Cognitive symptoms in Primary Biliary Cholangitis (PBC)

Dr Naw April Phaw (MB.BS, MRCP, ESEGH)

Thesis submitted for the degree of

Doctorate of Medicine

In

The School of Clinical Medical Sciences

University of Newcastle

STATEMENT OF ORIGINALITY

I certify that, to the best of my knowledge and belief, the work presented in this thesis is original and my own work except as acknowledged in the text. I certify that this thesis has not previously been submitted for any assessed qualification at this or another university.

LIST OF PUBLICATIONS AND PRESENTATIONS

Publications by candidate relevant to the thesis

Phaw NA, Dyson JK, Jones D. Emerging drugs for the treatment of primary biliary cholangitis. Expert Opinion Emerg Drugs. 2020 Jun;25(2):101-112.

Phaw NA, Leighton J, Dyson JK, Jones DE. Managing cognitive symptoms and fatigue in cholestatic liver disease. Expert Rev Gastroenterol Hepatol. 2021 Mar;15(3):235-241.

Poster Presentation

N A Phaw, J Dyson, D E Jones. Understanding of Central and Peripheral Fatigue in PBC patients. The abstract was an electronic poster presentation at ILC2020, The Digital International Liver Congress (EASL) and was selected for inclusion in the “Best of ILC summary slide deck”.

ACKNOWLEDGEMENTS

I am deeply thankful to my supervisors, Professor David Jones and Dr Jessica Dyson for their unwavering support, guidance, and valuable insights throughout the research process. Their expertise and encouragement have been instrumental in shaping and completion of this thesis. I am especially thankful to Dr. Dyson for kindly proof-reading the final manuscript.

I am thankful to my supervisors, Prof Andrew Blamire and Dr David Cousins for providing crucial input in cognitive measures and imaging findings.

I am grateful to Dr Jehill Prakash for DTI analysis and Kim Pearce for statistical support. I extend my appreciation to the members of my research team, Sister Kath Houghton and Jasmin Snowden for their help in recruitment of patients.

I want to express my gratitude to the patients for giving their time and dedication in this research project.

I acknowledge financial support from the Newcastle NIHR Biomedical Research Centre (BRC). Without this, the study would not have been possible.

This thesis would not have been possible without the collective support of these individuals and institutions. Thank you all for being an integral part of this academic endeavour.

DEDICATION

This thesis is dedicated to a few people for their unwavering support during my academic journey. Thank you, Maing, Saw, Ivy, and Si for your constant encouragement, understanding and solace during moments of doubt. To my parents whose boundless love and belief in me motivated me to finish this work. Last, to my Heavenly Father who is my source of wisdom and knowledge.

Abstract

Patients with primary biliary cholangitis (PBC) may suffer from cognitive symptoms in addition to the primary disease burden. These symptoms impact on quality of life but, to date, we have no pharmacological therapies demonstrated to improve these symptoms. It is crucial to understand the nature of cognitive symptoms and underlying neuropathological processes in PBC for the development of effective future clinical trials. In this thesis, neuropsychometric assessments and magnetic resonance Diffusion Tensor Imaging (DTI) measurements were used to determine the nature of cognitive symptoms in PBC, and to explore white matter pathology, as a potential explanation, respectively.

In this thesis, cognitive deficits in PBC were found in multiple domains with pronounced impairments in memory, attention, and emotional cognition. Neuropsychometric testing demonstrated cognitive deficits in both patients reporting significant cognitive difficulties and asymptomatic patients, compared to a normative population. Therefore, cognitive symptoms in PBC may be influenced by behavioural variation. Nevertheless, those reporting cognitive symptoms are typically the group with the highest unmet clinical need, and likely to be prioritized for interventional studies. We found that PBC-40 cognitive scores were not closely associated with fractional anisotropy (FA) values from deep white matter tracts, contrary to what we had hypothesised. This suggests that cognitive symptoms do not arise from white matter tract dysfunction, although we acknowledge that we only studied a small number of *a priori* regions. Only patients with cholestasis demonstrated significant cognitive deficits. Based on these findings, we propose that cognitive symptoms may only become apparent when cholestasis is established, and therefore aggressive treatment for cholestasis at an early stage may be needed.

Overall, the characterisation of cognitive symptoms in PBC, together with exploratory brain imaging, are important steps for informing the design of future PBC therapeutic trials by establishing reference endpoints for future studies.

ABBREVIATIONS

ADHD – Attention deficit hyperactivity disorders

AE2 – Anion exchanger 2

ALP – Alkaline phosphatase

ALT – Alanine transaminase

AMA – Anti-mitochondrial antibody

ANA – Anti-nuclear antibodies

ASBT - Apical sodium-dependent bile acid transporter

AST – Aspartate aminotransferase

BBB – Blood brain barrier

cAMP - cyclic adenosine monophosphate

CANTAB - Cambridge Neuropsychological Test Automated Battery

CNS – Central nervous system

COPD – Chronic obstructive airway disease

CPAP – Continuous positive airway pressure

CREST – Calcinosis, Raynaud, oesophageal dysfunction, sclerodactyly, telangiectasia

CRT - Continuous Reaction Time test

CT – Computed tomography

CYP7A1 - Cholesterol 7 Alpha-hydroxylase

DTI – Diffusion Tensor imaging

EEG - Electroencephalography

ELISA - Enzyme-linked immunosorbent assay

ERT – Emotional recognition task

ERTOMDRT – ERT overall median reaction time

ERTTH – ERT total hits

ESS – Epworth sleep score

FA - Fractional Anisotropy

FXR - Farnesoid X Receptor

HADS – Hospital Anxiety and Depression scale

HE – Hepatic Encephalopathy

IBAT – Ileal bile acid transporter inhibition

IL - Interleukin

ILF – Inferior longitudinal fasciculus

MAFLD – Metabolic dysfunction-associated fatty liver disease

mHE – minimal hepatic encephalopathy

MRI - Magnetic resonance imaging

MTT – Multitasking task

MTTLMTMD – MTT response latency median

MTTLCMD – MTT median congruent

NF-KB – nuclear factor-kappa-light-chain-enhancer

NTCP – Na+ -taurocholate co-transporting polypeptide

NVC – Neurovascular coupling

OCA – Obeticholic acid

OSA – Obstructive sleep apnoea

OTS – One touch stockings of Cambridge

PAL – Paired associate learning.

PBC – Primary biliary cholangitis

PALTEA – PAL total error

PALFAMS – PAL first attempt memory scores

PDC - Pyruvate dehydrogenase complex

PDE2 - Phosphodiesterase 2

PHES - Psychometric Hepatic Encephalopathy Score

PPAR - Peroxisome proliferator - activated receptor

QOL – Quality of life

RT – Reaction time

RVP – Rapid visual information processing

RVPMDL – RVP Median response latency

SLF – Superior longitudinal fasciculus

STAT - Signal transducer and activator of transcription

SWM – Spatial working memory

SWMBE – SWM between errors

SWMS – SWM strategy

TCD – Transcranial doppler

TNF-alpha - Tumour Necrosis Factor -alpha

Th – T helper

UDCA – Ursodeoxycholic acid

ULN – Upper limit of normal

WAIS – Wechsler adult intelligence scale (WAIS)

Table of Contents

LIST OF TABLES

LIST OF FIGURES

Chapter 1: Introduction and Literature Review

INTRODUCTION

1.1 Primary Biliary Cholangitis (PBC)

Primary biliary cholangitis (PBC) is a chronic autoimmune cholestatic liver disease characterised by biliary injury and inflammation of the intrahepatic bile ducts [1]. It occurs predominantly in women over the age of 40 years [1]. The disease is progressive and, without intervention, can often result in fibrosis, eventually leading to cirrhosis and its complications that may require liver transplantation or result in death [1, 2]. In addition to the progressive nature of the disease, patients experience significant symptom burden that is independent of disease severity [1, 3]. Ursodeoxycholic acid, and the second-line therapy, obeticholic acid, are the available licensed treatments for PBC [1]. Seladelpar and elafibranor have recently been approved for the treatment of PBC [4, 5]. Although there has been rapid development of novel therapies in recent years targeting risk reduction in PBC [2], there are very few symptom-directed therapies.

1.1.1 Epidemiology of PBC

It has been estimated that the incidence of PBC ranges between 0.3 and 5.8 per 100,000 population each year and its prevalence ranges between 1.9 and 40.2 per 100,000 population [6]. According to epidemiological data, there is worldwidegeographical variation in disease prevalence, with Europe and North America having the highest prevalence. [7]. The prevalence of PBC has been increasing, whereas the incidence has been constant, suggesting increased disease awareness [8]. Recent Swedish data estimated the prevalence of PBC to be 34.6 per 100,000 population and the incidence to be approximately 2.6 per 100,000 persons per annum [8].

1.1.2 Pathophysiology and Pathogenesis of cholestasis

The precise aetiology of PBC is not well understood, although complex interactions between environmental and genetic factors are thought to play a significant role [9]. A number of factors; genetic, dietary, and environmental including bacterial infection and xenobiotics may contribute to disease development [10]. The cross-reactivity between endogenous and

exogenous antigenic epitopes with E2 subunits of the mitochondrial 2-oxo-acid dehydrogenase complexes, principally the pyruvate dehydrogenase complex (PDC), activates the immune response [11]. The subsequent disruption of peripheral immune tolerance to the pyruvate dehydrogenase -E2 (PD-E2) antigen on the mitochondrial membrane, triggers the immune response targeting biliary epithelial cells (BEC) through molecular mimicry [11]. Although autoreactive immune responses clearly arise in PBC, a crucial second dimension to disease pathogenesis involves a self-propagating cycle of cholestatic injury.

The decreased expression of CL-/HCO-3 anion exchanger (AE2) on biliary epithelial cells in PBC patients is associated with biliary inflammation [12]. AE2 regulates biliary bicarbonate secretions and assists in maintaining a physiological bicarbonate-rich biliary "umbrella" that protects cholangiocytes against hydrophobic bile acid injury [12]. The accumulation of high concentrations of hydrophobic bile acids in cholestasis probably results in the progression of PBC. Hydrophobic bile acids are significantly more cytotoxic than hydrophilic bile acids [12, 13]. Recent findings showed that upregulation of MicroRNA-506 (miR-506) in cholangiocytes of PBC patients (that downregulate the CL-/HCO-3 AE2) and the subsequent defective activity of AE2 leads to reduced biliary bicarbonate secretion [13, 14]. This results in disruption of the biliary umbrella [13, 15]. Furthermore, downregulation of AE2 enhances cholangiocyte susceptibility to apoptosis by increasing the activity of adenylyl cyclase that may sensitize cholangiocytes to apoptotic insults [12, 13, 15].

Cholangiocytes produce various pro-inflammatory cytokines in response to biliary injury or stress and the increased expression of the pro-inflammatory cytokines, Tumour Necrosis Factor-alpha (TNF-alpha) and Interleukin-6 (IL-6), in damaged cholangiocytes has been demonstrated [16-18]. When combined with IL-6,TNF-alpha inhibits ductal secretion via cyclic adenosine monophosphate (cAMP), and also causes apoptosis or senescence of the biliary epithelium [19].

Biliary senescence is an arrested phase of damaged cells in the cell cycle that are not responsive to external stimuli and are ultimately eliminated from the local environment [20,

21]. The accumulation of senescent cells has been described in PBC, with the suggestion that the production of various proinflammatory cytokines (IL-1, IL6, chemokines (CCL2 and CXCL1)) may play a role in progressive biliary injury resulting in bile duct loss [22].

It has been postulated that biliary injury, resulting from impaired anion exchanger (AE) and apoptosis, is aggravated by inappropriate immune activation, leading to a progressive cycle of cholestasis and, ultimately, fibrosis [22]. Both T-helper 1 (Th1) and Th17 responses have been implicated in the immunopathogenesis of PBC [23, 24]. Data from genome-wide case-control association analysis by Hirschfield et al suggested the genetic association between IL12A, IL12RB2 and STAT4 variants in PBC [24] and implicated IL-12 in the autoimmunity of PBC. A variety of inflammatory responses trigger T-helper 1 (Th1) cells through IL-12 signalling and activation of signal transducer and activator of transcription (STAT4) [24]. This results in increased production of interferon-gamma, which promotes the apoptotic process; a known feature of biliary epithelial cell injury in PBC [23]. A role for Th17 responses in PBC has been postulated given the demonstration of elevated levels of IL17 and IL23 in the context of damaged bile ducts [25]. The exact mechanism by which Th17 responses arise in PBC remains unclear. It is possible that this may be a response to the micro-environment created by injured or stressed biliary epithelial cells [26]. Th1 responses arise as part of an upstream, triggering immune process in PBC, whilst the Th17 response is a secondary phenomenon resulting from bile duct injury [26]. Studies of murine models of PBC have confirmed potential roles for IL12 and IL23 signalling in PBC pathogenesis [26, 27].

The gut-liver axis, through the Farnesoid X Receptor (FXR) signalling pathway, is of interest in recent studies of bile acid metabolism. FXR physiologically regulates the genes involved in bile acid synthesis, absorption, uptake and transport [28-30]. Upon activation of the receptor, it inhibits bile acid synthesis by suppressing the transcription activity of genes encoding the enzyme cholesterol 7 Alpha-hydroxylase (CYP7A1) that initiate the bile acid production pathway [30, 31]. It also downregulates the expression of bile acid transporters (Na+ -taurocholate co-transporting polypeptide [NTCP] and Apical sodium-dependent bile acid transporter [ASBT] in the liver and intestine thereby regulating bile acid uptake and export [32]. Obeticholic acid, which targets FXR

receptors, shows promising results in treating PBC [33], despite the fact that the FXR signalling pathway has not been demonstrated in PBC.

1.1.3 Clinical presentation and diagnosis

Patients with cholestatic liver function tests may not have symptoms for years. PBC patients are may be identified following investigation of abnormal liver function tests found incidentally or due to PBC-associated symptoms including cholestatic itch, dry mouth, fatigue and abdominal discomfort [34].

Two out of three criteria are necessary to diagnose PBC: 1. persistently abnormal cholestatic liver function tests (with duration longer than 6 months); 2. presence of highly specific anti-mitochondrial antibody (AMA) or PBC-specific antinuclear antibody (antiglycoprotein 210 or anti-sp100); 3. histological evidence of chronic non-suppurative cholangitis or fibrosing obliterative cholangitis [34]. Due to the high sensitivity and specificity of the autoantibodies, liver biopsy is not routinely performed unless there is diagnostic uncertainty [34].

All patients should be stratified according to the likelihood of developing advanced liver disease and its complications. Patients who are diagnosed at a young age (< 45 years), symptomatic and of male sex are more likely biochemically to have an incomplete response to 1st-line therapy with ursodeoxycholic acid (UDCA) [35]. The presence of ductopenia on histology and advanced liver fibrosis (median liver stiffness >9.6 kPa) are independent risk factors for disease progression with increased risk of developing cirrhosis or requiring liver transplantation in the future [36, 37].

1.1.4 Symptoms of PBC

Pruritus

Cholestasis-related pruritus is a common symptom in patients with PBC and can often have a negative impact on quality of life (QOL) [34, 38]. It may present at any stage of the disease and sometimes precede the diagnosis of PBC. About 20-70% of PBC patients experience pruritus at some point in their disease course and its severity may diminish as the disease advances [38]. The pathogenesis of pruritus is not well defined. Increased concentration of

pruritogens such as bile salts, histamine, serotonin metabolites, and endogenous opioids are thought to be potential causes of pruritus in PBC [38]. The role of serum autotaxin activity and the effect of ileal bile acid transporter (IBAT) inhibition in PBC with cholestatic itch have been explored and may represent future therapeutic targets of anti-pruritic inventions. [39-41].

Cholestatic pruritus mostly occurs in the limbs, particularly the palms of the hands and soles of the feet, but can be generalised in nature [38, 42]. In most patients, itch intensity increases during the evening or night and worsens with heat or hot baths [38, 42]. A flare of symptoms is frequently associated with emotional stress, menstruation, and during pregnancy but sometimes may disappear for a period of time [38, 42]. Pruritus is often worse in patients with the premature ductopenic variant of PBC [42].

The currently available first line treatment for PBC, UDCA, has no evidence for improvement of itch whilst the commonest side effect of second line therapy, Obeticholic acid, is itch [38, 42, 43]. Bile acid sequestrants such as cholestyramine are commonly used as first-line treatment for itch [1, 43]. Patients whose itch fails to improve with bile acid sequestrants can be given rifampicin (a pregnane X receptor (PXR)-agonist and enzyme inducer) or naltrexone (opiate antagonist) as second- and third-line therapies, respectively [42, 43]. Sertraline (a selective serotonin reuptake inhibitor) and gabapentin may also be used but the evidence of their effectiveness is limited [42]. Physical management such as UV light therapy and intermittent nasobiliary drainage may be beneficial but the evidence is limited [42, 43]. Patients who are refractory to all conventional medical therapies may be considered for liver transplantation, regardless of stage of the disease [42, 43].

Sicca Complex

Sicca complex (dry eyes and dry mouth) is a common presentation of PBC patients with a report showing approximately 70% of PBC patients had these symptoms [1, 44]. This is sometimes associated with vaginal dryness, dysphagia, coughing, and hoarseness of voice [44, 45]. Patients with severe xerostomia may present with dental caries or oral candidiasis [44, 45]. Sicca complex is believed to be secondary to PBC rather than primary Sjogren's

syndrome, as patients with PBC do not often have the serological or immunological features seen in Primary Sjogren's syndrome [45]. The management of the Sicca complex is mainly symptom-directed with artificial tears or saliva [46]. Those who are refractory to topical treatment, or have a significant adverse impact on quality of life, should be referred for specialist management [47].

Fatigue

In recent years, fatigue has become an increasingly recognised problem in primary biliary cholangitis (PBC). It affects over 50% of patients with approximately 20% of patients reporting severe fatigue [48-52]. Through direct or indirect effects, this debilitating symptom contributes significantly to the poor quality-of-life (QoL) experienced by many patients with PBC, which can severely impair their social functioning [48-52].

The pathogenesis of fatigue in PBC is unknown and probably complex. It was thought to be centrally mediated [53]. Swain et al examined neurobehavioral changes in mouse models of induced cholestasis and demonstrated the presence of cholestasis related neurotransmission in bile duct ligated mice with an association with anhedonia and loss of social interest [54, 55]. In a study by Huang et al, bile duct ligated mice demonstrated significantly impaired spatial memory compared to those with sham ligation of the bile duct [56]. Patient descriptions, in conjunction with mechanistic studies, have suggested significant central nervous system (CNS) abnormalities. Abnormalities in Magnetic Resonance Imaging (MRI) measurements, such as magnetisation transfer ratios and diffusion-weighted metrics, appear early in the course of the disease [57]. Neurophysiological abnormalities (that do not improve following liver transplantation) and loss of cerebral autoregulation have also been demonstrated [58]. Due to possible overlapping mechanisms, fatigue in conjunction with cognitive symptoms is hypothesized to be centrally mediated.

Patient descriptions, such as "it feels like my batteries are running down", also suggest a peripheral component in a proportion of patients. This concept is supported by studies of peripheral muscle bioenergetics showing significant abnormalities related to fatigue severity

[59]. The role of AMA antibodies in PBC, and its inhibition of the pyruvate dehydrogenase enzyme, is also thought to be a possible cause of excessive anaerobic metabolism causing muscle fatigue [60].

Patients with severe fatigue, as compared to those without fatigue, frequently suffer with depression, sleep disturbances and autonomic symptoms [60]. The role of additional factors such as sleep, mood and cognition in perceived fatigue is difficult to assess. It was reported that fatigue in patients who describe “brain fog” and motivational fatigue were significantly correlated with cognitive impairment and autonomic dysfunction [61]. Data from the UK-PBC cohort showed that severity of fatigue was strongly correlated with the severity of cognitive symptoms [61]. Patients who had both severe fatigue and significant cognitive impairment (the central fatigue group) were younger [61]. The impact appears to be more global with higher symptom burden previously shown to be associated with fatigue in PBC, including social dysfunction, depression, anxiety, autonomic dysfunction and sleep disturbance [56, 59].

Despite its adverse impact and high prevalence, fatigue in PBC has no licensed or effective medications for its management. A placebo-controlled clinical trial employing the monoclonal anti-CD-20 antibody Rituximab (RIT-PBC) for PBC-related fatigue showed no statistically significant difference between the treatment and placebo arms [62]. However, the failure of the trial to show significance may be due to study design being based on peripheral muscle abnormality, but the study population had centrally associated fatigue. Modafinil has been shown to improve fatigue in patients with daytime somnolence but is not recommended for use routinely in patients with PBC-related fatigue associated sleep disorder due to its side effect profile [63]. In a small trial, plasmapheresis improved symptoms, including fatigue, but not without adverse effects [64]. The current interventions recommended in treatment guidelines for fatigue are non-pharmacological and mainly supportive [61]. In PBC-related fatigue, a "TrACE" approach has been suggested [60]. This includes treating the contributing co-morbid causes (e.g. hypothyroidism), improving the ameliorable causes such as sleep, mood disorders, coping strategies, and empathizing with patients [60].

Autonomic Dysfunction

As a complication of advanced liver disease, autonomic and sensory dysfunction is well documented, but is less frequently observed in those with PBC, in the absence of advanced liver disease. In recent years, studies have reported the presence of generalised neuropathy with autonomic dysfunction in PBC patients [65, 66]. The exact prevalence of autonomic symptoms in PBC patients is not known. PBC patients have been shown to have dysregulation of blood pressure, impairment of sympathetic and parasympathetic function and poor autonomic tone compared to normal controls [65, 66]. The exact mechanism of autonomic dysfunction in PBC remains elusive, but it is hypothesized to be a central mechanism or related to nutritional factors in PBC patients. Studies report autonomic dysfunction in PBC patients, and the length or severity of the disease may be associated with the symptoms [67, 68]. In a later study by Newton et al, both non-cirrhotic and cirrhotic PBC patients had autonomic dysregulation and the authors concluded that the symptom may be present at all stages of disease [65]. The correlation and close association of autonomic dysregulation with fatigue and cognitive symptoms in PBC may be significant in understanding the underlying pathological mechanisms.

Others

Raynaud's phenomenon occurs in about 25% of PBC patients and is linked to auto immunogenicity in PBC patients, although the exact pathophysiology is unclear [46]. Up to 30% of PBC patients suffer from restless leg syndrome and, once again, the underlying mechanism is unknown [46].

1.1.5 Symptom burden and health-related quality of life in PBC

Patients with PBC suffer from complex symptom phenotypes, which can negatively impact their health-related quality of life (HrQOL). There are many factors that contribute to quality of life in PBC, which are multifactorial and complex. In the UK-PBC cohort that recruited 2353 PBC patients from all clinical centres in the UK, 35% and 46% of patients reported perceived poor quality of life and impaired health status, respectively, as compared to 6% and 15% of healthy controls [50]. Although the manifestation of complex symptom burden (itch, cognition, fatigue, emotional, social, autonomic disturbance, anxiety and depression) appeared to be a factor, fatigue was a major contributor to poor quality of life for patients both through its direct and indirect effects [50]. Dyson et al further evaluated the severity of

the inter-relationship of symptoms and quality of life using the UK-PBC cohort [69]. They identified that younger patients were more affected and social dysfunction had the greatest impact on quality-of-life impairment [70]. European and North American studies have also reported a significant disease burden among PBC populations [71]. Symptoms and health-related quality of life do not appear to be influenced by genetic, ethnicity or cultural differences. In a multicentre observational study of 496 Japanese PBC patients, at least 25% experienced a substantial burden of symptoms, irrespective of severity of disease, with intensively impaired health related-quality of life [72]. The study reported younger age at presentation to be associated with greater symptom burden and female gender as a risk factor for severe fatigue. A study conducted in China also reported a significant burden on health-related quality of life among PBC patients [73]. Despite perceived poor quality of life in PBC patients being significant, symptom-directed treatment is very limited. One of the most pressing unmet clinical needs for PBC patients is the symptom burden and its impact.

1.1.6 Treatment

Until recently, the focus in PBC was on identifying patients with advanced liver disease, managing the complications of that disease state and considering liver transplantation at an appropriate time [74]. The advent of effective treatment in PBC for modifying disease risk has changed this perspective, and the focus is now on preventing disease progression and avoiding end-stage disease development in the first place. There is also increasing awareness of the importance of addressing symptoms as well as disease progression risk.

Current treatment guidelines recommend UDCA (13-15mg/kg/day) in all patients with PBC as first-line therapy and remains the bedrock of PBC therapy [74]. Current guidelines state that biochemical response to therapy should be evaluated after one year of standard therapy with UDCA using the proposed criteria (Table 1-1) [74].

Ursodeoxycholic acid (UDCA) is a secondary hydrophilic bile acid and its therapeutic actions are not fully understood, although contributing factors include cyto-protection of hepatocytes and cholangiocytes, enrichment of the hydrophilic bile acid pool and displacement of hydrophobic bile acids [74, 75]. UDCA has an excellent safety profile and true intolerance of UDCA is uncommon (under 5% of treated patients) [76]. The

most common side effects of UDCA are weight gain, hair loss and gastrointestinal side effects. However, approximately 40% of patients do not respond to treatment with UDCA [76]. Those patients who fail to respond to UDCA therapy should be considered for second line treatment with Obeticholic acid, elafibranor , seladelpar or off licence therapy with fibrates (Bezafibrate or Fenofibrate) or a clinical trial [74, 77].

Table 1-1: Criteria used to assess biochemical response in PBC.

Scoring System	Defined criteria for response to therapy	Time of assessment
Toronto [78]	ALP≤ 1.67 ULN	24 months
Poise	ALP ≤ 1.67 ULN, ≥ 15% reduction in ALP, and total bilirubin ≤ ULN	12 months
Barcelona [79]	40% decrease in initial level of ALP or normalisation of ALP	12 months
Paris II [80]	ALP≤ 1.5 ULN or total bilirubin > 1mg/dl or AST≤ 1.5 ULN	12 months
Paris I [81]	ALP <3x ULN and AST <2x ULN and bil <1mg/dl	12 months
Rotterdam [82]	Normalisation of bilirubin or albumin	12 months

[1]

ALP= alkaline phosphatase, ULN = upper limit of normal, AST= aspartate aminotransferase

Obeticholic acid (OCA) is a semi-synthetic bile acid derivative from the naturally occurring human bile acid chenodeoxycholic acid with an ethyl substituted group at the 6 alpha position [77]. It is a selective and potent agonist of the farnesoid X receptor (FXR) that regulates bile acid homeostasis by regulating genes involved in bile acid synthesis, absorption, uptake, and transport [74, 75, 77]. The most common side-effect of this medication is pruritus, affecting approximately 56-77% of patients [33, 83]. Pruritus is dose-dependent, and its exact pathophysiologic mechanism is not yet known but the accumulation of autotaxin and lysophosphatidic acid or potentiation of opioid receptors has been suggested [33, 83, 84].

Obeticholic acid should be considered as an adjunct therapy, although it can also be used as monotherapy in patients who are intolerant to UDCA. The starting dose for Obeticholic

acid is 5mg/day but it can be titrated to 10mg/day if patients fail to achieve biochemical response after 6 months of treatment [74, 75, 77]. OCA can be used in well compensated cirrhosis at a reduced dose but there is an increased risk of hepatic decompensation in patients with Child-Pugh A cirrhosis with portal hypertension and is contraindicated in Child-Pugh B/C cirrhosis [74].

Those patients who are not suitable for Obeticholic acid (e.g., severe itch) should be considered for fibrates, an off-label treatment option. Fibrates are peroxisome proliferator-activated receptor (PPAR) agonists, with different agents acting on different subtypes of receptors [77]. Bezafibrate is a pan-PPAR agonist, whilst fenofibrate, a licensed treatment for hypertriglyceridemia, acts as a pure PPAR alpha agonist. A growing body of evidence suggests fibrates are beneficial for PBC with significant improvements in liver biochemistry [85-87]. Similar to Obeticholic Acid, the long-term effects on transplant-free survival or liver - related deaths with fibrates have not been established. A recent UK-wide multicentre evaluation of current second-line therapies in PBC showed similar rates of biochemical response and drug discontinuation with fibric acid derivatives and OCA [88]. Recently, seladepar, potent selective PPAR delta agonist, and elafibranor, a dual PPAR alpha and delta agonist, have been approved for those who are not responded to UDCA therapy [4, 5]. The treatment algorithm is shown below in Figure 1-1.

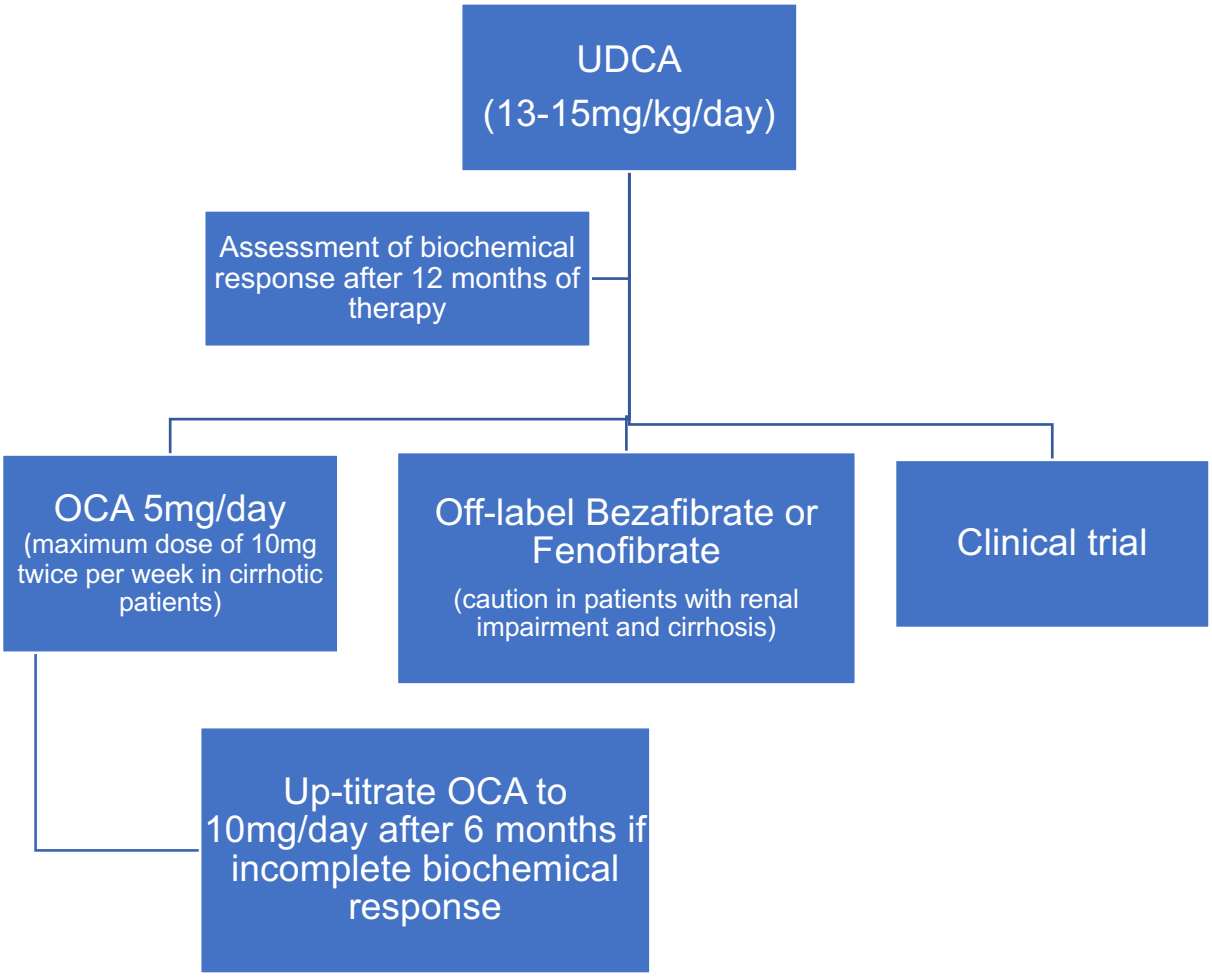

Figure 1-1: PBC treatment algorithm [77]

Liver transplantation

Liver transplantation should be considered in advanced liver disease with complications (worsening jaundice, ascites, spontaneous bacterial peritonitis, variceal bleeding, and hepatic encephalopathy) based on severity score (minimal listing criteria of UKELD greater than 49) [1, 9, 89]. The listing criteria for liver transplant in PBC in the UK are no different from those for other chronic liver diseases. Cholestatic pruritus usually subsides within 24 hours of transplantation and the current guidelines recommend that patients with severe, intractable pruritus refractory to medical treatment should be assessed for liver transplantation [1, 9, 89]. Other symptoms, such as fatigue, persist post liver transplant for PBC and they are not considered appropriate indications for liver transplant in this condition [1, 9, 89].

1.2 AMA positive without cholestasis

The literature on the characteristics of patients who have AMA antibodies in the absence of cholestatic liver function tests is limited. AMA is a highly specific antibody for PBC (>95%) and the diagnosis of PBC can be confidently made without a liver biopsy in the presence of abnormal liver function tests and the absence of any alternative explanation [1]. The presence of AMA antibodies is reported occasionally in other autoimmune conditions, including scleroderma and Sjogren's syndrome [90]. The clinical significance of the presence of AMA in the absence of clinical, biochemical, or histological features of PBC is unknown. There are different techniques used to detect AMA antibodies such as Western immunoblot with bovine sub-mitochondrial particles, indirect immunofluorescence, and ELISA (enzyme-linked immunosorbent assay) with AMA-specific recombinant protein [91]. The sensitivities for detecting AMA can vary depending on the laboratory techniques used [91]. The estimated prevalence of AMA positivity in the general population is between 0.07% and 9.9% [92, 93]. A study on annual health checks among corporate workers from Japanese companies reported a 0.64% prevalence of AMA antibodies (11/1714), with 6 subjects having abnormal liver blood tests [94]. It was estimated that 0.73% of Japanese people with AMA antibodies had symptoms of PBC [94]. Chen et al reported the prevalence of AMA antibody among healthy Xuhui District residents in Shanghai was 0.7% (133/19012) during routine health checks [95].

If AMA antibody positivity precedes cholestasis and ultimately PBC, it is interesting to know whether AMA antibodies are positive before cholestasis develops. In a small UK cohort of 29 patients found to be AMA antibody positive with completely normal alkaline phosphatase (ALP) and bilirubin during investigation for other autoimmune diseases, 12 had diagnostic features of PBC on liver biopsy. A further 12 had features compatible with PBC [96]. They reported that 11 of 16 patients developed cholestatic liver function tests after a mean follow up period of 8.7 years with 5 of them reporting PBC related-symptoms (itch and fatigue) [96]. In 10 years follow up of this cohort, 24 (83%) developed elevation of ALP and 22 reported PBC related-symptoms [97].

