Understanding the biological basis of symptoms in primary biliary cholangitis

Author: Dr Aaron Wetten

Supervisors: Dr Jessica Dyson, Professor David Jones

Translational and Clinical Research Institute, Newcastle University

Qualification applied for: Doctor of Medicine

Institution: Translational and clinical research institute, faculty medical sciences, Newcastle University.

Submitted: February 2024

Abstract:

Background and Aims:

Symptoms of pruritus, fatigue and cognitive impairment are common in Primary Biliary Cholangitis (PBC) with a significant impact on patients' quality of life. The biological mechanisms underpinning these are poorly understood. Management of symptomatic PBC remains a significant unmet need, with no effective treatment for fatigue or cognition and only partially effective treatments for pruritus. This study explored markers associated with the pathogenesis of PBC and their relationship to symptoms.

Method:

Study 1 (exploratory) investigated 19 O-link serum proteomic markers in 289 fully characterised PBC patients from the UK-PBC cohort. Markers were compared to 'clinically significant' (c/s) symptoms, as assessed using the PBC-40. Study 2 (validation) compared a multiplex of 11 markers to a symptom-heavy cohort of 160 PBC patients and 40 healthy controls. Study 3 explored the neurosteroid allopregnanolone in 120 PBC patients and 40 healthy controls, comparing serum levels to c/s symptoms.

Results:

Interleukin-6 (IL-6) was positively associated with c/s fatigue, as compared with 'not clinically significant' (nc/s) fatigue (*exploratory*: median 2.63(IQR 0.80) pg/ml vs 2.42(0.89); p=0.047; *validation*: 1.62(0.89) vs 1.06(0.86); p=0.01), while Interluekin-4 Receptor Alpha (IL-4RA) was associated with c/s pruritus, as compared with nc/s pruritus (*exploratory*: 3.27(1.30) pg/ml vs 3.09(1.01); p=0.032; *validation*: 1081(173) vs 1000(200); p=0.022). Allopregnanolone was elevated in c/s cognitive (0.037 (0.04) vs 0.023(0.036); p=0.044) and emotional symptoms (0.039(0.056) vs (0.027(0.036); p=0.01).

Conclusion:

IL-6 was elevated in two distinct PBC groups with fatigue and has previously been implicated in skeletal muscle fatigability. IL-6 blockade therapies may present a new therapeutic strategy for fatigue. IL-4RA demonstrated positive associations for pruritus across both groups. An ongoing IL-4RA receptor antagonist trial may prove effective in treating cholestatic pruritus. Elevated serum allopregnanolone is associated with severe

cognitive and emotional symptoms in PBC and may be amenable to novel compounds targeting GABA-A receptors.

Dedications

I would like to take the opportunity to thank both my supervisors Dr Jess Dyson and Prof. Dave Jones, as without their support and enthusiasm my journey into research would never have occurred. They have both opened my eyes to the incredible value that research adds to our practice in clinical medicine, the thrill in exploring new ideas, but most importantly the difference it can make to our patients. Jess in particular has been incredibly supportive and patient; keeping me driven and motivated to finish. I'm so very thankful for all her guidance and time spent reading, re-reading, and correcting.

I am very grateful to Jeremy Palmer and the extended lab team at Newcastle University for all their hard-work and support with the costings, logistics and running of analytes on serum samples in the laboratory. My sincere thanks to all the members of the clinical and research teams at the Freeman who have helped me tremendously over the last few years, there are too many to name individually, but sister Kath Houghton has been a rock.

I remain indebted to all the patients with PBC who have kindly contributed to the various cohort studies included in these works and to the individuals and teams of people who setup, maintained and shared these invaluable resources.

Throughout this work my family and friends have helped to keep me going. The support from my mum and stepdad (Paul) has been fantastic. During this thesis Paul was diagnosed with MND and he sadly passed away at the end of 2023. He was extremely proud that I was doing this.

Finally, my husband David and my daughter Isla have always given me their unconditional love and support. I know it hasn't been easy for them losing me to thesis writing during our family time, but they have bought me tea, cake, and cuddles to keep me going. Isla, your joy, laughter and love always lifts me up. David it is time to stoke up the firepit! I'm so very thankful to my family for their incredible encouragement and love.

Statement of funding

The laboratory work conducted in Study 3 was fully funded by Umecrine Cognition AB, Solna, Sweden. There were no additional external funding sources involved in the work presented here.

Contents

List of tables

List of figures

Chapter 1: Introduction

1.1 Background

Primary biliary cholangitis (PBC) is a slowly progressive immune-mediated, chronic liver disease that leads to inflammation and destruction of the small intrahepatic bile ducts, resulting in chronic cholestasis, that can lead to cirrhosis, liver decompensation and death (Carey, Ali and Lindor, 2015). With a prevalence in the United Kingdom of 35 per 100,000 population, PBC is considered a rare disease, with an annual incidence rate of 2-3 per 100,000 (James *et al.*, 1999). However, despite this, PBC still accounted for 7% of all adult elective liver transplantations in the UK in 2020 (*Annual report on liver transplantation: Report for 2019/2020*, 2020).

A clinical diagnosis of PBC can be made following persistent cholestatic liver function tests (greater than 6 months), without alternative explanation, and disease specific antibodies (anti-mitochondrial antibodies (AMA) > 1:40, or highly PBC-specific anti-nuclear antibodies (ANA)) and/or following compatible histological features on liver biopsy (Hirschfield *et al.*, 2018). International guidelines stipulate that the presence of all three features is defined as "definite" PBC; whilst meeting any 2 out of the 3 criteria is defined as "probable" PBC (Hirschfield *et al.*, 2018; Hirschfield *et al.*, 2017; Lindor *et al.*, 2018). The combination of persistent cholestatic liver function tests and positive AMA produces a diagnostic sensitivity and specificity for PBC of 95% and has thus largely removed the need for liver biopsy for diagnosis (Oertelt *et al.*, 2007).

There is a marked female predominance, with a prevalence ratio of 9 females to 1 male (Triger, Berg and Rodes, 2008), and with a median age of disease onset at 50 years (Griffiths, Dyson and Jones, 2014). Male gender and younger age at presentation for females (typically less than 45 years) carries a higher risk of treatment failure with standard first-line therapy, resulting in an increased risk of future disease progression (Carbone *et al.*, 2013b). In addition, the symptom burden in PBC, classically fatigue, cognitive symptoms (often described by patients as brain fog) and pruritus (itch) is often higher in younger females (Carbone *et al.*, 2013b).

There is significant geographical variation in the prevalence of PBC, with a higher incidence in Northern Europe, Scandinavian countries and the United States (Griffiths, Dyson and Jones, 2014). Even within countries, there can be significant geographical variation, such as seen within northern England where there is a higher prevalence of 240 cases per million, compared to 200 per million in southern Wales (James *et al.*, 1999; Metcalf *et al.*, 1997). Several epidemiological studies have suggested an apparent increase in the prevalence of PBC over time. Whilst this may represent a true increase, it is also likely that increased disease awareness and improved case-finding contribute to this, whilst variation in study populations and study design may also be an influence (Griffiths, Dyson and Jones, 2014).

1.2 Pathogenesis

The aetiopathogenesis of PBC is poorly understood. The clinical picture is of chronic cholestasis, with immune reactivity to AMA and/or specific ANA. Histologically there is chronic non-suppurative, granulomatous, lymphocytic cholangitis, with inflammation around the portal tracts and biliary epithelial cells (BEC) (Rubin, Schaffner and Popper, 1965; Selmi, Coppel and Gershwin, 2006). The majority of patients (95%) with PBC have AMA directed against the 2-oxoacid dehydrogenase enzyme complexes of the mitochondria, most commonly the pyruvate dehydrogenase complex (PDC), where reactivity is largely to the E2 and E3BP subunits, which share common lipoylated domains resulting in cross reactivity. However, reactivity is seen to some degree in other families of 2-oxoacid dehydrogenase (Jones, 2008; Fussey *et al.*, 1988; Yeaman *et al.*, 1988; Palmer *et al.*, 1999). Other characteristic antibodies include sp100 and gp210 and are listed in

Table 1.1**.** These lipoylated domains are covalent attachment sites for the essential cofactor, lipoic acid (Quinn et al., 1993). This cofactor is a catalyst for cell growth, oxidation of carbohydrates, amino acids and other fuels (Solmonson and Deberardinis, 2018). **See Figure** 1.

Table 1.1: Characteristic autoantibodies and their frequency in PBC (Jones, 2008)

Antibody group	Antigen	Patient Frequency
Mitochondria	PDC-E2*	95%
	PDC-E3BP*	95%
	PDC-E1a	40-60%
	PDC-E1b	10%
	OGDC-E2	40-90%
	BCOADC-E2	10%
Nuclear	Gp210	10-40%
	Sp100	10-30%
	P62	20-30%
	Centromere	10%
	Lamin B receptor	2%

*There is complete cross-reactivity from antibodies to these common lipoylated domains.

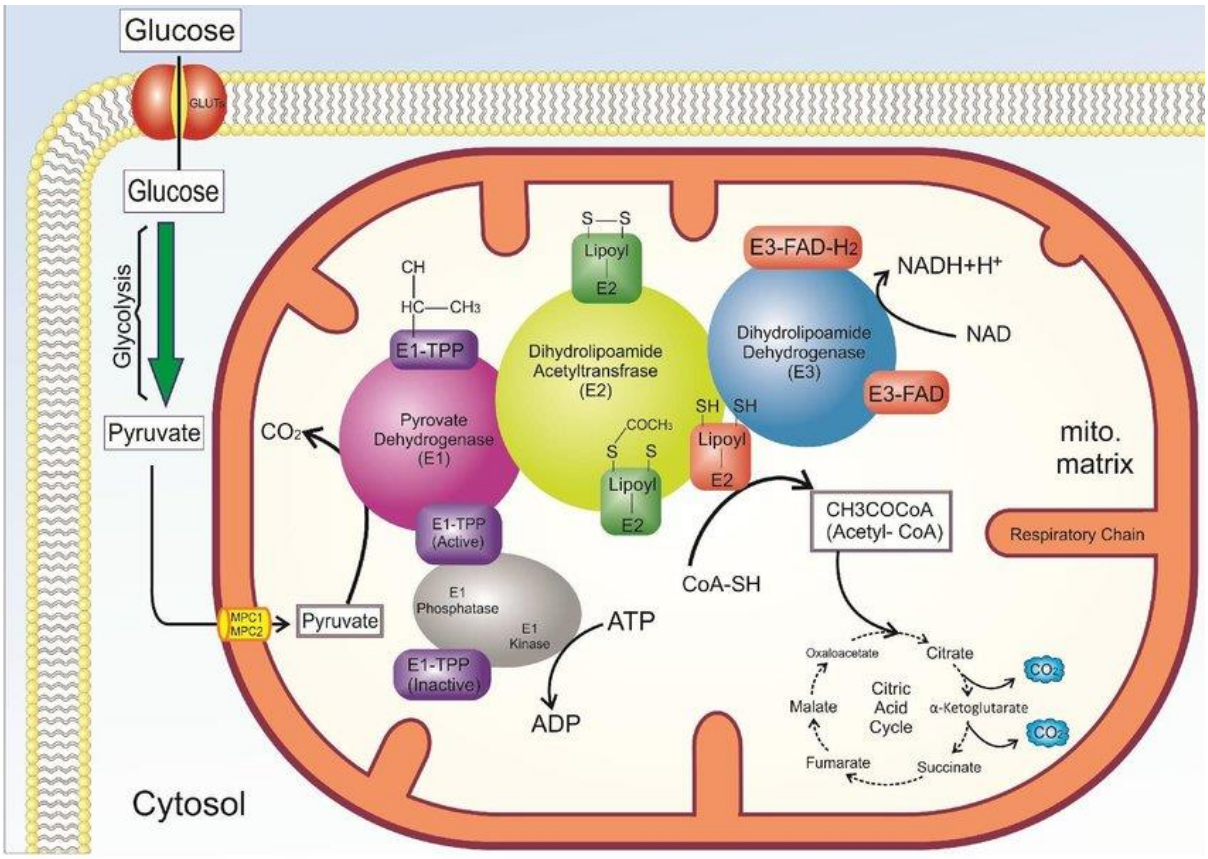


Figure 1.1 Overall reaction model of pyruvate dehydrogenase complex (PDC)
The three enzymes E1 (pyruvate dehydrogenase), E2 (dihydrolipoamide acetyl-transferase) and E3 (dihydrolipoamide dehydrogenase) links glycolysis to the Krebs cycle in mitochondria (Nasiri et al, 2019, page 3649)(Khodarahmi et al., 2020)

Although the discovery of serum antibodies in PBC, including AMA, have proven important in the diagnosis of PBC, the relevance of their presence in the underlying pathophysiology of disease is limited. Indeed, a large cohort study in Italy demonstrated that around 0.5% of the population had positive AMA serology, but without overt liver disease (Mattalia *et al.*, 1998). Studies from the 1980s demonstrated that a significant proportion of patients with AMA positivity go on to develop cholestatic liver function tests, and/or symptoms consistent with PBC during long-term follow up, with over a third of liver biopsies undertaken at the time of recruitment to these studies having some histological features that were consistent with PBC, while another third had histology that fulfilled diagnostic criteria, despite normal liver function tests. (Mitchison *et al.*, 1986; Metcalf *et al.*, 1996).

Individual susceptibility to PBC is due to complex interactions between genetics and environmental factors. Having a first degree relative with PBC is a strong independent risk factor for PBC, with an odds ratio of between 6.8-10.7 shown in several cohort studies (Gershwin *et al.*, 2005; Corpechot *et al.*, 2010; Jones *et al.*, 1999b), with a non-uniform distribution, and a predominance for daughters of affected patients (Jones *et al.*, 1999b). High concordance rates for PBC between monozygotic twins, compared to dizygotic twins, has previously been demonstrated, providing further evidence of a strong genetic component (Selmi *et al.*, 2004).

The Human Leukocyte Antigen (HLA) complex contains coding that is needed for antigen presentation. It is located on the short arm of chromosome 6 and includes groups of genes, related in structure and/or function, which encode heavy chain HLA class I (HLA-A, -B, and -C) molecules and HLA class II (HLA-DP, -DQ and DR) molecules used to present processed peptide antigens, whilst class III genes encode for a large number of other immune proteins, such as tumour necrosis factor (TNF)-α and complement (Gerussi *et al.*, 2021). Historically, many autoimmune diseases have been strongly associated with HLA regions (haplotypes), particularly involving HLA class II (Simmonds and Gough, 2007). Since 2006, high-risk HLA loci and low-risk non-HLA loci that predispose an individual to develop PBC have been identified by use of genome-wide association studies (GWAS) (Cordell *et al.*, 2015b; Mells *et al.*, 2011; Hirschfield *et al.*, 2009; Liu *et al.*, 2012). GWAS uses case-control association studies to find associations between a given phenotype and genetic loci across the genome. Stringent significance levels are applied to correct for false-positive associations and potential

significant associations should be replicated using a validation panel (Neuberger, 2020). The strongest HLA haplotype associations for PBC in European populations are shown in Table 1.2

Table 1.2 HLA locus associations in primary biliary cholangitis, adapted from autoimmune liver disease management and clinical practice, chapter 3 (Neuberger, 2020)

Population	**Effect**	**HLA allele/haplotype**
PBC (European)	Risk	B*39:06-DRB1*08:01-DQA1*04:01-DQB1*04:02
		DRB1*04:04-DQA1*03:01-DQB1*03:02
		DRB1*14-DQB1*05:03 (Italian population only)
		DPB1*03:01
		C8*04:01
		DPB1*10:01
		DPB1*17:01
		DPA*02:01
	Protective	DRB1*11:04-DRB1*11:01-DQA1*05:01-DQB1*03:01
		B*07:02-DRB1*15:01-DQA1*01:02-DQB1*06:02
		DPB1*04:01

Most autoimmune diseases arise as a result of abnormal activation of the immune system, as a consequence of individual genetic susceptibility, following exposure to a specific environmental factor, that results in failure of self-tolerance, leading to disease (Costenbader *et al.*, 2012). Evidence for loss of immune tolerance due to an environmental influence comes from studies outlining uneven spatial variation of PBC, along with space-time clustering that may suggest both transient and non-transient environmental causes. Examples of this include studies that identified clustering of PBC cases in the urban areas of Newcastle and Gateshead, along with marked space-time clustering between 1995 to 2003, with the strength of clustering being most significant when diagnosed within 1-4 months of each other, which the authors concluded might suggest a transient infective agent (Prince, 2001; Mcnally, Ducker and James, 2009; Griffiths, Dyson and Jones, 2014). A subsequent comprehensive follow-on study verified a high prevalence of PBC in the northeast of England associated with urbanisation, and in particular, demonstrated significant spatial clustering of cases in areas with a previous coal mining history, however, they did not replicate the temporal clustering identified previously (Dyson *et al.*, 2021). A study in New York City demonstrated the prevalence of

patients with PBC on the liver transplant waiting list was significantly higher in those who had zip codes that contained or were adjacent to superfund toxic waste sites (SFW) (Ala *et al.*, 2006). Other case control studies have consistently demonstrated an association between PBC and psoriasis, recurrent urinary tract infections and smoking (Corpechot *et al.*, 2010; Gershwin *et al.*, 2005; Howel *et al.*, 2000; Prince, Ducker and James, 2010), but other common risk factors remain inconsistent between studies, and challenges remain in identifying these due to lack a commonality between study methodologies, the possibility of multiple triggers and the potential latency between exposure, onset and diagnosis (Griffiths, Dyson and Jones, 2014).

Therefore, evidence suggests that on a permissive genetic background, environmental exposure to a trigger, such as a microbial, which acts as a PDC-E2 mimic, results in loss of immune tolerance (Gershwin and Mackay, 2008; Shimoda *et al.*, 2003). This activates an immune response, stimulating antigen presenting cells (APC) to form a T-cell complex, that results in the stimulation of B-cells to generate AMA, which binds to PDC-E2 present in BEC apoptotic blebs (Gershwin *et al.*, 2000; Kita *et al.*, 2002; Bassendine, Jones and Yeaman, 1997; Tsuneyama *et al.*, 1995). Subsequent E2 immune complexes are further presented to immune cells, resulting in the activation and expansion of CD8+ cells through major histocompatibility complex I (MHC), CD4+ cells (MHC II) and resulting in stimulation of B-cells and further AMA production, overall resulting in autoreactive inflammatory infiltrates to PDC-E2 around the portal tracts and BEC, causing chronic inflammation, cholestasis, and fibrosis (Byron and Lindsay, 2017; Hirschfield, Heathcote and Gershwin, 2010). **See Figure** 1.2 (Hirschfield, Heathcote and Gershwin, 2010)**.**

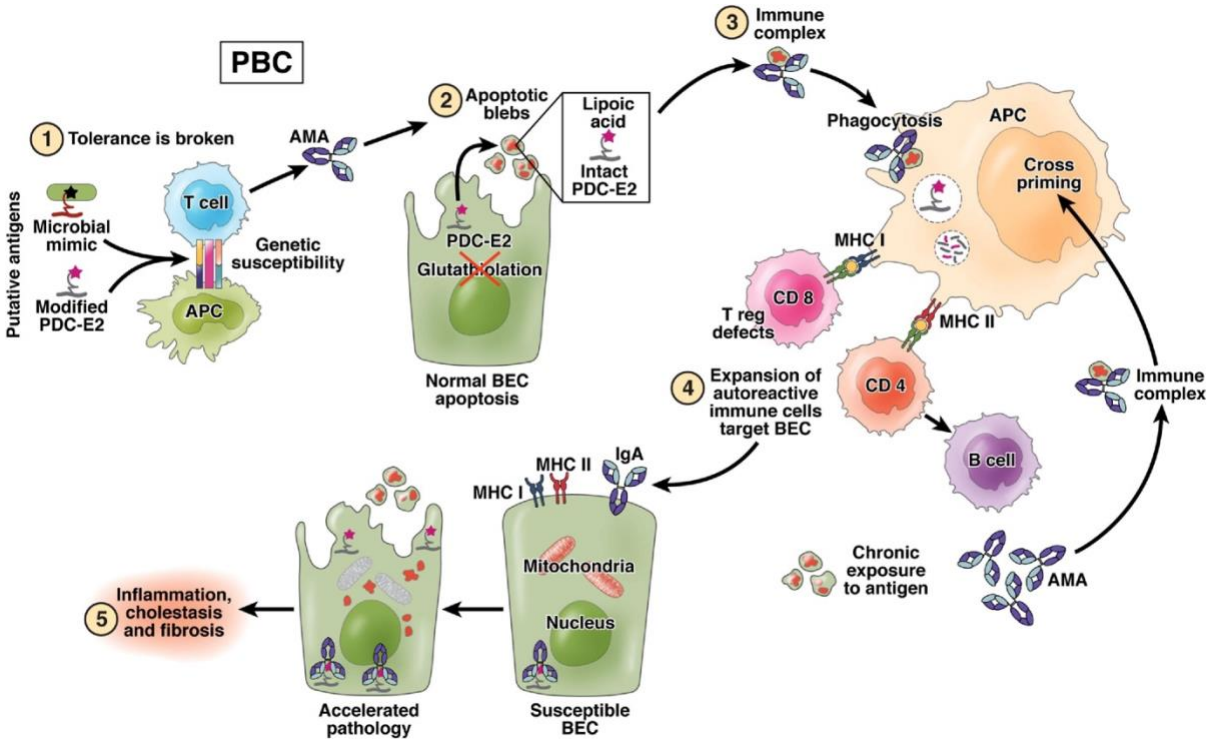


Figure 1.2: A proposed pathological model of primary biliary cholangitis (PBC).
Injury to biliary epithelial cells (BEC) results in apoptotic blebs containing the mitochondrial antigen, PDC-E2, being presented to immune cells. The development of immune complexes results in autoreactive immune cells that target BEC, resulting in chronic inflammation, cholestasis, and fibrosis. APC, antigen presenting cells; MHC, major histocompatibility complex (Hirschfield, 2010, page 1488) (Hirschfield, Heathcote and Gershwin, 2010)

Whilst there is an initial immune mediated "upstream" injury in PBC from loss of immune tolerance, it is apparent that there is further disease progression through a "downstream" injury. The initial injury to BEC and cholestasis results in abnormalities of the bile acid pool, which becomes enriched with cytotoxic hydrophobic bile acids, resulting in further BEC injury and apoptosis (Lamireau *et al.*, 2003). Sensitisation to apoptotic insult seems to occur by disruption of the normal protective "biliary bicarbonate umbrella" following downregulation of the anion exchanger 2 (AE2) in BEC as a response to biliary injury and exposure to hydrophobic bile acids (Chang *et al.*, 2016; Hisamoto *et al.*, 2016). This might explain why treatment with first-line therapy, ursodeoxycholic acid (UDCA), a hydrophilic, non-cytotoxic bile acid, is incomplete in its action; that is, it modifies the relative constitution of the bile acid pool, which reduces the cytotoxic concentration of bile, and can improve downstream injury, but does not alter the initiating upstream injury (Griffiths and Jones, 2014; Paumgartner and Beuers, 2004). In addition, there is evidence that injury to the small bile ducts results in senescence of BEC, which can result in the upregulation and release of chemokines and may contribute to the pathogenesis of PBC (Sasaki *et al.*, 2010b).

BEC of the small bile ducts in PBC demonstrate histological features of cellular senescence and that appears to occur following oxidative stress (Sasaki and Nakanuma, 2010; Sasaki *et al.*, 2010b; Sasaki *et al.*, 2010a; Sasaki *et al.*, 2005). Senescence, the process by which a cell arrests cellular proliferation but remains metabolically active, is likely an evolutionary response to cell insult that protects against abnormal proliferation (Sasaki *et al.*, 2005). Cells undergoing senescence can contribute to persistent inflammation and impaired tissue integrity. The pathogenesis of how cellular senescence of BEC results in bile duct loss is unclear, but it appears that injured cells undergoing senescence are not replaced, but rather remain in-situ and express a senescence-associated secretory phenotype (SASP) (Sasaki and Nakanuma, 2012; Sasaki *et al.*, 2005). Expression of SASP results in the secretion of a plethora of cytokines, such as interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF-α) and potent chemokines, such as interleukin-8 (IL-8), Chemokine CC motif ligand 20 (CCL20), along with growth factors and matrix metalloproteinases, which are involved in remodelling and in the accumulation of inflammatory cells, including T-cells (Yasoshima *et al.*, 1998; Sasaki *et al.*, 2010a; Sasaki *et al.*, 2010b). The ability of progenitor cells to proliferate and replace damaged BEC is likely impaired due to the occurrence of cellular senescence in ductular reaction (DR), which harbours progenitor cells (Sasaki *et al.*, 2005). It is likely that cellular senescence of BEC plays a significant role not only in the initial inflammatory cascade in PBC, but also contributing to the chronic inflammatory process.

1.3 Treatment

1.3.1 First-line treatment

First-line therapy for PBC is with the hydrophilic non-cytotoxic bile acid, ursodeoxycholic acid (UDCA) (Hirschfield *et al.*, 2018), which has been shown in clinical trials, at a therapeutic dose of 13-15mg/kg/day, to improve biochemistry, delay histological progression and improve long-term outcomes (Corpechot, Chazouilleres and Poupon, 2011). Current national and international guidelines stipulate assessment of biochemical 'response' following 12-months of treatment with UDCA at therapeutic doses, or intolerance of UDCA, before consideration of second-line therapy (Hirschfield *et al.*, 2018; Hirschfield *et al.*, 2017; Lindor *et al.*, 2018). Several criteria to assess response to UDCA in PBC have been developed (summarised in Table 1.3), all of which are largely based on assessment of liver biochemistry following 1 year of treatment with UDCA (Corpechot, Chazouilleres and Poupon, 2011; Pares, Caballeria and Rodes, 2006; Corpechot *et al.*, 2008; Kuiper *et al.*, 2009; Kumagi *et al.*, 2010a; Nevens *et al.*,

2016; Momah *et al.*, 2012). International guidelines do not currently recommend any criteria over another; however, it is clear from long-term data that those who meet any of the response criteria have a better long-term prognosis (Hirschfield *et al.*, 2018; Hirschfield *et al.*, 2017; Lindor *et al.*, 2018; Corpechot, Chazouilleres and Poupon, 2011; Pares, Caballeria and Rodes, 2006; Corpechot *et al.*, 2008; Kuiper *et al.*, 2009; Kumagi *et al.*, 2010a; Nevens *et al.*, 2016; Momah *et al.*, 2012)

Table 1.3 Criteria for assessing biochemical response in PBC

Criteria name	**Criteria definition**
Barcelona (Pares, Caballeria and Rodes, 2006)	ALP decrease > 40% of baseline values or normal levels after 12 months of UDCA treatment
PARIS I (Corpechot *et al.*, 2008)	All three of ALP < 3xULN, ALT < 2xULN, Bilirubin < ULN, after 12 months of UDCA treatment
Rotterdam (Kuiper *et al.*, 2009)	Normalisation of abnormal bilirubin and/or albumin values
Toronto (Kumagi *et al.*, 2010a)	ALP < 1.67xULN, after 2 years treatment with UDCA
PARIS II (Corpechot, Chazouilleres and Poupon, 2011)	ALP < 1.5xULN and ALT < 1.5ULN, after 12 months of UDCA treatment
Rochester II (Momah *et al.*, 2012)	ALP < 2xULN, after 12 months of UDCA treatment
POISE (Nevens *et al.*, 2016)	ALP < 1.67xULN and bilirubin < ULN after 12 months of UDCA treatment

ALP: Alkaline Phosphatase; ULN: Upper Limit of Normal; ALT: Alanine Aminotransferase; UDCA: Ursodeoxycholic Acid

1.3.1.1 Response criteria

The POISE criteria are commonly used in clinical practice to identify incomplete responders to first-line therapy. These are defined as an alkaline phosphatase (ALP) persistently > 1.67x upper limit of normal (ULN) and/or elevated bilirubin >1 x ULN after 12 months of treatment with UDCA, or for those intolerant to UDCA (Bowlus *et al.*, 2020). The origins for these criteria come from the POISE trial, a phase 3 study of the farnesoid X receptor (FXR) agonist, obeticholic acid (OCA), in patients with PBC, which used the described criteria to assess response to OCA (Nevens *et al.*, 2016). Those classified as "responders" are considered low risk for disease progression, whilst those with inadequate response are at increased risk of developing cirrhosis, resulting in the need for liver transplantation or death (Corpechot *et al.*, 2008). Up to 40% of patients have an incomplete response to UDCA and large-scale studies have demonstrated that earlier age at presentation and being male are associated with failure to respond to first-line treatment (Carbone *et al.*, 2013b). There is, however, increasing evidence, with a consensus from experts, that complete normalisation of liver function biochemistry is

required to reduce the risk of disease progression and improve long term outcomes (Jones *et al.*, 2022; Murillo Perez *et al.*, 2020; Lleo, 2022).

Age at presentation, response to treatment, along with serological markers of liver synthetic function, imaging, transient elastography and liver histology should all be used in the assessment of patients to aid in risk stratification (Hirschfield *et al.*, 2018). For those at a higher risk of disease progression, there are now two well-established second-line therapies with some evidence of long-term benefit from clinical trials (Corpechot *et al.*, 2018; Nevens *et al.*, 2016).

1.3.2 Second-line treatment

1.3.2.1 Farnesoid X receptor (FXR) agonists

The potent FXR agonist, obeticholic acid (OCA), was the first newly licensed therapeutic option in nearly 20 years for PBC. It was approved in 2017 in the UK by the national institute of clinical excellence (NICE) as an add-in therapy for those with an inadequate response to UDCA (or who are intolerant of UDCA) and are therefore considered to be high-risk for disease progression (Intercept, 2017). It is currently the only licensed second-line therapy for PBC.

FXR is mainly expressed in hepatocytes and enterocytes in humans. Naturally occurring bile acids, such as chenodeoxycholic acid (CDCA), act as ligands to FXR (Ali, Carey and Lindor, 2015). OCA is an agonist with a 100-fold higher affinity for FXR than CDCA, controlling de novo synthesis and uptake of bile through direct down regulation of the CYP7A1 gene. (Mousa *et al.*, 2015; Ali, Carey and Lindor, 2015). It indirectly releases fibroblast growth factor-19 (FGF-19), resulting in the regulation of hepatic bile flow, modulation of hepatic inflammation by reduction of cytokine production, and a reduction in fibrosis, with subsequent remodelling and regeneration (Mousa *et al.*, 2015; Ali, Carey and Lindor, 2015). Emerging evidence from murine models suggests that cellular senescence is reduced by exposure to OCA (Gee *et al.*, 2023).

Placebo-controlled trials have demonstrated sustained long-term biochemical improvement with OCA use, which is predictive of improved clinical outcomes (Nevens *et al.*, 2016; Bowlus *et al.*, 2020; Bowlus *et al.*, 2021). The 3-year interim analysis from the extension phase to the POISE trial showed that ALP remained significantly reduced compared to baseline, along with

stabilisation of total bilirubin at 12, 24, 36 and 48 months (Trauner *et al.*, 2019). However, evidence suggests that up to 50% of patients may have an inadequate response to combination therapy with OCA and UDCA and therefore remain at risk of disease progression (Trauner *et al.*, 2019) and this has been reflected in real-world reports (Roberts *et al.*, 2020; D'Amato *et al.*, 2021).

Exacerbation of pruritus with OCA is common, and can limit its use in some patients, with 29% of patients requiring treatment with bile acid sequestrants and 4% discontinuing treatment (Trauner *et al.*, 2019). Other common side effects include fatigue, gastrointestinal changes, dizziness, thyroid dysfunction, and arthralgia ((emc), 2018). OCA also increases serum small very low density lipoproteins (VLDL), small and large serum low density lipoproteins (LDL), with small reductions in high density lipoproteins (HDL), due to the reduction in the conversion of cholesterol to bile acids from down-regulation of cytochrome P450 (Dash *et al.*, 2017; Siddiqui *et al.*, 2020). The long-term risk of cardiovasular disease associated with these changes in patients with PBC is currently unknown (Hirschfield *et al.*, 2015; Nevens *et al.*, 2016), but has raised concerns in trials exploring the use of OCA for the treatment of non-alcohol related steatohepatitis (NASH) (Siddiqui *et al.*, 2020; Ratziu, 2021) and would be particularly concerning given the increased risk of cardiovascular disease in those with NASH and elevated LDL (Labenz *et al.*, 2019).

To date, there are no reported randomised control trials that report a significant benefit in symptoms following treatment with OCA (Nevens *et al.*, 2016; Samur *et al.*, 2017; Kowdley *et al.*, 2018; Trauner *et al.*, 2019; Bowlus *et al.*, 2021). However, these trials, with primary endpoints based on biochemical response to treatment, typically occur relatively late into the disease course, when the secondary downstream injury is well established, and symptoms are potentially irreversible. Murine models of cholestasis have demonstrated that early administration of OCA can improve short-term memory loss associated with cholestasis (Gee *et al.*, 2023). A Newcastle based clinical trial in the use of obeticholic acid for the amelioration of cognitive symptoms (OACS-1 and 2) in patients with PBC is currently ongoing (ISRCTN15223158).

1.3.2.2 Fibrates

Fibrates are potent peroxisome proliferator-activated receptor (PPAR) agonists. They act as ligands to PPAR to activate transcription factors, which results in the modification of bile acid and sterol synthesis, lipid storage, glucose metabolism and inflammatory pathways (Li and Chiang, 2009). There are 3 distinct PPAR isoforms: α, δ and γ (Li and Chiang, 2009), that have considerable crossover in functionality, but vary in their location. PPAR-α are highly expressed in hepatocytes, PPAR-δ are expressed universally (often at higher levels than α/γ) and PPAR-γ are predominantly expressed in adipose tissue and the immune system (Burns and Vandenheuvel, 2007).

Historically used to improve lipid profiles, fenofibrate (predominantly a PPAR-α agonist) and bezafibrate (a pan-PPAR agonist) are used off-licence as second-line agents for PBC to improve serum biochemistry through their anticholestatic properties (Corpechot *et al.*, 2018; Hosonuma *et al.*, 2015; Honda *et al.*, 2019; Levy *et al.*, 2011; Hegade *et al.*, 2016b). Their exact mechanism of action is uncertain, but it is thought they act by reducing the synthesis and uptake of bile acids, resulting in their detoxification, whilst protecting the biliary epithelium by increased production of biliary phospholipids and down regulation of cytokines involved in the immune response (Reig, Sese and Pares, 2018).

The strongest evidence for their use comes from the BEZURSO trial, a double-blind, placebo-controlled trial, where a combination of UDCA and Bezafibrate resulted in normalization of ALP in 67% of patients and complete normalization of liver biochemistry in 31% of patients (Corpechot *et al.*, 2018). Their effect on long-term outcomes in PBC patients is less certain. Survival models using a retrospective cohort suggest a reduction in all-cause and liver related deaths (Tanaka *et al.*, 2021), but long-term benefit from randomised control trials is not proven (Hosonuma *et al.*, 2015). There are several trials that demonstrate a beneficial effect on pruritus (De Vries *et al.*, 2020; Corpechot *et al.*, 2018), whilst newer fibrates, such as the potent and selective PPAR-δ agonist, seladelpar, and the dual PPARα/δ agonist, elafibranor, have shown promise in improving both liver biochemistry and pruritus, with seladelpar also showing some mild improvements in fatigue and sleep disturbance (Kremer *et al.*, 2022; Bowlus *et al.*, 2022; Levy *et al.*, 2020; 'ENHANCE: Safety and Efficacy of Seladelpar in Patients With Primary Biliary Cholangitis-A Phase 3, International, Randomized, Placebo-Controlled Study,' 2021; Schattenberg *et al.*, 2021).

There are some limitations with fibrates; lack of long term safety and outcome data, symptomatic myalgia (Hosonuma *et al.*, 2015), acute liver injury (Corpechot *et al.*, 2018) and renal dysfunction (Hosonuma *et al.*, 2015; Corpechot *et al.*, 2018) can occur and regular monitoring for these adverse events is recommended.

Currently there are no head-to-head trials to compare the efficacy of existing second line therapies. Such trials remain unlikely in the future, given the current available fibrates are generically produced and used off-label, and therefore unlikely to receive the pharmaceutical industry funding needed for large clinical trials. Newer fibrates remain under investigation and are likely to have the advantage of more robust underpinning evidence in the future (Wetten, Jones and Dyson, 2022; Bowlus *et al.*, 2022; Schattenberg *et al.*, 2021). There is emerging evidence to suggest that combination therapy with OCA and bezafibrate may improve normalisation of liver biochemistry further in those who fail to have an adequate response to the combination of UDCA and OCA, or UDCA and bezafibrate alone (Soret *et al.*, 2021). The active phase 2 randomised control trial, exploring the combination of OCA and bezafibrate, compared to bezafibrate alone, will provide more data on this (NCT04594694).

1.4 Symptoms in PBC

Prevention of disease progression (using biochemical improvement as a surrogate marker for long-term outcomes) has been the traditional primary emphasis of treatment in PBC, with a focus on disease control, rather than reversal/cure of disease. The burden of symptoms in non-cirrhotic PBC has historically been under-appreciated. The complex clinical phenotype of PBC, limitations in our understanding of the pathophysiology of symptoms and the lack of effective treatments can result in a failure by clinicians to adequately address symptoms with patients.

A significant proportion of patients with PBC develop symptoms that have a substantial effect on their quality of life (QOL) (Jacoby *et al.*, 2005). The most prevalent and well-described symptoms in PBC are those of pruritus, fatigue, and cognitive dysfunction. The interplay of these symptoms is complex, but ultimately can result in social dysfunction and marked social isolation (Mells *et al.*, 2013), with evidence that it has detrimental effects on family relationships and a reduced ability to work (Hale, Newton and Jones, 2012). Importantly,

symptom severity does not correlate with stage of disease and, except for pruritus, does not improve with any established treatments in PBC (Jopson and Jones, 2015; Poupon *et al.*, 2004).

Current treatment guidelines focus on biochemical control, and stipulate that patients are treated for 1 year to assess response with first-line therapy, prior to being considered for second-line therapies. However, it may be, that earlier initiation with more efficacious treatment may result in a reduction in the frequency and severity of symptoms. Overall, the approach to the management of PBC needs to be broader than biochemical response and disease progression and needs to consider morbidity and mortality in terms of quantity and quality of life, to provide the optimal care for our patients.

1.4.1 Impact of symptoms on quality of life in PBC

Several studies have demonstrated the impact of symptoms on QOL. Poupon et al assessed 276 unselected patients with PBC compared to age- and sex-matched controls using the Nottingham Health Profile (NHP), which assesses six major areas commonly associated with Health-Related Quality of Life (HRQOL). They noted a statistically significant difference in QOL between patients with PBC and controls (22.8 vs. 17.9 respectively, $P < 0.002$), with statistically significantly worse scores in energy (40.6 vs. 22.9, $P<0.0001$) and emotional reactions (22.2 vs. 16.1, $P<0.005$) domains compared to controls. Social isolation was strongly associated with fatigue ($P < 0.0001$). Liver biochemistry, histological stages (including cirrhosis) and disease duration were not statically associated with any of the NHP domains (Poupon *et al.*, 2004).

Over time, the impact of symptoms on patients with PBC has become more apparent. The largest published cohort study examining PBC and its impact on QOL was undertaken using the UK-PBC Patient Cohort, which was initially established to undertake a GWAS (Mells *et al.*, 2011; Liu *et al.*, 2012; Carbone *et al.*, 2013b), but has since evolved to help develop, evaluate and implement better approaches to treatment (consortium). This study included 2,353 participants with PBC, along with 196 community controls, case-matched (to the Newcastle PBC sub-cohort, using a best-friend approach) for age and sex. The PBC-40, a fully validated, disease-specific quality of life measure was utilised to assess symptom burden in the PBC population (Jacoby *et al.*, 2005) (**see section 3.4.1, table 3.1, appendix 1)**, whilst a modified version of the PBC-40 (PBC-40c), appropriate for use in non-PBC control subjects, was

developed and validated as part of the study. Other symptoms were assessed using the Epworth Sleepiness Scale (ESS) (Newton *et al.*, 2006c), Orthostatic Grading Scale (OGS) (Newton *et al.*, 2007a) and Hospital Anxiety and Depression Scale (HADS) (Blackburn *et al.*, 2007). A significant perceived QOL impairment in those with PBC, compared to controls (35% vs. 6% respectively, P<0.0001) was demonstrated. All symptom modalities had an impact on perceived QOL on univariate analysis, whilst multivariate analysis demonstrated fatigue and social symptoms were independently associated with impaired QOL. Overall, fatigue had the greatest overall impact on patients' QOL. Only 20% of PBC patients rated their health as very good or excellent, whilst 46% rated it as fair or poor, compared to 15% of the community controls (Mells *et al.*, 2013).

Overall, studies demonstrate that the burden of symptoms in PBC is high. Many patients experience symptoms in multiple domains, with a reduction in their overall QOL and with resultant social isolation and a significant impact on their daily living.

1.4.2 Fatigue

Fatigue associated with PBC can be debilitating and has been consistently demonstrated to be the symptom with the greatest impact on patients, resulting in a significant negative impact on patients' QOL (Jopson and Jones, 2015; Rice *et al.*, 2020; Goldblatt *et al.*, 2002; Mells *et al.*, 2013). The large cohort studies assessing symptoms with the PBC-40 have demonstrated that over 50% of patients with PBC suffer from moderate to severe fatigue (Mells *et al.*, 2013). Social isolation in particular is strongly associated with fatigue (Pells *et al.*, 2013b). Recent data exploring health utility confirms that the greatest impact on functional status in PBC comes from fatigue and cognitive symptoms (Rice *et al.*, 2020), with resultant social isolation giving rise to additional burden and worsening quality of life (Dyson *et al.*, 2016).

Typically, a younger age is associated with more severe symptoms of fatigue (Carbone *et al.*, 2013b; Phaw *et al.*, 2020). This can be particularly disabling as younger patients may have young families to take care of, many patients have active jobs and/or wish to continue an active social life but struggle with these everyday activities due to fatigue. Case reports from patients highlight the devastating impact that symptoms of fatigue can have on individuals and families (Hale, Newton and Jones, 2012). The need for adequate treatment to address these symptoms remains one of the major unmet clinical needs in the management of PBC.

The biological basis for why some patients with PBC experience significant symptoms of fatigue are complex and poorly understood. Unlike pruritus, there are currently no effective treatments for fatigue (Corpechot *et al.*, 2018; Mells *et al.*, 2013; Nevens *et al.*, 2016; Rudic *et al.*, 2012), with previous trials into rituximab (Khanna *et al.*, 2019), modafinil (Silveira *et al.*, 2017) and co-enzyme Q10 (Mehrabani *et al.*, 2019) demonstrating no significant benefit. Fatigue is not associated with disease severity (Cauch-Dudek *et al.*, 1998; Phaw *et al.*, 2020), does not improve following liver transplantation (Cauch-Dudek *et al.*, 1998; Pells *et al.*, 2013b), and is associated with increased mortality, particularly from cardiovascular disease (Jones, 2006). This may suggest that fatigue is not a direct consequence of the liver disease itself, but instead is a result of a liver-related process at the start of the disease and occurs as a consequence of an extra hepatic manifestation of the disease. Early control of disease with effective treatment may be essential to ensure that extrahepatic changes are not irreversible, as evidenced by the lack of improvement following liver transplantation.

Increasingly, fatigue in PBC is described as two distinct components: peripheral fatigue (neuromuscular in origin) and central fatigue (central nervous system [CNS] in origin). In peripheral fatigue, patients report feeling that their "batteries are running down" and this correlates with physiological changes where there is reduced recovery from acidosis following repeated exercise. In central fatigue, patients often describe symptoms of "brain fog" with impairments in memory and concentration that correlate with impaired performance on formal cognitive testing, along with functional and anatomical changes on magnetic resonance imaging (MRI) (Phaw *et al.*, 2020; Phaw *et al.*, 2021). There is significant overlap between cognitive symptoms and central fatigue (Phaw *et al.*, 2020).

One of the difficulties with understanding fatigue is its multifactorial nature. Poorly controlled pruritus may result in disturbed sleep that contributes to fatigue, whilst PBC is associated with other autoimmune conditions that may contribute to fatigue, including systemic lupus erythematosus (SLE), autoimmune thyroid disease, Raynaud's syndrome, Sjogren's syndrome, scleroderma, coeliac disease and pernicious anaemia; all of which are more common in patients with PBC compared to age and gender-matched controls (Gershwin *et al.*, 2005).

In addition, studies have pointed to the fact that given the average age of patients diagnosed with PBC is around 50, there is a high prevalence of non-immune mediated confounding co-

morbidities (Gershwin *et al.*, 2005), that are strongly associated with fatigue, such as type 2 diabetes, depression, sleep apnoea, or obesity which may in themselves contribute (Biagini *et al.*, 2008; Al-Harthy *et al.*, 2010) . International PBC guidelines highlight the importance of screening for other potential causes for fatigue (Hirschfield *et al.*, 2018; Hirschfield *et al.*, 2017; Lindor *et al.*, 2018).

Fatigue in PBC is often inadequately assessed by clinicians and therefore may be under reported in clinical practice. Al-Harthy et al assessed 327 patients from a single centre in Canada, where fatigue was verbally reported by 48% of patients during their consultation, but on subsequent formal assessment with the PBC-40, 25% of those who had not reported fatigue, scored moderate to severe on the PBC-40 fatigue domain. This illustrates the importance of using fully validated methods to assess fatigue in clinical practice.

Fatigue associated with PBC therefore remains a significant unmet need and understanding the pathophysiology of fatigue is essential to develop effective treatments and improve QOL.

1.4.2.1 Bioenergetic changes

Fatigue is complex. PBC patients not only describe a general fatigue, but also an extreme physically deep tiredness, and lack of energy (Phaw *et al.*, 2020). A strong inverse correlation between physical activity undertaken and levels of fatigue has been previously demonstrated (Newton *et al.*, 2006b). This has led to the theory that an organic component of the fatigue results in peripheral muscle dysfunction with several studies supporting an impairment in bioenergetic synthesis in PBC as a potential cause for peripheral fatigue.

In one such study, Goldblatt et al reported an association with PBC, fatigue and muscle fatigability. Peripheral muscle function, using grip strength, was measured in 14 healthy age and gender-matched controls and 18 female patients with PBC. The fatigue impact scale (FIS) was used as an objective assessment of fatigue (this consists of 40 items that evaluates the effect of fatigue on three domains: cognitive function, physical function, and psychosocial functioning). Severe fatigue was reported in 8 out of 18 patients with PBC (FIS score >84), whilst 10 reported minimal fatigue (FIS score <7). Recurrent grip strength measurements were made every 5 seconds for 5 minutes and the median percentage decrease in grip strength was calculated for every repeat measurement. Initial grip strength between controls, fatigued and

non-fatigued PBC patients showed no statistically significant difference. For controls and non-fatigued PBC patients, the median percentage decrease in grip strength was 0.7% and 1.1%, respectively (P=0.46) for repeated measurements. However, fatigued patients showed a statistically significantly greater rate of decrease than both controls and non-fatigued PBC patients (4% per repeat, P<0.005). A significant correlation was seen between fatigue severity (measured by FIS) and rate of decrease in muscle grip strength. There was no association between liver disease severity and muscle fatigability (Goldblatt, 2001). This suggests that PBC patients with fatigue, may have a lower threshold for peripheral muscle fatigability and that this contributes to the fatigue experienced by patients with PBC. However, the cause for this is unclear.

In vitro and animal studies have demonstrated that antibodies directed against mitochondria can become internalised to myocyte cells, by receptor mediated endocytosis, disrupting adenosine diphosphate (ADP) / adenosine triphosphate (ATP) exchange in myocyte mitochondria, potentially resulting in imbalances in energy metabolism (Kuhl, Ulrich and Schultheiss, 1987; Schulze *et al.*, 1990). As described previously, AMA antibodies in PBC show reactivity to PDC, largely to E2 and E3BP subunits. PDC mediates pyruvate oxidation after glycolysis, fuelling the Kreb cycle to meet energy demands (Park *et al.*, 2018; Byron and Lindsay, 2017). **See Figure** 1.3 (Byron and Lindsay, 2017). An association with AMA levels and fatigue have previously been demonstrated (Newton, Poyner and Jones, 2009; Hollingsworth *et al.*, 2008), but not replicated in other studies (Al-Harthy *et al.*, 2010). It is possible that cross-reactivity to PDC in PBC dysregulates these pathways for energy metabolism in cells, and it was this theory that resulted in the trial of the B-cell depleting therapy, Rituximab, in PBC patients with fatigue (Khanna *et al.*, 2018). The trial failed to show a significant benefit compared to placebo in treating fatigue, despite evidence to suggest it improved muscle metabolic dysfunction. The authors postulated that the trial may have failed to demonstrate a benefit due to the heterogeneity of the cohorts, but that it could still benefit a select subgroup with peripheral fatigue (Khanna *et al.*, 2019; Khanna *et al.*, 2018; Jopson *et al.*, 2015).

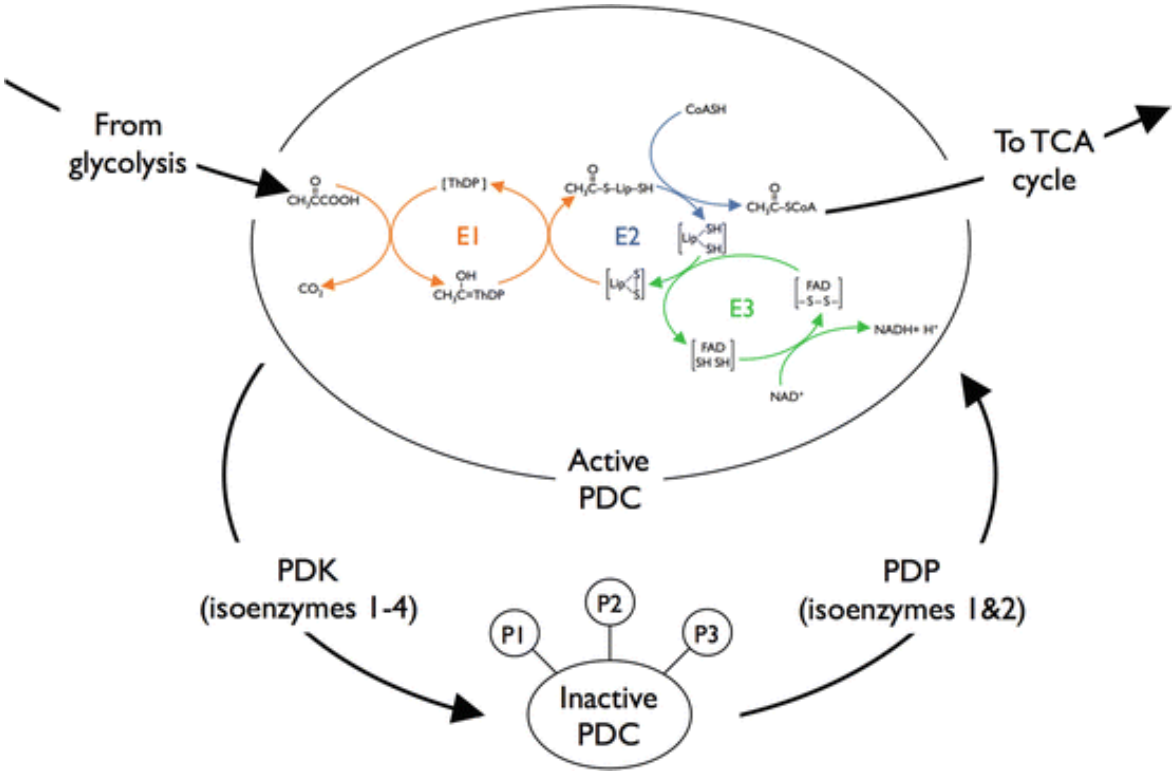


Figure 1.3 The pyruvate Dehydrogenase Complex (PDC) reaction cycle
PDC showing multi-enzyme complexes, including the E2 and E3 subunits, used in the formation of acetyl coenzyme A and nicotinamide adenine dinucleotide (Byron, 2017, page 525) (Byron and Lindsay, 2017)

Further evidence for impaired bioenergetics in PBC come from studies using magnetic resonance spectroscopy to understand the impact of anti-PDC on PDC function which has previously been shown to inhibit PDC in vitro. Magnetic resonance spectroscopy is an experimental approach that allows the real-time capture of metabolic information during and after mild exercise, to assess muscle tissue energy use, by measurements of ADP, ATP, phosphocreatine (PCr) (used as an immediate reserve for re-phosphorylation of ADP, **see Figure** 1.4 **(Dalal and Mishra, 2017)**), and cell pH (Hollingsworth *et al.*, 2008). The initial pilot study compared healthy controls, chronic fatigue/myalgic encephalomyelitis (ME) patients, PBC patients with and without fatigue (assessed using FIS) and primary sclerosing cholangitis (PSC) as a control cholestatic group. For the control, ME, and PSC groups there was a close correlation between PCr and ADP recovery following low-impact exercise, which would suggest a normal mitochondrial response to exercise associated accumulation of ADP. This correlation was not seen in either the fatigued or non-fatigued PBC groups, with statistically significantly higher ratios of PCr recovery ratio and ADP recovery compared to all non-PBC groups (Hollingsworth *et al.*, 2008). This would suggest that mitochondrial dysfunction, independent of levels of reported fatigue, exists in all patients with PBC.

However, the reason why some patients experience such profound fatigue, whilst others don't, is unclear.

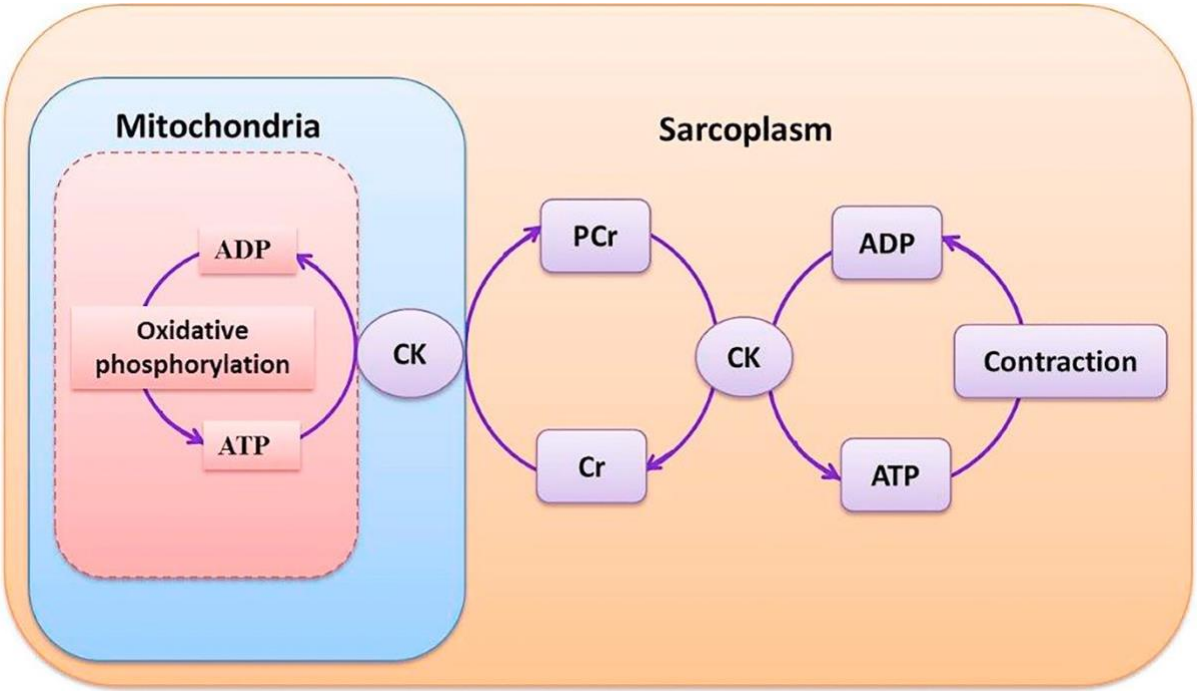


Figure 1.4 The phosphocreatine shuttle system
The high energy bond from adenosine triphosphate (ATP) is transferred to creatine by mitochondria creatinine kinase (CK) to form phosphocreatinine (PCr). PCr diffuse through the mitochondria membrane to the myofibrils of the muscles. The myofibrils CK catalyses the reformation of ATP from PCr. With increasing exercise and increased energy demands the PCr level decreases with a corresponding increase in adenosine diphosphate (ADP). The PCr:ATP ratio is a measure of muscle energetics (dalal, 2017, page 396) (Dalal and Mishra, 2017)

Other important findings of muscle metabolism from this study included that of cellular ph. In all groups, the resting pH was not significantly different. In the control, ME, and PSC groups there was a small decrease in muscle pH following exercise performed at 35% of maximum voluntary contraction (MVC), followed by a rapid return to baseline on cessation of exercise, whilst in PBC patients, regardless of FIS score, there was an early, more substantial decrease in pH following exercise, with a third of cases having a marked decrease. The correlation in PBC patients between mitochondrial function, minimum pH value and pH drop seen with exercise, suggested that mitochondrial dysfunction caused the level of post-exercise acidosis observed in muscles of PBC patients. In those patients reporting severe levels of fatigue, recovery of pH was significantly prolonged, although this was not related to mitochondrial function. Of note, in patients with PBC with a high PCr/ADP recovery time, a significantly higher serum Immunoglobulin G (IgG) anti-PDC titre was observed, suggestive that mitochondrial dysfunction is not only a consequence of serum anti-PDC, but that the degree of

dysfunction is related to serum levels. Other key findings were, as in previous studies (Goldblatt, 2001), that the initial maximum exercise capacity between all groups were similar, but that the limitation in PBC is the lack of capacity to sustain repeated exercise i.e. patients with PBC have the same initial capacity to perform a given muscle contraction, but they are unable to sustain repeated muscle contractions due to dysfunctional muscle metabolism (Hollingsworth *et al.*, 2008).

The follow-on study explored the effect of repeat exercise on muscle acid regulation between normal controls, PBC patients with mild and severe levels of fatigue, using measurements from the pilot study described previously. In normal subjects, the lowest pH seen after exercise was unchanged between successive periods of exercise of the same intensity. Recovery time to baseline pH was significantly prolonged in patients with PBC (PBC median 160s vs normal 25s) particularly those with high levels of fatigue when compared to normal controls. Normal controls showed a progressive (non-significant) shortening of recovery time to baseline pH following repeated exercise, which had previously been observed in the normal population. PBC patients with low levels of fatigue initially showed normal physiological shortening of recovery time, but the recovery time became blunted following repeated exercise, whilst those with high levels of fatigue showed prolongation of recovery much earlier during repeated exercise (Hollingsworth *et al.*, 2010b). This indicates that the normal adaptive physiological mechanisms related to pH recovery following repeated exercise are impaired in patients with PBC and this is particularly true for patients reporting severe fatigue. These findings potentially mirror the symptoms experienced by PBC patients with fatigue, whereby sustained activity results in the described feeling that their batteries have run down. As a follow on to the study, PBC patients continued a prescribed exercise program for 4 weeks, which was associated with improvements in fatigue, emotional and social domains as assessed by the PBC-40 and an overall higher level of functioning (Hollingsworth *et al.*, 2010b). This might suggest that a graded exercise program could readapt muscle metabolism to improve recovery time following repeated exercise, and this could be beneficial to patients with PBC and fatigue.

Impairment in muscle metabolism in PBC is not limited to skeletal muscles. Jones et al demonstrated altered myocardial dysfunction, with PBC patients demonstrating a lower cardiac muscle PCr/ADP ratio compared to controls, which correlated to an impaired left ventricular ejection time in response to standing (orthostasis). Of note, the PCr/ADP ratio in 40% of patients with PBC was below the clinically significant ratio of <1.6, which is associated with

an increased risk of cardiac mortality. There was no difference in cardiac structure or function between controls and the PBC group (Jones *et al.*, 2010b). Previous cohort studies have shown a correlation between fatigue and increased all-cause mortality, in particular with an increase in cardiac death in fatigued PBC patients (Jones, 2006; Jones *et al.*, 2010a).

Follow on studies have demonstrated disordered myocardial mechanics in PBC. Cardiac-tagged MRI (as a measure of myocardial torsion) demonstrated a reduced relative contribution of contraction from the sub-endocardium (measured by the torsion-to-shortening ratio (TSR)) in fatigued PBC patients, compared to non-fatigued PBC patients and controls. In all PBC patients there was an inverse correlation between reduced baroreflex effectiveness on standing and TSR, suggestive that the baroreflex (that regulates the sinus node on standing) was less effective in those with the most impaired TSR. The authors calculated, using previous studies in TSR and the results from their healthy controls, that the hearts of fatigued PBC patients had a mean age of 16 years above their chronological age (Hollingsworth *et al.*, 2012). It should be noted that this study was limited by the fact that tagging studies did not include the apex and base of the left ventricle, only small numbers (PBC fatigue n=7, PBC no fatigue n=6, and controls n=10) were examined, and the real-time resolution used for the cardiac-tagged MRI was low which may have adversely affected readings.

AMA-associated myositis is a rare cause of inflammatory myopathy affecting skeletal, cardiac and gastrointestinal muscles, which are associated with abnormalities of the muscle mitochondria in the presence of AMA positive antibodies in patients both with and without a clinical diagnosis of PBC (Varga *et al.*, 1993; Uenaka *et al.*, 2013; Albayda *et al.*, 2018; Maeda, Tsuji and Shimizu, 2012). It is commonly associated with longer disease duration prior to diagnosis and with histopathologic features of granulomatous inflammation on muscle biopsy and there is an increased association with cardiac involvement in those with a clinical diagnosis of PBC (Maeda, Tsuji and Shimizu, 2012). It should be noted that in many of these reported cases, the degree of myopathy was severe with muscle atrophy and overt clinical features such as winging of the scapula or cardiomyopathy. Whilst these are not commonly seen in clinical practice in patients with PBC, there may be a common pathophysiology involved in respect to the effect of AMA on muscle metabolism and this may represent a spectrum of disease, with these cases being at the extreme.

Taken together, these findings would suggest that a significant number of patients with PBC have disordered muscle metabolism and impaired aerobic exercise capacity, and that this is not limited simply to those who experience significant fatigue. Fatigue is experienced to varying degrees in PBC and it may be that disorders of muscle metabolism are also of a similar spectrum. AMA antibodies have been implicated as a rare, but potential cause of myositis in animal models, and whilst this should be interpreted with caution, it suggests that antibodies directed against mitochondria, which could include AMA, can penetrate the cell membrane and cause disruption of energy metabolism (Kuhl, Ulrich and Schultheiss, 1987; Schulze *et al.*, 1990). These bioenergetic changes may well be a contributor to peripheral fatigue in PBC.

1.4.3 Central fatigue and cognitive symptoms

Cognitive symptoms and central fatigue are complex and share many similar components. Characterised by neurophysiological irregularities, they encompass cognitive dysfunction, autonomic dysfunction, sleep disturbance and mood disorders. Here we include central fatigue as part of our discussion on cognitive symptoms.

1.4.3.1 Cognitive symptoms

In PBC, cognitive impairment largely relates to diminished memory and concentration, and whilst associated with fatigue, it is considered a discrete entity, having been derived and validated with development of the PBC-40 (Jacoby *et al.*, 2005). In cohort studies, 19% of patients reported severe cognitive symptoms, and 34% moderate symptoms (Newton *et al.*, 2008a). Cognitive symptoms remain independent of UDCA treatment, responder state and disease stage or severity (Mells *et al.*, 2013; Newton *et al.*, 2008a). The lack of symptom improvement following liver transplantation is highly suggestive that CNS damage becomes irreversible early in the disease process (Carbone *et al.*, 2013a; Mcdonald *et al.*, 2010; Pells *et al.*, 2013b).

Early objective, but not disease specific, neuro-psychometric tests have highlighted deficits in PBC patients related to memory and orientation (Floreani *et al.*, 1995). Full-scale Intelligence Quotient (IQ) tests have highlighted that PBC patients score significantly lower IQ global scores, when compared to age and gender-matched controls, however, these lower IQ scores are a consequence of abnormalities in domains relating to verbal fluency and cognitive processing (Newton *et al.*, 2008a). These inverse correlations in IQ scores to the PBC-40

cognitive domain scores substantiate self-reported cognitive symptoms to objective neuro-psychometric testing.

1.4.3.2 Neuroimaging

Resting-state functional magnetic resonance imaging (rsfMRI) is used to assess brain functional connections, i.e. how regions of the brain interact with each other. The strength of these functional connections can be measured using resting-state functional connectivity (rsFC) (Biswal *et al.*, 1995). Non-cirrhotic PBC patients exhibit markedly altered functional connections between brain deep grey matter compared to controls. Areas affected include the putamen, thalamus, amygdala and hippocampus and these changes correlate to symptom burden, particularly cognitive performance (Mosher *et al.*, 2017). The amygdala, hippocampus and putamen are important structures for memory, learning and emotions (Packard and Teather, 1998; McDonald and White, 1993; Poldrack and Packard, 2003) and these changes in functional connections may contribute to cognitive symptoms in PBC. Other abnormal functional connections have been observed between the hippocampus, amygdala and prefrontal cortex, which are involved in executive functions (memory, concentration and planning), whilst connections between the putamen, hippocampus, and motor and sensory cortexes have been associated with fatigue (Mosher *et al.*, 2017). Observations for functional connectivity changes involving the motor cortex in patients with fatigue may be a consequence of physiological adaption to dysfunction in post-contraction cortical depression that can occur in PBC patients with fatigue (Cerri *et al.*, 2010). Changes in connections between functional areas of the brain involved in memory, planning and learning may suggest a resultant mechanism for cognitive symptoms experienced by patients with PBC, but the initiating mechanism for changes in functional connections remains unclear.

