholic acid (TCDCA)	3.83 ± 8.85	0.33 ± 0.47	0.1712	0.42 ± 0.70	0.4141
Glycochenodeoxycholic acid (GCDCA)	13.4 ± 25.6	1.89 ± 2.94	**0.0285**	2.16 ± 1.6	0.5042
Deoxycholic acid (DCA)	0.33 ± 0.29	0.41 ± 0.43	0.5736	0.41 ± 0.47	0.8723
Taurodeoxycholic acid (TDCA)	0.33 ± 0.6	0.17 ± 0.38	0.2035	0.30 ± 0.66	0.5651
Glycodeoxycholic acid (GDCA)	1.97 ± 2.08	0.75 ± 0.84	0.0599	1.1 ± 1.69	0.2979
Lithocholic acid (LCA)	0.04 ± 0.03	0.03 ± 0.02	0.7646	0.01 ± 0.008	0.1517
Taurolithocholic acid (TLCA)	0.04 ± 0.06	0.01 ± 0.01	0.1677	0.01 ± 0.01	0.3987
Glycolithocholic acid (GLCA)	0.15 ± 0.18	0.07 ± 0.10	0.0997	0.06 ± 0.04	0.1661
Ursodeoxycholic acid (UDCA)	4.26 ± 4.5	5.08 ± 9.01	0.5538	0.04 ± 0.04	**<0.0001**
Tauroursodeoxycholic acid (TUDCA)	1.82 ± 4.5	0.20 ± 0.25	0.1554	0.01 ± 0.01	**0.0001**
Glycoursodeoxycholic acid (GUDCA)	9.16 ± 11.7	6.44 ± 10.7	0.9589	0.10 ± 0.05	0.1147

*Compared to PBC patients with pruritus. Statistical significance was determined by unpaired non-parametric test with Mann-Whitney test. Significant differences shown in bold. Total bile acid is the sum of 15 individual bile acids listed in the table. BA levels in μM (mean ± SD).

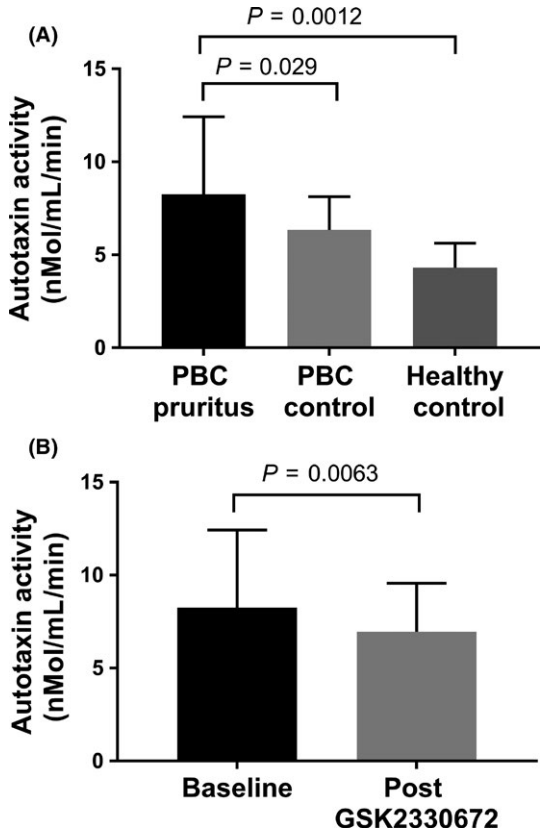

FIGURE 1 Serum autotaxin activity (A) in study cohorts, and (B) in PBC patients with pruritus at baseline and after treatment with GSK2330672. (Data in mean ± SD; Unpaired *t* test used in [A] and paired *t* test in [B])

3.3 | Serum autotaxin correlates with bile acids

In PBC patients with pruritus, significant correlations were observed between conjugated primary and secondary BA levels and serum ATX activity at baseline (Table 4). Also, following GSK2330672 treatment percentage (%) changes (Δ) in serum ATX activity from baseline correlated significantly with %Δ in serum BA levels from baseline (Table 4). However, %Δ in serum BAs (total or individual) or ATX activity did not significantly correlate with %Δ in 5-D itch, PBC-40 itch domain or NRS cores (Tables S5 and S7).

3.4 | Gut bacterial profile in PBC with pruritus

The faecal bacterial composition of PBC patients with pruritus was not significantly different from the two control cohorts. Compositional analysis performed on phylum, class and order levels showed relative abundance of faecal bacteria from PBC patients with pruritus was not significantly different from those of PBC-control or HC ($P > 0.05$ for all comparisons, ANOVA with Benjamini-Hochberg FDR). Comparison of diversity indices showed no significant differences in the Chao1 index ($P = 0.051$, Kruskal-Wallis test) or Shannon index ($P = 0.923$, Kruskal-Wallis test) between study cohorts (Figure S2).

3.5 | IBAT inhibition alters gut bacterial profile

Gut bacterial composition of PBC patients with pruritus was compared at baseline and after 14 days of treatment with GSK2330672 or placebo. For each subject, relative abundance of operational taxonomic units (OTUs) determined at the phylum level is shown in Figure S3. A non-metric multidimensional scaling (NMDS) plot showed clear separation of bacterial composition after GSK2330672 treatment (Figure S4A). Overall, GSK2330672 significantly changed the bacterial community composition at the phylum level (PERMANOVA $P = 0.027$), with a significant decrease in Bacteroidetes ($P = 0.033$) and increase in Firmicutes ($P = 0.033$) (figure S4B). Significant changes were also seen at the class and order levels with decrease in Bacteroidia ($P = 0.040$) and Bacteroidales ($P = 0.011$) and increase in Clostridia ($P = 0.040$) and Clostridiales ($P = 0.044$) respectively (Figure S4C,D). No significant changes were seen at other taxonomic levels.

Changes in faecal microbiota and faecal BA levels following GSK2330672 correlated with strongly positive correlation seen between phylum Firmicutes and CA ($r = 0.99$) and CDCA ($r = 0.95$) and negative correlation between phylum Bacteroidetes and CA ($r = -0.74$) and CDCA ($r = -0.68$) (Figure S5).

4 | DISCUSSION

We report the serum metabonomic profile and gut bacterial composition in PBC patients with pruritus and describe the effects of IBAT inhibition on serum and faecal BAs and compositional alterations in faecal bacteria in this patient group.

In this study, we found altered serum BA profile in PBC patients with pruritus compared to PBC patients without pruritus. In addition to significantly higher levels of total BA, GCA and GCDCA, we observed GCA and TCA correlated with 5-D itch scores in PBC patients with pruritus. A recent trial of NGM282 (an engineered analogue of FGF19) also found significant association between baseline 5-D itch scores and serum GCA and TCA in patients with PBC.[27] We acknowledge that our cohort of PBC patients with pruritus had higher baseline levels of ALP, GGT and bilirubin but FGF19 levels were similar to PBC patients without pruritus. Since serum FGF19 levels are linked to the severity of cholestasis,[28] the latter is unlikely to have biased our serum BA results.

We observed significant decrease in serum total and conjugated BAs following pharmacological IBAT inhibition with GSK2330672. In addition, we have recently reported that GSK2330672 treatment significantly improved pruritus scores in PBC patients with pruritus.[11] Therefore, the antipruritic effect of an IBAT inhibitor agent could be mediated by reduction in circulating BAs. However, reductions in serum BAs did not correlate with reductions in pruritus scores. In a historic study, fasting total BA levels were found to be higher in patients with pruritus compared to those without pruritus.[29] A positive relationship between pruritus and serum BAs has been shown[30] and improvement in pruritus with BA binding resin cholestyramine

TABLE 3 Changes in serum bile acid levels after treatment with GSK2330672 in PBC patients with pruritus

	PBC patients with pruritus (n = 16)		
	At baseline	After GSK2330672	*P* value*
Total BA	50.8 ± 51.3	32.1 ± 39.2	0.0577
Cholic acid (CA)	0.29 ± 0.38	0.10 ± 0.09	0.7820
Taurocholic acid (TCA)	4.46 ± 10.1	0.43 ± 0.88	**0.0002**
Glycocholic acid (GCA)	9.56 ± 16.7	1.72 ± 2.0	**<0.0001**
Chenodeoxycholic acid (CDCA)	0.21 ± 0.24	0.41 ± 0.40	**0.0290**
Taurochenodeoxycholic acid (TCDCA)	3.68 ± 8.83	0.60 ± 1.06	**0.0021**
Glycochenodeoxycholic acid (GCDCA)	11.7 ± 19.5	4.15 ± 4.99	**0.0131**
Deoxycholic acid (DCA)	0.35 ± 0.31	0.65 ± 0.65	**0.0110**
Taurodeoxycholic acid (TDCA)	0.40 ± 0.69	0.16 ± 0.19	**0.0125**
Glycodeoxycholic acid (GDCA)	2.26 ± 2.25	1.64 ± 1.93	**0.0214**
Lithocholic acid (LCA)	0.04 ± 0.03	0.03 ± 0.03	0.3755
Taurolithocholic acid (TLCA)	0.05 ± 0.07	0.01 ± 0.01	**0.0004**
Glycolithocholic acid (GLCA)	0.17 ± 0.20	0.06 ± 0.05	**0.0052**
Ursodeoxycholic acid (UDCA)	3.83 ± 4.0	5.07 ± 4.94	0.1591
Tauroursodeoxycholic acid (TUDCA)	2.46 ± 5.17	0.39 ± 0.56	**<0.0001**
Glycoursodeoxycholic acid (GUDCA)	11.3 ± 13.1	16.6 ± 29.7	0.6322

*Statistical significance determined by paired non-parametric test (Wilcoxon matched-pairs signed rank test). Significant differences shown in bold. Total bile acid is the sum of 15 individual bile acids listed in the table. BA levels in μM (mean ± SD).

further supports their role.[31] Taken together, our findings on differential BAs in PBC patients with pruritus and changes after IBAT inhibition therapy may suggest that serum (total or individual) BAs may have pathogenetic role in cholestatic pruritus.

We also studied serum ATX which drives enzymatic conversion of LPC into LPA, a novel proposed pruritogen in cholestatic diseases.[6,32] Similar to previous studies, we found elevated serum ATX activity in PBC patients with pruritus. Interestingly, we also observed correlations between serum BAs and ATX activity at baseline, with a strong correlation between GCDCA and ATX ($r = 0.80$, $P < 0.0001$). Also, reductions in tauro- and glyco-conjugated primary BAs and ATX levels after GSK2330672 treatment correlated significantly. Our observations on the association between serum BAs and ATX are novel. This, in addition to the recent intriguing finding of the inhibitory effect of GCDCA on ATX activity[33] merits further investigation into the complex interplay between BAs and ATX in cholestatic pruritus.

In the current literature there are only two reports on intestinal microbiota composition in PBC. In their study, Lv et al observed that early stage PBC patients had reductions of several potentially beneficial gut microbiota (such as Acidobacteria, *Lachnobacterium* sp, etc), and the enrichment of some opportunistic pathogens (such as γ-Proteobacteria, Enterobacteriaceae, etc).[19] Tang and co-workers observed reduced species richness and a lower level of microbial diversity in patients with PBC and partial restoration of these changes after UDCA treatment.[20] However, these investigators did not report gut microbiota in relation to pruritus associated with PBC. We hypothesised that pruritus in PBC is associated with specific gut bacterial dysbiosis. But our results did not show any significant difference in faecal bacterial composition or diversity between PBC patients with pruritus compared to the control group. This lack of difference may suggest that cholestatic pruritus may not be associated with a specific gut bacterial composition. However, since we did not study functional alterations in the gut microbiota, we cannot exclude the possibility of microbial metabolites contributing to cholestatic pruritus. Therefore, our negative findings on gut microbiota need to be confirmed in larger studies and additional studies are needed to investigate the role of gut microbial metabolites in cholestatic pruritus.

Evidence suggests that BAs are important in regulating gut microbial community structure[34,35] and animal data show regulatory effects of gut microbiota on BA homeostasis.[36,37] Although effects of IBAT inhibitor agents on serum and faecal BA levels have been studied in animal models of cholestasis,[38,39] to date, there are no human studies on the effect of IBAT inhibition on the gut microbiota. We observed that in PBC patients with pruritus treated with an IBAT inhibitor agent, faecal BA levels increased and faecal bacterial composition significantly changed from baseline. Increased faecal DCA levels could indicate increased conversion of CA to DCA by gut microbiota derived 7-α-dehydroxylase enzymes. Major

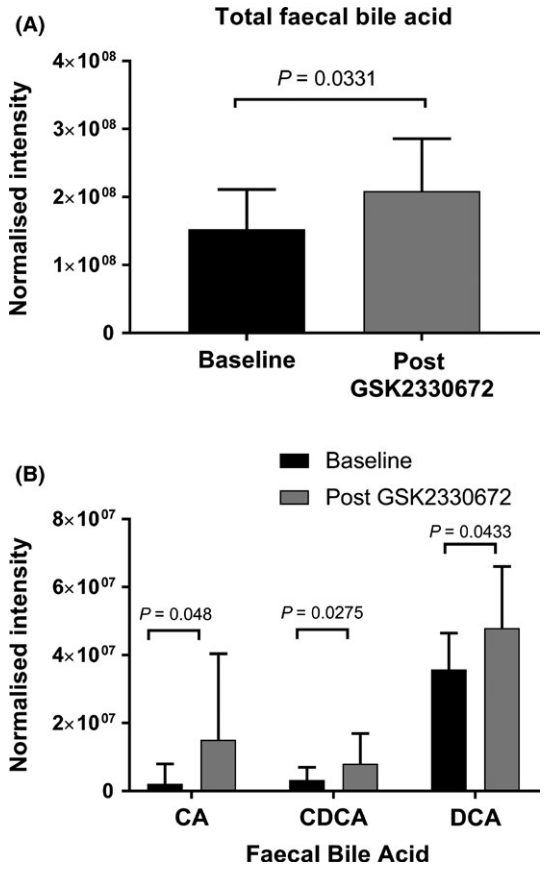

FIGURE 2 Faecal bile acid profile in PBC patients with pruritus at baseline and after treatment with GSK2330672. (A) Total and (B) individual bile acids. (Data in mean ± SD *P* values adjusted with FDR correction as described in method section)