According to a large French study using data from 63 immunology laboratories, AMA positivity can precede symptoms and abnormal liver tests [98]. There were 1318 patients

with positive AMA antibodies, of whom 229 (32%) did not have a definitive diagnosis of PBC, and 74% had a normal ALP [98]. Biopsies were performed in only a small number of patients (19%). Most patients with positive AMA without cholestasis were female (n=179, (78%) and 31 patients (24%) had another autoimmune disease. In follow-up data available for 92 patients up to 7 years, 1 in 6 patients were eventually diagnosed with PBC with a 5-year incidence of 16%. The study reported that AMA-antibody positive patients without cholestasis were slightly younger and had lower AMA titres and less frequent symptoms (itch, jaundice) compared to those with confirmed PBC (i.e. itch 3% in non-established PBC vs 24 % in established PBC) [98]. The presence of mild biochemical abnormalities, together with lower antibody titres, in the study raised the question of whether the disease process of PBC occurs for years in some patients with antibodies being present in a very early indolent inactive stage before becoming cholestatic.

Interestingly, rates of fatigue in AMA positive patients with normal liver function tests was similar to that of patients who had already been diagnosed with PBC (47% vs 45% respectively) [98]. This is in contrast to other symptoms such as itch [98]. It is thought that AMA antibodies in PBC patients directed against the pyruvate dehydrogenase complex (PDC) that regulates aerobic metabolism in muscles causing a metabolic insult, lead to fatigue [99]. It is known that fatigue in PBC does not correlate with the stage or severity of PBC. It would be intriguing to examine whether symptoms such as fatigue were present in cholestatic PBC earlier than was previously thought. To date, there is no study evaluating health related quality of life and symptom assessment in patients with positive AMA antibody without cholestasis.

1.3 Cognitive symptoms in liver disease

Chronic and acute liver disease can present with neuropsychological and central nervous system abnormalities. In addition to the disease specific burden and complications of advanced disease, patients also suffer from liver associated fatigue, cognitive decline, anhedonia and mood alternation regardless of severity and stage of disease [100, 101].

One of the neurological presentations in liver disease is hepatic encephalopathy, which is characterized by a wide range of neurological and psychiatric manifestations. It affects 30-40% of cirrhotic patients at some point in the disease course [102]. Chronic alcohol excess also contributes to cognitive impairment in alcohol related liver disease regardless of the severity of the disease [103] .There is growing evidence of cognitive impairment in Hepatitis C, Metabolic dysfunction-associated steatosis liver disease (MASLD) and cholestatic diseases, in particular Primary Biliary Cholangitis (PBC) in the absence of advanced liver disease [104-106].The possible mechanisms of cognitive dysfunction in those diseases without cirrhosis include systemic and neuro-inflammation, disturbed gut microbiota, cerebrovascular dysfunction and neurodegeneration [107]. The pathophysiology of cognitive dysfunction is not well understood.

Minimal hepatic encephalopathy (mHE) is a condition in which patients have coherent speech and positive engagement, but have cognitive impairment on advanced neuropsychiatric and neurophysiological testing [108]. This is felt to be restricted to cirrhotic patients [108]. The cognitive impairment experienced by patients with minimal hepatic encephalopathy is usually characterised by impaired attention, working memory deficit, visuospatial dysfunction and impaired executive function [108]. It may be subtle with patients themselves not being aware of it.

Although the prevalence of mHE is unknown, studies showd that it affects 20%-50% of patients with cirrhosis [102, 109]. mHE is prognostically significant as it is linked with an increased risk of progression to overt HE [110]. Hartmann et al reported 22/116 patients with proven cirrhosis (22%) had subclinical hepatic encephalopathy and they developed significantly more episodes of clinical encephalopathy during a median follow-up of 49 months [111]. Overt hepatic encephalopathy has a significant impact on socioeconomic status and quality of life [112, 113]. In addition, patients with minimal hepatic encephalopathy have impaired driving skills due to attention deficits and visual-motor deficits, which are associated with increased risk of motor vehicle accidents [114]. It was found that 45/130 (34.6%) patients with cirrhosis had minimal hepatic encephalopathy, with 40% of those with mHE reporting falls as compared to 12.9% in those without mHE (p= 0.001) (82) [115]. In

the same study, patients with mHE showed higher healthcare needs (8.8% vs 0% respectively, p <0.004) and hospitalizations (6.6% vs 2.3% respectively, p = 0.34) [115]. Although mHE may have a socioeconomic impact and impair quality of life, there is a general lack of consensus on who and how it should be screened for.

There are no current universally agreed diagnostic criteria for mHE and different studies apply different endpoints and criteria. Furthermore, there is no specific test that can be used to confirm the condition. Minimal encephalopathy impairs different components of cognitive function and should be assessed by trained psychologists utilizing different psychometric tools [110]. The commonly performed neuropsychological tests are Psychometric Hepatic Encephalopathy Score (PHES), Critical Flicker Frequency test, Continuous Reaction Time (CRT) test, Inhibitory Control Test and Stroop testing depend on local guidance, expertise and availability [116-118]. It is important to interpret these tests in conjunction with the clinical context. Although electroencephalography (EEG) may reveal changes in activity, it is not widely used.

Evidence for the treatment of minimal hepatic encephalopathy is lacking. Hepatic encephalopathy treatment may improve mHE or prevent the development of overt encephalopathy [119, 120]. Lactulose may reduce motor vehicle accidents [121] and rifaximin has been shown to improve quality of life and driving simulator performance [122]. However, the studies were short and used multiple endpoints and therefore further evidence from larger and longer duration trials is needed. As a result of the lack of solid data, current guidelines do not routinely recommend treating mHE. However, it may be beneficial to initiate treatment for patients with impaired driving, or those who experience issues with activities of daily living or cognitive complaints [102, 110].

1.4 Cambridge Neuropsychological Test Automated Battery (CANTAB)

The Cambridge Neuropsychological Test Automated Battery (CANTAB) was initially developed at Cambridge University for the assessment of subtypes of dementia in elderly people, but has since been used in various populations in many clinical trials such as

attention deficit hyperactivity disorders (ADHD), Alzheimer's dementia, Parkinson's disease, and schizophrenia [123-125]. To date, it has not been used to assess cognitive symptoms in PBC. CANTAB batteries are computerised measures of various neuropsychological functions with the key domains including memory, attention, executive function, cognition, and visuospatial function. The tests have available normative data from a healthy population which are matched by age,gender or educational background. One limitation of the tests is that they require a considerable amount of time to complete. CANTAB batteries are very easy to administer for researchers and offer precise objective assessment in a wide range of cognitive domains.

1.5 Literature Review of Cognitive Impairment in PBC

A literature search using the PubMed database was undertaken using the keywords "Primary biliary cholangitis/Primary biliary cirrhosis and cognitive impairment or Primary biliary cholangitis and symptoms impact or Primary biliary cholangitis/cirrhosis and quality of life measures or cognitive impairment and chronic liver disease or minimal hepatic encephalopathy". Only articles in English were included in our review.

1.5.1 Overview

There is emerging evidence that cognitive symptoms affect patients with primary biliary cholangitis (PBC) independent of disease stage and biochemical response status [126]. Approximately 50% of PBC patients demonstrate moderate to severe cognitive symptoms using the PBC–40 questionnaire; a fully validated, disease specific quality of life measure [126]. However, the exact prevalence of cognitive symptoms in the PBC population is unknown.

Cognitive impairment in PBC generally manifests itself as memory impairment, impaired attention or concentration and psychomotor dysfunction. These can occur in the early stages of PBC and are therefore unlikely to be a manifestation of hepatic encephalopathy that is seen in advanced liver disease [126]. Pre-cirrhotic PBC patients differ from healthy controls on MRI assessments highlighting that the disease process starts at an early stage [57]. The

possible association of white matter lesions of the brain with cognitive function in early PBC was previously reported [126]. Patients with chronic liver disease and cirrhosis have been reported to have low magnetization ratios, possibly due to low-grade cerebral oedema and hepatic encephalopathy [127]. Grovel et al found similar reduced magnetisation ratios and increased diffusivity in the thalamus, putamen and caudate in pre-cirrhotic PBC patients [57]. Prior studies have suggested a connection between reduced magnetisation ratios or increased manganese deposition and cholestasis [57]. However, the underlying mechanisms linking these MRI findings and fatigue or cognitive function in PBC remains to be proven. Overt hepatic encephalopathy resolves with liver transplantation. However, cognitive dysfunction, autonomic dysfunction and fatigue may persist even after transplantation suggesting that the underpinning mechanisms for these symptoms have become irreversible [58]. Although cognitive impairment is increasingly reported by patients, the scale of the socioeconomic impact and symptom burden due to these symptoms remains unknown.

1.5.2 *Possible mechanisms of cognitive impairment in cholestasis*

The exact aetiology and pathophysiology of cognitive impairment in PBC is not well understood. It has been hypothesized that impaired cerebral perfusion from reduced cerebral blood circulation may play a role in symptom pathophysiology [126, 128]. The correlation between autonomic dysregulation and cognitive impairment has also been established in non-liver diseases such as Parkinson's disease and heart failure [129-131]. A study conducted by Newton et al showed that up to 70% of PBC patients had autonomic dysfunction and suggested that the resultant bradycardia, low blood pressure and orthostatic hypotension may have effects on the cerebral circulation resulting in the development of cognitive impairment [65]. The presence of structural brain changes in the form of dense white matter lesions in the frontal lobes on MRI correlated with autonomic dysfunction and a degree of cognitive impairment in PBC patients [126]. Hollingsworth et al studied cerebral vascular flow using the Valsalva manoeuvre and cerebral blood flow using transcranial Doppler (TCD) in PBC patients [128]. They demonstrated the presence of upstream increased cerebrovascular resistance and impaired cerebral autoregulation and identified the association between these findings and the degree of structural changes in magnetic resonance imaging [128]. Manganese deposition in areas of the brain involved in

autonomic nervous system regulation (such as the basal ganglia) and a central inflammatory response secondary to cholestasis have been observed [66]. Although these phenomena were discussed as a possible association between autonomic regulation and cognitive symptoms in PBC patients, the exact mechanisms linking PBC to autonomic dysfunction remain poorly understood and further studies are needed.

The immune response seen in PBC occurs via peripheral autoimmune activation with the release of inflammatory cytokines such as interleukin 1 (IL1), interleukin 6 (IL6) and tumour necrosis factor α (TNFα) [23]. The cytokine and inflammatory reaction may induce disruption of the blood brain barrier (BBB) and disrupt neurovascular coupling [132, 133] with increased permeability of the BBB leading to transportation of immune cells into brain tissue. This may lead to local inflammatory reactions (neuroinflammation) that subsequently affect brain function and alter behaviour [132, 133]. Cerebral vascular endothelial cells are crucial for maintenance of the cerebral BBB and are involved in cerebral vasomotor regulation and perfusion [134]. Cholestasis is associated with increased numbers of systemic immune cells and may have a direct impact on cerebral endothelial cells. Kerfoot et al identified accumulation of TNFα secreting monocytes in the brains of bile duct resected cholestasis-induced mice activated by inflammatory cytokines [135]. The expression of TNFα and IL-6 on cerebral endothelial cells downregulates the expression of inter-endothelial junction proteins in the BBB and increases the generation of reactive oxygen species that may cause increased BBB permeability, which is a recognized feature of some neurodegenerative diseases [136]. Furthermore, the interaction of cerebral endothelial cells and TNFα producing monocytes is associated with microglia activation through expression of nitric oxide expression, which in turn decreases neural excitability causing behaviour alterations [137].

Neurovascular regulation is crucial for cerebral oxygen and glucose supply to meet the energy needs of brain tissue. The coordinated neuronal activity between astrocytes, microglia and cerebrovascular cells (endothelial and smooth muscle cells) is termed a neurovascular unit [138]. It plays an integral part in neurovascular coupling (NVC); the increase in cerebral blood flow triggered by neuronal activity to meet the energy needs of

the brain [138]. Various inflammatory stimuli or injuries may trigger the expression of adhesion modules and leucocyte activation resulting in the adherence of blood cells to the endothelium [139]. The resultant interaction of activated immune cells and cerebral endothelial cells may induce endothelial injury through cytotoxic release, causing inflammation and increased vascular permeability [139]. As a result, the adherence of activated leukocytes to cerebral endothelial cells may lead to the disruption of NVC. It has previously been demonstrated that there is marked leucocyte-cerebral endothelial cell adhesion mediated by P–selectins in bile duct ligated mice [140]. Therefore, increased adhesive interactions between leucocyte and cerebral endothelial cells triggered by cholestatic liver inflammation may cause NVC dysfunction leading to imbalance in neurovascular regulation and impaired cerebral oxygenation. Duszynski et al evaluated cerebral perfusion and oxygenation with near-infrared spectroscopy (NIRS) in 20 PBC patients showing that PBC patients had cerebral hypoxia with significant impaired cerebral oxygenation, elevated deoxygenated haemoglobin concentration and altered brain activity compared to normal healthy participants [141]. Thus, chronic liver inflammation associated with PBC may impact neurovascular regulation, thereby affecting brain function.

Activation of nuclear factor-kappa-light-chain-enhancer of activated B cells (NF-KB) in cholestasis induced bile duct ligated mice and other liver diseases has been reported [142, 143]. NF-KB is a key transcriptional regulator (controlling cytokine production and cell survival) in the inflammatory pathways. The retention and increased concentration of bile acids may induce NF-KB activation and trigger apoptosis and the inflammatory reaction during cholestasis [142][110]. Although the role of NF-KB in the central nervous system (CNS) is not well understood, there is increasing evidence of a link between neuroinflammation triggered by over activation of NF-KB and cognitive impairment in vivo in neurogenerative diseases [144, 145]. It is plausible that cognitive impairment in cholestasis may be secondary to NF-KB activation in the CNS but studies examining the association between NF-KB and cognitive dysfunction in cholestasis are needed.

1.5.3 Cognitive assessment in PBC patients

1.5.3.1 Subjective measures

There are no guidelines on how to identify which patients with PBC should be screened for cognitive impairment. These symptoms, as well as their impact on PBC patients, need to be explored by the clinician. In addition, studies to date that evaluate cognitive impairment have used different methodologies. Furthermore, there are no standardized diagnostic criteria or tools to assess the severity of cognitive impairment in cholestatic disease. There are 3 general approaches to assessing cognitive symptoms. These are subjective measures (e.g. questionnaires asking about the symptom), objective measures (e.g. neuropsychological tests) and mechanistic measures (e.g. magnetic resonance imaging).

PBC-40 questionnaire

The PBC-40 questionnaire is a robust, evidence-based, patient-derived quality of life (QoL) measure with 6 domains: fatigue, itch, cognitive symptoms, social function, emotional status, and general symptoms. It was developed by Jacoby et al exclusively for the PBC population and validated in 2005 to be used for both clinical and research purposes and it usually takes the patients about 10-20 minutes to complete [146]. It is accepted that the questionnaire reflects symptoms perceived by PBC patients. The cognitive domain in the PBC-40 self-assessment questionnaire has been widely used to assess cognitive impairment in PBC. This domain in the PBC-40 questionnaire contains 6 questions rated 1-5 (Table 1-2). The total cognitive score is obtained by summation of the individual scores from the 6 questions, giving a minimum score of 6. The questionnaire is easy to use, reproducible and relevant to patients' priorities in PBC. However, it does not discriminate between symptoms being related to PBC versus other associated conditions and it does not address the effect of comorbidities. Similar to other subjective questionnaire measures, it may be subject to halo effect, bias, difficulty in grading and fluctuation/inconsistency in scoring.

Table 1-2: PBC-40 cognitive domain scores

	Never	Rarely	Sometimes	Most of the time	Always
Because of PBC I had to make a lot of effort to remember things	1	2	3	4	5
Because of PBC I had difficulty remembering things from one day to the next	1	2	3	4	5
My concentration span was short because of PBC	1	2	3	4	5
Because of PBC, I had difficulty keeping up with conversations	1	2	3	4	5
Because of PBC, I found it difficult to concentrate on anything	1	2	3	4	5
Because of PBC, I found it difficult to remember what I wanted to do	1	2	3	4	5

1.5.3.2 Objective measures

Neuropsychometric measures

Neuropsychological test batteries are commonly used in clinical trials as an objective assessment of the different components of cognitive function. Different neuropsychometric tests have been used in clinical studies to assess cognitive symptoms in PBC. For cognitive assessments of PBC, however, there are no validated neuropsychological tests or computed batteries. Test batteries mostly measure memory, attention, executive function, and visuospatial motor skills. Raw scores from the test battery are compared against normative data from an age and sex-matched population. The interpretation of the results is

usually influenced by a number of variables including the patient's education level, substance use and age. Despite being considered a powerful tool to test cognitive function, they are difficult to access and rarely used in clinical practice.

(A): Wechsler Adult Intelligence Scale

Used in various cognitive and neuropsychiatric studies, the Wechsler Adult Intelligence Scale (WAIS) consists of different subtests to measure cognitive and intellectual abilities. After being developed in 1955, 4 revisions have been released for the WAIS [147, 148]. The shorter version of the WAIS III was used by Newton et al to assess the cognitive ability of PBC patients using the digit span test and a digit symbol search [126]. The digital span test measures short-term or working memory and assesses the ability to recall sequences of numbers [147, 148] .The sequence increases in number if correctly performed with the length of numbers going forward and backward. It also requires storage and manipulation of information to execute the task. In a symbol search test, participants mark one of two symbols in a row of five symbols in order to assess visual perception and information processing speed [147]. The tasks also include organisation of perception, working memory and motor responses. WAIS is readily available, widely used and effective in screening for cognitive impairment but education, intelligence variation and age can influence the results of the tests [147, 148].

(B): Trail-making test

A trail-making test is one of the most common neuropsychological tests used in research studies that evaluate patients with minimal or undiagnosed hepatic encephalopathy [118, 149]. There are two parts that assess visual attention, visual scanning speed, attention, information processing, and mental responses, which are components of executive function. Trail Making Tests have the advantages of being readily available, quick to administer, and sensitive for neuropsychological assessment. However, they can be influenced by educational status, age of the participants and recall.

Mechanistic Measures

Magnetic resonance imaging (MRI)

Magnetic Resonance Imaging (MRI) is non-invasive and generates high resolution images of the body without radiation exposure. It is a useful tool to study CNS changes but not a diagnostic tool for cognitive assessment in research studies. To date, there are few studies using different modalities of neuro MRI imaging in primary biliary cholangitis to explore cognitive symptoms.

Mosher et al used resting state functional MRI (rs-fMRI) to examine the functional connectivity of deep grey matter in PBC patients. They identified a reduction in hippocampal volume on volumetric MRI similar to that observed in neurodegenerative diseases [150-152]. Spontaneous fluctuations in signal intensity have been shown to correlate with spontaneous brain activity or functional connectivity of the brain [153]. Rs-fMRI can be used to evaluate the functional network activity of the brain relevant to the cognitive function of the brain at rest [153]. The advantage of rs-fMRI is that it does not require input from the patient or a requirement to train the subject. This means it is independent of learning or attenuation. In neurodegenerative diseases, this imaging modality has been extensively used to demonstrate functional connectivity of the brain and correlates with diminished cognitive function and mood disturbances [154, 155].

The association between mild cognitive impairment, fatigue and changes in Diffusion Tensor imaging (DTI) measurements has been reported in non- hepatic conditions e.g. Alzheimer's disease [156], traumatic brain injury [157] and chronic fatigue syndrome [158]. Contrast in diffusion-weighted MRI (dw-MRI) is generated by the diffusion of water and DTI (a variant of dw-MRI) and localises diffusion patterns to identify and chart white matter tract integrity in the brain. The axon and myelin sheaths restrict water movement, or diffusion, within the white matter tracts. This causes water molecules to move preferentially along the white matter tracts rather than across them and this is called anisotropic diffusion [159]. The degree of anisotropy increases with myelin formation. Therefore, in diseases with demyelination and axonal injury, the composition and architecture of the nervous system

may change. This affects the restriction of water diffusion, resulting in lower anisotropic measurements. [160]. To measure anisotropic diffusion, fractional anisotropy (FA), which is the difference between the tensor ellipsoid's shape and perfect sphere, is widely used and considered a measure of white matter integrity [160]. DTI has been used increasingly to study brain structural and compositional changes that may be associated with impairments in cognitive function in various neurodegenerative disease [161, 162]. However, data are limited in the PBC population. Studies using T1 weighted MRI and DTI to examine brain changes in PBC patients showed mixed findings. Newton et al reported white matter tract changes suggesting possible structural abnormalities in PBC patients. In contrast, Zenouzi et al found no structural alterations indicating either changes may be variable or differences in patient selection [126, 163]. These conflicting results highlight the need for further studies to better understand the brain changes and their impact on cognitive and fatigue symptoms in PBC patients.

Diffusion weighted MRI imaging with analysis of magnetisation transfer (MT) ratio was used by Hollingsworth et al [164] and Grover et al [57] . Magnetisation transfer effect is derived from continuous exchange of magnetisation between the bound pool (water molecules coupled to intracellular macromolecules) such as myelin and the free pool (free intracellular water) and analyses the shifting of signal between the two compartments [165]. The ratio is affected by the bound pool i.e. concentration of intracellular macromolecules bound to water. If there is accumulation of manganese in the brain, it may have an effect on the magnetisation transfer ratio and therefore it is used as a surrogate marker for myelin integrity of the brain [57]. Grover et al found that there was no association between the self-reported severity of cognitive impairment and the degree of reduction in magnetisation transfer in PBC patients compared to a control group [165].

Although MRI may not be diagnostic for cognitive impairment in PBC, it has shed some light on understanding brain changes in clinical studies. It is increasingly apparent that combined structural and diffusion weighted MRI are effective tools for research into brain changes in PBC populations and may help us to understand the behavioural symptoms of patients.A

summary of the different modalities used for cognitive assessment of PBC patients can be found in Table 1-3.

Table 1-3: A summary of the different modalities of cognitive evaluation used for cognitive assessment of PBC patients.

Study	Participants	Cognitive assessment Questionnaire and Findings	Neuropsychological assessment and findings	Imaging and Findings
Mosher et al [151]	15 non-cirrhotic PBC patients and 17 normal controls	PBC-40 questionnaires Findings: No association between total PBC score with MRI findings.	None	Tesla T1 – weighted MRI, resting-state functional MRI and quantitative susceptibility mapping. Findings: PBC patients exhibited reduced thalamic volume ($p< 0.01$) and reduced anterior insula activity ($p< 0.02$)
Grover et al [57]	13 newly diagnosed biopsy proven PBC pre-cirrhotic patients and 17 healthy volunteers	PBC – 40 questionnaires Findings – no severe cognitive impairment in study participants (mean value of cognitive domain = 15.9 (STD 4.6) compared to the whole PBC population.	None	3T Philips Intera MR system – T1 and T2 weighted MRI and Diffusion Weighted Imaging, Proton Magnetic Resonance Spectroscopy Findings: Cerebral magnetisation transfer ratio significantly

				reduced in thalamus (p<0.0001), putamen (p<0.0001), head of caudate (0.01) and mean diffusion coefficients increased (p <0.01) in thalamus of PBC patients.
Mosher et al [152]	17 non-cirrhotic PBC patients and 17 normal age/sex-matched controls	PBC-40 cognitive domain score – not calculated	None	Tesla T1 weighted MRI and quantitative susceptibility mapping Findings: significantly reduced hippocampal volume (p = 0.023) and increased hippocampal susceptibility in PBC patients (p= 0.048)
Zenouzi et al [163]	20 PBC patients and 20 normal controls		Digit Span, Digit ordering test A (DOT-A), DOT-B, German, Regensburger Wortflussigkeits test Findings: no differences with respect to working memory (digit span, digit	Tesla T1 – weighted MRI, voxel-based morphometry (VBM) and Diffusion Tensor imaging (DTI). Findings: No structural brain abnormalities

			ordering test A, B) and cognitive flexibility between the groups Significant worse verbal fluency in PBC patients ($p < 0.01$) Test battery for attention performance (TAP, PSYTEST, Herogenrath, Germany) Findings: No difference	
Mosher et al [150]	20 non-cirrhotic PBC patients and 21 age/gender matched normal controls.		Findings: Digit span tests for verbal/auditory working memory - significantly lower in PBC group ($p = 0.03$). Corsi Block-Tapping Test for spatial working memory: no difference ($p= 0.45$).	Resting-state-functional MRI (rsfMRI) Findings: increased altered resting state functional connectivity in brain deep grey matter (putamen, thalamus, amygdala, hippocampus) in PBC patients compared to normal controls

			Trial Making Test (A and B) for visual attention and task switching – no difference (p = 0.20)	
Newton et al. [126]	28 early stage PBC patients and 11 matched controls		Trail – making test scores - normal. Full scale IQ test scores – lower in PBC than in sex-matched controls (p=0.05) Verbal fluency and cognitive processing -lower in PBC than controls (p =0.009 and p=0.05, respectively)	Brain Magnetic Resonance Imaging n= 11 PBC patients Findings: lesions in white matter in frontal lobe in all PBC patients

1.5.4 Managing cognitive impairment in PBC patients

There is no treatment currently available that has been shown to improve cognitive impairment in PBC. The pathophysiology of cognitive impairment and systemic symptoms in PBC is complex and remains poorly understood, making it challenging to develop therapy for symptom improvement. There are very few studies on symptom-targeted therapies in PBC and, to date, they show no impact on the systemic symptoms of PBC, except pruritus [166]. The currently licensed anti-cholestatic therapy, ursodeoxycholic acid (UDCA), has not been shown to improve symptoms [166]. OCA is reported to improve short-term memory in bile duct ligated mice but there is no currenlty published human data about the effect of OCA on cognitive symptoms [167].

All patients with cognitive impairment should be assessed with a comprehensive history (description of symptoms, medical, psychiatric history, and medications) and examination (neurological signs and cognitive examination using PBC-40 cognitive questionnaire). It is imperative to rule out the reversible causes of cognitive impairment including thyroid disease, B12 and folate deficiency and diabetes. Neurological imaging, with MRI or computed tomography (CT), should be considered in patients with focal deficits or neurological symptoms, rapidly deteriorating cognitive function or head injury, to exclude structural causes. Neurology referral should be considered if there is diagnostic uncertainty. A full medication history, including over-the-counter medications, and recreational drug use should be obtained to rule out drugs that can impact cognitive function. Medications with sedative effects, such as benzodiazepines and opioids, may contribute to cognitive dysfunction and should be avoided if possible.

Cognitive impairment in cholestasis is closely linked with depression and sleep disturbance [70] and patients should be screened for these conditions. Self-reported daytime somnolence can be assessed using the Epworth Sleepiness Scale (ESS) with referral to the sleep clinic if appropriate. Obstructive sleep apnoea (OSA) is associated with fatigue and cognitive impairment and overnight continuous positive airway pressure (CPAP) ventilation may improve cognitive symptoms [168] but the evidence is still limited. Sleep hygiene should be encouraged, and depression should be identified and treated if present.

To manage cognitive impairment in cholestasis, the main objective is to optimisize the patients' function and general well-being [166]. Patients with cognitive impairment have complex inter-relationships with fatigue, emotional and social dysfunction and this often leads to poor perceived quality of life. It is crucial that patients understand that there are no licensed therapies available for cognitive symptoms at the moment. There should be an open discussion, empathy with patients and empower them to cope with their symptoms. The patient and their family should be educated to understand the scope and impact of symptoms as family and psychological support from social networks is of paramount importance. Patients should be encouraged to maintain social engagement, undertake exercise and use memory games to improve sensorimotor skills. The lack of acknowledgement, and negative perception, of these symptoms by clinicians is a barrier to optimal care. Thus, it is critical to acknowledge the impact of such symptoms on patients.

1.6 Rationale for research

Primary biliary cholangitis (PBC) is a chronic autoimmune disease predominantly seen in women over 40 years old [1]. Cirrhosis and its complications may occur as the disease progresses. In addition to disease risk, many PBC patients have a significant symptom burden leading to reduced quality of life [50]. Symptoms, particularly cognitive symptoms and fatigue, are closely associated with poor quality of life in PBC patients [50]. Although there has been significant recent progress in therapeutics for PBC for the management of cholestasis, the current treatments for PBC (including liver transplantation) do not improve systemic symptoms in the long-term.

Cognitive symptoms in PBC patients have been reported, but their exact prevalence is not clear. Despite discussions and hypotheses of brain changes caused by immune reactions or cholestasis, the underlying pathophysiology of cognitive symptoms remains unclear. Fatigue and cognitive symptoms in PBC are not related to disease severity [166]. In recent studies, brain changes were observed among patients with PBC in the early stages [151]. Importantly, these changes and symptoms do not improve with liver transplantation [58].

This suggests the disease may have started earlier than expected and by the time of liver transplantation, it appears irreversible.

To date, there is no available treatment to improve the systemic symptoms of PBC. The current first line treatment for PBC in terms of improving liver biochemistry, ursodeoxycholic acid (UDCA), is not effective in treating PBC symptoms [166]. A study showed that the second-line therapy, Obeticholic acid, improved short-term memory in bile duct – ligated mice [167]. However, it is given to those who fail to respond to UDCA after a year of therapy meaning it is only given to those with profound cholestasis. Therefore, if we believe the symptoms associated with PBC start at an earlier stage and become irreversible during a long and profound cholestatic stage, therapy as per the guidelines may be too late to reverse them.

To develop future therapeutic trials to improve symptoms, it is crucial to understand the nature of cognitive symptoms in PBC and how they impact the changes in the central nervous system (CNS). Neuropsychiatric batteries are useful tools to objectively assess cognitive function, but the batteries used in previous neurocognitive PBC studies were limited to determine the specific cognitive domains [126, 163]. The CANTAB neuropsychiatric battery has been extensively used for cognitive assessment in several studies in both neurological and mental health disorders [123-125]. It enables researchers to offer precise objective assessment in a wide range of cognitive domains including sustained memory, executive function, planning, attention, and social cognition [126]. Growing evidence indicates that a loss of white matter integrity plays a role in the development of cognitive impairment in Alzheimer's disease and other neurodegenerative diseases [156, 169]. In particular, impaired integrity of frontal and fronto-temporal white matter tracts – such as the forceps minor and uncinate fasciculus – has been linked to memory impairment, and attention /executive function deficits in conditions such as multiple sclerosis patients with depression, fatigue and Alzheimer's disease[169, 170]. Based on these findings, we hypothesize that similar white matter abnormalities may underlie cognitive dysfunction observed in PBC patients.

In spite of the potentially devastating effects of cognitive symptoms in PBC, very little is known about the nature of cognitive symptoms and why some PBC patients never develop these symptoms. Together with neuropsychiatric batteries, neuroimaging techniques allow for a comprehensive assessment of cognitive symptoms in PBC patients and enable better understanding of how these cognitive symptoms correlate with neural changes in the brain. This thesis will uniquely describe cognitive symptoms in PBC patients and investigate the subjective, cognitively symptomatic and asymptomatic PBC patients using the CANTAB neuropsychiatric battery and DTI MRI imaging and also assess the brain changes of PBC patients over different time points of PBC disease.

1.7 Aim of Study

The aim of the study was to define the nature of central nervous system (CNS) changes in cognitively symptomatic and asymptomatic patients. It also assessed brain changes in PBC patients over different time points in the PBC disease spectrum (i.e. AMA positive non cholestatic patients, non-cirrhotic PBC patients, and early cirrhotic PBC patients with no overt hepatic encephalopathy). In future PBC therapeutic trials, these findings may serve as reference endpoints.

1. To define the nature of cognitive symptoms, this study will explore:

- Whether there are differences in the clinical characteristics of PBC patients who are subjectively cognitively symptomatic and asymptomatic according to age, gender, biochemical characteristics and UDCA response status

- Whether there is a difference between the subjectively cognitively symptomatic and asymptomatic PBC patient groups using CANTAB testing
- whether there is a difference between subjectively symptomatic and asymptomatic groups in cerebral DTI measurements

2. To study the cognitive changes in PBC at different stages of disease:

- How does the phenotype of non-cirrhotic PBC patients compare to non-cholestatic AMA positive patients and cirrhotic PBC patients in terms of symptom phenotype, CANTAB test scores and DTI MRI imaging?

Chapter 2: Methodology

2.1 Brief Overview

In this section, I have outlined my personal contributions to the study and the areas where I have been supported by others to complete the Cognitive Primary Biliary Cholangitis (COG-PBC) project. This thesis was written while I was employed as a Clinical Research Associate in the Translational and Clinical Research Institute, Faculty of Medical Sciences, Newcastle University.

The conceptualisation, visualisation and methodology were developed by my supervisor, Professor David Jones. The project was funded by the Newcastle NIHR Biomedical Research Centre (BRC): BH Ref 172901/PDb064. Project funding was obtained from Professor David Jones in December 2017. Patient recruitment was from Freeman Hospital, Newcastle upon Tyne Hospitals NHS Foundation Trust and undertaken by me, Dr Amardeep Khanna and Dr Jessica Dyson. Consent and research visits were performed by me and Sister Kathryn Houghton. Sister Houghton trained me in the administration of the neuropsychological assessments, and helped administer the battery to the patients. I collected clinical and demographic data. Database construction was performed by me, Sister Houghton and Professor Dave Jones.Professor Jones and I collated demographic, questionnaire and CANTAB battery data. MRI data acquisition was performed by the radiographers at Newcastle Magnetic Resonance Centre, with MRI sequence selection, set-up and data analysis performed by Dr Jehill Parikh and Prof Andrew Blamire. Data interpretation was predominantly performed by me, Professor Dave Jones and Dr Jessica Dyson, with Dr Cousins contributing to analysis of cognitive measures and imaging findings.

The study was conducted in accordance with the study protocol and principles and ethical guidelines of the Declaration of Helsinki. Ethical approval was obtained from Northwest – Greater Manchester Central Research Ethics committee; REC reference (18/NW0119). The project was undertaken within Newcastle upon Tyne Hospitals NHS Foundation Trust and local research and development approval was obtained from the Research and Development Office. Each participant's data was pseudoanonymised and recorded using a unique identification number. All study records were kept in a secure, restricted-access site file with only approved members of the research team having access to the data.

2.2 Study Population

All patients in the study were recruited from Newcastle upon Tyne Hospitals NHS Foundation Trust between May 2018 and December 2019. Potential participants were identified and invited from the specialist autoimmune liver disease outpatient clinic. The study was explained to patients interested in participating by a member of the research team and they were provided with the participant information sheet (PIS). Those deciding to participate in the study were invited to a screening visit. Written informed consent was taken from all participants by a trained member of the research team.

2.3 Inclusion criteria

Participants were recruited into 5 pre-determined study groups:

- Group 1: PBC patients with moderate / severe cognitive impairment (i.e., PBC-40 cognitive domain score 16 and above) with no evidence of cirrhosis on transient elastography/liver biopsy within the previous 12 months
- Group 2: PBC patients with no or mild cognitive impairment (i.e., PBC-40 cognitive domain score <16) with no evidence of cirrhosis on elastography/biopsy within the previous 12 months
- Group 3: treatment naïve patients diagnosed with PBC within the previous 12 months
- Group 4: PBC patients with cirrhosis on transient elastography (median liver stiffness > 16.5 kPa) and/or liver biopsy within the previous 12 months
- Group 5: patients positive for AMA or PBC specific antibodies with normal liver blood tests.