Further support for abnormal functional connections in PBC comes from the use of trans-cranial magnetic stimulation (TMS) via paired pulse protocols to modulate cortical pathways and affect neuronal plasticity (Di Lazzaro, Rothwell and Capogna, 2018; Rossi *et al.*, 2009). Reduced CNS activation has been associated with both fatigue and cognitive dysfunction in many conditions (Zwarts, Bleijenberg and Van Engelen, 2008). Reduced central activation and abnormal intra-cortical inhibition have been demonstrated with TMS in both transplanted and non-transplanted PBC patients with cognitive dysfunction, fatigue and sleep disturbance, suggesting impaired intra-cortical inhibition as a potential cause (Mcdonald *et al.*, 2010).

Anatomical changes on MRI using T2-weighted images (sensitive to extracellular fluid that replaces cells that have lost integrity), have been observed in patients with PBC, with white matter lesions that correlate significantly with cognitive function being seen in a similar distribution to that detected in dementia (Mosher *et al.*, 2017; Kenny, Kalaria and Ballard, 2002; Siennicki-Lantz *et al.*, 1998). Despite these similarities, the burden of cognitive dysfunction in PBC is not comparable to that seen in dementia. The mechanism of injury is unclear, but white matter lesion load has been observed to correlate with the severity of cognitive dysfunction as well as autonomic regulation of blood pressure control, with a higher systolic baseline blood pressure being associated with improved cognitive function (as assessed using full-scale IQ) (Newton *et al.*, 2008a). Lesion density was noted to be highest in the frontal lobe, a region of the brain associated with higher executive function. These findings could be due to a lower baseline blood pressure resulting in a reduction in cerebral blood flow, and this could be further compounded by the autonomic dysfunction observed in PBC, resulting in further dysregulation of cerebral perfusion. Autonomic dysregulation, relating to postural blood pressure and heart rate variability, is well documented to pre-dispose non-liver patients to white matter lesions, which in turn is associated with cognitive dysfunction (Dadar *et al.*, 2020; Galluzzi *et al.*, 2009; Del Brutto *et al.*, 2022; Kenny, Kalaria and Ballard, 2002; Siennicki-Lantz *et al.*, 1998; Ballard *et al.*, 2000).

In normal human physiology, cerebral autoregulation maintains a consistent cerebral blood flow, even during periods of fluctuations in peripheral blood pressure. Impaired cerebral autoregulation can occur in many conditions, including advanced cirrhosis (O'Carroll *et al.*, 1991). Transcranial doppler, a non-invasive ultrasound technique, can be used to assess cerebral perfusion in real-time and the dynamic autoregulatory response (Bellapart and Fraser, 2009; Panerai, 2009). The Valsalva manoeuvre is a non-invasive procedure that results in peripheral haemodynamic and cerebral blood flow changes. In healthy individuals, a compensatory cardiovascular reflex should occur, with a slight dip in the middle cerebral artery flow observed during the procedure. Increased cerebral resistance and impaired cerebral autoregulation in response to the Valsalva manoeuvre has previously been identified in 80% of PBC patients, which correlated to indicators of autonomic dysfunction and increased MRI T2 relation time (an indicator of more gradual loss of cell integrity) of the globus pallidus (Hollingsworth *et al.*, 2010a). Whilst the study did not examine fatigue or cognitive symptoms

in the cohort, the study is highly suggestive that autonomic dysfunction results in impaired regulation of cerebral blood flow, resulting in structural changes in the brain.

Scintigraphy, a nuclear medicine diagnostic test that uses radioisotopes to assess tissue specific areas of the body, has been used to examine global cerebral flow and brain perfusion in patients with PBC. This technique demonstrated a positive correlation between the cognitive dimension of the FIS and right frontal lobe perfusion impairment (Raszeja-Wyszomirska, 2017). This suggests changes to the frontal lobe affect executive function (concentration and planning) leading to cognitive dysfunction, and that autonomic dysfunction in PBC maybe a contributory factor.

The hippocampus is a major element of the limbic system and involved in learning, memory and mood (Burgess, Maguire and O'Keefe, 2002). Hippocampal volume reduction is apparent on MRI scans of patients with PBC when compared to controls, with a reduction similar in magnitude to that seen in other patient populations, including major depressive disorder and neurodegenerative diseases (Mosher *et al.*, 2018). In particular, subregions of the hippocampus, involved in neurogenesis (important for cognition and brain repair) and regulation of the Hypothalamic-Pituitary-Adrenal (HPA) axis (see section 1.4.3.4) were significantly reduced. Increased brain iron deposition, seen in previous studies (Hollingsworth *et al.*, 2009), was highly suggestive of neuroinflammation and/or oxidative stress and may suggest a mechanistic cause. Importantly, the median time from first diagnosis in this group was 6 years, and there was no correlation in clinical characteristics, i.e., stage of disease, responder status etc. and the authors postulated that disease associated reduction in hippocampal volume, along with iron deposition occurred very early in the disease process.

Grover et al demonstrated early diffusion weighted imaging changes on MRI in the thalamus of patients newly diagnosed with PBC, as well as T1 weighted abnormalities of the thalamus, caudate putamen and global pallidus, compared to controls (Grover *et al.*, 2016). The study was not powered enough to associate symptoms to MRI changes (no participants had severe cognitive symptoms), but this study also strongly suggests that abnormalities in the brains of patients with PBC occur early, and that the underlying disease process, of inflammation and/or cholestasis, is the fundamental cause.

It has been postulated that manganese, a tiny mineral, usually found in miniscule amounts in the body, and which has been found in higher concentrations in the globus pallidus of cirrhotic patients (Rose *et al.*, 1999; Krieger *et al.*, 1995), may accumulate in patients with PBC (Hollingsworth *et al.*, 2009). Early stage PBC patients with elevated serum manganese, that correlates with fatigue, have been shown to have a significant reduction in magnetization transfer ratio (MTR); a sensitive means on MRI to quantify CNS myelin macromolecular content (Schmierer *et al.*, 2004). In the globus pallidus, this is thought to be secondary to manganese deposition (Forton, 2004). However, subsequent studies have failed to replicate the correlation between serum manganese, neuroimaging and/or symptoms (Hollingsworth *et al.*, 2009; Grover *et al.*, 2016). The previous association between manganese deposition may be due to the original study not controlling for the effect of age on MTR.

Overall, evidence suggests that anatomical and functional CNS changes relating to cognitive symptoms and autonomic dysfunction are observed even in early stage PBC (Keresztes, 2004; Kempler *et al.*, 1994; Newton *et al.*, 2006a; Newton *et al.*, 2007b; Newton *et al.*, 2007a; Horsfield, 2005), with pronounced MRI changes occurring as early as 6 months after disease diagnosis (Grover *et al.*, 2016). Whilst the mechanism for this is unclear, it confirms that cognitive symptoms occur independently of disease staging, and that a biological process early in the disease results in CNS changes. This has important implications for initiating effective second line treatment early to prevent irreversible CNS damage.

1.4.3.3 Neuroinflammation

Neuroinflammation, that is inflammation that affects the CNS, can include many causes including from peripheral organs and autoimmune disease. The mechanism of action may include disruption of the Blood Brain Barrier (BBB), activation of glial cells associated with systemic immune activation and disruption of the gut-brain axis relating to the autonomic nervous system (Sun, Koyama and Shimada, 2022). Studies suggest a relationship between systemic inflammation, microglial activation, and subsequent inflammation in the brain (Hoogland *et al.*, 2015). Chronic inflammation likely leads to degradation of the BBB, which in healthy individuals usually separates the central and peripheral circulatory system, but instead results in vulnerability of the CNS (Sun, Koyama and Shimada, 2022). Despite the breakdown in the BBB and resultant vulnerability, only specific regions of the brain appear to be susceptible to neuroinflammation, and this includes regions with a high distribution of

astrocytes (a subclass of glial cells) that include the hippocampus, amygdala, hypothalamus and basal ganglia (Sun, Koyama and Shimada, 2022).

IL-6, TNF-α and interleukein-1β (IL-1β) have been observed in animal models to impede tight junction regulation between endothelial cells, resulting in increased permeability of the BBB (Banks, 2005; Zhou *et al.*, 2006; Dantzer *et al.*, 2008). Elevated serum TNF-α has been previously associated in the development of sickness behaviours, including fatigue, social withdrawal, loss of motivation and cognitive dysfunction in animal models (D'Mello and Swain, 2014; D'Mello, Le and Swain, 2009; Capuron and Miller, 2011; D'Mello and Swain, 2016; Dantzer *et al.*, 2008) and has been observed in other non-liver chronic inflammatory conditions such as rheumatoid arthritis. Improvement in sickness behaviour is seen with anti-TNF-α treatment (D'Mello and Swain, 2014; Ban *et al.*, 2010), whilst cerebral spinal fluid (CSF) TNF-α and IL-6 levels have been implicated as a cause for neuroinflammation in major depressive disorder (Felger *et al.*, 2020).

Evidence for peripheral cytokine release during liver inflammation is apparent in animal and clinical models and these result in recruitment of immune cells, such as monocytes, by activation of microglia in the CNS which may contribute to disruption of the BBB and inflammation of the CNS (Barak *et al.*, 2009; D'Mello *et al.*, 2013; D'Mello and Swain, 2014; D'Mello, Le and Swain, 2009). **See Figure** 1.5 **(Swain and Jones, 2019)**

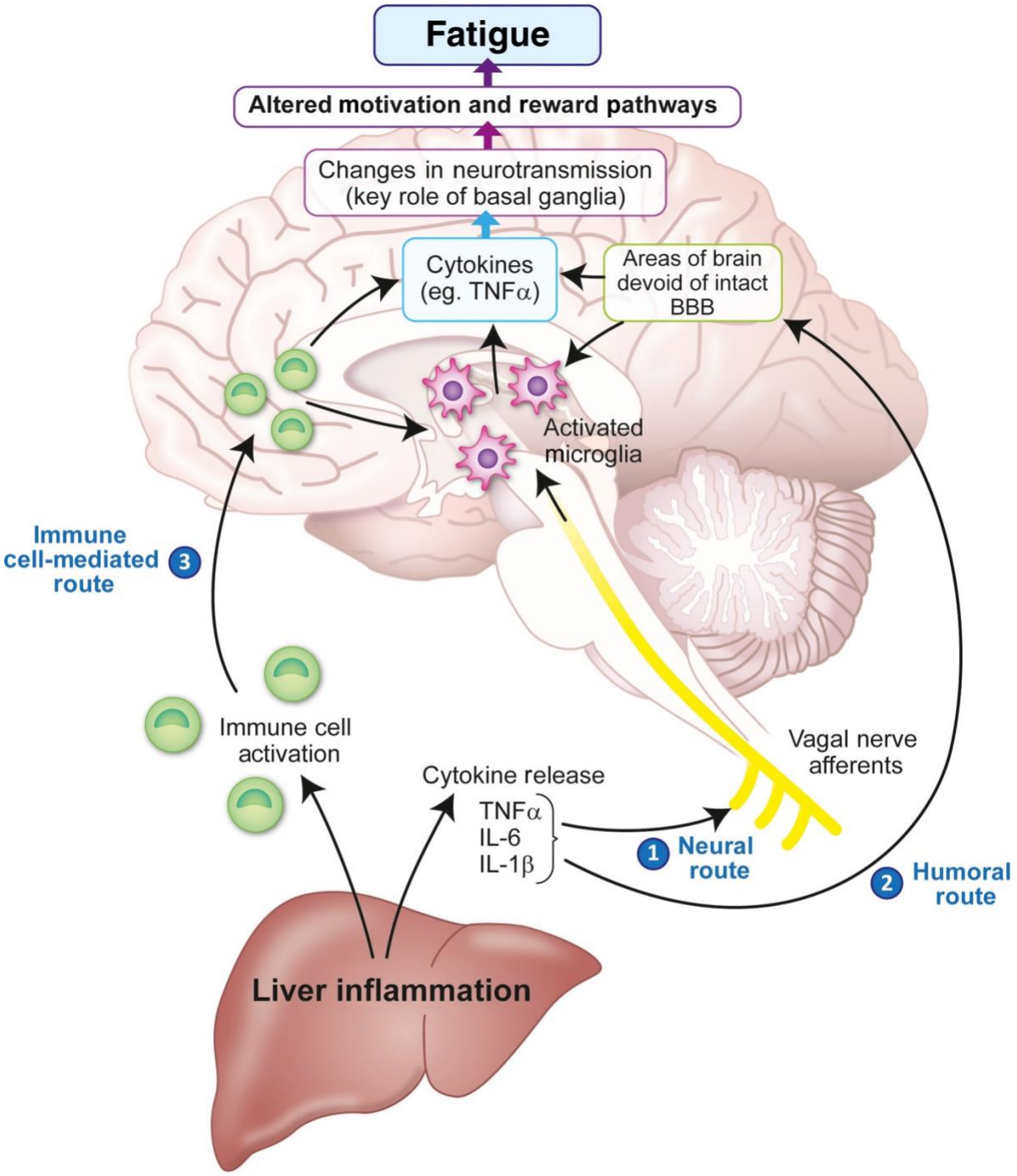


Figure 1.5. A model for central fatigue experienced in patients with primary biliary cholangitis.
Inflammation of the liver results in the production of inflammatory cytokines that can affect neurotransmission in the central nervous systems by direct or in direct pathways. 1) cytokines stimulate the vagal nerve afferents to signal via the spinal cord to the brain, causing direct or indirect alteration of neurotransmission in the brain. 2) Circulatory cytokines enter the brain via the disrupted blood brain barrier or directly stimulate cerebral endothelial cells, which release secondary neurotransmitters signals which stimulate microglia. 3) Activated immune cells such as monocytes are carried in the circulation and enter the brain parenchyma via endothelial cells, where they release further cytokines which directly or indirectly influence neurotransmission (Swain, Jones. 2018, page 9) (Swain and Jones, 2019).

In murine models of inflammatory liver injury, using bile duct ligation (BDL) techniques (an established means for mimicking cholestatic liver disease (Sato *et al.*, 2019)), activation of

microglia and infiltration of monocytes into the brain has been demonstrated to occur via TNF-α signalling (D'Mello, Le and Swain, 2009; Kerfoot *et al.*, 2006) which induces sickness behaviour, and are reversed following blockade of TNF-α (D'Mello *et al.*, 2013). Elevated circulatory TNF-α has previously been associated with PBC and disease response (Neuman *et al.*, 2002). Although they did not appear to contribute to the inflammatory proteome associated with PBC and UDCA response (Barron‐Millar *et al.*, 2021), TNF-α may still have a role in neuroinflammation in PBC that contributes to central symptoms, such as cognitive dysfunction.

A recent study provides strong evidence for cholestasis causing neuroinflammation and disruption of the BBB, resulting in cognitive dysfunction in PBC. BDL mice exhibited short-term memory loss (assessed by Y-maze), reduced astrocyte coverage of the BBB, along with hippocampal network deficits and neuronal senescence compared to controls (Gee *et al.*, 2023). These changes were reversed following administration of the FXR agonist, OCA (currently used as second line therapy in PBC), but not with UDCA. Cultured human neuronal cells also demonstrated neuronal senescence when exposed to cholestatic serum, which was again limited following exposure to OCA. This study is particularly important given the importance of astrocytes in regulating the BBB function and permeability (Preininger and Kaufer, 2022) and the demonstration of neuronal senescence in the hippocampus, a phenomenon associated with PBC and in particular UDCA non-responders. (Sasaki, Sato and Nakanuma, 2020) This is highly suggestive that senescence is linked to cholestasis, and that early treatment with OCA reversed many of these changes. The replication of these findings with human neurone cultures suggests that the reversal of cognitive dysfunction seen in rodent models, may translate into a clinically effective treatment for PBC patients, provided that treatment with OCA is initiated early in the disease course.

1.4.3.4 The hypothalamic-pituitary-adrenal axis

The HPA axis regulates physiological and psychological responses to acute and chronic stress. It has many influences, including the afferent pathways of the vagus nerve (Howland, 2014). The vagus nerve innervates many visceral organs including the liver (Jensen, Alpini and Glaser, 2013) and the afferent pathways not only express cytokine receptors but they also have macrophages located intermittently between fibres (D'Mello and Swain, 2016). Peripheral TNF-α, IL-1β, and IL-6 are all capable of stimulating the HPA axis, exciting the hypothalamus corticotropin-releasing hormone (CRH) neurone (Mastorakos, Chrousos and Weber, 1993;

Wan *et al.*, 1993). **See Figure** 1.5 (Swain and Jones, 2019). IL-6 in particular has been shown to have a profound and prolonged stimulatory effect on the HPA axis, resulting in elevated adrenocorticotrophic hormone (ACTH), that can result in fatigue (Mastorakos, Chrousos and Weber, 1993). Overstimulation of the HPA axis in patients with depression has also been observed (Maes *et al.*, 1993).

Not only is CRH a potent activator of the HPA axis, resulting in the release of ACTH but it has wide effects on the CNS due to the broad distribution of CRH containing nerve fibres in the CNS and plays a significant response in arousal and behaviour activation (Koob, 1999; Herman *et al.*, 2016). Defective release of CRH within the CNS has been implicated in the pathology of central fatigue in patients with atypical depression and chronic fatigue syndrome (Bearn *et al.*, 1995; DEMITRACK *et al.*, 1991). Central administration of CRH in rodents stimulates behavioural and locomotion activation (Sutton *et al.*, 1982), whilst low hypothalamic CRH (around 25% less than controls) and overexpression of CRH type 1 receptor expression has been shown to be present in rodent models of cholestatic liver disease resulting in a reduction in decreased motivation and locomotive activity (Burak, Le and Swain, 2002; Swain *et al.*, 1993; Swain and Maric, 1995). This suggests that defective release of CRH within the CNS may be a factor in the development of central fatigue.

1.4.4 Other associated symptoms

Fatigue in PBC has been shown to be strongly associated with several other clinical features of PBC, including autonomic dysfunction, sleep disturbance and depression (Mells *et al.*, 2013).

Mells et al explored the UK-PBC cohort of 2,353 patients to explore the impact of PBC on QOL. As well as using the PBC-40 to assess traditional symptoms associated with PBC, they also used validated measures including the ESS, OGS and HADS to characterize the extent and severity of other symptoms related to PBC. They found that 24% (575/2353) of patients suffered severe fatigue, with the incidence of sleep disturbance being higher in those suffering from severe fatigue (65% of this group as opposed to 10% in those with no or mild fatigue). Autonomic dysfunction was also significantly related to symptoms of severe fatigue (67%) compared to those with no or mild fatigue symptoms (15%). Co-existing sleep disturbance with autonomic dysfunction occurred in 28% of those with severe fatigue, whilst the combination

of depression and sleep disturbance was less common (6%). Autonomic dysfunction, sleep disturbance and depression co-existed in 19% of those with severe fatigue, suggesting that the complex interaction between these symptoms may be part of a CNS mediated process (Mells *et al.*, 2013). Significant daytime somnolence occurred in 28% of the PBC population (Mells *et al.*, 2013).

1.4.4.1 Autonomic dysfunction

Large UK-PBC cohort studies assessing autonomic dysfunction using the OGS suggest that around 29% of the PBC population are considered to have clinically significant autonomic dysfunction (Mells *et al.*, 2013). It is particularly associated with those with severe symptoms of fatigue (67%) compared to those with no or mild fatigue symptoms (15%) (Mells *et al.*, 2013). Symptoms of cardiovascular autonomic dysfunction have been more frequently reported, and more severe, when compared to matched healthy controls and patients with the autoimmune cholestatic disease, PSC, which independently correlated to the severity of fatigue and cognitive symptoms (Newton *et al.*, 2007b).

Heart rate variability (HRV) and baroreflex sensitivity (BRS), measures of autonomic dysfunction, have been demonstrated to be significantly reduced in patients with PBC, particularly in those reporting high levels of severe fatigue, but is unrelated to cirrhosis status (Newton *et al.*, 2006a). Fatigue, in particular, is related to very low frequency HRV, which has been implicated in abnormalities of neurohumoral and thermos-regulation ('Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology,' 1996; Shaffer and Ginsberg, 2017). These changes in autonomic function maybe important, given lower HRV is associated with an increased all-cause death (Fang, Wu and Tsai, 2020) and may give some insight into the non-liver/non-cancer excess standardised mortality rate associated with PBC. The mechanisms that result in reduced HRV and BRS are uncertain. Reduced HRV and fatigue have been previously noted in manganese alloy workers, with toxicity to manganese and deposition in the global pallidus (Barrington *et al.*, 1998; Roels *et al.*, 1987; Ferraz *et al.*, 1988), whilst deposition of manganese in the global pallidus that is associated with fatigue in patients with PBC has also been observed (Forton, 2004; Grover *et al.*, 2016). Autonomic dysregulation of blood pressure has been shown to be common in PBC,

correlating with moderate to severe scores on OGS and is associated with both fatigue and cognitive symptoms (Newton *et al.*, 2007b).

As well as HRV and BRS being manifestations of autonomic dysfunction, studies using MRI T2 imaging to identify structural differences, and transcranial doppler to monitor real time cerebral vascular flow have been used (Hollingsworth *et al.*, 2010a). Patients with PBC have been found to have markedly impaired cerebral autoregulation following the Valsalva manoeuvre, with increased cerebral resistance, and increased globus pallidus T2 relaxation time that correlated both with low frequency HRV and blood pressure, which is highly suggestive of autonomic failure (Hollingsworth *et al.*, 2010a). These support the existence of autonomic dysfunction in PBC, resulting in increased cerebral resistance and impaired cerebral autoregulation, which impairs cerebral blood flow. The underlying mechanisms for these findings remain unclear. Changes observed on MRI may suggest that this results in the cognitive dysfunction observed in PBC, but the causality is not proven.

A predictive model for fatigue, based on autonomic dysfunction and daytime somnolence has previously been validated (Newton *et al.*, 2008b). Scoring ≥4 on OGS and/or ≥10 on ESS is considered to be highly predictive of severe fatigue (sensitivity 71% and specificity 80%) (Newton *et al.*, 2008b), although fatigue is no more severe when patients score highly in both domains, suggesting that these are both independent factors for fatigue and that their underlying mechanisms may differ. In clinical practice this predictive model is rarely used.

1.4.4.2 Sleep disturbance

Sleep disturbance is commonly reported in patients with chronic liver disease and is prevalent in patients with PBC. It commonly occurs in those with severe fatigue and co-exists with autonomic dysfunction and daytime somnolence (Mells *et al.*, 2013; Marjot *et al.*, 2021). In previous cohort studies, the incidence of sleep disturbance has been found to be 65% in those suffering with severe fatigue, and 10% in those with no or mild fatigue. Co-existing sleep disturbance with autonomic dysfunction occurred in 28% of those with severe fatigue, whilst the combination of depression and sleep disturbance were less common (6%) (Mells *et al.*, 2013).

There is limited research specifically relating to PBC and sleep disturbance with research mostly focussing on cirrhosis and disruption of the circadian rhythm. Impaired hepatic metabolism of melatonin in particular is implicated as a potential mechanism in cirrhosis, but the evidence is unclear (Marjot *et al.*, 2021). It is likely that there is also a central mechanism underpinning this (based on evidence of disruption of circadian cycle related to melatonin and cortisol in cirrhosis) and it is apparent that hepatic encephalopathy has a role (Montagnese *et al.*, 2010; Montagnese *et al.*, 2014).

Behavioural, physiological, and biochemical rhythms in humans, including sleep regulation, are controlled by the master circadian clock, which is located in the anterior part of the hypothalamus, known as the suprachiasmatic nuclei (SCN). It is synchronized constantly by external influences: mainly light/dark time, but food also plays a role through the gut and liver. The pineal gland receives signals from the SCN and synthesises melatonin. Melatonin is considered a "neuroendocrine transducer" of the light/dark cycle, is increased before or during sleep and suppressed during daytime based on light exposure (Montagnese *et al.*, 2010). Melatonin activates two high-affinity G protein-coupled receptors, MT1 and MT2, to activate multiple signalling pathways that affect the circadian signal, in particular to exert an effect on the limbic system to regulate sleep (Dubocovich, 2007). Peripheral tissues (such as the gut and liver) also have their own circadian clocks, as well as the pineal gland and many other tissues (including the liver) producing melatonin (Sato *et al.*, 2020; Acuña-Castroviejo *et al.*, 2014).

There are two stages in human sleep: non-rapid eye movement sleep (NREM) and rapid eye movement (REM). NREM makes up 80% of sleep and is comprised of 4 stages, all of which demonstrate a specific synchronous cortical pattern termed "slow wave sleep" (SWS). This tends to occur in the first third of the night and correlates with the initiation of the sleep period and the time of wake up. During SWS there is minimal mental activity. As sleep progresses NREM moves to REM sleep, defined by electroencephalogram (EEG) activation, muscle atonia and episodes of rapid eye movements. This accounts for 20% of sleep and tends to occur in the last third of night, relating to circadian rhythm and body temperature (Plotogea *et al.*, 2021).

Studies exploring sleep disturbance in PBC, using fully validated, self-reported sleep measures such as the sleep timing and sleep quality screening questionnaire (STSQ), which records sleep quality and sleeping timings (i.e. bedtime, sleep onset-time, sleep latency and number of night

awakenings, wake up time and get up times), have consistently demonstrated that patients with PBC exhibit significantly greater sleep latency compared to controls (Newton *et al.*, 2006c; Montagnese *et al.*, 2013). The Pittsburgh sleep quality index (PSQI), which assesses global sleep quality, has identified that sleep quality is significantly lower in PBC patients compared to controls (Newton *et al.*, 2006c; Cauch-Dudek *et al.*, 1998), and that delayed sleep timing is associated with impaired sleep quality (Montagnese *et al.*, 2013; Cauch-Dudek *et al.*, 1998). Those with pruritus, not unexpectedly, have a longer sleep latency compared to those without, which also results in earlier wake up (Montagnese *et al.*, 2013). However, such disturbances in sleep cycle are apparent in the absence of pruritus and correlate strongly with fatigue severity.

Poor sleep quality has been associated with a significant increase in daytime somnolence as assessed by the ESS, with greater than 50% of PBC patients having an ESS score of >10 (the threshold for significant daytime somnolence) that was not associated with body mass index (BMI), in comparison to controls where an ESS score > 10 only occurred in 15% of cases (Newton *et al.*, 2006c). The pattern of sleep in PBC patients has been reported to be different to controls, with less time sleeping during the night and more time sleeping during the day, particularly for those with significant fatigue (Newton *et al.*, 2006c; Cauch-Dudek *et al.*, 1998).

Actigraphy, a validated and objective method of measuring sleep though use of a non-invasive accelerometer, has shown that overall, in 24 hours, the total amount of sleep appears to be the same, but in PBC patients there is a reduction in night-time sleep (increased sleep latency and time falling asleep after waking episodes) with an increase in daytime somnolence, that is associated with fatigue severity, but not disease severity (Newton *et al.*, 2006c; Montagnese *et al.*, 2013).

It has been postulated that the mechanism leading to sleep disturbance in PBC might be like that seen in cirrhosis, where there is a delayed melatonin rhythm, reduced sensitivity of the retinal-hypothalamic axis to light and delayed hepatic melatonin metabolism. It is theorised that if those with daytime sleepiness and fatigue have an inefficient waking period (i.e. the usual circadian rhythm mechanism of being awake/wakefulness for a period that then allows us to sleep later on) that this resulted in increased sleep latency (Montagnese *et al.*, 2013).

Non-PBC patients with delayed sleep-wake phase syndrome (a circadian sleep-wake disorder with a considerable delay in sleep onset/wake up times compared to a healthy population) have been shown to have improved sleep timing following treatment with morning light therapy that re-synchronises the circadian clock timing system (Rosenthal *et al.*, 1990; Richardson *et al.*, 2018). One interventional study, using a 15-day course of morning light therapy, showed a significant improvement in sleep onset and wake-up times in PBC patients compared to controls, with a reduction in the PSQI global score and a specific improvement in component 7 of the PSQI, related to sleep dysfunction due to sleepiness (Turco *et al.*, 2018). Unfortunately, this failed to demonstrate an improvement in QOL, as assessed using the health-related quality of life questionnaire, short form survey 36 (SF-36). However, the SF-36 is not specific or validated in PBC and it is difficult therefore to draw conclusions from this. In addition, due to the nature of the study the intervention could not be blinded, and this therefore may introduce bias.

It should be noted that the French PBC study group assessed the impact of PBC on QOL in 276 unselected with PBC using the NHP. They found no significant difference in sleep between PBC patients and age/sex matched controls (Poupon *et al.*, 2004). However, this study was limited as at the time (2004) this was the only QOL scoring system validated in French and therefore has not been validated in this context.

The SCN in the hypothalamus regulates rhythms and synchronises circadian clocks throughout the body based on the light-dark cycle, but some organs including the liver have their own circadian rhythms, which are mediated by activator genes like circadian locomotor output cycles kaput (CLOCK) and brain and muscle ARNT-like 1 (BMAL1) along with repressor genes such as period circadian protein homolog 1 (PER1) (Takahashi, 2017). Global removal of BMAL1 in murine models results in loss of circadian rhythms and expressions, but when these genes are introduced into murine hepatocytes and cholangiocytes, circadian expression and metabolism in the liver continues, but at a significantly reduced rate (Koronowski *et al.*, 2019). This suggests that full circadian function in the liver depends on signals from other tissues, and that disrupted circadian rhythms may play a role in liver disease (Marjot *et al.*, 2021; Mukherji *et al.*, 2019).

In the liver, there are high concentrations of melatonin in hepatocytes, and they release melatonin into human bile at concentrations 15 fold more than serum levels (Acuña-

Castroviejo *et al.*, 2014). Melatonin has been shown to suppress oxidative damage and ameliorate BDL-induced systemic oxidative stress in cholestatic rats. The melatonin receptors MT1 and MT2, along with the circadian genes CLOCK, BAML1, PER1 and CRY1, demonstrate up-regulation in cholangiocytes for rodents who have undergone BDL. Administration of melatonin in BDL rodents improves bilirubin, transaminases, and decreased expression of clock genes (Renzi *et al.*, 2011; Sato *et al.*, 2020). Similarly, pinealectomy or light treatment applied to BDL rats, results in reduced serum melatonin, and increased cytokines, such as IL-6, and has been shown to worsen ductular reaction and liver fibrosis, along with increasing biliary senescence (Chen *et al.*, 2019). The variations in the concentration of melatonin in human bile with patients with PBC is unknown, and could conceivably, based on the above studies, contribute to both liver inflammation and sleep disturbance. However, the interplay between melatonin and the liver is clearly complex and most studies are based in animals, particularly rodents, where there are significant differences, including the rodent expression of the MT3 receptor, which has not been found in human tissue.

Overall, disturbance of normal sleep patterns is common in liver disease (Mukherji *et al.*, 2019; Marjot *et al.*, 2021). In PBC, delayed sleep and reduced quality of sleep occurs frequently. In some it can result in daytime somnolence, which is strongly associated with fatigue. What is not clear, is if fatigue leads to disturbance of sleep, or, if sleep disturbance contributes to fatigue, but it is likely to be more complicated than one entity being the direct result of the other. The pathophysiology underlying sleep disturbance is unknown and there is currently only one study, with limitations, that has explored a therapeutic intervention (Turco *et al.*, 2018).

1.4.4.3 Depression

The psychological impact of fatigue in patients with PBC can be profound, with several studies showing an increased incidence of anxiety and depression in patients with PBC and fatigue (Biagini *et al.*, 2008; Blackburn *et al.*, 2007; Mells *et al.*, 2013; Huet *et al.*, 2000). Uncertainty exists as to whether depression is a potential cause of and contributor to fatigue, or if depression is a consequence of living with chronic and debilitating fatigue.

Earlier studies have assessed depressive symptoms using the Beck Depression Inventory (BDI), a self-reported mood severity scale, with around 40-45% of patients with PBC reporting

depressive symptoms (Huet *et al.*, 2000; van Os *et al.*, 2007), with a strong correlation associated with FIS scores (Huet *et al.*, 2000). However, when formally assessed for a depressive syndrome, as defined by the diagnostic and statistical manual of mental disorders IV (DSM-IV) criteria, the prevalence of depressive disorders has been found to be similar to that of the general population (van Os *et al.*, 2007). This should be interpreted with caution, however, given the heterogeneity in the clinical diagnosis of depression in current clinical practice; that is many people have depressive symptoms, a clinical diagnosis and treatment of depression, who have not been assessed (and may not fulfil) DSM-IV criteria.

Anxiety and depression were found to be higher in patients with severe fatigue in a 2007 study by Blackburn et al when 24 patients were assessed using the PBC-40 with a score of greater than 38 in the fatigue domain indicating severe fatigue (n=10). They found this severely fatigued group scored significantly higher for both anxiety and depression, as assessed by the HADS and had more frequent thoughts on the impact of fatigue on their life, as assessed by the Penn State Worry Questionnaire (PSWQ) (Blackburn *et al.*, 2007). This study demonstrated an association with fatigue, depression, and anxiety, but did not assess for associations with other symptom complexes (autonomic dysfunction, sleep disturbance).

The UK-PBC QOL cohort study demonstrated the prevalence of depression and fatigue to be present in 3% of those with moderate to severe symptomatic fatigue, compared to <1% for none or mild symptoms of fatigue (as assessed by HADS). Depression was more commonly seen in the context of a symptom complex, with a much higher prevalence occurring in those with fatigue, autonomic dysfunction, and daytime somnolence (19%) (Mells *et al.*, 2013) and similar symptom complexes have been replicated in other studies (Newton *et al.*, 2007b). Another large cohort study demonstrated levels of depression were similar between case-matched male and female patients with PBC, despite higher fatigue scores in the female PBC population, suggesting no direct correlation with fatigue (Carbone *et al.*, 2013b). This may suggest that the depression associated with PBC is not simply a consequence of living with a chronic illness, but rather is a result of a pathological mechanism resulting in a symptom complex.

A large cohort study of 1,177 PBC patients demonstrated that around 14% had a diagnosis of depression, with around 7% being diagnosed after their PBC diagnosis (Shaheen *et al.*, 2018), similar to previous studies (Al-Harthy *et al.*, 2010). Depression status was not a predictor of

poorer outcomes, although use of the anti-depressant mirtazapine was associated overall with better outcomes (mortality, liver transplantation and decompensation) (Shaheen *et al.*, 2018). The reason for this is unclear, but its complex pharmacology (with noradrenergic and serotonergic properties) may offer additional benefits compared to traditional selective serotonin reuptake inhibitors (SSRI). Other data on anti-depressants in PBC are largely limited to the use of SSRIs in relieving pruritus. Randomised placebo-controlled trials of SSRIs to improve fatigue have demonstrated no benefit in fatigue (ter Borg *et al.*, 2004) or HRQOL (Talwalkar *et al.*, 2006), suggesting that whilst there is an association between fatigue and depression, depression itself does not appear to be the primary driver for fatigue

Dysregulation of neurotransmitter synthesis has long been implicated as a cause for depressive disorders (Siever and Davis, 1985). Elevated serum amino acid concentrations of tyrosine (a pre-cursor to dopamine) and reduced serum tryptophan (a pre-cursor to serotonin) have previously been found in PBC patients, compared to controls, with a strong correlation with fatigue, cognitive symptoms and reduced QOL, as assessed by the FIS and SF-36 (Ter Borg *et al.*, 2005). Whilst depression was not formally assessed, PBC patients also demonstrated an association with the "mental health" component of the SF-36 and these findings may be of relevance given the link between neurotransmitter levels and mental health.

Animal models, using BDL rats to simulate cholestasis, have demonstrated anhedonia (loss of pleasure) and a loss of social interest, compared to controls (sham-resected rats), which was associated with elevated serum glucocorticoid levels, normal ACTH but a normal dexamethasone-induced suppression of HPA axis activity (Swain and Le, 1998). Elevated serum glucocorticoid levels have been shown to be present in humans with major depressive disorder, along with normal ACTH, but with an abnormal dexamethasone-induced suppression of HPA axis activity (Anacker *et al.*, 2011; Maes *et al.*, 1993). While caution should be taken when comparing animal models of disease to humans, this data may suggest that in cholestatic disease, the feedback for glucocorticoid is disrupted above the level of the pituitary. See section 1.4.3.4 for further discussion of the HPA axis in PBC.

Overall, many patients with PBC self-report depression and/or anxiety on established self-assessments, such as the HADS questionnaire. There appears to be a complex symptom association between fatigue, autonomic dysfunction, sleep disturbance and depression. Studies into depression in PBC are limited, as are data on the effectiveness of traditional treatments,

such as SSRIs to treat depression, but given the complex interplay of symptoms, it seems unlikely that traditional treatments would adequately address the currently poorly understood pathophysiology.

1.4.5 Pruritus

Cholestatic pruritus (itch) is the commonest symptom described in PBC, affecting up to 70% of patients during their disease course, with nearly a third of these experiencing persistent pruritus and approximately 15% reporting severe itch (Hegade *et al.*, 2015; Hegade *et al.*, 2014). Untreated, this can have a significant impact on QOL and can result in sleep deprivation, depression and in severe cases, suicidal ideation (Hegade *et al.*, 2016a; Jin and Khan, 2016). It is more prominent in younger patients (Hegade *et al.*, 2019), and in particular is associated with the aggressive ductopenic disease phenotype of PBC (Vleggaar *et al.*, 2001). Typically, pruritus associated with cholestasis follows a circadian rhythm, with patients reporting the highest intensity in the evening and early night (Kremer *et al.*, 2008b), but it also increases with increasing temperature. Females with cholestasis may have worsening pruritus following hormonal changes during the menstrual change, late in pregnancy and with hormone replacement therapy (Kremer *et al.*, 2008b; Bergasa, 2008). The limbs, soles and palms are typically affected (Kenyon *et al.*, 2010), but it can affect any area of the body. Primary skin lesions seen from pruritus in other dermatological disorders are absent, but secondary excoriations may occur from overt scratching (Beuers *et al.*, 2014).

Currently there are several therapeutic options available to treat cholestatic pruritus, but the use of these is largely empirical. Bile acid sequestrants (cholestyramine and colesevelam) (Hirschfield *et al.*, 2018; Hirschfield *et al.*, 2017; Lindor *et al.*, 2018) and rifampicin (a pregnane X receptor (PXR) agonist) (Prince, 2002) are the agents with the best evidence base, but are incomplete in their mode of action. Other treatment options include medications that modify neurotransmission (opiate antagonists, gabapentinoids and SSRIs) (Hirschfield *et al.*, 2018; Hirschfield *et al.*, 2017; Lindor *et al.*, 2018). For patients with pruritus that is intractable despite all medical interventions, liver transplantation remains an option even in the absence of liver failure (ELIAS and BURRA, 1991; Wood *et al.*, 1994). There is emerging evidence demonstrating fibrates to be beneficial in not only improving liver biochemistry and but also in improving pruritus in some patients (Wetten, Jones and Dyson, 2022; De Vries *et al.*, 2020; Reig, Sese and Pares, 2018; Soret *et al.*, 2021).

The biological basis for pruritus in PBC remains uncertain, but the partial response to current treatments hints at potential mechanisms (Langedijk, Beuers and Oude Elferink, 2021). It is likely that pruritogens accumulate in plasma and other tissues as a result of cholestasis (Bergasa, 2008), and is evidenced through limited relief by albumin dialysis (molecular adsorbent recirculating system (MARS) (Acevedo Ribó *et al.*, 2005; Montero *et al.*, 2006; Leckie *et al.*, 2012) and plasmapheresis (Alallam, Barth and Heathcote, 2008; Heerkens *et al.*, 2019). The improvement of symptoms in some patients following administration of oral bile acid sequestrants (Di Padova *et al.*, 1984), ileal bile acid transport inhibitors (Al-Dury and Marschall, 2018) and temporary relief by naso-biliary drainage (Beuers, Gerken and Pusl, 2006), would tend to suggest that pruritogens are either directly or indirectly excreted into the bile, and undergo enterohepatic circulation. Rifampicin, a potent PXR agonist, effective in relieving pruritus in around 77% of patients (Khurana and Singh, 2006), appears to downregulate transcription factors, possibly related to the glycoprotein autotaxin (ATX) (Kremer *et al.*, 2012), suggesting that pruritogens may be formed or transformed in the liver. Finally, it is likely that pruritogens exhibit an effect on opioid and serotonin receptors, given the partial effects of opioid antagonists (naltrexone) (Murray-Brown, 2021) and SSRIs (Kouwenhoven, van de Kerkhof and Kamsteeg, 2017).

Cholestatic pruritus is classified as a non-histaminergic itch with no provocation or activation of histamine receptor subtypes (Langedijk, Beuers and Oude Elferink, 2021). Itch signalling involves primary itch neurons in the skin that travel through the dorsal root ganglia, through several neurons and the spinothalamic tract, until it reaches the ventromedial nucleus of the thalamus (Langedijk, Beuers and Oude Elferink, 2021). The neurotransmitters glutamate, natriuretic peptide B and gastrin-releasing peptide are all involved at different points in the pathway. Pain has an inhibitory effect on pruritus, therefore the CNS creates an urge to cause a local pain by scratching behaviour (Ikoma *et al.*, 2006). Conversely, administration of opioids can result in general pruritus, or localised pruritus, if targeted opiates are given, such as via spinal injection (Ballantyne, Loach and Carr, 1988) and suggests a complex interplay between nociceptive and pruriceptive neurons. Whilst anatomical structures and pathways for pruritus are well defined, the activation, function and regulation of pruritus is poorly understood.

1.4.5.1 Pruritogens

Bile salts are the major organic solutes in bile, which breakdown and facilitate intestinal absorption of dietary fats (Boyer, 2013) and have previously been thought to be a potential pruritogen. Bile salt subtypes bind to numerous bile acid receptors including FXR, transmembrane G protein coupled receptor (TGR5), PXR and constitutive androstane receptor. Signalling of these receptors can affect bile acid synthesis, lipid synthesis, gluconeogenesis, inflammation and liver fibrosis (Schaap, Trauner and Jansen, 2014). Bile salt levels have been shown to be elevated in the serum of cholestatic patients (Trottier *et al.*, 2012), but do not appear to correlate to symptoms of pruritus (Kremer *et al.*, 2010; Freedman, Holzbach and Ferguson, 1981). Cholestatic disease may present with pruritus, at which point bile salts levels are often relatively low (Kremer *et al.*, 2008a). Despite the role of bile salts on numerous receptors within the liver, and the effect of bile acid sequestrants in relieving some patients' pruritus, the evidence suggests that bile salts do not play a significant role in cholestatic itch (Langedijk, Beuers and Oude Elferink, 2021).

Autotaxin (ATX) is a potent human motility-stimulating protein, that is required for angiogenesis, and neuronal development (Ptaszynska *et al.*, 2010). It has been linked to tumour cell proliferation in several cancers (Mills and Moolenaar, 2003). ATX catalyses extracellular lysophophatidylcholine to lysophosphatidic acid (LPA), a growth factor-like phospholipid with a wide variety of effects in many cells, and this mechanism is known as the ATX-LPA axis (She *et al.*, 2022). Interest in this as a mechanism for pruritus developed after it was noted that LPA plays a role in neuropathic pain. Kremer et al demonstrated that ATX activity was significantly higher in patients with cholestatic liver disease who experienced pruritus and that the degree of activity correlated strongly with the intensity of pruritus (Kremer *et al.*, 2008b; Kremer *et al.*, 2010). There was no correlation with pruritus to serum bile salts, opioid activity, or serum histamine. PBC patients with intractable pruritus, who have undergone nasobiliary drainage, have a significant reduction or resolution of pruritus, with a reduction in serum ATX activity, despite the ATX activity or protein not being present in the bile, whilst ATX levels are observed to return to pre-treatment levels on reoccurrence of pruritus (Kremer *et al.*, 2010; Kremer *et al.*, 2012). The mechanism for this remains unclear, but it is likely that a component of bile directly or indirectly increases ATX activity, and this is supported by the improvement in pruritus for some patients following treatment with bile acid resins and/or nasobiliary drainage. Reduced serum ATX activity, with a correlation in reduction in pruritus has been seen following treatment with rifampicin (Kremer *et al.*, 2012), bezafibrate (Kremer *et al.*,

2019), prednisolone (Sumida *et al.*, 2013) and plasmapheresis (Heerkens *et al.*, 2019), suggesting a strong relationship between the ATX-LPA axis and cholestatic pruritus. Despite this strong causal link, a recent study into serum ATX, pruritus and chronic liver disease failed to demonstrate a correlation between serum ATX, frequency or severity of pruritus in a sub-cohort of 119 patients with PBC, and instead found a correlation between serum ATX and markers of liver fibrosis (Fujino *et al.*, 2019). Overall, the evidence suggest that the ATX-LPA axis likely has a role in pruritus, but it is likely that other biological factors significantly contribute.

The CNS neurotransmitter, serotonin (5-HT), has previously been implicated in as a potential cause of cholestatic pruritus. It has been found to be synthesised and released by mast cells in the skin, resulting in pruritus (Kushnir-Sukhov *et al.*, 2007). Previous treatment with ondansetron (5-HT_3 antagonist) has been suggested to improve cholestatic itch (Schwörer, Hartmann and Ramadori, 1995), but subsequent meta-analysis of randomised control trials have failed to show a benefit (To *et al.*, 2012). Animal models using BDL techniques to simulate cholestasis have previously demonstrated elevated serotonin in the skin and spinal cord, which was associated with increased scratching behaviour following mechanical and heat stimuli, not seen in sham rats (Tian *et al.*, 2016). In addition, up and down regulation of 5HT subtypes occurred in BDL-rats, suggesting a dynamic response to cholestatic and that distinct subtypes may play a role in pruritus. Other studies have suggested differing roles for participation of 5HT subtypes in cholestatic pruritus, but there is not a clear relationship (Langedijk, Beuers and Oude Elferink, 2021). SSRIs are sometimes used as 3rd or 4th line therapy when 1st and 2nd line therapy has failed to adequately control cholestatic pruritus (Hirschfield *et al.*, 2018; Hirschfield *et al.*, 2017; Lindor *et al.*, 2018), with meta-analysis (albeit mostly from open-label trials) suggesting that they may benefit some patients (Kouwenhoven, van de Kerkhof and Kamsteeg, 2017).

Other theories that have previously been thought to play a role in cholestatic pruritus include endogenous opioids, histamine, bilirubin, and the gut microbiome. However, the evidence for these is lacking (Langedijk, Beuers and Oude Elferink, 2021; Kremer *et al.*, 2008a).

Our knowledge therefore of cholestatic pruritus is incomplete. Treatments offer effective therapy in some patients, but not all, and would suggest that these therapies do not target the main pathological mechanism that results in pruritus. Not only may more one than pruritogen

be involved but there is also evidence of multiorgan involvement. It remains unclear if those with cholestatic disease produce an excess of pruritogens or if pruritogens accumulate as a consequence of cholestasis, and while increased pruritogens have been implicated as the main underlying cause of pruritus, it is not known if increased sensitivity to these contributes.

Chapter 2: PBC and the serum proteome

2.1 Introduction

Proteomics, the large-scale analysis of proteins, has increasingly been used as a method to better gain an integrated understanding of human biological processes. Many different areas of study full into this guise, including protein expression profiling, proteome mining and protein-protein interactions (Graves and Haystead, 2002). Protein expression profiling, in particular, is a useful tool to better understand underlying disease mechanisms, identify potential future therapeutic targets and can be utilised to assess the impact of therapy on diseases.

2.2 PBC and the serum proteome

The UK-PBC consortium (www.UK-PBC.com) recently published a panel of 19 serum markers (**see Table** 2.1), identified using discovery proteomics, that remain elevated in patients with PBC following treatment with UDCA and that are credibly linked to disease pathogenesis (Barron‐Millar *et al.*, 2021). Further work was undertaken using 400 patients from the UK-PBC cohort to explore the relationship between these 19 serum markers and biochemical response to first line therapy with UDCA. The study demonstrated that any elevation in alkaline phosphatase levels in PBC, regardless of UDCA response criteria used (see Table 1.3), is associated with ongoing disease activity from the previously related inflammatory proteome (Jones *et al.*, 2022). Work to date on the serum proteome in PBC has largely focused on disease mechanism related to cholangitis in PBC and the biochemical response to treatment.

Table 2.1 Markers identified in the UK-PBC study as remaining elevated in UDCA treated PBC patients compared to healthy controls (Barron‐Millar et al., 2021)

Family	Abbreviation	Identity
Chemokines	CXCL9	Chemokine CXCL9
	CXCL10	Chemokine CXCL10
	CXCL11	Chemokine CXCL11
	CXCL13	Chemokine CXCL13
	CCL19	Chemokine CCL19
	CCL20	Chemokine CCL20
Cytokine Modulators	IL-4Rα	IL-4 Receptor Alpha Chain
	IL-18R1	IL-18 Receptor 1
Cell Surface & Structural Proteins	EpCam	Epithelial Cell Adhesion Molecule
	CD163	High Affinity Scavenger Receptor
	VIM	Vimentin
	KIM1	Kidney Injury Molecule 1
	SCAMP3	Secretory Carrier-Associated Membrane Protein 3
Metabolic Factors	HAOX1	Human Aldehyde Oxidase
	LEP	Leptin
	ACE2	Angiotensin Converting Enzyme 2
	CA5A	Carbonic Anhydrase 5A
	DECR1	2,4-Dienoyl-CoA Reductase 1
	AZU1	Azurocidin (Heparin Binding Protein)

2.3 Biomarkers associated with PBC

The role of the individual immune markers associated with PBC, including from the PBC inflammatory proteome, are reviewed below.

2.3.1 Cytokines

2.3.1.1 Interleukin-4 receptor α

Interleukin-4 receptor alpha (IL-4Rα) has recently been demonstrated to be differentially expressed between healthy controls and those with PBC, who have not undergone treatment with UDCA therapy and is thought to be a key component of the inflammatory PBC proteome (Barron‐Millar *et al.*, 2021). Further work from the UK-PBC consortium has demonstrated elevation of IL-4Rα in UDCA-treated patients across established UDCA-response criteria, with

increasing levels being associated with worse liver blood biochemistry (Jones *et al.*, 2022). Whilst there is an association with liver biochemistry, its incomplete suppression with UDCA and role in the immune response may suggest an association with symptomatic PBC.

There are two types of Interleukin-4 receptors (IL-4R). Both share the common IL- Rα subunit. IL-4R type-1 is composed of two subunits; IL-4Rα and the common gamma chain cytokine receptor (shared by IL-2, 7, 9, 15, 21). It is located on cells of haematopoietic stem cell origin. The receptor complex can only be formed by IL-4, and its activation results in active Th2 differentiation (LaPorte *et al.*, 2008). IL-4R type-2 is composed of the subunits IL-4Rα and IL-13Rα1. It is located on non-haematopoietic stem cells and can form a complex with both IL-4 and IL-13. It is not present on T-cells but is active in the regulation of cells related to mucus secretion and airway hypersensitivity (LaPorte *et al.*, 2008). Therefore, IL-4 can form a ligand to both types of receptors, whilst IL-13 may only form a ligand with type-2 IL-4R receptors.

IL4-R is largely involved in signalling pathways related to type-2 immune responses (Egholm *et al.*, 2019). IL-4 and IL-13 form complexes with IL-4R on B-cells to generate immunoglobulin E (IgE) antibody production, resulting in the "weep and sweep" response to expel parasites (Egholm *et al.*, 2019), with chronic infection resulting in the development of macrophage and eosinophil-rich granulomas (Van Dyken and Locksley, 2013). Granulomas are a hallmark of PBC histology, with eosinophils being present in PBC liver biopsies (Lewis, 2018; Terasaki *et al.*, 1993). Whilst chronic infection is not a known cause in PBC, there are similarities in the resultant chronic type-2 immune response.

IL-4 and IL-13 drive Th2 cell differentiation to alternatively activated macrophages (M2 macrophages), leading to inhibition of neutrophils and neutrophil chemotaxis, and shifting production away from a pro-inflammatory state to an anti-inflammatory profile, producing tissues repair factors and homeostasis maintenance (Egholm *et al.*, 2019; Van Dyken and Locksley, 2013; Rőszer, 2015). These mechanisms in the context of chronic inflammation are important as they can lead to the development of pathological fibrosis, which is thought to be secondary to persistent activation of the tissue repair pathways (Gieseck, Wilson and Wynn, 2018). In the liver this is demonstrated by chronic helminth infections such as schistosomiasis and liver flukes, which result in Th2 associated IL-4, 5, 13, along with IL-4 and IL-13 induced M2-macrophages (Gieseck, Wilson and Wynn, 2018).

Type-2 immunity has been shown to play a role in both acute and chronic liver disease (Roth *et al.*, 2010; McHedlidze *et al.*, 2013; Marvie *et al.*, 2009; Wang *et al.*, 2012; Gonzalez-Polo *et al.*, 2019). Elevated IL-33 results in the expression of IL13 (by activation type 2 innate lymphoid cells) and has been shown to be elevated in the sera of PBC patients, correlating with levels of ALP. In addition, expression of the primary receptor marker for IL-33 (soluble suppression of tumorigenicity 2 (sST2) receptor) was increased in PBC patients (Sun *et al.*, 2014). This suggests that type-2 immune pathways play a significant role in PBC. This is further supported by the study by Landi et al. who found PBC patients expressed increased levels of IL-1β, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, CXCL9, CXCL10, CCL4 and IFN-γ in sera compared with healthy controls and HCV (excluding CXCL10 and IL-10) (Landi *et al.*, 2014).

Overexpression of IL-13 and activation of IL-4R have been implicated as important causes for DR in animal models, resulting in down-regulation of the classical bile acid synthesis pathways, induction of cellular senescence, lipogenesis and recruitment of type 2 immune mediators in hepatocytes and BEC (Gieseck *et al.*, 2016). Whilst most of this work focused on cellular response to helminth infection, there are some parallels in the disruption of bile acid synthesis, activation of BEC senescence and altered lipogenesis seen in PBC.

Several therapies that target the effect of IL-4 and IL-13 on IL-4Rα are either licensed or undergoing phase II/III trials for use in a variety of immune-mediated diseases, including rheumatoid arthritis (Iwaszko, Biały and Bogunia-Kubik, 2021; Burmester *et al.*, 2018; Dhillon, 2017), psoriatic arthritis (Berekmeri *et al.*, 2018), asthma (Castro *et al.*, 2018), chronic rhinosinusitis, atopic eczema (Simpson *et al.*, 2016) and ulcerative colitis (Sandborn *et al.*, 2017). These therapies include Dupilumab (Harb and Chatila, 2020), a monoclonal antibody which blocks IL-4Rα; Upadacitinib (Burmester *et al.*, 2018) and tofacitinib (Dhillon, 2017; Sandborn *et al.*, 2017; Berekmeri *et al.*, 2018), both of which are small molecule Janus Kinase (JAK) inhibitors and block the downstream effect of IL-4R; Pitrakinra, a dual IL-4 and IL-13 antagonist, and Lebrikizumab, a monoclonal antibody that selectively blocks IL-13. There is limited experience in the use of these therapies in liver disease, but Dupilumab is currently undergoing a phase II open-label, 22-week, exploratory study to investigate its efficacy in the treatment of adults with moderate to severe chronic hepatic pruritus with a primary completion

estimated for September 2023 (Trial ID: NCT04256759; see: https://www.clinicaltrials.gov/ct2/show/NCT04256759).

Given the pathogenic mechanism of action between IL-4, IL-13 and IL-4Rα, the evidence for IL-4Rα as part of the inflammatory proteome of PBC and the fact that there licensed immune-modulating therapies in use for other immune-mediated disease, we further explored the role of these in symptomatic PBC.

2.3.1.2 Interleukin-6

The cytokine Interleukin-6 is tightly integrated in the human immune response. It has pleiotropic effects, as evidenced by it initially having several distinct molecular names based on its biological activity, until through DNA cloning, it was identified to be the same entity (Kawano *et al.*, 1988). Its roles include inflammation, immune response and haematopoiesis (Tanaka, Narazaki and Kishimoto, 2014). It is produced at the site of inflammation, mainly by macrophages and monocytes, and initially plays a key role in the acute phase response, including acting on hepatocytes to stimulate the production of acute phase proteins, such as C-reactive protein (CRP), hepcidin and fibrinogen (Gauldie *et al.*, 1987). Its haematopoietic action includes differentiation of CD4 T-cells to Th17 cells, CD8 T-Cells to cytotoxic T-cell differentiation, B-cell antibody production and thrombocytopenia (Tanaka, Narazaki and Kishimoto, 2014).

IL-6, in combination with transforming growth factor (TGF)-ß, causes Th17 differentiation (a CD4 T helper subset that defends against extra-cellular organisms, particularly in epithelial and mucosal barriers), but inhibits Treg differentiation (Bettelli *et al.*, 2006) (helps to maintain self-tolerance, loss of which results in autoimmune disease). Dysregulation and over production of IL-6 is therefore thought to result in the development of autoimmune and chronic inflammatory disease such as rheumatoid arthritis (Kimura and Kishimoto, 2010).

Acute inflammation during the immune response and corresponding wound healing is tightly regulated, whilst chronic inflammation is considered as an uncontrolled inflammatory response. Elevated IL-6 has been seen in several chronic inflammatory conditions, including the synovial fluid of RA (Houssiau *et al.*, 1988) and germinal centres of hyperplastic lymph nodes seen in Castleman disease (Yoshizaki *et al.*, 1989). In chronic inflammation, the IL-6

amplifier, a positive feedback loop for IL-6, occurs due to synergistic interactions between the transcription factor nuclear factor-kappa B (NF-κB) and signal transducer and activator of transcription 3 (STAT3) in immune and non-immune cells (Hirano, 2020). Initiation of the IL-6 amplifier can occur by local events, including cellular senescence (Kuilman *et al.*, 2008)

Naturally occurring cytokines that are both pro- and anti-inflammatory are induced by strenuous exercise. Commonly, there are significant increases with IL-6, whilst modest increases in plasma levels of TNF-α, IL-1Rα, TNF-receptors, IL-10, IL-8, Macrophage inflammatory protein (MIP-1) occur (Pedersen, Steensberg and Schjerling, 2001; Niemelä *et al.*, 2016). In extreme exercise, such as marathon running, IL-6 has been shown to increase by 100-fold (Ostrowski *et al.*, 1998). The majority of IL-6 during exercise is synthesised and released by contracting muscles in response to exercise, with the expression of IL-6 being dependent on the intensity and duration of exercise (Fischer, 2006; Pedersen and Febbraio, 2008). Activation of IL-6 transcription factors appears to be triggered in response to calcium homeostasis, impaired availability of glycogen/glucose and increased reactive oxygen species (ROS) (Fischer, 2006) – all of which are metabolic consequences to increased energy demand secondary to exercise. Released IL-6 regulates hepatic glucose metabolism (Bertholdt *et al.*, 2017), increases lipolysis (Wedell-Neergaard *et al.*, 2019) and stimulates cortisol via the HPA-axis (Pedersen *et al.*, 2004). Elevations of IL-6 during exercise are not linear, but are more akin to an exponential rise, with peak IL-6 occurring soon after exercise, followed by a rapid decline (Pedersen and Febbraio, 2008).

Animal models suggest that chronic exposure to IL-6 results in muscle fatiguability, with a reduction in not only the number of mitochondria but also their functioning, resulting in a reduction in ATP production via normal aerobic respiratory pathways (VanderVeen *et al.*, 2019; Abid *et al.*, 2020). In humans, elevated IL-6 (as well as IL-1Rα and CRP) has been shown to strongly correlate with poor physical performance and muscle strength in both genders in those over the age of 65 (Cesari *et al.*, 2004; Pereira *et al.*, 2009; Oliveira *et al.*, 2008; Coelho *et al.*, 2010), whilst elevated serum IL-6 in a large cohort of older women predicted a higher risk of developing physical disability and a greater decline in walking ability compared to those with lower levels (Ferrucci *et al.*, 2002).

Overall, the evidence suggests that IL-6 is vital in maintaining muscle metabolic homeostasis and providing an adaptive response to skeletal muscle exercise, and disruptions to this could result in a disturbance in muscle homeostasis.

A meta-analysis on sleep disturbance in inflammatory disease looked at 72 studies, with a total of over 3000 participants samples analysed for IL-6 levels. They found evidence that higher levels of IL-6 were associated with self-reported sleep disturbance and sleep disturbance as assessed by formal questionnaires, but not when using diagnostic criteria for insomnia. It was not associated with shortened sleep duration, but there was evidence that IL-6 was associated with a longer sleep duration. Meta-analysis suggested that sleep disturbance in females and younger age predicted higher IL-6 (Irwin, Olmstead and Carroll, 2016). The mechanism by which IL-6 may affect sleep is unclear, but sleep disturbance is common in PBC, and this therefore would be interesting to explore.

IL-6 (as well as IL-5) levels have been found to be elevated in liver tissue for patients with PBC compared to chronic hepatitis C (CHC) and healthy controls (Nagano *et al.*, 1999) whilst Il-6 and TNFα were elevated in serum samples in patients with PBC, compared to controls (Barak *et al.*, 2009). As described in the pathogenesis of PBC (see section 1.2), IL-6 contributes to the ongoing inflammatory response in PBC, being upregulated with other cytokines in response to the SASP, leading to tissue remodelling and the accumulation of immune cells (Yasoshima *et al.*, 1998; Sasaki *et al.*, 2010a; Sasaki *et al.*, 2010b). In PBC, as in other autoimmune diseases, IL-6 appears to contribute to the chronic inflammatory process.

Evidence suggests that IL-6 can activate the HPA axis which could contribute to fatigue (Vgontzas *et al.*, 1997; Vgontzas *et al.*, 1999; Mastorakos, Chrousos and Weber, 1993). Leptin appears to stimulate IL-6 (see section 2.3.3.1) and is increased in PBC (Breidert *et al.*, 2004). A previous small study exploring IL-6, IL-1β, TNF-α and IFN-γ demonstrated that these were elevated in the serum of PBC patients vs. controls, but, when assessed by the PBC-40, these did not correlate with severe fatigue (Pells *et al.*, 2013a).

Overall, IL-6 has pleiotropic effects on the human immune response. It certainly is a key factor in chronic inflammation observed in many autoimmune conditions. It has a significant role in muscle energy metabolism and chronic levels may alter mitochondrial function, leading to increased muscle fatigability. Bioenergetic changes in muscles are observed in PBC, whilst

elevated IL-6 is observed in PBC patients, making this a potential mechanism that is worthy of exploration.

2.3.1.3 Interleukin-18 Receptor

Interleukin-18 receptor 1 (IL-18R1) is a member of the interleukin-1 receptor (IL-1R) family, and specifically binds interleukin-18 (IL-18). IL-18 is similar in structure to IL-1β, but unlike IL-1β, it is synthesised as a biologically inactive precursor, and stored in the cytosol of most cells in healthy humans (Dinarello *et al.*, 2013). Following exposure to microbes and its activation by Toll-like receptors (such as lipopolysaccharide-induced pathways) on Th1 and Natural Killer (NK) cells, IL-18 is released via a large protein complex, known as an inflammasome (Rathinam, Vanaja and Fitzgerald, 2012; Meng and Lowell, 1997; Tsutsumi *et al.*, 2014). Released IL-18 binds to IL-18R1 on Th1 and facilitates the release of TNFγ, in conjunction with interleukin-12 (IL-12) (Yasuda, Nakanishi and Tsutsui, 2019). Co-stimulation of IL-12 and IL-18 (via IL-18R1) are essential components for the type 1 immune response.

In the absence of IL-12, production of IL-18 and activation of IL-18R1 stimulates non-polarised T-cells and NK cells to produce a type 2 inflammatory response, induce Th2 differentiation and stimulate established Th1 cells, resulting in the production of IL-3, IL-9 and IL-13, in addition to the stimulation of mast cells and basophils to produce IL-4 and IL-13 (Nakanishi *et al.*, 2001; Nakanishi, 2018). IL-18 therefore has pleiotropic functions and stimulates various cell types via IL-18R1. Upregulation of IL-18R1 occurs following exposure to IL-12 and TNFα in T cells and NK cells (Matikainen *et al.*, 2001), however, its regulation in other cells types is poorly understood (Yasuda, Nakanishi and Tsutsui, 2019).

Most research appears to focus on IL-18, rather than IL-18R1. On the basis that IL-18R1 can only be activated by IL-18, the role of IL-18 in disease has been included in our review.

IL-18 gene promotor polymorphisms along with elevated serum IL-18 have been associated with several autoimmune diseases, including SLE (Xiang *et al.*, 2021), RA (Gualberto Cardoso *et al.*, 2021), Bechet's disease(Yasuda, Nakanishi and Tsutsui, 2019), and inflammatory bowel disease (Yasuda, Nakanishi and Tsutsui, 2019; Naftali *et al.*, 2007). Large scale GWAS have identified IL-18R1 as a high-risk genetic locus for the development of PBC (Cordell *et al.*).

However, granuloma formation in several granulomatous diseases, appears to rely heavily on the presence of IL-18 and IL-18R1 (Sugawara *et al.*, 1999; Meda Spaccamela *et al.*, 2019; Shigehara *et al.*, 2000), with granulomas being a hallmark histological feature of PBC.

Overall, the role of IL-18 in PBC is unclear. In cholestatic mice models, IL-18 has been shown to have no significant effect on disease progress (Xu *et al.*, 2022b). However, human studies utilising PBC patients, suggest that serum IL-18 is elevated in PBC and associated with severity of disease (Yamano *et al.*, 2000). (Lewis, 2018). In the bile ducts of patients with PBC, the accumulation of mucosal-associated invariant T (MAIT) cells, rich in IL-18R1, have been previously identified (Toubal and Lehuen, 2016; Zhang, Kong and Wang, 2020). This is an area that warrants further investigation to better understand the immune pathophysiology of PBC.