TABLE 4 Correlations between (**A**) serum autotaxin activity and serum bile acid levels in PBC patients with pruritus at baseline, and **(B)** percentage (%) change (Δ) in serum autotaxin activity and bile acid levels after GSK2330672 treatment

	r	95% CI	p value
A) Correlation with ATX at baseline			
Glycochenodeoxycholic acid (GCDCA)	0.80	0.56 to 0.91	**<0.0001**
Glycoursodeoxycholic acid (GUDCA)	0.79	0.54 to 0.91	**<0.0001**
Taurochenodeoxycholic acid (TCDCA)	0.74	0.45 to 0.88	**<0.0001**
Glycodeoxycholic acid (GDCA)	0.71	0.41 to 0.87	**0.0002**
Glycocholic acid (GCA)	0.69	0.37 to 0.86	**0.0003**
Taurocholic acid (TCA)	0.68	0.35 to 0.86	**0.0005**
Taurodeoxycholic acid (TDCA)	0.68	0.36 to 0.86	**0.0004**
Tauroursodeoxycholic acid (TUDCA)	0.51	0.10 to 0.77	**0.0148**
Cholic acid (CA)	0.01	−0.42 to 0.44	0.9578
Chenodeoxycholic acid (CDCA)	0.01	−0.41 to 0.44	0.9364
B) Correlation with %Δ ATX post GSK2330672 treatment			
%Δ Tauroursodeoxycholic acid (TUDCA)	0.71	0.40 to 0.88	**0.0002**
%Δ Taurocholic acid (TCA)	0.60	0.22 to 0.8	**0.0034**
%Δ Taurochenodeoxycholic acid (TCDCA)	0.56	0.16 to 0.80	**0.0079**
%Δ Glycochenodeoxycholic acid (GCDCA)	0.55	0.15 to 0.80	**0.0084**
%Δ Glycocholic acid (GCA)	0.48	0.05 to 0.76	**0.0268**
%Δ Glycoursodeoxycholic acid (GUDCA)	0.46	0.02 to 0.75	0.0337
%Δ Taurodeoxycholic acid (TDCA)	0.39	−0.05 to 0.71	0.0754
%Δ Glycodeoxycholic acid (GDCA)	0.42	−0.02 to 0.72	0.0563
%Δ Cholic acid (CA)	0.15	−0.31 to 0.55	0.5058
%Δ Chenodeoxycholic acid (CDCA)	0.15	−0.30 to 0.55	0.5045

Significant differences shown in bold.

taxonomic alterations were seen at the phylum, class and order levels, respectively, with significant decreases in Bacteroidetes, Bacteroidia and Bacteroidales and increases in Firmicutes, Clostridia and Clostridiales. We hypothesise that these changes are at least in part because of the direct effect of increased BA load in the colon resulting from IBAT inhibition. This idea is supported by increased faecal CA and CDCA levels after GSK2330672 and their strong correlations with Firmicutes and Bacteroidetes. Interestingly, our findings are similar to Islam et al[21] study, where rats fed with high CA diet showed significant expansions in Firmicutes (from 54% to 93%-98%) and Clostridia (from 39% to 70%) and significant inhibition of the Bacteroidetes. However, an important question that remains unanswered by our study, but that merits further investigation is, whether the changes in the gut microbiome produced by the IBAT inhibitor contribute to its antipruritic effect in PBC via changes in faecal microbial metabolites.

Although we have attempted to provide a comprehensive insight into the serum metabonome and gut microbiota in cholestatic pruritus, our study has limitations to be addressed in future studies. First, our relatively small cohort may have resulted in insufficient statistical power to unravel all metabolic perturbations. To determine the complete metabonome profile and microbial diversities, a large cohort of PBC patients with pruritus is required. Ongoing clinical development of GSK2330672 (NCT02966834) may present the opportunity for further study of metabonomic and microbiomic profile in cholestatic pruritus. Second, we did not investigate the metagenome (functional composition profile) of microbiota which may help in the analysis of pathway(s) associated with cholestatic pruritus. Third, instead of mucosal microbiota, we opted to study stool samples, but it is known

that faecal bacterial profiles do not fully replicate mucosa associated profiles.[40] Also, we did not objectively assess stool consistency, which is recently shown to be strongly associated with gut microbiota composition.[41] Finally, although our cohort was matched for age, BMI and ethnicity, results could be influenced by other confounding effects such as environment and dietary factors.

In summary, in PBC patients with pruritus, we observed elevated serum bile acid and autotaxin levels which decreased after antipruritic treatment with an IBAT inhibitor agent. The strong correlation between serum bile acids and autotaxin at baseline and post IBAT inhibition suggests a complex interplay between bile acids and autotaxin in cholestatic pruritus is likely and may be modified by IBAT inhibition to reduce pruritus. Gut bacterial composition of PBC patients with pruritus was not different from control but altered significantly following IBAT inhibition. Our findings need to be confirmed in future studies which should focus on further dissecting the underlying molecular mechanism of cholestatic pruritus and clarifying the mechanisms of the antipruritic effect of IBAT inhibitor agents.

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CONFLICT OF INTEREST

SK is an employee of GlaxoSmithKline (GSK). GMH and DEJ are investigators on the UK-PBC consortium which has received research funding from GSK. All other authors declare no competing interests related to the study.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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REVIEW

A systematic approach to the management of cholestatic pruritus in primary biliary cirrhosis

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ABSTRACT

Pruritus (itch) is an important symptom of primary biliary cirrhosis (PBC), an archetypal cholestatic liver disease. Cholestatic pruritus can be a debilitating symptom causing significant deterioration in patients' quality of life. Effective management of pruritus in PBC involves awareness among clinicians to adequately assess its severity, and treatment with specific drug therapies in line with current practice guidelines. In PBC, antipruritic drugs are not universally effective and/or have significant side effects, and despite best efforts with various combinations of drugs, some patients remain significantly symptomatic, eventually opting for invasive or experimental treatments. Therefore, there is a clear unmet need for better alternative treatments for patients with refractory or intractable cholestatic pruritus. Recent advances in the understanding of pathogenesis of cholestatic pruritus and bile acid physiology have raised hopes for novel therapies, some of which are currently under trial. In this review, we aim to provide a practical guide to the management of this important and complex problem, discussing current knowledge and recent advances in the pathogenesis, summarise the evidence base for available therapeutic approaches and update potential novel future therapies for the management of pruritus in PBC.

INTRODUCTION

Primary biliary cirrhosis (PBC) is an archetypal autoimmune chronic cholestatic liver disease characterised biochemically by elevation in serum alkaline phosphatase (ALP) and gamma-glutamyl transferase (cholestatic liver function tests (LFTs)), serologically by presence of antimitochondrial antibodies and pathologically by apoptotic damage to the biliary epithelial cells lining the small intrahepatic ducts. In untreated patients, chronic immune-mediated injury results in cholestasis and parenchymal injury, which culminate in fibrosis and ultimately end-stage liver disease with associated complications such as portal hypertension, gastro-oesophageal varices, ascites and hepatocellular cancer. Like other autoimmune diseases, PBC predominantly affects women (female-to-male ratio 10:1)[1] and typically patients are women presenting at the age of ≥40. Patients are increasingly, however, diagnosed at a younger age and frequently in the asymptomatic stage of the disease. This is partly due to the increased awareness of the condition among clinicians and widespread availability of the non-invasive diagnostic serological tests.

Pruritus and fatigue are the extrahepatic symptoms accounting for the greatest burden for patients with PBC. In the majority of patients with PBC, pruritus remains mild and tolerable, but in a significant proportion of patients, it may be persistent, leading to severe sleep deprivation, depression, and ultimately, even suicidal ideations. For clinicians, pruritus in PBC and other cholestatic liver diseases is a relevant topic. Knowledge of the condition and specific drugs used for the management of cholestatic pruritus are curriculum requirements for UK trainees in gastroenterology (box 1). Also, a recent study of patients with PBC in the UK reported suboptimal use of antipruritic drugs in patients with itch and suggested a need for improvement in the awareness among clinicians for better management of cholestatic pruritus in PBC.[2]

NATURAL HISTORY OF PRURITUS IN PBC

There is scarcity of published literature on the epidemiology of pruritus in PBC.

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Box 1 Physician gastroenterology 2010 curriculum requirement for UK gastroenterology trainees applicable to the current review:

- Shows recognition of the potential complications of cholestasis, including pruritus.
- Aware of the investigations and treatment of cholestatic pruritus.
- Knows the therapeutic options and potential complications of colestyramine, rifampicin and naltrexone.

It is reported that pruritus occurs in 20–70% of patients with PBC at some point in their illness.[3 4] In the national UK-PBC cohort (n=2705) of patients recruited between 2007 and 2011, up to 70% reported some experience of itch and 46% reported persistent itch (frequent or all the time) since the diagnosis of PBC.[2] Pruritus can develop at any stage of the disease, and it may be the presenting symptom that leads to the eventual diagnosis of PBC. In one study, 75% of patients with PBC reported that pruritus preceded their PBC diagnosis.[5] Indeed, a significant number of initially asymptomatic patients subsequently develop pruritus in the course of their illness. For example, in a large cohort study of 770 patients with PBC from northeast England, the proportion of initially asymptomatic patients developing pruritus was 15%, 31% and 47% at 1, 5 and 10 years of follow-up, respectively.[6] Once pruritus develops, its severity often fluctuates from day to day and it may diminish over time, especially when the diseases becomes more advanced and liver synthetic function deteriorates.[3] However, in the majority of patients, it is unlikely to completely resolve unless effective treatment is started.[7] In a recent study of >2300 patients with PBC, pruritus severity was significantly higher in patients who were unresponsive to ursodeoxycholic acid (UDCA) therapy and showed no association with LFTs or with disease duration.[8] The same study also suggested that intensity of pruritus may be associated with the age at disease presentation. The pruritus score (measured on a visual analogue scale (VAS)) was 64% higher in patients with PBC who presented at younger than age 30 (n=24) in comparison to those presented at older than age 70 (n=178), suggesting that younger patients with PBC are more likely to have severe pruritus.[8]

PATHOGENESIS OF PRURITUS IN PBC

PBC is characterised by chronic immune-mediated destruction of small-sized intrahepatic bile ducts resulting in secondary hepatocyte secretory failure and cholestasis. Pruritus resulting from cholestasis is a complex field, and its pathophysiology remains incompletely understood. It is suggested that in cholestasis compounds (normally excreted in bile) are released into the systemic circulation. Among these compounds, one or more pruritogen(s) may diffuse from the plasma to the skin where they stimulate neural itch fibres. Subsequent transmission to the spinal cord and the brain then elicits a motor response of scratching. Although significant progress has been made in recent years to understand the pathogenesis of this symptom, to date no single substance has been conclusively shown to be the causative pruritogen in cholestasis. The role of bile salts (bile acids) has been controversially discussed, whereas that of endogenous opioids has been supported by several experimental and clinical studies. However, their role in the pathogenesis of cholestatic pruritus is not unequivocal as there are studies and observations to dispute the evidence. Recently, lysophosphatidic acid (LPA), a potent itch neuron activator,[9 10] was suggested as a potential pruritogen in cholestasis.[11 12] The bulk of circulating LPA is formed by autotaxin (ATX), a lysophospholipase D enzyme, and serum ATX activity correlates with itch intensity in cholestatic patients and the response to antipruritic treatments.[11 12] More importantly, rapid relief in itch and strong decline in the serum ATX activity were seen in patients with treatment-resistant cholestatic pruritus when they underwent endoscopic nasobiliary drainage (NBD).[12] It is proposed that in cholestasis an as-yet unidentified (probably biliary) factor increases circulating ATX activity, in turn increasing the levels of LPA, which mediates itch.[13] Very recently, it has been postulated that cholestatic itch may be caused by activation of the plasma membrane receptor TGR5 that, among various cell types, is also expressed on sensory neurons. It has been demonstrated that TGR5 is activated by bile salts and that intradermal injection of bile salts causes itch in wild-type mice but not in mice lacking the TGR5 receptor.[14 15]

A detailed description of pathogenesis of cholestatic itch and evidence for and against various candidate pruritogens in cholestasis is outside the scope of this review and has been comprehensively covered elsewhere.[16–18] Box 2 provides summary evidence supporting three common theories of potential pruritogens in cholestatic pruritus. There is experimental and clinical evidence to support that cholestatic pruritus results from a complex interplay of increased levels of LPA caused by increased ATX activity, increased opioidergic neurotransmission and direct or indirect actions of bile salts (bile acids) and their metabolites, resulting in triggering of pruritoceptive nerve fibres.

CLINICAL PRESENTATION

Clinicians treating patients with PBC should note that pruritus is independent of biochemical severity, duration of the disease and histological stage of PBC.[33] Therefore, it is not unusual to see a patient with

Box 2 Summary of current evidence of potential pruritogens in cholestatic pruritus

1. Bile acids:

Pros:

- Serum levels of bile salts elevated in cholestasis.[19]
- Feeding cholylsarcosine (synthetic bile acid) to cholestatic patients aggravates their pruritus.[20]
- Intradermal application of bile salts induces pruritus in healthy volunteers.[21 22]
- Dramatic reductions in pruritus seen in patients undergoing nasobiliary drainage (which removes bile salts from enterohepatic circulation) or extracorporeal albumin dialysis (which removes bile salts from systemic circulation).[23–25]
- Some antipruritic effects of colestyramine/colesevelam (bind to bile salts in the intestine and reduce serum levels of bile acids).[26–28]

Cons:

- No correlation has been found between plasma bile salt levels and itch intensity in cholestatic patients.
- Patients with bile salt synthesis defects, while cholestatic, generally do not suffer from itch.

2. Endogenous opioids:

Pros:

- Administration of opiate antagonists to patients with cholestasis is associated with an opiate withdrawal-like reaction and relief of pruritus.[29]
- Increased concentration of endogenous opioids in cholestasis.[30 31]
- Intraspinal administration of opioid agonists induces pruritus (eg, itching after epidural or spinal morphine).[32]

Con:

- No correlation has been found between plasma endogenous opioid levels and itch intensity in cholestatic patients.