A CONSORT diagram for patient recruitment is shown in Figure 2-1

2.4 Exclusion criteria

The exclusion criteria included pregnancy, significant medical conditions or risk behaviours that might affect cognition (such as stroke, dementia, uncontrolled diabetes mellitus (DM), hypertension, excessive alcohol consumption, malignancy, and decompensated cirrhosis i.e., variceal bleeding, hepatic encephalopathy, or ascites within the last 12 months), liver transplant recipients and those with contraindications to MRI (e.g., pacemakers or other implanted medical devices) were also excluded from the study.. Figure 2-2 outlines patients excluded from the study.

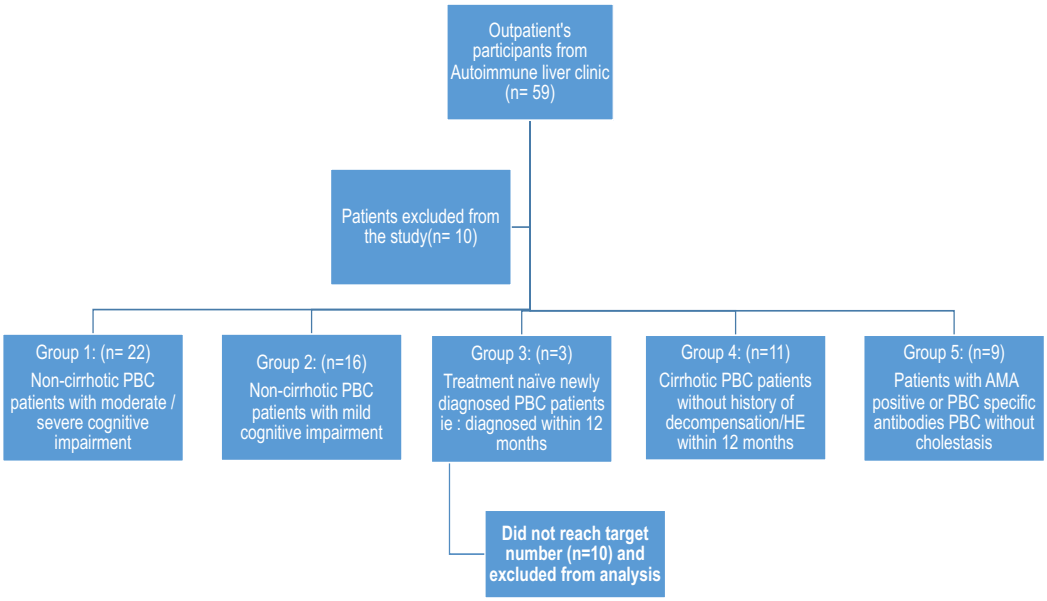

Figure 2-1: CONSORT diagram of recruitment of patients.

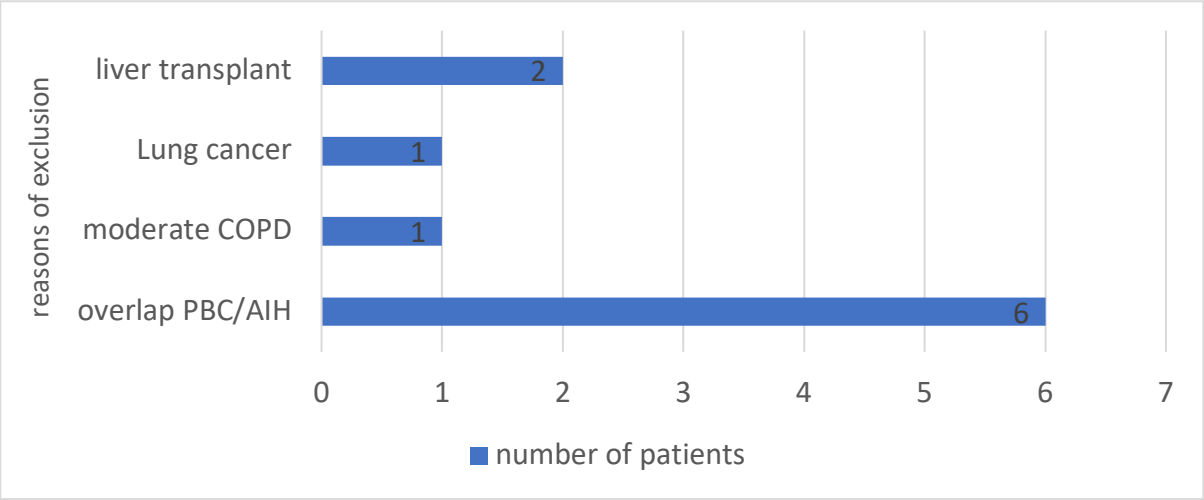

Figure 2-2 : Reasons for exclusion from the study.

2.5 Definition and Data collection

Primary Biliary Cholangitis

The diagnosis of PBC was made if patients fulfilled the European Association for the Study of the Liver (EASL) criteria for the diagnosis of PBC [1] i.e. the presence of two out of three of the following criteria:

1. persistently abnormal cholestatic liver function tests without an alternative explanation
2. presence of PBC specific antibodies (AMA, ANA; anti-sp100, anti-gp210)
3. histological evidence of chronic non-suppurative cholangitis.

Cirrhosis

In this study, cirrhosis was defined as transient elastography (median liver stiffness > 16.5 kPa) or liver biopsy proven diagnosis of cirrhosis.

Biochemical response

The Paris II criteria were used to assess biochemical response to UDCA (Alkaline phosphatase ≥ 1.5 upper limit of normal after 12 months of treatment) [1].

2.6 Demographic and laboratory data

The following demographic characteristics were collected from medical notes and hospital electronic records:

- Age at time of study enrolment
- Age at time of diagnosis
- Gender
- Duration of disease
- Pharmacotherapy
- Other medical conditions: respiratory disease, metabolic disease, renal disease, other autoimmune diseases, thyroid disease.

The following laboratory results were recorded at time of recruitment:

- PBC specific antibodies
- Alkaline phosphatase (IU/L)
- Alanine aminotransferase (IU/L)
- Bilirubin (µmol/L)
- Albumin (g/L)
- Platelet count (10^{*9}/L).

2.7 Data Handling and Record keeping

Each participant's data were recorded using a unique identification number, and study records were kept in a restricted-access site file.

2.8 Assessments

All participants were asked to complete a series of questionnaires for subjective cognitive assessment on the same day as their MRI assessment and CANTAB psychometric assessment.

2.8.1 Questionnaires

All partiticipants were subjectively assessed using the self-reported PBC-40 quality of life assessment tool, Epworth Sleepiness scale (ESS), and Hospital anxiety and depression scores (HADS). Questionnaire completion took approximately 20-30 minutes. Results were recorded and kept in the site file as an Excel Spreadsheet.

PBC-40 is a disease - specific, patient - derived multi-domain quality of life measure [146]. It has 6 domains related to fatigue, cognitive symptoms, itch,social function, emotional status and general symptoms. Each domain is calculated individually with the 40 items being scored 1-5 [146]. The cognitive domain includes 6 questionnaires. Each question uses a 5-point scale (1 = “never”, 2 = “rarely”, 3 = “sometimes”, 4 = “most of the time” and 5 = “always”). PBC-40 domains as per Newton et al are detailed in Table 2-1 [66]. In our study, patients with scores less than 16 points in the cognitive domain were defined as no or mild symptoms (Study Group 2), while those with 16 or more defined as having significant symptoms or moderate to severe symptoms (Study Group 1).

The Hospital Anxiety and Depression Scale (HADS) is a 14- item measurement tool used to assess anxiety and depression [171]. Each item is scored from 0 to 3. Seven items relate to the anxiety domain and the other seven to the depression domain. A score of eleven or greater in each domain inidcate presence of the symptom.

Epworth Sleepiness Scale (ESS) is a short questionnaire used to measure daytime sleepiness. It includes 8 questions with each answer rated from 0 to 3. Possible results range from 0 to 24. Scores below 9 are defined as normal while scores equal to or greater than 9 triggering specialist assessment for further investigation and treatment.

Table 2-1: Defined score ranges for the PBC-40 Domains [66]

PBC-40 domain	None	Mild	Moderate	Severe
Symptoms	<7	8-18	19 – 25	>26
Itch	<3	4-8	9 – 11	>12
Fatigue	<11	12 – 28	29 – 39	>40
Cognitive	<6	7 – 15	16 – 21	>22
Social and emotional symptoms	<13	14 – 34	35 – 49	>50

2.8.2 Psychometric assessment

Study participants underwent detailed and extensive neuropsychological assessment using the CANTAB battery of cognitive assessment tools. CANTAB is a comprehensive test battery that takes about 30-40 minutes to complete. The test application was installed on an iPad (Figure 2-3).The test battery was administered to each patient by trained research staff. The domains of function assessed were working memory, visuospatial learning, attention, and executive function.

The subtests included in the CANTAB battery in this study were:

- One Touch Stockings of Cambridge (OTS)
- Paired Associates Learning (PAL)
- Rapid visual information processing (RVP)
- Spatial Working Memory (SWM)
- Multitasking task (MTT)
- Emotional Recognition Task (ERT)
- Reaction Time (RTI).

Participants were administered seven CANTAB neuropsychological tasks integrated into a single seamless battery. The selected tasks were identified as potentially interesting and pre-specified according to recommendations from Cambridge Cognition Ltd, UK. The raw scores of all tests for each participant were provided in excel format by Cambridge Cognition

Ltd, UK. Each task consists of discrete tests (the first 3 letters of the abbreviation delineating the task and thereafter the test). Some tasks (SWM, PAL, ERT and RVP) included two subtests whilst a single test was used in OTS and RTI. The raw numerical scores for each cognitive subdomain of the participants were generated by the software. The values of the subjects are calculated based on the built-in normative data of the CANTAB battery. A z score < -1.5 standard deviation was considered mild cognitive deficit and z score < - 2 standard deviation as significant cognitive deficit [172].

The description of each task in CANTAB is as follows:

One touch stockings of Cambridge (OTS)

The task assesses planning ability. Subjects were given a number of trials and asked to choose the correct answer. The total number of trials where the correct answers were chosen on the first attempt was calculated and used as an outcome measure.

Paired Associates Learning (PAL)

This test assesses episodic memory. Subjects were tasked with recalling pattern locations within a display matrix in a number of trials. The total number of correct choices on the first attempt was calculated in all trials and used as an outcome measure.

Rapid visual information processing (RVP)

An assessment of sustained attention is provided by this test. The subjects were tasked with detecting strings of 3 digits (target number sequences) appearing in pseudo-random order as quickly as possible. The ratio of a subject's ability to identify target sequences by false alarm was used as a signal detection measure. The metric indicates the subject's sensitivity to detecting target sequences.

Spatial working memory (SWM)

In this test, executive function and working memory (the ability to retain and process spatial information temporarily) are assessed. The subjects were tasked with removing the token hidden in each of a number of boxes. The total number of times when the

subject revisited the box where the token was previously found and eliminated is considered “Between errors” and was used as an outcome measure.

Multitasking task (MTT)

The task assesses multitasking/attention or executive function. An arrow was displayed on either side of the screen (right or left) and can point in either direction (to the right or left). The subjects were tasked to select the right or left button as per the direction of the arrow pointing. The task may include congruent stimuli (an arrow on the right side pointing to the right) or incongruent stimuli (an arrow on the right side pointing to the left).

Emotional Recognition Task (ERT)

To assess emotional recognition, subjects were tasked with choosing an emotion word after a specific emotional facial feature was flashed onto the screen at intervals. The total number and percentage of correct selections were calculated and used as an outcome measure.

Reaction Time (RTI)

The test assesses psychomotor speed and provides measurement of the accuracy of the response. The subjects were tasked with holding the response button and releasing the button and selecting the target stimulus when it appeared on the screen (1 in the simple variant and 5 in the five-choice variant). The outcome measure was calculated across reaction time and movement for both modes in the trials where patients responded correctly.

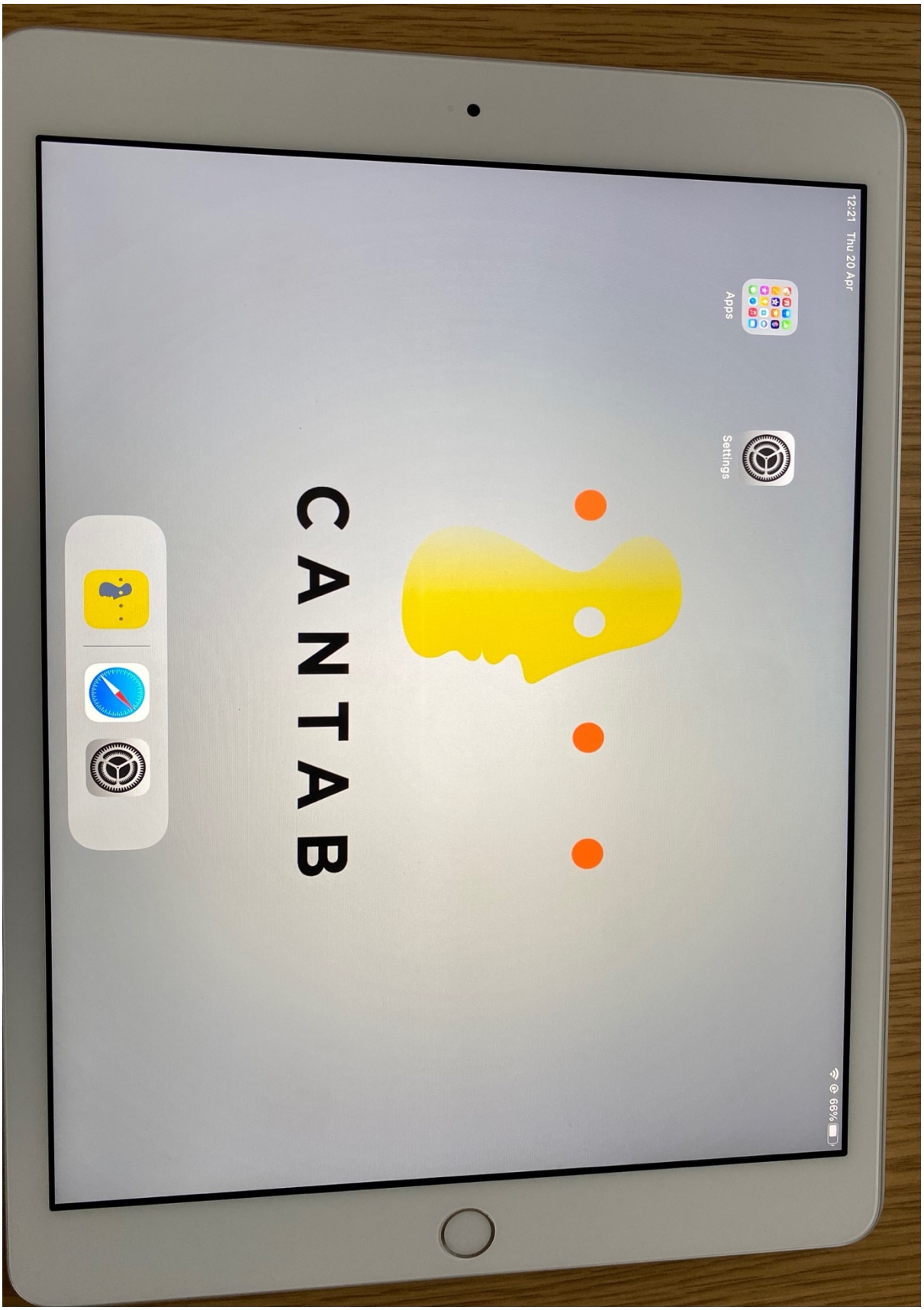

Figure 2-3 : IPAD installed with CANTAB neuropsychiatric battery software.

2.8.3 Mechanistic assessment

Magnetic Resonance Imaging (MRI)

Structural MRI and diffusor tensor imaging (DTI) sequence and analysis were used to obtain white matter fractional anisotropy (FA) values. All MRI data was acquired on a Philips Achieva 3T MRI scanner housed at the Newcastle Magnetic Resonance Centre using a 32 channel Head Coil. Structural images were acquired in a whole-brain 3D MPRAGE scan (magnetisation prepared gradient echo: sagittal acquisition, slice thickness 1.0 mm, in-plane resolution 1.0 x 1.0mm; TR = 8.3 ms, TE= 4.6 ms, flip angle = 8°, SENSE factor = 2). DTI acquisitions were based on a 2D diffusion-weighted, spin-echo, echo planar imaging sequence with 59 slices (TR=6100ms, TE=70ms, flip angle=90°, voxel size= 2.1x2.1mm, slice thickness = 2.1mm, FOV=270x270mm) with diffusion-weighting performed in 64 uniformly distributed directions (diffusion b=1000 s. mm-2) and in 6 acquisitions without diffusion weighting (b=0 s. mm-2) . We also collected an identical image with b=0 s. mm-2 but with the phase encoding direction reversed for distortion correction purposes.

DTI data were processed using FSL (FMRIB Software Library) (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki) (version 6) ,the program to correct susceptibility-induced distortions using the two b=0 s.mm-2 images with opposite phase encoding. The eddy package was then used to correct images for eddy current distortion, movement, and motion-induced signal dropout. FA was then calculated with the digital software, and the TBSS (tract-based spatial statistics) package used to align the FA images together, to create a white matter skeleton of major tracts, and extract FA values for each subject on the white matter skeleton. The images were visually inspected at each stage. FA values from forcep minor, superior longitudinal and inferior longitudinal fasciculus and uncinate fasciculus were extracted (Figure 2-4). The DTI processing and analysis was performed by an experienced MRI physicist (J Parikh) at Newcastle Magnetic Resonance Centre. The principles of diffusion and examples of fractional anisotropy (FA) images are shown in Figure 2-4 and Figure 2-5, respectively.

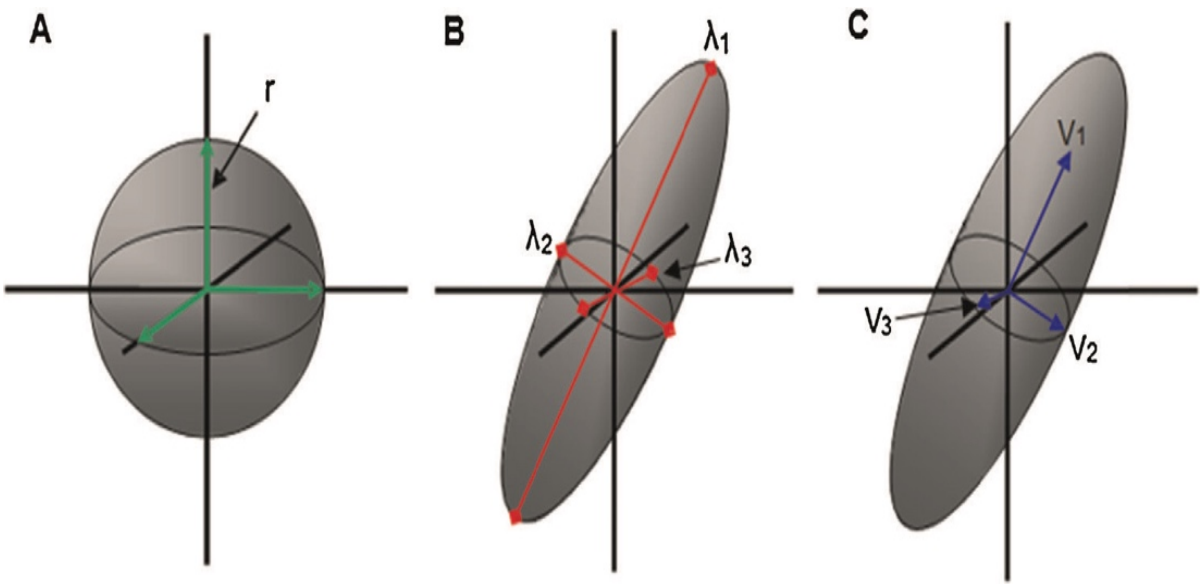

Figure 2-4 : Illustration of a Diffusion Tensor as a three-dimensional ellipsoid; the long axis represents the primary direction of motion. (A) the radius 'r' of the spherical range of motion defines the probability of motion in a given direction, (B and C) Anisotropic diffusion; three eigenvalues (λ_1, λ_2, λ_3) define shape of ellipsoid and three eigenvectors (ν_1,ν_2,ν_3) defines orientation or directions of ellipsoid. [173]

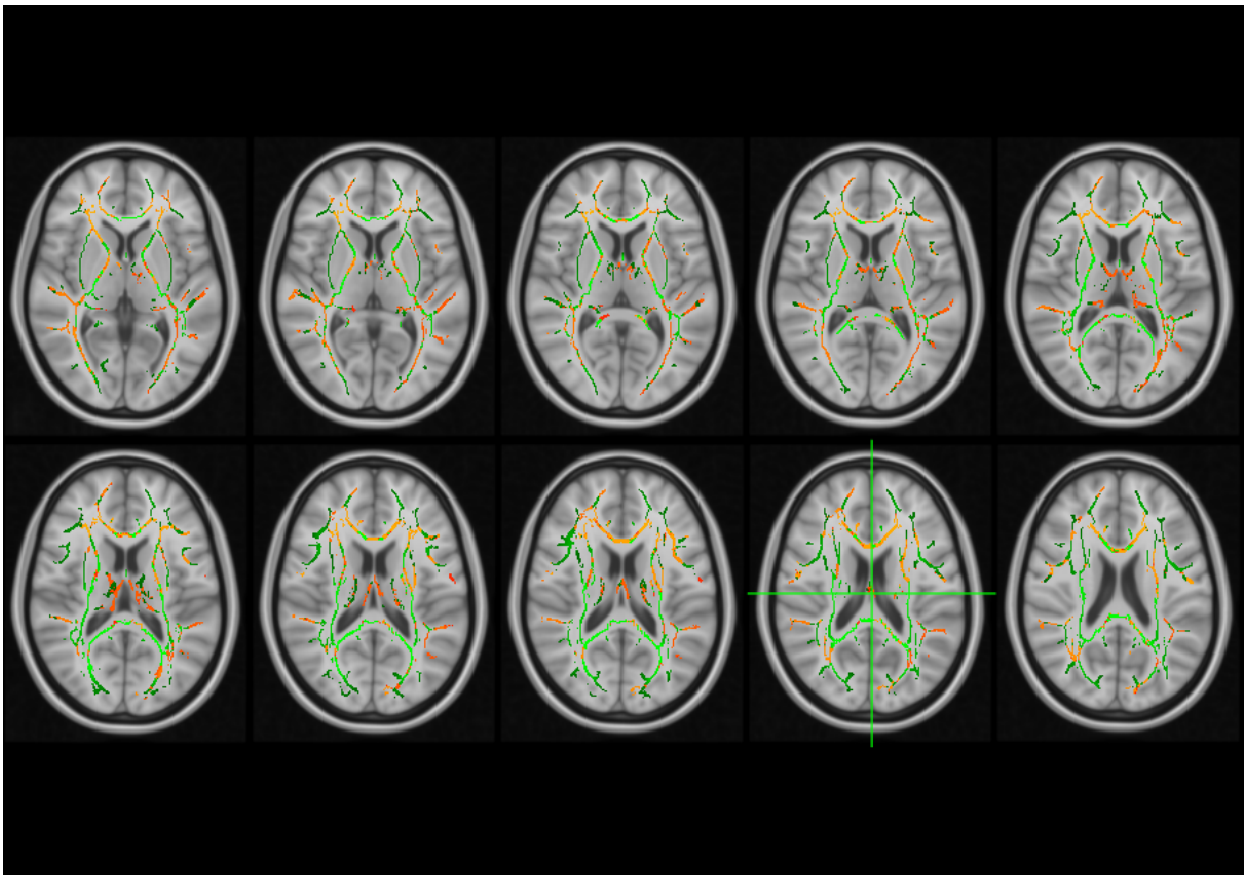

Figure 2-5: Example of fractional anisotropy (FA) image from a patient in the study. DTI using the direction of anisotropy and hence the direction of white matter tracts: green represents anterior-posterior, amber represents transverse and red represents caudo-cranial.

2.9 Statistical analysis

Statistical tests were performed in the IBM SPSS Statistics 21 program. Comparison of continuous variables was done using t-tests for normally distributed data and Man-Whitney U tests for non-normally distributed data. The percentage of differences in different domains in each or among the different groups was analysed using a cross-table test (chi-square). Correlations and assoications of different domains were analysed with Spearman's rank order and Univariate regression. To examine the differences in cognitive performance in each task between study subjects and the normative population, a one-sample z-test was applied with Minitab statistical software. Two-tailed tests were used to determine significance with $p < 0.05$ being deemed to be statistically significant.

Chapter 3: Clinical characteristics and cognitive assessment of non-cirrhotic PBC cohort

3.1 Introduction

This chapter will outline the clinical characteristics, symptom scores and objective neurocognitive symptoms assessed using the CANTAB battery in the different cognitive domains of the non-cirrhotic PBC cohort. The CANTAB battery has been used for cognitive assessment in several medical conditions (dementia, schizophrenia, etc) but to our knowledge this is the first time it has been used in PBC. CANTAB performance will be correlated with the demographic, symptom scores and laboratory data. Normative population data were provided by the CANTAB company. The aim is to characterise the cognitive dysfunction of non-cirrhotic PBC patients. Information about the study population, recruitment and data collection and the details of CANTAB testing were previously described in chapter 2.

3.2 Results

There were 38 PBC patients who did not have cirrhosis in the study. The normative population consisted of 731 sex-matched participants with median aged of 50.

3.2.1 Demographic characteristics of non-cirrhotic PBC patients

The mean age of the whole non-cirrhotic PBC cohort at time of study was 59.9 years and the majority of patients (37 out of 38 patients (97%)) were female. The mean age of patients at time of diagnosis of PBC was 51 years. 35 of 38 patients (92%) had AMA antibodies or PBC specific antinuclear antibodies (ANA) or both. The common medical comorbidities in non-cirrhotic PBC patients were hypertension, hypothyroidism and associated other autoimmune diseases. Approximately 95% of patients i.e. (36 of 38 of patients) were on Ursodeoxycholic acid (UDCA) and 6 of 38 patients (16%) were on second line treatment with Obeticholic acid. The details of the whole cohort, including laboratory data are described in Table 3-1.

Table 3-1: Demographic characteristics of non-cirrhotic PBC patients.

Demographics	**Total PBC without cirrhosis (n = 38)**
Age in years, Mean (SD)	59.9 (8.8)
Female gender, n (%)	37 (97)
Age at Diagnosis in years, Mean (SD)	51 (7.7)
Disease Duration in years, Mean (SD)	8.1 (6.6)
Diagnostic criteria	
AMA antibody positive, n (%)	35 (92%)
Liver histology, n (%)	10 (26%)
PBC specific anti-nuclear antibodies (%)	13 (40.5%)
Comorbidity	
Hypertension	6 (15.8%)
Type 2 Diabetes	3 (8%)
Hypothyroidism	5 (13%)
Depression	5 (13%)
Autoimmune diseases • Pernicious anaemia (1) • Psoriatic arthritis (1) • CREST (1) • Rheumatoid (1) • Sjogren syndrome (1)	5 (13%)
Respiratory disease • COPD (3) • Interstitial lung disease (1) • Bronchiectasis (1)	5 (13%)
Treatment	
UDCA Treatment, n (%)	36 (95%)
UDCA response rate, n (%)	28 (73%)
Obeticholic acid treatment, n (%)	6 (16%)
Laboratory parameters	
ALP Median (range) U/L	126 (52 -368)
ALT Median (range) IU/L	25.5 (10-76)
Bilirubin Median (range) μmol/L	6 (3-21)
Platelets Median(range) 10^9/L	280 (167 -468)
Albumin Median (range) g/L	44 (35-50)
SD,standard deviation;n,number;AMA,anti-mitochondrial antibodies;COPD,chronic obstructive airway disease;UDCA,Ursodeoxycholic acid;ALP, alkaline phosphatase; ALT,Alanine transaminase;CREST,calcinosisRaynaud, oesophageal dysfunction, sclerodactyly, telangiectasia.	

3.2.2 PBC-40 quality of life measure assessment scores

The number of non-cirrhotic PBC patients in the cohort according to the symptom severity of PBC–40 quality of life domains is shown in Figure 3-1. The mean scores in the non-cirrhotic PBC group for the PBC-40 domains were 16.61 ± 4.65 (possible range 7-35) for general symptom , 14.26± 6.37 (possible range 6-30) for cognitive, 5.97± 3.7 (possible range 3-15) for itch, 21.26± 9.24 (possible range 10-50) for social, 6.92± 3.31 (possible range 3-15) for emotional and 28.92± 11.07 (possible range 11-55) for fatigue.

3.2.3 Hospital Anxiety and depression scores (HADS) and Epworth sleepiness scores (ESS)

The mean hospital anxiety and depression scores of non-cirrhotic PBC patients were 5.53±4.7 and 5.29±3.9, respectively. Of the thirty-eight PBC non-cirrhotic patients, 16% (n=6) and 13 % (n=5) were identified to have anxiety and depression using HAD-A and HAD-D scores, respectively. For ESS diagnostic scores, the mean and standard deviation of the patients was 7.39±4.5 with 10 patients (26%) having possible daytime hypersomnolence.

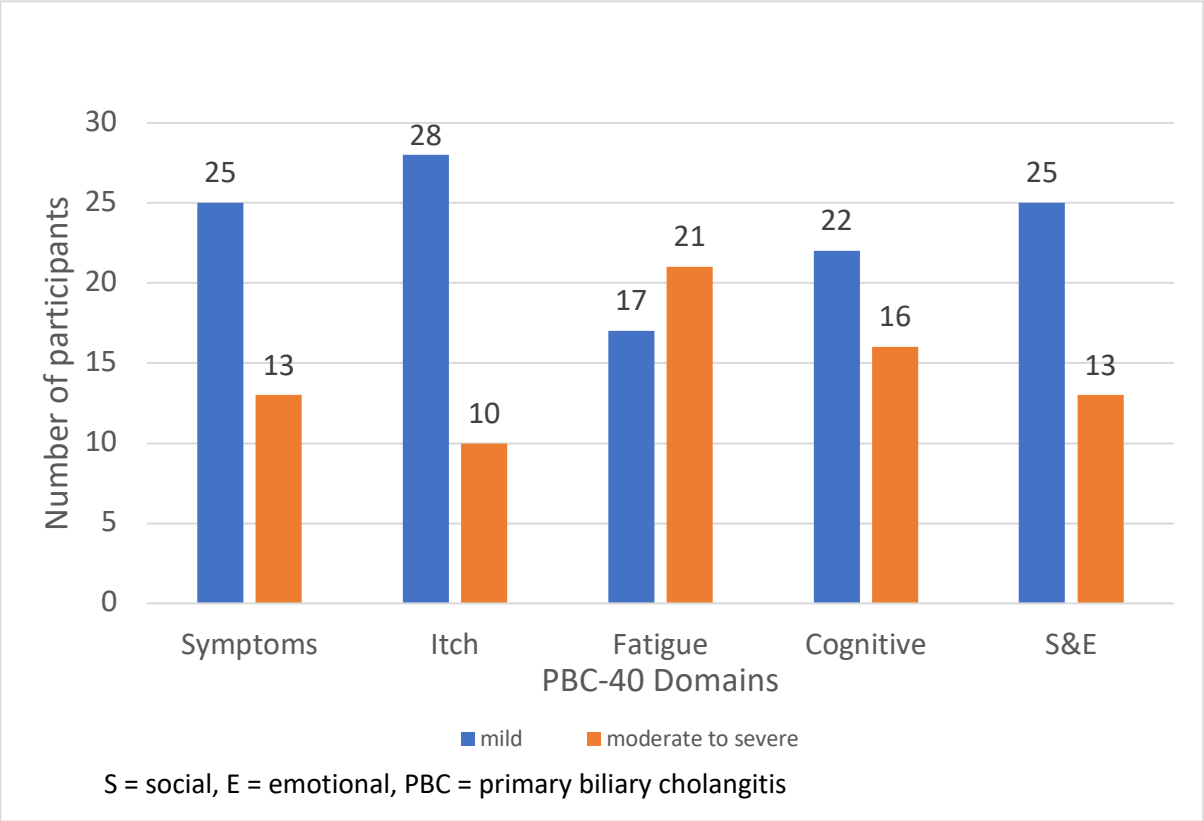

Figure 3-1 : 5 domains the PBC -40 questionnaire scores of non-cirrhotic PBC patients.

3.2.4 Neuropsychiatric assessment of non-cirrhotic PBC patients with CANTAB battery

3.2.4.1 Episodic memory

Paired Associates Learning (PAL) assesses episodic memory in the CANTAB battery. Of the 38 non-cirrhotic PBC patients, 36.9% (n= 14) and 42.1% (n = 16) demonstrated cognitive deficit in PALFAMS (PAL first attempt memory scores) and PALTEA (PAL total error) tests, respectively, suggesting that approximately a third of the patients in the non-cirrhotic PBC cohort had significant deficit in the memory cognitive domain of the CANTAB (Figure 3-2). Patients in the non-cirrhotic PBC cohort made more errors (PALTEA; $p = 0.001$) and had a lower number of correct trials on their first attempt (PALFAMS; $p = 0.001$),as compared to the normative population reference data in the Paired Associates Learning (PAL) test. There was no correlation between the performance of Paired Associates Learning (PAL) and the age of the patients, biochemical markers or UDCA treatment (Table 3-2).

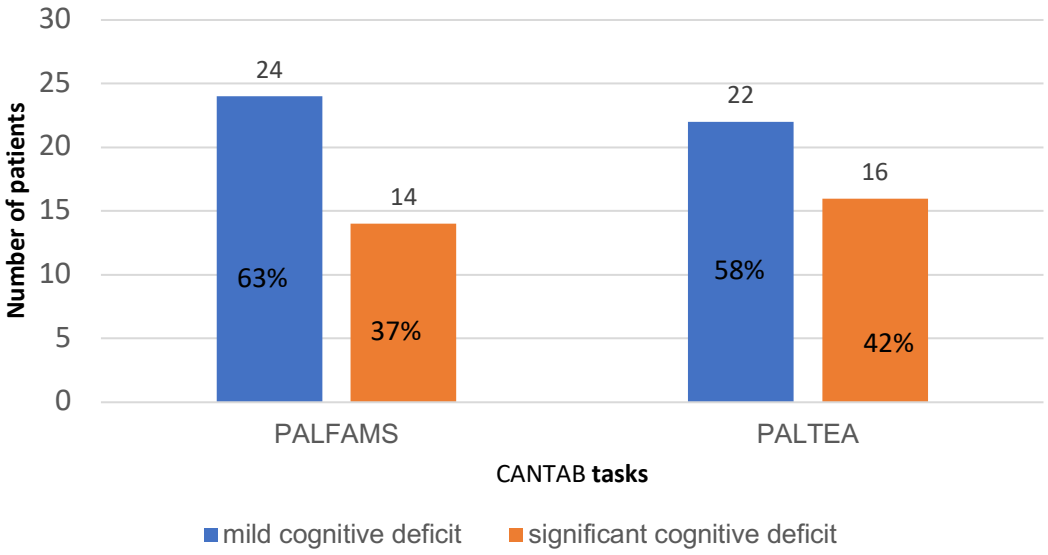

Figure 3-2 : Cognitive performance of CANTAB memory tests (PAL) in non-cirrhotic PBC patients

Table 3-2: Correlation between memory tasks and age, biochemistry and UDCA treatment in non-cirrhotic PBC patients

Memory function tasks	Age		Bilirubin		Albumin		UDCA		ALP	
	Pvalue	R^2	Pvalue	R^2	pvalue	R^2	pvalue	R^2	Pvalue	R^2
PALFAMS	0.14	0.05	0.67	0.02	0.69	0.00	0.10	0.04	0.1	0.00
PALTEA	0.07	0.08	0.61	0.72	0.97	0.00	0.06	0.00	0.09	0.00

UDCA,ursodeoxycholic acid; PALFAM,paired associate learning first attempt memory scores; PALTEA,PAL total error;ALP,alkaline phosphatase.