The role of IL-18 signalling in symptoms is unclear. IL-18 and IL-18R1 have been implicated as important factors in metabolic homeostasis. They, along with IL-6, activate adenosine monophosphate-activated protein kinase (AMPK), a protein which is crucial in identifying the intracellular energy status of skeletal muscle and helping maintain anabolic energy pathways (Lindegaard *et al.*, 2013; Kjøbsted *et al.*, 2018). Defective IL-18 is associated with impaired energy expenditure in the adipose tissue of mice (Zorrilla *et al.*, 2007), has been implicated in the development of myositis in autoimmune conditions such as dermatomyositis and polymyositis (Nagaraju and Morales, 2022; Chen *et al.*, 2018), and is associated with resistance to immunotherapy (Aguilar-Vazquez *et al.*, 2022). Given the role of IL-18 in energy metabolism, particularly in skeletal muscle and the effect of dysfunctional and chronic IL-18 signalling on these pathways, it is interesting to explore its role in PBC and peripheral fatigue.

Elevated IL-18 has been associated with the development of mild cognitive impairment (albeit in the presence of lower plasma vitamin D) in the elderly population (Cheng *et al.*, 2022). Elevated IL-18 from blood mononuclear cells has been positively associated with cognitive deficits in Alzheimer's disease (Bossù *et al.*, 2008). Although the association with MRI white matter lesions was not explored in this study, neuroimaging in PBC patients with cognitive symptoms appear to share many of the features seen in dementia (see section 1.4.3.3) (Newton *et al.*, 2008a).

Dysfunctional IL-18 signalling is thought to play a part in several diseases related to itch, based on its ability to promote Th1 and Th2 responses. This includes Th2 signalling in atopic dermatitis (Tanaka *et al.*, 2001), and Th1 signalling in psoriasis (Ohta, Hamada and Katsuoka, 2001) and cutaneous lupus erythematosus (Wang *et al.*, 2008a). Whilst these diseases all are associated with itch by different pathological mechanisms, it is interesting that IL-18 may contribute to these in its pleiotropic role.

Most therapies targeting IL-18 signalling pathways have either focused on the use of monoclonal antibodies that inhibit IL-18 by blocking its activation from its inactivated precursor form, or by administration of IL-18 binding protein (IL-18BP) which reduces circulating levels of serum IL-18 (Dinarello *et al.*, 2013; Yasuda, Nakanishi and Tsutsui, 2019). We are not aware of any therapies that target the IL-18R itself. Early trials of tadekinig alfa (a recombinant IL-18BP) in adult-onset Still's disease has shown some promise as a new therapy to control the disease (Gabay *et al.*, 2018).

2.3.1.4 Tumour necrosis factor alpha

Tumour necrosis factor alpha (TNFα) is a cytokine that is activated by macrophages, T cells and natural killer cells, and has pleiotropic effects on a plethora of cells (Horiuchi *et al.*, 2010). Originally named for its ability to cause necrosis of tumours, it has since been identified as a key regulator of the bodies inflammatory response (Bradley, 2008).

TNFα exerts biological activity via type 1 receptors, known as tumour necrosis factor receptor 1 (TNFR1) and 2 (TNFR2). TNFR1 is expressed on all human tissues and is considered the key signalling mechanism for TNFα, whilst TNFR2 is largely limited to cells of the immune lineage, with limited biological effect. Different signalling complexes are activated on binding of TNFR1, which determine the cellular response, including inflammation, host defence and cell proliferation, apoptosis or necroptosis (Jang *et al.*, 2021). Activation of TNFR2 mediates cell activation, migration and/or proliferation (Jang *et al.*, 2021).

TNFα is a critical regulatory component of the normal human immune response to pathogens. However, dysregulation of TNFα may lead to autoimmune disease. Examples of autoimmune diseases associated with elevated TNFα include rheumatoid arthritis (Choy and Panayi, 2001), inflammatory bowel disease (Van Deventer, 1997) and psoriatic arthritis (Mease, 2002). Anti-

TNFα agents are commonly used in the treatment of autoimmune diseases, including inflammatory bowel disease (Probert *et al.*, 2003; Laharie *et al.*, 2012; Adegbola *et al.*, 2018), rheumatoid arthritis (Feldmann, 2002) and ankylosing spondylitis (Brandt *et al.*, 2000).

Elevated levels of TNFα (and TNFβ) have previously been described in patients with PBC, with increased levels seen with more advanced disease, but with levels improving following treatment with UDCA (Neuman *et al.*, 2002). However, it is not clear whether elevated TNFα is an upstream driver of disease, or a secondary, downstream, effect of bile duct inflammation that is reduced following treatment with UDCA. Animal models of cholestasis suggest the latter; that elevated TNFα is a consequence, and contributes to, downstream, inflammation (Donner *et al.*, 2007). A recent small study of eighteen patients with PBC highlighted significantly elevated levels of IL-6, IL-8 and TNFα in the PBC cohort, compared to controls, but following logistic regression IL-8 was found to be the predominant cytokine (Akberova *et al.*, 2022). Increased frequency of the polymorphisms in the gene for TNFα have been associated with more advanced disease (Jones *et al.*, 1999a), suggesting it might be associated with increased risk of disease progression.

BDL models in rodents suggest that cholestasis results in the recruitment of TNFα expressing monocytes into the cerebral endothelium (Kerfoot *et al.*, 2006). This may contribute to sickness behaviours observed in cholestatic disease, including PBC.

2.3.2 Chemokines

Chemokines, or chemotactic cytokines, are small proteins that share structural similarity and represent a large family of cytokines that control leukocyte recruitment.

2.3.2.1 CXCL9, CXCL10 and CXCL11

C-X-C motif chemokine (CXC) ligand 9 (CXCL9), CXC ligand 10 (CXCL10) and CXC ligand 11 (CXCL11), are C-X-C Receptor 3 (CXCR3) agonists and potent chemotactic agents that activate T lymphocytes and NK cells (Liao *et al.*, 1995). CXCL9 and CXCL10 are predominantly expressed by human hepatic sinusoidal endothelial cells and hepatocytes (Shields *et al.*, 1999). Inflamed portal tracts of PBC patients demonstrate a high density of CD4+ Th1 cells expressing CXCL9 and CXCL10, in conjunction with over-expression of CXCR3 on CD4+cells in the peripheral blood and liver tissue (Manousou *et al.*, 2013; Ueno *et*

al., 2007) suggesting that these chemokines play a significant role in the upstream inflammatory process. Over expression of CXCL9 and CXCL10, but not CXCR3, in sisters and daughters of PBC patients has been observed and this may contribute to the familial risk associated with PBC (Chuang *et al.*, 2005).

The suppression of AE2 (which maintains the biliary bicarbonate umbrella, see section 1.2) is not only driven by exposure to hydrophobic bile acids, but also from the production of IL-6, IL-8 and CXCL10 from BEC in response to Toll-like receptors (Webb and Hirschfield, 2017) (receptors that recognise pathogens and endogenous molecules from damaged tissues (El-Zayat, Sibaii and Mannaa, 2019)). Treatment with UDCA therapy improves liver biochemistry and appears to result in a reduction in expression of CXCL9 and CXCL10 (Manousou *et al.*, 2013; Jones *et al.*, 2022).

An open-label, phase 2a study using NI-0801, a fully human monoclonal antibody against CXCL10, failed to show any improvement in liver biochemistry in PBC UDCA non-responders (De Graaf *et al.*, 2018), but this did not target CXCL9 which may have continued to contribute to the inflammatory process.

CXCL9, CXCL10 and CXCL11 have been implicated in neuroinflammatory and neurodegenerative conditions, such as multiple sclerosis, infective encephalitis, and Alzheimer's disease (Koper *et al.*, 2018). Reduced CXCL9 has been demonstrated in chronic fatigue syndrome (Landi *et al.*, 2016) but to the our knowledge there have been no studies exploring the impact of these chemokines on symptoms within PBC.

2.3.2.2 CCL19

Chemokine CC motif ligand 19 (CCL19) and Chemokine CC motif ligand 21 (CCL21) form an axis with C-C Chemokine receptor type 7 (CCR7). CCR7 is located on a number of immune cell subsets, and drives lymphocyte migration towards CCL19 and CCL21 as part of the adaptive immune response (Comerford *et al.*, 2013). CCL19 and CCL21 are both homeostatic and are not usually stimulated by inflammation. They typically are produced by stromal cells in both primary and secondary lymphoid tissues (Barone *et al.*, 2016). CCL19 is more readily available in a soluble form, whilst CCL21 is predominantly matrix bound (Comerford *et al.*, 2013). Functionally, CCL19 and CCL21 both activate integrin to activate cell adhesion of

endothelial surfaces, and facilitate migration of cells, such as dendritic cells (Hauser and Legler, 2016).

Dysfunction of these chemokines is associated with worse outcomes in Coronavirus Disease 2019 (COVID-19) infection and the increased risk of long COVID-19 (Qi, Li and Zhang, 2023). In autoimmune disease, overexpression of CCL19 and CCL21 have been implicated in primary Sjogren's syndrome (an autoimmune disease commonly associated with PBC) (Liu *et al.*, 2019) and maintenance of the chronic inflammation associated with multiple sclerosis (Columba-Cabezas, Elena and Aloisi, 2003).

In PBC, significant elevations in CCL19 (but not CCL21) have been observed (Mu *et al.*, 2020), particularly in non-responders to UDCA therapy (Barron‐Millar *et al.*, 2021).

2.3.2.3 CCL20

Chemokine CC motif ligand 20 (CCL20), also known as macrophage inflammatory protein 3α (MIP-3α) and Liver and activation-regulated chemokine (LARC) is the only known high affinity ligand for C-C Chemokine receptor type 6 (CCR6) (Schutyser, Struyf and Van Damme, 2003). Inflamed tissue that is dense in CCL20 promotes recruitment of Th17 cells and Tregs through expression of CCR6 (Kulkarni, Pathak and Lal, 2017). Bile ducts appear to be rich in CCL20, with increasing expression of CCL20 by TNF-α, among other chemokines, during inflammation (Iwamoto *et al.*, 2013; Oo *et al.*, 2012). Overexpression of CCL20 appears to result in disruption of normal homeostatic conditions, with inhibition of Treg, leading to an imbalance in the inflammatory Treg to Th17/Th1 ratio and resulting in a breakdown in immune tolerance (Kulkarni *et al.*, 2018; Kulkarni, Pathak and Lal, 2017), whilst promotion of Th17 differentiation results in chronic inflammation (Meitei, Jadhav and Lal, 2021).

The CCR6-CCL20 axis is an important contributor to many autoimmune diseases, including psoriasis (Hedrick *et al.*, 2009), rheumatoid arthritis (Hirota *et al.*, 2007) and inflammatory bowel disease (Kulkarni *et al.*, 2018). It is therefore an important therapeutic target in autoimmune disease (Meitei, Jadhav and Lal, 2021).

CCL20 biliary epithelium expression has been linked to advancing disease in PBC (Shi *et al.*, 2015) resulting in an increasing migration of Th17 cells, and subsequent shift away from Th1

differentiation, with an associated shift in IL-12 to IL-23 mediated signalling (Yang *et al.*, 2014). Identification of these pathways resulted in an open-label trial of the anti-IL-12/23 monoclonal antibody, ustekinumab, in adults with PBC and inadequate response to UDCA (Hirschfield *et al.*, 2016), but whilst modest reductions in ALP were observed, this was not sufficient to meet the primary outcome. Symptoms of fatigue and itch were assessed as secondary outcomes using FIS and the PBC-40, but no significant changes were reported.

CCL20 is recognised as a genetic risk locus for the development of PBC (Cordell *et al.*, 2015a).

2.3.3 Energy homeostasis

Energy homeostasis in humans and animals is largely controlled by the central nervous system using a highly integrated and complex neurohumoral system, operating to ensure that short-term fluctuations in energy requirements are managed with minimal impact on adipose mass (Elmquist *et al.*, 1998; Woods *et al.*, 1998). Two major hormones that contribute to this mechanism are leptin and ghrelin and are discussed next.

2.3.3.1 Leptin

Leptin is an amino-acid peptide hormone, that is largely expressed in white adipose tissue, but is also found in brown adipose tissue, skeletal muscle, gastric mucosa, the pituitary gland and the placenta (D'Souza *et al.*, 2017), however the contribution from these tissues is negligible (Odle *et al.*, 2014). It is one of the most important factors secreted by adipose tissues and is known as an "adipokine", contributing to low level inflammatory state in those overweight (Abella *et al.*, 2017).

Leptin follows a circadian rhythm and has diurnal variation, with higher levels in the evening and early morning (Licinio *et al.*, 1997). Major factors promoting leptin secretion include obesity and overfeeding (Kelesidis *et al.*, 2010). Evidence suggests that leptin is involved in regulation of energy homeostasis, metabolism, neuroendocrine function, regulation of immune function and bone metabolism (Kelesidis *et al.*, 2010; D'Souza *et al.*, 2017; Odle *et al.*, 2014; Bouassida *et al.*, 2010; Wein *et al.*, 2007; Cava and Matarese, 2004; Sahu, 2003; Ceddia, William Jr and Curi, 2001; Friedman and Halaas, 1998). Signalling the status of peripheral tissue remains its major function, whereby higher circulating leptin acts on the CNS to reduce

appetite and increases energy consumption when there is excess or increased adipose tissue (Friedman and Halaas, 1998).

Leptin has a complex relationship with the immune system with actions on both innate and adaptive immunity, affecting many different cell lineages, including the regulation of both Th17 and Treg cells, and has been implicated in the pathophysiology of autoimmune diseases such as SLE (Abella *et al.*, 2017). Its action on neutrophils, macrophages and eosinophils results in reduced apoptosis, increased cytokine production and chemotaxis (Abella *et al.*, 2017). Leptin mediated upregulation of IL-6, TNF-α, and IL-1ß expression pathways (via its primary signalling isoform Long OBR (OBRI)) is well documented (Yang *et al.*, 2013; Tazawa *et al.*, 2019; Vella and Allison, 2018; Abella *et al.*, 2017; Procaccini, Jirillo and Matarese, 2012).

Studies have demonstrated that there is accumulation of adipose tissue in skeletal muscle, known as intermuscular adipose tissue (IMAT), occurring in association with chronic inflammation (Tazawa *et al.*, 2019), chronic diseases (Goodpaster *et al.*, 2006) and increasing age (Vella and Allison, 2018), with resultant decreased muscle quality and strength (Addison *et al.*, 2014; Tuttle, Sinacore and Mueller, 2012). IMAT area has been associated with increased serum IL-6, Leptin and CRP (Vella and Allison, 2018; Hassler *et al.*, 2021). Evidence for IMAT in chronic liver disease is largely limited to non-alcoholic fatty liver disease (Kitajima *et al.*, 2013), post-operative outcomes in liver resection for hepatocellular carcinoma (Kaibori *et al.*, 2015) and liver transplantation (Hamaguchi *et al.*, 2017). The role of IMAT in PBC has not been explored.

Data regarding leptin in PBC are limited but appear to be controversial. Early studies found patients with PBC to have significantly lower serum leptin levels compared to controls, with no association with histological disease status (Rieger *et al.*, 2005; Ben-Ari *et al.*, 2002). However, lower serum leptin levels have also been found to be associated with PBC, but with elevated serum leptin correlating with advancing histological stage (García-Suárez *et al.*, 2004). In contrast, Breidert et al demonstrated elevated serum leptin in patients with PBC compared to controls (Breidert *et al.*, 2004). Comparisons are difficult to make as in Breidert's study all PBC patients were confirmed to have cirrhosis, whilst in the former studies the majority of patients were confirmed to have Ludwig stage (Ludwig, Dickson and Mcdonald, 1978) I and II disease i.e., not cirrhotic. Given that cirrhosis has been associated with higher

leptin levels this may explain these differences (Henriksen *et al.*, 1999; Shimizu *et al.*, 1998). In a normal population, leptin levels correlate with BMI, but this was not the case in those with PBC (Ben-Ari *et al.*, 2002). Lower levels of leptin have previously been associated with degree of liver blood test abnormality (as defined by decreasing stringency of response to first-line therapy, see section 1.3.1) (Jones *et al.*, 2022).

Elevated leptin has been associated with autoimmune liver disease, in particular with autoimmune hepatitis (AIH), where it was associated with histological inflammation, transaminitis and steroid responsiveness (Lal, Thakur and Bihari, 2020). Leptin was elevated in AIH, compared to PBC, CHC patients and healthy controls. Significance between serum leptin, PBC and healthy controls were not published, but median leptin levels in PBC patients were 126ng/ml (Interquartile range (IQR) 52-381) compared to 66ng/ml (40-157) in healthy controls, suggesting a higher serum concentration in PBC patients (Lal, Thakur and Bihari, 2020).

In certain aetiologies of liver disease where symptoms of fatigue are common, high circulating levels of serum leptin have been found to associate with severe fatigue. Piche et al compared serum leptin in 78 CHC patients, 13 PBC patients and 23 controls. Using the FIS, they found that both disease groups had significantly higher fatigue scores in all 3 domains (cognitive, physical, and psychosocial), and that these were more pronounced in females. When corrected for fat mass, leptin was significantly elevated in both CHC and PBC patients and correlated with fatigue score, particularly in the physical domain. There was no correlation between fatigue and age, liver function tests, viral load or meta-analysis of histological data in viral hepatitis (METAVIR) score (Piche, 2002). Resting energy expenditure was higher in CHC patients, consistent with previous studies (Piche *et al.*, 2000) and suggestive of hypermetabolism, but did not correlate with fatigue scores (Piche, 2002). The authors concluded that leptin may contribute to fatigue, but that the underlying mechanisms in fatigue are likely multi-factorial.

In another study, serum leptin was found to be elevated in 70 patients with CHC compared to 20 controls and when compared to fatigue, as assessed by the multidimensional assessment of fatigue (MAF), there was a statistically significant correlation between CHC and the presence and severity of fatigue, and this directly correlated to serum leptin levels for CHC, but not in controls (El-Gindy, Ali-Eldin and Meguid, 2012).

Studies relating to leptin and PBC are limited and controversial. Elevated leptin is associated with lipid oxidation, increased activity of PDH and the Kreb cycle, and a reduction in triglyceride content in in skeletal muscle (Wein *et al.*, 2007). Overall, leptin is more than just the "hunger hormone", but in fact demonstrates complex energy metabolism in skeletal muscle. Its role as an immunomodulator, with associations with autoimmune disease makes it an interesting candidate for further exploration.

2.3.3.2 Ghrelin

Ghrelin is a 28-amino acid peptide that contributes to the hypothalamic regulation of energy homeostasis (Sato *et al.*, 2012). Predominantly produced by oxyntic gastric cells, it can also be found in the hypothalamus, pituitary and pancreas (Kojima *et al.*, 1999). Ghrelin is a naturally occurring ligand of Growth Hormone Secretagogue (GHS) Receptor (2) and as such stimulates the release of Growth Hormone (GH) from the pituitary gland (Kojima *et al.*, 1999; Sato *et al.*, 2012).

Plasma concentrations tend to increase with caloric restriction, fat depletion and before a meal, whilst decreasing following the digestion of food (Cummings *et al.*, 2001). Levels tend to be to be inversely related to body weight and fat mass (Cummings, 2006). Crucially its function is linked to that of leptin, whereby fasting decreases leptin and increases ghrelin production, acting as an appetite stimulant (Inui, 2001). Its endocrine function includes influencing the secretion of enteric hormones like insulin and gastrin, linking the hypothalamus, pituitary and gastric axis (Baatar, Patel and Taub, 2011). Ghrelin has been demonstrated to induce tissue-specific changes in mitochondrial and lipid metabolism particularly in the liver and skeletal muscle (Barazzoni *et al.*, 2005).

Ghrelin is circulated in acylated (AG) and unacylated (UnAG) forms (Hosoda *et al.*, 2000; Akamizu *et al.*, 2005). The AG form represents approximately 10% of total plasma ghrelin (Hosoda *et al.*, 2000), which has an appetite-stimulating effect (Gil-Campos *et al.*, 2006), modulates insulin sensitivity (Poykko *et al.*, 2003), as well as stimulating liver gluconeogenesis (Gauna *et al.*, 2005), increasing adipose tissue (Perez-Tilve *et al.*, 2011), and improving skeletal muscle metabolism (Gortan Cappellari and Barazzoni, 2019), UnAG is reported to have no effect on appetite, but modulates skeletal muscle metabolism by improving

mitochondrial redox activities, and tissue inflammation (Gortan Cappellari and Barazzoni, 2019).

Ghrelin acylation is based on the degree of medium chain fatty acid and triglyceride availability (Nishi *et al.*, 2005; Barazzoni *et al.*, 2017) (Gortan Cappellari and Barazzoni, 2019). Significant elevations in serum fatty acids have been observed in patients with PBC, compared to controls, with treatment with UDCA but it is not clear if this relates to alterations in hepatic lipid metabolism, or a secondary effect of mitochondrial dysfunction with impaired fatty acid B oxidation (Bell *et al.*, 2015).

Ghrelin appears to influence metabolism in several tissues. In particular, peripheral administration of AG in rodents results in lipogenic and glucogenic patterns of gene expression in the liver, with an increase in triglyceride content and reduction in fatty acid oxidation, whilst increasing skeletal muscle mitochondrial oxidative activity (Barazzoni *et al.*, 2005). There is, therefore, evidence to suggest that ghrelin can induce tissue-specific changes in mitochondrial and lipid metabolism.

Over-expression of PPAR-γ in skeletal muscle in response to increased ghrelin, with a subsequent correlated increase in mitochondrial cytochrome c oxidase activity (Barazzoni *et al.*, 2005) (an important step to convert ADP to ATP during oxidative phosphorylation (*Cytochrome Oxidase*)) has been reported (Choi *et al.*, 2003). PPARs are important regulators of tissue fat metabolism (Gervois *et al.*, 2000) and changes in expression of these receptors may therefore influence skeletal muscle metabolic pathways. It is worth noting that PPAR agonists are currently used off-licence as second-line therapy for PBC non-responders although evidence is lacking to show that these improve peripheral fatigue in PBC (see section 1.3.2.2).

In vitro and in vivo studies demonstrate that AG and UnAG have potent anti-inflammatory effects and can bind to common sites on endothelial cells to mediate signals to inhibit cellular apoptosis (Baatar, Patel and Taub, 2011). In particular AG inhibits the expression of pro-inflammatory cytokines IL-1 ß, IL-6 and TNF-α (Dixit *et al.*, 2004)

Studies exploring ghrelin and PBC are few. Breidert et al explored both ghrelin and leptin in 22 female patients with liver cirrhosis (confirmed by biopsy or ultrasound) and found serum ghrelin levels to be significantly lower, and leptin to be significantly elevated, compared to age

and BMI matched controls (Breidert *et al.*, 2004). The authors noted that C-peptide was significantly increased in PBC patients, but that this did not correlate with circulating glucose. They postulated that these changes might be related to insulin resistance and could be a cause for cachexia that is often observed in patients with underlying cirrhosis.

2.3.4 Cell surface and structural proteins

2.3.4.1 Epithelial cell adhesion molecule

Epithelial cell adhesion molecule (EPCAM) is a glycoprotein that is expressed on normal epithelial cells to a variable level and was initially thought to be involved in cell-cell adhesion and originally identified as a marker for neoplasm of epithelial origin, where it is highly expressed on rapidly proliferating cells. However, its role is thought to be more diverse and is likely involved in signalling, cell migration, cell differentiation and increased expression is seen with active cell proliferation. (Trzpis *et al.*, 2007).

In the liver it is expressed solely in BEC, where its upregulation is associated with bile duct injury and ductular proliferation (de Boer *et al.*, 1999). Overexpression of EPCAM, associated with cholestatic liver fibrosis, has been demonstrated in liver histology of patients with PBC and PSC (Song *et al.*, 2017). Over-expression has also been associated with various liver diseases, including, but not limited to, alcoholic liver disease, viral hepatitis and autoimmune hepatitis, where it is again associated with inflammatory activity and fibrosis (Breuhahn *et al.*, 2006).

In the UK-PBC consortium proteomics study, EPCAM showed a positive association with non-response, that is, higher levels were observed those with more cholestatic liver blood tests (Jones *et al.*, 2022), in keeping with previous findings of overexpression in bile duct injury and cholestasis (de Boer *et al.*, 1999) (Song *et al.*, 2017).

Its utility in clinical practice has largely focused on its role in malignancy, with agents that target EPCAM activity, such as anti-EPCAM monoclonal antibodies, being explored as monotherapy and adjuvant treatment for neoplastic syndromes (Schmidt *et al.*, 2010; Armstrong and Eck, 2003). However, to date these have not shown significant benefit (Armstrong and Eck, 2003).

2.3.4.2 Kidney Injury Molecule-1/T-cell immunoglobulin and mucin domain-1

Kidney Injury Molecule-1 (KIM-1), also known as T-cell immunoglobulin and mucin domain-1 (TIM-1) is a type 1 transmembrane glycoprotein that was originally identified as a receptor for the hepatitis A virus (HAV) in monkeys (Kaplan *et al.*, 1996) and humans (Feigelstock *et al.*, 1998), but is commonly associated with acute tubular necrosis of the kidney (Han *et al.*, 2002). In normal physiology it is not expressed in the proximal tubule of the renal tract, but following renal epithelium injury, its synthesis occurs (Bonventre, 2009). Its accumulation in the apical membrane of the proximal tubule of the affected region, results in epithelial cells recognising apoptotic cells, which then direct and respond to lysosomes, which activates phagocytosis (Bonventre, 2009).

Outside of the renal system, it has an important immune regulatory role for Th2, where it may be activated by multiple ligands (Rodriguez-Manzanet *et al.*, 2009). Overall, there is minimal expression of KIM-1 on native CD4+ T cells. However, once activated, KIM-1 undergoes upregulation and expression, with ligand activation inducing a potent costimulatory effect for T cell activation and proliferation, with resulting differentiation to Th2 cells, production of IL-4, IL-5, IL-10 and IL-13 (Curtiss and Colgan, 2007) and a reduction Treg differentiation (Rodriguez-Manzanet *et al.*, 2009). Conversely Th1 and Th17 cells express very little KIM-1 (Rennert, 2011), however it is expressed on regulatory B-cells, that is a small proportion of B cells that act to suppress immune responses (Catalán *et al.*, 2021), where it helps maintain self-tolerance, by regulation of IL-10 production by regulatory B-cells (Xiao *et al.*, 2015).

Further evidence for the role in KIM-1 in autoimmune regulation is apparent with the discovery of an association with exposure to HAV and protection against the atopic diseases, asthma, allergic rhinitis and atopic dermatitis, in the presence of a gene variant that encodes KIM-1. (Mcintire *et al.*, 2003) Human KIM-1 has been associated with other autoimmune diseases, including rheumatoid arthritis (Chae *et al.*, 2005), asthma (Chae *et al.*, 2003) and SLE (Wang *et al.*, 2008b) .

In liver disease, much of the focus has been on the role of KIM-1 as a receptor for HAV. However, PBC patients have been shown to have a higher percentage of $CD19^{+}CD24^{hi}CD38^{hi}$ B-cells, which exhibit impaired KIM-1 expression. This increase in percentage of B-cells correlates with levels of cholestasis, and is associated with increased production of IL-6 and IL-12, with a corresponding reduction of IL-10 (Chen *et al.*, 2020). Overall, this appears to

result in impairment of the normal immune inhibition by Th2, with a corresponding promotion of proinflammatory Th1 cell differentiation and suggests that the B-cells in PBC patients may be tilted towards a proinflammatory state, for which KIM-1 may play a role.

To our knowledge there is no data exploring KIM-1 expression and disease specific symptoms.

2.3.4.3 CD163

CD163 is cell surface glycoprotein, expressed almost exclusively on monocytes and macrophages (Moestrup and Møller, 2004). It is a member of the Scavenger Receptor Cysteine-Rich (SRCR) family (Fabriek, Dijkstra and van den Berg, 2005) and acts as a primary haemoglobin scavenger receptor, regulating free haemoglobin clearance by facilitating the uptake of haptoglobin-haemoglobin (Hp-Hb) complexes (Weaver *et al.*, 2006).
Pathogen invasion of a host can result in haemolysis, providing an important source of iron for pathogen growth. Free haemoglobin clearance is therefore an important physiological response to infectious agents, reducing the source of iron, whilst also removing toxic redox-reactive iron from the circulation (Moestrup and Møller, 2004). Upregulation of CD163 occurs, following macrophage activation, through exposure to endotoxin and upregulation of proinflammatory cytokines, such as IL-6 (Buechler *et al.*, 2000). Shredding of CD163 following activation of cell surface toll-like receptors, results in soluble CD163 (Weaver *et al.*, 2006).

Elevation of soluble CD163 does not appear to be limited to pathogenic invasion. Upregulation of soluble CD163 by activated Kupffer cells (Grønbæk *et al.*, 2012) is associated with both acute and chronic liver disease with and without cirrhosis from various aetiologies, including alcohol related liver disease (ARLD) (Sandahl *et al.*, 2014), NAFLD (Kazankov *et al.*, 2015) and viral hepatitis (Kazankov *et al.*, 2014). In acute AIH, soluble CD163 was elevated sixfold, compared to patients with complete response to standard treatment, but remained elevated in those with incomplete response to therapy (Grønbaek *et al.*, 2016). CD163 activation therefore appears to occur across a spectrum of liver disease.

In a large Italian cohort, soluble CD163 was positively associated with elevated ALP in PBC, with higher CD163 levels at enrolment in UDCA naive patients being observed in those who were later defined as non-responders following UDCA treatment (Bossen *et al.*, 2020). The authors also reported an improvement in the accuracy of the UK-PBC risk score, (a risk

stratification tool used predict end-stage liver disease in PBC (Carbone *et al.*, 2016)) by inclusion of serum CD163. Elevation of CD163 in UDCA non-responders, and those with continued liver blood test abnormality have been mirrored in the UK-PBC consortium proteomic study (Jones *et al.*, 2022). There studies suggest that CD163 (a marker for macrophage activation) may represent both a marker of progression of disease and prognosis.

2.3.4.4 Vimentin

Vimentin (VIM) is part of the family of intermediate filaments. These are proteins that form elaborate networks of proteins in cells to anchor the nucleus and other organelles within the cell cytosol (Fuchs and Weber, 1994). As well as maintaining cell integrity and cell adhesion, VIM facilitates the attachment of lymphocytes to vascular endothelium, and its migration through endothelial cells (Ivaska *et al.*, 2007). Changes in vimentin organisation can alter the stability and metabolism of lipids in pre-adipose cells (Schweitzer and Evans, 1998).

Increased VIM has been observed in the cytoplasm of proliferating and damaged bile ductules secondary to chronic cholestasis and necro inflammatory disease, with evidence of aberrant expression and organisation of VIM in BEC in response to cellular damage (Nakanuma and Kono, 1992). Mice knock out studies suggest that cholangiocytes that are inhibited from expressing vimentin demonstrate reduced biliary senescence and liver fibrosis (Zhou *et al.*, 2019), and this may offer a therapeutic target in the treatment of cholangiopathies. Elevated serum VIM has been demonstrated in UDCA Paris-1 non-responders (Jones *et al.*, 2022). Studies in VIM and cholestatic liver disease are limited, but increased VIM may be a consequence of cellular damage in BEC and cholangiocytes following cholestasis and inflammation, that results in remodelling and the development of fibrosis.

2.3.4.5 Secretory carrier membrane proteins

The family of secretory carrier membrane proteins (SCAMP) are proteins with a tetraspanin structure. Four distinct isoforms (1-4) exist in mammalian cells, whilst a 5th isoform is found in neuronal cells (Castle and Castle, 2005). The 3rd isoform, SCAMP3, appears to be involved in endocytic membrane transport, that is, it facilitates the trafficking, sorting and budding of intraluminal vesicles following endocytosis (Falguières, Castle and Gruenberg, 2012). GWAS suggest an association between the gene loci for SCAMP3 and Crohn's disease (Lessard *et al.*, 2012; Cagliani *et al.*, 2013).

In liver disease, overexpression of SCAMP3 ribonucleic acid (RNA) has been identified in hepatocellular carcinoma (HCC) and is a predictor of poor prognosis (Zhang *et al.*, 2017; Han *et al.*, 2020). Elevated serum SCAMP3 was seen in PBC Paris 1 and Paris 2 non-responders, compared to responders in the UK-PBC consortium proteomics study, but did not show a significant trend across all responder classifications and normal liver blood tests (Corpechot *et al.*, 2022). Studies beyond these appear to be limited.

2.3.5 Metabolic factors

2.3.5.1 Angiotensin-converting enzyme 2 (ACE2)

Angiotensin-converting enzyme 2 (ACE2) plays an important role in the alternative renin angiotensin system (RAS). RAS, which is important for fluid and sodium homeostasis, which contributes to blood pressure management, is considered to have two arms – the classical arm and the alternative arm, which counterbalance each other (Chappell, 2012).

RAS is not only a system for maintaining circulation, but also exists as a local tissue-based system in several organs, including the liver, where it is upregulated in BDL studies (Paizis *et al.*, 2002). Upregulation of local RAS, and in particular, angiotensin II has been associated with the development of liver fibrosis and cirrhosis (Lubel *et al.*, 2009b; Paizis *et al.*, 2001).

ACE converts angiotensin I to the vasoconstrictor angiotensin II (Johnston, 1994). ACE2 is a homologue of ACE and converts angiotensin I to angiotensin 1-9, which is then converted by ACE to angiotensin 1-7, which can modulate and protect against angiotensin II mediated injury (Ferrario *et al.*, 1997; Paizis *et al.*, 2001). ACE2 is expressed in cardiovascular and renal tissues, but also throughout the gastrointestinal tract (Harmer *et al.*, 2002). It is also significantly expressed in the liver and upregulated in response to liver injury (Paizis, 2005; Paizis *et al.*, 2002). Angiotensin 1-7 are antifibrotic and therefore have been studied as therapeutic options in to treat fibrosis in liver disease (Lubel *et al.*, 2009a). Activation of ACE2 is also considered an attractive therapeutic target as it would reduce angiotensin II whilst increasing angiotensin 1-7 (Rajapaksha, Angus and Herath, 2019), potentially reducing injury and fibrosis.

An interesting area of research into ACE2 is as a chemoprophylactic approach for the COVID-19 disease. ACE2 acts as a main receptor for the virus SARS-CoV-2 (Lan *et al.*, 2020). FXR was shown to be a direct regulator of ACE2 transcription in several tissues affected by COVID-19, with administration of UDCA reducing FXR signalling, resulting in downregulation ACE2 in lung, bile ducts and intestines (Brevini *et al.*, 2023). This results in a reduction in susceptibility to SARS-CoV-2, which was then confirmed by exploring clinical outcomes in patients from the UK-PBC cohort (Brevini *et al.*, 2023). A reduction in ACE2 expression in UDCA responders was observed in the proteomics study that explored the relationship between disease activity and UDCA response (Jones *et al.*, 2022).

Further studies on PBC and ACE2 are limited. A recent study using mice and human livers suggested that ACE2 expression in cholangiocytes of PBC patients were reduced and associated with disease severity (Li *et al.*, 2023), but this should be interpreted with caution as sample sizes were small.

We are not aware of any other studies exploring ACE2 with symptoms in PBC. Studies on the effect of ACE2 and fatigue secondary to COVID-19 are conflicting (Malato *et al.*, 2021b; Malato *et al.*, 2021a; Fernández-de-las-Peñas *et al.*, 2022)

2.3.5.2 Hydroxyacid oxidase (HAOX1)

This protein is expressed primarily in the liver and pancreas and oxidises glycolic acid to glyoxylate (Jones, Morrell and Gould, 2000). It can result in the release H_2O_2 (hydrogen peroxide) (Angermüller, 1989) that can participate in the generation of ROS, which in high levels can result in a toxic effect on cells. Studies on the role of HAOX1 in disease appear to be limited. It has been implicated in the development of primary hyperoxaluria type 1, a rare disorder that causes a build-up of oxalate, leading to recurrent kidney stones, urinary tract infections and chronic kidney disease (Milliner *et al.*, 2022).

HOAX1 was shown to remain elevated in PBC patients with incomplete response to UDCA (Jones *et al.*, 2022).

2.3.5.3 Carbonic Anhydrase 5A (CA5A)

Carbonic anhydrases 5A (CA5A) catalyses the reversible hydration of carbon dioxide to bicarbonate (Dodgson, 1991). It is found primarily in the liver and within mitochondria. It plays an important role in gluconeogenesis, removal of ammonia and the production of amino acids. Deficiency in CA5A can result in hyperammonaemia, metabolic acidosis and hypoglycaemia (Sly and Hu, 1995).

CA5A showed very mild differences in PBC patients with normal LFTs compared to those with super-normal, but it showed no differences between PBC responders and non-responders, suggestive that CA5A was unlikely to be biologically relevant in PBC (Jones *et al.*, 2022).

2.3.5.4 2,4-Dienoyl-CoA Reductase 1 (DECR1)

2,4-Dienoyl-CoA Reductase 1 (DECR1) is a mitochondrial enzyme involved in the beta-oxidation of unsaturated fatty acids (Koivuranta, Hakkola and Hiltunen, 1994). Its overexpression has been demonstrated in the pancreas of murine models following chronic alcohol consumption (Satoh *et al.*, 2013). One study has suggested that in a very small subset of PBC patients (3%), antibodies against DECR1 were found and that this could contribute to a breakdown in apoptosis-related immune tolerance (Rong *et al.*, 2011) and DECR1 has been found to be present in BEC apoptotic blebs from PBC patients (Kawata *et al.*, 2012). DECR1 was found to be elevated in UDCA non-responders compared to responders (Jones *et al.*, 2022). Further studies on DECR1 in liver disease, PBC and symptoms are limited.

2.3.5.5 Azurocidin (Heparin Binding Protein)

Azurocidin (AZU1), also known as heparin binding protein, is a protein that is released from polymorphonuclear leukocytes (PMN) and has potent antimicrobial properties and is released in the early stage of PMN activation, and at a later stage when PMN reaches the site of inflammation (Soehnlein and Lindbom, 2008). As well as being a potent antimicrobial, it also acts an “alarm” system for the immune system, inducing recruitment of monocytes, activating monocytes and macrophages and enhancing bacterial phagocytosis (Soehnlein and Lindbom, 2008). Work on AZU1 has largely focused on its use as a biomarker in bacterial infection (Tverring *et al.*, 2020).

Its role in autoimmune disease, liver disease, PBC and symptoms is limited. In the PBC cohort study exploring disease activity and UDCA response, AZU1 was demonstrated to be elevated in UDCA POISE non-responders compared to responders, although AZU1 lost significance when stratifying by normal LFT and the various responder types (Jones *et al.*, 2022), perhaps suggesting a limited role in the pathophysiology of PBC.

2.4 Neurosteroids

Neurosteroids are endogenous steroids, which rapidly modulate neuronal excitability by non-genomic actions. Circulating steroid hormones, such as progesterone, act as precursors for the synthesis of neurosteroids in the brain independently of endocrine gland function (Reddy, 2010). Neurosteroids can be classified according to structural features and include: pregnane neurosteroids, androstane neurosteroids and sulphated neurosteroids (Reddy, 2010). Typically, neurosteroids exert brain excitability by interaction with neuronal membrane receptors and ion channels, rather than through regulation of gene transcription (Herd, Belelli and Lambert, 2007; Belelli and Lambert, 2005).

The amino acid, Gamma-aminobutyric acid (GABA), is one of the major primary inhibitory neurotransmitters in the CNS and exerts is action in synapses by binding to post synaptic GABA receptors, which in turn inhibit transmission of an action potential. The gamma-aminobutyric acid-A (GABA-A) receptor is one such receptor, and largely mediates synaptic inhibition within the CNS (Jewett and Sharma, 2022). GABA-A receptors are ligand-activated chloride channels that are comprised of five trans-membrane subunits which form the chloride ion channel. Different combinations of subunits result in different expressions of GABA-A receptors (Dunn, Bateson and Martin, 1994). Fundamental features of the GABA-A receptor, including sensitivity to GABA, channel kinetics, neuronal location and pharmacological properties are influenced by subunit composition (Turkmen *et al.*, 2011a).

While traditionally the GABA system is understood to be an inhibitory system, providing balance to excitatory transmission, this is an overgeneralised view. Instead, GABA receptors are organised into heterogeneous gene families with functional inhibition varying between different synapses (Birke and Draguhn, 2010). These inhibitory neurons have a significant role in higher brain functions, with alterations as a consequence of disease, resulting in inhibition of highly selective gene families with varying effects.

Neurosteroids can be positive or negative regulators of GABA-A receptor function (Reddy, 2003) and affect both synaptic and extra-synaptic GABA-A receptors (Reddy, 2010). Extra-synaptic receptors enable neurons to sense the ambient extra-cellular space GABA concentrations and provide a form of "tonic" inhibition; a sustained response during the course of a stimulus, whilst "phasic" firing refers to the fast activation and transient response of synaptic GABA-A receptors (Reddy, 2010).

2.4.1 Allopregnanolone

The progesterone derivative, allopregnanolone (3A-hydroxy-5A-pregnan-20-one), is one of the most studied neurosteroids and has potent positive allosteric effects on GABA-A receptors, contributing significantly to GABAergic tone (Jones, 2002; MacKenzie and Maguire, 2013). It is synthesized in the CNS either de novo from cholesterol or from steroid precursors like progesterone (Robel and Baulieu, 1994). **See Figure** 2.1 (Wetten *et al.*, 2022). The exact binding site for allopregnanolone is unknown, but it enhances the open probability of the GABA-A receptor chloride channel, increasing the mean opening time and the chloride current through the channel, resulting in a reduction in neuronal excitability (Reddy, 2010).

There has been increasing interest in neurosteroids as a potential therapeutic target for diseases relating to the CNS. Allopregnanolone has been implicated in impaired cognition and memory (Johansson *et al.*, 2002; Kask *et al.*, 2008), fatigue (Johansson *et al.*, 2018; Murphy, 2004), mood disorders, including premenstrual dysphoric disorder (Rupprecht, 2003; Walton and Maguire, 2019; Bäckström, Bixo and Strömberg, 2015), and dysregulation of sleep cycles (Rupprecht, 2003).

In liver disease, most studies have focused on the role of allopregnanolone in hepatic encephalopathy. This results in the concept of increased GABAergic tone as a result of increased ammonia levels, with ammonia increasing the affinity of GABA for GABA-A receptors, whilst also stimulating neurosteroid synthesis and release, further potentiating the effect of GABA on GABA-A receptors (Jones, 2002). Evidence for this comes from elevated levels of allopregnanolone in the cerebral tissue of patients with cirrhosis and hepatic encephalopathy (Ahboucha *et al.*, 2006; Ahboucha *et al.*, 2005).

Following the discovery that serum levels of allopregnanolone are elevated in patients with chronic fatigue syndrome (Murphy, 2004), it was theorised that allopregnanolone may have a role in fatigue in chronic liver disease. Ahboucha et al demonstrated that serum allopregnanolone is around 80% higher in both patients with PBC and chronic hepatitis C (CHC) compared to healthy controls, and was associated with symptoms of significant fatigue, as assessed using the FIS. PBC and CHC patients scored highly for all types of fatigue assessed by the FIS (cognitive, physical, and psychosocial) compared to controls. Analysis between serum allopregnanolone and fatigue type was not published, therefore it is impossible to conclude whether elevated allopregnanolone might contribute to central or peripheral fatigue.

Neuroinflammation, resulting in upregulation of translocator protein (TSPO), a mitochondrial neuroglial cholesterol transporter protein, has been shown to increase the synthesis of pregnenolone from cholesterol, ultimately resulting in the synthesis of allopregnanolone (Paul, Pinna and Guidotti, 2020). **See Figure** 2.1. This may suggest a potential mechanism for increased allopregnanolone levels in liver disease, such as PBC, that leads to neuroinflammation.

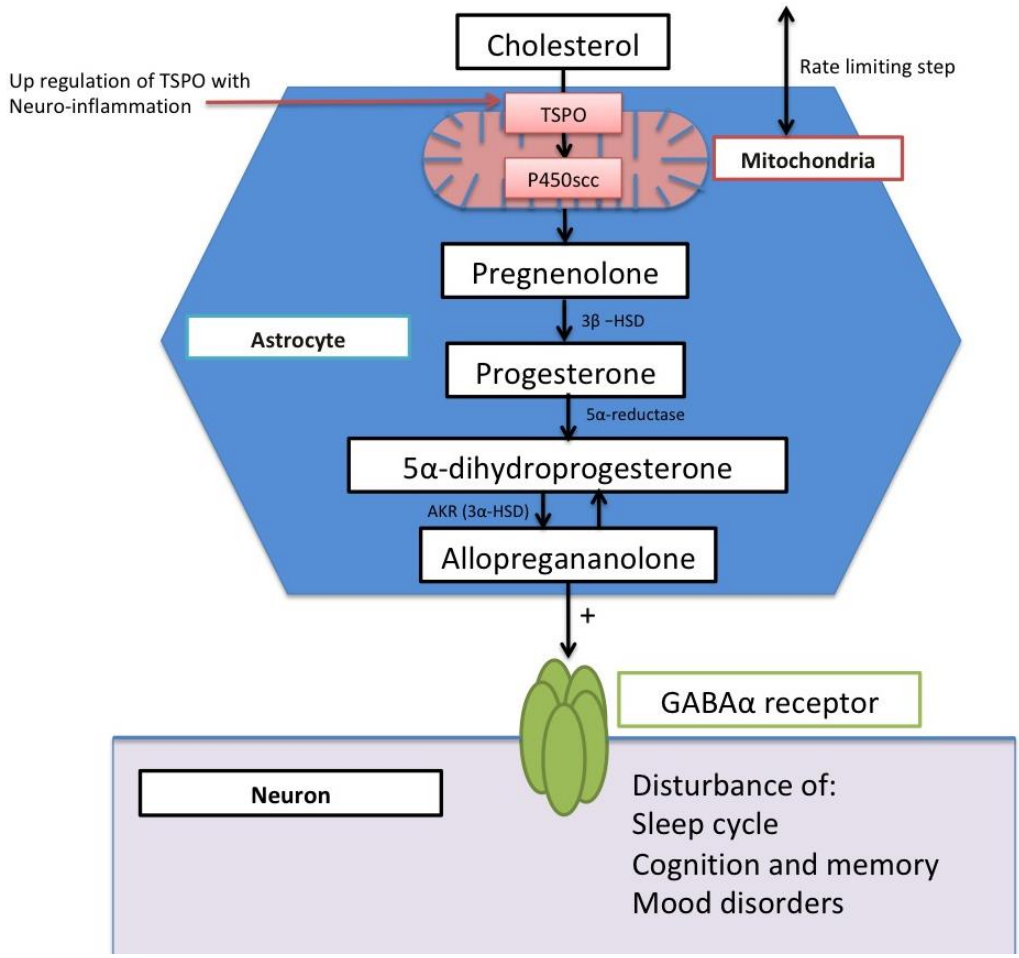


Figure 2.1 De novo synthesis of Allopregnanolone in the CNS.
"Cholesterol is transported across the mitochondrial membrane by the nucleoside transporter, translocator protein (TSPO) (Jacobs and Tavitian, 2012), and converted to pregnenolone within the mitochondria of glial cells, such as astrocytes, by side chain cleavage of cytochrome P450 (P450scc). Pregnenolone is subsequently converted to Progesterone by 3β-hydroxysteroid dehydrogenases (3β-HSD). 5α-reductase type 1, converts Progesterone to 5α-dihydroprogesterone (5α-DHP), 5α-DHP is then converted to Allopregnanolone by the aldo-keto reductases enzyme, 3α-hydroxysteroid dehydrogenase (3α-HSD). Allopregnanolone activates GABA-A receptor intracellular sites by lateral diffusion in the neuronal membrane, where it acts as a potent allosteric modulator. Allopregnanolone can eventually be reconverted back to 5α-DHP by 3α-HSD." (Wetten, 2022, page 10) (Paul, Pinna and Guidotti, 2020)

Chapter 3: Chapter 3 Methods

3.1 Overall study design

The aim of this work, undertaken in 3 sections, was to better understand the biological basis of symptoms in PBC based on recent mechanistic research into disease pathology. Patients and healthy controls were drawn from 4 pre-existing cohort studies (UK-PBC, BANC, RITPBC and EAILD). Details of these studies are provided in **Figure** 3.1**.**

First, we undertook an exploratory study (termed Study 1) exploring how PBC symptoms related to the 19 serum proteomic markers (**See**

Table 1.1**)** previously identified by the UK-PBC consortium to be associated with biochemical response in PBC (Barron‐Millar *et al.*, 2021; Jones *et al.*, 2022). Symptoms were assessed using the PBC-40; a disease-specific, fully-validated self-reported symptom measurement tool (Jacoby *et al.*, 2005). Patients were drawn from the UK-PBC Cohort study, described in **Section 3.3.1 and Figure** 3.1.

Secondly, a validation cohort (termed Study 2) was used to investigate a subgroup of the positive associations between serum proteomics and PBC-40 symptoms identified in the exploratory cohort (Study 1). This cohort was comprised of PBC patients from the RIT-PBC study (known to have a high symptom burden), UK-PBC and BANC and healthy controls from the E-AILD study (**See Section 3.3 and Figure** 3.1).

Thirdly, we undertook a proof-of-concept study (termed Study 3), exploring the relationship between the neurosteroid, allopregnanolone, and symptoms in PBC. This utilised a cross-sectional cohort of PBC patients from the UK-PBC and BANC cohorts, with healthy controls from the E-AILD cohort (**see Figure** 3.1**)**.

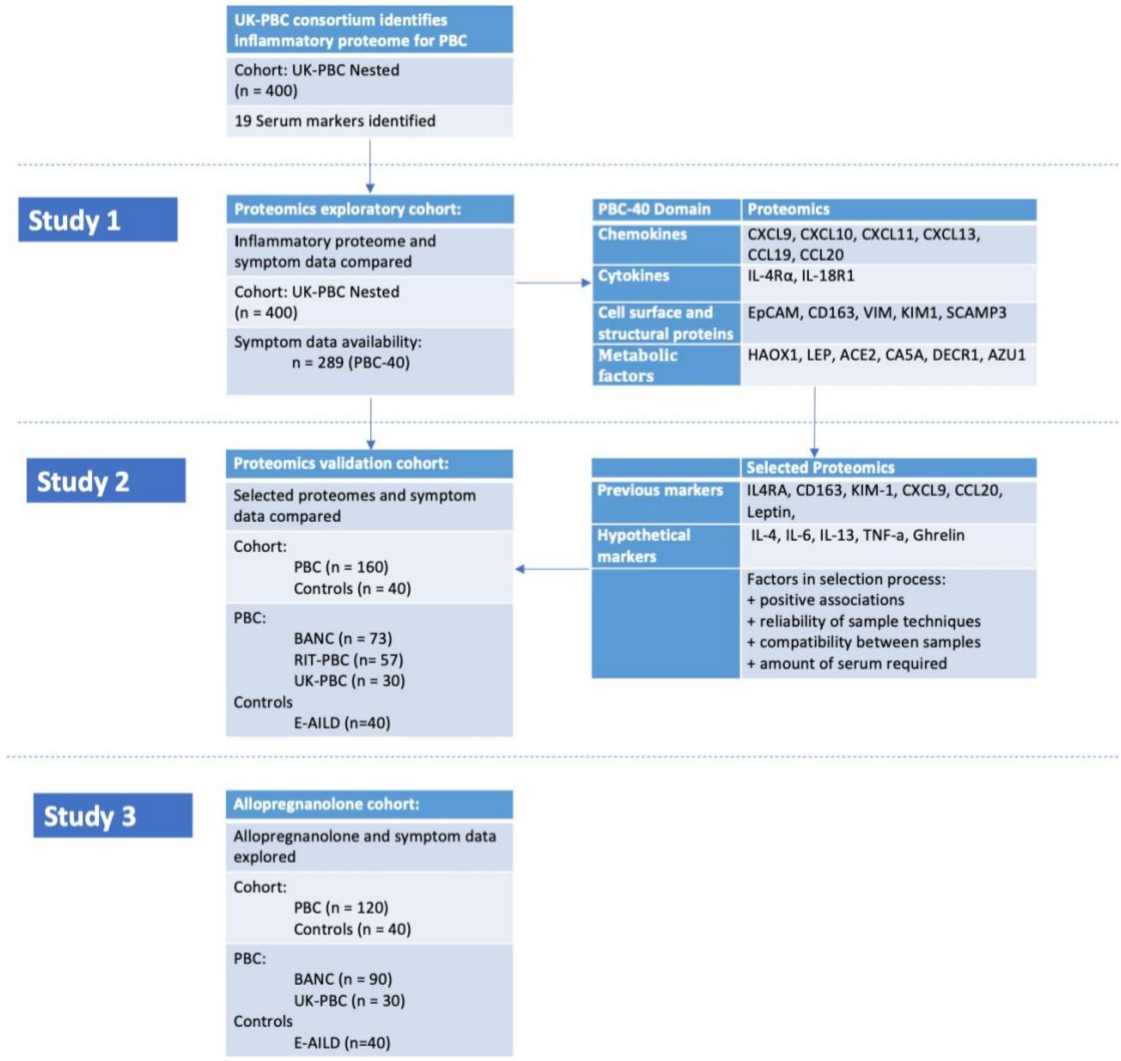


Figure 3.1 Summary of the 3 studies for understanding the biological basis of symptoms in PBC.

3.2 Ethical approval

All the pre-existing patient cohorts and serum samples included in this study had ethical approval in place with valid written informed consent obtained at the time of recruitment. As such, no additional ethical approvals were required for this work. This research was conducted in accordance with the International Conference on Harmonisation Good Clinical Practice Guidelines and the Declaration of Helsinki.

3.3 Pre-existing Cohorts

The cohorts utilised in these studies include detailed clinical information relating to diagnosis, treatment, biochemical response, and symptom assessment using well-validated scoring

systems. Clinical data and bio-fluid samples were collected at the time of recruitment to the relevant cohort.

3.3.1 UK-PBC Cohort

The UK-PBC Cohort (www.UK-PBC.com) is a large, prospective, national cross-sectional cohort of PBC patients that was established to undertake studies of treatment efficacy in PBC. The UK-PBC Nested Cohort, a sub-cohort within this study, was established to characterise cellular and molecular responses in PBC, stratify treatment response and disease progression and facilitate the development of second-line therapies (Liaskou *et al.*, 2018). The UK-PBC cohort study includes detailed clinical data collected at the time of recruitment, whilst the UK-PBC Nested Cohort also contains bio-fluid sampling that is paired with the clinical data. Recruitment was undertaken in 18 research centres over 5 years from 2014-2019 (REC reference number 14/NW/1146).

3.3.2 BANC Cohort

The Birmingham and Newcastle Cohort (BANC) was established as a proof-of-concept resource that recruited from Queen Elizabeth Hospital, University Hospitals Birmingham NHS Foundation Trust, Birmingham, UK and the Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, UK. It is a cross-sectional group of patients with an established diagnosis of PBC recruited between 2015 and 2017.

3.3.3 RIT-PBC Cohort

The RIT-PBC study was a phase II, single centre, randomised controlled, double-blinded trial comparing rituximab with placebo in fatigued PBC patients. It recruited 57 patients between October 2012 and October 2015, with detailed information gathered during the study period including medical and drug history, pre- and post-treatment physiological status using anaerobic threshold and muscle fatigue using magnetic resonance spectroscopy, serum samples and self-reported patient questionnaires (Jopson *et al.*, 2015; Khanna *et al.*, 2018; Khanna *et al.*, 2019). This trial explored the benefit of rituximab on PBC patients with fatigue, so the cohort population all had clinically significant levels of fatigue.

3.3.4 E-AILD Cohort

The E-AILD (Environmental factors in the aetiology of Autoimmune Liver Disease) cohort was established to search for potential environmental risk factors in patients within the North-East of England and North Cumbria with a confirmed diagnosis of autoimmune liver disease (PBC, autoimmune hepatitis, and primary sclerosing cholangitis). There were also 105 healthy age and gender-matched participants. Clinical data and serum samples were collected for all participants. Recruitment occurred between 2015 until 2017.

3.4 Symptom measurement

3.4.1 PBC-40

The PBC-40 is a fully validated, disease-specific quality of life measure, used to assess symptoms in PBC. It was developed using thematic analysis, following in-depth interviews with 30 demographically represented patients with PBC, to create a pool of questions related to their experience with symptoms. Reducing pools of questions were disseminated in several stages via national surveys to a total of 900 patients and cross-validated by other health-related QOL (HRQOL) measures. A final pool of questions was produced, comprising 40 questions across 6 domains: itch, fatigue, cognitive impairment, emotional, social, and general symptoms. The finalised PBC-40 was validated in a smaller subset of patients (40), using a blinded comparison with other HRQOL questionaries (Jacoby *et al.*, 2005).

Participants rate questions on a 5-point scale, with 1 = “never”, 2 = “rarely”, 3 = “sometimes”, 4 = “most of the time” and 5 = “always”. The number of questions for each domain varies. The answers related to each domain are summed and provide an overall score for that domain. The overall score then grades symptoms into a categorical group of none, mild, moderate, and severe symptoms for each domain, using well established cut-offs (Mells *et al.*, 2013), as defined in **Table** 3.1. **See appendix 1** for the full PBC-40 questionnaire.

Table 3.1 Defined scores and range cut-offs for PBC-40 domain (Phaw et al., 2020; Newton et al., 2007b)

PBC-40 domain	None	Mild	Moderate	Severe
Cognitive impairment	≤6	7-15	16-21	≥22
Emotional	≤3	4-7	8-11	≥12
Itch	≤3	4-8	9 - 11	≥12
Fatigue	≤11	12-28	29-39	≥40
Social	≤10	11-28	29-40	≥41
General Symptoms	≤7	8-18	19-25	≥26

3.4.2 Epworth sleepiness scale

The ESS is a short, self-completed questionnaire that evaluates the daytime sleepiness of individuals. Developed and published in 1991, it has been used extensively to assess symptoms in PBC studies, with meta-analysis demonstrating it to be a useful tool for group comparison (Kendzerska *et al.*, 2014; Johns, 1991; Newton *et al.*, 2006c; Newton *et al.*, 2008b; Phaw *et al.*, 2020; Mells *et al.*, 2013). The questionnaire includes 8 questions, with each answer rated from 0 to 3, giving a possible overall score of between 0 and 24. ESS scores have been demonstrated to significantly correlate with sleep latency and overnight polysomnography. Scores 0 to 9 are considered in the normal range, whilst scores greater or equal to 10 are considered clinically significant.

3.4.3 3.4.3 Orthostatic grading scale

The OGS is a 5 item, fully validated, self-reporting questionnaire that is used to quantify the frequency, severity, and response to orthostatic stressors. It has been demonstrated to accurately assess the severity of symptoms relating to orthostatic hypotension, and has been validated against more lengthy autonomic measures (Schrezenmaier *et al.*, 2005). Each item is rated on a scale of 0 to 4, with 0 representing no/rarely experiencing orthostatic symptoms, whilst a score of 4 correlates to always experiencing symptoms for that particular stressor. The total score is based on the sum of the scores for each item, with a score of between 4 to 9 being indicative of moderate orthostatic hypotension, and a score of greater than 9 is consistent with a formal diagnosis of orthostatic hypotension. It has been widely used in previous studies of PBC as a measure of autonomic dysfunction (Newton *et al.*, 2007b; Newton *et al.*, 2007a; Newton *et al.*, 2008a; Newton *et al.*, 2008b; Jones *et al.*, 2010b; Mells *et al.*, 2013).

3.4.4 Hospital anxiety and depression scale

The HADS is a self-assessment tool developed in 1983 to identify cases of anxiety and depressive disorders in non-psychiatric hospitals (Zigmond and Snaith, 1983). It has been extensively used in a wide variety of studies and disease pathologies, with meta-analysis demonstrating its usefulness in assessing caseness of anxiety and depression in the general population, as well as symptom severity (Bjelland *et al.*, 2002) . It is divided into anxiety (HADS-A) and depression (HADS-D) sub-domains, with 7 intermingled items in each sub-domain. Each item scores from 0 to 3. A score of 11 or greater for either domain is considered clinically significant and should prompt clinical assessment (Zigmond and Snaith, 1983). It has been used extensively as a tool for symptom assessment in the study of PBC (Mells *et al.*, 2013; Newton *et al.*, 2006b; Dyson *et al.*, 2016).

3.4.5 Clinically significant symptom cut-offs

The PBC consortium examined data from the large cross-sectional UK-PBC cohort study. Detailed symptom phenotyping was undertaken using the fully validated symptom measurement tools described above. Using a cohort of 2,353 patients, clinically significant cut-offs for symptoms in PBC were defined based on the normative data from a community-based control population and have been defined by using the mean +/- standard deviations for the control values, see **Table 3.2** (Mells *et al.*, 2013).

Table 3.2 Cut-offs for clinically significant symptoms in PBC (Mells et al., 2013)

Symptom tool	**Clinical cut-off**	**% Positive in PBC population**
PBC-40		
• Cognitive impairment	≥ 18	28
• Emotional	≥ 12	19
• Itch	≥ 7	38
• Fatigue	≥ 33	45
• Social	≥ 32	25
• General Symptoms	≥ 18	40
Daytime somnolence (ESS)	≥ 11	28
Autonomic dysfunction (OGS)	≥ 5	29

3.5 PBC clinical parameters

3.5.1 Diagnosis of PBC

All patients in each study cohort were diagnosed with "definite" or "probable" PBC using the standard diagnostic criteria used in clinical practice; the presence of at least two out of the three key characteristics of PBC: cholestatic liver biochemistry, positive serum AMA or PBC-specific ANA, with a titre of > 1 in 40 and/or diagnostic features of PBC on liver biopsy (Hirschfield *et al.*, 2018; Hirschfield *et al.*, 2017; Lindor *et al.*, 2018).

3.5.2 Biochemical response criteria

Several criteria to assess biochemical response to first-line therapy with UDCA in PBC have been developed and are summarised in **Table** 1.3 (Corpechot, Chazouilleres and Poupon, 2011; Pares, Caballeria and Rodes, 2006; Corpechot *et al.*, 2008; Kuiper *et al.*, 2009; Kumagi *et al.*, 2010a; Nevens *et al.*, 2016; Momah *et al.*, 2012). For the purposes of this work, the POISE criteria (Nevens *et al.*, 2016) were selected to stratify participants by response state as this is most widely used in clinical practice in the UK.

Using the POISE criteria, an adequate response is defined as the ALP < 1.67xULN and bilirubin < ULN after 12 months of UDCA treatment at the therapeutic dose of 13-15mg/kg/daily. Those failing to meet these criteria would be considered as non-responders and therefore at higher risk of adverse outcomes. In clinical practice, they would be considered eligible for second-line therapy with either obeticholic acid (OCA) or a fibrate.

3.5.3 Abnormal liver blood tests

Participants were considered to have abnormal liver blood tests if any of bilirubin, alkaline phosphatase or alanine transferases were elevated above the local laboratory defined upper limit of normal (ULN).

3.5.4 Cirrhosis

Participants were considered to have evidence of cirrhosis based on a liver stiffness measurement (LSM) > 16.9kpa on vibration-controlled transient elastography (VCTE) (Corpechot *et al.*, 2022), histological stage 4 on liver biopsy, a platelet count of <150 g/L in the absence of an alternative explanation or in the presence of complications secondary to liver cirrhosis, such as ascites, varices or hepatic encephalopathy (Corpechot *et al.*, 2012).

3.6 Study 1 - Exploratory proteomics

Study 1, summarised in **Figure** 3.1, was an exploratory study to try to identify if components of the PBC inflammatory proteome may contribute to symptoms in PBC. The UK-PBC consortium recently published data on discovery proteomics that were plausibly linked to disease pathogenesis in PBC (Barron‐Millar *et al.*, 2021). They identified biological markers of disease that remained elevated in treatment naïve patients, and then explored these in both responders and non-responders who were on therapeutic doses of UDCA (Barron‐Millar *et al.*, 2021). A total of 19 markers were identified as making up the PBC inflammatory proteome (**see Table** 2.1**)**. They further explored the role of those 19 markers according to biochemical response, using 400 patients from the UK-PBC nested cohort study (Jones *et al.*, 2022).

Study 1, the exploratory study, utilised a sub-population from the UK-PBC nested cohort, all of whom had received first-line treatment with UDCA for at least a year, with a therapeutic dose of 13-15mg/kg/day and represented a cross-sectional selection of patients with a diagnosis of PBC.

We utilised historical symptom data (assessed by the PBC-40, recorded at the time of study recruitment, contemporaneous with serum sampling) from the UK-PBC Nested cohort (Jones *et al.*, 2022).

Only participants with complete data for the PBC-40 questionnaire were included in analyses.

3.6.1 Proteomics analysis

Proteins were measured using the Olink® Target 96 Cardiovascular II & III, Inflammation and Oncology II panels (Olink Proteomics AB, Uppsala, Sweden) according to the manufacturer's instructions. The proximity extension assay (PEA) technology used for the Olink protocol has been well described (Assarsson *et al.*, 2014), and enables 92 analytes to be analysed simultaneously, using 1 µL of each sample. 4µL of serum was analysed for 368 analytes in this study (RRID:SCR_003899).

Pairs of oligonucleotide-labelled antibody probes bind to their targeted protein, and if the two probes are brought in close proximity the oligonucleotides will hybridize in a pair-wise manner. The addition of a deoxyribonucleic acid (DNA) polymerase leads to a proximity-dependent DNA polymerization event, generating a unique polymerase chain reaction (PCR) target sequence. The resulting DNA sequence is subsequently detected and quantified using a microfluidic real-time PCR instrument (Biomark HD, Fluidigm). The resulting C_T-data is then quality controlled and normalized using a set of internal and external controls. The final assay read-out is presented in normalized protein expression (NPX) values, which is an arbitrary unit on a log2-scale where a high value corresponds to a higher protein expression.

The internal controls are designed to mimic and monitor the different steps of the PEA. They consist of two incubation/immuno controls, an extension control, and a detection control. The internal controls are introduced to all samples as well as to the external controls and are used for quality control and normalization of the data. The external controls consist of a negative control used to calculate the limit of detection (LOD), as well as a triplicate of inter-plate controls (IPC) that are used for data normalization. Quality control of the data is performed in two steps. Firstly, the run is quality controlled by calculating the standard deviation for the detection control and the incubation/immuno controls. The standard deviation should be below 0.2 for a run to pass quality control. Secondly, each sample is quality controlled by comparing the results for the detection control and one of the incubation controls against the run median. Samples that fall more than 0.3 NPX from the run median with regards to these two internal controls will fail the quality control.