3. Autotaxin (ATX) and lysophosphatidic acid (LPA):

Pros:

- Serum LPA concentrations markedly increased in cholestatic patients.[11]
- LPA injected intradermally into mice induced dose-dependent scratch response.[11]
- Irrespective of the cause of cholestasis, serum ATX activity markedly elevated in patients with cholestatic pruritus (compared with cholestatic patients without pruritus and healthy controls).[12]
- Significant correlation between serum ATX activity and intensity of itch perception in cholestatic patients.[12]
- Serum ATX activity responds to and is closely correlated with effectiveness of therapeutic interventions.[12]

Cons:

- Causal relationship between ATX and itch remains to be proven.
- Serum ATX is also increased in other pathological conditions not all of which are associated with itch.

early-stage PBC and normal LFTs to present with severe itch, whereas patients with advanced PBC and liver synthetic dysfunction might have no pruritus. It is also important to be mindful of other differential diagnoses when a patient with PBC presents with pruritus. As pruritus is clearly not unique to cholestasis, other cutaneous (eg, psoriasis, atopic dermatitis) and systemic causes (eg, uraemia, lymphoma, myeloproliferative conditions) should be excluded.[34] However, patients with cholestatic pruritus usually describe some characteristic features that can aid in diagnosis. Majority describe their itch as a sensation of irritation deep under the skin—'creepy crawlies', 'bugs crawling' or 'deep itch'. Those with severe itch report that the itch is 'relentless' or so severe that it leads to wanting to 'tear their skin off' or 'scratching until bleeds'.[5] Unlike patients with dermatological conditions, PBC patients with pruritus do not develop primary pruritic skin lesions and scratching barely alleviates their itch.[16] However, intense scratching may produce excoriations, folliculitis, lichenification and rarely prurigo nodularis. For reasons that are not entirely clear, pruritus intensity in PBC shows seasonal (worse in the heat) and diurnal variations (worse at late evening and night time). It typically affects limbs, palms and soles of the feet (palmoplantar) but generalised itch may also occur. It is often exacerbated by psychological stress and contact with certain fabrics (eg, wool). Hormone replacement therapy, premenstrual period, menstruation and late-stage pregnancy may also worsen cholestatic pruritus, suggesting a role of female sex hormones.[16]

Assessment of pruritus

A patient with PBC presenting with pruritus needs systematic evaluation. Presence of skin lesions (other than scratch marks) should prompt referral to dermatology to rule out skin conditions contributing to pruritus.[35] Assessment of itch severity is useful not only to allow objective assessment of impact on patients' health and quality of life (QoL) but also to evaluate the effect of therapy. As itch is a sensation, its quantification is inherently difficult and subject to considerable inter-individual and intra-individual variation. Scratching,

Table 1 Tools used for the assessment of pruritus in PBC

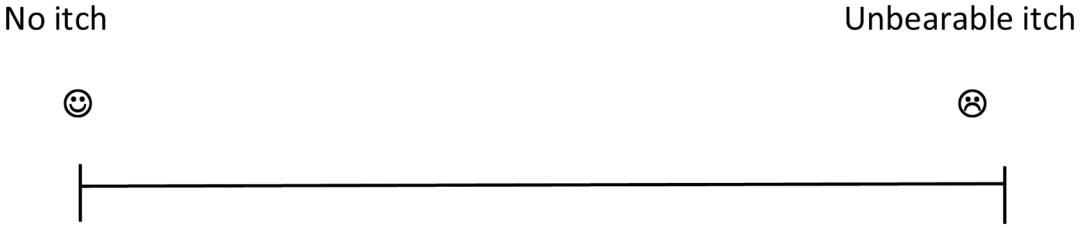
1. Grading scale

For example, on a scale of 0 to 10, where 0 is no itch and 10 is unbearable itch, how would you rate the worst itching you have experienced in the last seven days?

Worst itch: _____/10

2. Visual analogue scale (VAS)

For example, please mark on the scale below, indicating the worst itch you have experienced in the last seven days



3. PBC-40 itch score

	Never	Rarely	Occasionally	Frequently	Always
(i) Itching disturbed my sleep	1	2	3	4	5
(ii) I scratched so much, I made my skin raw	1	2	3	4	5
(iii) I have felt embarrassed because of the itching	1	2	3	4	5

Total score obtained by adding the individual scores. Maximum score 15, minimum score 3. Itch intensity: <3, none; 4–8, mild; 9–11, moderate; >12, severe.
PBC, primary biliary cirrhosis

the behavioural response to itch, can be quantified using piezo-film technology.[36] However, such complex techniques are used exclusively in clinical trials and are not routinely available in clinical practice. Instead, simple questionnaires that are not time consuming and do not burden healthcare providers and patients are preferred. These include the grading scale (GS), VAS, PBC-40 questionnaire (table 1) and 5D itch scale.

In the GS, patient is asked to rank the severity of itch on a scale of 0 (no itch) to 10 (worst itch). Similarly, in the VAS, itch severity is decoded into a point on a line from 0 to 10. GS and VAS are useful for detecting or monitoring change in an individual over time or with treatment. Both GS and VAS are simple tools but they are not specific to PBC. They require the patient to use thought processes to convert their itch severity to a mark on a continuum, and in the VAS the scoring requires manual measuring of the mark with a ruler.[37]

PBC-40 is a disease-specific QoL assessment tool developed and validated for self-completion by patients with PBC.[38] It consists of 40 items grouped into six domains of typical PBC symptoms (fatigue, itch, cognition, emotional, social and other symptoms). The itch domain consists of three questions framed as statements (table 1).

More recently, the 5D itch scale has been designed to characterise the extent of itch and its impact by defining five dimensions of itch. This single-page questionnaire grades the itch according to the duration, degree (severity), direction (getting better or worse), disability (impact on QoL) and distribution (skin sites affected). It is not validated for cholestatic itch specifically as only 63 of 234 patients included in this study had pruritus due to liver disease,[37] but it is easy to use and informative for clinical as well as research purposes.

Investigations

Blood tests usually show cholestatic LFT but serum bilirubin and alanine transaminase may be normal (particularly in early-stage PBC). Elevated serum bilirubin and jaundice usually suggest biliary obstruction (extrahepatic cholestasis) or advanced PBC. All patients with cholestasis and pruritus should have a trans-abdominal ultrasonography in which a finding of intrahepatic duct dilatation suggests an alternative process of biliary obstruction that may warrant further evaluation using CT and/or MRI/magnetic resonance cholangiopancreatography.[3] Generally, treatment of biliary obstruction (which involves endoscopic, surgical or transcutaneous biliary diversion depending on the aetiology) promptly relieves pruritus. Other systemic causes of pruritus (such as uraemia, lymphoma and myeloproliferative diseases) may coexist. These conditions should be considered, appropriately investigated and referred to relevant specialties for further management.

Currently, there is no diagnostic role of measuring serum ATX activity in PBC patients with pruritus.

Table 2 Currently available drugs for the treatment of pruritus in PBC

Approach	Drug	Dose (/day)	Proposed mechanism of action	Comments
First line	Colestyramine Colesevelam	4–16 g 3.75 g in 2–3 divided doses	Bile acid resins. Bind to the bile acids, reduce their reabsorption in the intestine and increase faecal excretion	▶ Morning and afternoon dose preferred ▶ Separate at least 4 h from other drugs ▶ Unpleasant taste (colestyramine) ▶ Bloating, constipation and diarrhoea (less with colesevelam)
Second line	Rifampicin (rifampin)	150–600 mg	▶ Pregnane X receptor (PXR) agonist ▶ PXR-mediated down regulation of ATX transcription ▶ Inducer of microsomal enzymes leading to increased metabolism of endogenous pruritogenic compounds (including opiates) ▶ Inhibition of bile salt uptake by hepatocytes ▶ Altered intestinal metabolism of pruritogens by antibiotic effect on the intestinal microbiota	▶ Fortnightly monitoring of blood count and liver biochemistry ▶ Hepatitis (~10% risk), liver failure and haemolysis
Third line	Naltrexone	50 mg	Mu opioid antagonist	▶ Start at 12.5 mg/day, increase every 2–3 days; monitor liver biochemistry ▶ Opioid withdrawal like reaction (abdominal pain, high blood pressure, tachycardia, goose bumps and nightmares)
Fourth line	Sertraline	100 mg	Serotonin reuptake inhibitor, antipruritic mechanism unclear	▶ Start at 25 mg/day, increase gradually

ATX, autotaxin; PBC, primary biliary cirrhosis.

However, serum ATX activity may be a useful diagnostic marker in intrahepatic cholestasis of pregnancy (ICP), a condition defined by pruritus but often with only mild cholestasis. As ICP is associated with serious adverse maternal and fetal outcomes,[39] accurate diagnosis of ICP in pregnant women presenting with pruritus is crucial. Serum ATX activity (cut-off value 27 nmol/mL/min) has been shown to accurately distinguish ICP from other pruritic disorders of pregnancy and pregnancy-related liver diseases.[40] In women with sustained pruritus after delivery, other underlying liver diseases, including PBC, should be investigated.

TREATMENT OF CHOLESTATIC PRURITUS

Physicians treating patients with itchy PBC should aim for effective symptom control as any improvement in the itch symptom is likely to have a positive effect on the patient's mood, quality of sleep and help his/her social function. Drugs endorsed by the European Association for the Study of the Liver (EASL) and the American Association for the Study of Liver Diseases (AASLD) guidelines (both in 2009) for the treatment of cholestatic pruritus include bile acid resins (colestyramine), rifampicin, opiate antagonists (naloxone, naltrexone) and sertraline.[3 4] Although the evidence base for some of these drugs is not high, the guidelines recommend their use in a step-wise manner. Table 2 summarises the current drug therapies, and figure 1 shows our approach to cholestatic pruritus ('treatment ladder').

UDCA, the current standard of care and so far the only disease-modifying therapy licensed for PBC, is not effective in treating pruritus.[4] In fact, it may exacerbate itch in some patients ('paradoxical itch'), which necessitates stopping or reducing UDCA. It is also noteworthy that antihistamines have no role as antipruritic agents in PBC and their routine use should be discouraged.[4] They can make sicca symptoms (eg, dry mouth) worse.[3] Use of sedative antihistamines should be reserved in those with significant nocturnal itch. General skin care should be encouraged and patients with mild, localised itch should be offered topical emollients with a coolant (eg, preparations containing aqueous cream and menthol).

Colestyramine (cholestyramine) is the guideline-recommended first-line therapy for pruritus in PBC.[3 4] Controlled trials and a meta-analysis have confirmed that it is a safe and effective therapy for cholestatic pruritus.[26–28 41 42] Its unpleasant taste limits its regular use. Colesevelam is a novel anion binding resin with a sevenfold higher bile acid-binding capacity and fewer side effects. Evidence to support colesevelam is scarce. The only published randomised placebo controlled trial of colesevelam (35 patients) in cholestatic pruritus was unable to demonstrate that it was more effective than a placebo in alleviating the severity of pruritus of cholestasis.[43] However, a trial of colesevelam should be offered to patients who respond to colestyramine but are unable to tolerate its side effects. Patients should be advised to take colestyramine and colesevelam at least 4 h apart from other medications as they might interfere with their intestinal uptake.

Patients intolerant to or not responding to bile acid resins should be switched to rifampicin (second-line therapy).[3 4] Rifampicin has the strongest evidence base for the treatment of cholestatic itch and is effective in about 70% of patients. Two meta-analyses reviewing five studies (n=62) on short-term (7–14 days) use of rifampicin have confirmed its safety and efficacy in treating cholestatic pruritus.[41 44] However, the majority of patients with PBC who

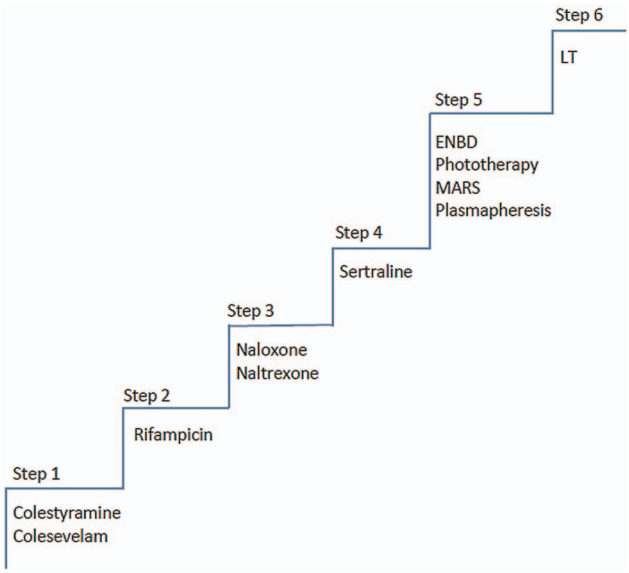


Figure 1 Cholestatic pruritus treatment ladder. If there is no response with one category of drugs, 'move up' the ladder. Patient may need combination of treatments to achieve and/or maintain symptom remission. Particular treatments may not be suitable for all patients. ENBD, endoscopic nasobiliary drainage; MARS, molecular adsorbent recirculating system; LT, liver transplantation.

develop rifampicin-induced liver injury ('rifampicin hepatitis')[45] have been shown to do so in the first two months of therapy. In the Bachs *et al*[46] study, 2/16 (12.5%) of patients with PBC treated with rifampicin (10 mg/kg/day) for mean 11.7 months developed hepatitis within two months of starting therapy. Similarly in the case report by Prince *et al*,[47] 3/41 (7.3%) of patients with PBC developed significant hepatitis and two of these were within first two months of rifampicin therapy. In clinical practice, it is now accepted that significant hepatotoxicity develops in only a limited number of patients with PBC receiving rifampicin. However, as the factors predicting 'at risk' patients are unknown, regular monitoring of LFTs in the first two months is recommended in all patients.[4] Also, as there is no clear evidence on the effect of rifampicin dose on hepatotoxicity, it should be started at 150 mg once daily and increased cautiously to maximum 600 mg daily based on clinical need.[4] In those who develop rifampicin hepatitis, prompt cessation of therapy usually improves LFTs.

Naloxone (intravenous) and naltrexone (oral) are the third-line drugs recommended for cholestatic pruritus.[3 4] They are generally well tolerated but can induce 'opioid withdrawal reaction'. This can be avoided (or reduced) by giving naloxone as a continuous intravenous infusion for 72 h followed by oral naltrexone started at 12.5 mg daily and discontinuation of the infusion. The dose of naltrexone can be gradually titrated to maximum 50 mg daily with regular monitoring of LFTs (hepatotoxicity is rare but has been reported).[48]

Sertraline is the fourth-line drug recommended by both EASL and AASLD guidelines. There is evidence to support its use,[49 50] but its mechanism of antipruritic action is not fully understood. However, experience with sertraline for pruritus treatment has been disappointing for many clinicians.[4]

Other drugs that are not guideline recommended but can be used in resistant pruritus are cimetidine and gabapentin. Patients with medically refractory or intractable pruritus despite maximal therapy with combination of the above-mentioned drugs should be referred to tertiary centres that have experience with experimental and/or more invasive interventions. Treatments that can provide immediate albeit short-term relief from cholestatic pruritus include ultraviolet B phototherapy,[51] NBD,[23 24] plasmapheresis[52 53] and extracorporeal albumin dialysis or molecular adsorbent recirculating system.[25 54–59] However, these invasive treatments can be associated with complications (eg, risk of pancreatitis with NBD). Finally, refractory cholestatic pruritus in PBC is a variant indication for liver transplantation and such patients should be offered referral to a transplant centre even in the absence of cirrhosis or liver synthetic dysfunction.[3 4]

NOVEL THERAPIES

Recent advances in the understanding of the bile acid physiology and pathophysiology of cholestatic pruritus have raised hopes for novel therapies to treat pruritus in PBC.