3.2.4.2 Executive function

Executive function involves high-level planning, implementation, and decision- making. The executive function tasks in CANTAB performed in our study were Multitasking Task (MTT) [i.e., cognitive inhibition], One-Touch Stocking of Cambridge (OTS) [i.e., assessment of planning] and Spatial Working Memory (SWM) [i.e., assessment of retention and manipulation of visuospatial information].

Of the 38 non-cirrhotic PBC patients, a small proportion (13.5% (n = 5)) had cognitive deficits in both MTT tests; MTTLMTMD (MTT Response Latency median) and MTTLCMD (MTT median congruent) tasks (Figure 3-3). A third of the patients (31.6% (n = 12)) demonstrated significant deficit in the OTS task, whilst 35% (n = 10) and 10.5% (n=4) showed significant cognitive deficit in the SWM tests; SWMBE and SWMS, respectively (Figure 3-3).

There was no significant difference in either subtasks of the multitasking test (MTT) between the non-cirrhotic PBC cohort and normative data (MTTLCMD; p = 0.42 and MTTLMTMD; p = 0.125) and this was therefore removed from further analysis. However, poor performance was observed in non-cirrhotic PBC patients in the One Touch Stockings of Cambridge (OTS) test (8.84±3.63 vs 10.82±2.7, p= 0.001). Non-cirrhotic PBC patients also underperformed in Spatial Working Memory (SWM) domain subtests; SWMBE (between errors); p = 0.000 and SWMS (strategy), p= 0.001. These findings suggest some components of executive function are impaired in non-cirrhotic PBC patients as compared to the normative population. In contrast to episodic memory, some correlation was found between patient age and executive function (OTS; p = 0.01, R^2 = - 0.17, SWMS; p= 0.03, R^2 = 0.12, SWMBE; p = 0.004, R^2 = 0.21). When analysis was restricted to those aged 60 and younger, no deficit in executive domain was observed between patients and the normative population. There was no correlation between executive tasks and biochemical markers, depression scores or UDCA treatment (Table 3-3).

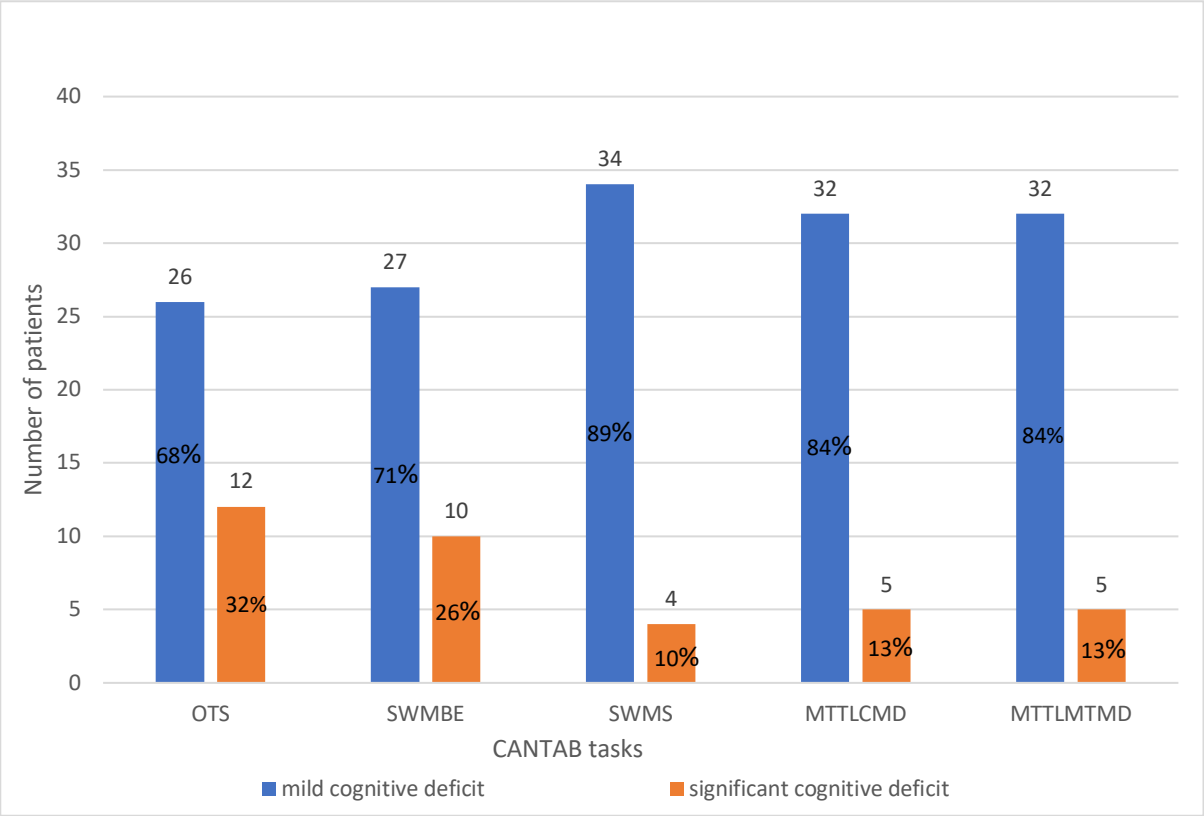

Figure 3-3 : Cognitive performance in CANTAB executive tests of PBC patients

Table 3-3: Correlation between biochemical markers (bilirubin and albumin) ,age, HAD-D scores, UDCA treatment and CANTAB tasks.

Executive function tasks	Age	Bilirubin	Albumin	HAD-D	UDCA	ALP
OTS Pvalue (R^2)	0.009*(-0.17)	0.22(0.04)	0.87(0.00)	0.22(0.04)	0.97(0.05)	0.75(0.01)
SWMS	0.03 *(0.12)	0.29(-0.17)	0.22(0.04)	0.84(0.00)	0.12(0.00)	0.44(0.00)
SWMBE	0.004 *(0.21)	0.78(0.00)	0.11(0.06)	0.38(0.01)	0.11(0.04)	0.46(0.01)
OTS,One-touch stockings of Cambridge; SWM,spatial working memory; SWMBE,SWM between error; SWMS, SWM strategy;HADS-D,Hospital Anxiety and Depression -Depression score;UDCA,ursodeoxycholic acid; ALP,alkaline phosphatase; * = the mean between the group differed significantly;						

Table 3-4: Executive Cognitive performance of non-cirrhotic PBC patients aged less than 60

Test		Normative population mean± SD (A)	PBC Patient aged ≥ 60 (n=19) mean± SD (B)	PBC Patient aged <60 (n=19) mean± SD (C)	P value
OTS (+)		10.82 ± 2.7	7.37 ± 3.4	10.16 ± 3.3	A vs C = 0.3 A vs B = 0.001*
SWM	SWMBE (-)	9.9 ± 9.2	22.26 ± 7.1	12.78 ± 8.2	A vs C =0.18 A vs B = 0.001*
	SWMS (-)	7.03 ± 2.8	10.42 ± 0.9	7.6 ± 2.8	A vs C = 0.37 A vs B = 0.001*

PBC,primary biliary cholangitis;OTS,One-touch stockings of Cambridge; SWM,spatial working memory; SWMBE,SWM between error; SWMS,SWM strategy;(+) = higher scores indicate better performance; (-) = lower scores indicate better performance, * = the mean between the group differed significantly.

3.2.4.3 Emotional cognition

Emotional cognitive function was assessed by the emotional recognition task (ERT). In the non-cirrhotic PBC patients, 44.8% (17/38) and 26.4% (10/38) had significant cognitive deficits in both the emotional recognition task overall median reaction time (ERTOMDRT) and emotional recognition task total hits (ERTTH), respectively (Figure 3-4). ERT performance in the non-cirrhotic PBC cohort showed a lower number of hits (ERTTH; p = 0.001) and a slower response time (ERTOMDRT; p = 0.001). There was no association between the PBC-40 emotional cognitive domain score or anxiety scores with either ERT test (Table 3-5). There was a degree of correlation between total hits (ERTTH) and depression (HADS-D), but no association was seen between time to response and depression scores (Table 3-5).

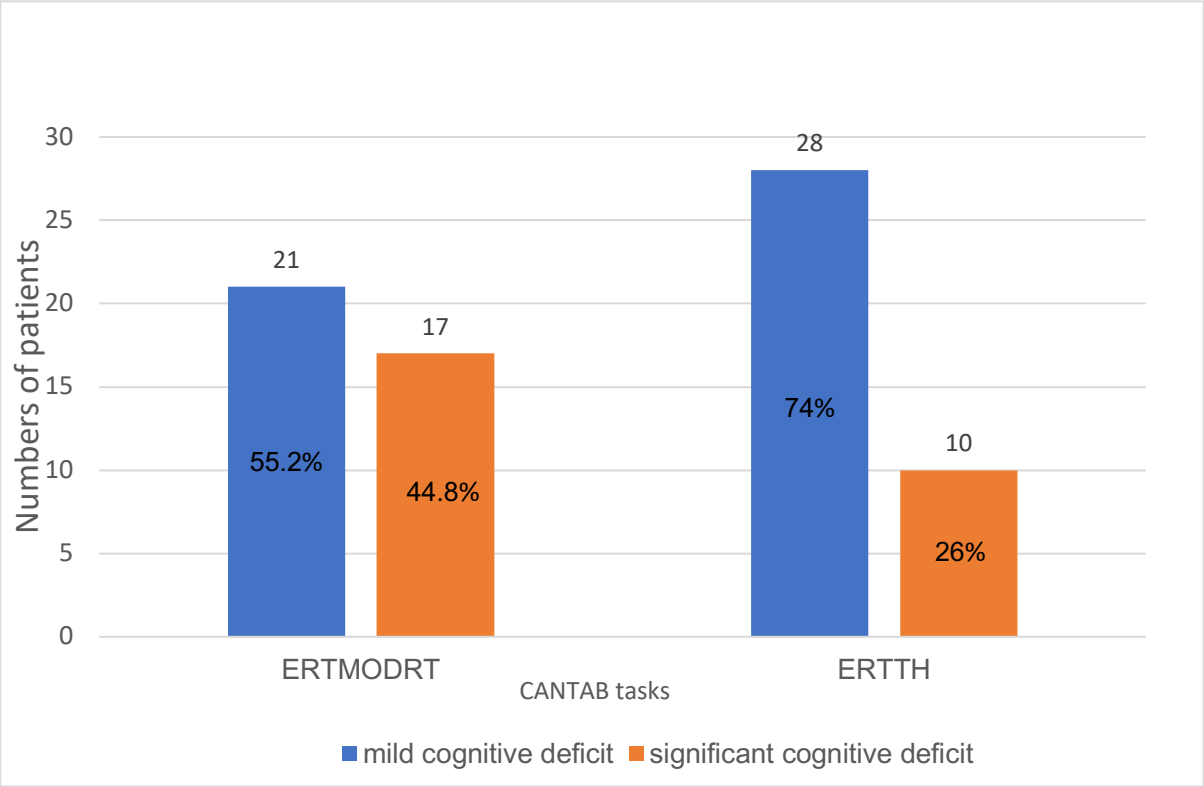

Figure 3-4 : Cognitive Performance of CANTAB emotional cognitive tests in PBC patients.

Table 3-5: Correlation between PBC - 40 emotional domain scores, HAD-D, HAD-A and ERT tasks.

ERT	PBC-40 emotional		HAD-A		HAD-D	
	P value	R^2	P value	R^2	P value	R^2
ERTTH	0.17	0.04	0.27	0.03	0.02*	0.15
ERTOMDRT	0.8	0.17	0.96	0.00	0.63	0.00
PBC,primary biliary cholangitis;HADS-D,Hospital Anxiety and Depression-Depression score;HADS-A,Hospital Anxiety and Depression-Anxiety score; ERTMODRT,ERT overall median reaction time;ERTTH,ERT total hits; * = the mean between the group differed significantly.						

3.2.4.4 Attention and psychomotor speed

In the CANTAB battery, the reaction time test (RTI) was used to test psychomotor speed. Only 5.2% (2/38) of the non-cirrhotic PBC patients had a significant deficit. In fact, the patients had significantly quicker reponse times than the normative population (401.28±52.15 vs 424±59.77, p = 0.017).

In the rapid visual information processing (RVP) task, 13.2% (5/38) and 7.9% (3/38) of non-cirrhotic PBC patients had significant deficits in sensitivity to correct responses to RVP (RVPA) and time to respond to stimuli (RVP median response latency, RVPMDL), respectively. Non-cirrhotic PBC patients had a lower number of correct responses on RVPA (p = 0.001), with slower response to stimuli (RVPMDL; p = 0.03) compared to the normative population. There was no association between CANTAB performance of attention task with age, biochemical markers or response to UDCA treatment (Table 3-6).

Table 3-6: Correlation between CANTAB attention and psychomotor speed and age/bilirubin, ALP and UDCA treatment.

Memory function tasks	**Age**		**Bilirubin**		**Albumin**		**UDCA**		**ALP**	
	Pvalue	R^2	Pvalue	R^2	pvalue	R^2	pvalue	R^2	Pvalue	R^2
RVPA'	0.32	0.02	0.92	0.02	0.94	0.00	0.65	0.00	0.86	0.17
RVPMADL	0.29	0.02	0.98	0.00	0.60	-0.0	0.58	0.00	0.26	-0.1
UDCA,ursodeoxycholic acid; ALP, alkaline phosphatase; RVP;rapid visual processing; RVPA,RVP A';RVPMDL,RVP Median response latency.										

3.3 Summary

The findings in this chapter demonstrate objective evidence of impaired cognitive function in non-cirrhotic PBC patients as compared to normative population data. There were 7 neurocognitive tests (PAL, OTS, MTT, SWM, ERT, RTI and RVP) used to assess learning memory, executive function, emotional recognition, attention and psychomotor speed.

Poor cognitive performance was observed in five out of the seven tasks (PAL, OTS, SWM, ERT and RVP) in non-cirrhotic PBC patients (Table 3-7). Whilst age-related cognitive impairment, such as dementia, is common over the age of 65, the mean age of our cohort was 59.9 years which suggests it is unlikely that all our positive findings can be explained by undiagnosed dementia. Among those five tasks, the most pronounced cognitive deficit was observed in memory cognitive tests (PAL) with a third of the non-cirrhotic PBC patients demonstrating significantly poor performance. Deficit in performance of memory tasks in PBC patients did not appear to be age-related, associated with biochemical markers or disease severity and was independent of UDCA treatment. The finding of a deficit in episodic memory is interesting as learning or episodic memory is believed to be closely linked to the temporo-hippocampal region [174, 175]. Previous MRI studies demonstrated structural changes in the hippocampus in PBC patients and increased altered resting state functional connectivity in brain deep grey matter [150-152]. Therefore, memory deficits in PBC patients may be associated with hippocampal changes in the brain. MRI imaging on PBC patients in future studies may shed light on the anatomical abnormalities that support this finding on CANTAB testing.

We found that not all components of executive function in non-cirrhotic PBC patients were impaired, with deficits shown in the One Touch Stockings (OTS) and Spatial Working Memory (SWM) tests but not multitasking test. The evaluation of executive function is complex as it may be affected by other factors. For example, working memory may decline with age [176]. In our study, we observed a significant association between increased age and declining performance on executive function tests in non-cirrhotic PBC patients. This was only seen in patients aged above 60 years. Further study to compare non-cirrhotic PBC patients with an age-matched normal control group would be valuable. Another

limitation when assessing executive performance is the lack of adjustment for intelligence testing or the level of patient education. This should also be explored in future studies. As seen with memory testing, executive function test was not affected by UDCA treatment or disease severity.

Emotional recognition is the ability to identify facial expressions and respond to emotional stimuli. Emotional recognition is a growing area of research in understanding mechanisms for processing mood-related information. PBC patients have been shown to have social and emotional dysfunction when assessed using the PBC-40 [61, 66]. Prior to this study, the emotional cognitive symptom domain has not been assessed using CANTAB battery in the PBC population. We demonstrated that PBC patients performed poorly in response to emotional stimuli with a quarter of patients underperforming in the accuracy test (ability to recognise the emotional stimulus). The association of inability to recognise the displays of facial emotions and impairment in emotional cognition is well recognized in mood disorders [177] and alcohol use disorder (AUD) [178]. In our study, we excluded patients with significant alcohol intake to avoid confounding factors. It has previously been reported that significant cognitive symptoms are closely linked to depression and anxiety in PBC patients [61]. The mechanism underlying this interrelationship is unknown, although hypothesised to be centrally driven. We observed an association between depression and ability to recognise emotional stimuli in PBC patients. Therefore, a close relationship between depression and cognitive function in PBC might explain the poor performance in the emotional cognitive domain in the CANTAB test.

Psychomotor speed of PBC patients was better than the normative reference population when only a direct motor response to a visual stimulus. However, tasks where there was a processing step between a visual stimulus and motor response (attention task) were consistently worse than normative population reference data suggesting the deficit in PBC patients' performance was due to information processing rather than abnormal speed of motor response.

In conclusion, we have demonstrated multi-domain cognitive dysfunction in non-cirrhotic PBC patients, affecting memory, some components of executive function, information processing, emotion and attention. The deficits were more pronounced in the memory and emotional domains and some of that impairment may be age-related or as a consequence of disrupted memory and attention. UDCA treatment did not impact cognitive function in our study. An improved understanding of cognitive function in PBC is important as the individual pattern of cognitive symptoms may correlate with brain structural or functional integrity. Clinically, this may help future therapeutic trials for PBC associated symptoms.

Table 3-7: Summary of CANTAB results for non-cirrhotic PBC patients.

Test subset		Non-cirrhotic PBC cohort (n=38) Mean ± SD	Normative cohort (n=731) Mean ± SD	P value
PAL	**PALFAMS (+)**	9.89 ± 3.9	13.32 ± 4.02	0.001*
	PALTEA (-)	25.21 ± 14.22	13.89 ± 12.17	0.001*
OTS	**OTSPSFC (+)**	8.84 ± 3.63	10.82 ± 2.70	0.001*
SWM	**SWMBE (-)**	17.65 ± 8.97	9.90 ± 9.23	0.001*
	SWMS (-)	9.05 ± 2.5	7.03 ± 2.77	0.001*
MTL	**MTTLCMD (-)**	703.43 ± 128.23	689.10 ± 109.39	0.42
	MTTLMTMD (-)	950.62 ± 193.82	909.62 ± 162.96	0.12
ERT	**ERTOMDRT (-)**	1579.89 ± 347.56	1267 ± 195.28	0.001*
	ERTTH (+)	50.5 ± 7.62	56.8 ± 6.84	0.001*
RTI	**RTIFMDRT (-)**	401.28 ± 52.15	424.34 ± 59.77	0.01 *
RVP	**RVPA (+)**	0.86 ± 0.056	0.91 ± 0.07	0.001*
	RVPMDL (-)	530.28 ± 175.73	483.88 ± 133.69	0.03 *

NP,normative population; PAL,Paired Associate Learning; PALFAM, PAL first attempt memory scores; PALTEA,PAL total error; OTS,One-touch stockings of Cambridge; SWM,spatial working memory; SWMBE,SWM between errors; SWMS,SWM strategy;ERT,Emotion Recognition Task; ERTMODRT,ERT overall median reaction time; ERTTH,ERT total hits;RVP,rapid visual processing; RVPA,RVP A'; RVPMDL,RVP Median response latency; (+) = higher scores indicate better performance; (-) = lower scores indicate better performance. *= the mean between the groups differed significantly.

Chapter 4: Symptom Assessment according to cognitive symptom severity in non-cirrhotic PBC patients

4.1 Introduction

This chapter examines the characteristics of non-cirrhotic primary biliary cholangitis (PBC) patients in relation to the severity of self-reported cognitive symptoms. Patients were stratified into two groups based on symptom severity: those with moderate to severe cognitive symptoms and those with mild or no symptoms. A score of 16 or higher on the cognitive domain of the PBC–40 questionnaire was used to define moderate to severe cognitive impairment, indicating clinically significant cognitive dysfunction.We explored demographics, laboratory results and subjective assessment tool scores (PBC-40, HADS and ESS). Detailed information about the study population, recruitment, data collection and questionnaires was described in Chapter 2.

4.2 Results

There were 16 non-cirrhotic PBC patients with clinically significant (moderate or severe) cognitive symptoms and 22 with mild or no cognitive symptoms.

4.2.1 Demographic and clinical characteristics of non-cirrhotic PBC patients according to severity of cognitive symptoms

PBC patients with reported clinically significant cognitive symptoms were younger (56 versus 61.5 years, p= 0.03) with 97% of this cohort being female. Moderate or severe cognitively symptomatic patients were diagnosed with PBC at a younger age with shorter median disease duration (4.5 vs 8 years) but neither of these were statistically significant (p=0.06 and p=0.40, respectively). All patients with significant cognitive symptoms and 86% in those with mild cognitive symptoms had positive AMA antibodies. Additionally, approximately 50% of all patients had other PBC-specific antibodies (anti-centromere or multiple nuclear dot ANA). Demographics and clinical characteristics are summarised in Table 4-1.

Depression was the most common comorbidity with 29% (n= 11/38), with 7 out 11 in the group with reported significant cognitive symptoms compared to cognitively mild or asymptomatic group (p= 0.14). Other comorbidities and associated autoimmune diseases are detailed in Table 4-2. Patients with significant respiratory disease with chronic hypoxemia or severe renal disease (eGFR <40) were excluded from the study.

There was no significant difference in biochemical parameters (alanine transaminase, alkaline phosphatase, albumin) according to symptom severity (Table 4-3). Most patients in the non-cirrhotic PBC cohort were on UDCA with 78% being on an appropriate dose (13-15 mg/kg/day). The reasons for sub-therapeutic dosing were not examined in this study due to a lack of available data.Approximately two thirds of patients were complete responders to UDCA. There was no difference in UDCA treatment response rate according to symptom severity (p = 0.52). A fifth of the non-cirrhotic PBC cohort were on second line treatment with Obeticholic acid but statistical analyses were not performed due to small sample size.

Table 4-1: Demographic characteristic of non-cirrhotic PBC patients according to severity of cognitive symptoms

	Total PBC patients (n = 38)	Moderate to severe cognitively symptomatic PBC (n =16)	Cognitively mild or asymptomatic PBC (n = 22)	P value
Age in years, mean (SD)	59.9 (8.8)	56.5 (8.4)	62.5(8.4)	0.03*
Female gender (number and %)	37 (97%)	16 (100%)	21 (95.5%)	NA
Age at diagnosis, mean (SD)	51.8 (7.7)	49 (7.6)	53.8 (7.4)	0.06
Disease duration, mean (SD)	8.1 (6.6)	7.4 (7.3)	8.6 (6.1)	0.404
AMA antibodies positive, n (%)	35 (92%)	16 (100%)	19 (86.3%)	NA
PBC specific anti-nuclear antibodies, n (%)	13 (40.5%)	8 (50 %)	5 (22%)	NA
UDCA Treatment n (%)	36 (95%)	15 (94%)	21 (95.5%)	0.67
UDCA recommended dose n (%)	30 (78%)	14 (87%)	16 (72%)	0.2
UDCA response rate n (%)	28 (73%)	11 (69%)	17 (77%)	0.52
Obeticholic acid n (%)	6 (16%)	4 (25%)	2 (9%)	
SD,standard deviation;UDCA,Ursodeoxycholic acid;AMA ,anti-mitochondrial antibodies;PBC, primary biliary cholangitis;* = the mean between the groups differed significantly				

Table 4-2: Co-morbid conditions in non-cirrhotic PBC patients according to severity of cognitive symptoms

	Total PBC patients (n = 38)	Moderate to severe cognitively symptomatic PBC (n =16)	Cognitively mild or asymptomatic PBC (n = 22)
Hypertension	6 (15.8%)	3	3
Diabetes	3 (8%)	2	1
Hypothyroid	5 (13%)	3	2
Depression	11 (29%)	7 (43%)	4 (18%)
Autoimmune diseases	5 (13%)	3	2
Cutaneous SLE		-	-
Pernicious anaemia	1	1	-
Psoriatic arthritis	1	1	-
CREST	1	1	-
Rheumatoid	1	-	1
Sjogren syndrome	1	-	1
Renal disease	1 (2.6%)	0	0
Respiratory disease	5 (13%)	4	1
Mild COPD	3	3	-
Interstitial lung disease	1	1	-
Mild bronchiectasis	1	-	1

PBC ,primary biliary cholangitis; SLE , systemic lupus erythematosus;CREST , Calcinosis, Raynaud's phenomenon, Oesophageal dysmotility, Sclerodactyly, Telangiectasia;COPD,chronic obstructive pulmonary disease.

Table 4-3: Laboratory characteristics of non-cirrhotic PBC patients according to severity of cognitive symptoms

	Total non-cirrhotic PBC patients (n = 38)	**Moderate to severe cognitively symptomatic PBC (n =16)**	**Cognitively mild or asymptomatic PBC (n = 22)**	**P value**
ALP median (range)	126 (52 -368)	150.50 (52 – 323)	116 (64-368)	0.326
ALT median (range)	25.5 (10-76)	21.50 (12 - 72)	27 (10 – 76)	0.45
Bilirubin median (range)	6 (3-21)	7 (4 – 16)	6 (3 – 21)	0.01*
Platelets median (range)	280 (167 -468)	279.5 (179-371)	280(167-468)	0.51
Albumin median (range)	44 (35-50)	43.50 (41-50)	44 (35 -47)	0.827

PBC,primary biliary cholangitis;ALT, Alanine Aminotransferase; ALP, alkaline phosphatase;* = the mean between the groups differed significantly

4.2.2 PBC-40 quality of life measure assessment tool scores

Patients with significant cognitive symptoms had higher fatigue scores than those with mild or no cognitive symptoms ($p < 0.001$). All 16 patients with significant cognitive symptoms experienced moderate to severe fatigue with a statistically significant correlation between cognitive impairment and fatigue severity ($p = 0.001$, $R^2 = 0.69$). Patients with significant cognitive symptoms also had higher social ($p < 0.001$), emotional ($p < 0.001$) and PBC-40 general symptoms scores ($p < 0.001$) than those with mild or no cognitive symptoms Table 4-4. No difference was seen for itch although this was one of the commonest symptoms reported ($p = 0.08$). The severity of cognitive symptoms and fatigue were significantly correlated with the other PBC-40 domains (Tables 4-4 and 4-5). The correlation between cognitive symptoms and other domains disappeared when the fatigue domain was controlled but this was not seen vice versa (Tables 4-4, 4-5 and 4-6).

Table 4-4: PBC-40 quality of life assessment tool scores of non-cirrhotic PBC patients according to severity of cognitive symptoms

Domains	Moderate to severecognitively symptomatic PBC (n =16)	Cognitively mild or asymptomatic PBC (n=22)	P value
PBC 40 general symptoms mean (SD)	19.63 (4.2)	14.41 (3.66)	0.001*
PBC 40 itch mean (SD)	7.19 (3.6)	5.09 (3.55)	0.08
PBC 40 fatigue mean (SD)	38.75 (3.06)	21.77 (9.07)	0.001*
PBC 40 social mean (SD)	28.38 (7.17)	16.09 (6.86)	0.001*
PBC 40 emotional mean (SD)	9.50 (2.60)	5.05 (2.4)	0.001*
SD,standard deviation; PBC,primary biliary cholangitis; * = the mean between the groups differed significantly			

Table 4-5: Correlation between cognitive symptoms and other PBC - 40 domains (*significant at $p < 0.05$)

Assessment domains	Correlation with Cognitive symptoms R^2 (p value)	Correlation with Cognitive symptoms with controlled fatigue domain R^2 (p value)
PBC-40 general symptom	0.4 (0.001) *	0.04 (0.22)
PBC-40 itch	0.07 (0.17) *	NA (0.23)
PBC-40 fatigue	0.69 (0.001)*	NA
PBC-40 social	0.4 (0.001)*	0.01 (0.45)
PBC-40 emotion	0.5 (0.001)*	0.06 (0.33)
PBC, primary biliary cholangitis; * = the mean between the groups differed significantly		

Table 4-6: Correlation between fatigue and other PBC -40 domains (*significant at $p < 0.05$)

Assessment domains	Correlation with fatigue symptoms R^2 (p value)	Correlation with fatigue with controlled cognitive domain R^2 (p value)
PBC-40 symptom	0.5 (0.001)*	0.16 (0.01)*
PBC-40 itch	0.17 (0.013)*	0.14 (0.01)*
PBC-40 cognitive symptom	0.69 (0.001)*	Na
PBC-40 social	0.5 (0.001)*	0.21 (0.001)*
PBC-40 emotion	0.5 (0.001)*	0.16 (0.003)*
PBC,primary biliary cholangitis; * = the mean between the groups differed significantly		

4.2.3 Hospital Anxiety and Depression Scale scores

Both anxiety and depression scores were higher in PBC patients who reported significant cognitive symptoms when compared to those without (p = 0.001 and p = 0.001, respectively) (Table 4-7). The severity of depression symptoms was significantly correlated with fatigue (p = 0.001, R^2 = 0.4) and cognitive symptoms (p= 0.001, R^2 = 0.3). When the fatigue domain was controlled for, the previously observed correlations between depression and cognitive symptoms (p=0.44, R^2 = 0.12) and anxiety and cognitive symptoms (p=0.69, R^2 = 0.06) disappeared.

4.2.4 Epworth sleep scores (ESS score)

Approximately 37% (n= 6) of non-cirrhotic PBC patients with moderate to severe cognitive symptoms had ESS scores diagnostic of pathological daytime somnolence compared to 18% (n= 4) of those with mild symptoms. The mean ESS scores between the 2 groups were not significantly different (Table 4-7). ESS scores were significantly correlated with fatigue (p=0.001, R 2 = 0.26) but not with cognition (p = 0.07, R 2 = 0.08).

Table 4-7 : Hospital Anxiety and Depression Scales and Epworth sleep scores of non-cirrhotic PBC patients according to severity of cognitive symptoms

Domains	Moderate to severe cognitively symptomatic PBC (n =16)	Mild or cognitively asymptomatic PBC (n = 22)	P value
HAD - D scores mean (SD)	7.63 ± 3.6	3.5 ± 3.3	0.001*
HAD - A scores mean (SD)	8.31 ± 4.5	3.5 ± 3.7	0.001*
ESS scores mean (SD)	8.5 ± 4.6	6.59 ± 4.8	0.21
HAD, Hospital Anxiety and Depression;HAD-D, depression;HAD- A, anxiety;ESS,Epworth sleep scores; SD, standard deviation, * = the mean between the groups differed significantly			

4.3 Summary

We have shown that younger PBC patients have a greater burden of cognitive symptoms and associated mental health issues (depression and anxiety) appear to influence cognitive symptoms. The central mechanism of PBC has previously been hypothesized as the cause of cognitive symptoms [61]. A limitation in this study was that depression was not further explored (e.g. timing of onset) so it is difficult to disentagle the complex relationship between depression and cognition in PBC. There was no relationship seen between disease severity (as assessed by biochemistry) and cognitive symptoms, as seen in previous work [126]. Although median bilirubin was numerically higher in those with significant cognitive symptom, maximum bilirubin in both groups was within normal range. We also found, similar to previous studies[53, 61, 150], that UDCA treatment was unrelated to cognitive symptoms, suggesting that the treatment itself does not influence symptom severity. A quarter of patients with significant cognitive symptoms (25%) were on second line treatment, as compared to 18% with mild or no symptoms. Therefore, patients with significant cognitive symptoms may have a more severe course of cholestasis. Alternatively, Obeticholic acid treatment may impact on cognitive symptoms and this warrants further investigation We observed that patients with moderate to severe cognitive symptoms had worse fatigue, social, emotional, depression and anxiety scores and sleep problems. The reasons for this are unclear. The correlation between cognitive and other PBC-40 symptom domains disappeared when controlled for fatigue. This suggests that fatigue may be the main driver for other symptoms. Another explanation for the inter-relationship between symptoms could be due to overlapping central mechanisms underlying PBC.

In summary, our study found that moderate to severe cognitively symptomatic patients were younger with no relationship to disease severity. Those with cognitive symptom burden were more likely to have high symptom burden across other domains.

Chapter 5: CANTAB neuropsychiatric assessment according to cognitive symptoms severity usingPBC-40 cognitive scores in non-cirrhotic PBC cohort

5.1 Introduction

This chapter will evaluate the characteristics of non-cirrhotic PBC patients with subjectively reported moderate to severe cognitive symptoms, and those without, using the objective CANTAB neuropsychiatric battery. Information about the study population, recruitment, data collection and CANTAB test are described in Chapter 2.

5.2 Results

There were 38 PBC non-cirrhotic patients in the study; 16 of whom had significant (moderate to severe) cognitive symptoms. All patients completed the CANTAB test battery.

Episodic memory

The non-cirrhotic PBC patients with significant cognitive symptoms underperformed on the PAL tests compared to the normative population reference data (PALFAMS; 9.13 ± 4.03 vs 13.32 ± 4.02, p = 0.001, 95% confidence interval (CI) = 7.16 – 11.09 and PALTEA; 26.13 ± 13.54, vs 13.89±12.17, p = 0.001, 95% CI = 20.16 – 32.09) [Table 5-1]. Those with mild or no cognitive symptoms also performed worse in comparison to the normative population reference data (z score PALFAMS; - 0.71 ± 0.94 vs -1.04 ± 1 and z score PALTEA; 0.87 ± 1.2 vs 1 ± 1.1) [Table 5-1]. We did not observe any significant difference between non-crrhotic PBC patients with significant cognitive symptoms and those without cognitive symptoms on either PALFAMS and PALTEA (PALFAMS; p=0.26 and PALTEA; p= 0.72)[Figure 5-1,Figure 5-2].

Table 5-1: CANTAB memory task (PAL) assessment of normative population and PBC patients according to severity of cognitive symptoms

Test		NP Mean ± SD	CS PBC (n=16) Mean ± SD	CAS PBC (n=22) Mean ± SD	P value
PAL	PALFAMs (+)	13.32 ± 4.0	9.1 ± 4	10.4 ± 3.8	NP vs CSPBC (p=0.001)* NP vs CAS PBC (p=0.001) * CS vs CAS (p= 0.26)
	PALTEA (-)	13.89 ± 12.2	26.1 ± 13.5	24.5 ± 14.9	NP vs CS PBC(p=0.001)* NP vs CAS PBC (p=0.000) * CS vs CAS (p= 0.72)
NP,normative population; CS ,cognitively symptomatic; CAS,cognitively asymptomatic; PAL,Paired Associate Learning; PALFAM,PAL first attempt memory scores; PALTEA,PAL total error; (+) = higher scores indicate better performance; (-) = lower scores indicate better performance. * = the mean between the groups differed significantly.					