All assay validation data (detection limits, intra- and inter-assay precision data, etc.) are available on the manufacturer's website (www.olink.com).

All proteomic assays in Study 1 were outsourced to Olink® Analysis Services.

3.7 Study 2 – Proteomics validation

Study 2 aimed to establish if any of the positive associations seen in Study 1 could be replicated using a second cohort.

3.7.1 Study group selection

Several different cohorts were used for patient selection in this validation study. This included healthy controls from E-AILD, a cross-sectional PBC population from UK-PBC and BANC, and a symptom heavy population from RIT-PBC. The RIT-PBC symptom cohort was selected based on the availability of stored serum samples and the abundance of symptom data, unanalysed and unpublished from the original study (Khanna *et al.*, 2019).

The selected participants from the UK-PBC and BANC cohort were age and gender matched to the E-AILD healthy controls. Participants were selected from the UK-PBC, and BANC cohort studies based on the availability and location of serum samples, and availability of relevant clinical data. As the RIT-PBC population was already defined, no age / gender matching was performed.

3.7.2 Selection of serum proteomic analytes

A subset of 6 serum proteomic markers from the exploratory study (Study 1) were selected for further analysis, and included, IL-4Rα**,** CXCL9, CCL20, Cd163, KIM-1 and Leptin. This selection was based on the degree of correlation to symptoms, our current understanding of biological pathways and symptomology, and feasibility of measuring serum markers.

An additional 5 analytes were included at this stage, selected based on literature review of the mechanisms involved in fatigue, cognitive symptoms and itch, and feasibility of measuring these markers. This included IL-4, IL-13, IL-6, TNF-α and ghrelin. Their inclusion into Study 2 is discussed in more detail in **section 5.2.9.**

Feasibility factors influencing the selection of serum markers included the accuracy of measuring a given marker in serum samples based on published data, the amount of serum required for the given analyte, the compatibility of analyte markers across multi-plex panels, the amount of serum samples available and the overall processing cost of a given analyte.

All analyte analysis for study 2, the validation study, was performed within the department of Translational and Clinical Research at Newcastle University by Dr Jeremy Palmer. The final serum analytes analysed for the validation study are listed in **Table** 3.3 and **Figure** 3.1

Table 3.3 Markers selected for further analysis in the validation study (Study 2)

Family	**Abbreviation**	**Identity**	**In exploratory study (Study 1)?**
Chemokines	CXCL9	Chemokine CXCL9	Yes
	CCL20	Chemokine CCL20	Yes
Cytokine Modulators	IL-4Rα	IL-4 Receptor Alpha Chain	Yes
	IL-4	Interleukin-4	No
	IL-6	Interleukin-6	No
	Il-13	Interleukin-13	No
	TNF-α	Tumour Necrosis Factor-α	No
Cell Surface & Structural Proteins	CD163	High Affinity Scavenger Receptor	Yes
	KIM1	Kidney Injury Molecule 1	Yes
Metabolic Factors	LEP	Leptin	Yes
	Ghrelin	Ghrelin	No

3.7.3 Proteomics sampling

In order to measure serum levels of the 11 analyte markers (IL-4Rα, CD163, KIM1, CXCL9, CCL20, IL-4, IL-6, IL-13, TNFα, leptin and ghrelin), we employed the Meso Scale Discovery® (MSD ((Rockville, MD, USA)) electrochemiluminescence assay platform. Using the U-PLEX Custom Biomarker format we created a custom 4-PLEX panel for detecting CD163, KIM1, CXCL9 and CCL20, a 5-PLEX panel for detecting IL-4, IL-6, IL-13, TNFα and leptin, and single-PLEX assays for ghrelin and IL-4Rα.

To retain uniformity and validity, the assays were performed exactly as per the manufacturer's instructions. Briefly, for each panel, biotinylated capture antibodies were covalently linked to specific spots within each well through use of unique linkers. Then, following a quenching step, plates were ready for sample application. Sera were thawed on ice and clarified by centrifugation at 18,000g for 10mins at 4°C. Sera were diluted 1:1 with supplied diluent and 25µl per well added in duplicate. Again, for each individual panel, a cocktail of the relevant analyte standards with known concentrations were prepared as a dose

calibration curve and 25µl per well was added in duplicate. Plates were covered and incubated for 2 hours at room temperature with shaking (200RPM). Following a wash step, for each panel a SULFO-TAG™ conjugated detection antibody cocktail (50µl) was added to all wells and plates incubated as before. After washing, read buffer was added (150µl) and the plates read on the MSD Sector Imager 6000 instrument.

The data were analysed using MSD Discovery Workbench v4.0. This software employs 4-parameter logistic (FourPL) calibration curve fitting from which sample values were derived. Each analyte had a lower limit of detection (LLOD) below all the sample values, so all samples were in range and measurable. All sample values for all analytes are given as pg/ml.

Mass spectrometry of analytes was performed by Dr Jeremy Palmer, team scientist, in the translational and clinical research institute at Newcastle University.

3.8 Study 3 Allopregnanolone

3.8.1 Study group selection

As detailed in **Section 3.3** and **Figure** 1., Study 3 was a proof-of-concept study that aimed to explore the relationship of the neurosteroid, allopregnanolone, with symptoms in PBC. PBC participants were selected from the UK-PBC and BANC cohort, whilst healthy controls were selected from the E-AILD cohort and were age and gender matched (**see Sections 3.3.1 3.3.2 and 3.3.4).** Participants were selected from the UK-PBC and BANC cohorts studies based on the availability and location of serum samples, and availability of relevant clinical data.

3.8.2 Allopregnanolone sampling

A Liquid Chromatography - Tandem Mass Spectrometry method was developed for the analysis of allopregnanolone in human plasma or serum (Admescope, Oulu, Finland). The samples were prepared for analysis by supported liquid extraction with ethyl acetate, followed by derivatization with hydroxylamine, as previously described (Keski-Rahkonen *et al.*, 2011).

Standard sample spiking solutions were prepared by diluting 1 mg/ml stock solution of allopregnanolone in dimethyl sulfoxide into acetonitrile at concentrations of 2pg/ml to 100 ng/ml of spiking solutions were added into 180µl of active charcoal purified human serum for standard and quality control samples in 2 ml 96-well plate. After spiking, 10µl of internal

standard solution (100ng/ml allopregnanolone-D5 in 50% methanol) was added into each sample and mixed for 5 minutes at 1000rpm. Finally, the plate was centrifuged at 2200g for 1 minute and 200µl of the sample was transferred onto Novum simplified liquid extraction max (Phenomenex) plate and the samples were absorbed into the plate material using vacuum at <5 inHg for 5 - 10 seconds. The plate was allowed to stand for 5 minutes (without vacuum), after which two-time 500µl of ethyl acetate was allowed to flow through each well. Finally, vacuum was applied for 20 - 30 seconds to allow the remaining ethyl acetate to flow through the plate. The collection plate was dried under nitrogen flow (20 L/h N2, 50 °C) for 20 minutes, until dry. Finally, 150µl of 100 mM hydroxylamine in 50% methanol was added into the wells and the plate was incubated at 65 °C for 60 minutes with shaking (600rpm) to derivatize allopregnanolone into oxime-derivative for improved analytical sensitivity (Keski-Rahkonen *et al.*, 2011). Finally, the plate was centrifuged for 5 minutes at 2200g, and the supernatants were transferred into clean 1 ml 96-well plate for analysis.

Increased sensitivity was reached by the use of UniSpray ion source in a XEVO-TQ-S triple quadrupole mass spectrometer. The obtained detection limit was 0.002ng/mL and the quantitation limit 0.005ng/mL, i.e., 0.006 and 0.015nmol/L, respectively. The method accuracies in the range 0.005 to 10ng/mL were 91.3 – 107.5% (n=4). Similarly, the precision (n=4) was determined to 10.5 – 2.5% in the concentration range 0.005 to 10ng/mL.

The extraction of serum allopregnanolone concentrations was outsourced to Admescope, located in Oulu, Finland.

3.9 Statistical analyses

Baseline characteristics of each of the cohorts included in this work are presented in the relevant results section and include basic demographics (age and gender), clinical characteristics of the PBC groups (including weight, treatment status with UDCA, biochemical response status, and presence of cirrhosis). All serum analytes were explored against the clinical characteristics of response status, abnormal liver blood tests and cirrhosis status.

Each PBC-40 domain was analysed against these clinical characteristics to explore whether there was an association between disease stage and symptoms. For the 3 parts of this work

(described in **Sections 3.7, 3.8 and 3.9**), a common methodology was employed to explore the association of a given serum analyte to symptoms.

For each PBC-40 domain, serum analytes were initially compared between those with "none/mild", "moderate" and "severe" symptoms. A post hoc analysis using Bonferroni pairwise correction was performed to correct for multiple comparisons i.e., comparing groups "none/mild", "moderate" and "severe", and to adjust for the fact that a single strong statistically significant association between 2 of these groups could demonstrate statistically significance overall between the three groups. This not only adjusts the P-value for multiple testing, but also explores pairwise associations between groups, that is "none/mild" vs "moderate", "none/mild" vs "severe", and "moderate" vs "severe" giving more information on where the association exists.

Following this, serum analytes were compared only between the "none/mild" versus "severe" symptom groups in each PBC-40 domain. "None/mild" symptoms are those that are typically found in the general population, whilst those who score "severe" represent those with the highest symptom burden.

Using this method, it is possible to identify which serum analytes continued to show a strong association across several groupings as well between the two extreme groups i.e., "none/mild", vs. "severe". The categorisation of scores by symptom severity ("none/mild", "moderate" and "severe") for each PBC-40 domain is described in **Table** 3.1**.**

Serum analytes were then tested against each PBC-40 domain using the predefined scores that have previously been deemed to be "clinically significant" in PBC. Applying this criterion identified those markers that were strongly associated with clinically significant symptoms. This methodology was particularly relevant for identifying serum proteomics from the exploratory cohort (Study1) that would be potentially informative in the validation cohort (Study 2).

All statistical analysis was undertaken using IBM SPSS statistics, version 28.0.0.0 (190) for macOS. All data was assessed for normality by graphical methods using histograms, P-P and Q-Q plots, and standard statistical tests for normality, including exploring Kolmogorov-Smirnov test, skewness, and kurtosis.

For all serum analytes, data did not follow a normal distribution, therefore are presented with median values and inter-quartile range (IQR) unless otherwise specified. Tests for statistical significance were undertaken using non-parametric methods; Mann-Whitney U, Kruskal-Wallis, Spearmen Rho Chi Square and Quads ANCOVA with the significance level set to $p < 0.05$ unless otherwise stated. Correction for multiple testing was undertaken using the Benjamini and Hochberg method (q-value of 0.05) for false detection rate (FDR) (Benjamini and Hochberg, 1995) to minimise the family wise error rates (Colquhoun, 2014).

All data collation, sorting, and analysis, including the statistical analyses presented herein, were performed by Dr. Aaron Wetten.

Chapter 4: Aims and Hypotheses

4.1 Overall Aim and Hypotheses

4.1.1 Overall Aim

This investigation seeks to enhance the understanding of the biological underpinnings of symptomatology in Primary Biliary Cholangitis (PBC) by building on recent mechanistic insights into disease pathology. Study 1 constitutes an exploratory analysis, while Study 2 serves as a validation study.

4.1.2 Overall Hypotheses

Consistent with present literature, it is hypothesised that younger patient age will correlate with a higher burden of severe symptoms encompassing fatigue, cognitive impairment, and emotional dysregulation. Conversely, except for pruritus, symptoms are not anticipated to exhibit a significant association with biochemical markers of disease activity, treatment response status, or the presence of cirrhosis.

4.2 Study 1 and Study 2: Aim and Hypotheses

4.2.1 Study 1 and Study 2: Aim

The primary aim of these studies is to investigate the relationship between symptoms in PBC and a panel of serum proteomic markers previously implicated in disease pathogenesis by the UK-PBC consortium. Furthermore, these studies aim to corroborate prior findings within a validation cohort.

4.2.2 Study 1 and Study 2: Hypotheses

It is hypothesised that in PBC patients experiencing more severe symptomatology, a significant correlation will be observed between a specific subset of proteomic markers and a given symptom domain. Specifically, based on current scientific evidence and known proteomic interactions in other disease contexts, it is predicted that fatigue will be associated with Leptin, CXCL9, KIM-1, and IL-6. Additionally, symptoms of pruritus are hypothesized to correlate with IL-4RA and IL-18R1.

4.3 Study 3: Aim and Hypothesis

4.3.1 Study 3: Aim

The aim of this study is to explore the relationship between the neurosteroid allopregnanolone and symptom manifestation in PBC.

4.3.2 Study 3: Hypothesis

It is hypothesised that serum allopregnanolone levels will be elevated in PBC patients compared to healthy controls, and that this increase will be associated with the presence and severity of cognitive symptoms.

Chapter 5: Study 1 exploratory proteomics: Results

5.1 Study 1 (exploratory study): Results

From the original 400 UK-PBC nested cohort study population (Jones *et al.*, 2022), symptom assessment using the PBC-40 was available for 289 participants. No other cases were included. Additional symptom data assessed using ESS, OGS and HADS were not available.

5.1.1 Study 1 (exploratory study): Demographics and clinical characteristics

Data on age and gender were available for 278 out 289 participants. In keeping with the epidemiology of PBC, 86% (248/289) of the cohort were female, with a median age of 65 years at the time of recruitment. The median age of the male patients was 67 years. There was no statistically significant difference in age between the male and female participants. **See Table** 5.1

Table 5.1 Gender and median ages in PBC group

Group	**Gender**	**Number (%)**	**Age in years* (IQR)**	**Significance across ages**
PBC	Total	278	66 (14)	
	Female	248 (86)	65 (14)	P = .246; $\chi^2(2) = 1.34$
	Male	30 (10)	67 (12)	
	Unknown	11 (4)		

**Median (Interquartile Range);* test for statistical significance Kruskal-Wallis; P < 0.05 considered statistically significant (2-sided test)

Clinical characteristics are summarised in **Table** 5.2**.** Median weight was 70 kg (IQR 23.4) with a median BMI of 26.6 (7.9). All participants were treated with UDCA at >12mg/kg/day for at least 12 months at the time of study recruitment as per the UK-PBC nested cohort inclusion criteria.

Median (Interquartile range) ALP at time of study entry was 138 (101) IU/L, ALT 28 (14) and bilirubin 9 (6). There were 100 participants (35%) with normal liver blood tests and 205 (71%) were biochemical responders according to the POISE criteria. There were 21 (7%) participants with a confirmed diagnosis of cirrhosis at the time of study recruitment.

Table 5.2 Clinical characteristics of the PBC study population

	All PBC (n=289)
Median weight, kg (IQR)	70 (23.4)
Median BMI, kg/m^2(IQR)	26.6 (7.9)
UDCA treated, number (%)	289 (100)
UDCA at therapeutic dose, number (%)	289 (100)
Median ALP (IQR) IU/L	138 (101)
Median ALT (IQR) IU/L	28 (24)
Median Bilirubin (IQR) umol/L	9 (6)
Median Albumin (IQR) g/L	No data
Normal LFT (%)	100 (35)
POISE Responder, number (%)	205 (71)
Cirrhosis present, number (%)	21 (7)

IQR – interquartile Range; BMI – body mass index; UDCA – ursodeoxycholic acid; ALP - alkaline phosphatase; ALT – alanine transaminase; LFT – liver function tests; n=number of cases in each group

The results from Study 1 were in keeping with the original UK-PBC proteomics work, with the majority of serum markers showing a statistically significant difference between POISE responders and non-responders (**see table 4.3**) (Jones *et al.*, 2022). A total of 16 out of 19 markers showed differences between the two groups, with SCAMP3 and VIM being non-significant in both the original work and here. CXCL13 was found to be statistically significantly different according to biochemical response in the original work but not in this study.

Data directly comparing serum proteomics between patients with normal and abnormal liver blood tests were not published in the previous proteomics study (Jones *et al.*, 2022), but in our study 16 out of 19 serum proteomics remained statistically significant across the two groups, with SCAMP3 and VIM again remaining not significant. CXCL13 remained significant between the normal and abnormal LFT groups, whilst AZU1 became non-significant. When stratified by cirrhosis status (i.e. cirrhotic versus non-cirrhotic), 9 of the 19 markers remained statistically significantly different (**See Table** 5.3**).**

Table 5.3 Serum proteomics according to POISE responder status, liver blood tests and cirrhosis status in Study 1

Marker	POISE			Liver blood tests			Cirrhosis		
	Responder (n=205) Median (IQR) pg/ml	Non-responder (n = 84) Median (IQR) pg/ml	Significance	Normal LFT (n = 100) Median (IQR) pg/ml	Abnormal LFT (n = 189) Median (IQR) pg/ml	Significance	No cirrhosis (n = 268) Median (IQR) pg/ml	Cirrhosis (n = 21) Median (IQR) pg/ml	Significance
IL-4Rα	2.94 (0.73)	4.06 (1.04)	P =.000 U = 14493	2.69 (0.60)	3.49 (1.13)	P =.000 U = 15436	3.11 (1.14)	3.90 (1.55)	P = .008 U = 3787
DECR1	4.05 (0.94)	4.56 (1.09)	P < .001 U = 11632	3.92 (0.72)	4.37 (1.18)	P < .001 U = 12855	4.17 (1.05)	3.97 (0.78)	P = .132 U = 2258
KIM-1	8.28 (1.07)	9.23 (1.23)	P < .001 U = 13136	8.24 (1.11)	8.60 (1.27)	P < .001 U = 12417	8.46 (1.23)	9.05 (2.20)	P = .009 U = 3778
ACE2	4.36 (1.26)	5.57 (1.90)	P < .001 U = 12789	4.09 (0.97)	5.08 (1.66)	P < .001 U =14145	4.58 (1.43)	6.28 (1.67)	P < .001 U = 4414
CA5A	3.43 (1.45)	4.54 (1.49)	P < .001 U = 12756	3.17 (1.05)	4.11 (1.54)	P < .001 U = 13984	3.67 (1.65)	4.07 (1.73)	P = .208 U = 3278
HAOX1	5.15 (2.31)	7.31 (2.12)	P < .001 U = 13424	4.67 (1.84)	6.63 (2.52)	P < .001 U =14904	5.72 (2.63)	6.24 (2.61)	P = .311 U = 3188
AZU1	6.62 (1.46)	6.25 (1.73)	P = .003 U = 6663	6.55 (1.62)	6.46 (1.53)	P = .405 U = 8887	6.57 (1.63)	6.05 (1.42)	P = .019 U = 1946
EP-CAM	4.85 (0.60)	5.22 (0.85)	P < .001 U = 11623	4.80 (0.64)	5.00 (0.80)	P = .025 U = 10970	4.94 (0.64)	5.22 (0.97)	P = .049 U = 3539
Leptin (weight adj.)	6.66 (1.39)	6.06 (1.62)	P < .001 U = 5783 P < .001	6.69 (1.27)	6.34 (1.64)	P < .002 U = 7384 P < .001	6.51 (1.45)	6.31 (1.52)	P = .395 U = 2500
CXCL11	8.96 (1.20)	9.27 (1.62)	P = .041 U = 9929	8.84 (0.91)	9.31 (1.49)	P < .001 U = 12678	9.05 (1.35)	8.70 (1.00)	P = .155 U = 2289
CXCL9	8.89 (1.28)	9.18 (1.05)	P = .014 U = 10193	8.62 (0.99)	9.19 (1.10)	P < .001 U = 12730	9.01 (1.23)	8.74 (1.15)	P = .698 U = 2671
CCL19	9.85 (1.19)	10.11 (1.41)	P = .010 U = 10271	9.74 (1.16)	10.07 (1.21)	P = .004 U = 11395	9.85 (1.16)	10.34 (1.64)	P = .002 U = 3946
IL-18R1	7.45 (0.77)	8.24 (0.85)	P < .001 U =13992	7.24 (0.51)	7.92 (0.78)	P < .001 U = 15093	7.63 (0.90)	8.05 (1.49)	P = .036 U = 3588
CXCL10	8.88 (1.15)	9.30 (1.11)	P = .002 U = 10643	8.70 (0.87)	9.22 (1.19)	P < .001 U = 12915	9.03 (1.18)	9.05 (0.74)	P = .911 U = 2855
CCL20	5.35 (1.53)	5.79 (1.43)	P = .007 U = 10352	5.15 (1.62)	5.66 (1.42)	P = .023 U = 10986	5.40 (1.45)	5.68 (2.62)	P = .128 U = 3376
SCAMP3	3.12 (1.37)	3.33 (1.27)	P =.214 U = 9412	2.89 (1.27)	3.28 (1.31)	P =.056 U = 10743	3.22 (1.38)	2.89 (1.19)	P = .071 U = 2147
VIM	4.64 (0.93)	4.60 (0.98)	P =.561 U = 8235	4.74 (1.09)	4.62 (0.97)	P =.649 U = 9758	4.64 (1.08)	4.61 (0.47)	P = .822 U = 2731
CXCL13	8.91 (0.75)	8.95 (0.75)	P =.180 U = 9474	8.82 (0.69)	8.96 (0.78)	P =.006 U = 11292	8.90 (0.73)	9.51 (1.27)	P < .001 U = 4294
CD163	8.14 (0.79)	8.56 (0.57)	P < .001 U = 12509	7.92 (0.75)	8.38 (0.63)	P < .001 U = 13089	8.24 (0.79)	8.89 (0.71)	P < .001 U = 4187

POISE criteria: ALP ≤ 1.67 x upper limit normal (ULN) and/or bilirubin ≤ 1 x ULN; LFT: Liver function tests; n=number of cases in each group; analysis using Mann-Whitney-U, P < 0.05 considered statistically significant (2-sided test); **red** denotes statistically significant

5.1.2 Study 1 (exploratory study): Clinical status and symptoms

To assess if the severity of liver disease, determined by biochemical response and by cirrhosis status had any impact on symptoms, we compared each of the PBC-40 domains between POISE responder status, normal or abnormal liver blood tests, and cirrhosis status. Severe itch on the PBC-40 domain was associated with biochemical non-response, but not with abnormal liver blood tests or cirrhosis, whilst severe scores in the fatigue, social and emotional domains were all statistically significantly associated with the presence of cirrhosis, but not biochemical status or abnormal liver blood tests (**See Table** 5.4**)**

Table 5.4: PBC-40 domain severity by POISE response status, liver bloods tests and cirrhosis status in Study 1

PBC-40 Domain	Severity	POISE Response			Liver blood tests			Cirrhosis		
		Responders (n=205)	Non responders (n=84)	Significance	Normal (n=100)	Abnormal (n=189)	Significance	Non-cirrhotic (n=268)	Cirrhotic (n=21)	Significance
Cognition	**None/mild**	147	55	P = .496 X(1) = 1.404	72	130	P = .554 X(1) = 1.180	189	13	P = .708 X(1) = 692
	Moderate	45	21		23	43		60	6	
	Severe	13	8		5	16		19	2	
Fatigue	**None/mild**	114	41	P = .338 X(1) = 2.170	53	102	P = .776 X(1) = .508	147	8	P = .023 X(1) = 7.583
	Moderate	61	25		32	54		81	5	
	Severe	30	18		15	33		40	8	
Itch	**None/mild**	147	53	P = .028 X(1) = 7.124	71	129	P = .833 X(1) = .365	190	10	P = .081 X(1) = 5.014
	Moderate	43	16		20	39		52	7	
	Severe	15	15		9	21		26	4	
Emotional	**None/mild**	145	49	P = .123 X(1) = 4.196	65	129	P = .191 X(1) = 3.311	185	9	P = .003 X(1) = 11.841
	Moderate	44	25		29	40		63	6	
	Severe	16	10		6	20		20	6	
Social	**None/mild**	142	53	P = .220 X(1) = 3.030	65	130	P = .139 X(1) = 3.945	184	11	P = .001 X(1) = 13.205
	Moderate	54	23		32	45		72	5	
	Severe	9	8		3	14		12	5	
General Symptoms	**None/mild**	154	60	P = .758 X(1) = .554	69	145	P = .313 X(1) = 2.325	201	13	P = .107 X(1) = 4.468
	Moderate	46	21		27	40		61	6	
	Severe	5	3		4	4		6	2	

POISE criteria: ALP ≤ 1.67 x upper limit normal (ULN) and/or bilirubin ≤ 1 x ULN; LFT: Liver function tests; n=number of cases in each group; analysis using Chi-square; P < 0.05 considered statistically significant (2-sided test); **red** denotes statistically significant

5.1.3 Study 1 (exploratory study): Age, symptoms, and serum proteomics

Age at time of study recruitment was available for 278 out of 289 (96%) patients. The impact of age on symptoms in PBC was analysed by comparing age in each of the PBC-40 domains according to symptom severity (**See Table** 5.5**)**. In all PBC-40 domains, median age was lower for increasing symptom severity. This was statistically significant in 4 of the PBC-40 domains: cognition, emotional, fatigue and social symptoms.

When analysing serum proteomics according to age, DECR1, HAOX1, CXCL11 and CCL19 showed a statistically significant negative correlation i.e., higher serum levels were associated with younger age. Conversely, KIM-1 and CXCL9 showed a positive correlation that reached statistical significance. When correcting for multiple testing CCL19 was considered a false positive. All other serum proteomics did not have a statistically significant correlation with age (**see Table** 5.6).

A statistically significant positive correlation to weight was observed for both leptin and CCL19, with leptin in particular showing a strong relationship to weight with a coefficient of 0.605 ($P < 0.001$), whilst a statistically significant negative relationship to weight was observed with KIM-1 and EP-CAM, however EP-CAM was considered a false positive when corrected for multiple testing with FDR (**seeTable** 5.7).

Table 5.5 PBC-40 domain severity by age (in years) in Study 1

PBC-40 Domain	**Severity**	Number	Age (years) [IQR]	Significance
Cognition	**None/mild**	194	67 [12]	P < .001 X(1) = 21.149
	Moderate	64	63 [14]	
	Severe	20	53 [13]	
Fatigue	**None/mild**	149	67 [13]	P = .016 X(1) = 8.297
	Moderate	82	63 [14]	
	Severe	47	62 [17]	
Itch	**None/mild**	193	66 [13]	P = .242 X(1) = 2.836
	Moderate	56	64 [5]	
	Severe	29	58 [22]	
Emotional	**None/mild**	187	66 [13]	P = .005 X(1) = 10.510
	Moderate	65	64 [15]	
	Severe	26	59 [13]	
Social	**None/mild**	185	67 [13]	P = .004 X(1) = 10.953
	Moderate	76	64 [13]	
	Severe	17	57 [15]	
General Symptoms	**None/mild**	206	66 [13]	P = .420 X(1) = 1.734
	Moderate	64	65 [13]	
	Severe	8	55 [26]	

n=number of cases in each group; analysis using Chi-square; P < 0.05 considered statistically significant (2-sided test); **red** denotes statistically significance

Table 5.6 Serum proteomics correlated by age (in years) in Study 1

Marker	Correlation coefficient	Significance
IL-4Rα*	-.077	P = .199
DECR1**	-.201	P < .001
KIM-1*	.144	P = .016
ACE2*	-.006	P =.919
CA5A**	-.127	P = .002
HAOX1**	-.296	P < .001
AZU1*	.042	P =.491
EP-CAM*	-.026	P =.665
Leptin*	-.042	P =.484
CXCL11**	-.215	P < .001
CXCL9*	1.46	P = .015
CCL19*	-.125	P = .037
IL-18R1**	-.169	P = .005
CXCL10*	-.042	P = .481
CCL20*	.014	P = .822
SCAMP3*	-.089	P = .138
VIM*	.090	P = .135
CXCL13*	.071	P = .241
CD163*	0.37	P = .535

analysis using Spearmen-rho; *P < 0.05 considered statistically significant (2-sided test); **P < 0.01 considered statistically significant (2-sided test), as defined by the sample size, and observed correlation; **red** denotes statistical significance; orange denotes considered false positive on FDR.

Table 5.7 Serum proteomics correlated by weight (in kg) in Study 1

Marker	Correlation coefficient	Significance
IL-4Rα*	-.080	P = .176
DECR1*	-.044	P = .449
KIM-1**	-.164	P = .005
ACE2*	-.058	P = .332
CA5A*	.045	P = .045
HAOX1*	-.086	P = .145
AZU1*	.061	P = .307
EP-CAM*	-.125	P = .034
Leptin**	.605	P < .001
CXCL11*	.010	P = .863
CXCL9*	-.050	P = .397
CCL19**	.159	P = .007
IL-18R1*	.-.001	P = .984
CXCL10*	.061	P = .301
CCL20*	.039	P = .515
SCAMP3*	-.030	P = .616
VIM*	.053	P = .369
CXCL13*	.023	P = .704
CD163*	.036	P = .539

analysis using Spearmen-rho; *P < 0.05 considered statistically significant (2-sided test); **P < 0.01 considered statistically significant (2-sided test); **red** denotes statistical significance; orange denotes considered false positive on FDR.

5.1.4 Study 1 (exploratory study): Serum proteomics and symptoms

After exploring the relationship between serum proteomics, age, symptoms and disease stage, the relationship between serum proteomics and individual PBC-40 domains was explored.

5.1.4.1 Serum proteomics and cognitive symptoms

For the PBC-40 cognitive domain, 202 out of 289 (70%) participants reported having "none/mild", 66 (23%) "moderate" and 21 (7%) "severe" symptoms. When stratified by clinically significant symptoms, defined as a score of 18 or more, 228 out of 289 (79%) participants were not considered to have clinically significant cognitive symptoms related to their PBC based on their PBC-40 cognitive domain scores, whilst 61 (21%) of participants cognitive symptoms were deemed to be clinically significant.

When comparing across the 3 cognitive symptom groups (none/mild, moderate, and severe), 5 analytes initially showed statistically significant associations, with increasing serum levels associated with increasing symptom severity. Following post hoc pairwise comparison using Bonferroni, persistent statistically significant associations were for DECR1 (none/mild vs. severe, P = .025), CCL19 (none/mild vs. severe, P = .035), IL-18R1 (none/mild vs. severe, P = .042), while IL-4Rα, and HAOX1 showed no statistical significance. In each case an increase in symptom severity was associated with increased serum analyte.

A total of 6 of the 19 serum proteomic markers were statistically significantly elevated in those with "severe" cognitive symptoms, when compared to those with "none/mild" symptoms: IL-4Rα (P=0.026), DECR1 (P=0.006), HAOX1 (P=0.021), IL-18R1 (0.013), CCL19 (P=0.010), and KIM-1 (P=0.031).

Using the pre-defined "clinically significant" score for the PBC-40 cognitive domain (a score of 18 or more being considered as significant; which incidentally lies midway in the moderate category), 4 of the 19 markers (IL-4Rα (P=0.023), KIM-1 (0.007), HAOX1 (P=0.018) and IL-18R (P=0.013)), were still significantly associated, with higher serum levels being associated with clinically significant cognitive symptoms.

Using each method of comparison, three serum analytes (IL-4Rα, HAOX1 and IL-18R1) were consistently statistically elevated in those with the worst cognitive symptoms (**See Table** 5.8**)**

Table 5.8 Serum markers (pg/ml) by cognitive domain severity as defined by PBC-40 (none/mild vs. severe) and by clinically significant cognitive score in Study 1

Marker	Cognitive Severity					Clinically* Significant Cognitive symptoms?		
	None/Mild (n=202) Median (IQR) pg/ml	**Moderate (n=66)** Median (IQR) pg/ml	**Severe (n=21)** Median (IQR) pg/ml	**Significance none/mild vs. moderate vs. severe**	**Significance None/mild vs. severe**	**No (n=228)** Median (IQR) pg/ml	**Yes (n=61)** Median (IQR) pg/ml	**Significance**
IL-4Rα	3.08 (1.03)	3.27 (1.31)	3.72 (1.69)	P = .031 $\chi^2(2) = 6.933$	P = .026 U = 2746	3.10 (1.03)	3.42 (1.38)	P = .023 U = 8275
DECR1	4.11 (0.97)	4.21 (1.43)	4.46 (1.24)	P = .008 $\chi^2(2) = 9.648$	P = .006 U = 2890	4.14 (0.97)	4.32 (1.30)	P = .053 U = 8078
KIM-1	8.47 (1.09)	8.44 (1.48)	8.96 (1.55)	P = .074 $\chi^2(2) = 5.212$	P = .031 U = 2727	8.45 (1.14)	8.96 (1.47)	P = .007 U = 8528
ACE2	4.65 (1.37)	4.49 (1.95)	5.33 (2.18)	P = .169 $\chi^2(2) = 3.551$	P = .057 U = 2657	4.64 (1.39)	4.88 (2.18)	P = .056 U = 8064
CA5A	3.59 (1.63)	3.78 (1.89)	4.13 (1.66)	P = .199 $\chi^2(2) = 3.225$	P = .072 U = 2627	3.62 (1.64)	4.10 (1.96)	P = .204 U = 7690
HAOX1	5.58 (2.46)	6.13 (2.92)	7.23 (3.21)	P = .021 $\chi^2(2) = 7.740$	P = .021 U = 2772	5.67 (2.43)	6.60 (3.12)	P = .018 U = 8327
AZU1	6.58 (1.54)	6.37 (1.62)	6.24 (1.78)	P = .798 $\chi^2(2) = 0.451$	P = .798 U = 2049	6.59 (1.56)	6.16 (1.60)	P = .140 U = 6098
EP-CAM	4.92 (0.65)	5.03 (0.67)	5.04 (1.04)	P = .446 $\chi^2(2) = 1.615$	P = .572 U = 2280	4.93 (0.63)	5.06 (0.83)	P = .126 U = 7842
Leptin	6.45 (1.43)	6.53 (1.30)	6.90 (1.97)	P = .236 $\chi^2(2) = 2.887$	P = .101 U =2582	6.46 (1.40)	6.61 (1.48)	P = .353 U = 7493
CXCL11	8.98 (1.23)	9.21 (1.36)	9.28 (1.48)	P = .458 $\chi^2(2) = 1.561$	P = .462 U = 2328	9.02 (1.27)	9.27 (1.36)	P = .490 U = 7354
CXCL9	8.90 (1.21)	9.14 (1.33)	9.18 (1.11)	P = .899 $\chi^2(2) = 0.213$	P = .685 U = 2235	8.94 (1.20)	9.05 (1.36)	P = .825 U = 6826
CCL19	9.85 (1.08)	10.08 (4.57)	10.50 (1.16)	P = .029 $\chi^2(2) = 7.113$	P = .010 U = 2844	9.88 (1.11)	10.09 (1.52)	P = .170 U = 7750
IL-18R1	7.60 (0.86)	7.88 (0.97)	7.90 (0.98)	P = .015 $\chi^2(2) = 8.417$	P = .013 U = 2816	7.62 (0.85)	7.90 (1.05)	P = .013 U = 8391
CXCL10	8.97 (1.09)	9.19 (1.36)	9.41 (0.81)	P = .219 $\chi^2(2) = 3.040$	P = .088 U = 2601	8.99 (1.11)	9.22 (1.34)	P = .437 U = 7405
CCL20	5.34 (1.44)	5.67 (1.75)	5.99 (1.72)	P = .063 $\chi^2(2) = 5.535$	P = .053 U = 2666	5.38 (1.44)	5.67 (1.55)	P = .175 U = 7740
SCAMP3	3.19 (1.20)	3.35 (1.74)	3.15 (1.14)	P = .485 $\chi^2(2) = 1.446$	P = .346 U = 2386	3.21 (1.30)	3.15 (1.57)	P = .791 U = 7108
VIM	4.66 (1.07)	4.66 (1.00)	4.46 (0.58)	P = .739 $\chi^2(2) = 0.605$	P = .434 U = 1901	4.66 (1.04)	4.60 (0.85)	P = .371 U = 6435
CXCL13	8.91 (0.76)	8.91 (0.71)	8.92 (0.77)	P = .988 $\chi^2(2) = 0.025$	P = .915 U = 2091	8.92 (0.75)	8.92 (0.74)	P = .622 U = 6668
CD163	8.26 (0.77)	8.24 (0.86)	8.48 (0.75)	P = 0.440 $\chi^2(2) = 1.641$	P = .227 U = 2461	8.26 (0.78)	8.33 (0.76)	P = .154 U = 7780

*A score of >= 18 in the cognitive domain of the PBC-40 is considered clinically significant; IQR – Interquartile Range; n=number of cases in each group; test for statistical significance using Mann-Whitney U and Kruskal-Wallis; P < 0.05 considered statistically significant (2-sided test); **red** denotes statistically significance; **yellow** denotes no statistical significance following post-hoc Bonferroni correction

KIM-1 previously showed a positive correlation with age and negative correlation with weight **(see Table** 5.6 **and Table** 5.7**)**. Therefore, ANCOVA was performed to ascertain the effect of these covariates on KIM-1 against clinically significant cognitive symptoms. When adjusted for age and weight, KIM-1 remained elevated in those with cognitive symptoms, with a statistically significant positive association observed with clinically significant cognitive symptoms (KIM-1: F = 10.536, P = 0.001) and between none/mild vs. severe groupings (KIM-1: F = 10.327, P = 0.002).

HAOX1, IL-18R1, and DECR1 showed significant correlation with age **(see Table** 5.8**)**. All 3 analytes failed to showed significance between the "none/mild" and "severe" groups when adjusted for age (HAOX1: F = 0.573, P = 0.450; IL-18R1: F 3.830, P = 0.051; DECR1: F = 3.081, P = 0.81). HAOX1 and IL-18R1 failed to show continued statistical significance with clinically significant cognitive symptoms (HAOX1: F = 2.023, P = 0.156; IL-18R1: F 2.631, P = 0.106). DECR1 did not previously show a significance with clinically significant cognitive symptoms and therefore no additional covariate adjustments were made.

When CCL19 was adjusted for by weight, it continued to show significance between "none/mild" and "severe" groups (CCL19: F = 5.959, P = .015), with higher levels associated with severe cognitive symptoms.

Therefore, following all post-hoc analyses, IL-4Rα, and KIM-1 continued to be statistically significant with higher values being associated with clinically significant cognitive symptoms (**see Figure** 5.1**).** Whilst IL-4Rα, KIM-1 and CCL19 showed statistically significant higher values between those with "none/mild" and "severe" cognitive symptoms.

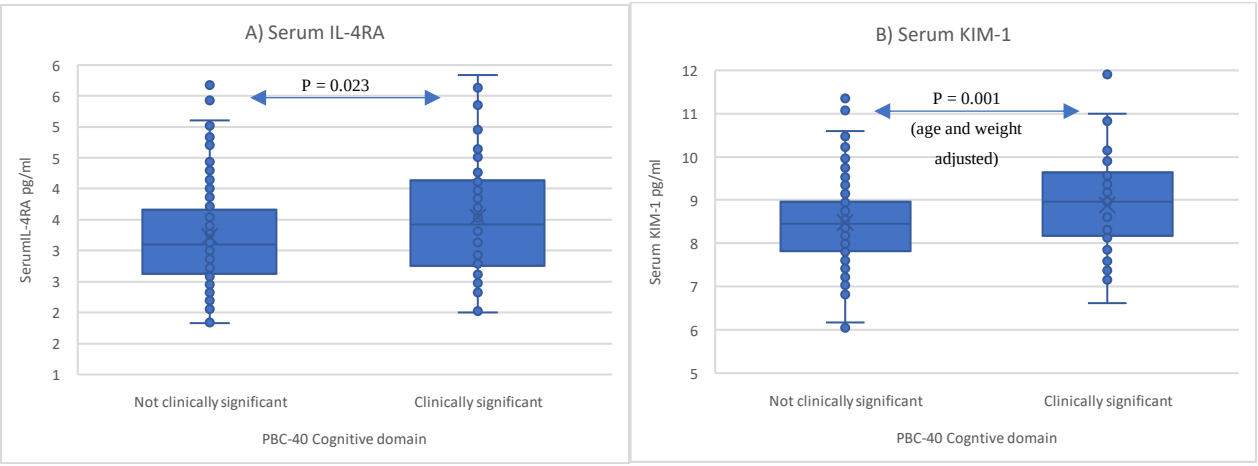


Figure 5.1 Serum markers (pg/ml) significantly associated with clinically significant cognitive symptoms as defined by the PBC-40 cognitive domain in Study 1: A) IL-4Rα, B) KIM-11

5.1.4.2 Serum proteomics and fatigue symptoms

Fatigue was more prevalent than cognitive symptoms in this cohort, with 155 out of 289 (53%) having "none/mild" fatigue, 86 (30%) having "moderate" fatigue and 48 (17%) having "severe" fatigue. Clinically significant fatigue, defined by a score of 33 or more in the PBC-40 fatigue domain, was present in 98 out of 289 (34%) of participants.

When comparing across the three PBC-40 fatigue symptom severity groups ("none/mild", "moderate" and "severe"), 3 proteomic markers remained statistically significant: leptin (P<0.001), KIM-1 (P=0.029), and CCL20 (P=0.023).

Following post-hoc Bonferroni pairwise correction, statistical significance was seen in the following pairwise groupings: KIM-1 (none/mild vs. severe, P = 0.037), leptin (none/mild vs. moderate, P = 0.014; none/mild vs. severe, P = 0.001) and CCL20 (none/mild vs. severe, P = 0.022). All other pairs failed to reach statistical significance.

A total of 6 of the 19 serum proteomic markers were statistically significantly elevated in those with "severe" fatigue, when compared to those with "none/mild" symptoms. The strongest association was with leptin (P<0.001), whilst IL-4Rα (P=0.018), KIM-1 (P=0.015), CCL19 (P=0.046), IL-18R1 (P=0.040) and CCL20 (P=0.008) were also significant. **See Table** 5.9**.**

In contrast to the cognitive domain, a greater number (7/19 for fatigue versus 4/19 for cognitive symptoms) of the serum proteomics were statistically significant when associations were tested using the clinically significant cut-off score of 33 or more. Of those 7, 5 were elevated when

comparing “none/mild” to “severe” symptoms: IL-4Rα (P=0.020), KIM-1 (P=0.004), IL-18R1 (P=0.027), CCL20 (P=0.022) and leptin (P<0.001). In addition, EP-CAM (P=0.013) and CD163 (P=0.045) were significantly elevated in those with clinically significant fatigue (**See Table** 5.9**).**

Table 5.9 Serum markers (pg/ml) according to PBC-40 fatigue domain severity (none/mild vs. severe) and by fatigue score deemed clinically significant in Study 1

Marker	Fatigue Severity					Clinically* Significant Fatigue symptoms?		
	None/Mild (n=155) Median (IQR) pg/ml	**Moderate (n=86)** Median (IQR) pg/ml	**Severe (n=48)** Median (IQR) pg/ml	**Significance none/mild vs. moderate vs. severe**	**Significance None/mild vs. severe**	**No (n=191)** Median (IQR) pg/ml	**Yes (n=98)** Median (IQR) pg/ml	**Significance**
IL-4Rα	3.08 (1.08)	3.17 (1.30)	3.38 (1.30)	P = .061 $\chi^2(2) = 5.609$	P = .018 U = 4564	3.08 (0.98)	3.30 (1.34)	P = .020 U = 10929
DECR1	4.12 (1.04)	4.17 (1.03)	4.22 (1.22)	P = .372 $\chi^2(2) = 1.979$	P = .266 U =4116	4.13 (1.01)	4.18 (1.11)	P = .226 U = 10174
KIM-1	8.36 (1.07)	8.60 (1.35)	8.73 (1.55)	P = .029 $\chi^2(2) = 7.098$	P = .015 U = 4586	8.41 (1.08)	8.71 (1.36)	P = .004 U = 11302
ACE2	4.64 (1.35)	4.64 (1.90)	4.87 (2.04)	P = .405 $\chi^2(2) = 1.806$	P = .180 U = 4197	4.64 (1.42)	4.76 (1.82)	P = .082 U = 10530
CA5A	3.67 (1.78)	3.62 (1.62)	3.91 (1.76)	P = .515 $\chi^2(2) = 1.329$	P = .303 U = 4086	3.67 (1.68)	3.73 (1.80)	P = .423 U = 9898
HAOX1	5.73 (2.30)	5.60 (2.70)	6.06 (3.22)	P = .316 $\chi^2(2) = 2.305$	P = .239 U = 4139	5.72 (2.49)	5.86 (2.91)	P = .496 U = 9817
AZU1	6.58 (1.50)	6.47 (1.66)	6.31 (1.71)	P = .997 $\chi^2(2) = 0.006$	P = .933 U = 3690	6.58 (1.55)	6.37 (1.68)	P = .342 U = 8720
EP-CAM	4.90 (0.68)	4.99 (0.57)	5.03 (0.76)	P = 2.97 $\chi^2(2) = 2.425$	P = .173 U = 4205	4.87 (0.65)	5.07 (0.70)	P = .013 U = 11022
Leptin	6.23 (1.52)	6.68 (1.61)	6.90 (1.16)	P < .001 $\chi^2(2) = 15.93$	P < .001 U = 4958	6.25 (1.42)	6.89 (1.49)	P < .001 U = 11573
CXCL11	8.96 (1.26)	9.12 (1.25)	9.08 (1.37)	P = .612 $\chi^2(2) = 0.981$	P = .591 U = 3911	9.01 (1.30)	9.09 (1.34)	P = .646 U = 9668
CXCL9	8.92 (1.27)	8.93 (1.23)	9.13 (1.25)	P = .667 $\chi^2(2) = 0.810$	P = .742 U = 3837	8.92 (1.23)	9.09 (1.19)	P = .673 U =9643
CCL19	9.85 (1.06)	9.83 (1.46)	10.1 (1.17)	*P = .159* $\chi^2(2) = 3.672$	P = .046 U = 4431	*9.85 (0.97)*	10.10 (1.64)	P = .137 U = 10360
IL-18R1	7.61 (0.80)	7.68 (1.01)	7.81 (0.94)	P = .120 $\chi^2(2) = 4.236$	P = .040 U = 4449	7.62 (0.77)	7.76 (0.98)	P = .027 U =10850
CXCL10	9.23 (0.96)	9.05 (1.26)	5.36 (1.51)	P = .386 $\chi^2(2) = 1.902$	P = .162 U = 4217	8.97 (1.13)	9.19 (1.08)	P = .235 U = 10158
CCL20	5.36 (1.51)	5.31 (1.77)	5.91 (1.67)	P = .023 $\chi^2(2) = 7.503$	P = .008 U = 4665	5.33 (1.33)	5.78 (1.93)	P = .022 U = 10897
SCAMP3	3.22 (1.27)	3.11 (1.30)	3.39 (1.35)	P = .290 $\chi^2(2) = 2.473$	P = .164 U = 4215	3.18 (1.23)	3.30 (1.55)	P = .257 U = 10122
VIM	4.64 (1.14)	4.76 (0.86)	4.46 (0.85)	P = .339 $\chi^2(2) = 2.165$	P = .805 U = 3632	4.65 (1.08)	4.61 (0.90)	P = .812 U = 9519
CXCL13	8.89 (0.76)	8.99 (0.73)	8.99 (0.78)	P = .503 $\chi^2(2) = 1.374$	P = .264 U = 4117	8.90 (0.76)	8.99 (0.73)	P = .403 U = 9922
CD163	8.26 (0.78)	8.25 (0.86)	8.38 (0.76)	P = .252 $\chi^2(2) = 2.758$	P = .093 U = 4317	8.23 (0.78)	8.35 (0.78)	P = .045 U =10705

*A score of >= 33 in the fatigue domain of the PBC-40 is considered clinically significant; IQR – Interquartile Range; n=number of cases in each group; test for statistical significance using Mann-Whitney U and Kruskal-Wallis; P < 0.05 considered statistically significant (2-sided test); **red** denotes statistically significant; **yellow** denotes no statistical significance following post-hoc Bonferroni correction

KIM-1 previously showed a significant correlation with age and weight. Adjusting KIM-1 for age and weight as covariates, using ANCOVA, demonstrated continued statistically significant elevations of KIM-1 with fatigue when stratifying by “none/mild” and “severe” groups (KIM-1: $F = 10.670$, $P = 0.001$) and clinically significant fatigue (KIM-1: $F = 11.972$, $P = 0.001$).

IL-18R1, when adjusted for by age, did not continue to show significance for clinically significant fatigue (IL-18R1: $F = 2.320$, $P = 0.129$) or between the “none/mild” vs. “severe” fatigue groups (IL-18R1: $F = 3.516$, $P = 0.062$).

Leptin, which showed a strong correlation to weight, was no longer significant when weight was included as a covariate for both clinically significant fatigue (Leptin: $F = 3.222$, $P = 0.074$) or between the “none/mild” and “severe” groups (Leptin: $F = 3.452$, $P = 0.065$). CCL19 was also no longer significant when corrected for weight for “none/mild” vs “severe” fatigue (CCL19: $F = 2.788$, $P = 0.097$).

Following post-hoc analyses, IL-4Rα, KIM-1, CD163 and CCL20 remained statistically significant with elevated levels being associated with clinically significant fatigue. **See Figure** 5.2

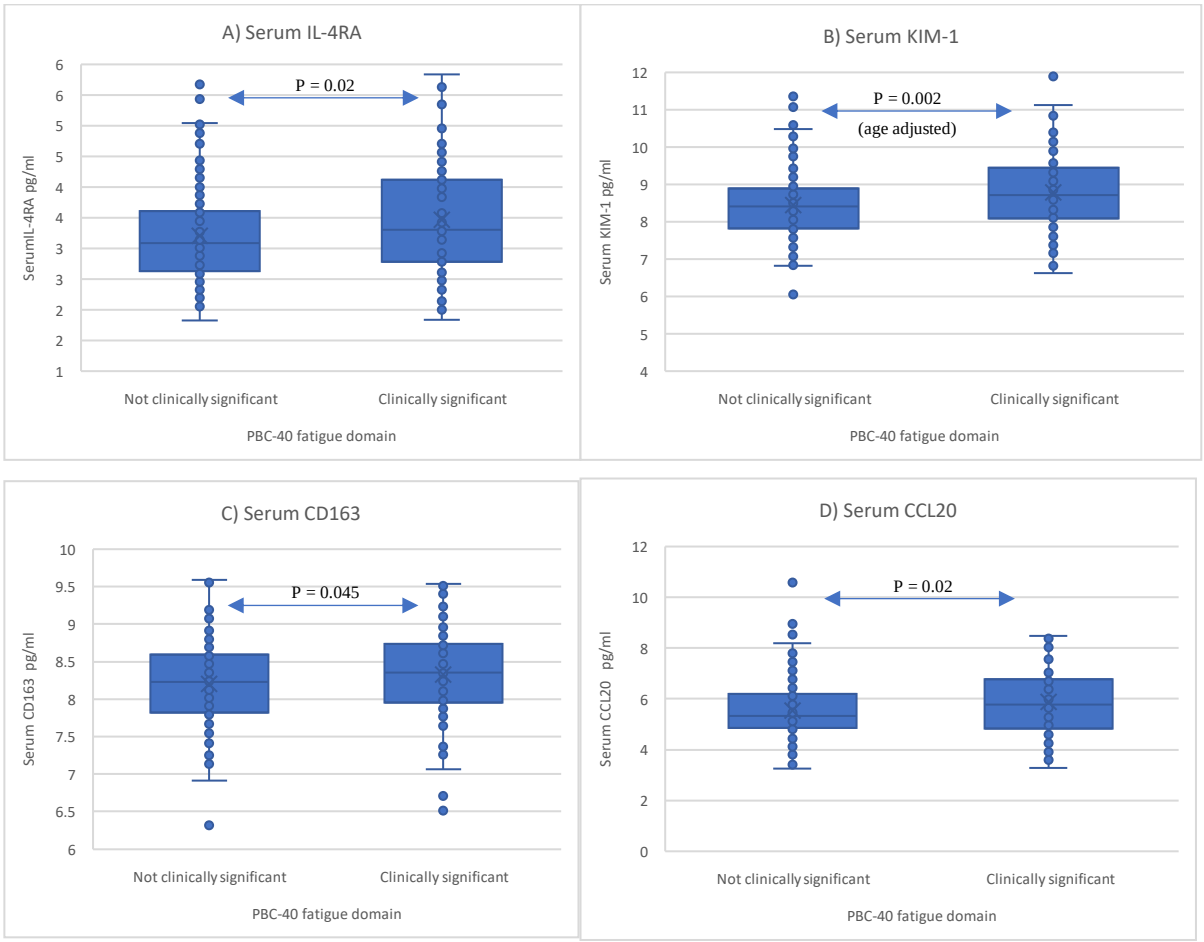


Figure 5.2 Serum markers (pg/ml) significantly associated with clinically significant fatigue symptoms as defined by the PBC-40 fatigue domain A) IL-4Rα, B) KIM1, C) CD163 D) CCL20

5.1.4.3 Serum proteomics and itch symptoms

"Severe" itch in the relevant PBC-40 domain was present in 30/289 (10%), "moderate" in 59/289 (21%), and 200/289 (69%) reported minimal or no itch and were categorised into the "none/mild" group. Clinically significant itch was defined by a PBC-40 itch domain score of 7 or more and using this definition, 143/289 (49%) had itch considered to be clinically significant. The number of participants with clinically significant itch is greater than the total number of participants reporting moderate/severe itch due to the pre-defined cut-off for clinically significant itch straddling the "none/mild" boundary (Mells *et al.*, 2013). **See section 3.4.5**.

When using the itch severity groups “none/mild”, “moderate” and “severe”, 6 markers (IL-4Rα (P=0.002), KIM1 (P=0.020), EPCAM (P=0.035), Leptin (P=0.016), IL-18R1 (P=0.003) and CD163 (P=0.007)) were higher in patients with more severe symptoms.

Following Bonferroni post-hoc pairwise analysis, statistically significant findings were observed for IL-4Rα (none/mild vs moderate, P = 0.025; none/mild vs severe, P = 0.023), KIM-1 (none/mild vs severe, P = 0.018), leptin (none/mild vs moderate, P = 0.045), IL-18R1 (none/mild vs moderate, P = 0.016) and CD163 (none/mild vs severe, P = 0.005). No significance was observed in EPCAM.

Comparing serum proteomics across the “none/mild” and “severe” categories of the PBC-40 itch domain, a statistically significant relationship was seen for 6 of the 19 markers, with higher serum values being associated with severe itch. The strongest positive associations were for IL-4Rα (P=0.008), KIM-1 (P=0.005) and CD163 (P=0.002), whilst there were also positive associations between ACE2 (P=0.044), EPCAM (P=0.023) and IL-18R1 (P=0.019) and worsening itch severity. Leptin was no longer significant.

Exploring serum proteomic markers based on clinically significant itch demonstrated 6 out of 19 markers to be statistically significant. These included 4 of the 6 previously observed significant markers: IL-4Rα (P=0.032), KIM-1 (P=0.032), IL-18R1 (P=0.015) and leptin (P<0.001), along with new associations with CCL19 (P=0.038) and CCL20 (P=0.027), where higher serum levels were observed in those with clinically significant itch. **See Table** 5.10 **and Figure** 5.3**.**

Table 5.10 Serum markers (pg/ml) by PBC-40 itch domain severity (none/mild vs. severe) and by clinically significant itch score in Study 1

Marker	Itch Severity					Clinically* Significant Itch symptoms?		
	None/Mild (n=200) Median (IQR) pg/ml	**Moderate (n=59)** Median (IQR) pg/ml	**Severe (n=30)** Median (IQR) pg/ml	**Significance none/mild vs. moderate vs. severe**	**Significance none/mild vs. severe**	**No (n=146)** Median (IQR) pg/ml	**Yes (n=143)** Median (IQR) pg/ml	**Significance**
IL-4Rα	3.05 (1.11)	3.38 (1.15)	3.40 (1.40)	P = .002 $\chi^2(2) = 11.99$	P = .008 U = 3899	3.09 (1.01)	3.27 (1.30)	P = .032 U = 11965
DECR1	4.15 (1.06)	4.10 (0.96)	4.17 (1.22)	P = .809 $\chi^2(2) = 0.424$	P = .516 U = 3221	4.13 (1.09)	4.18 (1.01)	P = .310 U = 11160
KIM-1	8.41 (1.15)	8.46 (1.75)	8.87 (1.22)	P = .020 $\chi^2(2) = 7.828$	P = .005 U = 3963	8.38 (1.15)	8.60 (1.29)	P = .032 U = 11965
ACE2	4.56 (1.43)	4.75 (1.71)	5.25 (2.03)	P = .088 $\chi^2(2) = 4.865$	P = .044 U = 3685	4.62 (1.36)	4.73 (1.82)	P = .450 U = 10975
CA5A	3.66 (1.71)	3.72 (1.82)	4.10 (1.70)	P = .266 $\chi^2(2) = 2.646$	P = .120 U = 3528	3.78 (1.79)	3.66 (1.71)	P = .442 U = 10985
HAOX1	5.68 (2.54)	5.95 (2.74)	6.26 (3.64)	P = .484 $\chi^2(2) = 1.452$	P = .379 U = 3299	5.67 (2.36)	5.89 (2.94)	P = .267 U = 11228
AZU1	6.48 (1.58)	6.59 (1.55)	6.25 (1.85)	P = .921 $\chi^2(2) = .165$	P = .709 U = 2873	6.45 (1.72)	6.52 (1.49)	P = .614 U = 10797
EP-CAM	4.87 (0.62)	5.02 (0.60)	5.20 (0.84)	P = .035 $\chi^2(2) = 6.700$	P = .023 U = 3770	4.91 (0.63)	5.01 (0.72)	P = .159 U = 11440
Leptin	6.36 (1.36)	6.86 (1.44)	6.93 (2.08)	P = .016 $\chi^2(2) = 8.238$	P = .066 U = 3624	6.26 (1.47)	6.76 (1.39)	P < .001 U = 12986
CXCL11	8.03 (1.35)	8.96 (1.20)	9.08 (1.87)	P = .902 $\chi^2(2) = .207$	P = .986 U = 3006	9.02 (1.40)	9.05 (1.27)	P = .788 U = 10630
CXCL9	8.93 (.125)	8.88 (1.07)	9.08 (0.94)	P = .817 $\chi^2(2) = .404$	P = .560 U = 3198	8.92 (1.20)	9.05 (1.28)	P = .980 U = 10421
CCL19	9.85 (1.13)	10.11 (1.39)	10.07 (1.32)	P = .143 $\chi^2(2) = 3.891$	P = .121 U = 3527	9.82 (1.13)	10.10 (1.34)	P = .038 U = 11909
IL-18R1	7.59 (0.89)	7.90 (0.89)	7.78 (0.97)	P = .003 $\chi^2(2) = 11.437$	P = .019 U = 3797	7.58 (0.78)	7.75 (0.95)	P = .015 U = 12166
CXCL10	9.01 (1.14)	8.96 (1.26)	9.23 (1.07)	P = .593 $\chi^2(2) = 1.047$	P = .313 U = 3343	8.99 (1.16)	9.09 (1.07)	P = .632 U = 10779
CCL20	5.35 (1.50)	5.88 (1.80)	5.47 (1.86)	P = .091 $\chi^2(2) = 4.794$	P = .349 U = 3318	5.35 (1.42)	5.66 (1.65)	P = .027 U = 12006
SCAMP3	3.18 (1.31)	3.33 (1.58)	2.96 (1.35)	P = .349 $\chi^2(2) = 2.103$	P = .740 U = 2887	3.15 (1.28)	3.22 (1.41)	P = .348 U = 11106
VIM	4.63 (1.05)	4.77 (1.11)	4.46 (0.68)	P = .668 $\chi^2(2) = .806$	P = .892 U = 2954	4.62 (1.02)	4.71 (0.96)	P = .140 U = 11487
CXCL13	8.89 (0.73)	9.01 (0.93)	8.94 (0.66)	P = .407 $\chi^2(2) = 1.796$	P = .930 U = 2970	8.90 (0.70)	8.94 (0.79)	P = .843 U = 10580
CD163	8.21 (0.79)	8.26 (0.84)	8.61 (0.62)	P = .007 $\chi^2(2) = 9.902$	P = .002 U = 4072	8.24 (0.76)	8.32 (0.82)	P = .201 U = 11347

*A score of >= 7 in the itch domain of the PBC-40 is considered clinically significant; IQR – Interquartile Range; n=number of cases in each group; test for statistical significance using Mann-Whitney U and Kruskal-Wallis; P < 0.05 considered statistically significant (2-sided test); **red** denotes statistically significant; **yellow** denotes no statistical significance following post-hoc Bonferroni correction

Adjusting for age and weight as a covariate for KIM-1, resulted in continued significant correlation of KIM-1 with clinically significant itch (KIM-1: F = 9.129, P = 0.003) and the "none/mild" and "severe" groups (KIM-1: F = 9.050, P = 0.003). CCL19, when adjusted for weight, failed to show significance (CCL19: F = 3.773, P = 0.053).

Leptin continued to show significant correlation to clinically significant itch when weight was included as a covariate (Leptin: F= 10.944, P = 0.001).

IL-18R1 remained significant when adjusted for by age with clinically significant itch (IL-18R1: F = 5.294, P = 0.022) and between "none/mild" and "severe" groups (IL-18R1: F = 3.825, P = 0.05)

Following post-hoc analyses, IL-4Rα, KIM-1, Leptin and IL-18R1 remained statistically significant with elevated levels being associated with clinically significant itch. **See Figure** 5.3

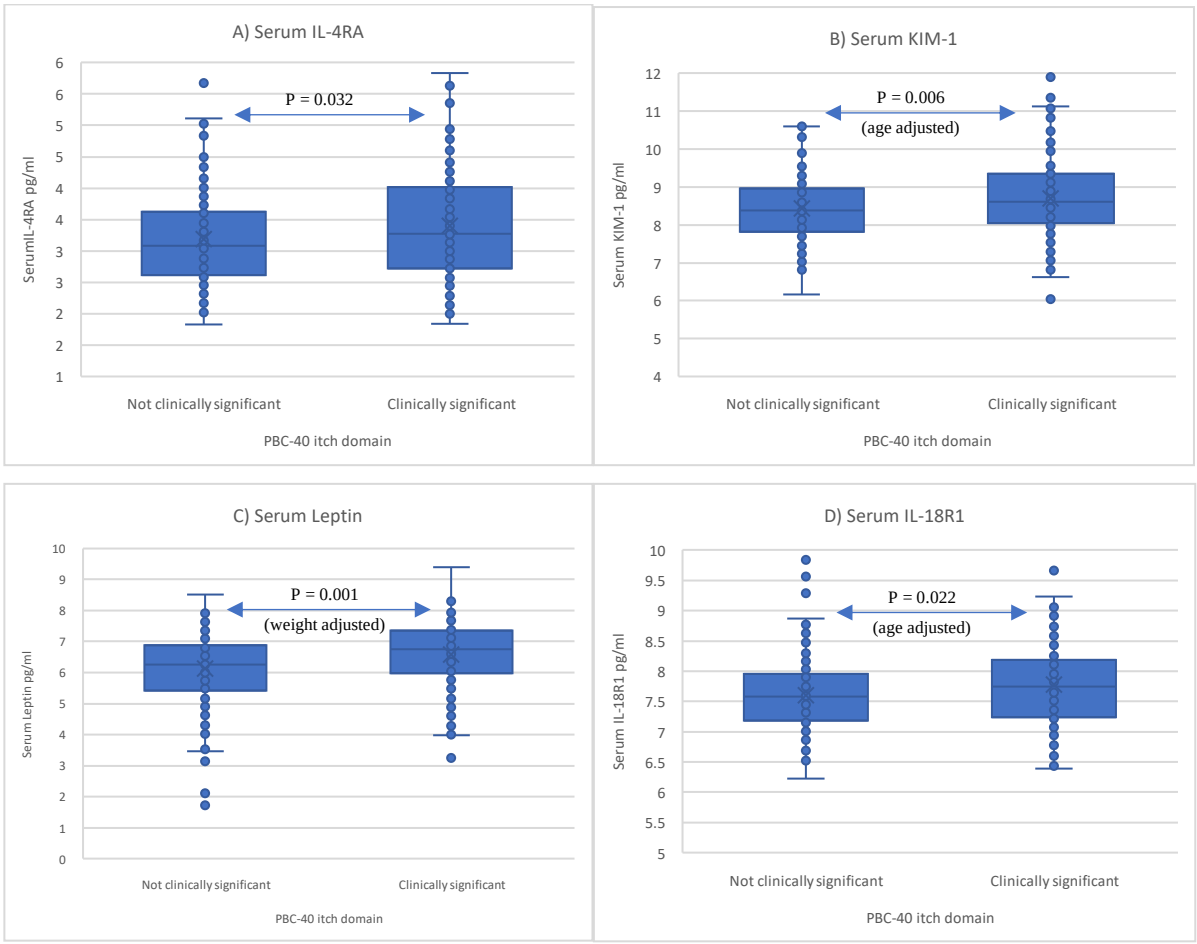


Figure 5.3 Serum markers (pg/ml) significantly associated with clinically significant itch symptoms as defined by the PBC-40 itch domain A) IL-4Rα, B) KIM1, C) Leptin D) IL-18R1.

5.1.4.4 Serum proteomics and emotional symptoms

From the PBC-40 emotional domain, 194 out 298 (67%) of participants were considered to have "none/mild" symptoms, 69 (24%) participants had "moderate" symptoms and 26 (9%) had "severe" symptoms. When split by clinical significance (using the pre-defined value of 12 or more), which is also the cut-off for "severe" symptoms, 26/289 (9%) had clinically significant emotional symptoms.

Using the categories of "none/mild", "moderate" and "severe", 4 out of 19 markers were considered statistically significant: IL-4Rα (P=0.04), ACE2 (P=0.016), HAOX1 (P=0.033) and IL-18R1 (P=0.037), with elevated analytes being associated with more severe emotional symptoms.