ASBT inhibitor

Currently, the main class of drugs being investigated in clinical trials as antipruritic agent is apical sodium-dependent bile acid transporter (ASBT) inhibitor (also called ileal bile acid transporter (IBAT) inhibitor). The scientific rationale for ASBT (IBAT) inhibition is based on its physiological role in the enterohepatic circulation of bile acids.[60] ASBT is predominantly located in the terminal part of the small intestine.[61] It mediates the uptake of conjugated bile acids across the brush border membrane of the ileal enterocyte. Subsequently, the bile acids are carried through the intestinal wall into the blood stream, where they are circulated to the liver via the portal vein. Pharmacological inhibition of IBAT is expected to block the uptake of bile acids in the terminal ileum, increase their excretion in the faeces and decrease the amount of bile acids returning to the liver via the enterohepatic circulation. An experimental study showed that inhibition of intestinal bile acid absorption by ASBT inhibitor A4250 attenuates bile acid-mediated cholestatic liver injury by reducing biliary bile acid output.[62] Therefore, pharmacological diversion of bile acids has a potential therapeutic role in cholestatic pruritus. By reducing the hepatic and systemic concentration of bile acids, ASBT inhibitor is

expected to improve pruritus. Currently, two phase II multicentre placebo-controlled randomised double-blind clinical trials are investigating the safety and efficacy of the ASBT inhibitors in PBC patients with pruritus (ClinicalTrials.gov identifier: NCT01899703 and NCT01904058).

Fibrates

Recently, there has been an increase in enthusiasm about fibrates (fenofibrate and bezafibrate) as novel anticholestatic therapy for PBC. Growing body of literature confirms that fibrates produce significant improvement in liver biochemistry (mainly ALP) when used in combination with UDCA in patients with PBC with suboptimal biochemical response to UDCA.[63] Of these studies, interestingly, two studies with bezafibrate and one study with fenofibrate reported improvement or disappearance of pruritus in patients with PBC treated with fibrates.[64–66] Also, anecdotal observations suggest that fibrates improve itch in some patients with PBC. The beneficial effect of fibrates as anticholestatic agents is primarily due to their peroxisome proliferator-activated receptor agonist action.[63] But the precise molecular mechanism(s) of action of fibrates in improving cholestatic itch, if any, is currently unknown. To elucidate the potential effect of fibrates on cholestatic itch, a European multicentre randomised placebo-controlled trial of bezafibrate is currently in preparation (Prof Beuers, personal communication).

ATX and LPA inhibitors

Recent experimental evidence for the role of ATX and LPA in the pathogenesis of cholestatic pruritus suggests that inhibiting ATX or blocking the LPA receptors could potentially improve pruritus in PBC. Therefore, both ATX and LPA are attractive medicinal targets in cholestatic pruritus. Although they have not reached clinical trials for this goal, ATX inhibitors are being developed and studied as anticancer drugs.[67] Whether or not they could be used in treating patients with cholestatic pruritus requires further experimental studies and clinical trials.

CONCLUSION

Pruritus is an unpleasant symptom that is prevalent and often significant complaint in patients with PBC. It should be assessed and treated as carefully as other aspects of the disease. Specific antipruritic therapy should complement disease-modifying drugs such as UDCA and comprise a step-up approach with guideline-recommended therapies. Ultimately, the possible risks and potential benefits of more invasive strategies might be considered to provide patients with an acceptable QoL. Currently ongoing research on the so far enigmatic pathophysiology of cholestatic itch may provide us with new therapeutic targets in the near future. Ileal bile acid transport inhibitors and fibrates are under investigation as potential future antipruritic agents in treating cholestatic pruritus.

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What Comes after Ursodeoxycholic Acid in Primary Biliary Cholangitis?

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Keywords
Non response · Obeticholic acid · Primary biliary cirrhosis/cholangitis · Symptom · Ursodeoxycholic acid

Abstract
Primary biliary cholangitis (PBC) is a rare autoimmune liver disease characterized by chronic cholestasis. Treatment with the accepted primary therapy ursodeoxycholic acid (UDCA) has been shown to be associated with delayed disease progression probably through reduced impact of cholestatic injury on the target biliary epithelial cells. Patients with inadequate response to UDCA (which can be identified through validated biochemical criteria) are at increased risk of disease progression, need for liver transplantation, and death. Obeticholic acid (OCA) is a farnesoid X receptor (FXR) agonist which has been evaluated as a second-line therapy in PBC and has been recently licensed by the Food and Drug Administration and European Medicines Agency for use in patients showing an inadequate response to UDCA or who are unable to tolerate it. Although evidence for biochemical improvement by OCA is compelling, there is, as yet, no evidence that OCA improves hard clinical outcomes or quality of life. In addition, OCA may not be suitable for PBC patients with pruritus as it can worsen the symptom. Other novel agents currently in clinical development may have better side-effect profile. Fibrates have the potential but currently lack high quality evidence to support their routine clinical use in PBC. Symptom management of PBC is challenging and ASBT inhibitors and rituximab are being evaluated for pruritus and fatigue, respectively.

Introduction

Primary biliary cholangitis (PBC), previously known as primary biliary cirrhosis, is a rare chronic autoimmune cholestatic liver disease which is characterized by lymphocytic cholangitis, gradual destruction of the small intrahepatic bile ducts, and progression to fibrosis, cirrhosis, and ultimately liver failure. It has a female preponderance with female to male ratio of 10:1 and usually presents in the fourth to sixth decade [1].

Patients with PBC can present with symptoms of fatigue or pruritus or, rarely, features of portal hypertension and/or advanced liver disease (ascites, hepatic encephalopathy, and esophageal variceal bleeding). A quarter of the patients are, however, asymptomatic at presentation and are identified through cholestatic liver function tests found on routine testing. PBC is currently diagnosed based on the fulfillment of 2 out of 3 criteria (elevated serum alkaline phosphatase [ALP], positive anti-mitochondrial antibody [AMA] or PBC-specific antinuclear antibody reactivity, and liver histology compatible with PBC) [2, 3]. As AMA is detected in 90–95% of patients with PBC and in less than 1% of normal controls liver histology is no longer a prerequisite for diagnosis of PBC [4]. Non-invasive methods such as transient elastography have mostly replaced liver biopsy for assessment of disease progression. Liver biopsy would be indicated in the absence of AMA and when considering other differential diagnosis [3, 5].

Ursodeoxycholic acid (UDCA) is the established first-line therapy and the current standard of care for

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PBC. However, up to 40% of patients have incomplete biochemical response to UDCA and this has been shown to be associated with the risk of progression to end-stage liver disease and worse transplant-free survival rates than UDCA-responsive patients [3, 6–9]. Additional therapeutic options are therefore needed to improve clinical outcomes for this group of patients. To this end, a number of second-line agents are being evaluated, and among them, obeticholic acid (OCA), a farnesoid X receptor (FXR) agonist, has been licensed as a second-line therapy in PBC by the Food and Drug Administration (FDA) and European Medicines Agency (EMA).

This review will elaborate on the development of UDCA as standard accepted therapy, UDCA treatment response criteria and associated prognostic factors, the development of OCA as second-line therapy. It will also touch on the role of fibrates and several new agents evaluated for symptoms of pruritus and fatigue.

UDCA as Standard Accepted Therapy in the Treatment of PBC

UDCA is the current standard of care for patients with PBC at the recommended adult dose range of 13–15 mg per kg/day [2, 3]. The mechanism of action of UDCA in PBC is thought to be through diluting toxic bile acids (BAs), promoting their excretion, upregulating the biliary bicarbonate "umbrella" which protects the biliary tract, and exerting immune modulatory and anti-inflammatory effects [8, 10].

UDCA was studied in 5 randomized, placebo-controlled trials in PBC patients (stages I–IV) with some positive or mixed findings. The first trial (n = 180 patients, Mayo Clinic) found that UDCA use was associated with delayed progression of the disease but there was a limited effect on symptoms, histology, and need for liver transplantation or survival [11]. A second multicenter trial (n = 222 patients, Canada) showed that UDCA significantly improved serum markers of cholestasis but had no effect on survival or need for liver transplantation [12]. A third trial in Europe (n = 145) found reduction in rates of disease progression, need for liver transplantation, and probability of death in the UDCA-treated group compared to placebo [13]. This was followed by a trial in the USA (n = 151) which found major improvements in liver biochemical parameters in patients with less severe PBC (bilirubin <2 mg/dL) as compared to patients with entry serum bilirubin more or equal to 2 mg/dL. Liver histology also improved in the former group but not in the latter group [14]. The Spanish trial (n = 192) found that UDCA improved liver histology, prevented histological stage progression but had no effect on survival or liver transplantation. The study concluded UDCA may be useful for preventing the progression of PBC [15].

However, the above studies did not show any survival benefit by UDCA treatment. Moreover, 2 meta-analyses of 11 and 16 randomized controlled trials (RCTs), respectively, did not show a positive effect on survival. This may be due to the inclusion of trials of short duration (3 months to 2 years) as well as studies using UDCA doses which are currently known to be ineffective [16, 17]. Further reports with long-term follow-up have, however, shown the beneficial effect of UDCA on long-term survival in patients with PBC on standard doses (13–15 mg/kg/day). The transplant-free survival for patients (with early-stage PBC) treated with UDCA were equivalent to an age- and gender-matched healthy control population [18–20]. Taken together, evidence suggests that UDCA improves biochemical markers of cholestasis, slows down the progression of PBC, and delays liver transplantation and death with improved survival [2, 3].

Non-Response to UDCA Increases Risk of PBC Disease Progression

UDCA is an effective treatment and UDCA treatment response has been shown to be strongly predictive of long-term outcome in PBC [21]. However, up to 40% of patients with PBC fail to respond adequately to UDCA and do not improve their liver biochemistry [6, 7, 9]. These patients with incomplete biochemical response to UDCA are referred to as "UDCA non-responders" and in comparison to UDCA responders, have poorer prognosis due to greater risk of disease progression, higher mortality risk, and likelihood to require liver transplantation [1]. Over the years, several treatment response criteria that have been developed based on various biochemical endpoints used in the clinical trials and are used as prognostic models (Table 1) [6, 22–24]. The Barcelona criteria for instance were developed following post-hoc analysis of the Spanish trial, evaluating patients based on their biochemical response to UDCA. This included total bilirubin, ALP, albumin, and prothrombin time. This analysis showed that in patients whose serum ALP reduced by 40% from baseline or normalized had similar survival to a standardized population. Patients who had incomplete

Table 1. Treatment response criteria

Criteria	Definition
Barcelona	Decrease in alkaline phosphatase level >40% of baseline level or a normal level
Paris I (all criteria met)	Alkaline phosphatase level ≤3 × ULN Aspartate aminotransferase level ≤2 × ULN Normal bilirubin level
Paris II (all criteria met)	Alkaline phosphatase level ≤1.5 × ULN Aspartate aminotransferase level ≤1.5 × ULN Normal bilirubin level
Toronto	Alkaline phosphatase level ≤1.67 × ULN

biochemical response had slightly better survival rates than predicted but worse than a comparable population group [23].

More recently, the United Kingdom PBC (UK-PBC) group studied a cohort of 2,353 patients and used all 4 treatment–response biochemical criteria (Barcelona, Paris I, Paris II, Toronto) to categorize patients as responders or non-responders to UDCA. Irrespective of the criteria used, there was a clear distinction in survival outcomes between responders and non-responders. Other predictive factors for nonresponse to UDCA include women diagnosed before age 50 years and men irrespective of age [7].

The same group compared 380 post-transplant patients and 2,300 non-transplant patients with PBC and found that more than 50% of patients presenting below the age of 50 had failed primary therapy (UDCA nonresponse) or already been transplanted at the time of the study. This further emphasized that PBC carries greater risk in younger patients and improved treatment options are urgently needed in this targeted group [25].

The Global PBC Study group, an international and multicenter collaboration between 15 liver centers in 8 North American and European countries performed a meta-analysis, combining individual patient data from major long-term follow-up cohorts. This showed the utility of serum levels of ALP and bilirubin as surrogate end points of outcomes in PBC. An elevated ALP greater than 1.67 × upper limit of normal (ULN) was independently associated with a 2–2.5 increase risk of transplantation or death compared to the risk associated with normal levels. A raised bilirubin above the ULN had a 5.1–10.7 times risk of liver transplantation or death compared to the risk associated with normal levels [26]. These 2 criteria (ALP >1.67 × ULN and bilirubin >ULN) have since been validated by the group and has become the de facto standard entry criteria for current trials of new treatments in PBC, notably the landmark POISE trial [27].

Obeticholic Acid

OCA (INT-747) is a semi-synthetic analog of the primary BA chenodeoxycholic acid that interferes with BA homeostasis through selective activation of the nuclear hormone receptor FXR [8, 28]. FXR activation reduces de novo synthesis of BAs in hepatocytes and increases transport of BAs out of hepatocytes leading to reduced exposure of the hepatocytes to BA. OCA has also been shown to have FXR-activation mediated anti-cholestatic, anti-inflammatory, and anti-fibrotic effects in pre-clinical and clinical studies [8, 28]. Due to these beneficial properties, it was postulated that OCA might exert a positive effect in patients with PBC.