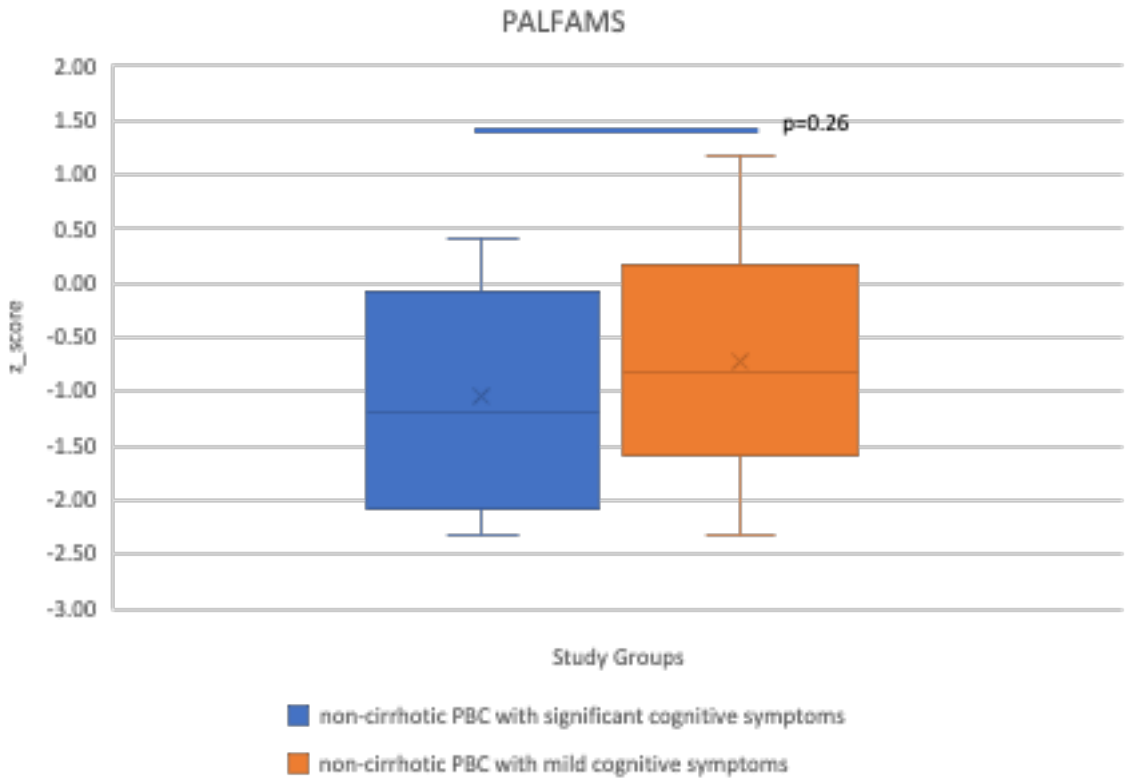

Figure 5-1: Comparison of PALFAMS scores between non-cirrhotic PBC with significant cognitive symptoms and non-cirrhotic PBC with mild cognitive symptoms

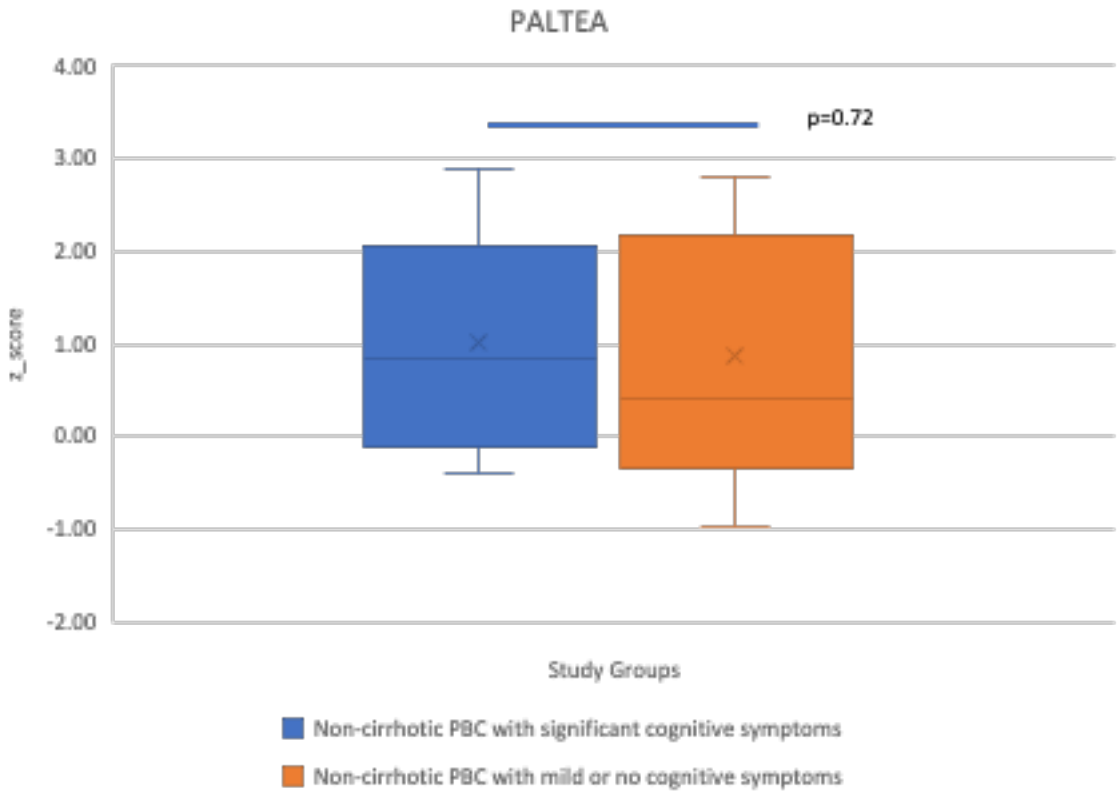

Figure 5-2:Comparison of PALTEA scores between non-cirrhotic PBC with significant cognitive symptoms and non-cirrhotic PBC with mild cognitive symptoms

Executive cognitive function

Executive function involves high-level planning, execution and decision-making. The executive function CANTAB tests performed in our study were Multitasking Task (MTT) [i.e., cognitive inhibition], One-Touch Stocking of Cambridge (OTS) [i.e., assessment of planning] and Spatial Working Memory (SWM) [i.e., assessment of retention and manipulation of visuospatial information].

No significant difference was seen in either subtask of theMTT between PBC patients and normative data (MTTLCMD; $p = 0.42$ and MTTLMTMD; $p = 0.125$) and therefore further analysis was not performed.

There was no difference in OTS between patients with moderate to severe cognitive symptoms when compared to the normative population reference data. However, those with mild or no cognitive symptoms had significant impairment on the OTS test compared to normative data (8.13±4.23 vs 10.82±2.7, $p = 0.001$, 95% CI = 7.008 – 9.265) [Table 5-2]. No statistical difference was observed in performance of OTS test between the PBC patients with and without significant cognitive symptoms ($p = 0.29$) Figure 5-3.In SWM tests, both PBC groups performed worse than the normative population Figure 5-3 and Figure 5-4.

Table 5-2 : CANTAB executive function (OTS, SWM) assessment of normative population, significant cognitively symptomatic PBC and cognitively asymptomatic PBC

Test		NP Mean ± SD	CS PBC (n=16) Mean ± SD	CAS PBC (n=22) Mean ± SD	P value
OTS (**+**)		10.82 ± 2.7	9.8 ± 2.4	8.1 ± 4.2	NP vs CS PBC (p=0.13) NP vs CAS PBC(p=0.001) * CS vs CAS (p= 0.29)
SWM	SWMBE (-)	9.9 ± 9.2	17.8 ± 6.5	17.5 ± 10.4	NP vs CS PBC (p=0.001) * NP vs CAS PBC(p=0.001) * CS vs CAS (p= 0.93)
	SWMS (-)	7.03 ± 2.8	9.3 ± 1.7	8.8 ± 2.9	NP vs CS PBC (p=0.001) * NP vs CAS PBC (p= 0.003) * CS vs CAS (p= 0.98)

NP;normative population; CS,cognitively symptomatic; CAS, cognitively asymptomatic; OTS,One-touch stockings of Cambridge; SWM,spatial working memory; SWMBE ,SWM between errors ; SWMS ,SWM strategy; (+) = higher scores indicate better performance; (-) = lower scores indicate better performance, *= the mean between the groups differed significantly.

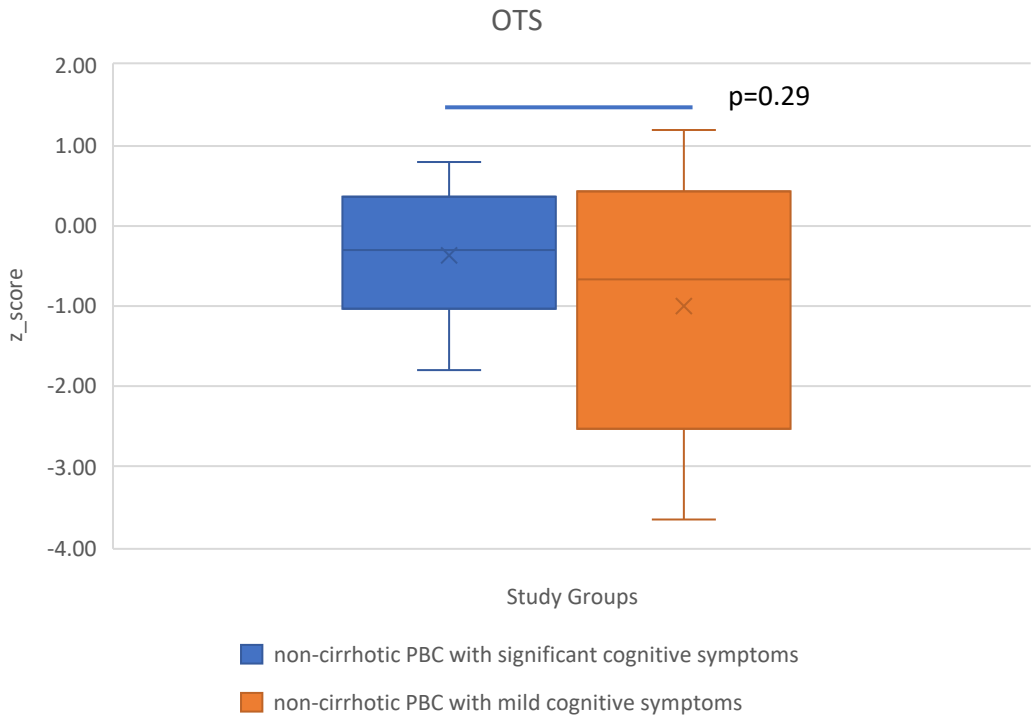

Figure 5-3: Comparison of OTS scores between non-cirrhotic PBC with significant cognitive symptoms and non-cirrhotic PBC with mild cognitive symptoms

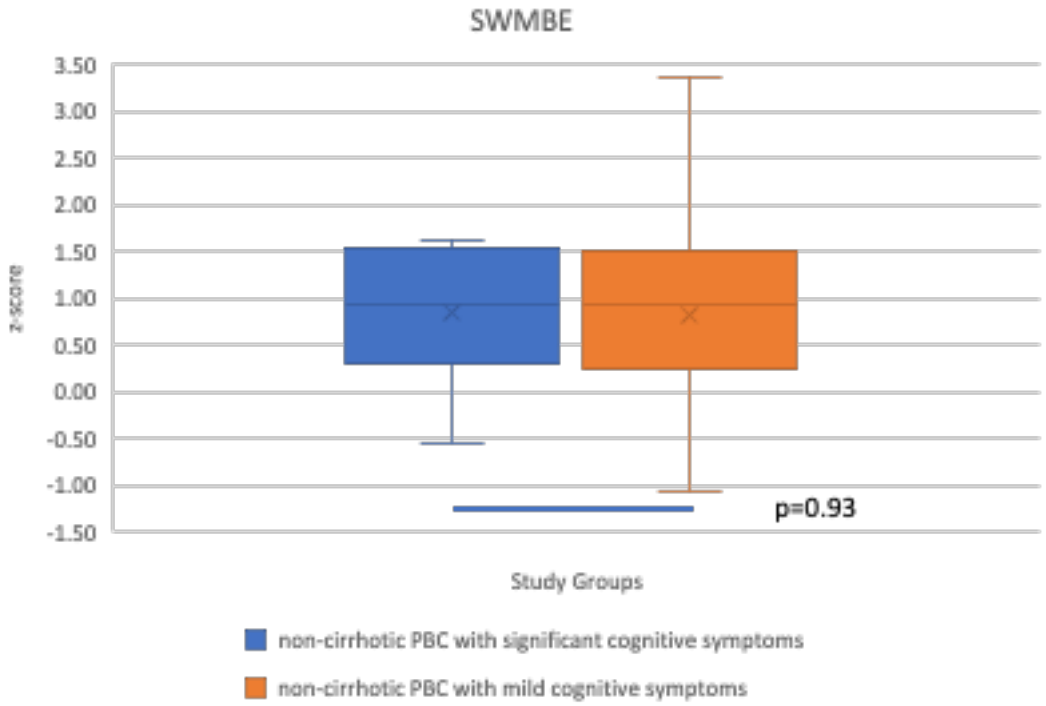

Figure 5-4:Comparison of SWMBE scores between non-cirrhotic PBC with significant cognitive symptoms and non-cirrhotic PBC with mild cognitive symptoms

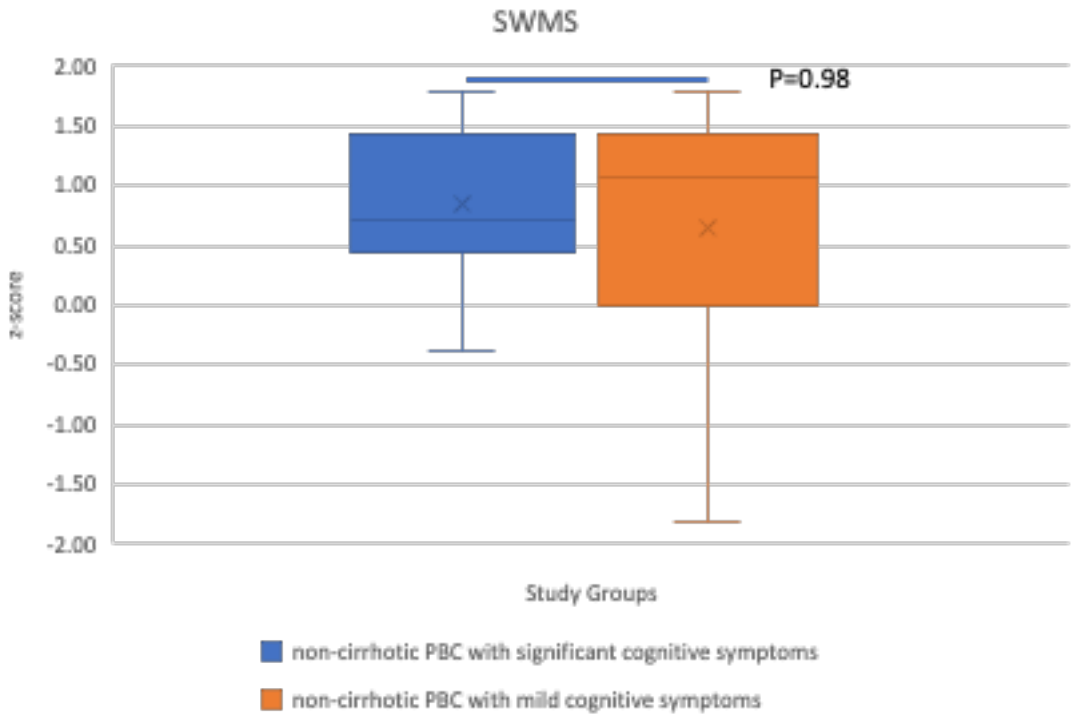

Figure:5-5:Comparison of SWMS scores between non-cirrhotic PBC with significant cognitive symptoms and non-cirrhotic PBC with mild cognitive symptoms

Emotional cognition

The non-cirrhotic PBC patients with significant cognitive dysfunction showed a lower number of hits (ERTTH) and slower response times (ERTOMDRT) compared to the normative population reference data (ER TOMDRT; 1674±385.2 vs 1267 ± 195.28, p = 0.001, 95% CI = 1578.5 – 1769.9 and ERTTH; 52.50±7.95 vs 56.8±6.84, p = 0.01, 95% CI = 49.15 – 55.85) (Table 5-3). The same was found in those with no or mild cognitive symptoms (p= 0.001 and p = 0.001, respectively) [Table 5-3]. No measurable difference was found between the two PBC groups, stratified by symptom severity, in either the ERTOMDRT and ERTTH tasks (p= 0.28 and p = 0.24, respectively) [Figure 5-6 and Figure 5-7].

Table 5-3 : CANTAB emotional cognitive function (ERT) assessment of normative population, significant cognitively symptomatic PBC and cognitively asymptomatic PBC and comparison across the groups

Test		NP Mean ± SD	CS PBC (n=16) Mean ± SD	CAS PBC (n=22) Mean ± SD	P value
ERT	ERTMODRT (-)	1267 ± 195.3	1674.2 ± 385.1	1511.2 ± 308.4	NP vs CS PBC (p=0.001) * NP vs CAS PBC p=0.001) * CS vs CAS (p= 0.28)
	ERTTH (+)	56.8 ± 6.8	52.5 ± 7.95	49 ± 7.2	NP vs CS PBC (p=0.01) * NP vs CAS PBC (p=0.001) * CS vs CAS (p= 0.24)
NP,normative population; CS,significant cognitively symptomatic; CAS,cognitively asymptomatic;ERT,Emotion Recognition Task; ERTMODRT,ERT overall median reaction time;ERTTH, ERT total hits(+) = higher scores indicate better performance; (-) = lower scores indicate better performance, * = the mean between the groups differed significantly.					

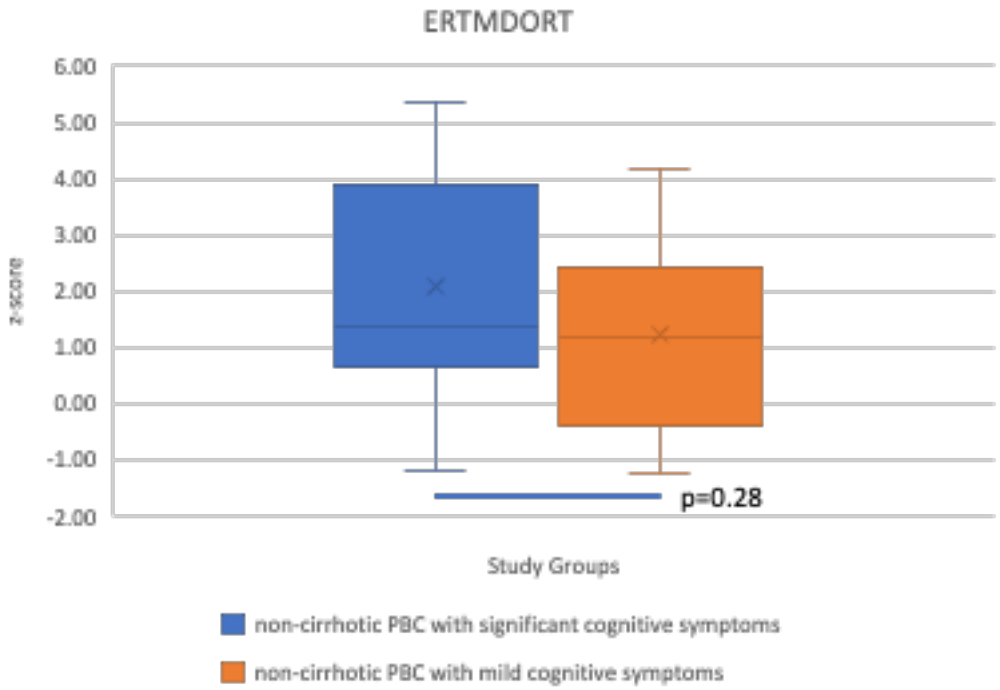

Figure 5-1: Comparison of ERTMDORT scores between non-cirrhotic PBC with significant cognitive symptoms and non-cirrhotic PBC with mild cognitive symptoms

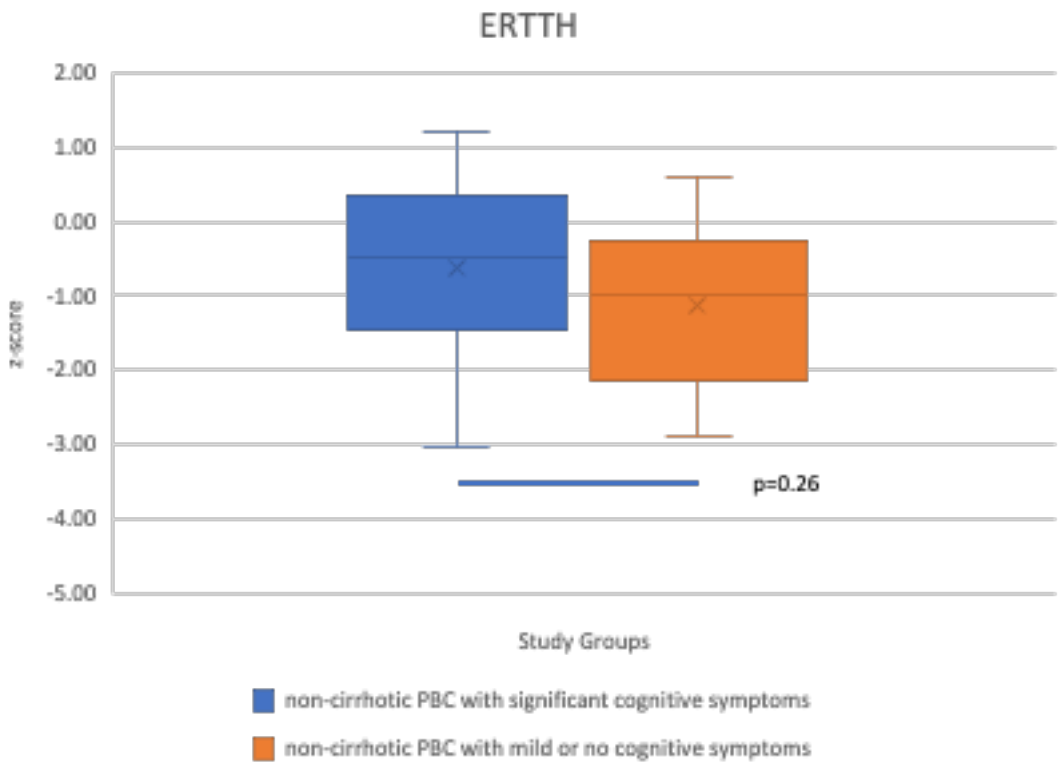

Figure 5-2: Comparison of ERTTH scores between non-cirrhotic PBC with significant cognitive symptoms and non-cirrhotic PBC with mild cognitive symptoms

Attention and psychomotor speed

Psychomotor speed was assessed using the reaction time test (RTI) in the CANTAB battery. Patients with non-cirrhotic PBC performed significantly better than the normative population and further analysis of the RTI test was not performed.

In the rapid visual information processing (RVP) task, both significantly cognitively impaired non-cirrhotic PBC patients and those without cognitive impairment had a lower number of correct responses on the RVPMD test (0.85±0.05 vs 0.91± 0.07, p = 0.003, 95% CI = 0.82 – 0.89) than the normative population reference data but this was not statistically significant (Table 5-4). There was no statistical difference between non-cirrhotic PBC patients with significant cognitive impairment and those without cognitive impairment on either RVPA or RVPMDL (p = 0.19 and p = 0.65, respectively) [Figure 5-8 and Figure 5-9].

Table 5-4: CANTAB attention and psychomotor cognitive function (RVP) assessment of normative population, significant cognitively symptomatic PBC and cognitively asymptomatic PBC and comparison across the groups.

Test		NP Mean ± SD	CS PBC (n=16) mean± SD	CAS PBC (n=22) mean± SD	P value
RVP **(+)**	RVPA (+)	0.9 ± 0.07	0.85 ± 0.05	0.87 ± 0.06	NP vs CS PBC (p=0.003) * NP vs CAS PBC (p=0.05) * CS vs CAS(p=0.63)
	RVPMDL (-)	483.8 ± 133.69	525.9 ± 111.6	533.5 ± 213.3	NP vs CS PBC (p=0.20) NP vs CAS PBC(p=0.08) CS vs CAS(p=0.19)
NP,normative population; CS ,cognitively symptomatic; CAS,cognitively asymptomatic; RVP,rapid visual processing; RVPA, RVP A';RVPMDL, RVP Median response latency; (+) = higher scores indicate better performance; (-) = lower scores indicate better performance, *= the mean between the groups differed significantly.					

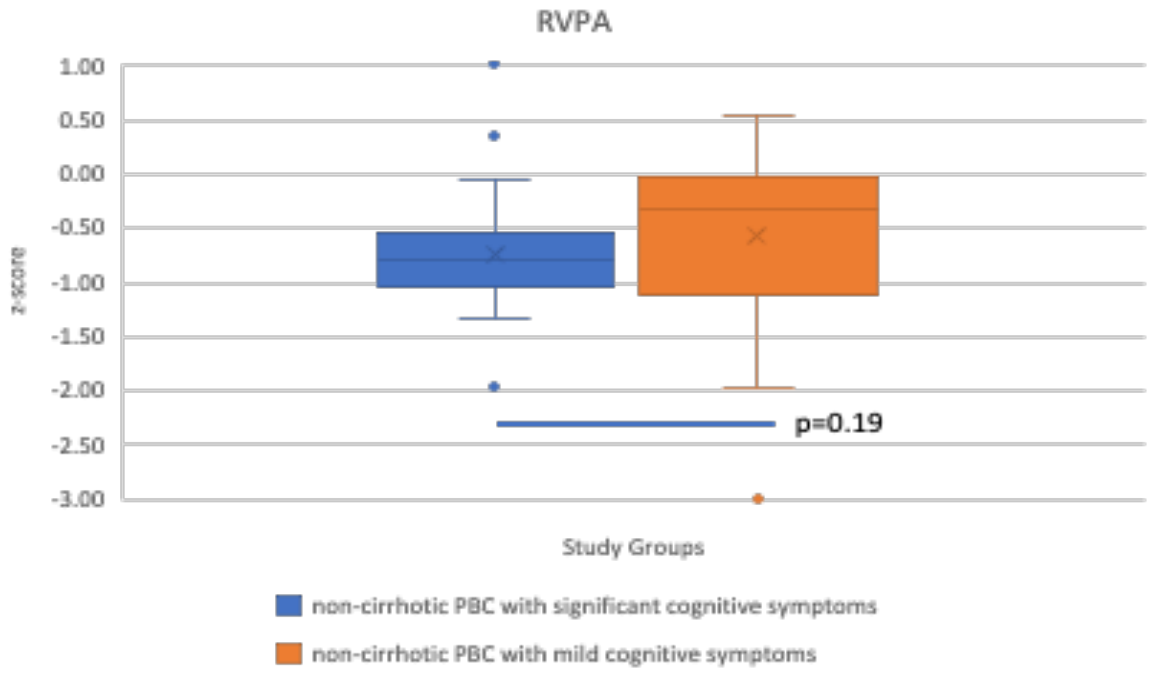

Figure 5-8: Comparison of RVPA scores between non-cirrhotic PBC with significant cognitive symptoms and non-cirrhotic PBC with mild cognitive symptoms

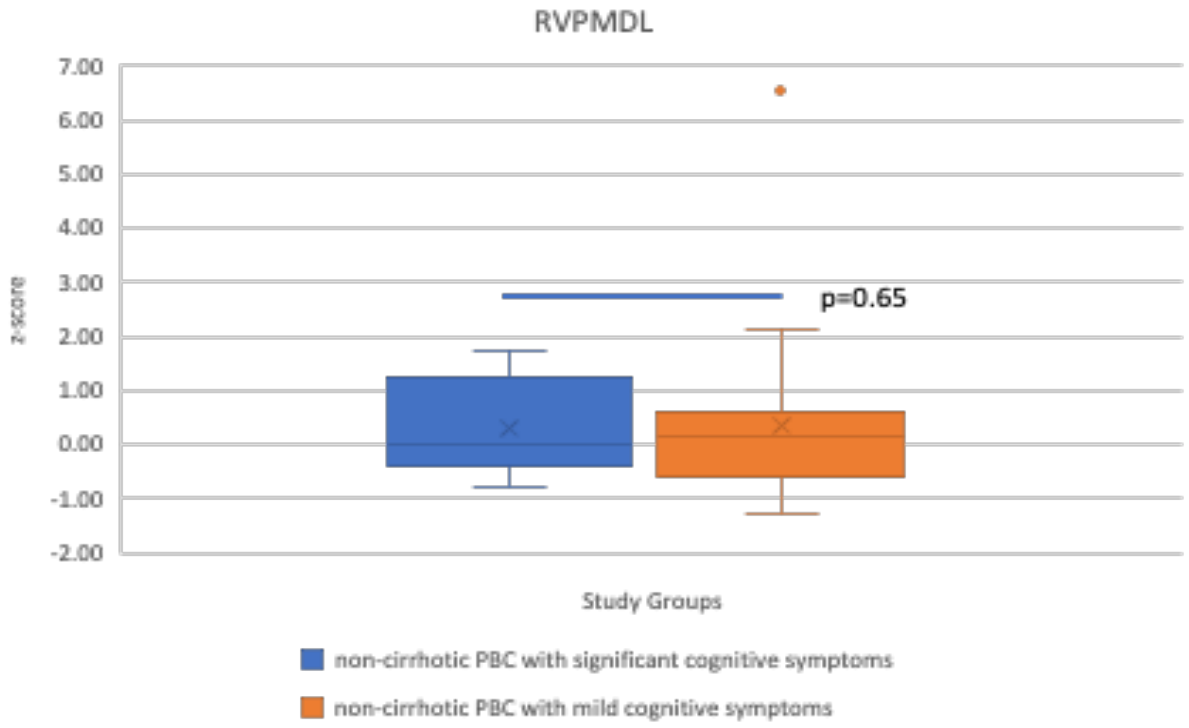

Figure 5-9: Comparison of RVPMDL scores between non-cirrhotic PBC with significant cognitive symtoms and non-cirrhotic PBC with mild cognitive symptoms

5.3 Summary

We used CANTAB neuropsychiatric assessment to assess the cognitive domains that were identified as being impaired in Chapter 3 (memory, executive function, attention, emotion) in non-cirrhotic PBC patients according to severity of cognitive symptoms (stratified using the PBC-40) [66].

PBC patients with significant cognitive symptoms underperformed in four cognitive tasks (PAL; learning memory, ERT; emotional cognition, SWM; working memory-executive function, and RVP; attention) when compared with a normative population. We had hypothesised that patients with mild cognitive symptoms would perform the same as a normal population but, in fact, we found poor performance on five cognitive tasks (PAL; learning memory, OTS; spatial planning task ERT; emotional cognition, SWM; working memory/executive function, RVP; attention). There were no differences between PBC patients in task performance when compared according to symptom severity. Since age and disease severity were the same in patients with or without clinically significant cognitive symptoms (Chapter 3 results) it is unlikely that these factors explain our findings. It may be that cognitive symptoms are subtle meaning that patients are unaware of them or that they lack insight into their symptoms. Another potential explanation is differences in coping strategies or adaptation to behavioural symptoms between patients.

Overall, objective cognitive deficits in memory, working memory, spatial planning, attention and emotional cognitive domains were present in patients with or without subjective perceived cognitive symptoms. However, we did not see significant differences in CANTAB cognitive performance according to symptom severity. Exhibition of cognitive symptoms in PBC patients may be influenced by behavioural adaptation/variation.

Chapter 6: DTI MRI imaging characteristics and the assessment according to Cognitive symptoms severity using PBC 40–cognitive scores in non-cirrhotic PBC cohort.

6.1 Introduction

This chapter will outline MRI characteristics of patients using Diffusion Tensor imaging (DTI) of specific deep white matter tracts, namely forceps minor, inferior longitudinal fasciculus, superior longitudinal fasciculus and uncinate tracts. White matter integrity was assessed by comparing fractional anisotropy (FA) values according to severity of cognitive symptoms, defined according to the PBC-40 cognitive domain cut-off scores. We will explore the association of DTI MRI measurements and CANTAB performance within this cohort. Information about the study population, recruitment, data collection, CANTAB testing and MRI methodology was described in chapter 2.

6.2 Results

There were 38 non-cirrhotic PBC patients in the study. One patient did not undergo MRI due to claustrophobia.

6.2.1 Differences between symptom severity groups

There were 15 non-cirrhotic PBC patients with significant cognitive symptoms and 22 with mild or no cognitive symptoms who underwent MRI assessment.

The mean FA scores of those with clinically significant cognitive symptoms as compared to those with no or mild symptoms were: 0.49. ± 0.03 and 0.48 ±0.02 in forceps minor, 0.5 ± 0.02 and 0.48 ± 0.02 in right inferior longitudinal fasciculus (ILF) region, 0.48 ± 0.02 and 0.48 ± 0.02 in left inferior longitudinal fasciculus (ILF), 0.5 ± 0.02 and 0.5 ± 0.02 in right uncinate, 0.5 ± 0.03 and 0.49 ± 0.02 in left uncinate, 0.49 ± 0.02 and 0.48 ± 0.02 in right superior longitudinal fasciculus (SLF), 0.49 ± 0.02 and 0.49 ± 0.02 in left superior longitudinal fasciculus (SLF) of the brain, respectively. None of these findings were statistically significant (Table 6-1).

Table 6-1: FA value of significantly cognitive symptomatic and mild or cognitively asymptomatic non-cirrhotic PBC patients.

	Significant cognitively symptomatic PBC (15)	mild or cognitively asymptomatic PBC (22)	P value
Forceps minor	0.49 ± 0.03	0.48 ± 0.02	0.28
Left ILF	0.48 ± 0.02	0.48 ± 0.02	0.68
Right ILF	0.5 ± 0.02	0.48 ± 0.02	0.13
Left SLF	0.49 ± 0.02	0.49 ± 0.02	0.63
Right SLF	0.49 ± 0.02	0.48 ± 0.02	0.60
Left Uncinate	0.50 ± 0.03	0.49 ± 0.02	0.35
Right Uncinate	0.50 ± 0.02	0.50 ± 0.02	0.65
PBC ,primary biliary cholangitis;ILF ,inferior longitudinal fasciculus;SLF, superior longitudinal fasciculus.			

6.2.2 Association with age in non-cirrhotic PBC patients

Age was not associated with FA values of any region of the brain: forcep minor (p = 0.1), right and left ILF (p= 0.87 and p =0.99), right and left SLF (p= 0.70 and p = 0.57) and right and left uncinate region (p= 0.35 and p = 0. 93) (Table 6-2).