Following post-hoc Bonferroni pairwise analysis, IL-4Rα did not remain significant, whilst HAOX1 (none/mild vs. moderate, P = 0.04, none/mild vs. severe, P = 0.04), IL-18R1 (none/mild vs. severe, P = 0.03) and ACE2 (none/mild vs. moderate, P = 0.018, none/mild vs. severe, P = 0.021) showed statistical significance between the described groups, with increased levels associated with increased emotional symptom severity.

When comparing "none/mild" vs. "severe" emotional symptoms 7 out of 19 proteome markers were significantly elevated. The strongest associations were for ACE2 (P=0.005) and IL-18R1 (P=0.009), whilst IL-4Rα (P=0.024), KIM-1 (P=0.036), CA5A (P=0.037), HAOX1 (P=0.012), IL-18R1 and CD163 (P=0.018) were also statistically significantly elevated in those with severe emotional symptoms.

Finally, applying the clinically significant cut-off for the PBC-40 emotional domain to the serum proteome resulted in a positive association with 5 out of 19 markers, with ACE2 (P=0.005) was the most significant, followed by HAOX1 (P=0.010), IL-18R1 (P=0.026), CD163 (P=0.026) and IL-4Rα (P=0.041). **See Table** 5.11**.**

Table 5.11 Serum markers (pg/ml) by PBC-40 emotional domain severity (none/mild vs. severe) and by clinically significant emotional score in Study 1

Marker	Emotional Severity					Clinically* Significant Emotional symptoms?		
	None/Mild (n=194) Median (IQR) pg/ml	**Moderate (n=69)** Median (IQR) pg/ml	**Severe (n=26)** Median (IQR) pg/ml	**Significance none/mild vs. moderate vs. severe**	**Significance none/mild vs. severe**	**No (n=263)** Median (IQR) pg/ml	**Yes (n=26)** Median (IQR) pg/ml	**Significance**
IL-4Rα	3.09 (1.04)	3.26 (1.35)	3.56 (1.61)	P = .040 $\chi^2(2) = 6.439$	P = .024 U = 3212	3.11 (1.20)	3.56 (1.61)	P = .041 U = 4251
DECR1	4.16 (1.01)	4.08 (0.96)	4.36 (1.39)	P = .269 $\chi^2(2) = 2.623$	P = .108 U = 3012	4.14 (0.99)	4.36 (1.39)	P = .106 U = 4076
KIM-1	8.41 (1.20)	8.59 (1.26)	8.96 (1.62)	P = .077 $\chi^2(2) = 5.115$	P = .036 U = 3160	8.46 (1.21)	8.96 (1.62)	P = .050 U = 4215
ACE2	4.68 (1.38)	4.43 (1.93)	5.50 (1.96)	P = .016 $\chi^2(2) = 8.216$	P = .005 U = 3370	4.60 (1.55)	5.50 (1.96)	P = .005 U = 4567
CA5A	3.62 (1.70)	3.73 (1.96)	4.15 (1.61)	P = .106 $\chi^2(2) = 4.484$	P = .037 U = 3159	3.66 (1.64)	4.15 (1.61)	P = .050 U = 4216
HAOX1	5.69 (2.49)	5.65 (3.04)	6.93 (2.83)	P = .033 $\chi^2(2) = 6.811$	P = .012 U = 3290	5.69 (2.61)	6.93 (2.83)	P = .010 U = 4469
AZU1	6.58 (1.59)	6.38 (1.73)	6.18 (1.43)	P = .407 $\chi^2(2) = 1.796$	P = .187 U = 2120	6.54 (1.58)	6.18 (1.43)	P = .201 U = 2899
EP-CAM	4.91 (0.64)	5.03 (0.61)	5.01 (0.87)	P = .377 $\chi^2(2) = 1.951$	P = .190 U = 2921	4.94 (0.63)	5.01 (0.87)	P = .224 U = 3913
Leptin	6.41 (1.38)	6.70 (1.39)	6.69 (1.69)	P = .147 $\chi^2(2) = 3.831$	P = .360 U = 2801	6.48 (1.44)	6.69 (1.69)	P = .521 U = 3680
CXCL11	9.03 (1.37)	9.02 (1.39)	9.00 (1.15)	P = .482 $\chi^2(2) = 1.458$	P = .684 U = 2398	9.03 (1.36)	9.00 (1.15)	P = .825 U = 3329
CXCL9	8.92 (1.30)	9.18 (1.14)	8.80 (1.31)	P = .655 $\chi^2(2) = 0.847$	P = .403 U = 2267	9.01 (1.20)	8.80 (1.31)	P = .369 U = 3054
CCL19	9.91 (1.11)	9.79 (1.43)	10.04 (1.22)	P = .176 $\chi^2(2) = 3.479$	P = .079 U = 3058	9.88 (1.19)	10.04 (1.22)	P = .068 U = 4162
IL-18R1	7.64 (0.85)	7.64 (1.07)	7.88 (1.17)	P = .037 $\chi^2(2) = 6.612$	P = .009 U = 3318	7.64 (0.93)	7.89 (1.17)	P = .011 U = 4449
CXCL10	8.97 (1.20)	9.15 (0.99)	9.13 (0.10)	P = .709 $\chi^2(2) = 0.689$	P = .527 U = 2715	9.01 (1.17)	9.13 (1.00)	P = .624 U = 3618
CCL20	5.38 (1.43)	5.38 (1.89)	5.50 (1.65)	P = .157 $\chi^2(2) = 3.701$	P = .399 U = 2779	5.47 (1.46)	5.50 (1.65)	P = .593 U = 3636
SCAMP3	3.23 (1.23)	3.30 (1.73)	4.00 (1.78)	P = .473 $\chi^2(2) = 1.496$	P = .301 U = 2207	3.23 (1.36)	3.00 (1.78)	P = .248 U = 2949
VIM	4.69 (1.11)	4.63 (1.01)	4.43 (0.45)	P = .210 $\chi^2(2) = 3.123$	P = .080 U =1989	4.67 (1.08)	4.43 (0.45)	P = .081 U = 2710
CXCL13	8.94 (0.71)	8.88 (0.77)	8.79 (1.01)	P = .981 $\chi^2(2) = 0.038$	P = .880 U = 2476	8.93 (0.74)	8.79 (1.02)	P = .896 U = 3366
CD163	8.25 (0.78)	8.24 (0.78)	8.39 (0.86)	P = .053 $\chi^2(2) = 5.867$	P = .018 U = 3241	8.26 (0.80)	8.39 (0.86)	P = .026 U =4326

*A score of >= 12 in the emotional domain of the PBC-40 is considered clinically significant; IQR – Interquartile Range; n=number of cases in each group; test for statistical significance using Mann-Whitney U and Kruskal-Wallis; P < 0.05 considered statistically significant (2-sided test); **red** denotes statistically significant; **yellow** denotes no statistical significance following post-hoc Bonferroni correction

KIM-1 showed significance between “none/mild” and “severe” emotional groups when adjusted for by age and weight (KIM-1: F = 7.818, P = 0.006) and for clinically significant emotional symptoms (KIM-1: F = 5.880, P = 0.016).

IL-18R1, when adjusted for age, showed significance for “none/mild” and “severe” emotional groups (IL-18R1: F = 4.602, P = 0.033) and those with clinically significant emotional symptoms (IL-18R1: F = 4.300, P = 0.039).

After adjusting HAOX1 for age as covariate against clinically significant emotional symptoms, and “none/mild” vs “severe” HAOX1 failed to show significance (HAOX1: F = 3.033, P = 0.083).

Therefore, after post-hoc analysis, IL-4Rα, KIM-1, ACE2, IL-18R1 and CD163 remained associated with clinically significant emotional symptoms. **See Figure** 5.4**.**

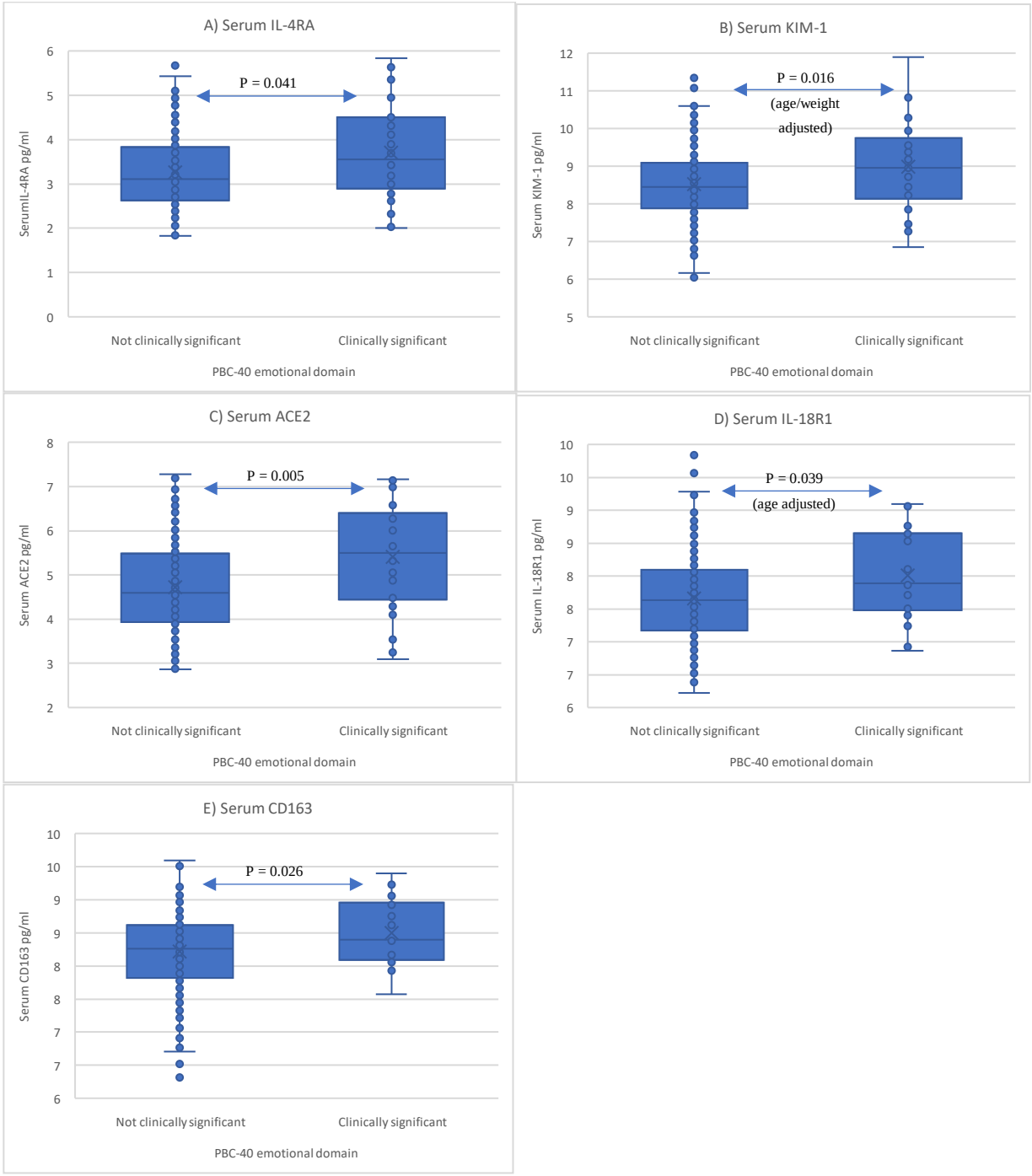


Figure 5.4 Serum markers (pg/ml) significantly associated with clinically significant emotional symptoms as defined by the PBC-40 emotional domain A) IL-4Rα, B) KIM-1 C) ACE2, D) IL-18R1 E) CD163

5.1.4.5 Serum proteomics and social symptoms

Applying the pre-defined severity cut-offs for the PBC-40 social domain, 195/298 (66%) participants had "none/mild" symptoms, 77/298 (27%) "moderate", and 17/298 (7%) "severe".

Splitting the cohort by clinically significant symptoms, defined by a PBC-40 social domain score of 32, resulted in 69/289 (24%) being considered to have clinically significant symptoms.

Only 3 of the 19 markers were statistically significant when analysed according to "none/mild", "moderate" and "severe" groups. These were KIM-1 (P=0.004), IL-4Rα (P=0.014), and leptin (P=0.007). KIM-1 and IL-4Rα demonstrated increasing serum levels with increased symptom severity, whilst for leptin, particularly high levels were observed in the "moderate" group compared to the "none/mild" and "severe" groups.

Following correction for multiple testing and with pairwise comparisons, IL-4Rα (none/mild vs. severe: P = 0.04), KIM-1 (none/mild vs. severe: P = 0.004) and leptin (none/mild vs. moderate: P = 0.005) remained significant, with significance observed between the described groups.

Exploring the serum proteome between the "none/mild" versus "severe" groups demonstrated statistical significance across 5 of the 19 markers. KIM-1 (P=0.002), IL-4Rα (P=0.014), ACE2 (P=0.032), CCL19 (P=0.023) and IL-18R1 (0.020). In all, cases higher serum levels were associated with severe symptoms.

Finally, using the pre-defined clinically significant social scores, 4 out of 19 markers reached significance. KIM-1 and leptin were elevated with clinically significant social symptoms: KIM-1 (P=0.003), leptin (P=.032) and were observed to be significant in the previous analysis, whilst 2 new markers AZU1 (P=0.014) and VIM (P=0.021)) were statistically significantly lower in those with clinically significant symptoms. **See Table** 5.12**.**

Table 5.12 Serum markers (pg/ml) by PBC-40 social domain severity (none/mild vs. severe) and by social score deemed clinically significant in Study 1

Marker	Social Severity					Clinically* Significant Social symptoms?		
	None/Mild (n=195) Median (IQR) pg/ml	**Moderate (n=77)** Median (IQR) pg/ml	**Severe (n=17)** Median (IQR) pg/ml	**Significance none/mild vs. moderate vs. severe**	**Significance none/mild vs. severe**	**No (n=220)** Median (IQR) pg/ml	**Yes (n=69)** Median (IQR) pg/ml	**Significance**
IL-4Rα	3.09 (1.08)	3.19 (1.35)	3.69 (1.53)	P = .039 $\chi^2(2) = 6.48$	P = .014 U = 2254	3.10 (1.08)	3.31 (1.54)	P = .096 U = 8599
DECR1	4.12 (0.99)	4.21 (1.09)	4.36 (1.67)	P = .235 $\chi^2(2) = 2.90$	P = .134 U = 2021	4.15 (0.99)	4.16 (1.15)	P = .657 U = 7859
KIM-1	8.42 (1.13)	8.60 (1.46)	9.56 (1.70)	P = .004 $\chi^2(2) = 11.142$	P = .002 U = 2422	8.41 (1.19)	8.69 (1.45)	P = .003 U = 9372
ACE2	4.64 (1.37)	4.72 (2.05)	5.60 (2.20)	P = .111 $\chi^2(2) = 4.397$	P = .032 U = 2177	4.60 (1.40)	4.87 (2.22)	P = .091 U = 8615
CA5A	3.67 (1.73)	3.73 (1.65)	4.20 (2.24)	P = .257 $\chi^2(2) = 2.717$	P = .116 U = 2039	3.66 (1.65)	3.83 (1.84)	P = .317 U = 8196
HAOX1	5.73 (2.54)	5.62 (2.82)	7.14 (3.27)	P = .138 $\chi^2(2) = 3.962$	P = .051 U = 2131	5.74 (2.53)	5.75 (3.03)	P = .593 U = 7914
AZU1	6.58 (1.55)	6.50 (1.72)	6.06 (1.22)	P = .434 $\chi^2(2) = 1.669$	P = .195 U = 1343	6.5 (1.46)	6.12 (1.54)	P = .014 U = 6097
EP-CAM	4.89 (0.64)	5.04 (0.53)	5.19 (0.94)	P = .062 $\chi^2(2) = 5.560$	P = .066 U = 2104	4.93 (0.64)	5.04 (0.68)	P = .216 U = 8339
Leptin	6.34 (1.38)	6.93 (1.34)	6.69 (2.14)	P = .007 $\chi^2(2) = 9.932$	P = .440 U = 1845	6.44 (1.40)	6.90 (1.45)	P = .032 U = 8892
CXCL11	9.02 (1.31)	9.15 (1.34)	8.73 (0.86)	P = .551 $\chi^2(2) = 1.193$	P = .408 U = 1457	9.03 (1.39)	9.00 (1.25)	P = .861 U = 7484
CXCL9	8.91 (1.25)	9.18 (1.13)	8.73 (1.26)	P = .582 $\chi^2(2) = 1.084$	P = .440 U = 1470	8.92 (1.27)	9.09 (1.11)	P = .799 U = 7436
CCL19	9.88 (1.05)	9.85 (1.55)	10.45 (1.26)	P = .070 $\chi^2(2) = 5.325$	P = .023 U =2209	9.88 (1.04)	10.07 (1.66)	P = .198 U = 8369
IL-18R1	7.64 (0.85)	7.71 (1.10)	7.90 (1.16)	P = .057 $\chi^2(2) = 5.714$	P = .020 U = 2222	7.64 (0.87)	7.71 (1.01)	P = .191 U = 8382
CXCL10	8.98 (1.16)	9.22 (1.10)	8.97 (1.04)	P = .386 $\chi^2(2) = 1.905$	P = .784 U = 1591	9.00 (1.19)	9.13 (1.01)	P = .650 U = 7864
CCL20	5.38 (1.45)	5.57 (1.85)	5.99 (1.15)	P = .177 $\chi^2(2) = 3.463$	P = .124 U = 2031	5.42 (1.41)	5.57 (1.86)	P = .161 U = 8438
SCAMP3	3.21 (1.22)	3.14 (1.65)	3.17 (1.49)	P = .938 $\chi^2(2) = 0.128$	P = .721 U = 1744	3.23 (1.30)	3.05 (1.58)	P = .452 U = 7134
VIM	4.69 (1.01)	4.47 (1.07)	4.46 (0.63)	P = .395 $\chi^2(2) = 1.859$	P = .182 U = 1334	4.71 (0.99)	4.44 (0.99)	P = .021 U = 6197
CXCL13	8.90 (0.77)	9.01 (0.71)	8.72 (0.94)	P = .404 $\chi^2(2) = 1.814$	P = .432 U =1467	8.90 (0.75)	9.01 (0.74)	P = .223 U = 8328
CD163	8.26 (0.78)	8.34 (0.84)	8.14 (0.86)	P = .472 $\chi^2(2) = 1.501$	P = .381 U = 1870	9.23 (0.78)	8.34 (0.87)	P = .205 U = 8358

*A score of >= 32 in the social domain of the PBC-40 is considered clinically significant; IQR – Interquartile Range; n=number of cases in each group; test for statistical significance using Mann-Whitney U and Kruskal-Wallis; P < 0.05 considered statistically significant (2-sided test); **red** denotes statistically significance

Adjusting for age and weight, KIM-1 continued to show statistical significance to clinically significant social symptoms, (KIM-1: F = 11.905, P = 0.001) and between the "none/mild" and "severe" groups (KIM-1: F = 15.657, P = < 0.001), with higher levels observed in severe/clinically significant symptoms. CCL19 failed to show continued significance (CCL19: F = 3.243, P = 0.073).

Leptin, when adjusted for weight, failed to show significant association with clinically significant social symptoms (Leptin: F 1.433, P = 0.232). IL-18R1 failed to show significance when corrected for by age (IL-18R1: F = 2.995, P = 0.085).

Figure 5.5 illustrates the analytes that are significantly associated with clinically significant social symptoms and include KIM-1, AZU1, and VIM, with KIM-1 being increased in clinically significant social symptoms, while AZU1 and VIM were reduced.

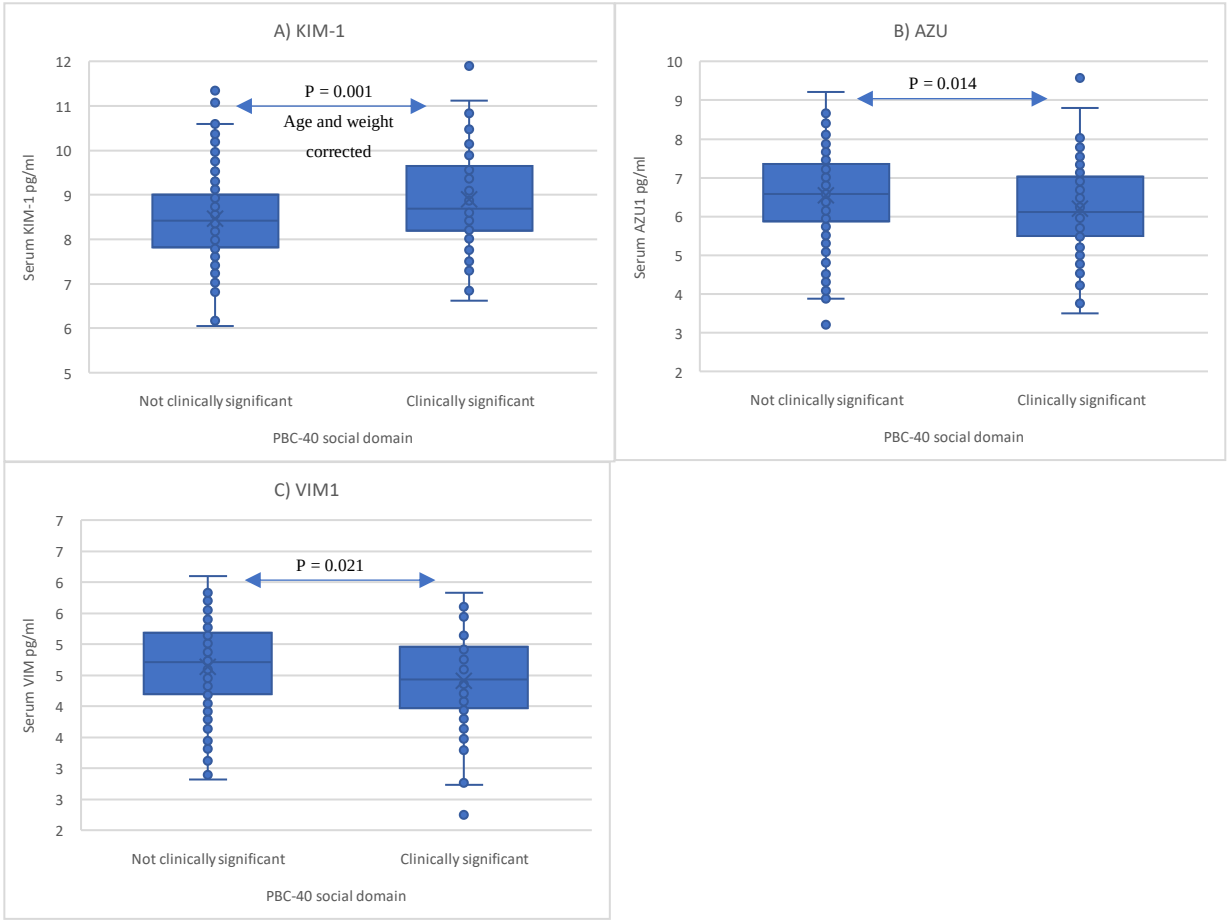


Figure 5.5 Serum markers (pg/ml) significantly associated with clinically significant social symptoms as defined by the PBC-40 social domain A) KIM-1, B) AZU1, C) VIM

5.1.4.6 Serum proteomics and general symptoms

A total of 214 out 289 (74%) of participants were classified as having "none/mild" symptoms in the PBC-40 general symptoms domain, whilst 67 out of 289 (23%) of participants had "moderate" symptoms. The number of participants considered to have "severe" symptoms was small, with only 8/289 (3%) in this category. When applying the pre-defined score of 18 or more to stratify by clinical significance, 99/289 (34%) participants had general symptoms that were considered clinically significant.

When comparing "none/mild", "moderate" and "severe" participants, only 2 out of the 19 markers, KIM-1 (P=0.020) and leptin (P=0.013), showed statistically significant differences, with higher serum levels being associated with severe symptoms. With post-hoc correction for multiple testing, pairwise comparison showed statistical significance for KIM-1 (none/mild vs. moderate, P = 0.023) and leptin (none/mild vs. moderate, P = 0.028), but not between the severe symptom groups.

No serum proteomic markers were statistically significantly different between the "none/mild" and "severe" categories.

When analysing according to clinically significant symptoms: KIM-1 (P=0.005) and leptin (P<0.001), and CCL20 (P=0.004) were significantly elevated in those with clinically significant symptoms. **See Table** 5.13**.**

Including the covariates age and weight for KIM-1 showed continued statistically significant levels in those with clinically significant symptoms (KIM-1: F = 11.750, P = 0.001). Leptin remained significantly elevated when corrected for by weight in clinically significant general symptoms (leptin: F = 10.390, P = 0.001). **See Figure** 5.6**.**

Table 5.13 Serum markers (pg/ml) by PBC40 general symptoms domain severity (none/mild vs. severe) and general symptoms score deemed clinically significant in Study 1

Marker	General Symptoms Severity					Clinically* Significant General Symptoms?		
	None/Mild (n=214) Median (IQR) pg/ml	**Moderate (n=67)** Median (IQR) pg/ml	**Severe (n=8)** Median (IQR) pg/ml	**Significance none/mild vs. moderate vs. severe**	**Significance none/mild vs. severe**	**No (n=190)** Median (IQR) pg/ml	**Yes (n=99)** Median (IQR) pg/ml	**Significance**
IL-4Rα	3.12 (1.08)	3.16 (1.58)	3.25 (1.64)	P = .601 $\chi^2(2) = 1.019$	P = .343 U = 1025	3.11 (1.03)	3.22 (1.49)	P = .236 U = 10204
DECR1	4.13 (1.04)	4.18 (1.04)	4.23 (1.23)	P = .626 $\chi^2(2) = 0.938$	P = .470 U = 985	4.13 (1.02)	4.34 (1.08)	P = .130 U = 10426
KIM-1	8.40 (1.17)	8.72 (1.55)	8.83 (1.75)	P = .020 $\chi^2(2) = 7.862$	P = .262 U = 1056	8.34 (1.09)	8.71 (1.41)	P = .005 U = 11279
ACE2	4.67 (1.46)	4.64 (2.38)	5.17 (2.65)	P = .356 $\chi^2(2) = 2.067$	P = .150 U = 1113	4.67 (1.46)	4.64 (1.83)	P = .635 U = 9725
CA5A	3.71 (1.64)	3.63 (1.92)	4.44 (1.75)	P = .518 $\chi^2(2) = 1.315$	P = .265 U =1055	3.69 (1.69)	3.73 (1.75)	P = .323 U = 10072
HAOX1	5.80 (2.48)	5.49 (3.12)	6.62 (4.09)	P = .941 $\chi^2(2) = 0.122$	P = .801 U = 811	5.77 (2.45)	5.69 (3.05)	P = .882 U = 9505
AZU1	6.57 (1.60)	6.41 (1.59)	6.23 (1.36)	P = .962 $\chi^2(2) = 0.078$	P = .805 U = 812	6.47 (1.58)	6.52 (1.59)	P = .528 U = 9830
EP-CAM	4.93 (0.61)	5.02 (0.91)	5.08 (0.62)	P = .577 $\chi^2(2) = 1.099$	P = .404 U = 1005	4.91 (0.60)	5.03 (0.83)	P = .119 U = 10457
Leptin	6.38 (1.36)	6.91 (1.35)	7.27 (2.64)	P = .013 $\chi^2(2) = 8.631$	P = .127 U =1128	6.34 (1.48)	6.91 (1.30)	P < .001 U = 12084
CXCL11	9.03 (1.37)	8.98 (1.09)	8.41 (1.14)	P = .129 $\chi^2(2) = 4.098$	P = .057 U = 516	9.01 (1.40)	9.07 (1.19)	P = .694 U = 9140
CXCL9	8.97 (1.29)	9.07 (1.06)	8.64 (1.20)	P = .486 $\chi^2(2) = 1.443$	P = .253 U = 652	8.92 (1.28)	9.07 (1.15)	P = .799 U = 9233
CCL19	9.89 (1.04)	9.98 (1.57)	10.09 (2.52)	P = .919 $\chi^2(2) = 0.170$	P = .920 U = 874	9.88 (1.06)	9.98 (1.58)	P = .481 U = 9880
IL-18R1	7.64 (0.85)	7.73 (1.15)	7.50 (1.19)	P = .600 $\chi^2(2) = 1.022$	P = .998 U = 856	7.64 (0.84)	7.75 (1.12)	P = .122 U = 10447
CXCL10	9.04 (1.18)	9.05 (1.01)	8.73 (1.07)	P = .443 $\chi^2(2) = 1.629$	P = .272 U = 660	9.00 (1.27)	9.12 (0.99)	P = .710 U = 9154
CCL20	5.32 (1.46)	5.70 (1.68)	5.81 (2.74)	P = .058 $\chi^2(2) = 5.680$	P = .594 U = 951	5.35 (1.49)	5.63 (1.65)	P = .004 U = 10761
SCAMP3	3.23 (1.31)	3.22 (1.59)	2.89 (0.30)	P = .762 $\chi^2(2) = .543$	P = .501 U = 736	3.22 (1.31)	3.17 (1.49)	P = .684 U = 9679
VIM	4.64 (1.10)	4.60 (0.82)	4.60 (0.76)	P = .853 $\chi^2(2) = .317$	P = .766 U = 803	4.63 (1.09)	4.64 (0.90)	P = .753 U = 9617
CXCL13	8.92 (0.71)	8.92 (0.86)	9.78 (1.17)	P = .756 $\chi^2(2) = 0.559$	P = .537 U = 746	8.97 (0.72)	8.85 (0.78)	P = .243 U = 8617
CD163	8.31 (0.75)	8.25 (0.91)	8.17 (0.81)	P = .951 $\chi^2(2) = 0.100$	P = .964 U =848	8.31 (0.75)	8.25 (0.91)	P = .563 U = 9795

*A score of >= 18 in the general symptom domain of the PBC-40 considered at clinically significant; IQR – Interquartile Range; n=number of cases in each group; test for statistical significance using Mann-Whitney U and Kruskal-Wallis; P < 0.05 considered statistically significant (2-sided test); **red** denotes statistically significance

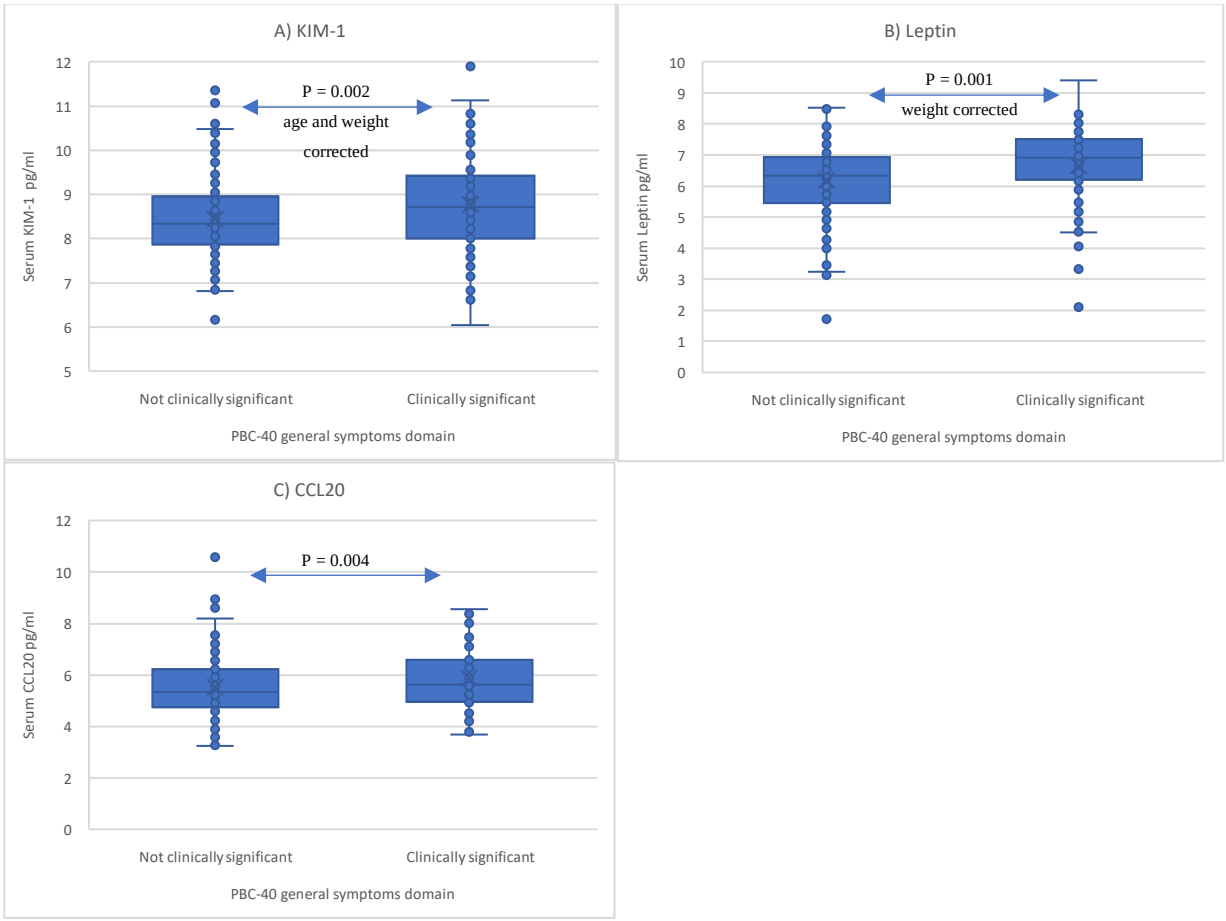


Figure 5.6 Serum markers (pg/ml) significantly associated with clinically significant general symptoms as defined by the PBC-40 general domain A) KIM-1, B) Leptin, C) CCL20

5.1.4.7 Serum proteomics and symptoms – summary

Following correction for multiple testing using FDR, several markers remained statistically significant for both comparisons according to "none/mild" vs "severe" symptoms and clinically significant symptoms. They are summarised in **Table** 5.14 and **Table** 5.15**.**

Notably, KIM-1 continued to show significance in both assessment methods for the cognitive, fatigue and itch domains, even when correcting for age, weight, and multiple testing. It was also significant for social symptoms in the "none/mild" vs. "severe" groupings, but not clinically significant symptoms, whilst for general symptoms it showed the opposite finding, i.e., was significant for clinically significant general symptoms.

Many of IL-4Rα's associations were considered false positives following correction for multiple testing. However, it continued to show significance for itch in the "none/mild" vs. "severe" group comparison. IL-18R1 and CD163 continued to remain significant for itch in the none/mild" vs. "severe" group comparisons, whilst leptin remained significant for clinically significant itch and clinically significant general symptoms.

Table 5.14 Summary table of statistical significance for median serum markers (pg/ml) by PBC-40 domain, split by symptom severity ("none/mild" vs "severe") for Study 1

Marker	PBC-40 Domain						
	Symptom Severity	**Cognition** Median (IQR) pg/ml	**Fatigue** Median (IQR) pg/ml	**Itch** Median (IQR) pg/ml	**Emotional** Median (IQR) pg/ml	**Social** Median (IQR) pg/ml	**General symptoms** Median (IQR) pg/ml
IL-4Rα	None/mild	3.08 (1.03)	3.08 (1.08)	3.05 (1.11)	3.09 (1.04)	3.09 (1.08)	3.12 (1.08)
	Severe	3.72 (1.69)	3.38 (1.30)	3.40 (1.40)	3.56 (1.61)	3.69 (1.53)	3.25 (1.64)
	Significance	P=.026	P = .018	P = .008	P = .024	P = .014	P = .343
DECR1	None/mild	4.11 (0.97)	4.12 (1.04)	4.15 (1.06)	4.16 (1.01)	4.12 (0.99)	4.13 (1.04)
	Severe	4.46 (1.24)	4.22 (1.22)	4.17 (1.22)	4.36 (1.39)	4.36 (1.67)	4.23 (1.23)
	Significance (age adjust.)	P=.006 P=.081	P = .266	P = .516	P = .108	P = .134	P = .470
KIM-1	None/mild	8.47 (1.09)	8.36 (1.07)	8.41 (1.15)	8.41 (1.20)	8.42 (1.13)	8.40 (1.17)
	Severe	8.96 (1.55)	8.73 (1.55)	8.87 (1.22)	8.96 (1.62)	9.56 (1.70)	8.83 (1.75)
	Significance (age/weight adjust)	P=.031 P=.002	P=.015 P= .001	P = .005 P = .003	P = .036 P = .006	P = .002 P = .001	P = .262
ACE2	None/mild	4.65 (1.37)	4.64 (1.35)	4.56 (1.43)	4.68 (1.38)	4.64 (1.37)	4.67 (1.46)
	Severe	5.33 (2.18)	4.87 (2.04)	5.25 (2.03)	5.50 (1.96)	5.60 (2.20)	5.17 (2.65)
	Significance	P=.057	P = .180	P = .044	P = .005	P = .032	P = .150
CA5A	None/mild	3.59 (1.63)	3.67 (1.78)	3.66 (1.71)	3.62 (1.70)	3.67 (1.73)	3.71 (1.64)
	Severe	4.13 (1.66)	3.91 (1.76)	4.10 (1.70)	4.15 (1.61)	4.20 (2.24)	4.44 (1.75)
	Significance	P=.072	P = .303	P = .120	P = .037	P = .116	P = .265
HAOX1	None/mild	5.58 (2.46)	5.73 (2.30)	5.68 (2.54)	5.69 (2.49)	5.73 (2.54)	5.80 (2.48)
	Severe	7.23 (3.21)	6.06 (3.22)	6.26 (3.64)	6.93 (2.83)	7.14 (3.27)	6.62 (4.09)
	Significance (age adjust.)	P=.021 P=.450	P = .239	P = .379	P = .012 P = .083	P = .051	P = .801
AZU1	None/mild	6.58 (1.54)	6.58 (1.50)	6.48 (1.58)	6.58 (1.59)	6.58 (1.55)	6.57 (1.60)
	Severe	6.24 (1.78)	6.31 (1.71)	6.25 (1.85)	6.18 (1.43)	6.06 (1.22)	6.23 (1.36)
	Significance	P=.798	P = .933	P = .709	P = .187	P = .195	P = .805
EP-CAM	None/mild	4.92 (0.65)	4.90 (0.68)	4.87 (0.62)	4.91 (0.64)	4.89 (0.64)	4.93 (0.61)
	Severe	5.04 (1.04)	5.03 (0.76)	5.20 (0.84)	5.01 (0.87)	5.19 (0.94)	5.08 (0.62)
	Significance	P=.572	P = .173	P = .023	P = .190	P = .066	P = .404
Leptin	None/mild	6.45 (1.43)	6.23 (1.52)	6.36 (1.36)	6.41 (1.38)	6.34 (1.38)	6.38 (1.36)
	Severe	6.90 (1.97)	6.90 (1.16)	6.93 (2.08)	6.69 (1.69)	6.69 (2.14)	7.27 (2.64)
	Significance (weight adjust.)	P=.101	P < .001 P = .065	P = .066	P = .360	P = .440	P = .127
CXCL11	None/mild	8.98 (1.23)	8.96 (1.26)	8.03 (1.35)	9.03 (1.37)	9.02 (1.31)	9.03 (1.37)
	Severe	9.28 (1.48)	9.08 (1.37)	9.08 (1.87)	9.00 (1.15)	8.73 (0.86)	8.41 (1.14)
	Significance	P=.462	P = .591	P = .986	P = .684	P = .408	P = .057
CXCL9	None/mild	8.90 (1.21)	8.92 (1.27)	8.93 (.125)	8.92 (1.30)	8.91 (1.25)	8.97 (1.29)
	Severe	9.18 (1.11)	9.13 (1.25)	9.08 (0.94)	8.80 (1.31)	8.73 (1.26)	8.64 (1.20)
	Significance	P=.685	P = .742	P = .560	P = .403	P = .440	P = .253
CCL19	None/mild	9.85 (1.08)	9.85 (1.06)	9.85 (1.13)	9.91 (1.11)	9.88 (1.05)	9.89 (1.04)
	Severe	10.50 (1.16)	10.1 (1.17)	10.07 (1.32)	10.04 (1.22)	10.45 (1.26)	10.09 (2.52)
	Significance weight	P= .010 P= .015	P = .046 P = .097	P = .121	P = .079	P = .023	P = .920
IL-18R1	None/mild	7.60 (0.86)	7.61 (0.80)	7.59 (0.89)	7.64 (0.85)	7.64 (0.85)	7.64 (0.85)
	Severe	7.90 (0.98)	7.81 (0.94)	7.78 (0.97)	7.88 (1.17)	7.90 (1.16)	7.50 (1.19)
	Significance (age adjust.)	P=.013 P=.051	P=.040 P= .062	P = .019 P = .005	P = .009 P = .033	P = .020 P = .085	P = .998
CXCL10	None/mild	8.97 (1.09)	9.23 (0.96)	9.01 (1.14)	8.97 (1.20)	8.98 (1.16)	9.04 (1.18)
	Severe	9.41 (0.81)	5.36 (1.51)	9.23 (1.07)	9.13 (0.10)	8.97 (1.04)	8.73 (1.07)
	Significance	P=.088	P = .162	P = .313	P = .527	P = .784	P = .272
CCL20	None/mild	5.34 (1.44)	5.36 (1.51)	5.35 (1.50)	5.38 (1.43)	5.38 (1.45)	5.32 (1.46)
	Severe	5.99 (1.72)	5.91 (1.67)	5.47 (1.86)	5.50 (1.65)	5.99 (1.15)	5.81 (2.74)
	Significance	P=.053	P = .008	P = .349	P = .399	P = .124	P = .594
SCAMP3	None/mild	3.19 (1.20)	3.22 (1.27)	3.18 (1.31)	3.23 (1.23)	3.21 (1.22)	3.23 (1.31)
	Severe	3.15 (1.14)	3.39 (1.35)	2.96 (1.35)	4.00 (1.78)	3.17 (1.49)	2.89 (0.30)
	Significance	P=.346	P = .164	P = .740	P = .301	P = .721	P = .501
VIM	None/mild	4.66 (1.07)	4.64 (1.14)	4.63 (1.05)	4.69 (1.11)	4.69 (1.01)	4.64 (1.10)
	Severe	4.46 (0.58)	4.46 (0.85)	4.46 (0.68)	4.43 (0.45)	4.46 (0.63)	4.60 (0.76)
	Significance	P=.434	P = .805	P = .892	P = .080	P = .182	P = .766
CXCL13	None/mild	8.91 (0.76)	8.89 (0.76)	8.89 (0.73)	8.94 (0.71)	8.90 (0.77)	8.92 (0.71)
	Severe	8.92 (0.77)	8.99 (0.78)	8.94 (0.66)	8.79 (1.01)	8.72 (0.94)	9.78 (1.17)
	Significance	P=.915	P = .264	P = .930	P = .880	P = .432	P = .537
CD163	None/mild	8.26 (0.77)	8.26 (0.78)	8.21 (0.79)	8.25 (0.78)	8.26 (0.78)	8.31 (0.75)
	Severe	8.48 (0.75)	8.38 (0.76)	8.61 (0.62)	8.39 (0.86)	8.14 (0.86)	8.17 (0.81)
	Significance	P=.227	P = .093	P = .002	P = .018	P = .381	P = .964

IQR – Interquartile Range; test for statistical significance using Mann-Whitney U; P < 0.05 considered statistically significant (2-sided test); **red** denotes statistically significance; orange denotes non-significant following correction for false detection rate.

Table 5.15 Summary table of statistical significance for median serum markers (pg/ml) by PBC-40 domain, split by clinically significant symptoms for Study 1

Marker	PBC-40 Domain						
	Clinically significant symptoms	**Cognition** Median (IQR) pg/ml	**Fatigue** Median (IQR) pg/ml	**Itch** Median (IQR) pg/ml	**Emotional** Median (IQR) pg/ml	**Social** Median (IQR) pg/ml	**General symptoms** Median (IQR) pg/ml
IL-4Rα	Not c/s	3.10 (1.03)	3.08 (0.98)	3.09 (1.01)	3.11 (1.20)	3.10 (1.08)	3.11 (1.03)
	c/s	3.42 (1.38)	3.30 (1.34)	3.27 (1.30)	3.56 (1.61)	3.31 (1.54)	3.22 (1.49)
	Significance	P=.023	P=.020	P=.032	P=.041	P=.096	P=.236
DECR1	Not c/s	4.14 (0.97)	4.13 (1.01)	4.13 (1.09)	4.14 (0.99)	4.15 (0.99)	4.13 (1.02)
	c/s	4.32 (1.30)	4.18 (1.11)	4.18 (1.01)	4.36 (1.39)	4.16 (1.15)	4.34 (1.08)
	Significance	P=.053	P=.226	P=.310	P=.106	P=.657	P=.130
KIM-1	Not c/s	8.45 (1.14)	8.41 (1.08)	8.38 (1.15)	8.46 (1.21)	8.41 (1.19)	8.34 (1.09)
	c/s	8.96 (1.47)	8.71 (1.36)	8.60 (1.29)	8.96 (1.62)	8.69 (1.45)	8.71 (1.41)
	Significance (age/weight adjust)	P=.007 P=.001	P=.004 P=.001	P=.032 P =.003	P=.050	P=.003 P=.001	P = .005 P = .001
ACE2	Not c/s	4.64 (1.39)	4.64 (1.42)	4.62 (1.36)	4.60 (1.55)	4.60 (1.40)	4.67 (1.46)
	c/s	4.88 (2.18)	4.76 (1.82)	4.73 (1.82)	5.50 (1.96)	4.87 (2.22)	4.64 (1.83)
	Significance	P=.056	P=.082	P=.450	P=.005	P=.091	P=.635
CA5A	Not c/s	3.62 (1.64)	3.67 (1.68)	3.78 (1.79)	3.66 (1.64)	3.66 (1.65)	3.69 (1.69)
	c/s	4.10 (1.96)	3.73 (1.80)	3.66 (1.71)	4.15 (1.61)	3.83 (1.84)	3.73 (1.75)
	Significance	P=.204	P=.423	P=.442	P=.050	P=.317	P=.323
HAOX1	Not c/s	5.67 (2.43)	5.72 (2.49)	5.67 (2.36)	5.69 (2.61)	5.74 (2.53)	5.77 (2.45)
	c/s	6.60 (3.12)	5.86 (2.91)	5.89 (2.94)	6.93 (2.83)	5.75 (3.03)	5.69 (3.05)
	Significance (age adjust)	P=.018 P=.156	P=.496	P=.267	P=.010	P=.593	P=.882
AZU1	Not c/s	6.59 (1.56)	6.58 (1.55)	6.45 (1.72)	6.54 (1.58)	6.5 (1.46)	6.47 (1.58)
	c/s	6.16 (1.60)	6.37 (1.68)	6.52 (1.49)	6.18 (1.43)	6.12 (1.54)	6.52 (1.59)
	Significance	P=.140	P=.342	P=.614	P=.201	P=.014	P=.528
EP-CAM	Not c/s	4.93 (0.63)	4.87 (0.65)	4.91 (0.63)	4.94 (0.63)	4.93 (0.64)	4.91 (0.60)
	c/s	5.06 (0.83)	5.07 (0.70)	5.01 (0.72)	5.01 (0.87)	5.04 (0.68)	5.03 (0.83)
	Significance	P=.126	P=.013	P=.159	P=.244	P=.216	P=.119
Leptin	Not c/s	6.46 (1.40)	6.25 (1.42)	6.26 (1.47)	6.48 (1.44)	6.44 (1.40)	6.34 (1.48)
	c/s	6.61 (1.48)	6.89 (1.49)	6.76 (1.39)	6.69 (1.69)	6.90 (1.45)	6.91 (1.30)
	Significance (weight adjust.)	P=.353	P< .001 P= .074	P<.001 P=.001	P=.521	P = .032 P = .232	P < .001 P = .001
CXCL11	Not c/s	9.02 (1.27)	9.01 (1.30)	9.02 (1.40)	9.03 (1.36)	9.03 (1.39)	9.01 (1.40)
	c/s	9.27 (1.36)	9.09 (1.34)	9.05 (1.27)	9.00 (1.15)	9.00 (1.25)	9.07 (1.19)
	Significance	P=.490	P=.646	P=.788	P=.825	P=.861	P=.694
CXCL9	Not c/s	8.94 (1.20)	8.92 (1.23)	8.92 (1.20)	9.01 (1.20)	8.92 (1.27)	8.92 (1.28)
	c/s	9.05 (1.36)	9.09 (1.19)	9.05 (1.28)	8.80 (1.31)	9.09 (1.11)	9.07 (1.15)
	Significance	P=.825	P=.673	P=.980	P=.369	P=.799	P=.799
CCL19	Not c/s	9.88 (1.11)	9.85 (0.97)	9.82 (1.13)	9.88 (1.19)	9.88 (1.04)	9.88 (1.06)
	c/s	10.09 (1.52)	10.10 (1.64)	10.10 (1.34)	10.04 (1.22)	10.07 (1.66)	9.98 (1.58)
	Significance weight	P=.170	P=.137	P = .038 P = .053	P = .068	P=.198	P=.481
IL-18R1	Not c/s	7.62 (0.85)	7.62 (0.77)	7.58 (0.78)	7.64 (0.93)	7.64 (0.87)	7.64 (0.84)
	c/s	7.90 (1.05)	7.76 (0.98)	7.75 (0.95)	7.89 (1.17)	7.71 (1.01)	7.75 (1.12)
	Significance (age adjust)	P=.013 P=.106	P=.027 P=.129	P=.015 P=.022	P = .011 P = .039	P=.191	P=.122
CXCL10	Not c/s	8.99 (1.11)	8.97 (1.13)	8.99 (1.16)	9.01 (1.17)	9.00 (1.19)	9.00 (1.27)
	c/s	9.22 (1.34)	9.19 (1.08)	9.09 (1.07)	9.13 (1.00)	9.13 (1.01)	9.12 (0.99)
	Significance	P=.437	P=.235	P=.632	P=.624	P=.650	P=.710
CCL20	Not c/s	5.38 (1.44)	5.33 (1.33)	5.35 (1.42)	5.47 (1.46)	5.42 (1.41)	5.35 (1.49)
	c/s	5.67 (1.55)	5.78 (1.93)	5.66 (1.65)	5.50 (1.65)	5.57 (1.86)	5.63 (1.65)
	Significance	P=.175	P=.022	P=.027	P=.593	P=.161	P=.004
SCAMP3	Not c/s	3.21 (1.30)	3.18 (1.23)	3.15 (1.28)	3.23 (1.36)	3.23 (1.30)	3.22 (1.31)
	c/s	3.15 (1.57)	3.30 (1.55)	3.22 (1.41)	3.00 (1.78)	3.05 (1.58)	3.17 (1.49)
	Significance	P=.791	P=.257	P=.348	P=.248	P=.452	P=.684
VIM	Not c/s	4.66 (1.04)	4.65 (1.08)	4.62 (1.02)	4.67 (1.08)	4.71 (0.99)	4.63 (1.09)
	c/s	4.60 (0.85)	4.61 (0.90)	4.71 (0.96)	4.43 (0.45)	4.44 (0.99)	4.64 (0.90)
	Significance	P=.371	P=.812	P=.140	P=.081	P=.021	P=.753
CXCL13	Not c/s	8.92 (0.75)	8.90 (0.76)	8.90 (0.70)	8.93 (0.74)	8.90 (0.75)	8.97 (0.72)
	c/s	8.92 (0.74)	8.99 (0.73)	8.94 (0.79)	8.79 (1.02)	9.01 (0.74)	8.85 (0.78)
	Significance	P=.622	P=.403	P=.843	P=.896	P=.223	P=.243
CD163	Not c/s	8.26 (0.78)	8.23 (0.78)	8.24 (0.76)	8.26 (0.80)	9.23 (0.78)	8.31 (0.75)
	c/s	8.33 (0.76)	8.35 (0.78)	8.32 (0.82)	8.39 (0.86)	8.34 (0.87)	8.25 (0.91)
	Significance	P=.154	P=.045	P=.201	P=.026	P=.205	P=.563

IQR – Interquartile Range; test for statistical significance using Mann-Whitney U; P < 0.05 considered statistically significant (2-sided test); **red** denotes statistically significance; orange denotes non-significant following correction for false detection rate.

5.2 Study 1 (exploratory study): Discussion

Our exploratory study, using the UK-PBC nested cohort, was representative of the general PBC population, with a median age of 65 years and 86% of participants being female. The average BMI was 26.6, which is slightly below the UK national average of 28.2 for this age group (digital, 2019). Data was available for UDCA treatment, with all participants having been treated with UDCA at 13-15mg/kg/day for at least 1 year.

Within the PBC cohort, 29% of participants were considered POISE non-responders to first-line therapy, which is lower than expected in the general PBC population, where up to 40% of patients may have an inadequate response (Corpechot *et al.*, 2008). Cirrhosis was confirmed in 7% of participants, as in seen in the original proteomics cohort study (Jones *et al.*, 2022).

Of the 19 serum markers explored in study 1, 16 markers were statistically significantly different between responders and non-responders to UDCA. SCAMP3 and VIM showed no significant difference according to POISE response status, which is mirrored in the proteomics study exploring UDCA response and disease status (Jones *et al.*, 2022). CXCL13, which was significant in the proteomics study, was not significant in our study when assessing using POISE response but it continued to be significantly elevated in those with abnormal liver blood tests.

Given that study 1 was a sub-cohort from the UK-PBC nested cohort (289 out of 400 participants), and therefore self-selected based on the availability of symptom data, it is reassuring that many of the main characteristics of the cohort were replicated, despite the cohort size being reduced by 28%.

5.2.1 Clinical characteristics and symptoms

In terms of the relationship between PBC-40 domains and clinical characteristics, non-responders to UDCA were more likely to experience severe itch, but this was not the case in those with any (and typically mild) degrees of LFT abnormality. This is in keeping with large cohort studies where more severe pruritus is associated with lack of response to UDCA (which may be a consequence of more aggressive ductopenic disease (Hegade *et al.*, 2019)), but does not show a direct relationship to degree of abnormality of LFTs or duration of diagnosis (Carbone *et al.*, 2013b). No other symptoms were associated with POISE response status or liver blood tests, in keeping with major cohort studies exploring symptomatic PBC (Jacoby *et al.*, 2005; Wetten, Jones and Dyson, 2021). Those with cirrhosis were more likely to be classified as having severe symptoms in the emotional, fatigue and social PBC-40 domains. Fatigue is a well-known phenomenon of cirrhosis (Swain, 2006; Swain and Jones, 2019), whilst

there is an increased prevalence of depression in cirrhotic patients (Abureesh *et al.*, 2022; Askgaard *et al.*, 2023) and an association with increased social isolation (Vaughn-Sandler *et al.*, 2014; Askgaard *et al.*, 2023).

Increased severity of symptoms in 4 of the PBC-40 domains (cognition, fatigue, emotional and social) were associated with younger age. More severe cognitive symptoms in particular were associated with a younger age, with the median age of those with severe symptoms being 53 years, compared to 67 in those with none or mild symptoms. This is in keeping with previous PBC cohort studies (Mells *et al.*, 2013; Rice *et al.*, 2020; Newton *et al.*, 2008a; Carbone *et al.*, 2013b). Similarly, severe fatigue has been associated with a younger age (Mells *et al.*, 2013; Rice *et al.*, 2020; Al-Harthy *et al.*, 2010; Carbone *et al.*, 2013b) and while direct data between age and emotional and/or social symptoms in PBC are lacking, both depressive symptoms and social isolation have been strongly linked to fatigue, which is in turn associated with a younger age (Shaheen *et al.*, 2018; Al-Harthy *et al.*, 2010; Mells *et al.*, 2013; Newton *et al.*, 2007b).

Typically, younger age is associated with increased severity of pruritus (Hegade *et al.*, 2019), but perhaps surprisingly, there was no correlation between age and pruritus in this cohort and the reason for this is not readily apparent. However, information on pruritus treatment was not available and given that participants were recruited via the UK-PBC network, it may be that a greater proportion of patients were recruited in specialist PBC centres with more experience in managing PBC, where the clinical assessment and treatment of pruritus is part of routine clinical practice. However, no data on the site of recruitment was available. In addition, only 29% of participants reported moderate to severe itch, which is fewer than expected in the PBC population from previous studies (Hegade *et al.*, 2015; Hegade *et al.*, 2014) but this may be due to effective itch treatment being delivered.

When serum analytes were assessed against age, 8 analytes showed an association with age, with KIM-1 and CXCL9 showing positive association with age, and DECR1, HAOX1, IL-18R1, CA5A, CXCL11 and CCL19 showing a negative association with age, i.e., lower levels with increasing age. Increased CXCL9 and CXCL10 (no correlation for CXCL10 was apparent here) have been associated with increasing age in some disease-specific cohort studies (de Araújo *et al.*, 2020; Bonfante *et al.*, 2017; Koper *et al.*, 2018), but do not appear to change significantly with age in the general population (Koper *et al.*, 2018; Samson *et al.*, 2022). HAOX1 does show considerable variability in humans, with many influences, including age, being a potential factor (Pryde *et al.*, 2010). CXCL11 has been demonstrated to be increased with increasing age (rather than decreased as seen here) in those with rheumatoid arthritis (Chen *et al.*, 2022) but not the general population (Samson *et al.*, 2022). KIM-1, DECR1, and

CCL19 do not appear to change with age (Stamova *et al.*, 2009; Kwok *et al.*, 2022) and when corrected for multiple testing, CCL19 was not considered significant. There was no previous data describing an association of age with IL-18R1 and CA5A. It is difficult to account for these variances with age, but whilst age may play a factor in these analytes in this cohort, it is also within the context of PBC, where response status and degree of liver blood test abnormality results in changes in expression of these analytes. In the context of this thesis, we have not explored further the impact of age as a covariate on analyte expression and disease severity, but this is worthy of further exploration in future studies.

5.2.2 Groupings

Three methods were consistently used to define and group participants for the analyses (see full description **in section 3.9).** In summary, the three methods to compare symptoms to analytes were:

1) none/mild versus moderate versus severe symptom groups
2) none/mild versus severe symptom groups (moderate group excluded)
3) According to pre-defined cut-offs for clinically significant symptoms. That is clinically significant symptoms versus not significant.

Due to these groupings, methods 1 and 3 resulted in all participants being included in the analyses, whilst method 2 excluded the moderate group (meaning that between 59 to 88 of the cohort were excluded depending on the PBC-40 symptom domain explored). Interestingly, using methods 2 (none/mild vs. severe) and 3 (clinically significant symptoms) generally resulted in more consistent common statistical associations between analytes, than using method 1 (all 3 groups). Pairwise analysis (undertaken with correction for multiple comparisons using Bonferroni) showed that statistical associations in all PBC-40 domains were largely between the none/mild and severe groups, with only some significant associations occurring between none/mild and moderate groups, mostly observed with Leptin (fatigue, itch, social, general symptoms domains). There were no associations between those who were considered to have moderate symptoms compared to those with severe symptoms.

This finding may be due to the very nature of the moderate group, which includes those who are both close to the none/mild or severe end of the symptom spectrum that could result in "dilution" of any signal seen due to the heterogeneity of symptoms within the moderate group. In contrast, none/mild and severe groupings consist of the two extreme groups and therefore provides a more homogenous symptom set that may give a clearer signal if one exists. Largely, the cut-offs for clinically significant symptoms (defined based on standard deviations from the mean in population studies) for each domain fall within the mid-range of the moderate groups. Whilst effectively splitting the cohort in half results in a more heterogenous group than using

none/mild and severe groupings, this split should produce two groups which are overall more phenotypically different to each other.

Given these considerations, the discussion will focus on the results from the none/mild vs. severe and clinically significant symptom groupings.

5.2.3 Cognitive symptoms

Cognitive symptoms were less prevalent in study 1's PBC population, with 23% and 21% reporting moderate and severe cognitive symptoms respectively, compared to 34% and 19% in the general population (Newton *et al.*, 2008a). This was also apparent when using the predefined clinically significant symptom threshold (18 or more in the PBC-40 cognitive domain) which resulted in 21% of the study population being deemed to have clinically significant symptoms, rather than the expected 28% (Mells *et al.*, 2013). Whilst the magnitude of these differences is not large, the reduced prevalence and resultant unbalanced groups (i.e., smaller numbers in the severe group) may influence our findings.

Three proteomic analytes showed consistent positive associations across cognitive symptoms, irrespective of the method used to categorise symptom severity. These were IL-4Rα, HAOX1 and IL-18R1. The associations for HAOX1 and IL-18R1, however, disappeared when adjusted for age. DECR1 and CCL19 were both statistically significantly elevated when comparing those with "none/mild" and "severe" symptoms, but the DECR1 association disappeared when adjusted for age. KIM-1 showed a strong relationship in those with clinically significant cognitive symptoms, and when adjusted for age and weight this remained statistically significant.

IL-4Rα had not previously shown a relationship to age and therefore was not adjusted for age.

Overall IL-4Rα, KIM-1 and CCL19 showed a continued statistically significant association with cognitive symptoms. However, when adjusted for multiple testing, only KIM-1 was considered likely to be a true positive result, with IL-4Rα and CLL9 considered to be false positives. **See Table** 5.14 **and Table** 5.15**.** Their relevance is discussed below.

5.2.4 Fatigue

The reported rate of fatigue in this study was in keeping with that seen in the general PBC population, with 47% of participants reporting moderate to severe fatigue (Mells *et al.*, 2013). However, only 34% of the cohort were considered to have clinically significant fatigue, which is below the expected prevalence of 45% when using a clinical cut-off score of 33 or more in

the PBC-40 fatigue domain (Mells *et al.*, 2013). This reduced prevalence in this unselected cohort could have influenced our findings.

Statistically significant associations were initially seen for IL-4Rα, KIM-1, leptin, IL-18R1 and CCL20 between the none/mild and severe groups, and those with clinically significant fatigue. KIM-1, CLL20 and leptin also showed association when comparing the non/mild, moderate, and severe groups. However, on pairwise analysis only leptin showed a significant relationship between all three groupings, whilst KIM-1 and CLL20 were only significant between "none/mild" and "severe" groups, but not the "moderate" group, and as discussed previously this may be due the "moderate" group diversity.

Including weight as a covariate for leptin resulted in a loss of significance. Given its strong association with body mass index in both this study (**see Table** 5.7) and the literature, this is not unexpected. However, it should be noted that the use of weight or BMI is not a perfect way to adjust for adipose tissue (the main producer of leptin) and the loss of significance may be a consequence of an imperfect approximation of adipose mass. Piche et al demonstrated a strong association with fatigue and PBC for leptin even after correcting for adipose tissue by measuring fat mass using electrical impedance (Piche, 2002). Given this limitation of the study (and its other positive associations), a more in-depth discussion of leptin has been provided. **See section 5.2.9.3**

Age was associated with KIM-1 and IL-18R1, so we included age as a covariate for these analytes. This resulted in a loss of statistical association for IL-18R1, whilst KIM-1 became more statistically significant. As discussed in **section 5.2.1,** KIM-1 does not appear to change with age in other studies, but a correlation was seen here. It is difficult to account for these differences, but it may be that there are other confounding factors involved. KIM-1 did have a positive association with higher levels seen with increasing age. Generally, symptoms show an inverse relationship with age in PBC so the increase in significance of KIM-1 with fatigue severity after inclusion of age as a covariate suggests a strong relationship between fatigue and KIM-1. KIM-1 is further discussed in **section 5.2.9.1**

Finally, when correcting for multiple testing using FDR, only KIM-1 was considered a true positive, whilst IL-4Rα, EP-CAM, and CCL20 were considered false positives. This does not mean that these analytes are not truly associated with fatigue but based on the balance of probabilities these are likely to be positive by chance.

5.2.5 Itch

Severe itch was present in 10% of the cohort, whilst moderate itch was present in 21%. Typically, a third of patients report persistent itch to varying degrees, with 15% reporting severe itch (Hegade *et al.*, 2015; Hegade *et al.*, 2014). This means that fewer patients in our cohort experienced severe itch as described in other PBC studies. This is difficult to interpret given that no information was available on current treatments for itch, however, as discussed in **section 5.2.1**, it maybe that the majority of patients were recruited from specialist hepatology centres, where it is likely that the assessment of itch and its subsequent treatment had been optimised during the year that UDCA was established, and this might account for the lower numbers in the severe category. This resulted in an imbalance of groups (200 vs 30), and this may increase the risk of spurious findings.

The pre-defined clinically significant cut-off of 7 or more, which straddles the upper end of the mild boundary (4-8), is based on +/-standard deviations from the mean from a control population (as discussed in **section 3.4.5**). This resulted in 49% of participants having itch that is considered clinically significant, whilst the expected prevalence in the PBC population using this cut-off would be 38%. This is still compatible with the theory that specialists' centres are more likely to manage itch effectively (hencefewer patients with severe itch), but typically with patients there is a crossover point where mild itch is less burdensome than the therapies used to treat itch. For example, bile acid sequestrants can be highly effective but unpalatable (Sinha *et al.*, 1998). Overall, clinically significant itch was more prevalent in this PBC cohort, resulting in two well balanced groups.

Overall, four analytes (IL-4Rα, KIM-1, IL-18R1 and CD163) remained significant for itch when comparing none/mild to severe itch, even after correcting for FDR. Three of these, IL-4Rα, KIM-1 and IL-18R1 were significant when stratifying by clinically significant itch, but only KIM-1 remained significant when correcting for FDR, whilst leptin became significant, even correcting for weight and FDR.

These results are potentially encouraging, given that the different grouping methods results in quite markedly different groups, that is, using none/mild compared to severe compares 69% to 10% of the cohort respectively, whilst using clinically significant symptoms compares 51% (not significant) to 49% (significant), yet despite this, a persistent signal for elevated IL-4Rα, KIM-1 and IL-18R1 remains. These markers are discussed in **section 5.2.9.1, 5.2.9.2 and 5.2.9.7**

ACE2, EP-CAM, and CCL20 demonstrated a positive association when stratified by clinically significant symptoms, but not when comparing the none/mild and severe groups. Based on correcting for multiple testing, these analytes were considered false positives. ACE2, and CCL20 are discussed further given their association in other domains.