OCA has been evaluated in both phase 2 and 3 trials. The first 12-week phase 2 trial (double-blind placebo-controlled parallel group dose–response study) randomized 59 patients with PBC (not on UDCA for 6 months with persistently raised ALP 1.5–10 × ULN) to placebo, OCA 10 mg, or 50 mg per day for 12 weeks. At the end of the study, the 10 mg group showed an ALP decrease from 3.9 × ULN pre-treatment to 1.9 × ULN. Pruritus was the most common adverse effect (AE; placebo – 30%, 10 mg – 70%, 50 mg – 94%) with severity and discontinuation rate increasing with dose [29]. The next key phase 2 trial (3-month duration with a 1-year open-label extension) randomized 165 patients with PBC who were on optimal UDCA dosage for 6 months and had an inadequate response to UDCA (persistently raised ALP 1.5–10 × ULN) to a range of OCA doses (10, 25, and 50 mg) or placebo. All OCA groups met the primary end point (relative percentage change in ALP values from baseline [day 0] to the end of the study [day 85] compared to placebo) with statistical significant reduction ($p < 0.0001$) of mean ALP values from baseline at the end of the study. The study concluded that daily OCA (10–50 mg) reduced ALP significantly compared with placebo in patients with PBC who had inadequate response to UDCA. Pruritus was again noted to be the main AE with incidence of 47% (no statistical significant difference), 87% ($p < 0.0003$), and 80% ($p < 0.006$) in the OCA 10, 25, and 50 mg group, respectively, compared to 50% in the placebo group [8].

More recently, a phase 3 randomized, double-blind, placebo-controlled trial (POISE trial) has been reported [27]. Patients were treated with OCA for over 12 months

with open-label extension data of a further 1 year. This landmark study recruited 216 patients who had inadequate response to at least 1 year of UDCA monotherapy (at optimal dosage) or were intolerant to UDCA. The biochemical entry criteria were ALP level more than 1.67 × ULN or abnormal total bilirubin level not more than 2 × ULN. The patients were randomly assigned to 10 mg OCA, 5 mg OCA with adjustment to 10 mg if applicable (5–10 mg group), or placebo. The primary end point was an ALP level <1.67 × ULN with a reduction of at least 15% from baseline and a normal total bilirubin level, an end point validated in both the UK-PBC and Global PBC patient cohorts. Out of the 216 patients, 93% were on UDCA therapy. The primary end point was met in 47% of the OCA 10 mg group and 46% of the titration group (5–10 mg) compared to 10% in the placebo with UDCA group ($p < 0.0001$). Secondary end points included reduction in ALP level which was greater in the OCA groups compared to placebo, with significant reduction from baseline at 1 year (least squares mean ± SE reduction, –113 ± 14 U/L in the titration group, and –130 ± 15 U/L in the 10 mg group compared to –14 ± 15 U/L in the placebo group; $p < 0.001$ for both comparisons). With regards to symptom control, there was no improvement in the PBC-40 questionnaire scores with patients in the 10 mg group scoring significantly worse itch-domain scores than those in the placebo group (during the first 3 months of the study). Of those who continued into the open-label extension of the study after completing the 12-month double-blind phase (i.e., 193 of 198), a sustained decrease in ALP and total bilirubin levels was seen in the OCA group. Those in the placebo group who received OCA in the open-label extension also had similar efficacy as those in the OCA group. Non-invasive parameters for liver fibrosis (transient elastography/Fibroscan, hyaluronic acid, and TIMP-1) did not change significantly between baseline to 12 months for both treatment group and placebo group.

Pruritus was the main AE occurring more in the OCA group (56% in the titration group and 68% in the 10 mg group) compared to placebo (38%). Treatment discontinuation occurred in 10% of the OCA 10 mg group ($n = 7$) and 1% ($n = 1$) of the 5–10 mg group. The study concluded that OCA in combination with UDCA or as single therapy for 12 months in patients with PBC resulted in reduction in ALP and total bilirubin levels that were significantly different from the chances observed with placebo [27].

A further double-blind randomized controlled phase 3b trial (COBALT study, NCT02308111) is currently in recruitment to study the clinical outcomes of patients with PBC, comparing patients randomized to taking OCA vs. placebo (standard of care). The primary end point events include death, liver transplant, MELD score ≥15, uncontrolled ascites, hepatocellular carcinoma, and hospitalization for new onset or recurrence of either variceal bleed or encephalopathy or spontaneous bacterial peritonitis.

In the meantime, the FDA has approved OCA as a second-line therapy for adult patients with PBC who have inadequate response to UDCA, used in combination with UDCA or as monotherapy in patients intolerant to UDCA.

Other Agents

In addition to OCA, a number of other novel drugs are emerging as potential second-line agents to treat PBC patients with UDCA non-response. Some of these are already in phase 2 clinical development. For instance, LJN452 (Novartis pharmaceuticals), a non-BA FXR agonist is currently being investigated in a multicenter double-blind study to assess the safety and efficacy in PBC patients with UDCA non-response (NCT02516605). Similarly, a phase 2 placebo-controlled RCT of NGM-282 (NGM Biopharmaceuticals, Inc.), a FGF-19 analog administered in combination with UDCA for 28 days (NCT02026401), and a further phase 2b study to evaluate 3 different doses of NGM-282 administered for 24 weeks (NCT02135536) have been completed and results are pending.

In a recent phase 2 double-blind placebo-controlled study (NCT02609048), MBX-8025 (CymaBay Therapeutics, Inc.), an orally administered, potent, and selective peroxisome proliferator-activated receptor (PPAR) delta agonist has been shown to markedly improve biochemical markers of cholestasis in patients with PBC. The unblinded data from 26 patients completing at least 2 weeks of treatment with MBX-8025 (or placebo) showed mean decreases of 57 and 62% from baseline in ALP for the 50 and 200 mg dose groups, respectively (vs. 0.37% for placebo; $p < 0.0001$ for both). Also, the responder rates for the placebo, 50 and 200 mg groups were 10, 67, and 100%, respectively. These early results are encouraging and suggest that MBX-8025 produces a rapid and potent anticholestatic effect in patients with PBC. However, this study was terminated early as 3 cases of asymptomatic increases in transaminases were observed (2 in the 200 mg MBX-8025 and 1 in the 50 mg MBX-8025 cohorts).

Fibrates (fenofibrate and bezafibrate) have long been proposed as adjunctive treatment for UDCA non-response in PBC. The anti-cholestatic effect of fibrates is pri-

marily due to the PPAR-mediated inhibition of hepatic BA synthesis. In addition, fibrates facilitate elimination of toxic BA, increase biliary phospholipid output, and exert immune modulator function via inhibition of NFκB [30]. A substantial body of circumstantial evidence (case series and small and/or short-term uncontrolled studies) have supported safety and efficacy of using fenofibrate and bezafibrate as adjunctive therapy to UDCA for up to a year in patients with PBC with incomplete response to UDCA [31, 32]. However, to date, there are no prospective RCTs of fibrates in PBC with hard end points and limited data exist on their use beyond 12 months [33]. Moreover, the effect of fibrates on disease progression is unclear. In a recent study, fenofibrate therapy was associated with significant improvements in decompensation-free and transplant-free survival in PBC patients with incomplete UDCA response but cirrhotic patients treated with fenofibrate had significantly higher elevation in serum bilirubin compared to the controls [34]. Therefore fenofibrate, should be used cautiously in PBC patients with cirrhosis with close monitoring for clinical/biochemical decompensation [34, 35]. Overall, there remains a considerable gap in our knowledge on fibrates in PBC and high quality RCTs are needed to address the effect of fibrates not only on biochemical markers, but also on histology, quality of life, need for transplantation, and survival [36]. Recently, bezafibrate has been subjected to a large multicenter, prospective, double-blind, placebo-controlled phase III study in combination with UDCA in PBC (BEZURSO study; NCT01654731) and results are pending.

New Treatments for PBC-Associated Symptoms

Pruritus and fatigue are the 2 most common symptoms of PBC, and their association with PBC is independent of biochemical severity or stage of the disease and seemingly unresponsive to both UDCA and OCA, the licensed therapies. Pruritus and fatigue are associated with significant impairment in the quality of life of PBC patients and they are often life altering when severe. The mechanisms driving these symptoms are complex and the treatment is challenging with few effective therapies available. UDCA, the primary therapy of PBC, has never been shown to reduce the severity of pruritus or fatigue. However, a cross-sectional study of 2,353 PBC patients showed that pruritus (but not fatigue) severity was significantly higher in patients who were unresponsive to UDCA therapy [37]. The same study also observed more severe pruritus and fatigue in patients presenting at an age younger than age 30 years.

Current pruritus treatment in PBC largely depends on the step-wise use of guideline recommended agents that include cholestyramine, rifampicin, naltrexone, and sertraline [3]. The potential newer agents for treating pruritus in PBC are ileal BA transporter (IBAT or ASBT) inhibitors and fibrates. The IBAT plays a key role in the enterohepatic circulation of BAs which are implicated in cholestatic pruritus [38]. Two RCTs of IBAT inhibitor drugs investigating their safety and efficacy in PBC patients with pruritus have been completed. The results of the CLARITY study (n = 61) where patients were randomized to daily oral lopixibat (LUM001) 10 mg, 20 mg, or placebo showed significant decrease in itch score from baseline in the within group comparison (26% lopixibat, $p < 0.0001$ and 23% placebo, $p < 0.0001$) but no significant difference between group comparison (lopixibat vs. placebo, p = 0.47) [39]. Another IBAT inhibitor drug GSK2330672 (GSK672) has been investigated in 21 PBC patients with pruritus. This was a phase 2 double-blind, randomized placebo-controlled crossover trial conducted over 14 days. No serious AEs were reported. GSK672 reduced itch intensity significantly more than placebo as measured by numeric rating scale (–23% [–1 to –45%]), PBC-40 itch domain (–14% [–1 to –26%]), and 5-D itch (–20% (–7 to –34%]). The study concluded that oral GSK672 was well tolerated and reduced itch intensity in PBC patients with pruritus. These results support further investigation of GSK672 as a potential treatment for cholestatic pruritus [38].

Although uncontrolled studies have suggested that fenofibrate and bezafibrate improve pruritus in PBC patients [40–42], there have been no prospective RCTs of fibrates with pruritus as the primary outcome. The BEZURSO study (NCT01654731) will hopefully shed more light on the effect of fibrates on pruritus associated with PBC.

OCA, although licensed for treating UDCA non-response PBC patients, is unfortunately not associated with significant abatement of symptoms as measured by the PBC-40 questionnaire. On the contrary, OCA therapy has been shown to be associated with dose-dependent increased frequency and severity of pruritus, as found in the phase 2 and 3 trials. Although the exact mechanism of pruritus related to OCA is unclear, TGR5 activation-induced pruritus (OCA is a weak TGR5 agonist) or activation of the autotaxin pathway have been proposed [8, 27].

Fatigue management in PBC is more challenging, and despite its high prevalence among PBC patients at present, there are no licensed treatments. Previous studies on oral supplementation with antioxidants (including coen-

zyme Q10) have shown mixed results at reducing fatigue levels [43, 44]. A strong association has been shown between abnormalities in sleep regulation, particularly excessive daytime somnolence and fatigue severity in PBC. Therefore, modafinil, a CNS-acting drug used in the treatment of excessive daytime somnolence in non-liver patients, has been investigated in PBC. An open-label study of 21 PBC patients treated with modafinil had shown objective, short-term benefit in daytime somnolence and fatigue [45] and a follow-up study of this cohort at 14 months showed sustained and statistically significant benefit in daytime somnolence but the fatigue scores were not significantly better than the baseline [46]. More recently, in a double-blind placebo-controlled study, PBC patients were randomized to receive modafinil (n = 20) or placebo (n = 20) for 12 weeks and fatigue was quantified by the Fisk Fatigue Impact Scale (FFIS). Although the treatment was found to be safe and well tolerated, modafinil did not significantly benefit fatigue as only 3 patients (17.6%) in the modafinil arm and 2 (12.5%) in the placebo arm (p = 1.00) met the primary end point of ≥50% reduction in FFIS score [47].

In the quest for effective treatment for fatigue, recent focus has been on rituximab, an anti-CD20 monoclonal antibody that selectively depletes B cell. Previously, 2 pilot studies that investigated rituximab (1,000 mg infusion) in UDCA non-responsive PBC showed it was safe and well tolerated in PBC and improved liver biochemistry [48, 49]. However, fatigue was not the primary outcome in these studies. A double-blind placebo-controlled study of rituximab (RIT-PBC study) aiming to investigate rituximab (given as two 1,000 mg intravenous infusion at 12 weeks apart) as a treatment for fatigue in PBC has been recently completed and a large number of PBC patients have been recruited in a single large UK center [50]. The first RCT of a treatment for fatigue in patients with PBC was likely to provide important data on the efficacy, safety, and tolerability of rituximab in PBC patients with significant fatigue.

Conclusion

The landscape of PBC therapy is changing rapidly with a recent surge in clinical trials of novel agents to treat UDCA non-response status and symptoms. OCA marks an important milestone in PBC and the data from phase 2 to 3 clinical trials on biochemical improvements are compelling, leading to its FDA approval for use in UDCA unresponsive patients. However, there is no evidence that OCA improves hard clinical outcomes or overall quality of life. In addition, OCA may not be suitable for PBC patients with pruritus as it may worsen the symptom. Other novel agents currently in clinical development may have better side effect profile. Fibrates have the potential but currently lack strong evidence to support their routine clinical use in PBC. Symptom management of PBC is challenging, and ASBT inhibitors and rituximab are being evaluated for pruritus and fatigue, respectively.

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Novel bile acid therapeutics for the treatment of chronic liver diseases

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***Abstract*:** Recent developments in understanding the role of bile acids (BAs) as signalling molecules in human metabolism and inflammation have opened new avenues in the field of hepatology research. BAs are no longer considered as simple molecules helping in fat digestion but as agents with real therapeutic value in treating complex autoimmune and metabolic liver diseases. BAs and their receptors such as farnesoid X receptor, transmembrane G protein-coupled receptor 5 and peroxisome proliferator-activated receptor have been identified as novel targets for drug development. Some of these novel pharmaceuticals are already in clinical evaluation with the most advanced drugs having reached phase III trials. Chronic liver diseases such as primary biliary cholangitis, primary sclerosing cholangitis and nonalcoholic fatty liver disease, for which there is no or limited pharmacotherapy, are most likely to gain from these developments. In this review we discuss recent and the most relevant basic and clinical research findings related to BAs and their implications for novel therapy for chronic liver diseases.