6.2.3 Association with fatigue and itch severity

There was no association between fatigue severity and FA values in any region of the brain studied: forceps minor (p = 0.66); right ILF (p = 0.34); left ILF (p = 0.67); right SLF (p = 0.98); left SLF (p = 0.75); right uncinate (p = 0.69) and left uncinate fasciculus (p = 0.61) (Table 6-2).

Itch severity was not associated with FA values in any region of the brain: forceps minor (p = 0.51), right ILF (p = 0.92), left ILF (p = 0.59), right SLF (p = 0.35), left SLF (p = 0.28), right uncinate (p = 0.8) and left uncinate (p = 0.41) [Table 6-2].

Table 6-2 : Correlation between FA values of brain regions and age, PBC–40 Fatigue and itch scores of non- cirrhotic PBC patients

	Correlation with age R^2 (p value)	Correlation with fatigue score R^2 (p value)	Correlation with itch score R^2 (p value)
Forceps minor	0.07 (0.10)	0.004 (0.66)	0.01 (0.51)
Left ILF	0.001 (0.99)	0.004 (0.67)	0.008 (0.59)
Right ILF	0.07 (0.87)	0.02 (0.34)	0.001 (0.92)
Left SLF	0.008 (0.57)	0.002 (0.75)	0.03 (0.28)
Right SLF	0.003 (0.70)	0.00 (0.98)	0.02 (0.35)
Left Uncinate	0.001 (0. 93)	0.006 (0.61)	0.016 (0.41)
Right Uncinate	0.02 (0.35)	0.003 (0.69)	0.18 (0.8)
ILF,inferior longitudinal fasciculus; SLF , superior longitudinal fasciculus; significant at $p < 0.05$			

6.2.4 Association with CANTAB performance of non-cirrhotic PBC patients

In non-cirrhotic PBC patients, higher RVPMDL scores were associated with decreased FA values in the left ILF. CANTAB performance in OTS, PAL, SWM were not associated with FA values in any of the brain regions studied. There was a trend towards association between the emotional cognitive performance task performance and FA values in forceps minor but this was not statistically significant. The results are summarised in Table 6-3

Table 6-3 : Correlation between the FA values of brain regions and CANTAB scores of different cognitive domains of non-cirrhotic PBC patients

	Forceps minor R^2 (p value)	Lt ILF R^2 (p value)	Rt ILF R^2 (p value)	Lt SLF R^2 (p value)	Rt SLF R^2 (p value)	Lt Uncinate R^2 (p value)	Rt Uncinate R^2 (p value)
ERTT	0.07 (0.08)	0.02 (0.34)	0.02 (0.31)	0.03 (0.27)	0.03 (0.26)	0.00 (0.83)	0.00 (0.82)
OTSPSFC	0.00 (0.68)	0.01 (0.50)	0.00 (0.81)	0.00 (0.83)	0.01 (0.53)	0.01 (0.43)	0.06 (0.12)
PALFAM	0.03 (0.27)	0.02 (0.34)	0.00 (0.60)	0.04 (0.23)	0.01 (0.40)	0.00 (0.81)	0.00 (0.83)
PALTEA	0.03 (0.25)	0.00 (0.60)	0.00 (0.83)	0.02 (0.31)	0.01 (0.50)	0.00 (0.97)	0.00 (0.72)
SWMS	0.01 (0.52)	0.01 (0.49)	0.00 (0.78)	0.00 (0.67)	0.00 (0.70)	0.00 (0.57)	0.00 (0.88)
SWMBE	0.02 (0.38)	0.00 (0.70)	0.00 (0.76)	0.00 (0.72)	0.00 (0.90)	0.01 (0.47)	0.00 (0.64)
RVPA	0.00 (0.72)	0.06 (0.11)	0.02 (0.31)	0.00 (0.68)	0.00 (0.58)	0.01 (0.51)	0.00 (0.78)
RVPMDL	0.04 (0.15)	0.14 (0.01) *	0.06 (0.10)	0.05 (0.16)	0.04 (0.15)	0.04 (0.19)	0.03 (0.25)
ERTOMDRT	0.01 (0.49)	0.03 (0.25)	0.00 (0.80)	0.01 (0.55)	0.01 (0.54)	0.04 (0.15)	0.07 (0.10)

ILF ,inferior longitudinal fasciculus;SLF,superior longitudinal fasciculus;OTS,One-touch stockings of Cambridge; SWM,spatial working memory; SWMBE,SWM between error; SWMS, SWM strategy;PAL, Paired Associate Learning; PALFAM,PAL first attempt memory scores; PALTEA,PAL total error;ERT ,Emotion Recognition Task; ERTMODRT, ERT overall median reaction time; ERTTH,ERT total hits; RVP,rapid visual processing; RVPA,RVP A'; RVPMDL, RVP Median response latency; * = the mean between the groups differed significantly.

6.3 Summary

Our DTI analysis showed no differences in fractional anisotropy (FA) between significant cognitively symptomatic non-cirrhotic PBC patients and those with mild or symptoms. Data showed that patient age, PBC-40 fatigue scores and itch severity were not associated with FA values in deep white matter tracts. Overall, we did not demonstrate any associations between white matter integrity and cognitive symptom severity in non-cirrhotic PBC patients.

The lack of differences in FA values in deep white matter tracts may simply show that there is no association between white matter integrity and cognitive symptoms. Previous data examining structural abnormalities in white matter in PBC patients is inconsistent. Newton et al demonstrated white matter lesions in the basal ganglia, infratentorial regions and frontal lobe in patients with early disease, with total lesion load correlating with cognitive decline [126]. Hollingsworth et al found white matter alteration in PBC patients compared to healthy subjects [164]. However, Zenouzi et al showed no major structural abnormalities in white matter in PBC patients with no correlation with cognitive symptoms or attention [163]. Grover et al demonstrated reduced hippocampal volume and grey matter changes in PBC patients [57] but no difference in diffusion coefficients in CNS white matter, except in the thalamus [57]. It is possible that structural abnormalities of the white matter tract may not play a significant role in the pathogenesis of cognitive symptoms in non-cirrhotic PBC patients. Our study did not include a healthy control group, meaning we were unable to determine whether the FA values in our patient group deviated from normal. Further studies, replicating investigation of different brain regions, compared with healthy normal subjects and significant cognitively symptomatic and asymptomatic non-cirrhotic PBC patients, may provide additional information.

The other explanation for the lack of FA difference according to severity of cognitive symptoms could be dissociation of subjective self-reported symptoms assessed by PBC-40 and abnormalities of white matter changes. Grover et al found that the decreased hippocampal volume they demonstrated was independent of symptom severity [57]. In our study, we also showed that neither fatigue nor itch severity were associated with FA values

of the brain regions studied. The cognitive symptom cut-off scores we used to stratify the symptomatic and asymptomatic cohorts were based on a subjective patient assessment questionnaire. We discussed in the previous chapter that the manifestation of cognitive symptoms may be influenced by variation in patients' behaviour and this could limit the validity of our DTI findings.

We found an association between CANTAB performance in the attention task and FA values in the left ILF region, with lower FA values being associated with poorer cognitive performance. A trend was seen between emotional cognitive performance and lower FA values in forceps minor. This could suggest that attention and emotional cognitive performance may be related to disrupted white matter tract function in PBC patients but we cannot make firm conclusions due to the small sample size and lack of a normal comparison group. This should be investigated in a larger study with healthy control subjects.

In summary, our DTI study revealed no difference in FA values in the white matter tracts investigated between the two groups (perceived significant cognitively symptomatic and asymptomatic non-cirrhotic PBC patients). This may be due to the absence of association between the white matter changes and cognitive symptoms or due to dissociation between the subjective self-reported cognitive symptoms and CNS changes as discussed above. Larger cohorts and a comparison with healthy control groups are needed to explore the results further.

Chapter 7: Clinical characteristics and cognitive assessment according to disease severity

7.1 Introduction

In this chapter, the demographic data, behavioural symptoms, and cognitive performance of cirrhotic PBC patients and those with AMA positivity, but normal liver function tests, will be described using both subjective and objective assessment. The clinical characteristics, behaviour symptoms score, and CANTAB performance details of non-cirrhotic PBC patients were described in chapter 3. We will then explore the phenotype of non-cirrhotic PBC patients as compared to those with cirrhosis and AMA positive patients with normal LFTs. We aim to characterise the cognitive symptoms at different disease stages in order to understand their natural history at different time points.

Patients with a history of hepatic decompensation (i.e., variceal bleeding, hepatic encephalopathy or ascites) within the last 12 months were excluded from the study.

7.2 Results

There were 11 cirrhotic PBC patients, 38 non-cirrhotic and 11 who were AMA positive with normal liver function tests (ie without cholestasis). Two patients from the AMA positive with normal LFTs group were excluded (one due to colon cancer on chemotherapy and the second due significant neurological conditions).

7.2.1 Demographic characteristics of cirrhotic PBC cohort

The mean age of cirrhotic PBC patients at time of study entry was 61± 9 years with the majority being female. The mean age at diagnosis was 50 years. Most patients had positive AMA antibodies and half of the cohort (6/11, 54%) underwent liver biopsy to confirm the PBC diagnosis and rule out other concomitant causes of liver disease. Depression and Type 2 Diabetes affected approximately 27% (3/11) of the cohort. The majority of the cohort were receiving UDCA with over half of them requiring second line therapy. Full demographic and laboratory details are described in Table 7-1.

7.2.2 Demographic characteristics of AMA positive patients with normal LFT

The mean age of study participants was 51 ± 11 years and all were female. Four out of nine patients (44%) had been diagnosed with depression. Demographics and laboratory parameters are presented in Table 7-2.

Table 7-1: Demographic and clinical characteristics of Cirrhotic PBC patients

Features	PBC patients with cirrhosis (n = 11)
Age; mean (SD)	61 (9)
Gender (% of Female)	10 (90%)
Age at Diagnosis Mean (SD)	47 (13)
Disease Duration (years) Mean (SD)	13.1 (7.7)
Diagnosis of PBC	
AMA antibodies	9 (82%)
Liver biopsy	6 (54.5%)
PBC specific ANA antibodies	3 (27%)
Comorbidity	
Hypertension	2 (18%)
Type 2 Diabetes	3 (27%)
Hypothyroid	1 (9%)
Depression	3 (27%)
Autoimmune diseases	
Cutaneous SLE	1 (9%)
Scleroderma	1 (9%)
Respiratory disease	
Sarcoid lung disease	1 (9%)
UDCA Treatment (%)	10 (91%)
UDCA response rate (%)	5 (45%)
2nd line treatment (%)	6 (55%)
Obeticholic acid	3 (27%)
Fibrate	3 (27%)
Other medications	
Antidepressant	1 (9%)
Antihistamine	0 (0%)
Laboratory	
ALP; mean (SD)	214 (179)
ALT; mean (SD)	41 (41)
Bilirubin; mean (SD)	11 (3)
Platelets; mean (SD)	175 (56)
Albumin; mean (SD)	43 (4)
SD,standard deviation;AMA,anti-mitochondrial antibodies; ALT ,Alanine Aminotransferase;ALP, alkaline phosphatase; UDCA,ursodeoxycholic acid; SLE, systemic lupus erythematosus	

Table 7-2 : Demographic and clinical characteristics of AMA positive, normal liver function test patients

Features	AMA positive normal LFT (n= 9)
Age; mean (SD)	51 (11)
Gender (% of Female)	9 (100%)
AMA antibodies	8
PBC specific ANA antibodies	1
Comorbidity	
Hypertension	2 (22%)
Diabetes	0
Hypothyroid	2 (22%)
Depression	4 (44%)
Autoimmune diseases	1 (11%)
Multi-system connective tissues disease	1
Laboratory	
ALP; mean (SD)	76 (24)
ALT; mean (SD)	19 (9)
Bilirubin; mean (SD)	8 (4)
Platelets; mean (SD)	289 (38)
Albumin; mean (SD)	46 (4)
SD,standard deviation;ALT,Alanine Aminotransferase;ALP,alkaline phosphatase; AMA, anti-mitochondrial antibody;LFT,liver function test.	

7.2.3 Comparison of demographic and laboratory parameters across the cohort

The mean age of AMA positive patients with normal LFT cohort was younger than that of non-cirrhotic and cirrhotic PBC. There was no difference in alanine transaminase and alkaline phosphatase between cirrhotic and non-cirrhotic PBC patients.The mean bilirubin was were higher in the cirrhotic than the non-cirrhotic PBC patients. The detailed findings are described in Table 7-3

Table 7-3: Comparison of clinical and laboratory of non-cirrhotic PBC, cirrhotic PBC and AMA positive normal liver function test patients

Features	Total N-C PBC (n = 38)	C PBC (n = 11)	AMA positive normal LFT (n = 9)	P value
Age; mean (SD)	59 (8)	61 (9)	51 (11)	Total N-C PBC vs C PBC (p= 0.6) Total N-C PBC vs AMA positive(p=0.04) * C PBC vs AMA positive (p= 0.03) *
Gender (% of Female)	37 (97%)	10 (90%)	9 (100%)	-
Age at Diagnosis Mean (SD)	51 (7)	47 (13)	-	Total N-C PBC vs C PBC (p= 0.31)
Disease Duration (years) Mean (SD)	8.1 (6.6)	13.1 (7.7)	-	Total N-C PBC vs C PBC (p= 0.04) *
UDCA Treatment (%)	36 (95%)	10 (91%)	-	-
UDCA response rate (%)	28 (73%)	5 (45%)	-	-
2nd line treatment (%)		6 (55%)	-	-
Obeticholic acid	6	3	-	-
Laboratory				
ALP; mean (SD)	148 (71)	214 (179)	76 (24)	Total N-C PBC vs C PBC (p= 0.92) Total N-C PBC vs AMA positive (p=0.001) * C PBC vs AMA positive (p= 0.002) *
ALT; mean (SD)	28 (15)	41 (41)	19 (9)	Total N-C PBC vs C PBC (p= 0.75) Total N-C PBC vs AMA positive (p= 0.04) * C PBC vs AMA positive (p= 0.26)
Bilirubin; mean (SD)	7 (3)	11 (3)	8 (4)	Total N-C PBC vs C PBC (p= 0.002) * Total N-C PBC vs AMA positive (p= 0.44) C PBC vs AMA positive (p=0.15)
Platelets; mean (SD)	286 (69)	175 (56)	289 (38)	Total N-C PBC vs C PBC (p= 0.001) * Total N-C PBC vs AMA positive (p= 0.86) C PBC vs AMA positive (p=0.001) *
Albumin; mean (SD)	43 (2)	43 (4)	46 (4)	Total N-C PBC vs C PBC (p= 0.93) Total N-C PBC vs AMA positive (p= 0.01) * C PBC vs AMA positive (p=0.03) *

SD ,standard deviation;PBC ,Primary biliary cholangitis; ALT ,Alanine Aminotransferase;ALP, alkaline phosphatase;UDCA ,ursodeoxycholic acid;Total N-C PBC, total non-cirrhotic PBC;C PBC ,cirrhotic PBC;AMA ,anti-mitochondrial antibody;LFT ,liver function test; significant at $p < 0.05$, *= the mean between the group differed significantly

7.2.4 Comparison of symptom assessment tools scores across the cohorts

The behavioural phenotype of non-cirrhotic PBC patients was explored in comparison to those with cirrhosis and those with positive AMA and normal liver function tests. The cirrhotic patients had moderate to severe scores using PBC-40 in the fatigue, cognitive, social and emotional domains. Conversely, all scores for the patients with positive AMA normal LFTs were within the mild range. Scores across all PBC-40 domains were higher in the cirrhotic cohort as compared to non-cirrhotic and patients with positive AMA and normal LFTs. However, the differences were not statistically significant. There were also no significant differences in levels of anxiety, depression or sleep disturbance between the different cohorts. The details findings were described in Table 7-3.

Table 7-4: Comparison of PBC-40 domains scores, HADS and ESS between non-cirrhotic PBC, cirrhotic PBC and AMA positive normal liver function test patients

Domains	Total N-C PBC Mean (SD)	C PBC Mean (SD)	AMA positive normal LFT Mean (SD)	P value
PBC-40 fatigue	28.92 (11.07)	32.27 (14.72)	30.78 (14.51)	Total N-C PBC vs C PBC (p= 0.50) Total N-C PBC vs AMA positive (p= 0.76) C PBC vs AMA positive (p= 0.71)
PBC-40 cognitive domain	14.26 (6.37)	17 (8.43)	15.78 (8.49)	Total N-C PBC vs C PBC (p= 0.25) Total N-C PBC vs AMA positive (p= 0.7) C PBC vs AMA positive (p= 0.65)
PBC-40 itch	5.97 (3.7)	6 (3.7)	4.89 (3.06)	Total N-C PBC vs C PBC (p= 0.88) Total N-C PBC vs AMA positive (p= 0.48) C PBC vs AMA positive (p= 0.71)
PBC social	21.26 (9.24)	28.18 (11.66)	20.78 (9.47)	Total N-C PBC vs C PBC (p= 0.74) Total N-C PBC vs AMA positive (p= 80) C PBC vs AMA positive (p= 0.15)
PBC emotional	6.92(3.316)	8.27 (4.02)	7.78 (3.80)	Total N-C PBC vs C PBC (p= 0.34) Total N-C PBC vs AMA positive (p= 0.62) C PBC vs AMA positive (p= 0.65)
PBC-40 general symptoms	16.61 (4.653)	15.45 (4.59)	16.22 (5.65)	Total N-C PBC vs C PBC (p= 0.47) Total N-C PBC vs AMA positive (p= 0.85) C PBC vs AMA positive (p= 0.94)
Hospital Anxiety score	5.5 (4.7)	6.1 (4.1)	6.1 (3.6)	Total N-C PBC vs C PBC (p= 0.55) Total N-C PBC vs AMA positive (p= 0.58) C PBC vs AMA positive (p= 0.94)

Hospital Depression score	5.2 (3.9)	7.1 (4.0)	6.5 (4)	Total N-C PBC vs C PBC (p= 0.11) Total N-C PBC vs AMA positive (p= 0.35) C PBC vs AMA positive (p= 0.65)
ESS score	7.3 (4.5)	7.9 (7.5)	8.4 (3.6)	Total N-C PBC vs C PBC (p= 0.75) Total N-C PBC vs AMA positive (p= 0.41) C PBC vs AMA positive (p= 0.63)
SD,standard deviation; PBC,Primary biliary cholangitis;ESS,Epworth sleep scores;Total N-C PBC ,total non-cirrhotic PBC;C PBC, cirrhotic PBC;AMA ,anti-mitochondrial antibody;LFT ,liver function test				

7.2.5 CANTAB neuropsychiatric testing of cirrhotic PBC and AMA positive with normal LFT patients

There was a significant cognitive deficit in the memory domain in the cirrhotic PBC patients compared to normative population. Of the 11 cirrhotic patients, 36.4% (n = 4) and 45.5 % (n = 5) showed significant deficits in PALFAMS (PAL First Attempt Memory Score) and PALTEA (PAL total errors) tests, respectively. They made more errors (PALTEA) and demonstrated lower number of correct trials on their first attempt (PALFAMS) than the normative population reference data (PALTEA; 25.21 ±14.22 vs 13.89 ±12.17, p = 0.001 and PALFAMS; 9.09 ± 5.10 vs 13.32 ± 4.02, p=0.001, respectively). They also had significant deficits in the emotional cognitive domain with slower response times (ERTOMDRT; p=0.001) and lower number of hits (ERTTH; p= 0.002) than the normative population reference data. Similar to the non-cirrhotic PBC cohort, there were abnormalities in attention and information processing (RVP; p = 0.01) but not in the speed of motor response (RTI; p= 0.37). They were no deficits in the other cognitive domains (executive function [OTS, SWM OTT] and psychomotor test [RTI]) in cirrhotic PBC patients.

In AMA positive patients with normal LFTs, CANTAB cognitive performance did not differ from the normative population reference data in memory, executive,emotional and attention domains (PALFAMs; p = 0.92. PALTEA; p =0.89, OTS; p = 0.24, MTTLCMD; p = 0.54, MTTLMTMD; p = 0.89, SWMBE; p = 0.52, SWMS; p = 0.09, ERTTH = 0.32, RVP; p = 0.17, RVPMDL; p = 0.97). They performed better in psychomotor task than normative population (RTI; p = 0.05). The detail summary of the findings was described in Table 7-4.

Table 7-5 : CANTAB cognitive assessment of normative population, cirrhotic PBC and AMA positive normal LFT patients and comparison across the groups.

Test		**NP mean± SD**	**cirrhotic PBC (n=11) mean ± SD**	**AMA positive (n=9) Mean ± SD**	**P value**
PAL	PALFAMs (+)	13.32 ± 4.0	9.09 ± 5.10	13.44 ± 3.64	NP vs Cirrhotic PBC (p = 0.001) * NP vs AMA positive (p = 0.92)
	PALTEA (-)	13.89 ± 12.2	27.64 ± 18.52	13.33 ± 13.45	NP vs Cirrhotic PBC (p=0.001) * NP vs AMA positive (p = 0.89)
OTS (+)		10.82 ± 2.7	9.4 ± 3.09	9.78 ± 1.39	NP vs Cirrhotic PBC (p = 0.09) NP vs AMA positive (p = 0.24)
MTT	MTTLCMD (-)	689.10 ± 109.39	716.86 ± 170.83	666.77 ± 78.57	NP vs Cirrhotic PBC (p = 0.4) NP vs AMA positive (p = 0.54)
	MTTLMTMD (-)	909.62 ± 162.96	911.86 ± 180.93	902.61 ± 178.18	NP vs Cirrhotic PBC (p = 0.96) NP vs AMA positive (p = 0.89)
SWM	SWMBE (-)	9.9 ± 9.2	12.55 ± 8.84	11.89 ± 10.22	NP vs Cirrhotic PBC (p =0.34) NP vs AMA positive (p = 0.52)
	SWMS (-)	7.03 ± 2.8	7.55 ± 2.42	8.56 ± 2.83	NP vs Cirrhotic PBC (p = 0.53) NP vs AMA positive (p = 0.09)
ERT	ERTMODRT (-)	1267 ± 195.3	1776.84 ± 626.91	1411.5 ± 319.5	NP vs Cirrhotic PBC (p = 0.001) * NP vs AMA positive (p = 0.02) *
	ERTTH (+)	56.8 ± 6.8	50.45 ± 7.188	54.56 ± 4.44	NP vs Cirrhotic PBC (p = 0.002) * NP vs AMA positive (p = 0.32)
RVP	RVPA (+)	0.9 ± 0.07	0.86 ± 0.05	0.87 ± 0.04	NP vs Cirrhotic PBC (p = 0.01) * NP vs AMA positive (p = 0.17)
	RVPMDL (-)	483.8 ± 133.69	492 .4 ± 143.6	482.11 ± 77.76	NP vs Cirrhotic PBC (p = 0.83) NP vs AMA positive (p = 0.97)

RTI	RTIFMDRT (-)	424.34 ± 59.77	408.5 ± 58.65	385.11 ± 34.94	NP vs Cirrhotic PBC (p = 0.37) NP vs AMA positive (p = 0.06)

SD,standard deviation; NP,normative population;PAL, Paired Associate Learning; PALFAM,PAL first attempt memory scores; PALTEA,PAL total error; OTS, One-touch stockings of Cambridge; SWM, spatial working memory; SWMBE, SWM between error; SWMS ,SWM strategy; ERT,Emotion recognition Task; ERTMODRT,ERT overall median reaction time; ERTTH, ERT total hits; RVP, rapid visual processing; RVPA, RVP A'; RVPMDL, RVP Median response latency; (+) = higher scores indicate better performance; (-) = lower scores indicate better performance, significant at $p < 0.05$, *= the mean between the group differed significantly

7.2.6 Comparison of CANTAB neuropsychiatric test results across the three cohorts

The details of the CANTAB cognitive performance of non-cirrhotic PBC are reported in chapter 3. There were no differences in performance on any CANTAB task between non-cirrhotic and cirrhotic PBC patients: episodic memory, executive function, emotion, attention, and psychomotor (PALFAM, p = 0.63; PALTEA, p = 0.70; OTS, p = 0.83; SWMBE, p = 0.12; ERTMODRT, p = 0.64; ERTTH, p= 0.14; RVPA, p = 0.64; RVPMDL, p = 0.33; RTIFMDRT, p = 0.69) These data are summarised in Table 7-5. AMA positive normal LFTs patients had better scores compared to non-cirrhotic PBC patients. This was statistically signigicant for episodic memory (PALFAMS; p = 0.01 and PALTEA; p = 0.02).

Table 7-6: CANTAB cognitive assessment of non-cirrhotic PBC, cirrhotic PBC and AMA positive normal LFT patients and comparison across the groups.

Test		**Total N-C PBC (n=38) mean± SD**	**C PBC (n=11) mean± SD**	**AMA positive (n=9) mean± SD**	**P value**
PAL	PALFAMs (+)	9.9 ± 3.9	9.09 ± 5.10	13.44 ± 3.64	Total N-C PBC vs C PBC (p= 0.63) Total N-C PBC vs AMA positive (p= 0.01) * C PBC vs AMA positive (p= 0.03) *
	PALTEA (-)	25.2 ± 14.2	27.64 ± 18.52	13.33 ± 13.45	Total N-C PBC vs C PBC (p= 0.70) Total N-C PBC vs AMA positive (p= 0.02) * C PBC vs AMA positive (p= 0.04) *
OTS (+)		8.8 ± 3.6	9.4 ± 3.09	9.78 ± 1.39	Total N-C PBC vs C PBC (p= 0.83) Total N-C PBC vs AMA positive (p= 0.75) C PBC vs AMA positive (p= 0.72)
SWM	SWMBE (-)	17.6 ± 8.9	12.55 ± 8.84	11.89 ± 10.22	Total N-C PBC vs C PBC (p= 0.12) Total N-C PBC vs AMA positive (p= 0.14) C PBC vs AMA positive (p= 0.88)
	SWMS (-)	9 ± 2.5	7.55 ± 2.42	8.56 ± 2.83	Total N-C PBC vs C PBC (p= 0.04) * Total N-C PBC vs AMA positive (p= 0.57) C PBC vs AMA positive (p= 0.50)
ERT	ERTMODRT (-)	1579.89 ± 347.5	1776.84 ± 626.91	1411.5 ± 319.5	Total N-C PBC vs C PBC (p= 0.64) Total N-C PBC vs AMA positive (p= 0.24) C PBC vs AMA positive (p= 0.17)
	ERTTH (+)	50.5 ± 7.6	50.45 ± 7.188	54.56 ± 4.44	Total N-C PBC vs C PBC (p= 0.99) Total N-C PBC vs AMA positive (p= 0.14) C PBC vs AMA positive (p= 0.23)

RVP	RVPA (+)	0.86 ± 0.05	0.86 ± 0.05	0.87 ± 0.04	Total N-C PBC vs C PBC (p= 0.64) Total N-C PBC vs AMA positive (p=0.52) C PBC vs AMA positive (p= 0.33)
	RVPMDL (-)	530.2 ± 175.7	492 .4 ± 143.6	482.11 ± 77.76	Total N-C PBC vs C PBC (p= 0.33) Total N-C PBC vs AMA positive (p= 0.60) C PBC vs AMA positive (p= 0.71)
RTI	RTIFMDRT (-)	398.3 ± 47.8	408.5 ± 58.65	385.11 ± 34.94	Total N-C PBC vs C PBC (p= 0.69) Total N-C PBC vs AMA positive (p= 0.29) C PBC vs AMA positive (p= 0.23)
Total N-C PBC ,Total non-cirrhotic PBC; C PBC,Cirrhotic PBC; PAL, Paired Associate Learning; PALFAM, PAL first attempt memory scores; PALTEA,PAL total error; OTS, One-touch stockings of Cambridge; SWM, spatial working memory; SWMBE,SWM between error; SWMS, SWM strategy; ERT, Emotion recognition Task; ERTMODRT, ERT overall median reaction time; ERTTH, ERT total hits; RVP, rapid visual processing; RVPA, RVP A'; RVPMD, RVP Median response latency; (+) = higher scores indicate better performance; (-) = lower scores indicate better performance, significant at $p < 0.05$, * = the mean between the group differed significantly.					

Table 7-7 : CANTAB cognitive testing of PBC patients who were on obeticholic acid and those who were not.

Test		Normative population (A) Mean ± SD	PBC patient on OCA (B) mean ± SD (n=9)	PBC patient not on OCA (C) Mean ± SD (n = 35)	P value
PAL	PALFAMs (+)	13.32 ± 4.0	9 ± 4.9	9.6 ± 4	A vs B (p = 0.001) * A vs C (p = 0.001) * B vs C (p= 0.7)
	PALTEA (-)	13.89 ± 12.2	28.67 ± 19.2	25.57 ± 14.1	A vs B (p = 0.001) * A vs C (p = 0.001) * B vs C (p = 0.58)
OTS (+)		10.82 ± 2.7	9.7 ± 3.4	8.5 ± 3.3	A vs B (p = 0.24) A vs C (p = 0.001) * B vs C (p = 0.32)
SWM	SWMBE (-)	9.9 ± 9.2	13 ± 8.2	17.9 ± 8.9	A vs B (p = 0.31) A vs C (p = 0.001) * B vs C (p = 0.14)
	SWMS (-)	7.03 ± 2.8	8.4 ± 3.0	8.8 ± 2.3	A vs B (p = 0.13) A vs C (p = 0.001) * B vs C (p = 0.65)
ERT	ERTMODRT (-)	1267 ± 195.3	1538.1 ± 507.2	1676.9 ± 418	A vs B (p = 0.001) * A vs C (p = 0.001) * B vs C (p = 0.39)
	ERTTH (+)	56.8 ± 6.8	53.11 ± 6.1	49.2 ± 7.8	A vs B (p = 0.1) A vs C (p = 0.001) * B vs C (p = 0.18)

RVP (+)	RVPA (+)	0.9 ± 0.07	0.87 ± 0.03	0.85 ± 0.06	A vs B (p = 0.34) A vs C (p = 0.001) * B vs C (p = 0.39)
	RVPMDL (-)	483.8 ± 133.69	484 ± 73.9	540 ± 192	A vs B (p = 0.99) A vs C (p = 0.01) * B vs C (p = 0.39)

SD,standard deviation; OCA,Obeticholic acid;PAL,Paired Associate Learning; PALFAM,PAL first attempt memory scores; PALTEA,PAL total error;OTS,One-touch stockings of Cambridge; SWM, spatial working memory; SWMBE,SWM between error; SWMS,SWM strategy;ERT,Emotion recognition Task; ERTMODRT, ERT overall median reaction time; ERTTH ,ERT total hits; RVP, rapid visual processing; RVPA ,RVP A';RVPMDL,RVP Median response latency; (+) = higher scores indicate better performance; (-) = lower scores indicate better performance

significant at $p < 0.05$, *= the mean between the group differed significantly.

7.2.7 CANTAB cognitive performance of PBC patients receiving Obeticholic acid compared to normative population

There were nine PBC patients who were on Obeticholic acid (OCA) in whole PBC population in the study. Of them, six patients were non-cirrhotic. Patients not on treatment with OCA had significant cognitive deficits in memory, executive, emotional and attention domains compared to normative population data, whilst those receiving OCA only had abnormal paired associate learning. There was no statistically significant difference in any of the CANTAB domains when comparing PBC patients according to OCA treatment. The results are summarised in Table 7-7.

7.2.8 DTI MRI imaging assessment across the groups

There were 38 non-cirrhotic PBC, nine cirrhotic PBC and eight AMA positive normal LFTs patients who underwent MRI. One AMA positive normal LFTs and one non-cirrhotic PBC patient did not undergo MRI assessment due to claustrophobia. The mean FA values of each group in forceps minor, right and left superior longitudinal fasciculus, right and left inferior longitudinal fasciculus, right and left uncinate fasciculus are shown in Table 7-7. There was no difference in FA values in any of regions between the groups.

Table 7-8 : FA values of non-cirrhotic PBC, cirrhotic and AMA positive normal LFT patients and comparison across the groups

	Non-cirrhotic PBC (n =37)	Cirrhotic PBC (n =9)	AMA positive (n = 8)	P value
Forceps minor	0.48 ± 0.03	0.48 ± 0.04	0.50 ± 0.03	N-CPBC vs AMA = 0.14 N-CPBC vs C-PBC = 0.97 C-PBC vs AMA = 0.36
Left ILF	0.48 ± 0.02	0.48 ± 0.02	0.49 ± 0.02	N-CPBC vs AMA = 0.30 N-CPBC vs C-PBC = 0.99 C-PBC vs AMA = 0.39
Right ILF	0.49 ± 0.02	0.49 ± 0.02	0.50 ± 0.01	N-CPBC vs AMA = 0.24 N-CPBC vs C-PBC = 0.92 C-PBC vs AMA = 0.33
Left SLF	0.49 ± 0.02	0.48 ± 0.03	0.48 ± 0.03	N-CPBC vs AMA = 0.41 N-CPBC vs C-PBC = 0.42 C-PBC vs AMA = 0.98
Right SLF	0.49 ± 0.02	0.49 ± 0.02	0.47 ± 0.03	N-CPBC vs AMA = 0.26 N-CPBC vs C-PBC =0.98 C-PBC vs AMA = 0.42
Left Uncinate Fasciculus	0.49 ± 0.03	0.47 ± 0.02	0.49 ± 0.04	N-CPBC vs AMA = 0.88 N-CPBC vs C-PBC = 0.09 C-PBC vs AMA = 0.23
Right Uncinate Fasciculus	0.50 ± 0.02	0.49 ± 0.03	0.50 ± 0.04	N-CPBC vs AMA = 0.97 N-CPBC vs C-PBC = 0.58 C-PBC vs AMA = 0.79
ILF, inferior longitudinal fasciculus;SLF,superior longitudinal fasciculus; N-CPBC, non cirrhotic Primary biliary cholangitis; AMA,anti-mitochondrial antibody; C – PBC,cirrhotic primary biliary cholangitis; LFT = liver function test.				

7.3 Summary

This chapter has shown that patients with positive AMA antibodies with normal LFTs have a pronounced self-reported symptom burden including fatigue, cognitive symptoms and sleep disturbance. The severity of these symptoms is comparable to patients who meet diagnostic criteria for PBC. Despite subjective assessment showing significant cognitive symptom burden, there was no significant difference in objective assessment using CANTAB between AMA positive normal LFTs patients and normative population reference data. This may suggest that cognitive symptoms in AMA positive normal LFTs patients (i.e. without the presence of cholestasis) are influenced by complex psychological factors and not necessarily linked to biological processes underlying PBC. A significant number had a history of depression that may potentially contribute to fatigue and other symptoms. Of note, we assumed the AMA positive normal LFTs population to be a 'pre-PBC' group and therefore used the PBC-40 questionnaire to assess their symptom burden. However, this questionnaire has not been validated in this patient group so findings should be interpreted with caution.

It is recognised that some cirrhotic patients have debilitating symptoms with a negative impact on their quality of life [179]. We identified a significant symptom burden in the cirrhotic PBC patients in our study even in the absence of complications of advanced liver disease. There was no statistically significant difference in the perceived cognitive symptom burden depending on cirrhosis status, also consistent with previous studies demonstrating that PBC-associated symptoms are independent of disease severity [66, 126].