5.2.6 Emotional

The majority of patients in our cohort had none or mild emotional symptoms (67%), 24% reported moderate emotional symptoms and 9% severe emotional symptoms. The bar for clinically significant symptoms for the emotional domain is set quite high, matching the threshold for the severe symptom category. In the general PBC population this would be expected to affect around 19% of patients so emotional symptoms would appear to be slightly under-represented in our cohort. The high number of patients falling into the none/mild category compared to severe resulted in unequal groups, which was further exacerbated when assessed using clinically significant cut-offs (which included none/mild and moderate vs. severe, i.e., n =263, vs. n = 26), this can lead to unequal variance between samples and can affect statistical testing.

It is difficult to draw direct comparisons from previous PBC cohort studies on symptoms of depression and anxiety and our data using the emotional domain. The emotional domain is not a specific questionnaire to assess symptoms of depression and/or anxiety such as the HADS. It is comprised of only three questions: "because of PBC I get more stressed about things than I used to", "having PBC gets me down" and "I worry about how my PBC will be in the future" (**see appendix 1)** and therefore broadly highlights patients that may be more likely to have anxiety or depression. Unfortunately, more detailed data on depression and anxiety from the validated self-reporting questionnaires such as HADS were not available in this cohort.

There were 6 analytes that were statistically significant for the emotional domain using none/mild vs severe (IL-4Rα, KIM-1, ACE2, CA5A, IL-18RA and CD163). However, following correction for multiple testing with FDR, none were considered significant. This is also true when assessing for clinically significant symptoms, with IL-4Rα, ACE, IL-18R1, and CD163 not considered as significant following correction for FDR (this is due to how the Benjamini-Hochberg method effectively ranks P values). Given the limited scope of the emotional domain in assessing mood, and therefore the difficulty in plausibly associating a biological process to this, this is potentially reassuring.

5.2.7 Social

The effect of PBC on participants ability to socialise appeared to be limited in this study. The majority of participants met the none/mild criteria (66%), with only 17 participants (7%) meeting the severe criteria. From a statistical point of view this again resulted in unequal groupings. Splitting the group by those considered as clinically significant social symptoms, resulted in 24% of participants being considered as having social symptoms that are clinically significant, and this is similar to that expected in the general PBC population (25%) (Mells *et al.*, 2013).

Interestingly, IL-4Rα, KIM-1 and ACE2 were statistically significant when comparing none/mild to severe groups, but only KIM-1 remained significant when adjusting for multiple testing. Given the imbalance in the groups, and the risk of increased unequal variance, these results should be interpreted with caution. When explored using the clinically significant cut-off, which utilises larger group sizes (220 and 69 participants for clinical significance, compared to 195 and 17 for none/mild vs. severe groups), KIM-1, AZU1 and VIM had a p value less than 0.05, but were considered false positives when adjusted for FDR. In the context of the larger group sizes using the clinically significant symptoms this is likely to be a more reliable method and the results related to this are likely to be more plausible.

The social domain in the PBC-40 recognises the impact of PBC on the ability of patients to carry out normal social functioning, with statements such as “my social life has almost stopped” and “PBC has reduced the quality of my life”. Such impacts may be an indirect consequence of PBC and its associated symptoms such as fatigue, rather than a direct a consequence of a biological process. As such, it might be expected that no analytes would have shown a positive association. However, our analyses are limited in that they do not consider the complex interplay of fatigue, cognition, and itch with social functioning. It is well established that fatigue in PBC significantly impairs patients QOL (Poupon *et al.*, 2004) (Mells *et al.*, 2013). It is likely in this cohort that those with a high symptom burden in one or more domains (cognition, fatigue, itch) will also score more highly in the social domain. This may explain why some analytes show a positive association in the social domain, reflecting part of a biological process that has resulted in a symptom that has led to social isolation.

5.2.8 General symptoms

In the general symptoms domain only 8 participants (3%) had severe symptoms compared to 214 who had none or mild symptoms, which resulted in unequal group sizes that may have affected our results. No analytes showed a positive association when comparing these two groups but given the small numbers in the severe group this should be interpreted with caution.

When stratified by clinical significance, 34% had general symptoms that were clinically significant, which is lower than that expected for the general population of 40% (Mells *et al.*, 2013). KIM-1, leptin and CCL20 all showed a positive association with symptoms, even when corrected for age or weight and following correction for multiple testing.

The general symptoms domain encompasses a range of symptoms, with statements about gastrointestinal (GI) symptoms, sicca syndrome, right upper quadrant discomfort and bone pain. It is difficult to identify a common biological phenomenon that would link all these symptoms. These analyses did not explore if individual symptoms, i.e. sicca syndrome, were associated with specific analytes and this would be worthy of consideration, as for example, GI symptoms could potentially be associated with one analyte, such as leptin. Not including this in our study is a limitation.

5.2.9 Biomarkers

From the 19 original analytes, 6 were selected to be included in the validation study, these are discussed individually.

5.2.9.1 KIM-1

KIM-1 showed a strong positive association across several symptom domains, including cognitive, fatigue and itch, even when adjusting for age as a covariate and correcting for multiple testing. The fact that this analyte spans several domains may suggest that this marker is not a direct cause for symptoms, given its apparent non-specificity. Fatigue and cognitive symptoms are often interlinked, but there is a not strong association between fatigue, cognitive symptoms, and itch. This may suggest that there are confounding factors.

KIM-1, also known as T-cell immunoglobulin and mucin domain-1, is an important immune regulator for Th2 (Rodriguez-Manzanet *et al.*, 2009). Its activation may occur through multiple ligands (Rodriguez-Manzanet *et al.*, 2009) and it has been associated with other autoimmune diseases including rheumatoid arthritis (Chae *et al.*, 2005), asthma (Chae *et al.*, 2003) and systemic lupus erythematosus (Wang *et al.*, 2008b). In PBC, impaired KIM-1 expression on B-cells has been associated with promotion of a proinflammatory state (Chen *et al.*, 2020). KIM-1 activation is associated with a potent costimulatory effect for T cell activation and proliferation, with resultant production of IL-4 and IL-13 (Curtiss and Colgan, 2007). There has been no research on the role of KIM-1 and symptom expression in disease, but given its mechanism of action, current association with autoimmune disease and positive findings in study 1, KIM-1 was selected for further study in study 2 (validation study).

5.2.9.2 IL-4Rα

Like KIM-1, IL-4Rα showed positive associations across several PBC domains, including cognition, fatigue, and itch. Following correction for multiple testing, many of these associations were considered likely false positives, with the exception being in the itch domain.

IL-4Rα is the common subunit for IL-4R type 1 and type 2 receptors, with both receptors playing a pivotal role with the type-2 immune response (Egholm *et al.*, 2019). This is discussed further in **section 2.3.1.1**. Key points from this are that these receptors are activated by either IL-4 or IL13. These have distinct and overlapping roles, including the formation of macrophage and eosinophil-rich granulomas (Van Dyken and Locksley, 2013). Ductal granulomas are a frequent, and hallmark, histological appearance in PBC (Lewis, 2018), with eosinophilic infiltration of the portal tracts observed in around a third of PBC liver biopsies (Terasaki *et al.*, 1993).

Dysregulation of the IL-4, IL-13 and IL-4Rα pathways are apparent in immune mediated disease, including rheumatoid arthritis (Iwaszko, Biały and Bogunia-Kubik, 2021), ulcerative colitis (Kasaian *et al.*, 2014; Roda *et al.*, 2011) and asthma (Shirakawa *et al.*, 2000). Several therapies that target and block IL-4Rα pathways have proven effective in disease control and are described in section 5.3.3.1 (Harb and Chatila, 2020; Dhillon, 2017; Sandborn *et al.*, 2017; Berekmeri *et al.*, 2018).

Given IL-4Rα's observed potential association with itch, its activation by IL-4 and IL-13 (both of which are also upregulated following co-stimulation of T-cells by KIM-1 (Curtiss and Colgan, 2007)) and the availability of novel therapies to target these pathways, IL-4Rα was included in the validation study, along with IL-4 and IL-13.

5.2.9.3 Leptin

Levels of leptin are promoted with increasing fat mass (Kelesidis *et al.*, 2010) and typically follow a circadian rhythm (Licinio *et al.*, 1997). Data on the timings of serum samples were not available although weight (kg) was available for all PBC patients in study 1.

Changes in serum leptin were observed in our study according to POISE responder status and those with abnormal liver blood tests, with lower levels associated with non-response and those with abnormal liver biochemistry, even when weight adjusted. This is in keeping with the underpinning proteomics PBC studies (Barron‐Millar *et al.*, 2021; Jones *et al.*, 2022). Results relating to serum leptin and responder status/abnormal liver blood tests were not published by

Piche et al who explored the association of PBC, fatigue and serum leptin, but previous studies have failed to show a difference in serum leptin according to the level of abnormality of liver blood tests (Rieger *et al.*, 2005).

Initially leptin appeared to show a strong positive association for fatigue. However, this disappeared after including weight as a covariate. We were, therefore, unable to replicate the results of Piche et al who observed increased serum leptin levels in PBC and CHC patients who scored highly on the physical domain of the FIS (even when adjusted for fat mass (Piche, 2002)). As discussed in **section 5.2.4** a limitation to our study is the use of weight as an approximation of individual fat mass.

Leptin did show a positive association with itch but only when assessed using the clinically significant symptoms. No studies have previously explored itch and serum leptin in PBC. However, one study found that pregnant women with intra-hepatic cholestasis (ICP) and itch had significant differences in serum leptin (along with other adipokines including ghrelin) compared to controls, with lower leptin observed with worsening ICP (Yurtcu *et al.*, 2022). This study should be interpreted with caution as the authors have not reported the relationship between serum adipokines and fat mass, or any potential adjustments for this. Studies related to PBC and leptin, along with its relationship to immunity, are discussed in **section 2.3.3.1**

There are limited data on the role of leptin in itch in PBC but given its association with immune regulation, energy homeostasis and previous findings in PBC, leptin was selected to be explored in study 2 (validation), along with its opposing adipokine, ghrelin.

5.2.9.4 CD163

CD163 is primarily a haemoglobin scavenger receptor (Weaver *et al.*, 2006), and is upregulated following macrophage activation and release of proinflammatory cytokines (Buechler *et al.*, 2000). It is associated with a spectrum of liver disease by activated Kupffer cells (Grønbæk *et al.*, 2012). In PBC it has been associated with elevated ALP and non-response to UDCA (Bossen *et al.*, 2020). See **section 2.3.4.3** for more details.

In study 1, CD163 showed a positive association with itch when assessed by none/mild and severe groups, which persisted when corrected for multiple testing. It also suggested an association with fatigue, but this was considered likely a false positive. However, given that itch is associated with more aggressive ductopenic disease (Vleggaar *et al.*, 2001) and given the potential association of elevated CD163 with non-response to UDCA (more common in

aggressive ductopenic disease (Kumagi *et al.*, 2010b)), CD163 was selected to be explored further in the validation study.

5.2.9.5 CCL20

CCR6-CCL20 has been implicated in the pathogenesis of PBC, particularly in advancing disease (Shi *et al.*, 2015) and CCL20 is recognised as a genetic risk locus for the development of PBC (Cordell *et al.*, 2015a). Genetic upregulation of CCL20, and subsequent overexpression, has been linked to severe and persistent fatigue following breast cancer survival (Bower *et al.*, 2011). CCL20 has found to be a potential contributor to inflammatory myopathies (Dalakas, 2004).

In study 1, CCL20 showed a positive association with fatigue when assessed by none/mild and severe groups, or clinically significant findings, although with correction for multiple testing, these results were considered false positives. However, we felt that this was worthy of further exploration, and given the association of upregulation of CCL20 with TNFα (Iwamoto *et al.*, 2013; Schutyser, Struyf and Van Damme, 2003), TNFα was included. IL-6 was also included in the validation study given its interlinked role with TNFα and role in fatigue. **See section 1.4.2.1, 2.3.1.2 and 2.3.1.4**

5.2.9.6 CXCL9

CXCL9 demonstrated no positive association in study 1. However, CXCL9 was selected for inclusion in the validation study (study 2) due to its strong association with the pathophysiology of PBC (**see section 2.3.2.1**). It required very little additional resource (serum and cost) to include.

5.2.9.7 Other biomarkers

Other biomarkers that would have been worthy of further exploration include IL-18R1 and ACE2. Unfortunately, it was not logistically possible to measure these with resources available.

5.2.10 Proteomic validation

To further validate the observed associations between proteomes and symptoms, proteome mapping, a process encompassing the exploration of proteome expression, localisation, and interactions, could have been implemented. This approach has the potential to elucidate the strength of the relationship between specific proteomes and symptom domains, thereby providing additional support to our findings if significant correlations were demonstrated. This was beyond the scope of this thesis.

Chapter 6: Study 2 validation proteomics: Results

6.1 Study 2 (validation study): Results

A total of 200 serum samples were successfully analysed: 160 patients with a probable/definite diagnosis of PBC from the BANC, RITPBC and UK-PBC cohorts and 40 healthy controls from the E-AILD cohort.

6.1.1 Study 2 (validation study): Demographics and clinical characteristics

The median age between the whole PBC cohort and healthy control group was not significantly different (58 vs. 60 years; P = 0.070). However, within the individual PBC sub-cohorts there was a statistically significant difference between the 3 PBC cohorts: RITPBC 55 years, in comparison to 59 for both BANC and UK-PBC (P < .015). This is not unexpected, given the nature of the individual cohorts i.e. BANC and UK-PBC are both cohort studies whose only inclusion criterion is a probable or definite diagnosis of PBC, whilst the RITPBC study specifically recruited participants with severe fatigue, which is typically associated with younger age. The median age between male and females between the PBC subgroups is not included given the small numbers of males in each sub-group (mirroring classical PBC epidemiology). The gender split was similar to that seen in the general PBC population, with the control group having 36 out 40 (90%) females, whilst the PBC group was comprised of 153 females out of 160 (95%). **See Table** 6.1

Table 6.1 Median age in healthy control and PBC groups, with median age in each PBC sub-cohort in Study 2

Group	**Gender**	**Number (%)**	**Age* (IQR)**	**Significance across ages**
Healthy Controls	Total	40	60 (16)	P = .070; U = 2606
	Female	36 (90)		
	Male	4 (10)		
PBC	Total	160	58 (12)	
	Female	153 (95)		
	Male	7 (5)		
PBC Subgroup				
BANC (n=73)	Total	73	59 (14)	P = .015; $\chi^2(2) = 8.39$
	Female	69 (94)		
	Male	4 (6)		
RITPBC (n=57)	Total	57	55 (12)	
	Female	55 (96)		
	Male	2 (4)		
UK-PBC (n=30)	Total	30	59 (13)	
	Female	29 (96)		
	Male	1 (4)		

Median (Interquartile Range);* test for statistical significance either Mann-Whitney U or Kruskal-Wallis; P < 0.05 considered statistically significant (2-sided test); **red denotes statistically significance

The clinical characteristics of all the PBC participants and according to the individual cohorts are described in **Table** 6.2**.** BMI data was not available for the BANC cohort due to height not being part of the standard data collection in this study. In all the cohorts, around a 1/4 of the participants did not have a dose recorded for UDCA. Data on cirrhosis status was not available for RITPBC, however, all those recruited to the study required a Child-Pugh score of fewer than 7 to be included in the trial.

Table 6.2 Clinical characteristics of the whole PBC study population and according to subgroup

	All PBC (n=160)	**PBC Subgroup**		
		BANC (n=73)	**RITPBC (n=57)**	**UK-PBC (n=30)**
Median weight, kg (IQR)	72.9 (23.2)	71 (29)	70 (19.8)	76 (16.8)
Median BMI, kg/m^2(IQR)	28.4 (6.9)	No data	27 (7.5)	29 (6.6)
UDCA treated, Number (%)	Y = 125 (78) N = 24 (15) Unknown = 11 (7)	Y = 71 (98) N = 1 (1) Unknown = 1 (1)	Y = 44 (77) N = 13 (23)	Y= 10 (33) N = 10 (33) Unknown = 10 (33)
UDCA therapeutic, Number (%)	Y = 65 (41) N = 53 (33) Unknown = 42 (26)	Y = 25 (34) N = 29 (40) Unknown = 19 (26)	Y = 30 (53) N = 14 (25) Unknown = 13 (22)	Y = 10 (33) N = 10 (33) Unknown = 10 (33)
Median ALP (IQR) IU/L	136.5 (130)	134 (93)	134 (146)	174 (141)
Median ALT (IQR) IU/L	34 (34)	28 (28)	36 (34)	37.5 (40)
Median Bilirubin (IQR) umol/L	7 (5)	7 (5)	6 (4)	9 (8)
Median Albumin (IQR) g/L	45 (4)	45 (4)	44 (2)	40 (7)
Normal LFT (%)	62 (39)	32 (44)	22 (39)	8 (26)
POISE Responder, number (%)	113 (70)	56 (77)	38 (67)	19 (63)
Cirrhosis present number (%) n = 103	13 (14)	6 (8)	No data	8 (26)

IQR – interquartile Range; BMI – body mass index; UDCA – ursodeoxycholic acid; ALP - alkaline phosphatase; ALT – alanine transaminase; LFT – liver function tests; n=number of cases in each group

We first compared all serum proteomic markers between healthy controls and all PBC patients, demonstrating a statistically significant elevation for all proteomics in the PBC group, apart from leptin, IL-13, and ghrelin (**See Table** 6.3). The significant findings persisted when correcting for multiple testing with FDR. Leptin was previously significant in the UK-PBC discovery proteomics work (Barron‐Millar *et al.*, 2021), whilst IL-13 was not previously significant, and ghrelin was not explored.

Table 6.3 Median serum markers (pg/ml) by Healthy controls vs. PBC group in Study 2

Marker	**Group**		**Significance**
	Controls (n=40) Median (IQR) pg/ml	PBC (n=160) Median (IQR) pg/ml	
IL-4R	879.9 (113.9)	1031.6 (198.7)	P < .001 U = 4888
CD163	367919.8 (197467.5)	499943.3 (282984)	P < .001 U = 4734
KIM-1	49.4 (31.3)	75.7 (75.2)	P < .001 U = 4996
CXCL9	607.7 (437.6)	1239 (866.1)	P < .001 U = 5416
CCL20	9.5 (10.8)	17.1 (19.6)	P < .001 U = 4318
IL-13	0.21 (0.39)	0.21 (0.91)	P = .312 U = 3495
IL-4	0.08 (0.0)	0.08 (0.10)	P = .033 U = 3818
IL-6	0.98 (0.71)	1.52 (0.94)	P < .001 U = 4439
Leptin	25097 (19250)	25109 (34883)	P = .954 U = 3219
TNF-α	1.01 (0.61)	1.50 (1.34)	P < .001 U = 4370
Ghrelin	**(n=40)** 439.5 (633.3)	**(n=152)** 356.5	P = .607 U = 2879

IQR – Interquartile Range; n=number of cases in each group; test for statistical significance using Mann-Whitney U; P < 0.05 considered statistically significant (2-sided test)

Exploring serum proteomic markers within the individual PBC cohorts demonstrated no significant differences between 8 out of the 11 analytes. However, there were significant differences between the 3 PBC cohorts for CXCL9 (P = .002), TNF-α (P < .001) and ghrelin (P < .001). The most striking difference was observed in the RITPBC group, with the median serum level for ghrelin being 79.4pg/ml, compared to 538pg/ml and 617pg/ml in the BANC and UK-PBC groups, respectively (P < 0.001). Similarly, TNF-α was significantly lower in the RITPBC group (1.02pg/ml versus 1.74pg/ml in BANC and 1.70pg/ml in UK-PBC; P < 0.001). CXCL9 was significantly higher in the RITPBC group (1516pg/ml compared to 1135pg/ml in BANC and 1024pg/ml in UK-PBC; P = 0.002). **See Table** 6.4**.** This is of particular interest given that the RITPBC group is predominantly a cohort with a high symptom burden (especially fatigue).

Table 6.4 Median serum markers (pg/ml) by PBC subgroups in Study 2

Marker	**PBC subgroups**			**Significance**
	BANC (n=73) median (IQR) pg/ml	**RITPBC (n=57) median (IQR) pg/ml**	**UK-PBC (n=30) median (IQR) pg/ml**	
IL-4R	1030 (210)	1013 (198)	1075 (188)	P = .636 $\chi^2(2) = .905$
CD163	525281 (3277758)	478404 (248266)	445633 (298629)	P = .441 $\chi^2(2) = 1.636$
KIM-1	76.7 (68.8)	73.5 (81.1)	77.0 (118.1)	P = .557 $\chi^2(2) = 1.172$
CXCL9	1136 (855)	1516 (744)	1024 (744)	P = .002 $\chi^2(2) = 12.894$
CCL20	15.2 (20.4)	15.7 (18.7)	18.6 (21.5)	P = .598 $\chi^2(2) = 1.027$
IL-13	0.21 (0.86)	0.21 (0.78)	0.21 (0.13)	P = .803 $\chi^2(2) = .439$
IL-4	0.08 (0.13)	0.08 (0.06)	0.08 (0.12)	P = .499 $\chi^2(2) = 1.389$
IL-6	1.49 (0.92)	1.53 (1.22)	1.52 (0.85)	P = .673 $\chi^2(2) = .792$
Leptin	31820 (36529)	21672 (29815)	23414 (29523)	P = .154 $\chi^2(2) = 3.741$
TNF-α	1.74 (1.02)	1.02 (0.94)	1.70 (2.31)	P < .001 $\chi^2(2) = 22.119$
Ghrelin	**(n=73)** 538.5 (750.7)	**(n=55)** 79.4 (156.0)	**(n=24)** 617.4 (675.5)	P < .001 $\chi^2(2) = 63.927$

IQR – Interquartile Range; n=number of cases in each group; test for statistical significance using Kruskal-Wallis; P < 0.05 considered statistically significant (2-sided test)

There were no significant differences in serum proteomic markers between males and females across healthy controls and the PBC cohorts (**See Table** 6.5), Given the low total numbers of males, gender differences were not explored between in the individual cohorts.

Table 6.5 Median serum markers (pg/ml) by gender for healthy controls and PBC

Marker	**Healthy and PBC cohort**		**Significance**
	Female (n=189) Median (IQR) pg/ml	Male (n=11) Median (IQR) pg/ml	
IL-4R	1012.9 (214.6)	922.7 (213.6)	P = .221 U = 811
CD163	439071.3 (2578723.1)	449754.2 (306004.2)	P = .572 U = 1145
KIM-1	69.3 (55.9)	83.8 (143.4)	P = .381 U = 1203
CXCL9	1088.0 (839.9)	1136.8 (1150.4)	P = .547 U = 1152
CCL20	15.2 (17.1)	10.0 (32.1)	P = .557 U = 930
IL-13	0.21 (0.85)	0.21 (0.31)	P = .442 U = 912
IL-4	0.08 (0.90)	0.08 (0.00)	P = .100 U = 767
IL-6	1.39 (0.96)	1.47 (0.84)	P = .694 U = 1113
Leptin	25484.0 (33052.4)	14488.0 (25840.0)	P = .141 U = 765
TNF-α	1.36 (1.18)	1.43 (0.46)	P = .594 U = 1139
Ghrelin	**(n=181)** 375.1 (672.3)	**(n=11)** 410.8 (669.4)	P = .765 U = 942

IQR – Interquartile Range; n=number of cases in each group; test for statistical significance using Mann-Whitney U; P < 0.05 considered statistically significant (2-sided test)

As with the exploratory cohort (Study 1), many of the symptoms associated with PBC are strongly and significantly associated with a younger age. In particular, younger people report more severe symptoms in the cognitive, fatigue and emotional domains as assessed with the PBC-40, and this is in keeping with previous large PBC cohort studies (Mells *et al.*, 2013). **See Table** 6.6 **and Figure** 6.1**.** A younger age was significantly associated with higher scores in the general symptom domain. However, younger patients tended to report moderate rather than severe symptoms. It should be noted that the number of patients reporting severe general symptoms was small (n=8).

Table 6.6 PBC-40 domain severity by age (in years) in Study 2

PBC-40 Domain	Severity	Number	Age (years) [IQR]	Significance
Cognition	**None/mild**	60	60 [12]	P < .001 X(1) = 20.307
	Moderate	57	57 [11]	
	Severe	41	50 [13]	
Fatigue	**None/mild**	32	60 [9]	P = .003 X(1) = 11.382
	Moderate	52	58 [14]	
	Severe	76	54.5 [12]	
Itch	**None/mild**	128	58 [12]	P = .155 X(1) = 3.728
	Moderate	18	57.5 [16]	
	Severe	14	52 [13]	
Emotional	**None/mild**	56	60 [10]	P < .001 X(1) = 17.634
	Moderate	51	55 [14]	
	Severe	51	54 [12]	
Social	**None/mild**	83	59 [12]	P = .087 X(1) = 4.873
	Moderate	65	55 [15]	
	Severe	10	57 [10]	
General Symptoms	**None/mild**	85	59 [12]	P < .001 X(1) = 13.598
	Moderate	66	53 [13]	
	Severe	8	57 [8]	

n=number of cases in each group; analysis using Chi-square; P < 0.05 considered statistically significant (2-sided test); **red** denotes statistically significance

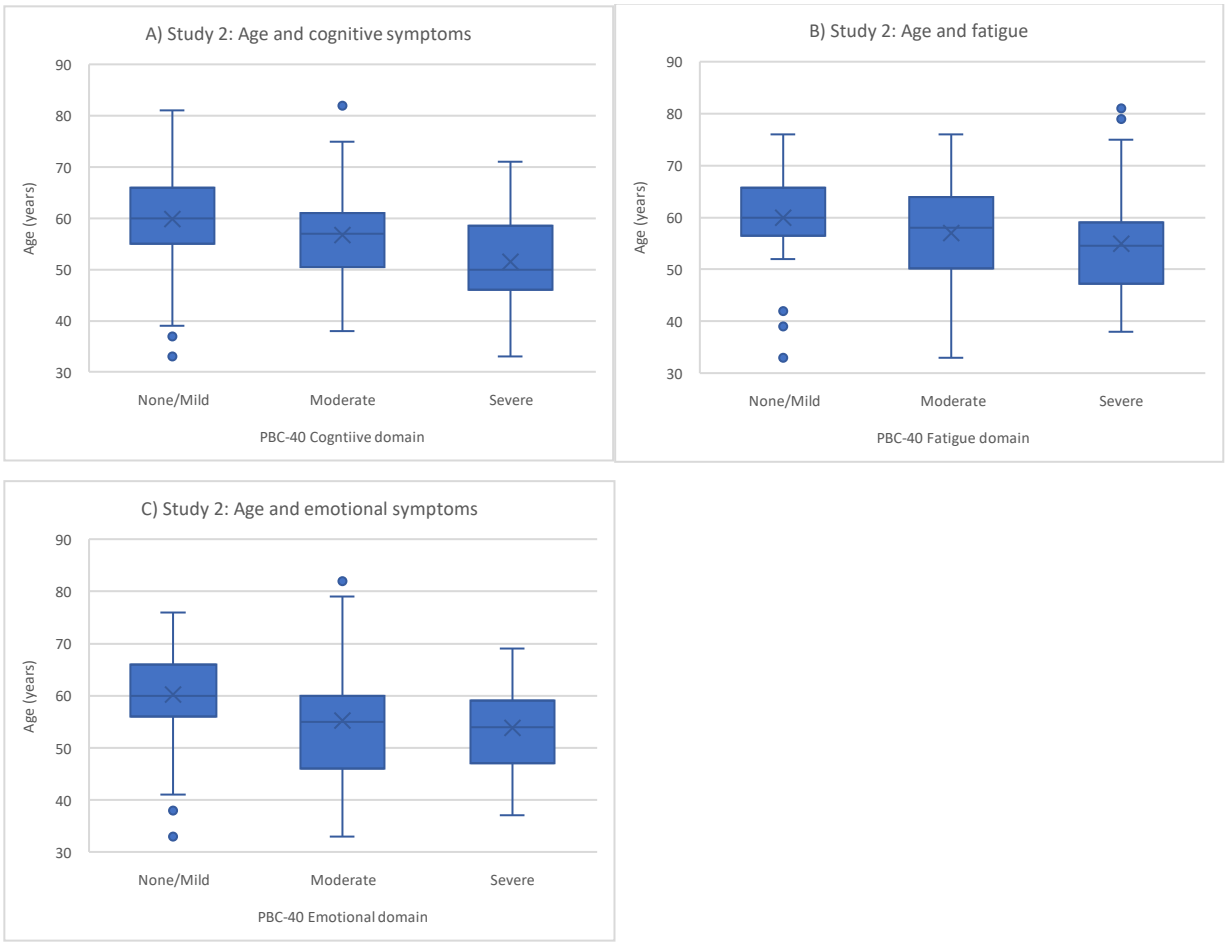


Figure 6.1 PBC-40 domain severity by age (in years) in Study 2 A) Cognitive domain, B) fatigue domain, C) emotional domain

Only 3 analytes showed a significant correlation to age. IL-4 and IL-6 showed a negative correlation, i.e. higher levels with younger ages, whilst ghrelin showed a positive correlation (**see Table** 6.7**).**

Table 6.7 Serum proteomics correlated by age (in years) in Study 2

Marker	**Correlation coefficient**	**Significance**
IL-4R	-.006	P = .937
CD163	.121	P = .089
KIM-1	.095	P = .180
CXCL9	-.066	P = .355
CCL20	.018	P = .803
IL-13	-.126	P = .076
IL-4*	-.164	P = .020
IL-6*	-.165	P = .020
Leptin	-.027	P = .709
TNF-α	-.057	P = .422
Ghrelin*	.179	P = .013

analysis using Spearmen-rho; *P < .05 considered statistically significant (2-sided test); ** P < .01 considered statistically significant (2-sided test); **red** denotes statistical significance; orange denotes considered false positive on FDR.

Given the role of ghrelin and leptin in energy metabolism, and particularly given their influence by adipose tissue and therefore body weight, correlation of body weight with all the serum analytes was performed. Only leptin, ghrelin and CCL20 were associated with body weight,

with higher levels of ghrelin observed in lower body weights, whilst leptin and CCL20 showed the opposite effect, **see Table** 6.8

Table 6.8 Serum proteomics correlated by weight in Study 2

Marker	Correlation coefficient	Significance
IL-4R	-.009	P = .873
CD163	.055	P = .307
KIM-1	-.001	P = .989
CXCL9	-.035	P = .513
CCL20	.109	P = .042
IL-13	.029	P = .616
IL-4	-.002	P = .972
IL-6	.081	P = .130
Leptin	4.64	P < .001
TNF-α	.075	P = .162
Ghrelin**	-2.32	P = .004

analysis using Spearmen-rho; *P < .05 considered statistically significant (2-sided test); ** P < .01 considered statistically significant (2-sided test); **red** denotes statistical significance; orange denotes considered false positive on FDR.

6.1.2 Study 2 (validation study): Clinical status and symptoms

When exploring the effect of biochemical responder status on the serum proteome, using the previously described POISE criteria, 113/160 (71%) of PBC participants were POISE responders and therefore at low risk of disease progression. There was a strong positive association between response status, with higher levels of IL-4R, CD163, KIM-1, CXCL9 and CCL20 in non-responders, whilst leptin was significantly lower in comparison to responders. This mirrors the findings in our exploratory work in Study 1 (**see section 5.1.2)**. IL-13, IL-4, IL-6, TNF-α and ghrelin were not significantly different between responders and non-responders, and this is in keeping with the published data on the inflammatory proteome in PBC. **See table 5.7.**

For abnormal liver function tests (LFT), the same profile of markers that were elevated in non-responders, showed significant differences between those with normal and abnormal LFT, with increased IL-4R, CD163, KIM-1, CXCL9 and CCL20 in the abnormal LFT group, and leptin being significantly lower (the same as in Study 1). In addition, though, when using the more stringent analysis of normal versus abnormal LFT, IL-6 and TNF-α also showed a significant association with abnormal LFT, but this disappeared when correcting for multiple testing. Finally, in those with cirrhosis, IL-4R, and KIM-1 were elevated, in keeping with Study 1, although CD163 failed to show a significant difference according to cirrhosis status, unlike in Study 1. **See Table** 6.9**.**

Table 6.9. Median serum markers (pg/ml) by biochemical response status (POISE criteria) and normal versus abnormal liver function tests (LFT)

Marker	**PBC Group Responder status**			**PBC Group Abnormal LFT**			**Cirrhosis**		
	Responder (n=113) Median (IQR)	Non-responder (n=47) Median (IQR)	Significance	Normal LFT (n=62) Median (IQR)	Abnormal LFT (n=98) Median (IQR)	Significance	Non-cirrhotic (n=89)	Cirrhotic (n=14)	Significance
IL-4R	991.1 (170)	1118 (156.6)	P <.001 U = 3971	955.2 (157.5)	1083.9 (177.9)	P <.001 U = 4322.5	1027 (184)	1166 (373)	P = .003 U = 936
CD163	438001 (242482)	563956 (258832)	P =.006 U = 3392	417894 (241826)	541508 (268248)	P =.019 U = 3706	496988 (288664)	604873 (354971)	P = .070 U = 811
KIM-1	63.6 (44)	157.9 (134.5)	P <.001 U = 4416	60.9 (29.6)	91.8 (111.6)	P <.001 U = 4434	59.6 (75.2)	205.9 (174.8)	P = .004 U = 919
CXCL9	1156 (731.8)	1573 (1164)	P =.011 U = 3334	1090 (704)	1436 (902)	P =.004 U = 3852	1058 (779)	1251 (1360)	P = .408 U = 709
CCL20	15.2 (14.5)	20.8 (35.3)	P <.001 U = 3544	14.9 (14.9)	18.0 (22.5)	P = .016 U = 3728	17.0 (19.4)	24.4 (29.4)	P = .413 U = 708
IL-13	0.21 (0.97)	0.21 (0.90)	P = .532 U = 2505.5	0.21 (0.72)	0.21 (0.95)	P = .699 U = 3137.5	0.21 (1.16)	0.21 (0.08)	P = .125 U = 481
IL-4	0.08 (0.12)	0.08 (0.08)	P = .719 U = 2568	0.08 (0.13)	0.08 (0.92)	P = .945 U = 3020	0.09 (0.13)	0.08 (0.11)	P = .488 U = 556
IL-6	1.51 (0.88)	1.52 (0.99)	P = .540 U = 2819	1.35 (0.70)	1.63 (1.07)	P = .042 U = 3618	1.42 (0.86)	1.71 (0.91)	P = .027 U = 853
Leptin	28101 (36085)	21192 (31125)	P = .041 U = 2111	35341 (34594)	20624 (29866)	P = .005 U = 2244	31339 (36529)	21234 (32266)	P = .985 U = 621
TNF-α	1.49 (1.14)	1.55 (1.57)	P = .295 U = 2935	1.40 (1.13)	1.58 (1.39)	P = .041 U = 3622	1.74 (1.18)	1.68 (2.54)	P = .931 U = 632
Ghrelin^	**(n=107)** 375.1 (691.6)	**(n=45)** 291.3 (599.4)	P = .286 U = 2143	**(n=58)** 319.3 (695.9)	**(n=94)** 385.9 (675.5)	P = .997 U = 2725	**(n=58)** 634 (790)	**(n=58)** 477 (471)	P = .278 U = 411

IQR – Interquartile Range; n=number of cases in each group; test for statistical significance using Mann-Whitney U; P < 0.05 considered statistically significant (2-sided test); **red** denotes statistically significance; orange denotes considered false positive on FDR

6.1.3 Study 2 (validation study): Serum proteomics and symptoms

After establishing the relationship between serum proteomics, age, symptoms and disease characteristics, the relationship between serum proteomics and the individual PBC-40 domains was explored.

6.1.3.1 Study 2 (validation study): cognitive symptoms

Assessment using the PBC-40 cognitive domain showed 62/160 participants (39%) with "none/mild" symptoms, 57 (36%) with moderate and 41 (25%) with severe. When split according to the pre-defined clinically significant cut-off for cognitive symptoms (a PBC-40 score of greater or equal to 18, mid-way in the moderate score), 86/160 (54%) of participants were classified as having clinically significant cognitive symptoms, which is higher than the expected 28% in the general PBC population. **See and Table** 3.2

Of the 11 serum markers explored, only 3 reached statistical significance when stratified by the PBC-40 cognitive domain. CXCL9 and IL-6 were elevated in those experiencing high levels of cognitive symptoms, (CXCL9 severe: 1471pg/ml vs. none/mild: 1025pg/ml, P = 0.002; IL-6 severe: 1.72pg/ml vs. none: 1.37pg/ml, P = 0.006), whilst ghrelin was lower in those with more severe symptoms (Ghrelin severe: 276pg/ml vs. none/mild 527pg/ml, P = 0.003).

Using pairwise comparison with Bonferroni and correcting for multiple testing, the only statistically significant associations were CXCL9 (none/mild vs. severe, P = 0.004), IL-6 (none/mild vs. severe, P = 0.014, moderate vs. severe, P = 0.047) and ghrelin (none/mild vs. severe, P = 0.07, none/mild vs. moderate, P = 0.001).

CXCL9 and ghrelin both maintained their association with cognitive symptoms when stratified by clinical significance of symptoms, but IL-6 failed to maintain an association, whilst IL-4 showed a weak positive association. **See Table** 6.10

Table 6.10 Serum markers (pg/ml) by cognitive domain severity as defined by PBC-40 (none/mild vs. severe) and by cognitive score deemed clinically significant in Study 2

Marker	**Cognitive Severity**					**Clinically* Significant Cognitive Symptoms?**		
	None/Mild (n=62) Median (IQR) pg/ml	**Moderate (n=57)** Median (IQR) pg/ml	**Severe (n=41)** Median (IQR) pg/ml	**Significance none/mild vs. moderate vs. severe**	**Significance none/mild vs. severe**	**No (n=74)** Median (IQR) pg/ml	**Yes (n=86)** Median (IQR) pg/ml	**Significance**
IL-4R	1026 (191)	1039 (223)	1021 (181)	P = .990 $\chi^2(2) = 0.021$	P =.956 U = 1222	1035 (215)	1030 (187)	P = .566 U = 3014
CD163	533491 (316127)	500282 (287692)	452653 (213056)	P = .400 $\chi^2(2) = 1.831$	P =.194 U = 1041	529741 (330760)	457441 (235127)	P = .269 U = 2859
KIM-1	74.6 (71.6)	76.0 (78.0)	80.3 (113.2)	P = .884 $\chi^2(2) = 0.246$	P =.633 U = 1299	73.4 (69.9)	81.5 (81.6)	P = .479 U = 3389
CXCL9	1025 (769)	1339 (802)	1471 (917)	P = .004 $\chi^2(2) = 11.088$	P =.002 U = 1688	1037 (755)	1445 (966)	P = .002 U = 4066
CCL20	17.3 (20.4)	15.7 (13.4)	17.7 (24.1)	P = .820 $\chi^2(2) = 0.397$	P =.683 U = 1171	17.6 (18.9)	15.8 (20.7)	P = .684 U = 3063
IL-13	0.21 (0.58)	0.21 (0.85)	0.21 (1.16)	P = .669 $\chi^2(2) = 0.803$	P =.839 U = 1257	0.21 (0.49)	0.21 (1.14)	P = .414 U = 3397
IL-4	0.08 (0.10)	008 (0.06)	0.08 (0.16)	P = .113 $\chi^2(2) = 4.361$	P =.065 U = 1474	0.08 (0.07)	0.09 (0.09)	P = .047 U = 3709
IL-6	1.37 (0.70)	1.47 (0.91)	1.72 (0.96)	P = .012 $\chi^2(2) = 8.823$	P =.006 U = 1628	1.41 (0.91)	1.61 (0.95)	P = .370 U = 3444
Leptin	21914 (25559)	27212 (42054)	25625 (39863)	P = .390 $\chi^2(2) = 1.885$	P =.830 U = 1261	23933 (31379)	25654 (37288)	P = .654 U = 3313
TNF-α	1.65 (1.51)	1.36 (1.17)	1.43 (1.44)	P = .111 $\chi^2(2) = 4.400$	P =.226 U = 1055	1.55 (1.36)	1.46 (1.27)	P = .529 U = 2998
Ghrelin^	**(n=59)** 527 (751)	**(n=54)** 226 (505)	**(n=39)** 276 (607)	P = <0.01 $\chi^2(2) = 15.553$	P = .003 U = 719	**(n=71)** 491.4 (646.0)	**(n=81)** 234 (568)	P = .005 U = 2109

*A score of >= 18 in the cognitive domain of the PBC-40 is considered clinically significant; IQR – Interquartile Range; n=number of cases in each group; test for statistical significance using Mann-Whitney U or Kruskal-Wallis; P < 0.05 considered statistically significant (2-sided test); **red** denotes statistically significance; ^note for ghrelin serum levels were only available for a subset of patients

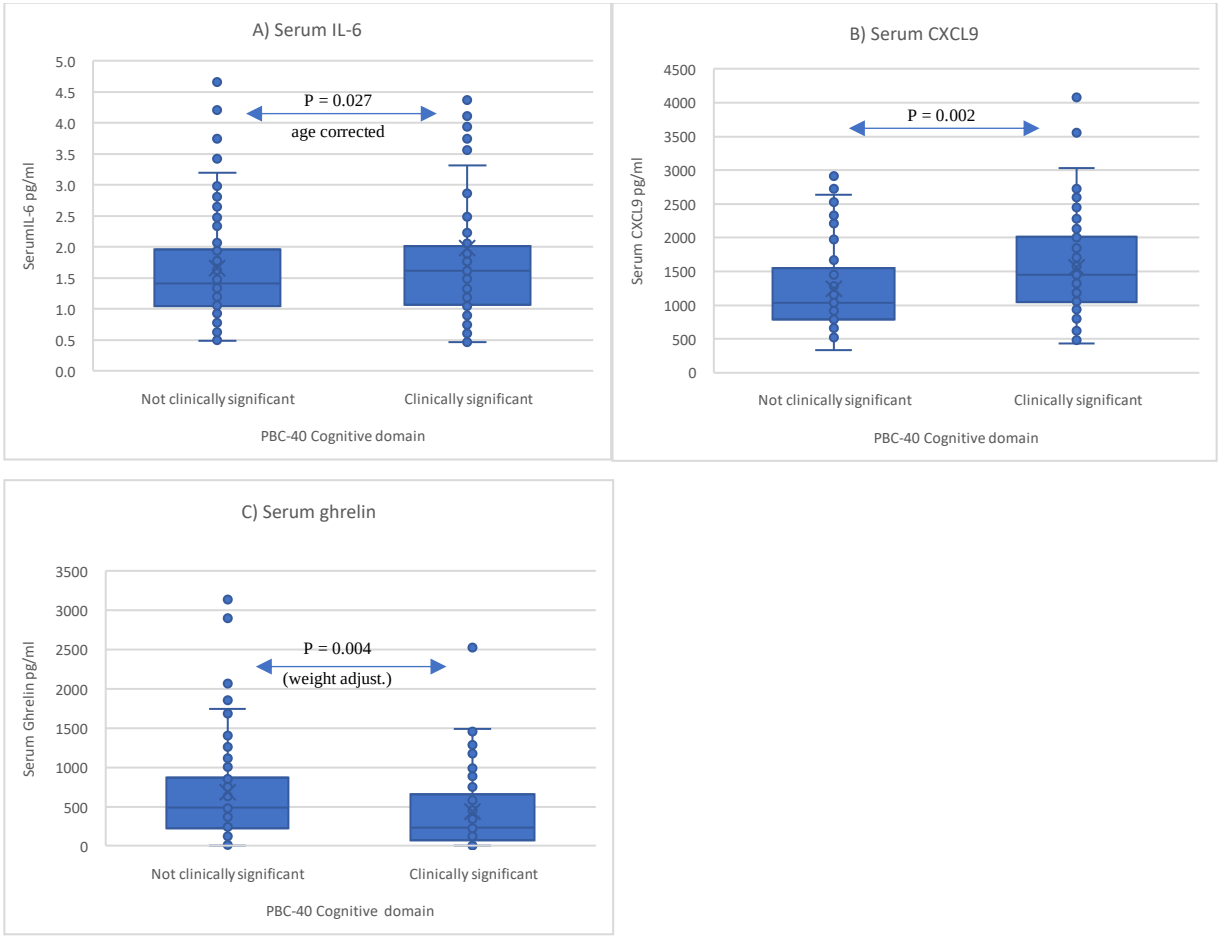


Figure 6.2 Serum markers (pg/ml) significantly associated with clinically significant cognitive symptoms as defined by the PBC-40 cognitive domain A) IL-6, B) CXCL9, C) ghrelin.

As ghrelin levels were associated with weight, an ANCOVA covariate analysis was performed. It remained statistically significant (none/mild vs. severe: F = 8.922, P = 0.004; Clinically significant: F = 7.418, P = 0.007). When IL-6 was corrected for age as a covariate, significance remained between the "none/mild" and "severe" cognitive groups (IL-6: F = 5.009, P = 0.027). **See Figure** 6.2**.**

6.1.3.2 Study 2 (validation study): fatigue symptoms

The burden of fatigue in this cohort was high when assessed using the PBC-40 fatigue domain; 77/160 (49%) with "severe" symptoms, 51 (32%) "moderate" and only 32 (19%) with "none/mild". Overall, this resulted in 122/160 (76%) experiencing clinically significant fatigue (defined by a PBC-40 score of 33 or more in the fatigue domain). **See Table** 6.11**.** This is higher than found in the general PBC population using this cut-off score, where fatigue is expected to be clinically significant in 45% of the PBC population. **See Table** 3.2

As with cognitive symptoms, the 3 markers that showed a statistically significant difference between the “none/mild” and “severe” groups were CXCL9 (severe: 1451pg/ml versus none/mild: 999, P=0.006) and IL-6 (severe: 1.64pg/ml versus none/mild: 1.05pg/ml, P=0.003). These both increased with more severe symptoms, whilst ghrelin (severe: 253pg/ml, none/mild: 559pg/ml, P=0.002) decreased with increased severity of fatigue.

This finding persisted when comparing between “none/mild”, “moderate” and “severe” groups. Pairwise correction with Bonferroni demonstrated significance for CXCL9 (none/mild vs. severe, P = 0.012), IL-6 (none/mild vs. severe, P = 0.013) and ghrelin (none/mild vs. severe, P = 0.005; none/mild vs. moderate, P = 0.034).

CXCL9, IL-6 and ghrelin remained statistically significant when stratified by clinically significant fatigue. No other analytes were statistically significant in any group comparisons. **See Table** 6.11**.**

Table 6.11 Serum markers (pg/ml) by fatigue domain severity as defined by PBC-40 (none/mild vs. severe) and by fatigue score deemed clinically significant in Study 2

Marker	**Fatigue Severity**					**Clinically* Significant Fatigue symptoms?**		
	None/Mild (n=32) Median (IQR) pg/ml	**Moderate (n=51)** Median (IQR) pg/ml	**Severe (n=77)** Median (IQR) pg/ml	**Significance none/mild vs. moderate vs. severe**	**Significance none/mild vs. severe**	**No (n=38)** Median (IQR) pg/ml	**Yes (n=122)** Median (IQR) pg/ml	**Significance**
IL-4R	1016 (279)	1035 (246.9)	1035 (158.8)	P = .923 $\chi^2(2) = 0.160$	P = .665 U = 1297	1016 (263)	1038 (181)	P = .758 U = 2395
CD163	535349 (329224)	529741 (307066)	484459 (237394)	P = .573 $\chi^2(2) = 1.113$	P = .328 U = 1085	547167 (318938)	481431 (281223)	P = .137 U = 1947
KIM-1	87.6 (77.4)	79.9 (73.4)	74.1 (105.1)	P = .737 $\chi^2(2) = 0.610$	P = .775 U = 1189	87.6 (77.1)	74.1 (77.1)	P = .569 U = 2176
CXCL9	999 (874)	1134.9 (815.1)	1451 (1041)	P = .011 $\chi^2(2) = 9.031$	P = .006 U = 1646	1048 (737)	1345 (834)	P = .027 U = 2868
CCL20	17.8 (21.9)	16.05 (20.8)	17.3 (17.0)	P = .700 $\chi^2(2) = 0.714$	P = .762 U = 1277	16.9 (21.6)	17.1 (19.2)	P = .643 U = 2433
IL-13	0.21 (1.08)	0.21 (0.92)	0.21 (0.73)	P = .836 $\chi^2(2) = 0.358$	P = .639 U = 1296	0.21 (0.62)	0.21 (0.95)	P = .717 U = 1296
IL-4	0.08 (0.08)	0.08 (0.14)	0.08 (0.09)	P = .622 $\chi^2(2) = 0.950$	P = .739 U = 1277	0.08 (0.07)	0.08 (0.10)	P = .898 U = 2347
IL-6	1.05 (0.82)	1.52 (0.79)	1.64 (0.96)	P = .017 $\chi^2(2) = 8.099$	P = .003 U = 1681	1.06 (0.86)	1.62 (0.89)	P = .010 U = 2959
Leptin	28134 (33073)	22196 (25908)	25625 (41412)	P = .941 $\chi^2(2) = 0.121$	P = .942 U = 1243	24740 (29815)	25358 (35238)	P = .712 U = 2410
TNF-α	1.69 (1.00)	1.20 (1.64)	1.49 (1.24)	P = .200 $\chi^2(2) = 3.215$	P = .464 U = 1122	1.74 (1.20)	1.43 (1.28)	P = .078 U = 1878
Ghrelin^	**(n=31)** 559.5 (767.4)	**(n=48)** 297.6 (646.2)	**(n=73)** 253.5 (588.5)	P = .005 $\chi^2(2) = 10.431$	P = .002 U - 686	**(n=37)** 559.5 (654.9)	**(n=115)** 274 (579.9)	P = .002 U = 1416

*A score of >= 33 in the fatigue domain of the PBC-40 is considered clinically significant; IQR – Interquartile Range; n=number of cases in each group; test for statistical significance using Mann-Whitney U or Kruskal-Wallis; P < 0.05 considered statistically significant (2-sided test); **red** denotes statistically significance; ^note for ghrelin serum levels were only available for a subset of patients.

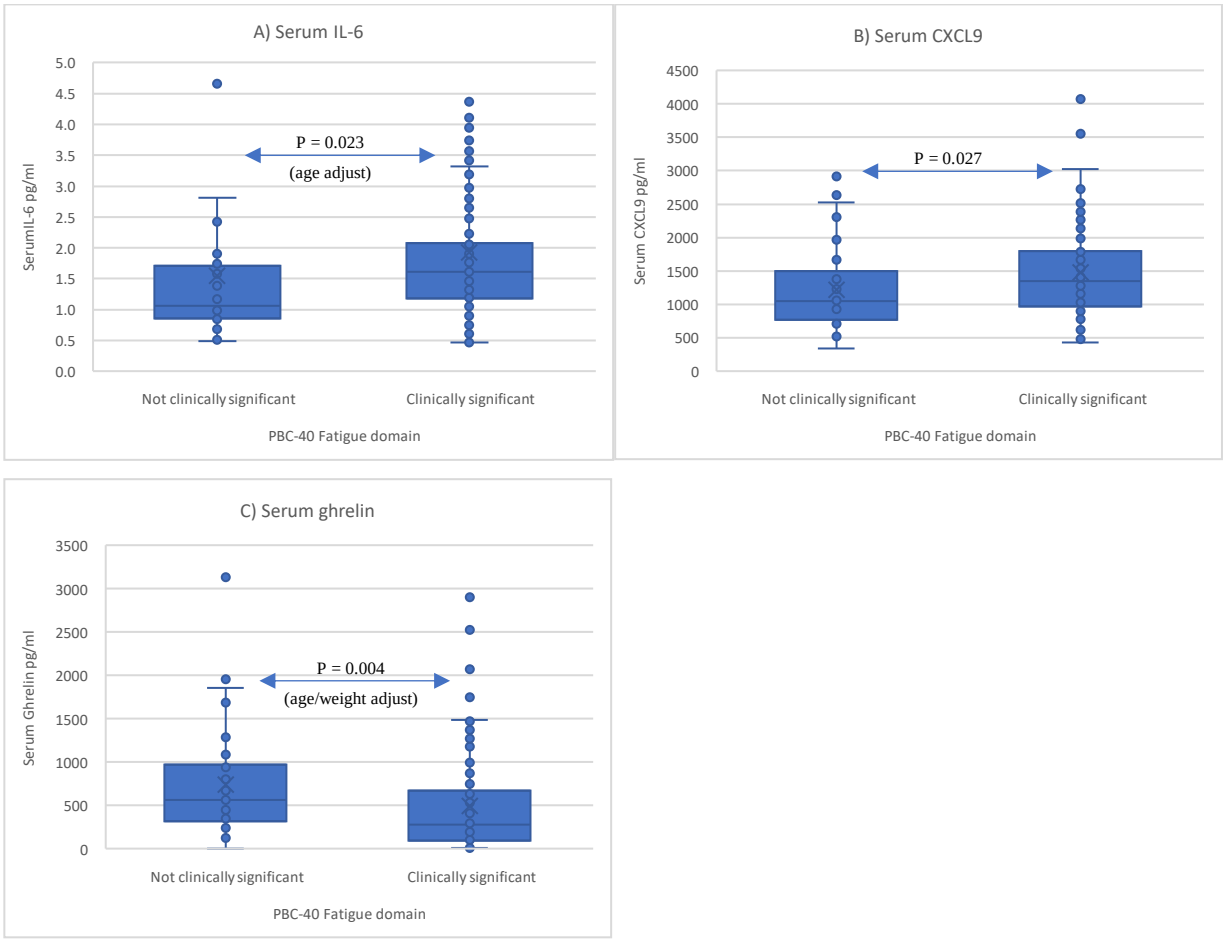


Figure 6.3 Serum markers (pg/ml) significantly associated with clinically significant fatigue as defined by the PBC-40 fatigue domain A) IL-6, B) CXCL9, C) ghrelin.

Given that IL-6 levels showed an association with age, an ANCOVA analysis was undertaken to include age as a covariate, stratified by "none/mild" vs "severe" groups and clinically significant fatigue. IL-6 continued to show a statistically significant correlation with fatigue severity when adjusting for age as a covariate (none/mild vs. severe: F = 6.103, P = 0.015; clinically significant: F = 5.240, P= 0.023)

Including weight as a covariate for ghrelin, resulted in continued statistical significance for none/mild vs. severe groups and clinically significant fatigue, (none/mild vs. severe: F = 8.169, P = 0.005; clinically significant: F = 8.426, P = 0.004). **See Figure** 6.3**.**

6.1.3.3 Study 2 (validation study): itch symptoms

Symptom assessment using the PBC-40 itch domain demonstrated that 128 out 160 participants (80%) experienced "none/mild" symptoms of itch, 18 (11%) were categorised as having moderate itch, and 14 (9%) had severe itch. When splitting the group using the previously

published PBC-40 clinically significant itch score (a score of 7 or more, which includes 25% of those experiencing mild symptoms [mild range 4-8]), 57/160 (36%) had clinically significant itch. **See Table** 6.12**.** This is akin to published data where a cut-off of 7 in the itch domain yielded clinically significant symptoms in 38% of the PBC population). **See Table** 3.2

TNF-α was significantly elevated in the "severe" group when compared to the "none/mild" group (P=0.022) and when comparing "none/mild", "moderate" and "severe" (P=0.043).
It should be noted that whilst the median serum TNF-α in the "severe" group was higher than the "none/mild" group (TNF-α: severe: 2.30pg/ml versus none/mild 1.47pg/ml), the severe group was small, and the median serum TNF-α in the moderate group was lower than the none/mild group (moderate: 1.39pg/ml). When corrected for pairwise comparison using Bonferroni, these associations became non-significant between all symptom severity groups. No other serum markers were significantly different between these groupings. **See Table** 6.12
Using the clinically significant cut off for itch, the association with TNF-α disappeared, whilst IL-4Rα showed statistical significance (P=0.022) with an elevation in IL-4Rα being associated with clinically significant itch symptoms. No other serum marker reached significance. **See Figure** 6.4

Table 6.12 Serum markers (pg/ml) by itch domain severity as defined by PBC-40 (none/mild vs. severe) and by itch score deemed clinically significant in Study 2

Marker	**Itch Severity**					**Clinically* Significant Itch Symptoms**?		
	None/Mild (n=128) Median (IQR) pg/ml	**Moderate (n=18)** Median (IQR) pg/ml	**Severe (n=14)** Median (IQR) pg/ml	**Significance none/mild vs. moderate vs. severe**	**Significance none/mild vs. severe**	**No (n=103)** Median (IQR) pg/ml	**Yes (n=57)** Median (IQR) pg/ml	**Significance**
IL-4Rα	1013 (212)	1093 (130.7)	1077 (100)	P = .071 $\chi^2(2) = 5.292$	P =.145 U = 1109	1000 (200)	1081 (173)	P =.022 U = 3578
CD163	483589 (268253)	619324 (782698)	484592 (295419)	P = .311 $\chi^2(2) = 2.336$	P =0.80 U = 859	500282 (280825)	499603 (296441)	P =.970 U = 2946
KIM-1	74.8 (71.9)	93.6 (153)	82.7 (111.6)	P = .219 $\chi^2(2) = 3.036$	P =.191 U = 1087	75.4 (79.2)	80.3 (68.8)	P =.770 U = 3017
CXCL9	1262 (825)	1262 (825)	1456 (1253)	P = .198 $\chi^2(2) = 3.238$	P =.821 U = 929	1256 (845)	1202 (866)	P =.655 U = 2810
CCL20	16.9 (20.7)	16.9 (11.2)	19.7 (23.7)	P = .960 $\chi^2(2) = 0.082$	P =.795 U = 934	17.0 (19.3)	17.3 (19.8)	P =.836 U = 2993
IL-13	0.21 (0.92)	0.21 (0.51)	0.49 (0.97)	P = .758 $\chi^2(2) = 0.555$	P =.744 U = 939	0.21 (1.07)	0.21 (0.91)	P =.604 U = 2804
IL-4	0.08 (0.09)	0.08 (0.01)	0.13 (0.14)	P = .341 $\chi^2(2) = 2.150$	P =.451 U = 997	0.08 (0.94)	0.08 (0.10)	P =.973 U = 2944
IL-6	1.49 (0.90)	1.64 (1.21)	1.87 (1.38)	P = .104 $\chi^2(2) = 4.535$	P =.067 U = 1164	1.50 (0.95)	1.52 (0.86)	P =.374 U = 3185
Leptin	25583 (35548)	21625 (20123)	25457 (45536)	P = .417 $\chi^2(2) = 1.751$	P =.460 U = 1004	28101 (35683)	23961 (33543)	P =.854 U = 2987
TNF-α	1.47 (1.27)	1.39 (1.33)	2.30 (2.42)	P = .043 $\chi^2(2) = 6.298$	P =.022 U = 1230	1.55 (1.24)	1.43 (1.56)	P = .580 U = 2780
Ghrelin^	**(n=122)** 315 (621)	**(n=16)** 540 (677)	**(n=14)** 618 (946)	P = .185 $\chi^2(2) = 3.377$	P = .071 U = 1106	**(n=98)** 356 (632)	**(n=54)** 378 (701)	P = .908 U = 2676

*A score of >= 7 in the itch domain of the PBC-40 is considered clinically significant; IQR – Interquartile Range; n=number of cases in each group; test for statistical significance using Mann-Whitney U or Kruskal-Wallis; P < 0.05 considered statistically significant (2-sided test); **red** denotes statistically significance; **yellow** denotes no statistical significance following post-hoc Bonferroni correction; ^note for ghrelin serum levels were only available for a subset of patients

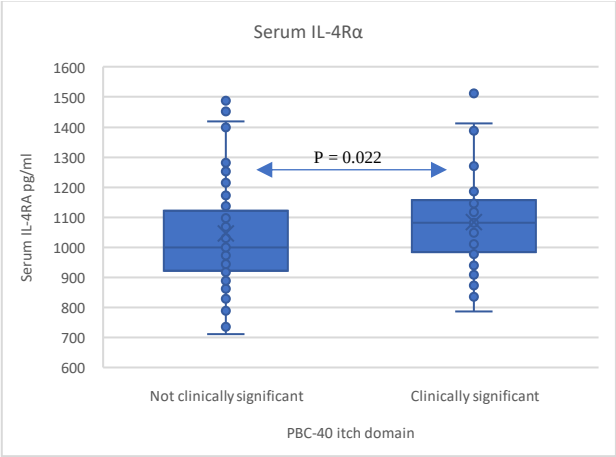


Figure 6.4 Serum IL-4Rα (pg/ml) and clinically significant itch as defined by the PBC-40 itch domain.

6.1.3.4 Study 2 (validation study): emotional symptoms

Exploring the cohort with the PBC-40 domain emotional cohort resulted in a relatively even split between "none/mild", "moderate" and "severe" symptoms, with 57/160 (35%) classified as "none/mild", 52 (33%) "moderate" and 51 (32%) severe. Clinically significant symptoms as defined by a score of 12 or more, which is expected to be seen in 19% of the PBC population (Mells *et al.*, 2013), was seen in 51/160 (32%) of participants.

IL-6 was statistically significantly elevated when comparing those with "none/mild" and "severe" symptoms (P = 0.009) and between all groups (P = 0.026). When correcting for multiple pairwise testing, significance occurred between "none/mild" and "severe" groups (P = 0.023), but not other groupings. When using the clinically significant cut-off score of 12 or more, IL-6 remained statistically significant (P = 0.012).

Ghrelin was significantly lower with increasing symptoms when comparing across the "none/mild", "moderate" and "severe" symptoms (P = 0.017), and when corrected for by pairwise comparison, remained significant between "none/mild" and "moderate" groups (P = 0.021), but not the other groupings. No other markers showed a statistical association. **See Table** 6.13 **and Figure** 6.5**.**

Table 6.13 Serum markers (pg/ml) by Emotional domain severity as defined by PBC-40 (none/mild vs. severe) and by Emotional score deemed clinically significant in Study 2

Marker	**Emotional Severity**					**Clinically* Significant Emotional Symptoms?**		
	None/Mild (n=57) Median (IQR) pg/ml	**Moderate (n=52)** Median (IQR) pg/ml	**Severe (n=51)** Median (IQR) pg/ml	**Significance none/mild vs. moderate vs. severe**	**Significance none/mild vs. severe**	**No (n=109)** Median (IQR) pg/ml	**Yes (n=51)** Median (IQR) pg/ml	**Significance**
IL-4R	1017 (246)	1013 (194)	1068 (171)	P = .254 $\chi^2(2) = 2.739$	P =.210 U = 1629	1015 (225)	1068 (171)	P =.106 U = 3220
CD163	524935 (317987)	472473 (303931)	478404 (254582)	P = .704 $\chi^2(2) = .701$	P =.417 U = 1298	511216 (308060)	478404 (252582)	P =.565 U = 2622
KIM-1	74.2 (76.1)	77.2 (80.1)	77.8 (104.8)	P = .700 $\chi^2(2) = .712$	P =.891 U = 1450	74.2 (75.8)	77.8 (104)	P = .587 U = 2928
CXCL9	1136 (876)	1522 (1192)	1159 (736)	P = .266 $\chi^2(2) = 2.649$	P =.681 U = 1494	1277 (906)	1159 (736)	P =.694 U = 2672
CCL20	12.7 (15.0)	17.8 (19.3)	15.7 (17.7)	P = .161 $\chi^2(2) = 3.652$	P =.425 U = 1556	17.1 (19.7)	15.7 (17.6)	P =.905 U = 2747
IL-13	0.21 (0.48)	0.26 (1.25)	0.21 (0.92)	P = .274 $\chi^2(2) = 2.586$	P =.346 U = 1561	0.21 (0.91)	0.21 (0.92)	P =.818 U = 2836
IL-4	0.08 (0.07)	0.08 (0.10)	0.08 (0.14)	P = .386 $\chi^2(2) = 1.904$	P =.198 U = 1615	0.08 (0.08)	0.08 (0.14)	P =.337 U = 3018
IL-6	1.36 (0.90)	1.54 (0.75)	1.67 (0.91)	P = .026 $\chi^2(2) = 7.305$	P =.009 U = 1848	1.45 (0.80)	1.67 (0.91)	P = .012 U = 3464
Leptin	25428 (31821)	20903 (33595)	32898 (45571)	P = .261 $\chi^2(2) = 2.683$	P =.312 U = 1590	23961 (30899)	32898 (45571)	P = .126 U = 3197
TNF-α	1.53 (1.10)	1.43 (1.48)	1.60 (1.71)	P = .697 $\chi^2(2) = 0.721$	P =.587 U = 1515	1.45 (1.26)	1.60 (1.71)	P = .365 U = 3027
Ghrelin^	**(n=56)** 469 (799)	**(n=49)** 234 (449)	**(n=47)** 343 (725)	P = .017 $\chi^2(2) = 8.127$	P = .297 U = 1137	**(n=105)** 369 (675)	**(n=47)** 343 (725)	P = .809 U = 2528

*A score of >= 12 in the emotional domain of the PBC-40 is considered clinically significant; IQR – Interquartile Range; n=number of cases in each group; test for statistical significance using Mann-Whitney U or Kruskal-Wallis; P < 0.05 considered statistically significant (2-sided test); **red** denotes statistically significance; ^note for ghrelin serum levels were only available for a subset of patients

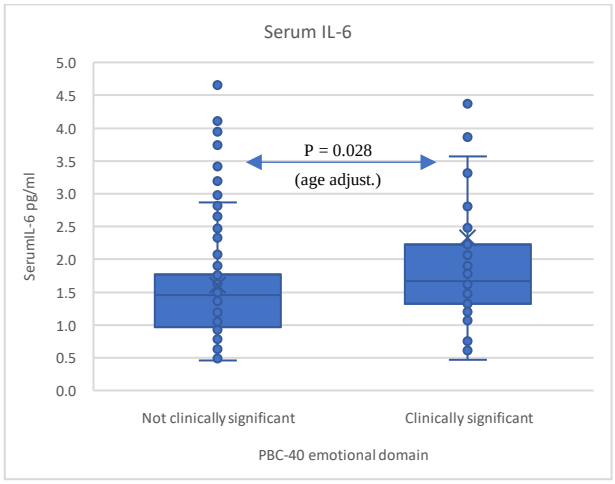


Figure 6.5 *Serum IL-6 (pg/ml) and clinically significant emotional symptoms as defined by the PBC-40 emotional domain.*

Including age as a covariate for IL-6 in emotional symptoms demonstrated persistent statistical significance (none/mild vs. severe: F = 5.992, P = 0.016; clinically significant: F = 4.904, P = 0.028).