Keywords: bile acids, drug therapy, liver disease, nonalcoholic fatty liver disease, primary biliary cholangitis, primary sclerosing cholangitis

Introduction

The incidence and prevalence of liver diseases in general is rising and significantly contributing to the increasing burden on health care [Williams and Horton, 2013]. To deal with the challenge of treating patients with liver diseases, clinicians need effective therapies that target the underlying disease pathology as well as symptoms and complications associated with the disease. Whilst great success has been achieved in the last few years in the pharmacological treatment of viral hepatitis [Lam *et al.* 2015], the need for effective drug therapies in metabolic and cholestatic liver diseases has been only partially met. As obesity is becoming increasingly prevalent, there is a clear and pressing need for drug therapies in patients with nonalcoholic fatty liver diseases (NAFLDs) and nonalcoholic steatohepatitis (NASH). At the same time, patients with primary sclerosing cholangitis (PSC) and high-risk primary biliary cholangitis (PBC) are also in need of better second-line disease-modifying drugs as well as effective therapies to manage associated symptoms (e.g. pruritus).

Bile acids (BAs) have always been of interest to gastroenterologists and hepatologists with their traditional role in fat digestion known for more than 50 years [Borgstrom *et al.* 1957]. In recent years, there has been a growing interest in BAs as signalling molecules and they are emerging as the key players in the quest for novel drug therapies in liver diseases. Major developments achieved in the basic and clinical research related to BAs have augmented our interest in exploiting their physiological role for therapeutic benefit in liver diseases. This has effectively set the stage to identify novel targets for treating patients with PBC, PSC and NAFLD for which there is currently limited or no effective drug therapy.

An in-depth review of BAs with respect to their chemistry, synthesis, transport and regulation is beyond the scope of this article and has been extensively reviewed elsewhere [Dawson *et al.* 2009; Monte *et al.* 2009; Schaap *et al.* 2014]. Therefore, in the first part of this review we provide an overview of BA synthesis, transport, signalling and regulation. We then describe recent advances in the understanding of BA receptors in relation to cholestasis, glucose and lipid metabolism, immune function and antifibrotic actions. These relevant updates will help the reader to

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better understand the therapeutic benefits of BAs in chronic liver diseases covered in the later part of this paper. In this review of potential novel BA-based therapeutic agents we mainly focus on the treatment of patients with PBC, PSC and NAFLD.

BA synthesis and transport

BAs along with phospholipids and cholesterol are major constituents of bile. BAs are amphipathic molecules (i.e. with both hydrophilic and hydrophobic regions) with detergent-like actions and are synthesized from enzymatic catabolism of cholesterol by the hepatocytes [Monte *et al.* 2009]. BA synthesis is a complex process involving at least 17 different enzymes but can be summarized into three main steps: modification of the steroid ring, cleavage of the side chain and conjugation with glycine or taurine [Russell, 2003]. Two pathways exist for BA synthesis. The classical ('neutral') pathway is responsible for the production of cholic acid (CA) and chenodeoxycholic acid (CDCA) which accounts for 90% of primary BA synthesis in humans [Anderson *et al.* 1972]. The other 10% is produced by the alternative ('acidic') pathway which can only produce CDCA. Cytochrome P450 7A1 (CYP7A1) is the rate-limiting enzyme in BA synthesis. After their synthesis, unconjugated CA and CDCA are targeted to the peroxisomes where they are conjugated (amidation) with glycine and taurine that renders them more hydrophilic and more readily secretable in the bile.

In humans, predominant conjugated BAs are glycoconjugates and under physiological pH conditions these conjugated BAs exist as anionic salts and are therefore called 'bile salts' (BS). These BS are stored in the gallbladder and upon ingestion of a meal they are released into the intestinal lumen where they facilitate absorption of fat and fat-soluble vitamins. Conjugated primary BAs present in the intestinal lumen are modified by the intestinal bacteria by deconjugation, oxidation and dehydroxylation to produce secondary BAs: lithocholic acid (LCA) and deoxycholic acid (DCA) [Ridlon *et al.* 2006]. Human bile predominantly contains CDCA and DCA and a very small amount of ursodeoxycholic acid (UDCA). Hydrophobicity of the BAs is a determinant of their cytotoxicity which increases in the order of LCA > DCA > CDCA > CA > UDCA [Carey, 1983].

After their normal physiological function is completed in the intestine, BAs reach the ileum where most are reabsorbed efficiently *via* a sodium-dependent process. The apical sodium-dependent BA transporter (ASBT, gene symbol *SLC10A2*) expressed in the distal ileum is the predominant transporter mediating the ileal uptake of conjugated BAs [Craddock *et al.* 1998]. ASBT mediates active transfer of BAs across the luminal plasma membrane to an intracellular protein called ileal BA binding protein (IBABP) that facilitates intracellular diffusion of BAs to the basolateral membrane (Figure 1). Then, BAs exit the enterocyte across the basolateral plasma membrane mediated by the organic solute transporter (OSTα/β) and enter the portal bloodstream [Rao *et al.* 2008]. BAs circulating in the portal circulation are transported across the basolateral membranes of the hepatocytes *via* sodium taurocholate cotransporting polypeptide transporter (NTCP, gene symbol *SLC10A1*) [Hagenbuch and Meier, 1994]. Finally, BAs are transported across the canalicular plasma membrane of the hepatocytes *via* the bile salt export pump (BSEP) and secreted into bile. This efficient cycle between small intestine and liver is repeated several times a day to ensure 95% of BAs re-enter the liver, leaving only approximately 5% (or approximately 0.5 g/day) in the intestinal lumen [Hofmann, 1984].

BA regulation

BA homeostasis is under tight regulation mediated by the negative feedback effect of BAs on the activity and expression of CYP7A1 as well as signalling *via* farnesoid X receptor (FXR) and fibroblast growth factor (FGF19).

FXR

FXR was first identified in 1995 as an orphan receptor. It is a member of the nuclear receptor superfamily and acts as key regulator in a diverse range of cell functions including development, differentiation and metabolism [Mangelsdorf *et al.* 1995]. FXR was so named because a supraphysiological concentration of farnesol, an intermediate in the mevalonate pathway, was found to demonstrate weak agonism [Modica *et al.* 2010]. The discovery that primary BAs were the natural, endogenous ligands for FXR was reported at the turn of the century [Makishima *et al.* 1999; Parks *et al.* 1999]. Soon FXR was implicated in BA homeostasis following the discovery that FXR

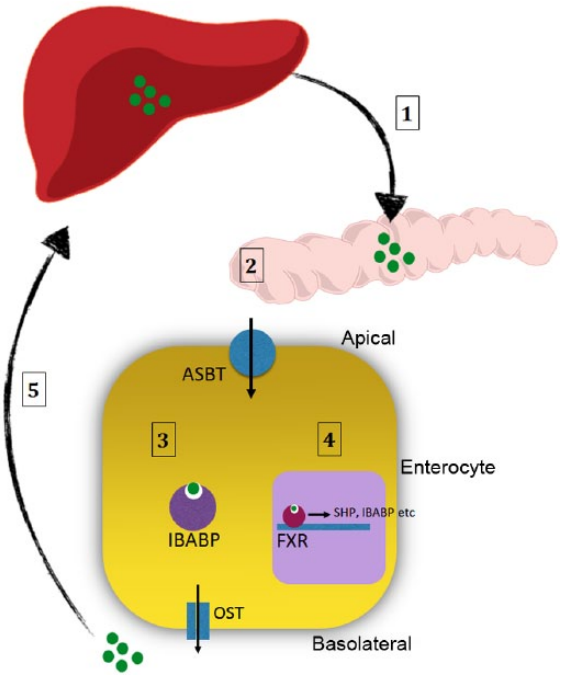

Figure 1. Enterohepatic circulation of bile acids (BAs) *via* enterocyte in terminal ileum. (1) Primary BAs synthesized in liver and excreted into duodenum as constituent of bile; (2) BAs avidly and actively reabsorbed in the terminal ileum *via* ASBT (apical sodium bile acid transporter); (3) BAs transported intracellularly by IBABP (intracytosolic bile acid binding protein); (4) BAs free to bind with nuclear receptor FXR (farnesoid X receptor); (5) BAs released into portal venous circulation *via* organic solute transporter (OST) α/β and circulated back to liver. SHP, small heterodimer partner.

knockout (*FXR*$^{-/-}$) mice showed diminished ability to downregulate CYP7A1 mRNA in response to BAs [Sinal *et al.* 2000]. Although FXR is expressed in the ileum, liver, adrenal glands and the kidneys, the intestine (mainly ileum) seems to have the most intense FXR expression [Inagaki *et al.* 2006]. Indeed, among all the nuclear hormone receptors, FXR is the most dedicated to BA signalling [Schaap *et al.* 2014]. The activation of FXR by primary BAs has several downstream effects on both hepatocytes and enterocytes. In the hepatocytes, FXR activation induces small heterodimer partner (SHP) that downregulates the synthesis of BAs by inhibiting CYP7A1 [Kir *et al.* 2012]. Also, FXR activation downregulates NTCP (reduced BA uptake) and upregulates BSEP (increased export of BAs) [Martinez-Augustin and Sanchez De Medina, 2008]. In the enterocytes, FXR activation reduces ASBT expression (inhibits BA absorption) and increases expression of IBABP and OSTα/β (prevents intracellular BA accumulation) [Martinez-Augustin and Sanchez De Medina, 2008]. FXR activation also prevents BA toxicity by transcriptional induction of detoxification enzymes and canalicular secretion of BAs via upregulation of BSEP.

FGF19

A second FXR-dependent mechanism to reduce primary BA synthesis is through the production of enterokine FGF15 (in rodents) or FGF19 (in humans) [Holt *et al.* 2003; Inagaki *et al.* 2005]. When there is a high BA load in the ileum, activated FXR induces transcription of the FGF19 in the ileum. FGF19 is able to travel in the bloodstream and bind to its receptor hepatocyte FGF receptor 4 (FGFR4) and initiate a SHP-independent downregulation of CYP7A1, resulting in inhibition of BA synthesis [Jones, 2012; Kir *et al.* 2012].

In summary, FXR and FGF19 activation reduces endogenous BA synthesis, protects hepatocytes from BA toxicity and promotes secretion of BAs. Therefore the 'anticholestatic' effects of the BA-FXR-FGF19 signalling cascade have potential therapeutic implications in cholestatic diseases.

Transmembrane G protein coupled receptor 5

Transmembrane G protein coupled receptor 5 (TGR5, also called Gpbar-1) is a G protein coupled BA receptor that was identified as the first cell surface receptor for BAs [Maruyama *et al.* 2002; Kawamata *et al.* 2003]. TGR5 is not expressed in the hepatocytes but found in the cholangiocytes as well as a variety of other cell types such as brown adipose tissue, brain, gall bladder epithelium, intestines, spleen, endothelial cells, Kupffer cells and CD14+ cells [Maruyama *et al.* 2002; Kawamata *et al.* 2003; Keitel *et al.* 2007]. Although many BAs are capable of activating TGR5, the most potent natural ligands are taurine-conjugated secondary BAs, such as taurolithocholate [Keitel *et al.* 2008]. It has been shown that BA-induced TGR5 activity plays a major role in glucose homeostasis, increased energy expenditure, oxygen consumption and gallbladder filling [Katsuma *et al.* 2005; Watanabe *et al.* 2006; Thomas *et al.* 2009; Li *et al.* 2011]. In addition, TGR5 activation improves hepatic steatosis and

insulin sensitivity and protects biliary epithelium against the detergent effect of BAs. More recently, BA-mediated TGR5 activation has also been shown to have an anti-inflammatory role by reducing nuclear factor κB (NFκB) translocation [Li *et al.* 2011]. Due to its diverse and favourable effects, TGR5 is an emerging target for drug discovery with a potentially beneficial role of TGR5 agonists in the treatment of type 2 diabetes mellitus (T2DM) and inflammation-driven metabolic diseases such as NASH.

Scientific rationale for BA-based therapy

In addition to their key role in BA homeostasis, FXR activity and FGF19 signalling are involved in diverse biological pathways. Essentially, FXR exerts its functions by eliciting transcriptional alterations and controls a number of important metabolic pathways [Schaap *et al.* 2014]. In this section, we briefly review the key biological processes modulated by FXR and FGF19 with respect to their attractive therapeutic implications.

Glucose and lipid metabolism

Activation of FXR inhibits the expression of hepatic sterol regulatory element-binding protein 1c (SREBP-1c) [Watanabe *et al.* 2004]. SREBPs are transcription factors that act as master regulators of lipid metabolism. They act to control the biogenesis of cholesterol and also control the expression of genes involved in lipogenesis. In an animal model, activation of FXR by CA inhibited hepatic SREBP-1c expression in a SHP-dependent manner, leading to reduction in serum triglyceride levels [Watanabe *et al.* 2004]. In addition, FXR can induce the expression of apolipoprotein C-II [Houten *et al.* 2006] which is a coactivator for lipoprotein lipase that acts to clear serum triglyceride from the circulation. FGF19 is also shown to regulate key enzymes in hepatic lipid synthesis [Miyata *et al.* 2011].

FXR signalling is also essential to maintain glucose homeostasis. In an *FXR*$^{-/-}$ mouse model, elevated serum glucose and impaired glucose and insulin tolerance were demonstrated by Ma and colleagues [Ma *et al.* 2006]. This study also demonstrated that administration of CA repressed the expression of gluconeogenic genes and decreased serum glucose in wild-type mice. In addition, FGF19 is involved in glucose metabolism with its actions resembling that of insulin; that is, inhibition of gluconeogenesis and stimulation of glycogen synthesis [Potthoff *et al.* 2011].

Immune functions

It has long been recognized that BAs are bacteriostatic (but weakly bactericidal) and that decrease in BAs within the small bowel leads to bacterial overgrowth [Floch *et al.* 1971; Berg, 1995]. This has been confirmed recently by the experimental studies of obstructive cholestasis and cirrhosis that showed oral administration of BAs reduced bacterial overgrowth as well as maintained the intestinal barrier function and prevented endotoxaemia [Lorenzo-Zuniga *et al.* 2003; Ogata *et al.* 2003]. BA-FXR signalling has been proposed as the key mechanism by which BAs control bacterial overgrowth and maintain the epithelial barrier. A landmark study showed that BAs regulate an anti-inflammatory response via FXR in the terminal ileum [Inagaki *et al.* 2006]. In this study of a rodent model of cholestasis FXR agonist treatment protected the epithelial barrier by increasing the expression of several genes associated with intestinal mucosal defence pathways and decreased the number of bacteria isolated from mesenteric lymph nodes. Similarly, treatment with a FXR agonist has been shown to maintain the epithelial barrier in an animal model of colitis in wild type, but not *FXR*$^{-/-}$ mice [Gadaleta *et al.* 2011]. In addition, FXR activation rendered several different immune cell types refractory to stimulation with lipopolysaccharide (LPS), a bacterial cell wall component. More recent evidence shows FXR agonism reduces the LPS-induced production of proinflammatory cytokines by macrophages, whilst maintaining the production of anti-inflammatory interleukin (IL)-10 [Haselow *et al.* 2013] and attenuates the chemoattractant IL-8 response to stimulation with tumour necrosis factor α (TNFα) [Speight *et al.* 2015]. Finally, FXR and FGF19 exert anti-inflammatory activity *via* suppression of NFκB, which is a key nuclear receptor in both acute and chronic inflammatory processes. This is supported by observations that FXR and NFκB mutually antagonize each other [Wang *et al.* 2008] and activation of FXR and FGF19 inhibit the expression of NFκB controlled inflammatory genes [Drafahl *et al.* 2010; Gadaleta *et al.* 2010; Zhou *et al.* 2014].