Using CANTAB for objective cognitive assessment, we identified significant deficits in the memory and emotional domains in cirrhotic PBC patients. Hepatic encephalopathy (HE) is usually a late sign in PBC and associated with synthetic dysfunction. The cirrhotic patients in our study had well compensated disease with no history of decompensation, hepatic encephalopathy, variceal bleeding or ascites. Cirrhotic patients were found to have cognitive deficits in three cognitive domains: memory (PAL), attention (RVP) and emotion (ERT) as compared to five domains in non-cirrhotic patients (PAL, OTS, SWM, ERT and RVP). This suggests that progression of cognitive symptoms does not mirror disease progression in

PBC, unlike in neurodegenerative conditions such as Alzheimer's or Parkinson's disease. It is not impossible that the cognitive deficits observed in the cirrhotic patients represent minimal HE but it seems more likely that they are related to PBC itself. This study did not explore the pathogenic mechanisms underlying cognitive symptoms in PBC, which is an important area for future study.

When using CANTAB to compare the groups, both cirrhotic and non-cirrhotic PBC patients performed worse than AMA positive LFTs patients in the memory domain. In DTI analysis, there were no significant differences between FA values across the 3 groups in any of the brain regions studied. The AMA positive group with normal liver function test was closely resembled normal healthy controls,suggesting no detectable white matter tract changes in PBC patients in this study. However, these analyses were limited by the small sample size and need to be replicated in the larger studies.

In conclusion, we have demonstrated that both non-cirrhotic and cirrhotic PBC patients have significant cognitive deficits when objectively assessed using CANTAB but this is not seen in those with AMA positive normal LFTs. Therefore, objective symptoms may only become apparent with established cholestasis. There was no association between symptoms and white matter assessment using MRI.

Chapter 8: General Discussion

8.1 Review of thesis objectives

A high burden of cognitive symptoms in PBC patients has been previously reported [126] but despite some hypotheses attributing this to structural changes in the brain due to immune reactions or cholestasis, the underlying pathophysiology remains unclear. There are reports of perceived cognitive dysfunction in PBC but only a few studies examined the characteristics of cognitive symptoms[61, 66, 126]. Improved understanding of the nature of cognitive symptoms and CNS changes in PBC throughout the course of disease is crucial to the development of future therapeutic trials. This study aimed to define the nature of CNS changes in PBC patients according to symptom severity and during different time points in the disease process (i.e., AMA positive normal LFTs, non-cirrhotic, cirrhotic patients with no evidence of decompensation). These findings may serve as valuable endpoints for future PBC trials and be important in appropriate trial design.

8.2 Discussion of findings

Cognitive symptoms in PBC disease are increasingly recognised but there is little data concerning the specifc nature of the cognitive deficits in PBC. The two studies using formal neuropsychometric testing reported contrary findings. One reported global cognitive impairment with pronounced deficits in verbal fluency and cognitive processing whilst the other study evaluated attention function and showed no deficit [126, 163]. Using a comprehensive neuropsychometric battery (CANTAB), we extensively evaluated different domains of cognitive function by employing 7 cognitive tasks. We demonstrated multidomain cognitive dysfunction in non-cirrhotic PBC patients with deficits in episodic memory, information processing and emotional cognition whilst executive and psychomotor function were relatively preserved. The impairment of executive function (OTS and SWM) was age-related and may also be influenced by patients' IQ and education level, making interpretation of results more difficult. When assessing attention and psychomotor speed, direct response to visual stimuli remained intact but there was impaired processing between visual stimuli and motor response. This demontsrates abnormal attention and information processing but normal motor response speed. We believe that using CANTAB to objectively assess cognitive function could be valuable as end points for treatment response in therapeutic trials of agents targeting cognitive symptoms in PBC.

The profound cognitive deficits in memory, emotion and attention domains could potentially be explained by anatomical connections. Abnormalities of the hippocampus and deep grey matter have been previously reported in PBC patients [150-152], comparable to brain changes in neurodegenerative disease. Both areas have a major impact on memory and emotions [174]. In animal experiments, bile duct-ligated mice show cholestatic-induced neuronal senescence within the hippocampal network and were destabilized due to cholestatic senescence [167]. The hippocampus is a core area of study in various psychological disorders and neurodegenerative diseases (e.g. Alzheimer's) [175, 180, 181]. It is vulnerable to various stresses and stimuli. Overall, the cognitive deficits identified in our study may enhance the ability to detect and characterise anatomical abnormalities that are potentially associated with cognitive dysfunction in PBC.

Whilst many PBC patients describe marked cognitive symptoms, they are not present in all patients. Both central and peripheral signalling pathways have been hypothesised as links between underlying cholestasis and brain changes [166] but the exact mechanism is unknown. We explored the characteristics of PBC patients both with and without marked cognitive symptoms using subjective and objective assessments.

We found that significantly symptomatic and asymptomatic non-cirrhotic PBC patients were phenotypically very similar with respect to biochemistry, liver synthetic function and disease duration. Patients with high perceived cognitive symptom burden were younger and had more fatigue, emotional and social problems, anxiety, depression and sleep disturbance. This is consistent with previous findings with regards to the interrelationship between these symptoms [70]. Fatigue has previously been reported to be interlinked with other behavioural symptoms such as insomnia, emotional and social dysfunction, and autonomic dysfunction [61]. Our study suggests that it may be fatigue, rather than cognitive symptoms, that is the main driver of other behavioural symptoms. However, the relationship between fatigue and cognitive symptoms, and the mechanism underlying their development, remain unclear. Our findings suggest the underlying mechanisms of both

symptoms may overlap and potentially be centrally driven. This is an important area for future studies.

Patients with and without reported cognitive symptoms showed similar objective deficits on the CANTAB battery compared to normative population, suggesting a disconnect between subjective reports and objective cognitive performance.This may be due to variations in coping mechanisms, adaptation, or lack of insight into symptoms. For example, if an individual never engages or performs a task, they won't know they are unable to perform the task. We used the PBC-40, which is a subjective patient-reported questionnaire, to define study groups with significant or mild/no cognitive symptoms. This tool effectively captures PBC-related symptoms and reflects patients' experience, but it does not quantify cognitive symptoms and may not reliably identify patients with cognitive impairment. The current management of cognitive symptoms in PBC relies on non-pharmacological techniques (coping strategies, social engagement, memory games and empathy) But these have not been formally studied. It would be valuable to assess the impact of these strategies on cognitive symptoms in PBC patients.

Similar to the neuropsychometric results, the findings in our DTI analysis showed no association between white matter integrity and severity of cognitive symptoms in PBC. The role of white matter lesions in cognitive dysfunction has been increasingly reported in neurodegenerative diseases including Alzheimer's disease, and chronic traumatic encephalopathy [156, 157]. Data regarding structural abnormalities of white matter in PBC is limited, with studies yielding contradictory findings [126, 163]. It was known that hippocampal dysfunction was associated with cognitive impairment [174]. In combination with our findings on CANTAB neuropsychometric testing, we hypothesize that white matter integrity does not seem to play a significant role in the pathophysiology of cognitive symptoms in PBC and is to be a useful trial end-point. However, this conclusion is limited by the absence of a normal healthy control group in our study. Further studies looking at brain regions in partnership with comprehensive objective cognitive assessment in both patients and healthy controls may provide important additional information.

It has been shown that cognitive symptoms are present in early PBC, but their natural history is unclear. We explored cognitive symptoms in various stages of PBC (i.e., AMA positive normal LFTs, non-cirrhotic PBC and early cirrhotic PBC). We showed that both non-cirrhotic and cirrhotic PBC patients had equally significant cognitive symptoms both subjectively and objectively. We excluded patients with decompensated cirrhosis to avoid bias in the results due to the possibility of hepatic encephalopathy. Our data suggest that PBC patients experience a high cognitive burden early in disease and irrespective of disease severity. This is in contrast to neurodegenerative diseases, such as Alzheimer's, in which cognitive function declines progressively with global cognitive dysfunction in later stages. Using CANTAB neuropsychometric assessment, cirrhotic patients had cognitive deficit in three tasks: memory (PAL), emotion (ERT) and attention (RVP), whilst 5 tasks were affected in non-cirrhotic patients (PAL, OTS, SWM, ERT and RVP) as compared to the normative population.

Interestingly, AMA positive patients with normal LFTs self-report cognitive symptoms comparable to cirrhotic and non-cirrhotic PBC patients although we acknowledge that the PBC-40 questionnaire is not validated in this group. Using CANTAB for objective assessment, we did not identify cognitive deficit in AMA positive normal LFTs patients as compared to the normative population. This may suggest that perceived cognitive symptoms described by AMA positive normal LFTs are due to complex psychological factors or other unknown mechanisms. Proinflammatory cytokines are released in response to biliary injury [1]. This along with secondary immunological responses are believed to drive cognition problems in PBC[132, 137]. We hypothesise that cognitive symptoms in PBC may be due to the sequelae of cholestasis or a pro-inflammatory cytokine response in the CNS in response to biliary injury rather than the immunological pathogenesis of PBC. This means that cognitive deficit appears when cholestasis becomes overt. It would be interesting to examine whether cognitive deficits are also present in other cholestatic diseases such as primary sclerosing cholangitis (PSC). Recent murine data from bile duct ligated (BDL) mice demonstrated blood brain barrier disruption from reduced astrocyte coverage and neuronal senescence [167] with cholestasis triggering the systemic immune reaction [167]. This may result in local CNS inflammation that subsequently affects brain function and behaviour. If our hypothesis is correct, then aggressive and early treatment of

cholestasis may improve or reverse cognitive deficits in PBC. Further studies evaluating the role of cholestasis and its effect on cognitive decline in PBC should be undertaken.

Anticholestatic treatments and FXR agonists lead to significant reduction in cognitive dysfunction in BDL mice by enhancing the neurovascular unit in the blood brain barrier (BBB) [167]. It has been proposed that Obeticholic acid (OCA) may improve the integrity of the BBB,thereby improving short term memory in cholestatic rats. Our study found that patients on OCA had cognitive performance comparable to the normative population in most cognitive tasks (OTS, MTL, SWM, ERT, RVP, RTI). However, this study was not designed to specifically evaluate the effect of OCA on cognitive function and the sample size was too small to draw any definitive conclusions. An important piece of future work would be to examine whether OCA, given at an early stage in disease to aggressively treat cholestasis, can change the course of cognitive symptoms in PBC.

8.3 Study limitations

The study was successful in terms of reaching its objectives but it important that we acknowledge its limitations. Time constraints and shielding restrictions during the COVID-19 pandemic resulted into fewer patients than planned being recruited into the perceived cognitive asymptomatic (16/20) and AMA positive normal LFTs cohorts (9/10). We also found it challenging to recruit treatment naive PBC patients (3/10 recruited), due to PBC being a rare disease and also the majority had been commenced on UDCA primary to referral to our service. This led to this study group not being included in analyses.

The overall study size was small, introducing the possibility of type 2 errors (false negatives) due to reduced statistical power. This also limited multiple comparisons, and therefore, confounding factors could not be excluded in the findings. Larger numbers of participants in further studies may produce more robust findings. Acknowledging this limitation, however, this study was exploratory with a view to developing proof of concept data to inform future research.

DTI analysis did not include a matched healthy control group. Although we achieved our objective of comparing perceived significant cognitively symptomatic and asymptomatic cohorts, the absence of a control group limits broader interpretation of these findings . AMA positive patients with normal LFTs could serve as a near-normal comparison group; however,their small number in the MRI assessment may limit the validity of the findings.

This was a cross-sectional study of groups of patients (AMA positive normal LFTs, non-cirrhotic, and cirrhotic PBC patients) at a single time point. However, in order to measure changes in cognitive symptoms in PBC patients, we would need serial measures at different disease stages in the same patient using a prospective longitudinal study design. This is practically very difficult given that PBC is a chronic, slowly progressive disease. To our knowledge, this is the first study to compare neuropsychiatric symptoms at different disease stages and the CANTAB findings demonstrate that objectively cognitive symptoms only become apparent in patients with established PBC.

Cognitive performance interpretation using neuropsychiatric batteries can be limited by internal and external factors such as intelligence, education level and motivation. In addition, cognitive symptoms in PBC are closely related to other neuropsychiatric symptoms. The possibility of confounding influences on neuropsychiatric battery performance factors such as fatigue, depression, anxiety, and sleep abnormalities cannot be ruled out. It is, unfortunately, challenging to conduct an ideal clinical trial with a homogeneous PBC population without confounding factors.

Overall, although there were limitations in the study, this work has characterised the cognitive function and investigated the natural history of cognitive symptoms at different time points in PBC. As this is a proof of concept study, the results in the study should be considered preliminary and as a starting point for further studies.

8.4 Recommendation for further study

I would suggest a number of further studies to build on this work. First, validation of characteristics and pattern recognition of cognitive symptoms in PBC patients using a larger sample. This would provide a clearer understanding and recognition of cognitive symptoms in PBC, potentially enabling improved management strategies and acting as the basis for pharmacological and non-pharmacological studies.

Second, an exploratory study with neuroimaging in larger cohorts using the characteristics and pattern recognition of cognitive symptoms. This may lead to improved understanding of anatomical associations and MRI characteristics of cognitive symptoms. We would suggest that future trial design should try to take account of behavioural variation and lack of insight from patients into their symptoms.

Finally, this study demonstrated that cognitive symptoms only become apparent objectively in established cholestasis. Since cognitive symptoms did not appear to progress, aggressive treatment for cholestasis at earlier stage may improve symptoms. Therefore, further studies targeting anti-cholestasis are recommended.

Chapter 9: Conclusion

This thesis provides meaningful insights and improved understanding of cognitive symptoms in PBC patients and may ultimately help future trial design. The main findings of this study and take-home messages are as follows:

1) There was multi-domain cognitive impairment in PBC patients, involving memory, emotional and attention deficits with relative preservation of executive function and psychomotor function.

2) PBC patients with perceived significant cognitive symptoms had higher levels of fatigue, depression, anxiety and sleep disturbance. However, cognitive symptoms with fatigue rather than cognitive symptoms alone may be the main driver of other behavioural symptoms.

3) There was no difference in biochemical parameters, synthetic function or disease duration between perceived cognitively symptomatic and asymptomatic PBC patients.

4) There was no difference in CANTAB neuropsychiatric battery cognitive performance between perceived significant cognitively symptomatic and asymptomatic PBC patients. Thus, cognitive symptoms may be influenced by patient insight or behavioural adaptations. It is important to remember that PBC with significant perceived cognitive symptoms and fatigue are the group with the highest unmet clinical need and should be a priority for interventional studies.

5) There was no correlation between white matter integrity (FA measurement) of the brain and cognitive symptoms in PBC patients. This suggests that white matter

integrity may not play a significant role in the pathogenesis of cognitive symptoms and therefore may not be helpful as an end point for future studies.

6) Objective cognitive deficits were found only in established PBC patients using CANTAB. Therefore, we propose that cognitive symptoms may become apparent only when cholestasis is established. Aggressive anti-cholestatic intervention in early disease may improve cognitive symptoms and this is an important area of further work.

Chapter 10: References

1. EASL Clinical Practice Guidelines: The diagnosis and management of patients with primary biliary cholangitis. J Hepatol, 2017. **67**(1): p. 145-172.
2. Selmi, C., C.L. Bowlus, M.E. Gershwin, and R.L. Coppel, Primary biliary cirrhosis. Lancet, 2011. **377**(9777): p. 1600-9.
3. Lee, J.Y., et al., Treatment of Fatigue in Primary Biliary Cholangitis: A Systematic Review and Meta-Analysis. Dig Dis Sci, 2019. **64**(8): p. 2338-2350.
4. GOV.UK. Seladelpar approved to treat adult patients in the UK with liver illness. 2025 [cited 2025 July]; Available from: https://www.gov.uk/government/news/seladelpar-approved-to-treat-adult-patients-in-the-uk-with-liver-illness.
5. Ahmed, I.F., et al., Elafibranor (Iqirvo) unveiled: a groundbreaking FDA-approved therapy revolutionizing primary biliary cholangitis treatment. Ann Med Surg (Lond), 2024. **86**(12): p. 6910-6912.
6. Boonstra, K., et al., Rising incidence and prevalence of primary biliary cirrhosis: a large population-based study. Liver Int, 2014. **34**(6): p. e31-8.
7. Chascsa, D., E.J. Carey, and K.D. Lindor, Old and new treatments for primary biliary cholangitis. Liver Int, 2017. **37**(4): p. 490-499.
8. Marschall, H.U., et al., Incidence, prevalence, and outcome of primary biliary cholangitis in a nationwide Swedish population-based cohort. Sci Rep, 2019. **9**(1): p. 11525.
9. Hirschfield, G.M., et al., The British Society of Gastroenterology/UK-PBC primary biliary cholangitis treatment and management guidelines. Gut, 2018. **67**(9): p. 1568-1594.
10. Tanaka, A., P.S. Leung, and M.E. Gershwin, Environmental basis of primary biliary cholangitis. Exp Biol Med (Maywood), 2018. **243**(2): p. 184-189.
11. Odin, J.A., et al., Bcl-2-dependent oxidation of pyruvate dehydrogenase-E2, a primary biliary cirrhosis autoantigen, during apoptosis. J Clin Invest, 2001. **108**(2): p. 223-32.
12. Medina, J.F., Role of the anion exchanger 2 in the pathogenesis and treatment of primary biliary cirrhosis. Dig Dis, 2011. **29**(1): p. 103-12.
13. Banales, J.M., et al., Up-regulation of microRNA 506 leads to decreased Cl-/HCO3- anion exchanger 2 expression in biliary epithelium of patients with primary biliary cirrhosis. Hepatology, 2012. **56**(2): p. 687-97.
14. Deutschmann, K., et al., Bile acid receptors in the biliary tree: TGR5 in physiology and disease. Biochim Biophys Acta Mol Basis Dis, 2018. **1864**(4 Pt B): p. 1319-1325.

15. Beuers, U., et al., The biliary HCO(3)(-) umbrella: a unifying hypothesis on pathogenetic and therapeutic aspects of fibrosing cholangiopathies. Hepatology, 2010. **52**(4): p. 1489-96.
16. Yasoshima, M., et al., Increased expression of interleukin-6 and tumor necrosis factor-alpha in pathologic biliary epithelial cells: in situ and culture study. Lab Invest, 1998. **78**(1): p. 89-100.
17. O'Hara, S.P., J.H. Tabibian, P.L. Splinter, and N.F. LaRusso, The dynamic biliary epithelia: molecules, pathways, and disease. J Hepatol, 2013. **58**(3): p. 575-82.
18. Gulamhusein, A.F. and G.M. Hirschfield, Pathophysiology of primary biliary cholangitis. Best Pract Res Clin Gastroenterol, 2018. **34-35**: p. 17-25.
19. Bour, E.S., L.K. Ward, G.A. Cornman, and H.C. Isom, Tumor necrosis factor-alpha-induced apoptosis in hepatocytes in long-term culture. Am J Pathol, 1996. **148**(2): p. 485-95.
20. Sasaki, M., et al., Telomere shortening in the damaged small bile ducts in primary biliary cirrhosis reflects ongoing cellular senescence. Hepatology, 2008. **48**(1): p. 186-95.
21. Campisi, J. and F. d'Adda di Fagagna, Cellular senescence: when bad things happen to good cells. Nat Rev Mol Cell Biol, 2007. **8**(9): p. 729-40.
22. Sasaki, M., M. Miyakoshi, Y. Sato, and Y. Nakanuma, Modulation of the microenvironment by senescent biliary epithelial cells may be involved in the pathogenesis of primary biliary cirrhosis. J Hepatol, 2010. **53**(2): p. 318-25.
23. Nagano, T., et al., Cytokine profile in the liver of primary biliary cirrhosis. J Clin Immunol, 1999. **19**(6): p. 422-7.
24. Hirschfield, G.M., et al., Primary biliary cirrhosis associated with HLA, IL12A, and IL12RB2 variants. N Engl J Med, 2009. **360**(24): p. 2544-55.
25. Yang, C.Y., et al., IL-12/Th1 and IL-23/Th17 biliary microenvironment in primary biliary cirrhosis: implications for therapy. Hepatology, 2014. **59**(5): p. 1944-53.
26. Harada, K., et al., Periductal interleukin-17 production in association with biliary innate immunity contributes to the pathogenesis of cholangiopathy in primary biliary cirrhosis. Clin Exp Immunol, 2009. **157**(2): p. 261-70.
27. Yoshida, K., et al., Deletion of interleukin-12p40 suppresses autoimmune cholangitis in dominant negative transforming growth factor beta receptor type II mice. Hepatology, 2009. **50**(5): p. 1494-500.

28. Dyson, J.K., et al., Novel therapeutic targets in primary biliary cirrhosis. Nat Rev Gastroenterol Hepatol, 2015. **12**(3): p. 147-58.
29. Pellicciari, R., et al., 6alpha-ethyl-chenodeoxycholic acid (6-ECDCA), a potent and selective FXR agonist endowed with anticholestatic activity. J Med Chem, 2002. **45**(17): p. 3569-72.
30. Fiorucci, S., et al., The nuclear receptor SHP mediates inhibition of hepatic stellate cells by FXR and protects against liver fibrosis. Gastroenterology, 2004. **127**(5): p. 1497-512.
31. Goodwin, B., et al., A regulatory cascade of the nuclear receptors FXR, SHP-1, and LRH-1 represses bile acid biosynthesis. Mol Cell, 2000. **6**(3): p. 517-26.
32. Shin, D.J. and L. Wang, Bile Acid-Activated Receptors: A Review on FXR and Other Nuclear Receptors. Handb Exp Pharmacol, 2019. **256**: p. 51-72.
33. Nevens, F., et al., A Placebo-Controlled Trial of Obeticholic Acid in Primary Biliary Cholangitis. N Engl J Med, 2016. **375**(7): p. 631-43.
34. Lindor, K.D., et al., Primary biliary cirrhosis. Hepatology, 2009. **50**(1): p. 291-308.
35. Carbone, M., et al., Sex and age are determinants of the clinical phenotype of primary biliary cirrhosis and response to ursodeoxycholic acid. Gastroenterology, 2013. **144**(3): p. 560-569.e7; quiz e13-4.
36. Corpechot, C., et al., Noninvasive elastography-based assessment of liver fibrosis progression and prognosis in primary biliary cirrhosis. Hepatology, 2012. **56**(1): p. 198-208.
37. Roll, J., J.L. Boyer, D. Barry, and G. Klatskin, The prognostic importance of clinical and histologic features in asymptomatic and symptomatic primary biliary cirrhosis. N Engl J Med, 1983. **308**(1): p. 1-7.
38. Khanna, A., J. Leighton, L. Lee Wong, and D.E. Jones, Symptoms of PBC - Pathophysiology and management. Best Pract Res Clin Gastroenterol, 2018. **34-35**: p. 41-47.
39. Beuers, U., A.E. Kremer, R. Bolier, and R.P. Elferink, Pruritus in cholestasis: facts and fiction. Hepatology, 2014. **60**(1): p. 399-407.
40. Hegade, V.S., et al., Autotaxin, bile acid profile and effect of ileal bile acid transporter inhibition in primary biliary cholangitis patients with pruritus. Liver Int, 2019. **39**(5): p. 967-975.
41. Kremer, A.E., et al., Serum Autotaxin Activity Correlates With Pruritus in Pediatric Cholestatic Disorders. J Pediatr Gastroenterol Nutr, 2016. **62**(4): p. 530-5.

42. Hegade, V.S., et al., A systematic approach to the management of cholestatic pruritus in primary biliary cirrhosis. Frontline Gastroenterol, 2016. **7**(3): p. 158-166.
43. Kremer, A.E., et al., Pathogenesis and Management of Pruritus in PBC and PSC. Dig Dis, 2015. **33 Suppl 2**: p. 164-75.
44. Mang, F.W., et al., Primary biliary cirrhosis, sicca complex, and dysphagia. Dysphagia, 1997. **12**(3): p. 167-70.
45. Golding, P.L., R. Bown, A.M. Mason, and E. Taylor, "Sicca complex" in liver disease. Br Med J, 1970. **4**(5731): p. 340-2.
46. Watt, F.E., O.F. James, and D.E. Jones, Patterns of autoimmunity in primary biliary cirrhosis patients and their families: a population-based cohort study. Qjm, 2004. **97**(7): p. 397-406.
47. Kruszka, P. and R.J. O'Brian, Diagnosis and management of Sjögren syndrome. Am Fam Physician, 2009. **79**(6): p. 465-70.
48. Goldblatt, J., et al., The true impact of fatigue in primary biliary cirrhosis: a population study. Gastroenterology, 2002. **122**(5): p. 1235-41.
49. Jopson, L. and D.E. Jones, Fatigue in Primary Biliary Cirrhosis: Prevalence, Pathogenesis and Management. Dig Dis, 2015. **33 Suppl 2**: p. 109-14.
50. Mells, G.F., et al., Impact of primary biliary cirrhosis on perceived quality of life: the UK-PBC national study. Hepatology, 2013. **58**(1): p. 273-83.
51. Cauch-Dudek, K., S. Abbey, D.E. Stewart, and E.J. Heathcote, Fatigue in primary biliary cirrhosis. Gut, 1998. **43**(5): p. 705-10.
52. Hale, M., J.L. Newton, and D.E. Jones, Fatigue in primary biliary cirrhosis. Bmj, 2012. **345**: p. e7004.
53. Khanna, A., J. Leighton, L. Lee Wong, and D.E. Jones, Symptoms of PBC - Pathophysiology and management. Best practice & research. Clinical gastroenterology, 2018. **34-35**: p. 41-47.
54. Swain, M.G. and M. Maric, Defective corticotropin-releasing hormone mediated neuroendocrine and behavioral responses in cholestatic rats: implications for cholestatic liver disease-related sickness behaviors. Hepatology, 1995. **22**(5): p. 1560-4.
55. Swain, M.G. and T. Le, Chronic cholestasis in rats induces anhedonia and a loss of social interest. Hepatology, 1998. **28**(1): p. 6-10.
56. Huang, L.T., et al., Obstructive jaundice in rats: cause of spatial memory deficits with recovery after biliary decompression. World J Surg, 2004. **28**(3): p. 283-7.

57. Grover, V.P., et al., Early primary biliary cholangitis is characterised by brain abnormalities on cerebral magnetic resonance imaging. Aliment Pharmacol Ther, 2016. **44**(9): p. 936-945.
58. Pells, G., et al., The impact of liver transplantation on the phenotype of primary biliary cirrhosis patients in the UK-PBC cohort. J Hepatol, 2013. **59**(1): p. 67-73.
59. Hollingsworth, K.G., et al., Loss of capacity to recover from acidosis in repeat exercise is strongly associated with fatigue in primary biliary cirrhosis. J Hepatol, 2010. **53**(1): p. 155-61.
60. Swain, M.G. and D.E.J. Jones, Fatigue in chronic liver disease: New insights and therapeutic approaches. Liver Int, 2019. **39**(1): p. 6-19.
61. Phaw, N.A., J.K. Dyson, G. Mells, and D. Jones, Understanding Fatigue in Primary Biliary Cholangitis. Dig Dis Sci, 2021. **66**(7): p. 2380-2386.
62. Khanna, A., et al., Rituximab Is Ineffective for Treatment of Fatigue in Primary Biliary Cholangitis: A Phase 2 Randomized Controlled Trial. Hepatology, 2019. **70**(5): p. 1646-1657.
63. Jones, D.E. and J.L. Newton, An open study of modafinil for the treatment of daytime somnolence and fatigue in primary biliary cirrhosis. Aliment Pharmacol Ther, 2007. **25**(4): p. 471-6.
64. Surrenti, C., et al., Effects of plasma exchange (PE) in primary biliary cirrhosis (PBC). A pilot study. Hepatogastroenterology, 1990. **37**(1): p. 128-30.
65. Newton, J.L., J. Allen, S. Kerr, and D.E. Jones, Reduced heart rate variability and baroreflex sensitivity in primary biliary cirrhosis. Liver Int, 2006. **26**(2): p. 197-202.
66. Newton, J.L., et al., Population prevalence and symptom associations of autonomic dysfunction in primary biliary cirrhosis. Hepatology, 2007. **45**(6): p. 1496-505.
67. Hendrickse, M.T. and D.R. Triger, Autonomic and peripheral neuropathy in primary biliary cirrhosis. J Hepatol, 1993. **19**(3): p. 401-7.
68. Keresztes, K., et al., Autonomic and sensory nerve dysfunction in primary biliary cirrhosis. World J Gastroenterol, 2004. **10**(20): p. 3039-43.
69. Dyson, J., et al., The inter-relationship of symptom severity and quality of life in 2055 patients with primary biliary cholangitis. Aliment Pharmacol Ther, 2016. **44**(10): p. 1039-1050.
70. Dyson, J.K., et al., The inter-relationship of symptom severity and quality of life in 2055 patients with primary biliary cholangitis. Aliment Pharmacol Ther, 2016. **44**(10): p. 1039-1050.

71. Gungabissoon, U., et al., Disease burden of primary biliary cholangitis and associated pruritus based on a cross-sectional US claims analysis. BMJ Open Gastroenterol, 2022. **9**(1).
72. Yagi, M., et al., Symptoms and health-related quality of life in Japanese patients with primary biliary cholangitis. Sci Rep, 2018. **8**(1): p. 12542.
73. Liu, Y., et al., Symptoms Burden and Health-related Quality of Life in Chinese Patients with Primary Biliary Cholangitis. J Clin Transl Hepatol, 2021. **9**(6): p. 860-867.
74. Goldstein, J. and C. Levy, Novel and emerging therapies for cholestatic liver diseases. Liver Int, 2018. **38**(9): p. 1520-1535.
75. Shah, R.A. and K.V. Kowdley, Current and potential treatments for primary biliary cholangitis. Lancet Gastroenterol Hepatol, 2020. **5**(3): p. 306-315.
76. Carbone, M., et al., Pretreatment prediction of response to ursodeoxycholic acid in primary biliary cholangitis: development and validation of the UDCA Response Score. Lancet Gastroenterol Hepatol, 2018. **3**(9): p. 626-634.
77. Phaw, N.A., J.K. Dyson, and D. Jones, Emerging drugs for the treatment of primary biliary cholangitis. Expert Opin Emerg Drugs, 2020. **25**(2): p. 101-112.
78. Kumagi, T., et al., Baseline Ductopenia and Treatment Response Predict Long-Term Histological Progression in Primary Biliary Cirrhosis. Official journal of the American College of Gastroenterology | ACG, 2010. **105**(10): p. 2186-2194.
79. Parés, A., L. Caballería, and J. Rodés, Excellent Long-Term Survival in Patients With Primary Biliary Cirrhosis and Biochemical Response to Ursodeoxycholic Acid. Gastroenterology, 2006. **130**(3): p. 715-720.
80. Corpechot, C., O. Chazouillères, and R. Poupon, Early primary biliary cirrhosis: Biochemical response to treatment and prediction of long-term outcome. Journal of Hepatology, 2011. **55**(6): p. 1361-1367.
81. Corpechot, C., et al., Biochemical response to ursodeoxycholic acid and long-term prognosis in primary biliary cirrhosis. Hepatology, 2008. **48**(3): p. 871-877.
82. Kuiper, E.M.M., et al., Improved Prognosis of Patients With Primary Biliary Cirrhosis That Have a Biochemical Response to Ursodeoxycholic Acid. Gastroenterology, 2009. **136**(4): p. 1281-1287.
83. Trauner, M., et al., Long-term efficacy and safety of obeticholic acid for patients with primary biliary cholangitis: 3-year results of an international open-label extension study. Lancet Gastroenterol Hepatol, 2019. **4**(6): p. 445-453.

84. Jones, D.E., Pathogenesis of cholestatic itch: old questions, new answers, and future opportunities. Hepatology, 2012. **56**(4): p. 1194-6.
85. Grigorian, A.Y., et al., Fenofibrate is effective adjunctive therapy in the treatment of primary biliary cirrhosis: A meta-analysis. Clin Res Hepatol Gastroenterol, 2015. **39**(3): p. 296-306.
86. Suraweera, D., et al., Treatment of primary biliary cholangitis ursodeoxycholic acid non-responders: A systematic review. Liver Int, 2017. **37**(12): p. 1877-1886.
87. Corpechot, C., et al., A Placebo-Controlled Trial of Bezafibrate in Primary Biliary Cholangitis. N Engl J Med, 2018. **378**(23): p. 2171-2181.
88. Abbas, N., et al., UK-Wide Multicenter Evaluation of Second-line Therapies in Primary Biliary Cholangitis. Clin Gastroenterol Hepatol, 2022.
89. Carbone, M. and J. Neuberger, Liver transplantation in PBC and PSC: indications and disease recurrence. Clin Res Hepatol Gastroenterol, 2011. **35**(6-7): p. 446-54.
90. Skopouli, F.N., C. Barbatis, and H.M. Moutsopoulos, Liver involvement in primary Sjögren's syndrome. Br J Rheumatol, 1994. **33**(8): p. 745-8.
91. Muratori, P., et al., 'True' antimitochondrial antibody-negative primary biliary cirrhosis, low sensitivity of the routine assays, or both? Clin Exp Immunol, 2004. **135**(1): p. 154-8.
92. Mattalia, A., et al., Characterization of antimitochondrial antibodies in health adults. Hepatology, 1998. **27**(3): p. 656-61.
93. Turchany, J.M., et al., A study of antimitochondrial antibodies in a random population in Estonia. Am J Gastroenterol, 1997. **92**(1): p. 124-6.
94. Shibata, M., et al., Prevalence of antimitochondrial antibody in Japanese corporate workers in Kanagawa prefecture. J Gastroenterol, 2004. **39**(3): p. 255-9.
95. Chen, B.H., et al., Screening of anti-mitochondrial antibody subtype M2 in residents at least 18 years of age in an urban district of Shanghai, China. Eur Rev Med Pharmacol Sci, 2016. **20**(10): p. 2052-60.
96. Mitchison, H.C., et al., Positive antimitochondrial antibody but normal alkaline phosphatase: is this primary biliary cirrhosis? Hepatology, 1986. **6**(6): p. 1279-84.
97. Metcalf, J.V., et al., Natural history of early primary biliary cirrhosis. Lancet, 1996. **348**(9039): p. 1399-402.
98. Dahlqvist, G., et al., Large-scale characterization study of patients with antimitochondrial antibodies but nonestablished primary biliary cholangitis. Hepatology, 2017. **65**(1): p. 152-163.