6.1.3.5 Study 2 (validation study): social symptoms

According to the PBC-40 social domain, 83 out of 160 participants (52%) reported "none/mild' symptoms, 67 (42%) reported "moderate" symptoms, and 10 (6%) reported severe symptoms. When stratifying by clinical significance using a cut of 32 or more, 50 (32%) had social symptoms that were considered clinically significant. This is slightly higher than the 25% observed in the general PBC population and might be related to the high fatigue and cognitive symptom burden in this group. **See Table** 6.14**.**

Ghrelin was the only analyte that was significantly associated with social symptom severity, being elevated in those with "severe" symptoms when compared to "none/mild" (P=.026). Ghrelin was lowest in those with "moderate" symptoms, with pairwise analysis showing significance only between "moderate" and "severe" social symptoms (P = 0.026), but not other paired groups. Ghrelin was not significantly elevated when stratifying by clinically significant social symptoms.

Table 6.14 Serum markers (pg/ml) by social domain severity as defined by PBC-40 (none/mild vs. severe) and by social score deemed clinically significant in Study 2

Marker	**Social Severity**					**Clinically* Significant Social Symptoms**?		
	None/Mild (n=83) Median (IQR) pg/ml	**Moderate (n=67)** Median (IQR) pg/ml	**Severe (n=10)** Median (IQR) pg/ml	**Significance none/mild vs. moderate vs. severe**	**Significance none/mild vs. severe**	**No (n=110)** Median (IQR) pg/ml	**Yes (n=50)** Median (IQR) pg/ml	**Significance**
IL-4R	1015 (266)	1055 (148)	1013 (212)	P = .701 $\chi^2(2) = 0.710$	P = .340 U = 492	1020 (233)	1035 (158)	P =.276 U = 3046
CD163	525281 (334326)	480561 (281914)	462228 (208364)	P = .332 $\chi^2(2) = 2.207$	P = .480 U = 358	526561 (332720)	450889 (233185)	P =.525 U = 2577
KIM-1	75.2 (70.5)	73.8 (82.1)	80.3 (161.3)	P = .277 $\chi^2(2) = 2.567$	P =.173 U = 525	75.3 (71.3)	78.3 (110.1)	P = .322 U = 3019
CXCL9	1136 (876)	1331 (801)	1455 (1048)	P = .394 $\chi^2(2) = 1.863$	P = .254 U = 323	1214 (856)	1331 (924)	P =.296 U = 2466
CCL20	16.7 (20.7)	17.7 (24.0)	15.8 (12.9)	P = .347 $\chi^2(2) = 2.114$	P = .655 U = 451	17.1 (21.0)	16.8 (18.1)	P =.647 U = 2874
IL-13	0.21 (0.80)	0.21 (1.15)	0.21 (0.65)	P = .173 $\chi^2(2) = 3.506$	P =.919 U = 422	0.21 (0.95)	0.21 (0.82)	P =.497 U = 2916
IL-4	0.08 (0.09)	0.08 (0.10)	0.08 (0.10)	P = .337 $\chi^2(2) = 2.175$	P = .146 U = 522	0.08 (0.10)	0.08 (0.11)	P =.193 U = 3072
IL-6	1.37 (0.77)	1.66 (0.92)	1.61 (0.81)	P = .242 $\chi^2(2) = 2.839$	P = .264 U = 505	1.38 (0.85)	1.65 (0.86)	P = .385 U = 2986
Leptin	23904 (24276)	29396 (38469)	29284 (41738)	P = .835 $\chi^2(2) = 0.361$	P = .527 U = 466	23933 (29878)	30433 (44527)	P = .229 U = 3077
TNF-α	1.53 (1.24)	1.60 (1.69)	1.20 (0.92)	P = .683 $\chi^2(2) = 0.763$	P = .406 U = 482	1.56 (1.31)	1.43 (1.33)	P = .819 U = 2812
Ghrelin^	**(n=79)** 392 (731)	**(n=65)** 301 (501)	**(n=9)** 884 (925)	P = .032 $\chi^2(2) = 6.863$	P = .026 U = 517	**(n=104)** 315 (718)	**(n=48)** 429 (538)	P = .265 U = 2777

*A score of >= 32 in the social domain of the PBC-40 is considered clinically significant; IQR – Interquartile Range; n=number of cases in each group; test for statistical significance using Mann-Whitney U or Kruskal-Wallis; P < 0.05 considered statistically significant (2-sided test); **red** denotes statistically significance; ^note for ghrelin serum levels were only available for a subset of patients

When corrected for by weight, ghrelin remained statistically significant for “none/mild” vs “severe” groups (F = 5.184, P = 0.025).

6.1.3.6 Study 2 (validation study): general symptoms

Only 8/160 (5%) of PBC participants were categorised as having “severe” general symptoms using the relevant PBC-40 domain. There were 85/160 (53%) with “none/mild” symptoms and 67 (42%) had “moderate” symptoms. Using the pre-defined cut-off for clinically significant symptoms (a score of 18 or more), resulted in 78 (55%) being considered clinically significant. This is in comparison to 40% seen in the general PBC population.

Several analytes were significant depending on which method was used to correlate symptom severity. CCL20 was significantly lower in those with “severe” general symptoms compared to those with “none/mild” and “moderate” symptoms, where serum levels were similar (“severe”: 6.6pg/ml, “moderate”: 17.3, “none/mild”: 17.1). Correcting for multiple comparisons with pairwise analysis, showed statistical significance between “none/mild” vs. “severe” groups (P = 0.014), and “moderate” to “severe” (P = 0.009).

Leptin was also significant when comparing “none/mild”, “moderate” and “severe”, with those with “moderate” symptoms having the lowest levels, whilst those with “severe” symptoms had the highest. Correcting for multiple comparisons, showed only a significance between the “none/mild” and “moderate” groups (P=0.016).

For both CCL20 and leptin, these correlations did not persist when using the clinically significant cut-off score.

Ghrelin and TNF-α were both significantly lower in those with clinically significant general symptoms, but only ghrelin showed the same relationship when comparing between “none/mild”, “moderate” and “severe” groups. Neither showed significance when comparing “none/mild” to “severe”. The unbalanced group numbers between “none/mild”, “moderate” and “severe” should be noted. **See Table** 6.15 **and Figure** 6.6

Ghrelin did not remain significant for clinically significant general symptoms when adjusted for weight and age (F: 2.286; P = 0.133). CCL20 remained significant when corrected for weight in the “none/mild” vs “severe” group (F: 10.225; P = 0.002)

Table 6.15 Serum markers (pg/ml) by general symptoms domain severity as defined by PBC-40 (none/mild vs. severe) and by general symptoms score deemed clinically significant in Study 2

Marker	General Symptom Severity					Clinically* Significant General Symptom Symptoms?		
	None/Mild (n=85) Median (IQR) pg/ml	**Moderate (n=67)** Median (IQR) pg/ml	**Severe (n=8)** Median (IQR) pg/ml	**Significance none/mild vs. moderate vs. severe**	**Significance none/mild vs. severe**	**No (n=72)** Median (IQR) pg/ml	**Yes (n=88)** Median (IQR) pg/ml	**Significance**
IL-4R	1017 (220)	1046 (190)	1064 (360)	P = .994 $\chi^2(2) = 0.012$	P = .995 U = 339	1021 (228)	1038 (183)	P =.898 U = 3205
CD163	500282 (275443)	465921 (292493)	553243 (346224)	P = .587 $\chi^2(2) = 1.064$	P =.476 U = 288	517594 (271537)	480561 (296661)	P =.054 U = 2607
KIM-1	74.2 (73.0)	83.1 (105.8)	71.0 (93.8)	P = .589 $\chi^2(2) = 1.060$	P =.403 U = 401	76.1 (70.9)	75.1 (84.4)	P = .929 U = 3142
CXCL9	1219 (845)	1401 (860)	792 (582)	P = .608 $\chi^2(2) = 0.995$	P = .805 U = 322	1156 (798)	1363 (798)	P = .241 U = 3510
CCL20	17.1 (21.3)	17.3 (18.1)	6.6 (5.9)	P = .011 $\chi^2(2) = 8.984$	P = .005 U = 133	17.6 (21.1)	16.6 (18.3)	P = .672 U = 3044
IL-13	0.21 (1.25)	0.21 (0.65)	0.544 (0.92)	P = .857 $\chi^2(2) = .310$	P = .877 U = 350	0.21 (1.26)	0.21 (0.69)	P = .553 U = 3323
IL-4	0.08 (0.08)	0.08 (0.10)	0.21 (0.20)	P = .830 $\chi^2(2) = 0.373$	P =.561 U = 379	0.08 (0.09)	0.08 (0.10)	P = .740 U = 3080
IL-6	1.50 (0.95)	1.64 (0.85)	1.63 (0.81)	P = .314 $\chi^2(2) = 2.319$	P =.584 U = 380	1.48 (0.81)	1.63 (1.03)	P = .161 U = 3577
Leptin	32898 (38222)	21352 (25896)	38293 (133944)	P = .004 $\chi^2(2) = 11.078$	P = .179 U = 438	33342 (38900)	22933 (30658)	P = .130 U = 2727
TNF-α	1.65 (1.44)	1.35 (1.17)	1.47 (1.39)	P = .202 $\chi^2(2) = 3.199$	P =.593 U = 301	1.72 (1.54)	1.39 (1.13)	P = .042 U = 2576
Ghrelin^	**(n=80)** 404 (684)	**(n=64)** 219 (578)	**(n=7)** 406 (1433)	P = .013 $\chi^2(2) = 8.726$	P = .357 U = 221	**(n=69)** 471 (652)	**(n=83)** 252 (570)	P = .010 U = 2165

*A score of >= 18 in the general symptom domain of the PBC-40 is considered clinically significant; IQR – Interquartile Range; n=number of cases in each group; test for statistical significance using Mann-Whitney U or Kruskal-Wallis; P < 0.05 considered statistically significant (2-sided test); **red** denotes statistically significance; ^note for ghrelin serum levels were only available for a subset of patients

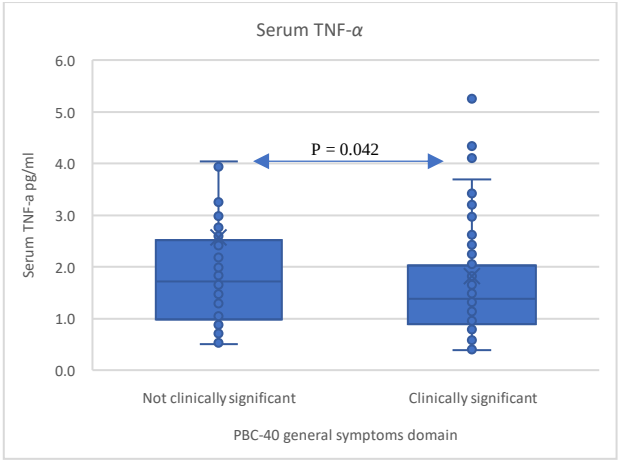


Figure 6.6 Serum markers (pg/ml) significantly associated with clinically significant general symptoms as defined by the PBC-40 general symptoms domain TNF-α B)

6.1.3.7 Study 2 (validation study): serum proteomics and symptoms – summary

A summary of the significant proteomics markers associated with symptoms as assessed and ordered by the PBC-40 domains are in **Table** 6.16 **and Table** 6.17

CXCL9, IL-6 and ghrelin showed consistent associations across both cognitive and fatigue domains. When corrected for multiple testing, both CXCL9 and ghrelin continued to be considered statistically significant results for "none/mild" vs. "severe" comparisons, but CXCL9 was considered a false positive for clinically significant fatigue.

IL-6 remained significant for clinically significant fatigue even when corrected for FDR but was considered a false positive on correction for multiple testing for the domains fatigue, cognition and emotional when comparing "none/mild" vs. "severe".

CXCL9 whilst showing strong associations with cognition and fatigue in Study 2 was not elevated in the cognitive or fatigue domain in Study 1. **See section 5.1.4.7.**

Reduced serum ghrelin was strongly associated with fatigue and cognitive symptoms (P=.002 and P=.007 respectively), but this analyte was not used in the exploratory study (Study 1). It should be noted that leptin, which shares a strong biological relationship with ghrelin, and was previously associated with fatigue in the exploratory study (study 1), showed no significant correlation in the validation study (study 2).

IL-4Rα demonstrated a positive association with itch when assessed by clinical significance, but not when comparing "none/mild" and "severe" symptoms. However, it should be noted, that using this categorisation resulted in only 14 participants being classified as "severe", as opposed to 54 considered to have clinically significant symptoms. On correcting for multiple testing, IL-4Rα was considered a false positive but it was positively associated with significant itch in the exploratory study (Study 1).

TNF-α demonstrated a positive relationship for itch when assessed by "none/mild" and "severe" symptoms, but this disappeared when correcting for multiple testing and there was no correlation when exploring by clinically significant symptoms.

No other markers showed a significant relationship in the other PBC-40 domains.

Table 6.16 Summary table of statistical significance for median serum markers (pg/ml) by PBC-40 domain, split by none/mild and severe symptoms for Study 2

Marker	PBC-40 Domain						
	Symptom severity	**Cognition** Median (IQR) pg/ml	**Fatigue** Median (IQR) pg/ml	**Itch** Median (IQR) pg/ml	**Emotional** Median (IQR) pg/ml	**Social** Median (IQR) pg/ml	**General symptoms** Median (IQR) pg/ml
IL-4Rα	None/mild	1026 (191)	1016 (279)	1013 (212)	1017 (246)	1015 (266)	1017 (220)
	Severe	1021 (181)	1035 (158.8)	1077 (100)	1068 (171)	1013 (212)	1064 (360)
	Significance	P=.956	P = .665	P =.145	P = .210	P = .340	P = .995
CD163	None/mild	533491 (316127)	535349 (329224)	483589 (268253)	524935 (317987)	525281 (334326)	500282 (275443)
	Severe	452653 (213056)	484459 (237394)	484592 (295419)	478404 (254582)	462228 (208364)	553243 (346224)
	Significance	P =.194	P = .328	P =0.80	P = .417	P = .480	P = .476
KIM-1	None/mild	74.6 (71.6)	87.6 (77.4)	74.8 (71.9)	74.2 (76.1)	75.2 (70.5)	74.2 (73.0)
	Severe	80.3 (113.2)	74.1 (105.1)	82.7 (111.6)	77.8 (104.8)	80.3 (161.3)	71.0 (93.8)
	Significance	P =.633	P = .775	P =.191	P = .891	P =.173	P = .403
CXCL9	None/mild	1025 (769)	999 (874)	1262 (825)	1136 (876)	1136 (876)	1219 (845)
	Severe	1471 (917)	1451 (1041)	1456 (1253)	1159 (736)	1455 (1048)	792 (582)
	Significance	P =.002	P = .006	P =.821	P = .681	P = .254	P = .805
CCL20	None/mild	17.3 (20.4)	17.8 (21.9)	16.9 (20.7)	12.7 (15.0)	16.7 (20.7)	16.7 (20.7)
	Severe	17.7 (24.1)	17.3 (17.0)	19.7 (23.7)	15.7 (17.7)	15.8 (12.9)	10.3 (11.6)
	Significance	P =.683	P = .762	P =.795	P = .425	P = .655	P = .005
IL-13	None/mild	0.21 (0.58)	0.21 (1.08)	0.21 (0.92)	0.21 (0.48)	0.21 (0.80)	0.21 (1.25)
	Severe	0.21 (1.16)	0.21 (0.73)	0.49 (0.97)	0.21 (0.92)	0.21 (0.65)	0.544 (0.92)
	Significance	P =.839	P = .639	P =.744	P = .346	P = .919	P = .877
IL-4	None/mild	0.08 (0.10)	0.08 (0.08)	0.08 (0.09)	0.08 (0.07)	0.08 (0.09)	0.08 (0.08)
	Severe	0.08 (0.16)	0.08 (0.09)	0.13 (0.14)	0.08 (0.14)	0.08 (0.10)	0.21 (0.20)
	Significance	P =.065	P = .739	P =.451	P = .198	P = .146	P = .561
IL-6	None/mild	1.37 (0.70)	1.05 (0.82)	1.49 (0.90)	1.36 (0.90)	1.37 (0.77)	1.50 (0.95)
	Severe	1.72 (0.96)	1.64 (0.96)	1.87 (1.38)	1.67 (0.91)	1.61 (0.81)	1.63 (0.81)
	Significance	P = .006	P = .003	P =.067	P = .009	P = .264	P = .584
	(age adjust.)	P = .027	P = .015		P = .016		
Leptin	None/mild	21914 (25559)	28134 (33073)	25583 (35548)	25428 (31821)	23904 (24276)	31235 (35246)
	Severe	25625 (39863)	25625 (41412)	25457 (45536)	32898 (45571)	29284 (41738)	23904 (144619)
	Significance	P =.830	P = .942	P =.460	P = .312	P = .527	P = .179
TNF-a	None/mild	1.65 (1.51)	1.69 (1.00)	1.47 (1.27)	1.53 (1.10)	1.53 (1.24)	1.57 (1.41)
	Severe	1.43 (1.44)	1.49 (1.24)	2.30 (2.42)	1.60 (1.71)	1.20 (0.92)	1.53 (1.31)
	Significance	P =.226	P = .464	P =.022	P = .587	P = .406	P = .593
Ghrelin ^	None/mild	527 (751)	559.5 (767.4)	315 (621)	469 (799)	392 (731)	404 (684)
	Severe	276 (607)	253.5 (588.5)	618 (946)	343 (725)	884 (925)	406 (1433)
	Significance	P = .003	P = .002	P = .071	P = .297	P = .026	P = .357
	(weight adjust.)	P = .004	P = .005			P = .025	

IQR – Interquartile Range; test for statistical significance using Mann-Whitney U; P < 0.05 considered statistically significant (2-sided test); **red** denotes statistically significance; orange denotes non-significant following correction for false detection rate

Table 6.17 Summary table of statistical significance for median serum markers (pg/ml) by PBC-40 domain, split by clinically significant symptoms for Study 2

Marker	PBC-40 Domain						
	Clinically significant symptoms	**Cognition** Median (IQR) pg/ml	**Fatigue** Median (IQR) pg/ml	**Itch** Median (IQR) pg/ml	**Emotional** Median (IQR) pg/ml	**Social** Median (IQR) pg/ml	**General symptoms** Median (IQR) pg/ml
IL-4Rα	**Not c/s**	1035 (215)	1016 (263)	1000 (200)	1015 (225)	1020 (233)	1021 (228)
	c/s	1030 (187)	1038 (181)	1081 (173)	1068 (171)	1035 (158)	1038 (183)
	Significance	P = .566	P=.758	P=.022	P=.106	P = .276	P = .898
CD163	**Not c/s**	529741 (330760)	547167 (318938)	500282 (280825)	511216 (308060)	526561 (332720)	517594 (271537)
	c/s	457441 (235127)	481431 (281223)	499603 (296441)	478404 (252582)	450889 (233185)	480561 (296661)
	Significance	P = .269	P=.137	P=.970	P=.565	P = .525	P = .054
KIM-1	**Not c/s**	73.4 (69.9)	87.6 (77.1)	75.4 (79.2)	74.2 (75.8)	75.3 (71.2)	76.1 (70.9)
	c/s	81.5 (81.6)	74.1 (77.1)	80.3 (68.8)	77.8 (104)	78.3 (110.1)	75.1 (84.4)
	Significance	P = .479	P=.569	P=.770	P=.587	P = .322	P = .929
CXCL9	**Not c/s**	1037 (755)	1048 (737)	1256 (845)	1277 (906)	1214 (856)	1156 (798)
	c/s	1445 (966)	1345 (834)	1202 (866)	1159 (736)	1331 (924)	1363 (798)
	Significance	P = .002	P=.027	P=.655	P=.694	P = .296	P = .241
CCL20	**Not c/s**	17.6 (18.9)	16.9 (21.6)	17.0 (19.3)	17.1 (19.7)	17.1 (21.0)	17.6 (21.1)
	c/s	15.8 (20.7)	17.1 (19.2)	17.3 (19.8)	15.7 (17.6)	16.8 (18.1)	16.6 (18.3)
	Significance	P = .684	P=.643	P=.836	P=.905	P = .647	P = .672
IL-13	**Not c/s**	0.21 (0.49)	0.21 (0.62)	0.21 (1.07)	0.21 (0.91)	0.21 (0.95)	0.21 (1.26)
	c/s	0.21 (1.14)	0.21 (0.95)	0.21 (0.91)	0.21 (0.92)	0.21 (0.82)	0.21 (0.69)
	Significance	P = .414	P=.717	P=.604	P=.818	P = .497	P = .553
IL-4	**Not c/s**	0.08 (0.07)	0.08 (0.07)	0.08 (0.94)	0.08 (0.08)	0.08 (0.10)	0.08 (0.09)
	c/s	0.09 (0.09)	0.08 (0.10)	0.08 (0.10)	0.08 (0.14)	0.08 (0.11)	0.08 (0.10)
	Significance	P = .047	P=.898	P.973	P=.337	P = .193	P = .740
IL-6	**Not c/s**	1.41 (0.91)	1.06 (0.86)	1.50 (0.95)	1.45 (0.80)	1.38 (0.85)	1.48 (0.81)
	c/s	1.61 (0.95)	1.62 (0.89)	1.52 (0.86)	1.67 (0.91)	1.65 (0.86)	1.63 (1.03)
	Significance (age adjust.)	P = .370	P = .010 P = .023	P=.374	P = .012 P = .028	P = .385	P = .161
Leptin	**Not c/s**	23933 (31379)	24740 (29815)	28101 (35683)	23961 (30899)	23933 (29878)	33342 (38900)
	c/s	25654 (37288)	25358 (35238)	23961 (33543)	32898 (45571)	30433 (44527)	22933 (30658)
	Significance	P = .654	P=.712	P=.854	P=.126	P = .229	P = .130
TNF-a	**Not c/s**	1.55 (1.36)	1.74 (1.20)	1.55 (1.24)	1.45 (1.26)	1.56 (1.31)	1.72 (1.54)
	c/s	1.46 (1.27)	1.43 (1.28)	1.43 (1.56)	1.60 (1.71)	1.43 (1.33)	1.39 (1.13)
	Significance	P = .529	P=.078	P=.580	P=.365	P = .819	P = .042
Ghrelin ^	**Not c/s**	491.4 (646)	559.5 (654.9)	356 (632)	369 (675)	404 (734)	407 (672)
	c/s	234 (568)	274 (579.9)	378 (701)	343 (725)	287 (529)	279 (649)
	Significance Weight adjust.	P = .005 P = .007	P = .002 P = .004	P=.908	P=.809	P = .265	P = .010 P = .011

IQR – Interquartile Range; test for statistical significance using Mann-Whitney U; P < 0.05 considered statistically significant (2-sided test); **red** denotes statistically significance; orange denotes non-significant following correction for false detection rate

6.1.4 Study 2 (validation study): Serum proteomics: other symptom measures

Other symptom measures (ESS, OGS and HADS) are often used in PBC clinic trials but data for these scores was only available for the RIT-PBC cohort used here. These symptom measures were not collected at the time of recruitment to UK-PBC or BANC. Results for ESS, OGS and HADS were therefore available for the 57 participants from the RITPBC trial.

6.1.4.1 Study 2 (validation study): Epworth sleepiness scale (ESS)

The ESS is defined as either clinically significant or clinically not significant based on a score of 11 or more. Clinically significant day-time somnolence was seen in 35 of the 57 (61%) participants from the RITPBC group. This is higher than the 28% that would be expected in the general PBC population. **See Table** 3.2

CCL20 was significantly elevated in those with clinically significant daytime somnolence (not clinically significant (median): 12.6pg/ml versus clinically significant: 17.4pg/ml; P=0.037), but this disappeared on correcting for multiple testing. No other markers were significantly elevated. **See Table** 6.18

Table 6.18 Epworth sleepiness scale by serum proteome markers in Study 2

Marker	Epworth Sleepiness Scale		
	Not clinically significant (score <10) (n=22) Median (IQR) pg/ml	**Clinically significant (score >=10) (n=35)** Median (IQR) pg/ml	**Significance**
IL-4R	965 (174)	1022 (192)	P = .082 U = 491
CD163	467106 (256980)	478404 (258411)	P =.896 U = 393
KIM-1	63.8 (61.7)	82.7 (85.9)	P =.394 U = 437
CXCL9	1415 (1305)	1583 (951)	P = .244 U = 456
CCL20	12.6 (13.8)	17.4 (19.1)	P = .037 U = 512
IL-13	0.21 (0.54)	0.21 (0.93)	P = .452 U = 427
IL-4	0.08 (0.03)	0.08 (0.86)	P =.526 U = 418
IL-6	1.41 (1.38)	1.63 (1.28)	P =.258 U = 454
Leptin	22807 (31882)	20211 (29963)	P = .806 U = 400
TNF-α	0.99 (0.74)	1.07 (0.99)	P =.376 U = 439
Ghrelin^	**(n=21)** 136.8 (192.1)	**(n=34)** 60.7 (146.3)	P = .150 U = 274

IQR – Interquartile Range; n=number of cases in each group; test for statistical significance using Mann-Whitney U or Kruskal-Wallis; P < 0.05 considered statistically significant (2-sided test); **red** denotes statistically significance; orange denotes non-significant following correction for false detection rate; ^note for ghrelin serum levels were only available for a subset of patients

6.1.4.2 Study 2 (validation study): Orthostatic grading scale (OGS)

Results for OGS were available for 56 out of the 57 RITPBC participants. There were 27/56 (49%) with "none/mild" symptoms, 26/56 (46%) with "moderate" and 3/56 (5%) with "severe" symptoms.

Comparing those with none/mild to moderate and severe symptoms demonstrated a significant difference in CXCL9 (P=0.018). However, it should be noted that those with moderate symptoms had the highest median serum level, whilst those with severe scores had the lowest (none/mild: 1426pg/ml, moderate 1629pg/ml, severe 632pg/ml). This may be due to the small number (n=3) of participants in the severe group, so we then combined the moderate and severe groups, resulting in CD163 becoming statistically significant, with a decrease in serum levels as severity of orthostatic symptoms increased (none/mild: 520061pg/ml, moderate:

388240pg/ml, severe 374422pg/ml; P=0.048). However, this disappeared on correction for multiple testing. **See Table** 6.19

Table 6.19 Orthostatic grading scale by serum proteome markers in Study 2

Marker	Orthostatic Grading Scale				
	None/mild (score < 9) (n=27) Median (IQR) pg/ml	**Moderate (score = 4-9) (n=26)** Median (IQR) pg/ml	**Severe (score > 9) (n=3)** Median (IQR) pg/ml	**Significance (None/mild vs. moderate vs/ severe)**	**Significance (none/mild vs. moderate & severe)**
IL-4R	1022 (263)	1013 (263)	954 (-)	P = .646 $\chi^2(2) = .875$	P = .363 U = 336
CD163	520061 (261517)	388240 (275051)	374422 (-)	P = .141 $\chi^2(2) = 3.917$	P = .048 U = 271
KIM-1	71.7 (38.9)	81.5 (118)	59.6 (-)	P = .709 $\chi^2(2) = .687$	P = .799 U = 407
CXCL9	1426 (1011)	1628 (979)	632 (-)	P = .018 $\chi^2(2) = .7.988$	P = .517 U = 431
CCL20	17.3 (16.2)	15.4 (19.9)	3.8 (-)	P = .104 $\chi^2(2) = .4.533$	P = .605 U = 360
IL-13	0.21 (0.32)	0.75 (1.35)	0.21 (-)	P = .066 $\chi^2(2) = 5.431$	P =.163 U = 469
IL-4	0.08 (0.01)	0.08 (0.16)	0.08 (-)	P = .376 $\chi^2(2) = 1.957$	P = .203 U = 459
IL-6	1.48 (1.34)	1.64 (1.43)	1.36 (-)	P = .757 $\chi^2(2) = .558$	P = .899 U = 383
Leptin	20211 (30999)	20745 (37062)	27212 (-)	P = .318 $\chi^2(2) = .2.294$	P = .426 U = 440
TNF-α	0.90 (1.01)	1.16 (0.76)	0.96 (-)	P = .299 $\chi^2(2) = 2.416$	P = .248 U = 462
Ghrelin^	**(n=27)** 85.2 (156.1)	**(n=22)** 63.3 (122.0)	**(n=3)** 149.3 (-)	P = .630 $\chi^2(2) = .923$	P = .551 U = 330

IQR – Interquartile Range; n=number of cases in each group; test for statistical significance using Mann-Whitney U or Kruskal-Wallis; P < 0.05 considered statistically significant (2-sided test); **red** denotes statistically significance; orange denotes non-significant following correction for false detection rate; ^note for ghrelin serum levels were only available for a subset of patients

6.1.4.3 Study 2 (validation study): Hospital anxiety and depression scale (HADS)

Results for the HADS score were available for all RITPBC participants (n=57). There were 35/57 (62%) with none/mild symptoms of anxiety, 34 (60%) with none/mild symptoms of depression, 10/57 (18%) with moderate symptoms of anxiety, 17/57 (30%) with moderate symptoms of depression, 11/57 (20%) with severe symptoms of anxiety and 5/57 (9%) with severe features of depression.

Both KIM-1 and IL-6 were statistically significant between the none/mild, moderate and severe anxiety groups, but in both instances the median serum level was highest in the moderate groups (KIM-1: none/mild 69.4pg/ml, moderate 147.2pg/ml, severe 62.3pg/ml; P=0.008 and IL-6: none/mild 1.37pg/ml, moderate 2.61pg/ml, severe 1.48pg/ml; P=0.031). These were

considered false positives on correction for multiple testing. No serum markers were statistically significantly different between the severity of depression symptoms. **Table** 6.20

Table 6.20 Hospital anxiety and depression scale by serum proteome markers in Study 2

Marker	HADS - Anxiety				HADS - Depression			
	None/mild (n=35)	**Moderate (n=10)**	**Severe (n=11)**	**Significance**	**None/mild (n=34)**	**Moderate (n=17)**	**Severe (n=5)**	**Significance**
IL-4R	1006 (182)	1135 (284)	1012 (161)	P = .177 $\chi^2(2) = 3.467$	1002 (188)	1022 (213)	1067 (213)	P = .502 $\chi^2(2) = 1.379$
CD163	462228 (256521)	576253 (349115)	439071 (189533)	P = .415 $\chi^2(2) = 1.761$	455991 (243054)	533012 (227070)	617272 (533445)	P = .576 $\chi^2(2) = 1.104$
KIM-1	69.4 (60.9)	147.2 (130.9)	62.3 (47.1)	P = .008 $\chi^2(2) = 9.757$	71.6 (52.1)	82.7 (106.5)	163.5 (186.0)	P = .541 $\chi^2(2) = 1.228$
CXCL9	1445 (978)	1731 (850)	1451 (1512)	P = .382 $\chi^2(2) = 1.924$	1485 (1094)	1451 (806)	1778 (2676)	P = .920 $\chi^2(2) = .167$
CCL20	15.1 (16.2)	30.7 (43.2)	14.3 (11.1)	P = .080 $\chi^2(2) = 5.056$	14.9 (17.3)	21.7 (15.8)	12.3 (23.0)	P = .316 $\chi^2(2) = 2.303$
IL-13	0.21 (0.57)	0.21 (1.30)	0.77 (3.45)	P = .614 $\chi^2(2) = .976$	0.27 (0.91)	0.21 (2.67)	0.21 (0.28)	P = .407 $\chi^2(2) = 1.795$
IL-4	0.08 (0.67)	0.08(0.21)	0.08 (0.02)	P = .875 $\chi^2(2) = .266$	0.08 (0.46)	0.09 (0.19)	0.08 (0.01)	P = .205 $\chi^2(2) = 3.173$
IL-6	1.37 (0.88)	2.61 (2.30)	1.48 (1.44)	P = .031 $\chi^2(2) = 6.978$	1.48 (1.18)	1.63 (2.34)	1.82 (1.02)	P = .584 $\chi^2(2) = 1.077$
Leptin	21672 (36026)	17425 (24423)	23314 (38957)	P = .836 $\chi^2(2) = .359$	21776 (32484)	17370 (34176)	27212 (46115)	P = .738 $\chi^2(2) = .607$
TNF-α	0.98 (0.75)	1.48 (2.67)	0.96 (1.05)	P = .222 $\chi^2(2) = 3.013$	0.96 (0.73)	1.60 (1.97)	1.02 (.273)	P = .336 $\chi^2(2) = 2.180$
Ghrelin ^	**(n=34)** 94.3 (165.4)	**(n=10)** 97.4 (203.5)	**(n=10)** 50.0 (217.7)	P = .893 $\chi^2(2) = .227$	**(n=34)** 94.4 (142.5)	**(n=15)** 79.4 (158.0)	**(n=5)** 38.9 (286.7)	P = .852 $\chi^2(2) = .321$

IQR – Interquartile Range; n=number of cases in each group; test for statistical significance using Kruskal-Wallis; P < 0.05 considered statistically significant (2-sided test); **red** denotes statistically significance; orange denotes non-significant following correction for false detection rate; ^note for ghrelin serum levels were only available for a subset of patients

6.2 Study 2 (validation study): Review of IL-6 in exploratory cohort

Given the statistically significant findings in the validation study (Study 2) of elevated IL-6 associating with severe scores for the fatigue, cognition and emotional domains of the PBC-40, we obtained the IL-6 data that was generated during by the initial PBC discovery serum proteomics work (paper entitled "The Serum Proteome and Ursodeoxycholic Acid Response in Primary biliary Cholangitis", by Barron-Millar et al)(Barron‐Millar *et al.*, 2021)).

In the discovery proteomics study, IL-6 was noted to show a significant differential expression between study groups (PBC UDCA treatment naïve vs. healthy controls), and remained significantly elevated in UDCA treated PBC patients, but was not (as assessed by log-fold change) elevated to the same degree compared to the final published 19 serum markers (Barron‐Millar *et al.*, 2021). Data on IL-6 exploring the relationship between disease activity and UDCA response was therefore not published in the second PBC proteomics paper (Jones *et al.*, 2022). As IL-6 was not included in the 19 serum markers, it was not initially used in our exploratory study (Study 1).

6.2.1 Study 1 (exploratory study): IL6 and symptoms results

In the study 1 (exploratory study) cohort, a statistically significant positive association between IL-6 and the PBC-40 fatigue domain was observed, when comparing those with "none/mild" symptoms to those with "severe" symptoms (P = .035) and when using the pre-defined clinically significant cut-off scores for fatigue (P = .047). **See Table** *6.21***.** There were no statistically significant associations with IL-6 across any of the other PBC-40 domains, see **Appendix 2**. However, it should be noted that for all the other PBC-40 domains, the number of participants suffering "severe" symptoms was low.

A Spearman's rank-order correlation was run to determine the relationship between age and IL-6. There was a weakly positive correlation between age and IL-6, which was statistically significant ($r_s(278) = .149$, P = .014). Adjusting for age as covariate, using ANOCVA to explore IL-6 levels, resulted in a continued statistically significant relationship between IL-6 and fatigue (none/mild vs. severe: F = 5.710, P = 0.018; clinically significant fatigue: F = 4.831, P = .029). **See Figure** 6.7. There was no observed correlation between weight and serum IL-6 ($r_s(286) = .073$, P = 0.116).

Table 6.21 Serum IL-6 markers (pg/ml) by fatigue domain severity as defined by PBC-40 (none/mild vs. severe) and by fatigue score deemed clinically significant in the exploratory cohort.

Marker	Fatigue Severity					Clinically* Significant Fatigue symptoms?		
	None/Mild (n=155) Median (IQR) pg/ml	**Moderate (n=86)** Median (IQR) pg/ml	**Severe (n=48)** Median (IQR) pg/ml	**Significance None/mild vs. severe**	**Significance none/mild vs. moderate vs. severe**	**No (n=191)** Median (IQR) pg/ml	**Yes (n=98)** Median (IQR) pg/ml	**Significance**
IL-6	2.42 (0.89)	2.56 (0.88)	2.65 (0.75)	P = .035 U = 4469	P = .0.86 $\chi^2(2) = 4.913$	2.42 (0.89)	2.63 (0.80)	P = .047 U = 10697

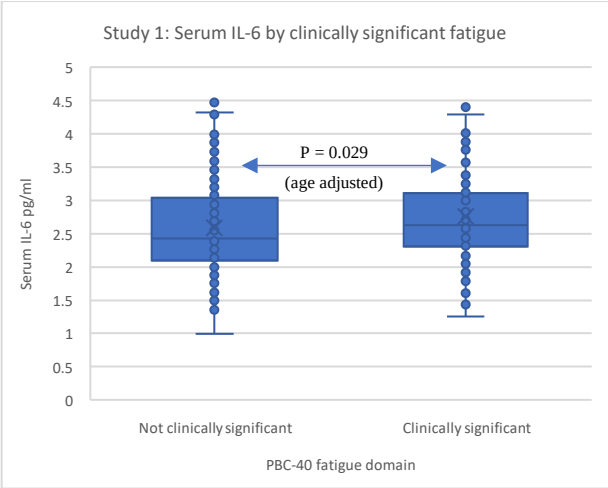


Figure 6.7 Serum IL-6 (pg/ml) relationship with clinically significant fatigue symptoms as defined by the PBC-40 fatigue domain.

6.3 Study 1 (exploratory study) and study 2 (validation study): results summary

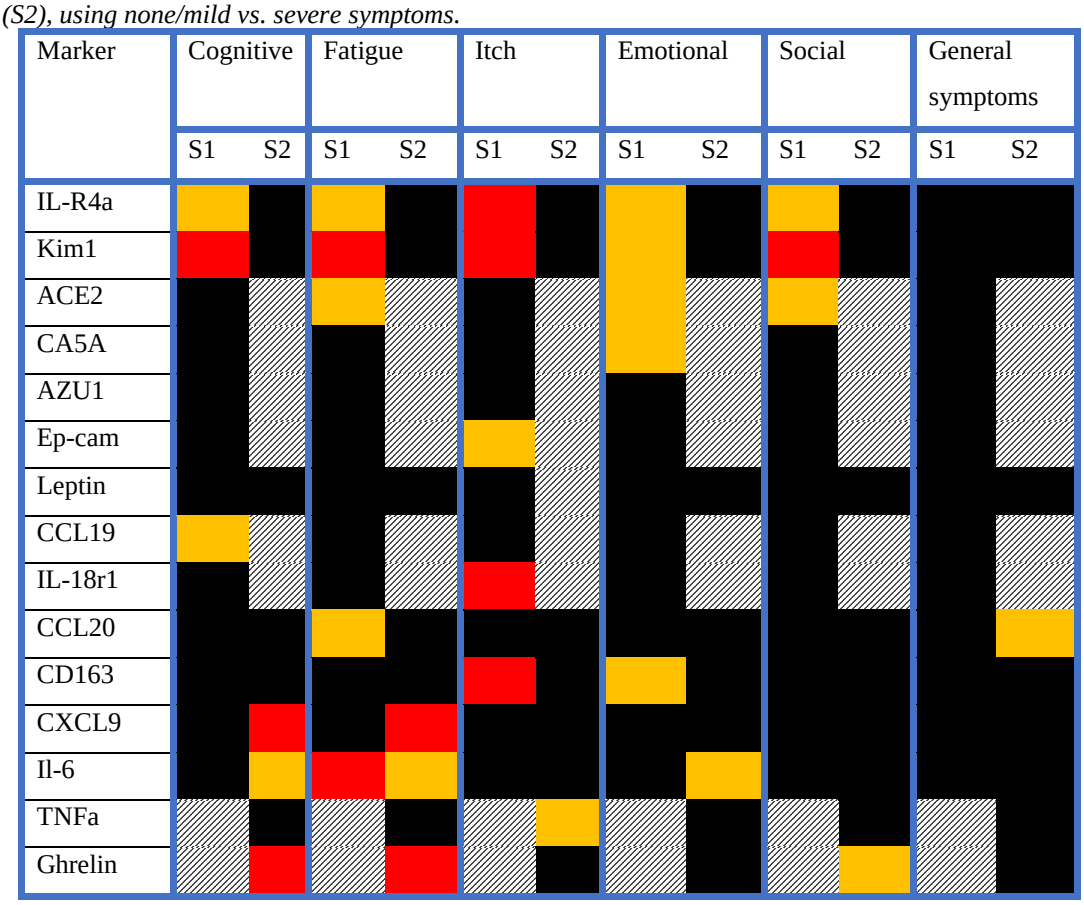
A summary of the overall results for study 1 and study 2 are presented in **Table** 6.22**, Table** 6.23 **and Table** 6.24**.** Study 2 results are discussed in full in **section 6.4**, with an overall discussion for the two studies presented in **Chapter 8:**

Table 6.22: Significant associations between serum analytes and symptom domains across study 1 (S1) and study 2 (S2)

	Category	**Cognition**	**Fatigue**	**Itch**	**Emotional**	**Social**	**General symptoms**
Study 1	None/Mild vs. Severe	IL-4Rα KIM-1 CCL19	IL-4Rα KIM-1 ACE2 CCL20 IL-6	IL-4Rα KIM-1 EP-CAM IL-18R1 CD163	IL-4Rα KIM-1 ACE2 CA5A CD163	IL-4Rα KIM-1 ACE2	
	Clinically significant	IL-4Rα KIM-1	IL-4Rα KIM-1 EPCAM CCL20 CD163 IL-6	IL-4Rα KIM-1 Leptin IL-18R1 CCL20	IL-4Rα ACE2 IL-18R1 CD163	KIM-1 AZU1 VIM	KIM-1 Leptin CCL20
Study 2	None/Mild vs. Severe	CXCL9 IL-6 Ghrelin	CXCL9 IL-6 Ghrelin	TNF-a	IL-6	Ghrelin	CCL20
	Clinically significant	CXCL9 Ghrelin	CXCL9 IL-6 Ghrelin	IL-4Rα	IL-6		TNF-a Ghrelin

Red denotes statistically significant following correction for multiple testing.
Yellow denotes statistically significant result disappeared following correction for multiple testing.

Table 6.23: Significant associations between serum analytes and symptom domain across study 1 (S1) and study 2 (S2), using none/mild vs. severe symptoms.



Red box denotes statistically significant following correction for multiple testing.
Yellow box denotes statistically significant result disappeared following correction for multiple testing.
Black box denotes no statistical significance.
Shaded box denotes analyte not tested in corresponding study.

Table 6.24: Significant associations between serum analytes and symptom domain across study 1 (S1) and study 2 (S2), using clinically significant symptoms.

Marker	Cognitive		Fatigue		Itch		Emotional		Social		General symptoms	
	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
IL-R4a												
Kim1												
ACE2												
CA5A												
AZU1												
Ep-cam												
Leptin												
CCL19												
IL-18r1												
CCL20												
CD163												
CXCL9												
Il-6												
TNFa												
Ghrelin												

Red box denotes statistically significant following correction for multiple testing.
Yellow box denotes statistically significant result disappeared following correction for multiple testing.
Black box denotes no statistical significance.
Shaded box denotes analyte not tested in corresponding study.

6.4 Study 2 (validation study): Discussion

As a whole, the PBC cohort and healthy controls were well balanced in terms of age and gender, and representative of the epidemiology of PBC. However, exploring the PBC by subgroups, i.e., the original cohort they were recruited from, demonstrated a statistically significant reduction in age in the RITPBC cohort (with a difference of a median of 4 years between RITPBC and the UK-PBC and BANC cohorts). Given that RITPBC specifically recruited PBC patients with significant fatigue and given that fatigue is more apparent in younger patients, this finding is not surprising. Given that analytes were tested to see if there was a change with age (with none found to be significant) this is unlikely to have markedly affected the results.

BMI measurements were missing for all the BANC cohort (the largest cohort contributor to the PBC group). For this reason, weight (rather than BMI) was used as a covariate for ghrelin and leptin. No other analytes showed a statistical association with weight.

Other than leptin, ghrelin and IL-13, all other analytes were significantly elevated in the PBC group compared to controls. IL-6 and TNFα were not included in the preceding PBC inflammatory proteome studies that explored the impact of UDCA response and responder status (Barron-Millar *et al.*, 2021; Jones *et al.*, 2022). When stratified by disease status, both were elevated in those with abnormal liver blood tests. However, these associations disappeared when correcting for multiple testing. This might suggest that these proinflammatory chemo/cytokines are elevated in PBC but act independently of biochemical disease activity. A strong association with PBC, leptin and UDCA response was previously identified in the PBC inflammatory proteomics study (Jones *et al.*, 2022), but was not apparent here. It is unclear why this is the case.

KIM-1 and IL-4Rα were both positively associated with cirrhosis in study 2, and this mirrors what was seen in study 1. In total, 13% had an underlying diagnosis of cirrhosis (compared to 7% in study 1), but data on cirrhosis was not available for the RITPBC study. This could have unduly affected some of our findings.

6.4.1 Study 2 (validation study): Clinical characteristics and symptoms

As with study 1, more severe symptoms relating to cognition, fatigue and emotions were prevalent in younger patients. This is particularly striking in the cognitive domain, where the median age for severe cognitive symptoms was 50, compared to 60 for those with none or mild symptoms. This highlights that many patients with cognitive dysfunction and fatigue may be

at an age whereby they are juggling families and careers whilst dealing with these symptoms. Itch was less prevalent and was not associated with a younger age.

6.4.2 Study 2 (validation study): Cognitive and fatigue symptoms

As expected, the PBC group comprised of 3 cohorts, was particularly symptom heavy, with 47% reporting severe fatigue, 33% reporting severe cognitive symptoms, and 61% reporting moderate to severe cognitive symptoms. This may provide more confidence in our findings, given that having a greater number of participants with severe symptoms is likely to give a stronger biochemical signal, if one exists.

For the cognitive symptoms, this resulted in more balanced groups than that seen in study 1, which overall is likely to produce statistically stronger results, by reducing the risk of unequal variance.

A common theme for analytes were observed in the cognitive and fatigue domain with three analytes initially showing significant associations. These three analytes are discussed next.

6.4.2.1 Study 2 (validation study): IL-6

IL-6 demonstrated a positive association for fatigue in both the none/mild vs severe group and clinically significant fatigue, even once adjusted for age. Correcting for multiple testing resulted in the association with clinically significant fatigue remaining a significant finding, whilst in the none/mild vs. severe group, this was considered a false positive. A positive association was found in those with severe cognitive symptoms compared to those with none or mild symptoms, but this was not seen when assessed by clinically significant cognitive symptoms.

Based on this association in study 2 and given evidence for IL-6 in the homeostasis of muscle metabolism, the retrospective application of IL-6 to symptom data in study 1 was undertaken, as described in section 5.2.1. This demonstrated a statistically significant positive correlation between patients with none/mild vs severe symptoms and those with clinically significant fatigue, even when adjusted for age in study 1.

We have demonstrated a statistically significant positive association between IL-6 and fatigue (none/mild vs. severe and clinically significant fatigue) in two distinct PBC cohorts, which

points not only to a potential mechanistic link to fatigue, but also a potential new therapeutic target.

IL-6's role in the development of autoimmune disease and chronic inflammatory disease is well documented (Kimura and Kishimoto, 2010; Houssiau *et al.*, 1988; Yoshizaki *et al.*, 1989; Fragiadaki *et al.*, 2012; Bonifati *et al.*, 1994). Several anti-IL-6 agents exist, including the licenced medications tocilizumab (Burmester *et al.*, 2011; Smolen *et al.*, 2008) and siltuximab (Deisseroth *et al.*, 2015), whilst additional agents including olokizumab (Evgeniy *et al.*, 2022) and clazakizumab (Doberer *et al.*, 2021) are under evaluation. Blockade of the IL-6 receptor by tocilizumab has proven an effective strategy in controlling autoimmune diseases such rheumatoid arthritis (Burmester *et al.*, 2011), castleman disease (Deisseroth *et al.*, 2015), systemic juvenile inflammatory arthritis (Stone *et al.*, 2017), and giant cell arteritis (Stone *et al.*, 2017; Choy *et al.*, 2020).

IL-6 is not only important in the immune response, but also has a significant anti-inflammatory role, contributing to the acute phase response and providing an adaptive response to skeletal muscle exercise (Fischer, 2006; Pedersen and Febbraio, 2008), in particular by maintaining muscle metabolic homeostasis (Febbraio *et al.*, 2004; Febbraio and Pedersen, 2002; Steensberg *et al.*, 2000). **See section 2.3.1.2.** Elevated levels, particularly with aging, have been associated with muscle fatigability (Cesari *et al.*, 2004; Pereira *et al.*, 2009; Oliveira *et al.*, 2008; Coelho *et al.*, 2010; Ferrucci *et al.*, 2002). Prolonged exposure to systemic IL-6 has been shown to increase skeletal muscle fatigability, decrease mitochondrial content and function resulting in a reduction in aerobic respiration and ATP production (VanderVeen *et al.*, 2019; Abid *et al.*, 2020).

Sleep disturbance and daytime somnolence are associated with elevated IL-6 in a large meta-analysis, and females are more vulnerable to sleep disturbance than males (Irwin, Olmstead and Carroll, 2016). Sleep deprived individuals have been shown to experience daytime over secretion and night time under secretion of IL-6 (Vgontzas *et al.*, 1999). Sleep disturbance is common in PBC, whilst the vulnerability of the female gender to disruption of sleep may help to explain the high prevalence in PBC given that 90% of patients are female. Despite this, there was no significant association with ESS and IL-6 observed in this study, although the number of participants who had completed the ESS was low (n=57).

Fatigue, which is common in rheumatoid arthritis, has been shown to significantly improve following treatment with Tocilizumab (Abu-Shakra *et al.*, 2018; Hammer, Agular and Terslev, 2022; Corominas *et al.*, 2019; Gossec *et al.*, 2015; Furst, Thenkondar and Townes, 2012). In particular the TAMARA (Burmester *et al.*, 2011) and OPTION (Smolen *et al.*, 2008) studies demonstrated a rapid improvement during initial treatment, which occurred well before there was a reduction in joint inflammation. In addition, treatment with tocilizumab results in a reduction of sleep disturbance prior to improvements in disease activity (Fragiadaki *et al.*, 2012). Elevated IL-6 has been associated with chronic fatigue syndrome (Nas *et al.*, 2011; Lattie *et al.*, 2012).

Fatigue in RA, much like PBC, is complex. Inflammation has an unclear relationship to fatigue, whilst pain, physical function and depression have all been found to be contributory factors in RA (Nikolaus *et al.*, 2013; Katz, 2017). We must be careful in drawing direct comparisons. However, the findings from this study, linked to the improvements in fatigue in other inflammatory conditions may offer a new potential therapy for our patients.

6.4.2.2 Study 2 (validation study): CXCL9

CXCL9 demonstrated a positive association with both fatigue and cognitive symptoms when assessed by none/mild vs. severe groups, which persisted when assessed by clinically significant symptoms. The positive association with clinically significant fatigue was however considered a false positive when corrected for multiple testing.

CXCL9 had been included in the validation study to enable it to be used in other studies related to this cohort (BANC, RITPBC and UK-PBC). In study 1, the exploratory study, CXCL9 failed to associate with any symptoms. There are two possibilities for this. The first is that the observation of an increase in serum CXCL9 seen in study 2 with cognitive symptoms and fatigue is spurious and has occurred due to random chance. Having tested 11 different serum markers, against 6 different domains, there is a high risk of type I errors. The fact that the elevation is seen across the two domains of fatigue and cognition is perhaps not surprising given that there is considerable overlap between the fatigue and cognitive symptoms, with many patients experiencing both in tandem. This means that the two groups (none/mild vs. severe or clinically significant vs not clinically significant) in each domain will have a degree of overlap. The other possibility is that the association between CXCL9 and fatigue and/or cognitive symptoms seen in study 2 does exist, but that the signal has not been strong enough in study 1, resulting in a type II error. The number of patients in study 1 with severe cognitive

symptoms was only 7% of participants, whilst severe fatigue was present in 16%. In study 2, both symptoms were much more prevalent (25% and 48% respectively). The small number of severe symptoms in study 1 and resultant imbalanced groups could therefore have introduced type 1 errors into this study.

CXCL9 and its associated receptor, CXCR3, are both integral in neuronal-glial interactions (Xia *et al.*, 2000), and CXCL9 is expressed in microvascular endothelial cells and astrocytes, particularly in proinflammatory states, such as seen in multiple sclerosis (MS) (Salmaggi *et al.*, 2002). In relapsing MS, elevated CXCL9 has been detected in cerebral spinal fluid (CSF) which decreases following treatment with the monoclonal anti-integrin antibody, natalizumab (Mellergård *et al.*, 2010). CXCL9, CXCL10 and CXCL11 have all been shown to be elevated during CNS inflammation from infection (Campanella *et al.*, 2008; Klein *et al.*, 2005). Neuroinflammation resulting in disruption of the BBB associated endothelium cells is postulated as a mechanism that results in cognitive symptoms in PBC. See **section 1.4.3.3**. Our findings in study 2 may suggest part of the underlying pathophysiology for neuroinflammation and associated cognitive symptoms in PBC. In addition CXCL9 has been shown to be elevated in a large cross-sectional cohort of older multi-ethnic participants who demonstrated impaired cognitive performance in memory, language, processing speed and executive function (Elkind *et al.*, 2021). In mouse models of Alzheimer's disease, elevated CXCL9 has been associated with plaque formation and poor cognitive performance (Krauthausen *et al.*, 2015; Duan *et al.*, 2008). There is therefore, increasing evidence that CXCL9 can contribute to not only to neuroinflammation, but also impairment in cognitive performance.

Studies into CXCL9 and fatigue are limited. An inverse relationship between CXCL9 (and CXCL10) and aerobic energy metabolism was observed in animal models of chronic obstructive pulmonary disorder (frequently associated with skeletal muscle wasting) (Davidsen *et al.*, 2014). Elevated CXCL9 has been found to be associated with increased muscle fatiguability in older adults (Ramírez-Vélez *et al.*, 2020). However, this was a relatively small, selected population (n=38), of older patients taken from an acute medical ward (although the authors attempted to adjust for the confounder). Interestingly, one study found lower CXCL9 levels in the CSF of patients with atypical CFS (Hornig *et al.*, 2017). The cause for this was unclear but might suggest a different pathophysiology in atypical CFS. The role of CXCL9 in fatigue, particularly its effects on skeletal muscle, is worthy of further exploration.

CXCL9 is implicated in the upstream inflammation seen in PBC, with a high degree of expression in the inflamed portal tracts of PBC patients (Manousou *et al.*, 2013; Ueno *et al.*, 2007). It is associated with UDCA non-response (Jones *et al.*, 2022). In study 2 it has strongly been associated with cognitive and fatigue symptoms but was not seen in study 1. This would be interesting to explore further. Currently there are no immune therapies known to successfully target the CXCR3-CXCL9 axis. However, it is thought that this axis may account for resistance in some cancer immunotherapies (Tokunaga *et al.*, 2018) (anti programmed cell death-1) and therefore research into this is likely to expand.

6.4.2.3 Study 2 (validation study): Ghrelin

Ghrelin demonstrated a strong negative association for cognitive symptoms and fatigue even when corrected for weight. This negative association was present when assessed by none/mild vs. severe and clinically significant symptoms. There were weaker positive associations with social symptoms in the none/mild vs. severe grouping, but this was not clinically significant. Whilst general symptoms showed a negative association in the clinically significant symptom group, both of these disappeared when correcting for multiple testing.

As discussed in **section 2.3.3.2,** its main function is considered as an appetite stimulant that is closely linked to leptin, with an inverse relationship, increasing with fasting, whilst leptin decreases (Inui, 2001). However, ghrelin also appears to induce mitochondrial changes in skeletal muscle (Barazzoni *et al.*, 2005), with higher levels being associated with enhanced mitochondrial redox activities, resulting in improved muscle metabolism (Gortan Cappellari and Barazzoni, 2019). Elevated ghrelin results in over-expression of PPARγ leading to increased mitochondrial cytochrome c oxidase activity, essential to convert ADP to ATP (Barazzoni *et al.*, 2005; Choi *et al.*, 2003). In this study, lower levels of ghrelin were associated with more severe fatigue, and this is therefore in keeping with the described mechanisms of action for ghrelin on skeletal muscle metabolism. That is, lower levels of ghrelin are likely to lead to a reduction in the traditional aerobic pathways in skeletal muscle, essential for the generation of ATP. In PBC, skeletal muscle bioenergetics appear to be impaired, with disordered aerobic pathways and pH recovery following repeated or sustained exercise (Hollingsworth *et al.*, 2008; Goldblatt, 2001; Hollingsworth *et al.*, 2010b). The role of ghrelin in over-expression of PPARγ may be particularly relevant given the role of PPAR agonists in PBC (**see section 1.3.2.2),** however current fibrates do not typically target PPARγ (other than Bezafibrate a PAN-PPAR agonist) and have not been shown to improve fatigue (Corpechot *et al.*, 2018; Honda *et al.*, 2019; Kremer *et al.*, 2022).

In vitro and in vivo studies demonstrate that ghrelin has potent anti-inflammatory effects, binding to endothelial cells and mediating signals that inhibit cellular apoptosis (Baatar, Patel and Taub, 2011). In particular the acylate form inhibits the expression of the pro-inflammatory cytokines IL-1B, IL-6 and TNF-α (Dixit *et al.*, 2004). The significance of elevated IL-6 is discussed in **section 6.4.2.1**. The findings of elevated IL-6, but lower ghrelin associated with fatigue in our study, would therefore be consistent with these previous studies.

Ghrelin can cross the BBB and stimulates the release of GH by GHS (Kojima *et al.*, 1999; Sato *et al.*, 2012). **See section 2.3.3.2**. In addition, ghrelin, along with insulin, insulin-response substrates (IRSs), insulin-like growth factor-I (IGF-I) and glucagon-like peptide-1 (GLP-1) appears to affect neuronal plasticity (synaptic potentiation and depression) as well as neurogenesis (Mainardi, Fusco and Grassi, 2015). Given the importance of glucose homeostasis in the brain and the fact that neurons are high energy-consuming cells the influence of these metabolic hormones is perhaps not surprising. These hormones, including ghrelin, exert an effect on the hippocampus, the major centre involved in memory and learning (Bliss and Collingridge, 1993). Evidence suggests that activation of ghrelin receptor growth hormone secretagogue type 1a (GHS-R1a) by ghrelin in the hippocampus significantly enhances memory retention and spatial learning (Ribeiro *et al.*, 2014; Diano *et al.*, 2006; Carlini *et al.*, 2002; Carlini *et al.*, 2004). In PBC, hippocampal volume reduction has been observed (Mosher *et al.*, 2018) along with a reduction in neurogenesis (Mosher *et al.*, 2018; Hollingsworth *et al.*, 2009). In our study, lower levels of ghrelin were observed in patients with significant cognitive symptoms (which typically affect executive functions memory, concentration, and spatial learning (Mosher *et al.*, 2017; Gee *et al.*, 2023)). Given higher levels of ghrelin enhance memory and spatial learning in the hippocampus, the findings in this study would be consistent with the action of ghrelin on the hippocampus and suggest a mechanistic reason for cognitive symptoms in PBC.

One other important consideration is that sleep disturbance is common in PBC. **See section 1.4.4.2**. Ghrelin has been linked to sleep disturbance (Kim *et al.*, 2009), with lower levels observed in participants with disordered sleep (Motivala *et al.*, 2009; Dzaja *et al.*, 2004; Unger *et al.*, 2011)

Given the observations of lower serum ghrelin levels in those with significant fatigue and cognitive symptoms and our current understanding of the central and peripheral effects of

ghrelin, this is an area that should be explored further. Unfortunately ghrelin samples were not included in the initial PBC discovery proteomics work (Barron‐Millar *et al.*, 2021) and we were therefore unable to retrospectively explore this data using the study 1 cohort. However, given that study 1 is comprised from the UK-PBC cohort, further aliquots of original serum would be available to analyse, and this could be used to validate our findings from study 2.

6.4.3 Study 2 (validation study): Itch

In comparison to study 1, fewer participants had clinically significant itch (study 1: n=143 (49%), study 2: n=57 (36%)), whilst the percentage of patients with severe itch was similar. The smaller number of participants in study 2 leads it to be more susceptible to unequal variance with resultant type 1 and type 2 errors (study 1: n=30 (10%), study 2: n=14 (%)). These results should therefore interpretated with caution.

Two serum markers showed significance on initial tests. IL-4Rα showed a positive association when assessing clinically significant symptoms, but not when comparing none/mild vs. severe groups. IL-4Rα had showed positive associations in study 1 when assessed using none/mild vs. severe groupings and clinically significant symptoms. This positive association with itch disappeared on correction for multiple testing using FDR. However, given that a statistically significant positive association was seen in study 1, it is not unreasonable to consider that the association found in study 2 remains scientifically significant.

6.4.3.1 Study 2 (validation study): IL-4Rα

The role of IL-4Rα in the immune system and PBC is discussed in **section 2.3.1.1 and section 5.2.9.2.**

It is difficult to postulate a mechanism by which IL-4Rα contributes to cholestatic itch as studies on this are sparse. Atopic dermatitis is an immune mediated inflammatory skin condition that is predominantly driven by type 2 immunity (Chiricozzi *et al.*, 2020). Whilst having significant differences to cholestatic itch (including disruption of the epidermal barrier resulting in eczematous eruptions), it does share some similarities to cholestatic itch; anti-histamines are not effective (He, Feldman and Fleischer, 2018), and itch severity follows a circadian cycle (typically being worse at night) (Fishbein *et al.*, 2015). IL-31 is considered a critical mediator of itch via central pathways in atopic dermatitis (Meng *et al.*, 2018), and elevated IL-31 has been shown to correlate with degree of pruritus in PBC and PSC patients with cholestatic itch due to increased hepatocyte expression (Xu *et al.*, 2022a). IL-4, IL-13 and

IL-31 pathways are considered essential factors in the pathogenesis of atopic dermatitis (Dubin, Del Duca and Guttman-Yassky, 2021). IL-31 is expressed by Th2 cells and is induced by IL-4, via IL-4Rα (Stott *et al.*, 2013). It maybe that these two conditions exhibit some common immune pathways that results in the central perception of itch.

Several therapies that target the effect of IL-4 and IL-13 on IL-4Rα are either licensed or undergoing phase II/III trials for use in a variety of immune-mediated diseases, including rheumatoid arthritis (Iwaszko, Biały and Bogunia-Kubik, 2021; Burmester *et al.*, 2018; Dhillon, 2017), psoriatic arthritis (Berekmeri *et al.*, 2018), asthma (Castro *et al.*, 2018), chronic rhinosinusitis, atopic eczema (Simpson *et al.*, 2016) and ulcerative colitis (Sandborn *et al.*, 2017). These therapies include Dupilumab (Harb and Chatila, 2020), a monoclonal antibody which blocks IL-4Rα; Upadacitinib (Burmester *et al.*, 2018) and tofacitinib (Dhillon, 2017; Sandborn *et al.*, 2017; Berekmeri *et al.*, 2018), both of which are small molecule Janus Kinase (JAK) inhibitors and block the downstream effect of IL-4R; Pitrakinra, a dual IL-4 and IL-13 antagonist (Antoniu, 2010), and Lebrikizumab, a monoclonal antibody that selectively blocks IL-13 (Silverberg *et al.*, 2023).

Given the consistent findings from study 1 and study 2 of elevated IL-4Rα with cholestatic itch this is an important area to explore further. There is limited experience in the use of these therapies in liver disease, but Dupilumab is currently undergoing a phase II open-label, 22-week, exploratory study to investigate its efficacy in the treatment of adults with moderate to severe chronic hepatic pruritus with a primary completion estimated in September 2023 (Trial ID: NCT04256759; see: https://www.clinicaltrials.gov/ct2/show/NCT04256759). In addition, given the described IL-4/IL-31 pathways, and elevated IL31 levels in cholestatic itch and atopic dermatitis it would be interesting to correlate IL-31 levels to itch in our cohorts.

6.4.4 Study 2 (validation study): Serum analytes detection

An important aspect to note with our serum analyses are the limitations in relation to the lower limits of detection (LLOD). In total, 120 samples for IL-4 and 118 samples for IL-13 were reported at the threshold for LLOD, meaning that for IL-4, 0.080pg/ml was the lowest level that could be reliably detected, whilst for IL-13 this was 0.210 pg/ml. This is likely a reflection of the precision of the equipment used. Therefore, no meaningful data can be determined from the IL-4 and IL-13 assays. For all other assays, the LLOD occurred in 5% or fewer of cases.

Another important finding was that following subgroup analysis, ghrelin levels were significantly lower in patients from the RITPBC group compared to the BANC and UK-PBC groups (median 79.4pg/ml, vs. 538.5 and 617.4 respectively). It is difficult to account for these differences. Given that the RITPBC study, with the inclusion criteria requiring all participants to report significant peripheral fatigue, it could be that the observed lower ghrelin levels may be a reflection of the severity of fatigue in this group. Ghrelin typically increases following fasting. However, patients were not asked to fast prior to any of their RITPBC trial visits. Another possibility could be a technical issue with the stored serum samples from the RITPBC study. However, if this were the case then we would have expected other significant differences would occur across analytes. Variations between TNFα and CXCL9 in the RITPBC did occur, but not to the degree observed with ghrelin.