Taken together, these results suggest FXR agonists have anti-inflammatory actions and may have potential therapeutic utility in preventing bacterial translocation and reducing spontaneous

bacterial peritonitis in patients with cholestasis and cirrhosis.

Liver fibrosis and carcinogenesis

Evidence shows that FGF19 increases proliferation of hepatocytes through activation of FGFR4, and BA-FXR-FGF19 signalling is essential for normal liver regenerative process [Huang *et al.* 2006; Wu *et al.* 2010; Zhang *et al.* 2012; Uriarte *et al.* 2013; Kong *et al.* 2014]. Therefore, inhibition of FGF19 signalling could be a potential therapy for hepatocellular carcinoma (HCC). Also the observation that *FXR*$^{-/-}$ mice have high incidence of HCC [Kim *et al.* 2007] suggests the potential regulatory role of FXR in tumour suppression. This has recently been corroborated by two animal studies in which FXR agonist treatment prevented development of liver cancer and reduced liver tumour size and metastasis [Deuschle *et al.* 2012; Jiang *et al.* 2013].

FXR activation is also implicated in the inhibition of fibrotic mechanisms within the liver *via* hepatic stellate cells (HSCs) [Fiorucci *et al.* 2005; Renga *et al.* 2011]. In a mouse model, administration of a FXR agonist for 12 weeks promoted resolution of liver fibrosis [Fiorucci *et al.* 2004]. However, a more recent study has contradicted this by showing a low level of FXR expression in HSCs in liver fibrosis and suggesting HSCs may not represent direct therapeutic targets for FXR ligands [Fickert *et al.* 2009]. Therefore, the current evidence on the antifibrotic effect of FXR is equivocal and merits further investigation.

Novel BA-based therapies

PBC, PSC and NASH represent complex, multifactorial diseases in which effective drug management remains an unmet clinical need. There is a clear need beyond UDCA in patients with PBC and PSC and beyond diet and lifestyle modifications in patients with NASH. In this section we review innovative BA-based therapeutic approaches being investigated for these diseases (summary in Table 1).

UDCA: 'the current BA therapy'

UDCA is the only US Food and Drug Administration (FDA) approved drug for PBC and it is the current standard of care for patients with this condition. The mechanism of action of UDCA has been well established and comprehensively explained in an excellent recent review [Beuers *et al.* 2015]. In brief, UDCA has potent anticholestatic, antiapoptotic and anti-inflammatory properties. Notably, UDCA is a weak FXR and TGR5 ligand [Parks *et al.* 1999; Halilbasic *et al.* 2013]. The optimum dose of UDCA in treating patients with PBC is 13–15 mg/kg/day and guidelines recommend initiating treatment at a low dose and increasing it gradually to the optimum dose [Beuers *et al.* 2009; Lindor *et al.* 2009].

Multiple lines of evidence confirm that UDCA improves biochemical markers of cholestasis and may delay the progression of PBC [Beuers *et al.* 2009; Lindor *et al.* 2009]. However, unfortunately the response to UDCA is not universal and up to 40% of patients with PBC do not improve their liver biochemistry on UDCA. Indeed a substantial proportion of patients have disease progression despite UDCA therapy. Patients with incomplete biochemical response to UDCA are referred to as 'UDCA nonresponders' based on different treatment response biochemical criteria (e.g. Paris and Barcelona criteria) [Corpechot, 2012]. UDCA nonresponders are at higher risk of disease progression, symptom burden and poor prognosis compared with 'UDCA responders'. In addition, the Global PBC Study Group recently demonstrated that serum levels of alkaline phosphatase (ALP) and bilirubin are surrogate endpoints of outcomes in PBC and patients with an ALP greater than 1.67 $\times$ upper limit of normal (ULN) or bilirubin greater than ULN have increased risk of transplantation or death [hazard ratio (95% confidence interval, CI): 2.83 (2.4–3.4); $p < 1 \times 10^{-34}$] [Lammers *et al.* 2014].

In PSC patients the conventional dose of UDCA (10–15 mg/kg/day) is safe but high dose (28–30 mg/kg/day) has been shown to be harmful [Lindor *et al.* 2009]. Moreover, the long term efficacy of UDCA therapy in PSC is unclear as the evidence suggests that UDCA improves liver biochemistry but has no significant effect in slowing disease progression [Poropat *et al.* 2011]. Therefore, the current guidelines recommend against the use of UDCA as medical therapy in PSC [Beuers *et al.* 2009; Chapman *et al.* 2010].

In PBC and PSC, UDCA is not effective in improving cholestasis-associated symptoms such as pruritus and fatigue. Due to these limitations

Table 1. Novel bile acid based therapeutic approaches in chronic liver diseases.

Class of molecule	Example molecules	Therapeutic rationale	Target disease	Phase of development	ClinicalTrials.gov identifier
FXR agonists	INT-747, INT-767, GW4064, GSK2324, PX-102, Way362450, fexaramine, LJN452	↓BA synthesis and promotion of BA excretion	PBC	Phase II completed	NCT00570765
				Phase II completed	NCT00550862
				Phase III (POISE)	NCT01473524
				Phase III	NCT02308111
				Phase II	NCT02516605
				Phase II	NCT02516605
			PSC	Phase II (AESOP)	NCT02177136
		↓lipogenesis, gluconeogenesis and liver inflammation	T2DM with NAFLD	Phase II completed	NCT00501592
			NASH	Phase II	NCT01265498
		↑tumour suppression and ↓liver fibrosis	HCC	–	–
TGR5 agonist	INT-767, INT-777	↓BA synthesis, ↑bile flow, anti-inflammatory	PSC		
		↑energy expenditure, anti-inflammatory	NASH	–	–
FGF-19 analogue	NGM-282	↓BA synthesis, anti-inflammatory, antifibrotic	PBC	Phase II	NCT02026401
ASBT inhibitor	LUM-001, GSK2330672	↓enterohepatic circulation of BAs and ↑faecal excretion	Pruritus in PBC	Phase II (CLARITY)	NCT01904058
				Phase II	NCT01899703
PPAR agonists	Fenofibrate, bezafibrate	↓BA synthesis and promotion of BA excretion, anti-inflammatory, may ↑FXR activity	PBC, PSC	Phase III (BEZURSO)	NCT01654731
	GFT505	↑insulin sensitivity and glucose homeostasis, hepatoprotective, anti-inflammatory	NASH	Phase II (GOLDEN)	NCT01694849
UDCA related	norUDCA	cholangioprotective, stabilizes 'biliary bicarbonate umbrella'	PSC	Phase II (NUC-3)	NCT01755507
	Tauroursodeoxycholate (TUDCA)	more hydrophilic than UDCA	PBC	Phase III	NCT01857284
Fatty acid–bile acid conjugate	Aramchol	Stearoyl coenzyme A desaturase 1 (SCD1) inhibitor, ↓liver fat	NASH	Phase II (ARREST)	NCT02279524

ASBT, apical sodium dependent BA transporter; BA, bile acid; FGF, fibroblast growth factor; FXR, farnesoid X receptor; HCC, hepatocellular carcinoma; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; PBC, primary biliary cholangitis; POISE, PBC OCA International Study of Efficacy; PPAR, peroxisome proliferator-activated receptor; PSC, primary sclerosing cholangitis; T2DM, type 2 diabetes mellitus; TGR, transmembrane G protein-coupled receptor; UDCA, ursodeoxycholic acid.

of UDCA, there is an urgent need for novel therapy in PSC and second line therapies in patients with PBC with UDCA nonresponse status. The potential therapeutic role of UDCA in other but rare liver diseases is also being explored. Recent experimental models of polycystic liver disease (PLD) have shown that UDCA inhibits hepatic cystogenesis by inhibiting the proliferation of polycystic cholangiocytes [Munoz-Garrido *et al.* 2015]. Therefore, UDCA may be an effective therapeutic option in reducing liver volume in PLD and help to improve symptoms caused by

the mass effect of polycystic liver. An international, multicentre, randomised controlled trial is currently recruiting patients to assess the efficacy of UDCA (15–20 mg/kg/day UDCA for 24 weeks) in reducing total liver volume in PLD patients (CURSOR study; NCT02021110).

FXR agonists

As noted above, among all nuclear receptors FXR has emerged as a prime therapeutic target due to its diverse functions in the regulation of BAs, metabolism of glucose and lipids, and anti-inflammatory activity. Several FXR agonists have been developed with two basic structures: small molecule, steroidal semisynthetic ligands and nonsteroidal, fully synthetic ligands. The most clinically advanced therapeutic FXR agonist is INT-747 [obeticholic acid (OCA), 6α-ethyl-chenodeoxycholic acid, Intercept Pharmaceuticals, New York, USA]. OCA is a steroidal semisynthetic BA in which CDCA has been modified by the addition of an alkyl group to form a more potent FXR agonist [Pellicciari *et al.* 2002].

GW4064 (GlaxoSmithKline, NC, USA) is a nonsteroidal fully synthetic FXR agonist first developed in 2000 [Crawley, 2010]. Animal studies have shown that GW4064 prevents diet-induced hepatic steatosis and insulin resistance, and attenuates endotoxin-induced hepatic inflammation by repressing macrophage activation [Ma *et al.* 2013; Yao *et al.* 2014]. As an FXR agonist, GW4064 has greater potency than CDCA but is currently not being evaluated for clinical use. It can be commercially obtained from Sigma Aldrich (St. Louis, USA) for experimental use.

PX-102 is a therapeutic nonsteroidal compound manufactured as a modification of GW4064 (Phenex Pharmaceuticals AG, Ludwigshafen, Germany). It has demonstrated some efficacy in the mouse models of NAFLD by decreasing levels of serum and liver cholesterol and triglyceride [Hambruch *et al.* 2013].

WAY-362450 (Exelixis Inc., California) is a fully synthetic agonist manufactured as an azepino derivative [Flatt *et al.* 2009]. Studies on animal models of NAFLD and NASH demonstrated that treatment with WAY-362450 reduced liver inflammation and fibrosis with an associated decrease in serum liver enzymes [Zhang *et al.* 2009].

Finally, fexaramine is another nonsteroidal FXR ligand shown to have distinct genomic targets and favourable metabolic effects in a mice model but currently is not being studied in humans [Downes *et al.* 2003].

FXR therapeutics in PBC. By virtue of its potent FXR agonist action and resulting effects on BAs, OCA is emerging as a promising second-line agent for treating patients with PBC who are 'UDCA nonresponders'. Two phase II randomized placebo-controlled trials have evaluated OCA in PBC with improvement in liver enzymes as the primary endpoint and as a surrogate marker for patient outcome. The first study [ClinicalTrials.gov identifier: NCT00570765] showed that in comparison to placebo, monotherapy with OCA 10 or 50 mg daily for 12 weeks ($n = 59$) reduced ALP from a mean of 3.9 × ULN to 1.9 × ULN [Kowdley *et al.* 2011]. Significant improvements were also seen in the levels of serum γ-glutamyl transpeptidase (γ-GT), bilirubin, C-reactive protein, immunoglobulin M (IgM) and TNFα. More recently, in a phase II multicentre double-blind efficacy trial, 165 patients with PBC and inadequate response to UDCA (serum alkaline phosphatase levels > 1.5 × ULN) were randomly treated with 10 mg, 25 mg or 50 mg doses of OCA or placebo once daily for 3 months [ClinicalTrials.gov identifier: NCT00550862] [Hirschfield *et al.* 2015]. Compared with placebo, OCA significantly decreased ALP levels with average decline of 20–25% from baseline. Significant improvements were also seen in the levels of serum bilirubin, γ-GT and ALT. The biochemical benefit of OCA was maintained in 61 patients who had completed a 12-month open-label extension of the study. Currently two phase III studies of OCA in PBC are ongoing [ClinicalTrials.gov identifiers: NCT01473524, NCT02308111] with early results from the first phase III PBC OCA International Study of Efficacy (POISE) study showing clinically meaningful biochemical improvement [Nevens *et al.* 2014].

LJN452 (Novartis, Basel, Switzerland) is a non-BA FXR agonist currently entering a phase II trial to assess safety, tolerability and efficacy in patients with PBC [ClinicalTrials.gov identifier: NCT02516605].

FXR therapeutics in NAFLD. NAFLD is the most common cause of chronic liver disease in the developed world, affecting up to a third of the

population. It is characterized by the accumulation of hepatic fat and presents as a spectrum from simple steatosis to NASH, fibrosis and eventually cirrhosis [Sattar *et al.* 2014]. NAFLD is strongly associated with T2DM and patients with NASH have an increased risk for the development of progressive fibrosis, cirrhosis and HCC. So far, OCA is the only FXR agonist studied in patients with NAFLD, with encouraging results from the FLINT (Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis) study reported recently [Neuschwander-Tetri *et al.* 2015]. In this double-blind, randomized, placebo-controlled trial, patients with NASH without cirrhosis were randomized to receive either 25 mg of OCA (n = 141) or placebo (n = 142) for 72 weeks. The primary outcome measure was reduction in the histological score for fibrosis (NAFLD activity score) from baseline to the end of treatment. The trial was stopped early as 45% of patients in the OCA group had reached the primary endpoint compared with 21% in the placebo group (relative risk 1.9; 95% CI 1·3–2·8). The OCA intervention also significantly improved the serum ALT levels. These results suggest that the FXR agonist OCA may be beneficial in NAFLD and prevent progression of fibrosis in patients with NASH.