99. Yeaman, S.J., J.A. Kirby, and D.E. Jones, Autoreactive responses to pyruvate dehydrogenase complex in the pathogenesis of primary biliary cirrhosis. Immunol Rev, 2000. **174**: p. 238-49.
100. Weinstein, G., et al., Non-alcoholic fatty liver disease, liver fibrosis score and cognitive function in middle-aged adults: The Framingham Study. Liver Int, 2019. **39**(9): p. 1713-1721.
101. Swain, M.G., Fatigue in liver disease: pathophysiology and clinical management. Can J Gastroenterol, 2006. **20**(3): p. 181-8.
102. Hepatic encephalopathy in chronic liver disease: 2014 practice guideline by the European Association for the Study of the Liver and the American Association for the Study of Liver Diseases. J Hepatol, 2014. **61**(3): p. 642-59.
103. Elliott, C., et al., Functional impairment in alcoholic liver disease and non-alcoholic fatty liver disease is significant and persists over 3 years of follow-up. Dig Dis Sci, 2013. **58**(8): p. 2383-91.
104. Celikbilek, A., M. Celikbilek, and G. Bozkurt, Cognitive assessment of patients with nonalcoholic fatty liver disease. Eur J Gastroenterol Hepatol, 2018. **30**(8): p. 944-950.
105. Lombardi, R., S. Fargion, and A.L. Fracanzani, Brain involvement in non-alcoholic fatty liver disease (NAFLD): A systematic review. Dig Liver Dis, 2019. **51**(9): p. 1214-1222.
106. Forton, D.M., et al., Hepatitis C and cognitive impairment in a cohort of patients with mild liver disease. Hepatology, 2002. **35**(2): p. 433-9.
107. Kjærgaard, K., et al., Cognitive Dysfunction in Non-Alcoholic Fatty Liver Disease-Current Knowledge, Mechanisms and Perspectives. J Clin Med, 2021. **10**(4).
108. Ortiz, M., C. Jacas, and J. Córdoba, Minimal hepatic encephalopathy: diagnosis, clinical significance and recommendations. J Hepatol, 2005. **42 Suppl**(1): p. S45-53.
109. Amodio, P., S. Montagnese, A. Gatta, and M.Y. Morgan, Characteristics of minimal hepatic encephalopathy. Metab Brain Dis, 2004. **19**(3-4): p. 253-67.
110. EASL Clinical Practice Guidelines on the management of hepatic encephalopathy. J Hepatol, 2022. **77**(3): p. 807-824.
111. Hartmann, I.J., et al., The prognostic significance of subclinical hepatic encephalopathy. Am J Gastroenterol, 2000. **95**(8): p. 2029-34.
112. Schomerus, H. and W. Hamster, Quality of life in cirrhotics with minimal hepatic encephalopathy. Metab Brain Dis, 2001. **16**(1-2): p. 37-41.

113. Marchesini, G., et al., Factors associated with poor health-related quality of life of patients with cirrhosis. Gastroenterology, 2001. **120**(1): p. 170-8.
114. Bajaj, J.S., et al., Minimal hepatic encephalopathy is associated with motor vehicle crashes: the reality beyond the driving test. Hepatology, 2009. **50**(4): p. 1175-83.
115. Román, E., et al., Minimal hepatic encephalopathy is associated with falls. Am J Gastroenterol, 2011. **106**(3): p. 476-82.
116. Lauridsen, M.M., P. Jepsen, and H. Vilstrup, Critical flicker frequency and continuous reaction times for the diagnosis of minimal hepatic encephalopathy: a comparative study of 154 patients with liver disease. Metab Brain Dis, 2011. **26**(2): p. 135-9.
117. Amodio, P., et al., Prevalence and prognostic value of quantified electroencephalogram (EEG) alterations in cirrhotic patients. J Hepatol, 2001. **35**(1): p. 37-45.
118. Nabi, E. and J.S. Bajaj, Useful tests for hepatic encephalopathy in clinical practice. Curr Gastroenterol Rep, 2014. **16**(1): p. 362.
119. Mittal, V.V., B.C. Sharma, P. Sharma, and S.K. Sarin, A randomized controlled trial comparing lactulose, probiotics, and L-ornithine L-aspartate in treatment of minimal hepatic encephalopathy. Eur J Gastroenterol Hepatol, 2011. **23**(8): p. 725-32.
120. Sharma, P., B.C. Sharma, A. Agrawal, and S.K. Sarin, Primary prophylaxis of overt hepatic encephalopathy in patients with cirrhosis: an open labeled randomized controlled trial of lactulose versus no lactulose. J Gastroenterol Hepatol, 2012. **27**(8): p. 1329-35.
121. Bajaj, J.S., S.D. Pinkerton, A.J. Sanyal, and D.M. Heuman, Diagnosis and treatment of minimal hepatic encephalopathy to prevent motor vehicle accidents: a cost-effectiveness analysis. Hepatology, 2012. **55**(4): p. 1164-71.
122. Bajaj, J.S., et al., Rifaximin improves driving simulator performance in a randomized trial of patients with minimal hepatic encephalopathy. Gastroenterology, 2011. **140**(2): p. 478-487.e1.
123. Sharma, A., Cambridge Neuropsychological Test Automated Battery, in Encyclopedia of Autism Spectrum Disorders, F.R. Volkmar, Editor. 2013, Springer New York: New York, NY. p. 498-515.
124. Chamberlain, S.R., et al., Translational approaches to frontostriatal dysfunction in attention-deficit/hyperactivity disorder using a computerized neuropsychological battery. Biol Psychiatry, 2011. **69**(12): p. 1192-203.

125. Fowler, K.S., et al., Computerized delayed matching to sample and paired associate performance in the early detection of dementia. Appl Neuropsychol, 1995. **2**(2): p. 72-8.
126. Newton, J.L., et al., Cognitive impairment in primary biliary cirrhosis: symptom impact and potential etiology. Hepatology (Baltimore, Md.), 2008. **48**(2): p. 541-9.
127. Iwasa, M., et al., Magnetization transfer contrast of various regions of the brain in liver cirrhosis. AJNR Am J Neuroradiol, 1999. **20**(4): p. 652-4.
128. Hollingsworth, K.G., et al., Impaired cerebral autoregulation in primary biliary cirrhosis: implications for the pathogenesis of cognitive decline. Liver Int, 2010. **30**(6): p. 878-85.
129. Zuccalà, G., et al., Hypotension and cognitive impairment: Selective association in patients with heart failure. Neurology, 2001. **57**(11): p. 1986-92.
130. van Boxtel, M.P., et al., Is nondipping in 24 h ambulatory blood pressure related to cognitive dysfunction? J Hypertens, 1998. **16**(10): p. 1425-32.
131. Matsubayashi, K., et al., Postural dysregulation in systolic blood pressure is associated with worsened scoring on neurobehavioral function tests and leukoaraiosis in the older elderly living in a community. Stroke, 1997. **28**(11): p. 2169-73.
132. Dantzer, R. and K.W. Kelley, Twenty years of research on cytokine-induced sickness behavior. Brain Behav Immun, 2007. **21**(2): p. 153-60.
133. Kisler, K., A.R. Nelson, A. Montagne, and B.V. Zlokovic, Cerebral blood flow regulation and neurovascular dysfunction in Alzheimer disease. Nat Rev Neurosci, 2017. **18**(7): p. 419-434.
134. Abbott, N.J., et al., Structure and function of the blood-brain barrier. Neurobiol Dis, 2010. **37**(1): p. 13-25.
135. Kerfoot, S.M., et al., TNF-alpha-secreting monocytes are recruited into the brain of cholestatic mice. Hepatology, 2006. **43**(1): p. 154-62.
136. Rochfort, K.D., L.E. Collins, R.P. Murphy, and P.M. Cummins, Downregulation of blood-brain barrier phenotype by proinflammatory cytokines involves NADPH oxidase-dependent ROS generation: consequences for interendothelial adherens and tight junctions. PLoS One, 2014. **9**(7): p. e101815.
137. D'Mello, C., T. Le, and M.G. Swain, Cerebral microglia recruit monocytes into the brain in response to tumor necrosis factoralpha signaling during peripheral organ inflammation. J Neurosci, 2009. **29**(7): p. 2089-102.

138. Girouard, H. and C. Iadecola, Neurovascular coupling in the normal brain and in hypertension, stroke, and Alzheimer disease. J Appl Physiol (1985), 2006. **100**(1): p. 328-35.
139. Akopov, S., R. Sercombe, and J. Seylaz, Cerebrovascular reactivity: role of endothelium/platelet/leukocyte interactions. Cerebrovasc Brain Metab Rev, 1996. **8**(1): p. 11-94.
140. D'Mello, C., et al., P-selectin-mediated monocyte-cerebral endothelium adhesive interactions link peripheral organ inflammation to sickness behaviors. J Neurosci, 2013. **33**(37): p. 14878-88.
141. Duszynski, C.C., et al., Near-Infrared Spectroscopy Reveals Brain Hypoxia and Cerebrovascular Dysregulation in Primary Biliary Cholangitis. Hepatology, 2020. **71**(4): p. 1408-1420.
142. Miyoshi, H., C. Rust, M.E. Guicciardi, and G.J. Gores, NF-kappaB is activated in cholestasis and functions to reduce liver injury. Am J Pathol, 2001. **158**(3): p. 967-75.
143. Luedde, T. and R.F. Schwabe, NF-κB in the liver--linking injury, fibrosis and hepatocellular carcinoma. Nat Rev Gastroenterol Hepatol, 2011. **8**(2): p. 108-18.
144. Saggu, R., et al., Astroglial NF-kB contributes to white matter damage and cognitive impairment in a mouse model of vascular dementia. Acta Neuropathol Commun, 2016. **4**(1): p. 76.
145. Li, Z., et al., Calcineurin/nuclear factor-κB signaling mediates isoflurane-induced hippocampal neuroinflammation and subsequent cognitive impairment in aged rats. Mol Med Rep, 2017. **15**(1): p. 201-209.
146. Jacoby, A., et al., Development, validation, and evaluation of the PBC-40, a disease specific health related quality of life measure for primary biliary cirrhosis. Gut, 2005. **54**(11): p. 1622-9.
147. Saklofske, D.H. and M.R. Schoenberg, Wechsler Adult Intelligence Scale (All Versions), in Encyclopedia of Clinical Neuropsychology, J.S. Kreutzer, J. DeLuca, and B. Caplan, Editors. 2011, Springer New York: New York, NY. p. 2675-2680.
148. Axelrod, B.N., Validity of the Wechsler abbreviated scale of intelligence and other very short forms of estimating intellectual functioning. Assessment, 2002. **9**(1): p. 17-23.

149. Weissenborn, K., N. Rückert, H. Hecker, and M.P. Manns, The number connection tests A and B: interindividual variability and use for the assessment of early hepatic encephalopathy. J Hepatol, 1998. **28**(4): p. 646-53.
150. Mosher, V., et al., Primary Biliary Cholangitis Alters Functional Connections of the Brain's Deep Gray Matter. Clin Transl Gastroenterol, 2017. **8**(7): p. e107.
151. Mosher, V., et al., Primary biliary cholangitis patients exhibit MRI changes in structure and function of interoceptive brain regions. PLoS One, 2019. **14**(2): p. e0211906.
152. Mosher, V.A.L., et al., Magnetic resonance imaging evidence of hippocampal structural changes in patients with primary biliary cholangitis. Clin Transl Gastroenterol, 2018. **9**(7): p. 169.
153. Biswal, B., F.Z. Yetkin, V.M. Haughton, and J.S. Hyde, Functional connectivity in the motor cortex of resting human brain using echo-planar MRI. Magn Reson Med, 1995. **34**(4): p. 537-41.
154. Miller, A.H., et al., Decreased basal ganglia activation in subjects with chronic fatigue syndrome: association with symptoms of fatigue. PLoS One, 2014. **9**(5): p. e98156.
155. Carballedo, A., et al., Functional connectivity of emotional processing in depression. J Affect Disord, 2011. **134**(1-3): p. 272-9.
156. Medina, D., et al., White matter changes in mild cognitive impairment and AD: A diffusion tensor imaging study. Neurobiology of Aging, 2006. **27**(5): p. 663-672.
157. Palacios, E.M., et al., Diffusion tensor imaging differences relate to memory deficits in diffuse traumatic brain injury. BMC Neurology, 2011. **11**(1): p. 24.
158. Thapaliya, K., S. Marshall-Gradisnik, D. Staines, and L. Barnden, Diffusion tensor imaging reveals neuronal microstructural changes in myalgic encephalomyelitis/chronic fatigue syndrome. Eur J Neurosci, 2021. **54**(6): p. 6214-6228.
159. Alexander, A.L., J.E. Lee, M. Lazar, and A.S. Field, Diffusion tensor imaging of the brain. Neurotherapeutics, 2007. **4**(3): p. 316-29.
160. O'Donnell, L.J. and C.F. Westin, An introduction to diffusion tensor image analysis. Neurosurg Clin N Am, 2011. **22**(2): p. 185-96, viii.
161. Mayo, C.D., et al., Relationship Between DTI Metrics and Cognitive Function in Alzheimer's Disease. Front Aging Neurosci, 2018. **10**: p. 436.
162. Auning, E., et al., White matter integrity and cognition in Parkinson's disease: a cross-sectional study. BMJ Open, 2014. **4**(1): p. e003976.

163. Zenouzi, R., et al., Patients with primary biliary cholangitis and fatigue present with depressive symptoms and selected cognitive deficits, but with normal attention performance and brain structure. PLoS One, 2018. **13**(1): p. e0190005.
164. Hollingsworth, K.G., et al., Globus pallidus magnetization transfer ratio, T(1) and T(2) in primary biliary cirrhosis: relationship with disease stage and age. J Magn Reson Imaging, 2009. **29**(4): p. 780-4.
165. York, E.N., et al., Quantitative magnetization transfer imaging in relapsing-remitting multiple sclerosis: a systematic review and meta-analysis. Brain Communications, 2022. **4**(2).
166. Phaw, N.A., J. Leighton, J.K. Dyson, and D.E. Jones, Managing cognitive symptoms and fatigue in cholestatic liver disease. Expert Rev Gastroenterol Hepatol, 2021. **15**(3): p. 235-241.
167. Gee, L.M.V., et al., Anti-Cholestatic Therapy with Obeticholic Acid Improves Short-Term Memory in Bile Duct-Ligated Mice. Am J Pathol, 2023. **193**(1): p. 11-26.
168. Naegele, B., et al., Cognitive executive dysfunction in patients with obstructive sleep apnea syndrome (OSAS) after CPAP treatment. Sleep, 1998. **21**(4): p. 392-7.
169. Luo, C., et al., White Matter Microstructural Damage as an Early Sign of Subjective Cognitive Decline. Front Aging Neurosci, 2019. **11**: p. 378.
170. Gobbi, C., et al., Forceps minor damage and co-occurrence of depression and fatigue in multiple sclerosis. Mult Scler, 2014. **20**(12): p. 1633-40.
171. Wu, Y., et al., Accuracy of the Hospital Anxiety and Depression Scale Depression subscale (HADS-D) to screen for major depression: systematic review and individual participant data meta-analysis. Bmj, 2021. **373**: p. n972.
172. Abbott, R.A., et al., Normative data from linear and nonlinear quantile regression in CANTAB: Cognition in mid-to-late life in an epidemiological sample. Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring, 2019. **11**(1): p. 36-44.
173. Grover, V.P., et al., Magnetic Resonance Imaging: Principles and Techniques: Lessons for Clinicians. J Clin Exp Hepatol, 2015. **5**(3): p. 246-55.
174. Maguire, E.A., Hippocampal involvement in human topographical memory: evidence from functional imaging. Philos Trans R Soc Lond B Biol Sci, 1997. **352**(1360): p. 1475-80.
175. de Rover, M., et al., Hippocampal dysfunction in patients with mild cognitive impairment: a functional neuroimaging study of a visuospatial paired associates learning task. Neuropsychologia, 2011. **49**(7): p. 2060-70.

176. Wang, M., et al., Neuronal basis of age-related working memory decline. Nature, 2011. **476**(7359): p. 210-3.
177. Gur, R.C., et al., Facial emotion discrimination: II. Behavioral findings in depression. Psychiatry Res, 1992. **42**(3): p. 241-51.
178. Freeman, C.R., et al., Emotion Recognition Biases in Alcohol Use Disorder. Alcohol Clin Exp Res, 2018.
179. Orr, J.G., et al., Health related quality of life in people with advanced chronic liver disease. J Hepatol, 2014. **61**(5): p. 1158-65.
180. Mu, Y. and F.H. Gage, Adult hippocampal neurogenesis and its role in Alzheimer's disease. Mol Neurodegener, 2011. **6**: p. 85.
181. Drevets, W.C., J.L. Price, and M.L. Furey, Brain structural and functional abnormalities in mood disorders: implications for neurocircuitry models of depression. Brain Struct Funct, 2008. **213**(1-2): p. 93-118.

Chapter 11: Appendix

A. Participant invitation letter

The Newcastle upon Tyne Hospitals NHS
NHS Foundation Trust

Date:

Patient's name and Address:

Assessing cognitive impairment in primary biliary cirrhosis (COG-PBC)

Dear.....

RE: **Assessing cognitive impairment in primary biliary cirrhosis (COG-PBC)**

I am contacting you because you are a patient with Primary Billiary Cirrhosis (PBC). You are being invited to take part in this study as your doctor has identified you as being eligible.

The purpose of the research is to find out more about the symptoms that some PBC patients get called cognitive symptoms. These are things like memory problems, recalling words and following a list of instructions. We also want to work out whether having these symptoms means that changes have happened to the brain. If the brain does change we want to see if it happens before or after the cognitive symptoms start.

Agreeing to take part in the study would involve 1 extra visits to the hospital. You wouldn't need to stay overnight but visit may take 3-4 hours. You would fill in questionnaires, do computerised tests and puzzles and have a MRI scans of your head.

I have enclosed a detailed participant information sheet which provides you with more information about the study. If you are interested in knowing more about this study, please read this information and feel free to discuss the study with your family and/or friends.

If after reading the participant information sheet you are interested in taking part in the study, please contact;

Kath Houghton (Senior Research Nurse)

Tel: 0191 2829885 or email: Kathryn.Houghton@newcastle.ac.uk

B. Consent form

The Newcastle upon Tyne Hospitals NHS
NHS Foundation Trust

IRAS REF: 237954

Centre no:

Informed Consent Form for Participants

Study Title: **Assessing cognitive impairment in primary biliary cirrhosis (COG-PBC)**

Name of Researcher: **Professor David Jones**

Participant identification number for this study:

Please initial each box if you are in agreement with each statement.

1. I confirm that I have read and understood the participant information sheet, dated ******** for the above study. I understand the purpose of the study. I have had the opportunity to consider the information, have had the chance to ask questions and all of my questions have been answered satisfactorily in a way I understand. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason or my legal rights or usual care being affected. ☐

3. I agree to confidential data collected about me as part of this study being stored securely on systems within the NHS and Newcastle University. ☐

4. I agree to undergo a magnetic resonance imaging (MRI) scan of my head ☐

5. I understand that relevant sections of my medical notes and data collected during this study may be looked at by individuals from regulatory authorities or from the NHS ☐

C. MRI Safety Questionnaire

Newcastle University
Campus for Ageing and Vitality
Newcastle upon Tyne, NE4 5PL
Tel: 0191 208 1250, Fax: 0191 208 1151, www.ncl.ac.uk/civi

Newcastle University
Centre for In Vivo Imaging

MR Safety Questionnaire

Name _____ Date of birth ___ /___ / _____

Weight: _____ Height: _____

Please check the following carefully. Some items can interfere with MRI examinations, and may also be hazardous to your safety. Clearly mark your answer with a circle.

Do you have a **cardiac pacemaker, defibrillator or pacing wires**?	YES	NO
Have you had **ANY** surgery to your heart (heart valve replacement/stents)? **Details:**	YES	NO
Have you **EVER** had any surgery to your head (including eyes/ears/brain), neck or spine? **Details:**	YES	NO
Do you have **ANY** implantable devices such as: programmable hydrocephalus shunt, nerve stimulators, cochlear implants, aneurysm clip, contraceptive device? **Details:**	YES	NO
Have you had any operation involving metallic pins /plates /screws/wires? **Details:**	YES	NO
Have you had **ANY** other surgical procedure or operations of any kind? **Details:**	YES	NO
Have you **EVER** sustained any injuries involving metal fragments to your eyes or any other part of the body?	YES	NO
Have you ever had a fit or blackout, or do you suffer from epilepsy or diabetes?	YES	NO
Do you have any of the following (if yes please circle): Dentures with metal — Hearing Aid — Hairclips or hairpieces/wigs Body piercing/ jewelry — Tattoos — Artificial limb or prosthesis Trans dermal patches		
Could you be pregnant?	YES	NO

IMPORTANT: Remove ALL loose metal objects prior to entering the magnet room

I understand the procedure of a MRI examination. I also understand the above questions.

Signature: _____ Date: _____

Staff Signature: _____ Date: _____

D. Ethic approval letter

Health Research Authority

North West - Greater Manchester Central Research Ethics Committee
3rd Floor
Barlow House
4 Minshull Street
Manchester
M1 3DZ

Please note: This is the favourable opinion of the REC only and does not allow you to start your study at NHS sites in England until you receive HRA Approval

22 March 2018

Professor David Jones
Professor of Liver Immunology
Newcastle University
Institiute Of Cellular Medicine
William Leech Building, 4th floor
Newcastle upon Tyne
NE27DN

Dear Professor Jones

Study title:	**Assessing cognitive impairment in primary biliary cirrhosis (COG-PBC)**
REC reference:	**18/NW/0119**
IRAS project ID:	**237954**

Thank you for your correspondence of 20 March 18, responding to the Proportionate Review Sub-Committee's request for changes to the documentation for the above study.

The revised documentation has been reviewed and approved by the sub-committee.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this favourable opinion letter. The expectation is that this information will be published for all studies that receive an ethical opinion but should you wish to provide a substitute contact point,

E. NIHR Newcastle Biochemical Research Centre (BRC) Funding

The Newcastle upon Tyne Hospitals NHS
NHS Foundation Trust

Newcastle upon Tyne Hospitals
Accounts Payable Department
Research Finance
Regent Point,
Regent Farm Road,
Gosforth
NE3 3HD

Our ref: MJ/ML/1217

Professor David Jones
Institute of Cellular Medicine
4th Floor Leech Building

19th December 2017

Dear Dave

NIHR Newcastle Biomedical Research Centre (BRC) Funding

Re: Chronic Cognitive Impairment in Cholestatic Liver Disease: Impact, Pathogenesis, Treatment and Relevance to Dementia
BH Ref 172901 / PDB064

The Newcastle upon Tyne Hospitals NHS Foundation Trust on behalf of the NIHR Newcastle Biomedical Research Centre is pleased to award a total of £277,942. University costs £262,788, Trust Costs £15,154

Comprising:

University Costs

Salary:	166,336.00
Consumables:	94,004.00
Travel:	2,448.00
Total:	**£262,788.00**

Trust Costs

Consumables:	473.00
Fees:	8,275.00
Other:	6,406.00
Total:	**£15,154.00**

Project Start Date:	**1st March 2018**
Project Duration (Months):	**24**

From: Ternent, Victoria
To: Dave Jones
Cc: Amardeep Khanna; Russell, Heather; Finance Research and Development Team; McShane, Lesley
Subject: 08695 - Confirmation of Capacity and Capability - 2018-05-03
Date: 03 May 2018 14:56:32
Attachments: image001.png
08695 - Statement of Activities - 2018-05-03.pdf

Dear Professor Jones

Confirmation of Capacity and Capability at The Newcastle upon Tyne Hospitals NHS Foundation Trust

-

Study Title: Chronic Cognitive Impairment in Cholestatic Liver Disease: Impact, Pathogenesis, Treatment and Relevance to Dementia
IRAS ID: 237954
R&D Ref: 8695
Site for Recruitment to CPMS: CAMPUS FOR AGEING AND VITALITY - RTD03

This email confirms that **The Newcastle upon Tyne Hospitals NHS Foundation Trust** has the capacity and capability to deliver the above referenced study. Please find attached the completed Statement of Activities.

You now may begin work on this study.

Best Wishes
Vicky

Victoria Ternent | Research & Development Officer | Joint Research Office | The Newcastle Upon Tyne Hospitals NHS Foundation Trust
Regent Point (Level 1)
Regent Farm Road
Gosforth
Newcastle upon Tyne
NE3 3HD

Telephone (direct line): 0191 2826773
Reception: 0191 2825959

https://microsites.ncl.ac.uk/njro/

F. HRA approval letter

NHS
Health Research Authority

Professor David Jones
Professor of Liver Immunology
Newcastle University
Institute Of Cellular Medicine
William Leech Building, 4th floor
Newcastle upon Tyne
NE27DN
david.jones@ncl.ac.uk

Email: hra.approval@nhs.net

04 April 2018

Dear Professor Jones

Letter of HRA Approval

Study title:	**Assessing cognitive impairment in primary biliary cirrhosis (COG-PBC)**
IRAS project ID:	**237954**
REC reference:	**18/NW/0119**
Sponsor	**Newcastle upon Type Hospitals NHS Foundation Trust**

I am pleased to confirm that **HRA Approval** has been given for the above referenced study, on the basis described in the application form, protocol, supporting documentation and any clarifications noted in this letter.

Participation of NHS Organisations in England

The sponsor should now provide a copy of this letter to all participating NHS organisations in England.

Appendix B provides important information for sponsors and participating NHS organisations in England for arranging and confirming capacity and capability. **Please read *Appendix B* carefully**, in particular the following sections:

- *Participating NHS organisations in England* – this clarifies the types of participating organisations in the study and whether or not all organisations will be undertaking the same activities
- *Confirmation of capacity and capability* - this confirms whether or not each type of participating NHS organisation in England is expected to give formal confirmation of capacity and capability. Where formal confirmation is not expected, the section also provides details on the time limit given to participating organisations to opt out of the study, or request additional time, before their participation is assumed.
- *Allocation of responsibilities and rights are agreed and documented (4.1 of HRA assessment criteria)* - this provides detail on the form of agreement to be used in the study to confirm capacity and capability, where applicable.

Further information on funding, HR processes, and compliance with HRA criteria and standards is also provided.

G. PBC- 40 Questionnaires

Patient ID	Date:

For each statement, please circle the response that comes closest to how you feel. If any of the statements do not apply to you please circle 'does not apply'.

Can you say how often the following statements about digestion and diet applied to you IN THE LAST FOUR WEEKS?

1	I was able to eat what I liked	Never	Rarely	Sometimes	Most of the time	Always	
2	I ate or drank only a small amount, and still felt bloated	Never	Rarely	Sometimes	Most of the time	Always	
3	I felt unwell when I drank alcohol	Never	Rarely	Sometimes	Most of the time	Always	Did not apply /never drink alcohol

And IN THE LAST FOUR WEEKS, how often did you experience any of the following?

4	I had discomfort in my right side	Never	Rarely	Sometimes	Most of the time	Always	
5	I had dry eyes	Never	Rarely	Sometimes	Most of the time	Always	
6	My mouth was very dry	Never	Rarely	Sometimes	Most of the time	Always	
7	I had aches in the long bones of my arms and legs	Never	Rarely	Sometimes	Most of the time	Always	

Some people with PBC experience itching. How often did you experience itching IN THE LAST FOUR WEEKS? If you did not itch, please circle 'does not apply'

8	Itching disturbed my sleep	Never	Rarely	Sometimes	Most of the time	Always	Did not apply/ no itch
9	I scratched so much I made my skin raw	Never	Rarely	Sometimes	Most of the time	Always	Did not apply/no itch
10	I felt embarrassed because of the itching	Never	Rarely	Sometimes	Most of the time	Always	Did not apply/no itch

Fatigue can also be a problem for many people with PBC. How often did the following statements apply to you IN THE LAST FOUR WEEKS?

11	I had to force myself to get out of bed	Never	Rarely	Sometimes	Most of the time	Always
12	I had to have a sleep during the day	Never	Rarely	Sometimes	Most of the time	Always
13	Fatigue interfered with my daily routine	Never	Rarely	Sometimes	Most of the time	Always
14	I felt worn out	Never	Rarely	Sometimes	Most of the time	Always
15	I felt so tired, I had to force myself to do the things I needed to do	Never	Rarely	Sometimes	Most of the time	Always
16	I felt so tired, I had to go to bed early	Never	Rarely	Sometimes	Most of the time	Always
17	Fatigue just suddenly hit me	Never	Rarely	Sometimes	Most of the time	Always
18	PBC drained every ounce of energy out of me	Never	Rarely	Sometimes	Most of the time	Always

The next section is about the effort and planning that can be involved in living with PBC. Thinking about THE LAST FOUR WEEKS, how often did the following statements apply to you?

19	Some days it took me a long time to do anything	Never	Rarely	Sometimes	Most of the time	Always
20	If I was busy one day I needed at least another day to recover	Never	Rarely	Sometimes	Most of the time	Always
21	I had to pace myself for day-to-day things	Never	Rarely	Sometimes	Most of the time	Always

The following statements are about the effects that PBC may have on things like memory and concentration. Thinking about THE LAST FOUR WEEKS, how often did the following statements apply to you?

22	Because of PBC I had to make a lot of effort to remember things	Never	Rarely	Sometimes	Most of the time	Always
23	Because of PBC I had difficulty remembering things from one day to the next	Never	Rarely	Sometimes	Most of the time	Always
24	My concentration span was short because of PBC	Never	Rarely	Sometimes	Most of the time	Always

25	Because of PBC, I had difficulty keeping up with conversations	Never	Rarely	Sometimes	Most of the time	Always	
26	Because of PBC, I found it difficult to concentrate on anything	Never	Rarely	Sometimes	Most of the time	Always	
27	Because of PBC, I found it difficult to remember what I wanted to do	Never	Rarely	Sometimes	Most of the time	Always	

Now some more general statements about how PBC may be affecting you as a person. How much do the following statements apply to you?

28	Because of PBC, I get more stressed about things than I used to	Not at all	A little	Somewhat	Quite a bit	Very much	
29	My sex life has been affected because of PBC	Not at all	A little	Somewhat	Quite a bit	Very much	Does not apply
30	Having PBC gets me down	Not at all	A little	Somewhat	Quite a bit	Very much	
31	I feel I neglect my family because of having PBC	Not at all	A little	Somewhat	Quite a bit	Very much	Does not apply
32	I feel guilty that I can't do what I used to do because of having PBC	Not at all	A little	Somewhat	Quite a bit	Very much	
33	I worry about how my PBC will be in the future	Not at all	A little	Somewhat	Quite a bit	Very much	

These statements relate to the possible effects of PBC on your social life. Thinking of your own situation, how much do you agree or disagree with them?

34	I sometimes feel frustrated that I can't go out and enjoy myself	Strongly agree	Agree	Neither agree nor disagree	Disagree	Strongly disagree
35	I tend to keep the fact that I have PBC to myself	Strongly agree	Agree	Neither agree nor disagree	Disagree	Strongly disagree
36	I can't plan holidays because of having PBC	Strongly agree	Agree	Neither agree nor disagree	Disagree	Strongly disagree
37	My social life has almost stopped	Strongly agree	Agree	Neither agree nor disagree	Disagree	Strongly disagree

The next section is about the impact that PBC may be having on your life overall. How much do you agree or disagree with the following statements?

38	Everything in my life is affected by PBC	Strongly agree	Agree	Neither agree nor disagree	Disagree	Strongly disagree
39	PBC has reduced the quality of my life	Strongly agree	Agree	Neither agree nor disagree	Disagree	Strongly disagree
40	I can still lead a normal life, despite having PBC	Strongly agree	Agree	Neither agree nor disagree	Disagree	Strongly disagree

The next few questions are about your general health and well being:

A	In general, would you say your health is:	Excellent	Very good	Good	Fair	Poor
B	And how would you have rated it before you had PBC?	Excellent	Very good	Good	Fair	Poor
C	COMPARED TO ONE YEAR AGO, how would you rate your health in general now?	Much better	Somewhat better	About the same	Somewhat worse	Much worse

THANK YOU FOR TAKING THE TIME TO COMPLETE

H. Hospital Anxiety Depression Scale (HADS) Questionnaire

Hospital Anxiety and Depression Scale (HADS)

Tick the box beside the reply that is closest to how you have been feeling in the past week.
Don't take too long over you replies: your immediate is best.

D	A		D	A	
		I feel tense or 'wound up':			**I feel as if I am slowed down:**
	3	Most of the time	3		Nearly all the time
	2	A lot of the time	2		Very often
	1	From time to time, occasionally	1		Sometimes
	0	Not at all	0		Not at all
		I still enjoy the things I used to enjoy:			**I get a sort of frightened feeling like 'butterflies' in the stomach:**
0		Definitely as much		0	Not at all
1		Not quite so much		1	Occasionally
2		Only a little		2	Quite Often
3		Hardly at all		3	Very Often
		I get a sort of frightened feeling as if something awful is about to happen:			**I have lost interest in my appearance:**
	3	Very definitely and quite badly	3		Definitely
	2	Yes, but not too badly	2		I don't take as much care as I should
	1	A little, but it doesn't worry me	1		I may not take quite as much care
	0	Not at all	0		I take just as much care as ever
		I can laugh and see the funny side of things:			**I feel restless as I have to be on the move:**
0		As much as I always could		3	Very much indeed
1		Not quite so much now		2	Quite a lot
2		Definitely not so much now		1	Not very much
3		Not at all		0	Not at all
		Worrying thoughts go through my mind:			**I look forward with enjoyment to things:**
	3	A great deal of the time	0		As much as I ever did
	2	A lot of the time	1		Rather less than I used to
	1	From time to time, but not too often	2		Definitely less than I used to
	0	Only occasionally	3		Hardly at all
		I feel cheerful:			**I get sudden feelings of panic:**
3		Not at all		3	Very often indeed
2		Not often		2	Quite often
1		Sometimes		1	Not very often
0		Most of the time		0	Not at all
		I can sit at ease and feel relaxed:			**I can enjoy a good book or radio or TV program:**
	0	Definitely	0		Often
	1	Usually	1		Sometimes
	2	Not Often	2		Not often
	3	Not at all	3		Very seldom

Please check you have answered all the questions

Scoring:
Total score: Depression (D) _____ Anxiety (A) _____
0-7 = Normal
8-10 = Borderline abnormal (borderline case)
11-21 = Abnormal (case)

I. Epworth Sleepiness Scale

The Epworth Sleepiness Scale

The Epworth Sleepiness Scale is widely used in the field of sleep medicine as a subjective measure of a patient's sleepiness. The test is a list of eight situations in which you rate your tendency to become sleepy on a scale of 0, no chance of dozing, to 3, high chance of dozing. When you finish the test, add up the values of your responses. Your total score is based on a scale of 0 to 24. The scale estimates whether you are experiencing excessive sleepiness that possibly requires medical attention.

How Sleepy Are You?
How likely are you to doze off or fall asleep in the following situations? You should rate your chances of dozing off, not just feeling tired. Even if you have not done some of these things recently try to determine how they would have affected you. For each situation, decide whether or not you would have:

- No chance of dozing =0
- Slight chance of dozing =1
- Moderate chance of dozing =2
- High chance of dozing =3

Write down the number corresponding to your choice in the right hand column. Total your score below.

Situation	Chance of Dozing
Sitting and reading	•
Watching TV	•
Sitting inactive in a public place (e.g., a theater or a meeting)	•
As a passenger in a car for an hour without a break	•
Lying down to rest in the afternoon when circumstances permit	•
Sitting and talking to someone	•
Sitting quietly after a lunch without alcohol	•
In a car, while stopped for a few minutes in traffic	•

Total Score = _____

Analyze Your Score

Interpretation:

0-7: It is unlikely that you are abnormally sleepy.
8-9: You have an average amount of daytime sleepiness.
10-15: You may be excessively sleepy depending on the situation. You may want to consider seeking medical attention.
16-24: You are excessively sleepy and should consider seeking medical attention.

Reference: Johns MW. A new method for measuring daytime sleepiness: The Epworth Sleepiness Scale. *Sleep* 1991; 14(6):540-5.