Safety of FXR therapy. Pruritus is the most common adverse event reported to be associated with OCA in PBC and NAFLD trials. In the phase II PBC trial, frequency of pruritus was 47% with 10 mg, 87% with 25 mg and 80% with 50 mg of OCA [Hirschfield *et al.* 2015]. Similarly in the FLINT study, 23% of patients in the OCA arm developed pruritus compared with 6% in the placebo group [Neuschwander-Tetri *et al.* 2015]. Data from both trials suggested that pruritus was directly related to the OCA dose. This issue is being investigated in the phase III (POISE) study, with early results suggesting the frequency of pruritus is lowest when OCA is started at 5 mg and titrated upwards [Nevens *et al.* 2014].

A potentially more concerning adverse event in the FLINT study was dyslipidaemia seen in patients treated with OCA. Increase in low-density lipoprotein and decrease in high-density lipoprotein (HDL) levels were observed in the OCA group. Although dyslipidaemia is a well-known cardiovascular risk factor, whether this is true for patients treated with an FXR agonist is unclear. Interestingly animal models of atherosclerosis have provided some evidence to suggest that FXR agonists are protective [Hartman *et al.* 2009]. Clearly more research is needed to assess the cardiovascular risk of FXR agonist therapy in NASH. Interestingly in the PBC study OCA treatment was associated with decrease in serum levels of total and HDL cholesterol [Hirschfield *et al.* 2015].

An unproven safety concern is the potential for FXR agonists to predispose people to the development of HCC induced by FXR overexpression and elevation of circulating FGF19 levels. FGF19 transgenic mice develop HCC [Nicholes *et al.* 2002] and overexpression of FGF19 has been associated with development of HCC in both animal models and potentially in humans [Lin and Desnoyers, 2012; Schaap *et al.* 2015]. Therefore, it is feasible that overpromotion of FXR with FXR agonist therapy could lead to high levels of FGF19, which in turn is carcinogenic. This concern merits further clinical evaluation. Similar procarcinogenic concern applies to FGF19 analogues. One proposal to overcome this undesirable effect is to engineer FGF19 variants that have lost the mitotic activity but still are effective in their metabolic activity [Wu *et al.* 2010].

INT-767

INT-767, a semisynthetic BA analogue, is a 23-sulphate derivative of OCA with dual FXR and TGR5 agonist actions but with a higher affinity to FXR [Rizzo *et al.* 2010]. Prominent features of INT-767 include inhibition of BA synthesis, simulation of bicarbonate-rich choleresis by enhancing biliary bicarbonate secretion and immune modulation *via* inhibition of NFκB. In the mouse model of sclerosing cholangitis, INT-767 reduced serum levels of ALT, ALP as well as liver inflammation and fibrosis [Baghdasaryan *et al.* 2011]. Interestingly these effects were shown to be mediated exclusively by FXR and not by TGR5. Therefore, INT-767 may be a potential therapeutic agent in treating patients with cholestasis and it is currently entering phase I clinical trials.

INT-777/TGR5 agonists

INT-777 [6α-ethyl-23(S)-methylcholic acid] is a potent, semisynthetic and selective TGR5 agonist [Pellicciari *et al.* 2009]. Animal studies have shown that INT-777 increases bile flow, produces significant reduction in weight gain and adiposity as well as improvement of liver function with concomitant reductions in steatosis and fibrosis [Pellicciari *et al.* 2009; Thomas *et al.* 2009].

These results suggest promising therapeutic potential for INT-777 in the treatment of obesity and related disorders such as NASH. A theoretical concern with the use of TGR5 agonists is the potential for aggravating pruritus, a common symptom in patients with cholestatic liver disease. Although animal studies have shown that activation of TGR5 can induce itch [Alemi *et al.* 2013; Lieu *et al.* 2014] it remains unknown whether therapeutic use of TGR5 agonists will have a similar effect on pruritus. Currently there are no ongoing clinical trials with INT-777.

NGM-282

NGM-282 (NGM Biopharmaceuticals, Inc., San Francisco, CA, USA), a biological drug, is a recombinant variant of FGF19 with potential anticholestatic properties. A phase II clinical trial evaluating the safety and tolerability of 28 days of treatment of NGM-282 with UDCA in patients with PBC has recently been completed [ClinicalTrials.gov identifier: NCT02026401] and results are awaited.

Norursodeoxycholic acid

24-norUrsodeoxycholic acid (norUDCA) is a synthetic, side-chain-shortened UDCA homologue. With its hepatocyte and cholangiocyte protective properties it has recently emerged as an attractive therapeutic candidate for cholestatic liver diseases, especially for PSC. NorUDCA differs from UDCA in metabolism and therapeutic mechanisms, with important clinical consequences. Like UDCA, norUDCA is not a direct FXR or TGR5 ligand but it is significantly more hydrophilic and less toxic than UDCA [Fickert *et al.* 2013]. NorUDCA is superior to UDCA in the treatment of sclerosing cholangitis, attributed largely to its ability to increase the hydrophilicity of biliary BAs, stimulate bile flow with flushing of injured bile ducts, and induce detoxification and elimination routes for BAs [Hofmann *et al.* 2005; Fickert *et al.* 2006]. Because of its shortened side chain norUDCA is not conjugated with taurine or glycine in the liver and is secreted into the bile in its unconjugated form. Human studies have shown norUDCA induces a sustained bicarbonate-rich hypercholeresis, the increased bile flow being attributed mainly to 'cholehepatic shunting' [Hofmann *et al.* 2005]. The cholangioprotective effect is mainly due to profound alkalinization of bile which stabilizes the 'biliary bicarbonate umbrella' [Hohenester *et al.* 2012] and in turn reduces ductular reaction, inflammation and fibrosis [Halilbasic *et al.* 2009; Fickert *et al.* 2013]. In addition to the anticholestatic and cholangioprotective mechanisms, rodent studies have suggested that norUDCA has potential antiproliferative, anti-inflammatory and antifibrotic properties [Fickert *et al.* 2006; Halilbasic *et al.* 2009] which could be beneficial in both cholestatic and noncholestatic conditions. Recent data also suggest norUDCA produces significant suppression of lipogenesis and normalization of BA metabolism through mechanisms involving crosstalk between CYP7A1 and SHP [Beraza *et al.* 2011].

Due to its multiple beneficial properties suggested by experimental data, norUDCA is a promising drug therapy to attenuate the progression of complex disorders such as PSC and NASH. Also the potential beneficial effects of combined therapy with norUDCA (bicarbonate-rich choleresis) and FXR agonist (suppression of BA synthesis) in cholestatic liver disease merits further exploration. Currently the optimal dose of norUDCA for therapeutic benefit is not known and a large, multicentre, double-blind, placebo-controlled, randomized dose-finding phase II trial (Dr Falk Pharma GmbH, Freiburg im Breisgau, Germany) is evaluating the efficacy of three different doses of norUDCA for the treatment of PSC [ClinicalTrials.gov identifier: NCT01755507].

Aramchol

Aramchol [(3β-arachidyl-amido, 7α-12α-dihydroxy, 5β-cholan-24-oic acid), Trima Israel Pharmaceutical Products, Maabarot, Israel] is a fatty acid–BA conjugate currently being investigated for NAFLD and NASH. Aramchol is a novel synthetic lipid molecule obtained by conjugating two natural components, CA and arachidic acid (saturated fatty acid). The main mechanism of action of Aramchol is *via* inhibition of the stearoyl coenzyme A desaturase 1 (SCD1) activity which is a key enzyme modulating fatty acid metabolism in the liver [Dobrzyn and Ntambi, 2005; Leikin-Frenkel *et al.* 2008]. SCD1 inhibition decreases the synthesis and increases β oxidation of fatty acids, resulting in decreased hepatic storage of triglycerides and fatty acid esters.

Aramchol has received a fast-track status from the FDA. In a phase IIa placebo-controlled trial of 58 patients [ClinicalTrials.gov identifier: NCT01094158], 3 months of treatment with single daily dose of aramchol (100 or 300 mg) was

safe and well tolerated and produced significant and dose-dependent reduction in liver fat [Safadi *et al.* 2014]. A large multicentre, double-blind, placebo-controlled phase IIb study (ARREST trial) is currently evaluating the safety and efficacy of two aramchol doses in patients with NASH without cirrhosis [ClinicalTrials.gov identifier NCT02279524] with reduction in hepatic steatosis as the primary endpoint.

ASBT inhibitor

As noted above, ASBT is a key protein involved in the enterohepatic circulation of BAs and maintaining the BA pool. Physiological effects of ASBT inhibition include lack of ileal BA uptake, increased faecal BAs, reduced FXR stimulation and reduced FGF19 levels. In animal studies, SC-435 (an ASBT inhibitor) produced BA malabsorption (resulting in diarrhoea) and lowered plasma cholesterol [West *et al.* 2003]. As ileal BA uptake is upregulated in PBC [Lanzini *et al.* 2003], pharmacological inhibition of ASBT on the circulating levels of BAs in PBC has generated considerable interest. Recently in a bile duct ligated mice model, treatment with ASBT inhibitor A4250 was shown to attenuate BA-mediated cholestatic liver injury by reducing biliary BA output [Baghdasaryan *et al.* 2014].

Since BAs have been proposed as potential direct or indirect pruritogens in cholestasis, ASBT inhibitors may also have a role in treating pruritus. Recently two large multicentre, randomized phase II clinical trials evaluating the safety and efficacy of ASBT inhibitor drugs (GSK2330672 and LUM001) in patients with PBC and pruritus have completed recruitment [ClinicalTrials.gov identifiers: NCT01899703, NCT01904058]. The results of these studies are likely to inform the safety and therapeutic potential of ASBT inhibitors in the treatment of cholestatic pruritus.

Fibrates

Fenofibrate and bezafibrate are fibric acid derivatives that have been in use for over two decades primarily to treat hyperlipidaemia in patients with cardiovascular and metabolic diseases. Following the first study in 1993 that suggested fibrates improve liver biochemistry [Day *et al.* 1993], they have been actively pursued as potential adjuvants to UDCA therapy to improve cholestasis. The primary mechanism of anticholestatic effect of fibrates is through inhibition of BA synthesis mediated *via* nuclear receptor peroxisome proliferator-activated receptor (PPAR). Fenofibrate is a PPARα selective agonist and bezafibrate is a 'pan-PPAR' agonist as it activates all three isoforms (α, γ and δ). PPARα plays a key role in maintaining BA homeostasis by regulating genes responsible for BA synthesis and transport [Ghonem *et al.* 2015]. Therefore, by activating PPARα, fenofibrate and bezafibrate reduce BA synthesis (downregulate CYP7A1), decrease BA secretion into bile, facilitate elimination of toxic BA and increase biliary phospholipid output. Fibrates also have immune modulator function *via* inhibition of NFκB, increased apolipoprotein AII (inhibits lymphocyte activation), and suppression of lymphocyte proliferation [Vu-Dac *et al.* 1995; Schoonjans *et al.* 1996]. In addition, the crosstalk between PPARα and FXR may enhance PPARα transcription in HSCs, leading to decreased liver fibrosis [Pineda Torra *et al.* 2003].

To date, a number of studies (mostly uncontrolled and small case series) with bezafibrate and fenofibrate have consistently reported significant improvement in ALP, ALT and IgM in patients with PBC. Recent systematic reviews and meta-analyses also support the use of fibrates as adjuvant treatment in patients with PBC who are UDCA nonresponders despite treatment with the optimum dose of UDCA for 12 months [Grigorian *et al.* 2015; Zhang *et al.* 2015]. However, fibrates currently carry contraindications for use in patients with hepatic or severe renal dysfunction, including in patients with PBC. Therefore, safety and efficacy of fibrates need further evaluation in prospective studies before they can be used in routine clinical practice for PBC. Currently, a large multicentre, prospective, double-blind, placebo-controlled phase III study of bezafibrate in combination with UDCA (BEZURSO study) in PBC is recruiting [ClinicalTrials.gov identifier: NCT01654731] and the results are eagerly awaited to clarify the true effect of fibrates in cholestasis.

GFT505

GFT505 (GENFIT, Loos, France) a novel PPARα/δ agonist, is currently being developed as a novel therapy for NASH and has received FDA fast-track status. Preclinical studies showed hepatoprotective effects of GFT505 as it decreased hepatic lipid accumulation, improved liver dysfunction markers, and inhibited proinflammatory gene expression [Staels *et al.* 2013]. GFT505

treatment also decreased plasma concentrations of ALT, ALP and γ-GT. In addition, GFT505 has an insulin-sensitizing effect which in combination with hepatoprotective effects makes it a potential therapy for NAFLD [Cariou and Staels, 2014]. Currently an international placebo-controlled phase IIb study (GOLDEN trial) is investigating the safety and efficacy of GFT505 (80 and 120 mg once daily for 52 weeks) in patients with NASH without cirrhosis, with resolution of NASH without worsening of fibrosis as the primary endpoint [ClinicalTrials.gov identifier: NCT01694849].

BA therapy in malignancy

A few years ago BAs were proposed as potential shuttles to deliver chemotherapy agents to treat tumours such as HCC, cholangiocarcinoma (CCA) and colorectal carcinoma [Kramer *et al.* 1992]. The rationale for this proposal was that the tumours in the enterohepatic circulation maintain good expression of BA transporter proteins (especially ASBT) and the 'BA-drug couple' would be efficiently taken up by carrier proteins expressed in the tumour cells. Therefore specific organotropic cytostatic BA derivatives (called 'Bamets') were developed to enhance anti-tumour drug delivery and increase tumour sensitivity to chemotherapy [Criado *et al.* 1997]. Conjugates of cisplatin with glycocholate (Bamet-R2) and with UDCA (Bamet-UD2) have been proved useful in the chemotherapy of experimental models of HCC [Macias *et al.* 1998; Larena *et al.* 2002]. More recently, Bamet-UD2 has been shown to inhibit tumour growth in CCA cells expressing ASBT [Lozano *et al.* 2015]. Results of these experimental models are encouraging but the possibility of using BA derivatives to treat malignancies needs further clinical evaluation.

Conclusion

The landscape of treating chronic liver diseases is rapidly changing largely due to recent advances in unravelling the role of BAs as signalling molecules in metabolic and cholestatic diseases. The understanding of diverse role of BAs in biological pathways is not complete and is continuing to evolve. Novel BA-based pharmaceuticals have raised hope for availability of better therapies in the near future with several compounds already in clinical trials. The current and future development of drugs based on the therapeutic concept of BAs is most likely to benefit patients with PBC, PSC and NASH.

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Conflict of interest statement

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