6.4.5 Study 2 (validation study): Serum analytes selection

The selection of six serum proteomic markers for further analysis from the exploratory study (Study 1) – IL-4Rα, CXCL9, CCL20, CD163, KIM-1, and Leptin – was driven by their correlation with observed symptoms, our current understanding of relevant biological pathways and symptomology, and the practical considerations of measuring these markers in serum. Regrettably, IL-18R1 and ACE2, which also showed promise in Study 1, could not be included due to technical limitations related to multiplex panel compatibility, the required serum volume, and the associated processing costs.

Building upon this, Study 2 incorporated an additional five analytes (IL-4, IL-13, IL-6, TNF-α, and ghrelin) based on a literature review highlighting their involvement in fatigue, cognitive symptoms, and itch, alongside an assessment of their measurability. A more comprehensive discussion of the rationale behind their inclusion in Study 2 is provided in **section 5.2.9.**

If not restricted by the forementioned factors, then it would have been informative to include an analysis in studies 1 and 2 for IL-31 (given its connection to the IL-4 and IL-13 pathway and recent association with pruritus in PBC and PSC); ghrelin, growth hormone (GH), insulin-like growth factor-I (IGF-I) and glucagon-like peptide-1 (GLP-1) due to the evidence for their relationship to neuronal plasticity; CCR6 (due to its role in the CCR6-CCL20 axis) and CXCR3 (part of the CXCR3-CXCL9 axis) due to their involvement in the pathogenesis of PBC, as well evidence for fatigue in other diseases.

Chapter 7: Study 3 Allopregnanolone: Results

7.1 Study 3 (allopregnanolone)

A total of 160 samples were successfully analysed: 120 with a probable/definite diagnosis of PBC from the UK-PBC, and BANC cohorts, and 40 healthy controls from the E-AILD cohort. However, one sample from the healthy controls was excluded from further analysis due to significant variance.

7.1.1 Study 3 (allopregnanolone): Demographics and clinical characteristics

The basic demographics of the two groups are summarised in **Table** 7.1**.** There was no significant difference in age between PBC patients and healthy controls (median 59 vs. 60 years; P = 0.33) or gender (U=2236.5, P = 0.35) and within the PBC sub-cohorts there was no significant difference in age (U = 1381, P = 0.851). Given the small number of males in both the healthy controls and PBC cohort, no sub-analysis was performed between gender and age. The gender split reflects that seen in the general PBC population (113/120 (94%) female, with the healthy control group being both age- and gender-matched (36/40 (90%) females).

In the healthy control group, age was not significantly associated with allopregnanolone levels (r = -0.21, P = 0.21). However, in the PBC group, a younger age was significantly associated with higher serum allopregnanolone levels (r= -0.53, P < 0.001). **See Table** 7.1 **and Figure** 7.1**.**

Table 7.1 Allopregnanolone levels (ng/ml) by, group, gender, and age for Study 3

Group	**Gender**	**Number**	**Age (IQR)**	**Significance between cohort ages (males and females)**	**Median Allopregnanolone ng/ml (IQR)**	**Significance between allopregnanolone and age in each cohort**
Healthy	Male	4 (10%)	60 [20]		0.042 (0.088)	
	Female	35 (90%)	60 [15]		0.030 (0.023)	
	Both	39	60 (15)	P = .33	0.030 (0.025)	r(39) = -0.21 p = 0.21
PBC	Male	7 (5.8%)	59 [18]	U = 2096	0.025 (0.045)	
	Female	113 (94.2%)	59 [13]		0.032 (0.042)	
	Both	120	59 (13)		0.031 (0.042)	r(120) = -0.53 p < 0.001

Median (Interquartile Range);* test for statistical significance Mann-Whitney U and Spearman's rho; P < 0.05 considered statistically significant (2-sided test); **red denotes statistically significance

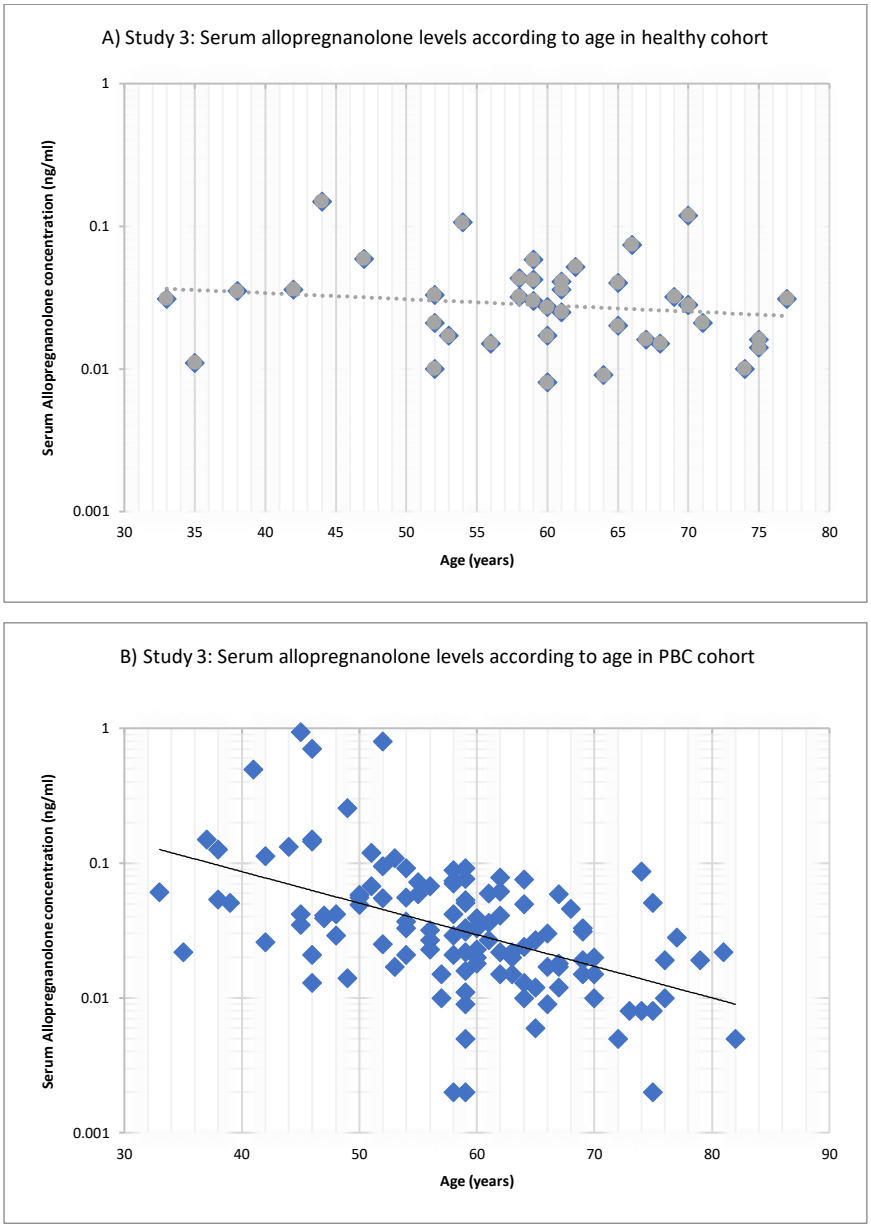


Figure 7.1 Serum allopregnanolone levels (ng/ml) according to age A) Healthy cohort B) PBC cohort

The clinical characteristics of the PBC cohort and its sub-cohorts, are detailed in **table 6.2**. For the BANC cohort, biochemical and cirrhosis data were unavailable for 10 participants that were recruited in Birmingham. In addition, BMI data was not available for the BANC cohort as height was not recorded as part of the standard data collection. Treatment with UDCA status was unknown in 9% of participants and 19% were not treated with UDCA. Only 30% were on a therapeutic dose of UDCA, 47% were subtherapeutic, and in 23% of cases the dose was unknown. The majority of participants were POISE responders (72%), but only 38% of cases had normal LFTs. **See Table** 7.2**.**

Table 7.2 Clinical characteristics of the PBC study population, including subgroups for Study 3

	All PBC (n=120)	**PBC Subgroup**	
		BANC (n=90)	**UK-PBC (n=30)**
Median weight, kg (IQR)	76 (22.1)	75 (22.8)	76 (16.8)
Median BMI, kg/m^2(IQR)	29 (6.6)	No data	29 (6.6)
UDCA treated, Number (%)	Y = 86 (72) N = 23 (19) Unknown = 11 (9)	Y = 78 (8) N = 1 (1) Unknown = 11 (12)	Y= 8 (27) N = 22 (73)
UDCA therapeutic, Number (%)	Y = 37 (30) N = 56 (47) Unknown = 27 (23)	Y = 27 (30) N = 46 (51) Unknown = 17 (19)	Y = 10 (33) N = 10 (33) Unknown = 10 (33)
Median ALP (IQR) IU/L n = 110	143 (127)	135 (114)	174 (141)
Median ALT (IQR) IU/L n = 110	32.5 (31)	27 (28)	37.5 (40)
Median Bilirubin (IQR) umol/L n = 110	7 (4)	7 (4)	9 (8)
Median Albumin (IQR) g/L n = 110	44 (4)	45 (4)	40 (7)
Normal LFT (%) n = 110	42 (38)	34 (38)	8 (26)
POISE Responder, number (%) n = 110	79 (72)	60 (67)	19 (63)
Cirrhosis present number (%) n = 110	14 (11)	6 (7)	8 (26)

IQR – interquartile Range; BMI – body mass index; UDCA – ursodeoxycholic acid; ALP - alkaline phosphatase; ALT – alanine transaminase; LFT – liver function tests; n=number of cases in each group

Serum allopregnanolone levels were analysed according to PBC clinical characteristics. There were no significant differences in serum allopregnanolone levels in those on a therapeutic versus subtherapeutic dose of UDCA, according to POISE responder status, those with abnormal liver function tests or in cirrhotic patients. **See Table** 7.3**.**

Table 7.3 Allopregnanolone (ng/ml) levels, by UDCA therapy, POISE criteria, liver blood tests and cirrhosis for Study 3

Status	**Number of participants**	**Median (IQR) Allopregnanolone ng/ml**	**Significance**
UDCA therapy	93		
Therapeutic dose	37 (40%)	0.033 [0.044]	p = .580 u = 965
Sub-therapeutic dose	56 (60%)	0.031 [0.045]	
POISE Criteria	110		
Responder	79 (72%)	0.025 [0.043]	p = .141 u = 1446
Non-responder	31 (28%)	0.037 [0.49]	
Abnormal LFTs	110		
Normal	42 (38%)	0.022 [0.032]	p = .075 u = 1717
Abnormal	68 (62%)	0.036 [0.048]	
Cirrhosis	110		
Non-cirrhotic	96 (90%)	0.031 [0.041]	p = .098 u = 379
Cirrhotic	14 (10%)	0.012 [0.049]	

POISE criteria: ALP ≤ 1.67 x upper limit normal (ULN) and/or bilirubin ≤ 1 x ULN; LFT: Liver function tests; n=number of cases in each group; analysis using Mann-Whitney-U, P < 0.05 considered statistically significant (2-sided test); **red** denotes statistically significance

7.1.2 Study 3 (allopregnanolone): Clinical status and symptoms

To assess the impact of severity of liver disease on symptoms in PBC, we explored biochemical response (by the POISE criteria and abnormal liver function tests) and cirrhosis status against all the PBC-40 domains, split by “none/mild”, “moderate” and “severe” symptoms. POISE non-responders and those with abnormal liver blood tests were more likely to have moderate to severe itch, but this trend was not seen in those with cirrhosis. Those with cirrhosis were statistically significantly more likely to suffer with fatigue, particularly “moderate” fatigue. No other PBC-40 domains were associated with POISE responder status, liver function tests or cirrhosis status. **See Table** 7.4

Table 7.4 POISE Response, liver bloods tests and Cirrhosis by PBC-40 domain severity for Study 3

PBC-40 Domain	Severity	POISE Response (n=110)			Liver blood tests (n=110)			Cirrhosis (n=110)		
		Responders (n=79)	Non-responders (n=31)	Significance+	Normal (n=42)	Abnormal (n=68)	Significance+	Non-cirrhotic (n=96)	Cirrhotic (n=14)	Significance+
Cognition	**None/mild**	43	15	p = .516 X(1) = 1.324	20	38	p = .410 x(1) = 6.368	51	7	p = .517 X(1) = 3.249
	Moderate	21	7		16	12		25	3	
	Severe	15	9		6	18		20	4	
Fatigue	**None/mild**	25	9	p = .937 X(1) = .152	13	21	p = .961 x(1) = .080	32	2	p = .037 X(1) = 10.21
	Moderate	24	9		12	21		25	8	
	Severe	30	13		17	26		39	4	
Itch	**None/mild**	71	18	p < .001 X(1) = 14.760	39	50	p = .042 x(1) = 6.346	80	9	p = .058 X(1) = 9.143
	Moderate	3	6		1	8		6	3	
	Severe	5	7		2	10		10	2	
Emotional	**None/mild**	35	11	p = .641 X(1) = .888	17	29	p = .276 x(1) = 2.573	40	6	p = .776 X(1) = 1.782
	Moderate	20	8		14	14		25	3	
	Severe	24	12		11	25		31	5	
Social	**None/mild**	41	13	p = .634 X(1) = .912	19	35	p = .596 x(1) = 1.036	49	5	p = .239 X(1) = 5.510
	Moderate	31	15		20	26		40	6	
	Severe	7	3		3	7		7	3	
General Symptoms	**None/mild**	54	19	p = .452 X(1) = 1.590	30	43	p = .464 x(1) = 1.535	65	8	p = .512 X(1) = 3.282
	Moderate	22	9		11	20		27	4	
	Severe	3	3		1	5		4	2	

POISE criteria: ALP ≤ 1.67 x upper limit normal (ULN) and/or bilirubin ≤ 1 x ULN; LFT: Liver function tests; n=number of cases in each group; analysis using Chi-square; P < 0.05 considered statistically significant (2-sided test); **red** denotes statistically significance

7.1.3 Study 3 (allopregnanolone): Age, symptoms, and serum allopregnanolone

We next explored age and symptoms, as assessed by the PBC-40 domains. In keeping with large PBC cohort studies, more severe symptoms were generally associated with a younger age (Mells *et al.*, 2013). In particular, cognitive and emotional symptoms were strongly inversely associated with age, particularly when using the two extreme categorisations, that is "none/mild" and "severe" (cognitive domain: P < .001, emotional domain: P < .001). Itch, social and fatigue symptoms were also more severe in younger patients, although the correlation was less strong, and fatigue lost statistical significance when stratifying by "none/mild", "moderate" and "severe". **See Table** 7.5**.**

*Table 7.5**:** Age (years) PBC-40 domain and symptom severity in Study 3*

PBC-40 Domain	**Severity**	**Number**	**Median Age years (IQR)**	**Significance none/mild vs. moderate vs. severe**	**Significance none/mild vs. severe**
Cognitive	None/Mild	65	60 (13)	P = .003	P < .001
	Moderate	31	59 (10)	$\chi^2(2) = 11.458$	U = 423
	Severe	24	50.5 (12)		
Fatigue	None/Mild	37	60 (10)	P = .126	P = .045
	Moderate	39	59 (15)	$\chi^2(2) = 4.137$	U = 602
	Severe	44	57.5 (13)		
Itch	None/Mild	96	59 (13)	P = .030	P = .011
	Moderate	12	58.5 (9)	$\chi^2(2) = 6.982$	U = 315
	Severe	12	50.5 (13)		
Emotional	None/Mild	52	64 (11)	p < .001	P < .001
	Moderate	29	55 (14)	$\chi^2(2) = 17.617$	U = 536
	Severe	39	57 (12)		
Social	None/Mild	61	64 (19)	P = .043	P = .04
	Moderate	49	58 (14)	$\chi^2(2) = 6.286$	U = 184
	Severe	10	57 (10)		
General symptoms	None/Mild	80	59 (15)	P = .279	P = .6
	Moderate	34	58 (13)	$\chi^2(2) = 2.556$	U = 205
	Severe	6	57.5 (7)		

IQR – denotes interquartile; analysis using Kruskal-Wallis and Mann-Whitney U; P < 0.05 considered statistically significant (2-sided test); **red** denotes statistically significance.

When assessing PBC-40 domains and serum allopregnanolone, the cognitive, itch and emotional domains showed a positive association with more severe symptoms, when assessed using "none/mild" vs. "severe" (cognitive domain: P =.019, itch domain: P = .032, emotional

domain: P = .004). However, only the emotional domain continued to show a positive association when stratified by "none/mild", "moderate" and "severe" symptoms, with increasing serum allopregnanolone levels correlating with increased severity of symptoms. **See Table** 7.6 **and Figure** 7.2

The PBC-40 social domain, having previously not shown significance for "none/mild" vs "severe" symptoms, did demonstrate a significant difference in allopregnanolone levels when the "moderate" group were included. However, it should be noted that the "moderate" group had the highest serum allopregnanolone levels, followed by the "none/mild" group, then "severe" group. **See Table** 7.6

Table 7.6 Serum allopregnanolone by PBC-40 domain and symptom severity in Study 3

PBC-40 Domain	**Severity**	**Number**	**Median allopregnanolone ng/ml (IQR)**	**Significance none/mild vs. moderate vs. severe**	**Significance None/mild vs. severe**
Cognitive	None/Mild	65	0.024 (0.036)	P = .058	P = .019
	Moderate	31	0.029 (0.046)	$\chi^2(2) = 5.710$	U = 1034
	Severe	24	0.042 (0.044)		
Fatigue	None/Mild	37	0.027 (0.035)	P = .496	P = .501
	Moderate	39	0.027 (0.046)	$\chi^2(2) = 1.403$	U = 885
	Severe	44	0.035 (0.047)		
Itch	None/Mild	96	0.031 (0.039)	P = .065	P = .032
	Moderate	12	0.024 (0.045)	$\chi^2(2) = 5.465$	U = 795
	Severe	12	0.050 (0.101)		
Emotional	None/Mild	52	0.024 (0.038)	P = .015	P = .004
	Moderate	29	0.031 (0.046)	$\chi^2(2) = 8.356$	U = 1374
	Severe	39	0.039 (0.056)		
Social	None/Mild	61	0.025 (0.036)	P = .020	P = .643
	Moderate	49	0.039 (0.063)	$\chi^2(2) = 7.781$	U = 277
	Severe	10	0.022 (0.031)		
General symptoms	None/Mild	80	0.029 (0.040)	P = .455	P = 665
	Moderate	34	0.032 (0.048)	$\chi^2(2) = 1.577$	U = 214
	Severe	6	0.026 (0.084)		

n=number of cases in each group; analysis using Kruskal-Wallis; P < 0.05 considered statistically significant (2-sided test); **red** denotes statistically significance

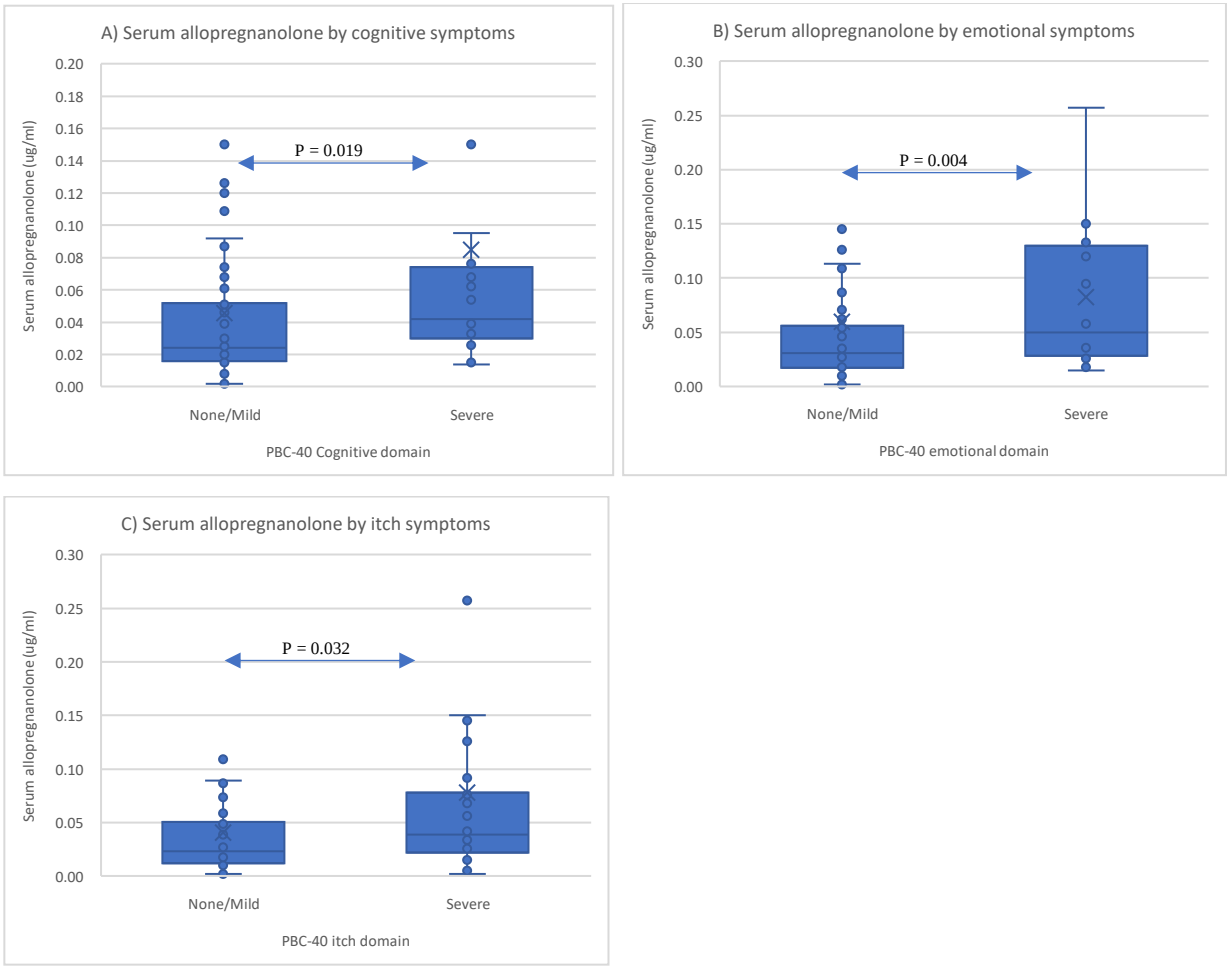


Figure 7.2 Serum allopregnanolone by PBC-40 domain and symptom severity in Study 3 A) Cognitive B) Emotional C) Itch

Further assessment of serum allopregnanolone levels against previously defined PBC-40 cut-off scores considered to be clinically significant (see section 3.4.5) is shown in **Table** 7.7. Using this method, both cognitive and emotional symptoms continued to show a positive association, with higher allopregnanolone levels being significantly associated with clinically significant symptoms. Clinically significant social symptoms also showed a positive association. **See Figure** 7.3**.**

Itch, having shown a positive correlation when comparing "none/mild" to "severe" symptoms, failed to show significance for clinically significant symptoms. However, only 12 participants (10%) were categorised as having "severe" pruritus, whilst 37 (30%) were considered to have clinically significant pruritus where no correlation was found.

Table 7.7 Serum allopregnanolone by PBC-40 domain and clinically significant symptoms in Study 3

PBC-40 Domain	Clinically significant symptoms	number	Median allopregnanolone ng/ml (IQR)	Significance+
Cognitive	Not c/s	71	0.023 (0.036)	P = .044
	c/s	49	0.037 (0.043)	U = 2117
Fatigue	Not c/s	45	0.031 (0.037)	P = 858
	c/s	75	0.031 (0.045)	U = 1654
Itch	Not c/s	83	0.030 (0.037)	P = .506
	c/s	37	0.036 (0.060)	U = 1652
Emotional	Not c/s	81	0.027 (0.036)	P = .010
	c/s	39	0.039 (0.056)	U = 2041
Social	Not c/s	76	0.027 (0.036)	P = .037
	c/s	44	0.040 (0.050)	U = 2055
General symptoms	Not c/s	72	0.026 (0.041)	P = .241
	c/s	48	0.034 (0.040)	U = 1947

n=number of cases in each group; analysis using Mann Whitney U; P < 0.05 considered statistically significant (2-sided test); **red** denotes statistically significance

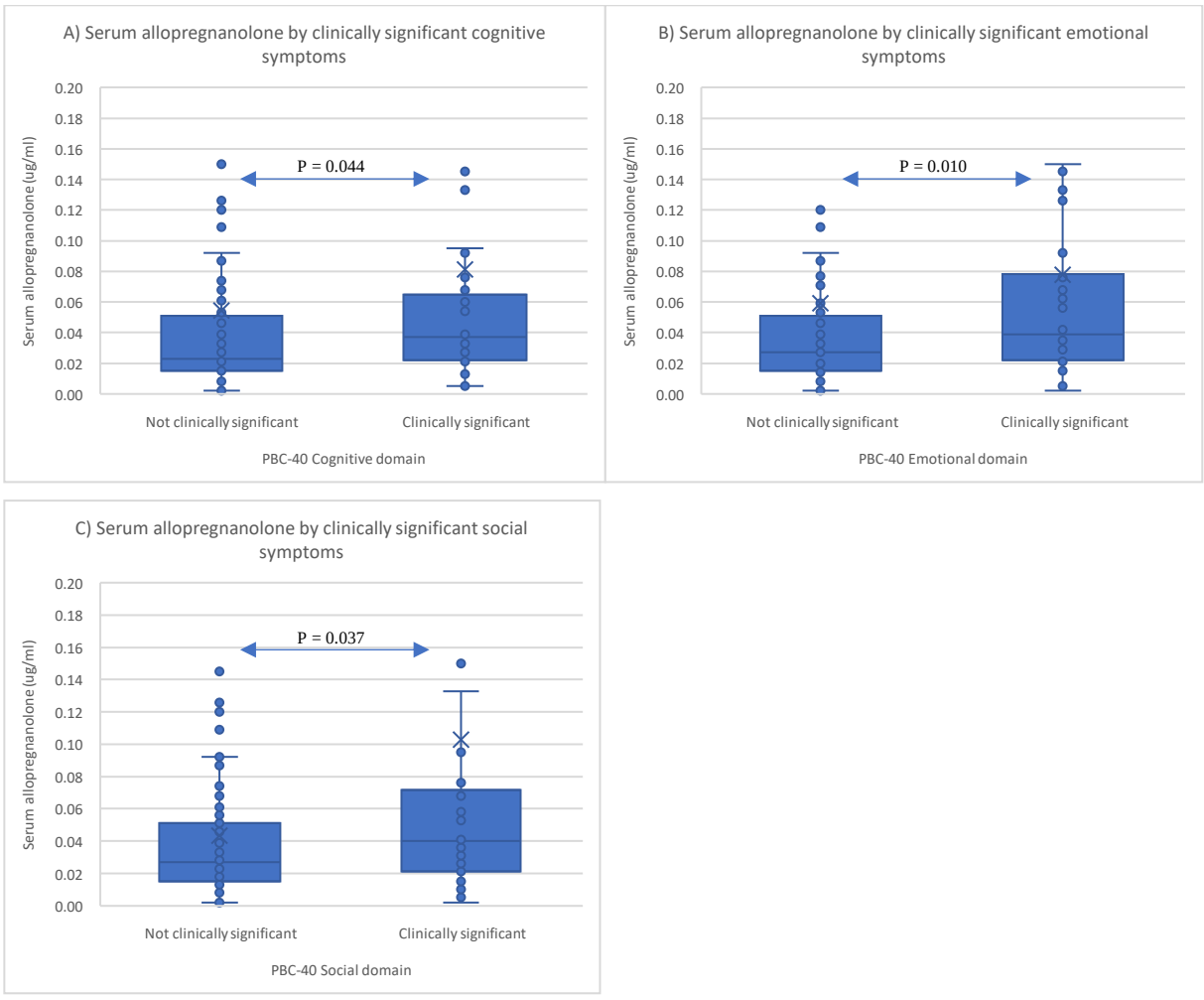


Figure 7.3 Serum allopregnanolone by clinically significant symptoms in Study 3 A) Cognitive B) Emotional C) Social

7.2 Study 3 (allopregnanolone): Discussion

This study has demonstrated an association between elevated serum allopregnanolone levels and increased symptom severity in PBC. Key findings from study 3 were that higher serum allopregnanolone associated with not only a younger age, but also with significant cognitive, emotional and itch symptoms in the PBC group. This may offer new novel therapeutic opportunities, through the application of allopregnanolone antagonists that alter the functioning of GABA-A receptors to improve symptomatic PBC.

7.2.1 Study 3 (allopregnanolone): Clinical characteristics

There were no significant differences between the healthy controls and PBC group in terms of age, with a median age of 59 in the PBC cohort and 60 in the control group. However, in the PBC group higher allopregnanolone levels were significantly associated with a younger age, whilst in the control group, there was no significant change with age or gender, and this is in keeping with previous general population studies in allopregnanolone (Genazzani *et al.*, 1998). **See figure 6.1.** Whilst our findings in the control group mirror that seen in other studies, allopregnanolone levels can vary with gender and physiological stress (Genazzani *et al.*, 1998; Luisi *et al.*, 2000; MacKenzie and Maguire, 2013; Reddy, 2003) (as well as pathological conditions such as seen in mood disorders (Bäckström *et al.*, 2014)). It is worth noting that in the control group the median allopregnanolone levels for males were 0.042ng/ml with a wide IRQ (0.088) compared to the PBC male group median levels of 0.0025ng/ml (IQR 0.045). The number of males in both groups were small (healthy: n=4, PBC: n=7) and it is likely that both these groups were not sufficiently powered and would be strongly influenced by outliers. For this reason, we only explored the significance in allopregnanolone levels between healthy controls and PBC patients as whole, but not by gender.

A younger age was associated with worse liver biochemistry, and non-response to UDCA therapy, which is typical of PBC (Carbone *et al.*, 2013b) where more aggressive disease is seen in younger patients, denoting higher risk disease (Hirschfield *et al.*, 2018). Overall, a younger age was significantly associated with more severe symptoms in all PBC-40 domains, excluding general symptoms, with statistical significance being particularly strong in the cognitive and emotional symptom domains. Only itch was significantly associated with disease status, with more severe itch occurring in non-responders and those with abnormal liver blood tests, in keeping with previous cohort studies (Hegade *et al.*, 2019; Carbone *et al.*, 2013b). Fatigue was associated with cirrhosis, a common phenomenon encountered (Kalaitzakis *et al.*, 2012; Bhandari and Kapoor, 2022). This is in keeping with previous studies in relation to

symptomatic PBC (Mells *et al.*, 2013; Al-Harthy *et al.*, 2010; Forton, 2004; Jones *et al.*, 2010a; Jones, 2006; Jopson and Jones, 2015; Newton *et al.*, 2008b). Crucially, serum allopregnanolone levels were not associated with disease status (responder status, abnormal liver bloods tests, or cirrhosis) suggesting that it is symptoms, not disease severity, that is associated with allopregnanolone levels.

7.2.2 Study 3 (allopregnanolone): Allopregnanolone and symptoms

In our study, significantly elevated allopregnanolone levels were seen in patients with severe cognitive symptoms compared to those with none or mild symptoms. Despite the moderate symptom group having higher median allopregnanolone levels than the none/mild group this failed to reach statistical significance. When stratified by clinically significant cognitive symptoms statistically significant difference was still observed.

Previous studies have demonstrated elevation of the neurosteroids pregnanolone and allopregnanolone in PBC, with a statistically significant relationship observed between allopregnanolone and fatigue (Ahboucha *et al.*, 2008a). We did not demonstrate a relationship with allopregnanolone and fatigue in this study. However, the study undertaken by Albоucha et al. used the FIS to assess fatigue. Unlike the PBC-40, the FIS is not disease specific and includes domains related to physical, cognitive and psychosocial domains, with questions such as “because of fatigue I feel slowed down in my thinking” and therefore assesses both central and peripheral components of fatigue (Fisk *et al.*, 1994). Data on which domains patients scored highly on were not published and therefore it is difficult make comparisons between our study findings and those of Albоucha et al (Ahboucha *et al.*, 2008a). Much of the focus on the role of allopregnanolone in disease has been its effects on the CNS, including its role in mood disorders (Porcu *et al.*, 2012; Walton and Maguire, 2019; Bäckström *et al.*, 2014), memory and learning (Johansson *et al.*, 2002; Johansson *et al.*, 2015; Kask *et al.*, 2008), and hepatic encephalopathy (Ahboucha and Butterworth, 2007; Ahboucha *et al.*, 2008b; Ahboucha *et al.*, 2006; Johansson *et al.*, 2015). It seems likely based on its site and mechanism of action that its effects, and therefore symptoms, are largely central in nature.

Chronic exposure to elevated allopregnanolone has been shown to result in accelerated Alzheimer’s disease (with observed impaired memory and learning), which is associated with increased β-amyloid proteins. (Wang, 2013) β-amyloid proteins are reliable diagnostic markers for disease severity and are associated with the formation of amyloid plaques (Gouras, Olsson and Hansson, 2015). This may be particularly relevant given the observation of white

matter lesions seen on MRI images of PBC patients with cognitive symptoms, distributed in a pattern observed in dementia (Mosher *et al.*, 2017; Kenny, Kalaria and Ballard, 2002; Siennicki-Lantz *et al.*, 1998).

The strongest signal for allopregnanolone was observed in the emotional domain of the PBC-40. Here, elevated allopregnanolone levels were observed in both the moderate and severe groups, with the highest levels observed in the severe group. When assessing by clinically significant emotional symptoms this trend continued. Due to how the clinically significant threshold is set this essentially compared the severe group to the none/mild and moderate group together (see section 3.4.5). The fact that a significant trend continued to be observed when comparing these groups may suggest that the trend for elevated allopregnanolone in emotional symptoms is particularly strong. However, the emotional domain of the PBC-40 is restricted in its assessment of symptoms of depression and anxiety, comprising of only 3 questions. It gives a general overview of patients' emotional state but is not as detailed as other mood-specific screening questionnaires such as the HADS. Unfortunately, this data was not available for this cohort.

Levels of allopregnanolone are controversial in mood disorders. The strongest evidence for allopregnanolone comes from post-partum depression (PPD) where there is a significant increase in allopregnanolone levels during the third trimester and then a rapid fall following birth and during the post-partum period (Bäckström, 2021; Bäckström *et al.*, 2014; Bäckström *et al.*, 2021; Genazzani *et al.*, 1998; Paul, Pinna and Guidotti, 2020; Walton and Maguire, 2019). In some females in the post-partum period, changes in the concentration and distribution of GABA-A receptors, along with rapid reduction in allopregnanolone levels, results in changes in resting-state functional connectivity and is linked to the development of PPD (Bäckström *et al.*, 2014; Deligiannidis *et al.*, 2019). Brexanolone, a synthetic allopregnanolone agonist, is licenced for treatment of severe PPD (Walkery *et al.*, 2021). Yet, there is a paradoxical effect on mood observed with allopregnanolone. During the luteal phase of the menstrual cycle there is an increase in allopregnanolone that has been linked to negative emotions in 3-8% of females, resulting in premenstrual dysphoric disorder (PMDD) (Bäckström *et al.*, 2014) Treatments that antagonise the effect of allopregnanolone are known to alleviate the symptoms of PMDD (Bäckström, 2021). The effect of allopregnanolone on emotions is therefore complex, and likely varies with the concentration and distribution of GABAergic receptors, synthesis of allopregnanolone in both functional and anatomical connections, as well as sites of abnormal pathology, including neuroinflammation.

Altered functional connections between functional regions of the brain involved in memory, concentration, planning, and emotions are well documented in PBC and are evidenced by multiple techniques including MRI and TMS (Mosher *et al.*, 2017; Di Lazzaro, Rothwell and Capogna, 2018; Rossi *et al.*, 2009) (see section 1.4.3.2). Reduced CNS activation is associated with cognitive dysfunction in many conditions (Zwarts, Bleijenberg and Van Engelen, 2008). Importantly, GABA-A receptor modulation is observed using paired pulsed protocols with TMS (Di Lazzaro, Rothwell and Capogna, 2018), and both reduced central activation and abnormal intra-cortical inhibition have been demonstrated using TMS in PBC patients with cognitive dysfunction, fatigue and sleep disturbance. (Mcdonald *et al.*, 2010) These findings support impaired intra-cortical inhibition as a potential cause. Allopregnanolone exerts a positive allosteric action on GABA-A receptors, with increasing levels resulting in increased inhibition of CNS pathways, a concept known as "GABAergic tone" (Cauli *et al.*, 2009). The association of increased allopregnanolone in those with cognitive and emotional symptoms observed in this study could suggest a potential underlying mechanism for central symptoms in PBC.

The association between itch and allopregnanolone was an unanticipated finding in this study. A review of the literature highlights that there is limited research into the effect of serum allopregnanolone and pruritus of any aetiology. Atopic dermatitis mice have been observed to develop marked dose-dependent scratching behaviour following injections of allopregnanolone, which was almost completely reversed following administration of a tryptamine derivative, 2-methyl-5-HT (Fujii *et al.*, 2019). 2-methyl-5-HT is closely related to serotonin and acts as a moderate 5HT3 agonist. Given that SSRIs can be beneficial to some patients with PBC associated itch (Hirschfield *et al.*, 2018), this might provide a partial insight into the mechanism of PBC-associated itch. This is further supported by the fact that allopregnanolone appears to have no influence on histamine induced scratching (Fujii *et al.*, 2019) (anti-histamines having no effect in cholestatic itch (Kremer *et al.*, 2008a)), whilst central mechanisms for itch in PBC are apparent, with associations between itch severity and functional connections of the sensory and premotor cortices having been observed (Mosher *et al.*, 2017).

Mechanisms for elevated allopregnanolone levels in PBC remain unclear. The biosynthesis of neurosteroids occurs de novo within the CNS from cholesterol, which is largely synthesised in the endoplasmic reticulum of hepatic cells and the adrenal glands (Cerqueira *et al.*, 2016).

Fundamental to this process is the rate-limiting transport of cholesterol across the mitochondrial membrane in glial cells by the nucleoside transporter, translocator protein (TSPO) (Jacobs and Tavitian, 2012). After this step, cholesterol is converted to pregnenolone following side chain cleavage by cytochrome P450 (P450scc) within the mitochondria. Several further chemical reductions occur within the glial cell cytoplasm that result in the conversion of progesterone to 5α-dihydroprogesterone, followed by conversion to allopregnanolone (Paul, Pinna and Guidotti, 2020). **See Figure** 2.1**.** Studies demonstrate that TSPO is upregulated in the CNS in response to neuroinflammation, especially in microglial cells (Papadopoulos *et al.*, 2006; Jacobs and Tavitian, 2012), while a mechanism for neuroinflammation has been postulated in PBC patients with central fatigue (Swain and Jones, 2019) and further supported by animal models using BDL that results in disruption of the BBB and hippocampal dysfunction (Gee *et al.*, 2022). It is apparent that the CNS changes that occur in PBC become irreversible, given the lack of improvement in symptoms following liver transplantation (Carbone *et al.*, 2013a; Mcdonald *et al.*, 2010; Pells *et al.*, 2013b). This phenomenon of irreversible cognitive impairment has been replicated in wild type mice receiving continuous allopregnanolone treatment (Bengtsson, Johansson and Backstrom, 2016) and may suggest a mechanism for irreversibility in PBC.

It is worth noting that elevated serum cholesterol is commonly seen in PBC patients (Jahn *et al.*, 1985), but we are not aware that a relationship between cholesterol, allopregnanolone and CNS symptoms has been explored before. Unfortunately, data on our cohort's lipid profiles were not available for this study, but this would be worthy of further future investigation.

Excess accumulation of steroids in the CNS, such as progesterone, can also result in the increased production of allopregnanolone (Paul, Pinna and Guidotti, 2020). Progesterone and oestradiol are both metabolised in the liver, and typically increase in end-stage liver disease. This may contribute to increased allopregnanolone production. However, progesterone is observed to fall again following liver transplantation (Aller *et al.*, 2001), but improvement in cognitive symptoms and fatigue do not typically occur post-transplantation (Carbone *et al.*, 2013a; Mcdonald *et al.*, 2010; Pells *et al.*, 2013b). This suggests that the CNS damage is irreversible. It is important to remember that cognitive symptoms are independent of disease stage or severity in PBC (Mells *et al.*, 2013; Newton *et al.*, 2008a) and whilst it is plausible that elevated pregnanolone secondary to liver dysfunction may contribute to allopregnanolone levels in chronic liver disease, it is unlikely to be the primary mechanism in PBC.

7.2.3 Study 3 (allopregnanolone): Allopregnanolone a potential therapeutic target?

The study findings detailed here and, current evidence supporting the association allopregnanolone with cognitive, emotional and itch symptoms offer a new potential therapeutic target for symptomatic PBC.

The selective antagonist, previously known as GR3027, and subsequently branded as golexanolone, has been postulated to reverse the positive allosteric effect of allopregnanolone on GABA-A receptor, resulting a reduction in GABAergic tone (Johansson *et al.*, 2015). Reversal of allopregnanolone induced dysfunction in spatial learning, memory and motor co-ordination has been demonstrated in rodent models following administration of golexanolone (Johansson *et al.*, 2015).

In human studies, adverse effects on memory, somnolence and saccadic eye velocity (SEV) (strongly controlled by GABAergic transmission and an established objective evaluation of a pharmacological treatment targeting this (Bäckström, Das and Bixo, 2022)) have been observed following administration of allopregnanolone. These effects are reversed following administration of golexanolone (Kask *et al.*, 2008; Johansson *et al.*, 2018). A pilot study using golexanolone in patients with covert hepatic encephalopathy (HE) secondary to cirrhosis demonstrated statistically significant improvements in daytime somnolence and electroencephalogram readings in the delta and theta waves (slowing of which are associated with HE (Amodio and Montagnese, 2015)) compared to placebo (Montagnese *et al.*, 2021). Crucially, golexanolone was found to be safe and well tolerated.

Other support for allopregnanolone antagonism as a therapeutic option for CNS disorders, include isoallopregnanolone, an isomer of allopregnanolone, but without any functional effects on GABA-A receptors (Gyermek, Iriarte and Crabbe, 1968). Following administration of allopregnanolone in healthy female volunteers, a decrease in SEV and sedative effects were again observed, with reversal of these following administration of isoallopregnanolone (Bengtsson *et al.*, 2015). Isoallopregnanolone (in a subcutaneous preparation known as sepranolone) was subsequently shown in a phase II RCT to reduce symptoms of PMDD significantly when compared to placebo, and was safe and well tolerated (Bäckström, Das and Bixo, 2022; Bäckström *et al.*, 2021). Further trials are pending, as are trials exploring the use of sepranolone in menstrual associated migraines and Tourette syndrome (Bäckström, Das and Bixo, 2022).

Given the emerging evidence for allopregnanolone antagonists that target GABA-A receptors, reduce GABAergic tone, and improve symptoms related to mood, somnolence and cognitive symptoms, a further study should be undertaken to confirm the findings presented here. If validated, then a proof-of-concept trial to evaluate the effectiveness of allopregnanolone antagonists, such as golexanolone, on symptomatic PBC should be explored.

7.2.4 Study 3 (allopregnanolone): limitations

The limitations in study 3 mainly relate to potential confounding factors that can affect allopregnanolone levels. Fluctuations in levels are apparent during normal physiological conditions, including the menstrual cycle, pregnancy (including post-partum period) and the menopause, whilst acute stress, depression and social isolation are also associated with changes in allopregnanolone (Paul, Pinna and Guidotti, 2020; Turkmen *et al.*, 2011b). Given the epidemiology of PBC, it is likely that the majority of female patients in this cohort would have entered the menopause/post-menopause era of their lives. However, we did not control for this and additional data on these potential influences were not available. Data on concomitant medications, such as SSRIs, antipruritic treatments etc. were not available, which may have influenced allopregnanolone levels and/or symptomatology. The study could not draw conclusions on gender influence in allopregnanolone levels and/or symptomatic PBC due to the smaller numbers of males included in the study (in keeping with the epidemiology of PBC).

Exploration of the pre-curser steroids required for the synthesis of allopregnanolone, such as isopregnanolone, as well as the associated degradation molecules were beyond the scope of this project. Previous studies exploring the relationship between symptomatic PBC and neurosteroids are limited but have failed to show a relationship with the pregnanolone metabolites isopregnanolone, epipregnanolone, and tetrahydrodoxycorticosterone (Ahboucha *et al.*, 2008a; Ahboucha *et al.*, 2008c). This would be worthy of further exploration in the future.

Chapter 8: Overall discussion

8.1 PBC and symptoms

Fatigue and cognitive symptoms have a significant detrimental effect on patients QOL, yet despite the significant improvements in disease-modifying therapies for PBC in recent years, there have been no therapeutic advances to treat these symptoms (Wetten, Jones and Dyson, 2021). Traditional treatments for itch are effective in some, but not all, patients. Younger patients classically have more inflammatory and aggressive disease, with an increased risk of complications (Quarneti *et al.*, 2015). Typically, symptoms of fatigue, cognition and itch are also worse in younger patients (despite there being no link with disease severity and symptoms). Prognostically, fatigue related to PBC is independently associated with an increased all-cause mortality (Jones *et al.*, 2010a), whilst health utility studies confirm the greatest impact on functional status in PBC comes from fatigue and cognitive symptoms (Rice *et al.*, 2020).

Many therapeutic studies in PBC focus on improvement in liver function tests as their primary outcome, with symptomology often (but not always) being a secondary outcome. These studies improve hard clinical outcomes, including time to death or transplantation, but in general fail to address the significant burden patients experience from symptoms. New therapeutics for managing cholestatic itch, including I-BAT inhibitors (Al-Dury and Marschall, 2018; Hegade *et al.*, 2017), and selective-PPAR agonists, such as seladelpar, show promise ('ENHANCE: Safety and Efficacy of Seladelpar in Patients With Primary Biliary Cholangitis-A Phase 3, International, Randomized, Placebo-Controlled Study,' 2021; Boudes *et al.*, 2018; Bowlus *et al.*, 2022; Kremer *et al.*, 2022; Wetten, Jones and Dyson, 2022), but their mode of action is incomplete and thus ineffective in some patients, while their use can be limited by side effects.

8.2 Potential targets amenable to novel therapeutics to treat symptoms in PBC

In the work presented here, we have identified several potential mechanisms that might contribute to fatigue, pruritus, cognitive and emotional symptoms in PBC. We have consistently demonstrated elevated serum IL-6 in two distinct PBC groups with clinically significant fatigue, whilst elevated IL-4Rα has similarly been identified and validated in PBC patients with significant itch. Elevated allopregnanolone has been associated with central symptoms of cognition and emotional symptoms, in keeping with its known mechanism of action. Ghrelin, an important regulator of energy homeostasis, shows significant associations with central and peripheral fatigue. Crucially, there are novel therapeutics available that can target these pathways.

IL-6 receptor blockade, by monoclonal antibodies such as tocilizumab is licenced in several autoimmune diseases (see section 5.3.2.1) (Burmester *et al.*, 2011; Smolen *et al.*, 2008). Of significance, is the association in rheumatoid arthritis with fatigue, with rheumatoid patients describing "feeling drained" and a significant lack of energy (Nicklin *et al.*, 2010; Stamm *et al.*, 2005), similar to that described by PBC patients. Like PBC, fatigue is not always associated with disease activity in rheumatoid arthritis (Hammer, Agular and Terslev, 2022). Treatment with tocilizumab seems to be effective in treating fatigue in rheumatoid arthritis, with symptoms of fatigue improving rapidly following treatment and well before an improvement in disease activity (Abu-Shakra *et al.*, 2018; Hammer, Agular and Terslev, 2022; Corominas *et al.*, 2019; Gossec *et al.*, 2015; Furst, Thenkondar and Townes, 2012). This suggests that IL-6 may be a significant contributor to fatigue in chronic inflammatory conditions, and given the known effects of IL-6 on skeletal muscle fatiguability and mitochondrial dysfunction (VanderVeen *et al.*, 2019; Abid *et al.*, 2020), the evidence for dysfunctional skeletal muscle metabolism in PBC (Hollingsworth *et al.*, 2008; Hollingsworth *et al.*, 2010b) and the findings in study 1 and 2 of elevated IL-6 associated with fatigue, a proof-of-concept trial evaluating anti-IL-6 therapy in PBC with fatigue should be considered.

Future work from this thesis should also focus on further exploring the link with IL-6, fatigue, and muscle metabolism in PBC. The RITPBC trial collected data on muscle metabolism using novel magnetic resonance spectroscopy as well as data on Anaerobic thresholds (AT), using integrated cardiopulmonary exercise testing on a stationary ergometer. There was not scope in this thesis to explore this data, but this would be worthy of further exploration.

Therapies that block IL-4Rα pathways are already used in clinical practice for many autoimmune diseases, see section 5.3.3.1. Similarities between itch in atopic dermatitis and PBC exist, not only in their clinical manifestation (Fishbein *et al.*, 2015; Bergasa, 2008), but also in the IL-4/IL-31 immunological pathways that have been associated with itch in the two diseases (Meng *et al.*, 2018; Xu *et al.*, 2022a). The anti-IL-4Rα therapy, dupilumab, has proven effective in treating atopic dermatitis (Simpson *et al.*, 2016) and would be worthy of further exploration in managing itch in PBC. It will be interesting to see the results of the phase II open-label study investigating the efficacy of dupilumab in the treatment of adults with chronic hepatic pruritus (Trial ID: NCT04256759; see: https://www.clinicaltrials.gov/ct2/show/NCT04256759). Allopregnanolone was associated with itch in study 3 and interestingly there is an association with elevated allopregnanolone

and scratching behaviour in atopic dermatitis (Fujii *et al.*, 2019), see section 6.2. This again demonstrates biochemical similarities between these two conditions and the reversibility of allopregnanolone in atopic dermatitis by a serotonin agonist might explain why SSRI are effective in some PBC patients with pruritus.

In addition to pruritus, elevated serum allopregnanolone was associated with cognitive and emotional symptoms, in keeping with its known mechanism of action on GABAergic tone (see section 2.5.1). There is increasing interest in the agent golexanolone as a potential treatment for central symptoms (cognition, emotional, sleep disturbance) in PBC. The pharmaceutical company, Umecrine Cognition, have recently completed a phase I clinical trial for golexanolone (unpublished data), whilst recruitment has started to their phase II double-blind randomised control trial evaluating the pharmacokinetics, safety, tolerability and preliminary efficacy of golexanolone in PBC patients (EudraCT number: 2022-000422-16, available at https://www.clinicaltrialsregister.eu/ctr-search/trial/2022-000422-16/HU). This may offer significant hope for PBC patients with cognitive symptoms.

Lower levels of ghrelin were associated with clinically significant fatigue and cognitive symptoms in this study. There is increasing evidence for the role of ghrelin on energy homeostasis, particularly in the hippocampus, where it has a critical impact on memory retention and spatial learning (Ribeiro *et al.*, 2014; Diano *et al.*, 2006; Carlini *et al.*, 2002; Carlini *et al.*, 2004). Its reported inhibitory effects on IL-6 are in keeping with the findings of our study 2 (Dixit *et al.*, 2004). It is important to validate the results from study 2 in a second cohort of PBC patients as if validated, these findings would potentially offer an opportunity for development of a proof-of-concept trial exploring the efficacy of parentally administered ghrelin (considered to have a good safety profile (Garin *et al.*, 2013)) on fatigue and cognitive symptoms in PBC.

8.3 Overall study strengths and limitations

The work presented here has potentially provided insight into partial mechanistic reasons for symptoms in PBC. The overall limitations and strengths common to these three studies are discussed here.

A potential criticism of study 1 and study 2 is that two distinct and separate mechanisms were used in detecting analytes. In study 1, proximity extension assays were undertaken by O-link, using minimal volumes of serum, to detect protein immunoassays. Study 2 used

electrochemiluminescence assay techniques, with custom made MSD-multiplex panels, to identify serum analytes. In some instances, this resulted in marked differences in detection levels between the two studies. For example, in study 1, all analytes were between 2pg/ml and 10 pg/ml, whilst in study 2, most analyte values ranged between 1pg/ml to 2ng/ml, with CD163 going as high as 600ng/ml. Within the individual two studies (study 1 and 2), the concentration of analytes was consistent within cohorts, and whilst different methods were used, it is the trend of individual analytes across symptoms, rather than the absolute values, that these studies have focused on. Nevertheless, given the different detection methods used between studies, care should be taken in these comparisons.

A potential limitation to this study relates to the age of a subgroup of serum samples. The RITPBC trial started recruiting in 2015, therefore some samples used for this study were over 8 years old {Jopson, 2015 #85}. Whilst these samples have been processed, stored, and subsequently thawed using well established techniques within a standardised operating procedure, it is possible that this may have adversely affected the stability of analytes.

In these all 3 studies well-established cohorts were utilised. However, detailed information on co-existing disease, especially other autoimmune disease, along with non-PBC related therapy was not available. These could act as potential cofounders to these studies.

For studies 1 and 2, correction for multiple testing was undertaken using a false discovery rate. This may have increased the risk of type I errors, but this method was selected as it is typically less conservative than the Bonferroni method. Incorporating an exploratory (study 1) and validation (study 2) study increased the robustness of this methodology, by requiring any positive results to be validated in the validation study, thus reducing the risk of type 1 errors. Irrespective of methods used for multiple testing, there also remains a risk of type II errors. i.e., missing truly positive results across the two studies.

All three studies utilised a large cohort of real-life PBC patients. Due to the nature of PBC being a rare disease, this can be problematic in many PBC studies, and this is one of the reasons that these national PBC cohorts were established (Carbone *et al.*, 2013). Study 1 utilised 289 PBC patients, study 2, 160 PBC patients and study 3, 120 PBC patients, whilst study 2 and study 3 used 40 healthy controls. This is a significant number of participants for each study. In addition, the use of an exploratory (study 1) and validation (study 2) study for the serum proteomics strengthens the validity of the finding of these two studies.

Another key strength from these cohorts was that these represented a large cross-sectional cohort of patients in the UK with PBC. The majority of patients used were unselected, with the exception of the RITPBC cohort, which was fatigue heavy, in keeping with RITPBC study protocol (Jopson *et al.*, 2015). Given the unselected nature of the participants used, this suggests that the findings from these studies are more likely to be translatable to clinical practice and real-life PBC patients.

8.4 Conclusion

The lack of effective treatment for the systemic symptoms of PBC is hindered by our lack of understanding of the underlying pathways and mechanisms that results in some patients experiencing devastating symptoms. Addressing these burdens of disease remains a significant unmet need in PBC. The findings presented in these three studies offer evidence for tangible and plausible immunological pathways that may contribute to symptomology in PBC, providing a foundation for the application of several novel therapeutics to target specific pathways, improve symptoms and address these significant unmet needs.

List of abbreviations

Abbreviation	Term
(TGF)-ß	transforming growth factor
5-HT	serotonin
ACE2	angiotensin-converting enzyme 2
ACTH	adrenocorticotrophic hormone
ADP	adenosine diphosphate
AE2	anion exchanger 2
AG	acylated ghrelin
AIH	autoimmune hepatitis
ALP	alkaline phosphatase
AMA	anti-mitochondrial antibodies
AMPK	adenosine monophosphate-activated protein kinase
ANA	anti-nuclear antibodies
APC	antigen presenting cells
ARLD	alcohol related liver disease
ATP	adenosine triphosphate
ATX	autotaxin
AZU1	azurocidin
BANC	Birmingham and Newcastle cohort
BBB	blood brain barrier
BDI	beck depression inventory
BDL	bile duct ligation
BEC	biliary epithelial cells
BMAL1	brain and muscle ARNT-like 1
BMI	body mass index
BRS	baroreflex sensitivity
CA5A	carbonic anhydrases 5A
CCL19	chemokine CC motif ligand 19
CCL20	chemokine CC motif ligand 20
CCL20	chemokine CC motif ligand 20
CCL21	chemokine CC motif ligand 21
CCR6	C-C Chemokine receptor type 6
CCR7	C-C Chemokine receptor type 7
CDCA	chenodeoxycholic acid
CHC	chronic hepatitis C
CLOCK	circadian locomotor output cycles kaput
CNS	central nervous system
COVID-19	coronavirus disease 2019
CRH	corticotropin-releasing hormone
CRP	C-reactive protein
CSF	cerebral spinal fluid
CXCL10	C-X-C motif chemokine ligand 10
CXCL11	C-X-C motif chemokine ligand 11

CXCL9	C-X-C motif chemokine ligand 9
CXCR3	C-X-C Receptor 3
DECR1	2,4-Dienoyl-CoA Reductase 1
DNA	deoxyribonucleic acid
DSM-IV	diagnostic and statistical manual of mental disorders IV
E-AILD	environmental factors in the aetiology of autoimmune Liver disease
EEG	electroencephalogram
EPCAM	Epithelial cell adhesion molecule
ESS	Epworth Sleepiness Scale
FDR	false detection rate
FGF-19	fibroblast growth factor-19
FIS	fatigue impact scale
FXR	farnesoid X receptor
GABA-A	gamma-aminobutyric acid-A
GH	growth hormone
GHS	growth hormone secretagogue
GHS-R1a	ghrelin receptor growth hormone secretagogue type 1a
GI	gastrointestinal
GLP-1	glucagon-like peptide-1
GWAS	genome-wide association studies
HADS	hospital anxiety and depression scale
HAOX1	hydroxy acid oxidase
HAV	hepatitis A virus
HCC	hepatocellular carcinoma
HDL	high density lipoproteins
HLA	human leukocyte antigen
Hp-Hb	haptoglobin-haemoglobin
HPA	hypothalamic-pituitary-adrenal
HRQOL	health-related quality of life
HRQOL	health-Related quality of life
HRV	heart rate variability
IgE	immunoglobulin E
IGF-I	insulin-like growth factor-I
IgG	immunoglobulin G
IL-18	interleukin-18
IL-18BP	IL-18 binding protein
IL-18R1	Interleukin-18 receptor 1
IL-1β	interleukein-1β
IL-4R	Interleukin-4 receptor
IL-4Rα	Interleukin-4 receptor alpha
IL-6	interleukin-6
IL-8	interleukin-8
IMAT	intermuscular adipose tissue
IPC	Inter-plate controls

IQ	intelligence Quotient
IQR	interquartile range
IRSs	insulin-response substrates
JAK	janus kinase
KIM-1	kidney injury molecule-1
LARC	liver and activation-regulated chemokine
LDL	low density lipoproteins
LLOD	lower limit of detection
LOD	limit of detection
LPA	lysophosphatidic acid
LSM	liver stiffness measurement
MAF	multidimensional assessment of fatigue
MAIT	mucosal-associated invariant T
MARS	molecular adsorbent recirculating system
ME	myalgic encephalomyelitis
METAVIR	meta-analysis of histological data in viral hepatitis
MHC	major histocompatibility complex
MIP-3α	macrophage inflammatory protein 3α
MRI	magnetic resonance imaging
MTR	magnetization transfer ratio
MVC	maximum voluntary contraction
NASH	non-alcohol related steatohepatitis
NF-κB	nuclear factor-kappa B
NHP	Nottingham health profile
NICE	national institute of clinical excellence
NK	natural killer
NPX	normalized protein expression
NREM	non-rapid eye movement sleep
OACS-1 and 2	obeticholic acid for the amelioration of cognitive symptoms
OCA	obeticholic acid
OGS	orthostatic grading scale
PBC	primary biliary cholangitis
PCr	phosphocreatine
PCR	polymerase chain reaction
PDC	pyruvate dehydrogenase complex
PEA	proximity extension assay
PER1	period circadian protein homolog 1
PMN	polymorphonuclear leukocytes
PPAR	peroxisome proliferator-activated receptor
PSC	primary sclerosing cholangitis
PSQI	Pittsburgh sleep quality index
PSWQ	Penn state worry questionnaire
PXR	pregnane X receptor
QOL	quality of life
RAS	renin angiotensin system

REM	rapid eye movement
RNA	ribonucleic acid
ROS	reactive oxygen species
rsFC	resting-State Functional Connectivity
rsfMRI	resting-state functional magnetic resonance imaging
SASP	senescence-associated secretory phenotype
SCAMP	secretory carrier membrane proteins
SCN	suprachiasmatic nuclei
SF-36	short form survey 36
SFW	superfund toxic waste
SLE	systemic lupus erythematosus
SRCR	scavenger receptor cysteine-rich
SSRI	selective serotonin reuptake inhibitors
sST2	soluble suppression of tumorigenicity 2
STAT3	signal transducer and activator of transcription 3
STSQ	sleep timing and sleep quality screening questionnaire
SWS	slow wave sleep
TGR5	transmembrane G protein coupled receptor
TIM-1	T-cell immunoglobulin and mucin domain-1
TMS	trans-cranial magnetic stimulation
TNF	tumour necrosis factor
TNF-α	tumour necrosis factor-alpha
TNFR1	tumour necrosis factor receptor 1
TNFR2	tumour necrosis factor receptor 2
TSR	torsion-to-shortening ratio
UDCA	ursodeoxycholic acid
ULN	upper limit of normal
UnAG	unacylated ghrelin
VCTE	vibration-controlled transient elastography
VIM	Vimentin
VLDL	very low-density lipoproteins

Appendix 1.

PBC-40 Questionnaire (coded)

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 Symptoms	Never 5	Rarely 4	Sometimes 3	Most of the time 2	Always 1	
2	I ate or drank only a small amount, and still felt bloated Symptoms	Never 1	Rarely 2	Sometimes 3	Most of the time 4	Always 5	
3	I felt unwell when I drank alcohol Symptoms	Never 1	Rarely 2	Sometimes 3	Most of the time 4	Always 5	Did not apply /never drink alcohol 0

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

4	I had discomfort in my right side Symptoms	Never 1	Rarely 2	Sometimes 3	Most of the time 4	Always 5
5	I had dry eyes Symptoms	Never 1	Rarely 2	Sometimes 3	Most of the time 4	Always 5
6	My mouth was very dry Symptoms	Never 1	Rarely 2	Sometimes 3	Most of the time 4	Always 5
7	I had aches in the long bones of my arms and legs Symptoms	Never 1	Rarely 2	Sometimes 3	Most of the time 4	Always 5

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 Itch	Never 1	Rarely 2	Sometimes 3	Most of the time 4	Always 5	Did not apply/ no itch 0
	I scratched so much I made my skin raw Itch	Never 1	Rarely 2	Sometimes 3	Most of the time 4	Always 5	Did not apply/no itch 0
10	I felt embarrassed because of the itching Itch	Never 1	Rarely 2	Sometimes 3	Most of the time 4	Always 5	Did not apply/no itch 0

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

11	I had to force myself to get out of bed Fatigue	Never 1	Rarely 2	Sometimes 3	Most of the time 4	Always 5
12	I had to have a sleep during the day Fatigue	Never 1	Rarely 2	Sometimes 3	Most of the time 4	Always 5
13	Fatigue interfered with my daily routine Fatigue	Never 1	Rarely 2	Sometimes 3	Most of the time 4	Always 5
14	I felt worn out Fatigue	Never 1	Rarely 2	Sometimes 3	Most of the time 4	Always 5
15	I felt so tired, I had to force myself to do the things I needed to do Fatigue	Never 1	Rarely 2	Sometimes 3	Most of the time 4	Always 5
16	I felt so tired, I had to go to bed early Fatigue	Never 1	Rarely 2	Sometimes 3	Most of the time 4	Always 5
17	Fatigue just suddenly hit me Fatigue	Never 1	Rarely 2	Sometimes 3	Most of the time 4	Always 5
18	PBC drained every ounce of energy out of me Fatigue	Never 1	Rarely 2	Sometimes 3	Most of the time 4	Always 5

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 Fatigue	Never 1	Rarely 2	Sometimes 3	Most of the time 4	Always 5
20	If I was busy one day I needed at least another day to recover Fatigue	Never 1	Rarely 2	Sometimes 3	Most of the time 4	Always 5
21	I had to pace myself for day-to-day things Fatigue	Never 1	Rarely 2	Sometimes 3	Most of the time 4	Always 5

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

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 Emotional	Not at all 1	A little 2	Somewhat 3	Quite a bit 4	Very much 5

29	My sex life has been affected because of PBC Social	Not at all 1	A little 2	Somewhat 3	Quite a bit 4	Very much 5	Does not apply 0
30	Having PBC gets me down Emotional	Not at all 1	A little 2	Somewhat 3	Quite a bit 4	Very much 5	
31	I feel I neglect my family because of having PBC Social	Not at all 1	A little 2	Somewhat 3	Quite a bit 4	Very much 5	Does not apply 0
32	I feel guilty that I can't do what I used to do because of having PBC Social	Not at all 1	A little 2	Somewhat 3	Quite a bit 4	Very much 5	
33	I worry about how my PBC will be in the future Emotional	Not at all 1	A little 2	Somewhat 3	Quite a bit 4	Very much 5	

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 Social	Strongly agree 5	Agree 4	Neither agree nor disagree 3	Disagree 2	Strongly disagree 1
35	I tend to keep the fact that I have PBC to myself Social	Strongly agree 5	Agree 4	Neither agree nor disagree 3	Disagree 2	Strongly disagree 1
36	I can't plan holidays because of having PBC Social	Strongly agree 5	Agree 4	Neither agree nor disagree 3	Disagree 2	Strongly disagree 1
37	My social life has almost stopped Social	Strongly agree 5	Agree 4	Neither agree nor disagree 3	Disagree 2	Strongly disagree 1

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

38	My life is affected by PBC Social	Strongly agree 5	Agree 4	Neither agree nor disagree 3	Disagree 2	Strongly disagree 1

39	PBC has reduced the quality of my life Social	Strongly agree 5	Agree 4	Neither agree nor disagree 3	Disagree 2	Strongly disagree 1
40	I can still lead a normal life, despite having PBC Social	Strongly agree 1	Agree 2	Neither agree nor disagree 3	Disagree 4	Strongly disagree 5

Appendix 2.

Table A2.1. *Serum IL-6 markers (pg/ml) by PBC-40 domain scores none/mild vs severe for study 1.*

PBC-40 Domain	None/mild vs severe	number	Median IL-6 ng/ml (IQR)	Significance+
Cognitive	None/mild	202	2.49 (0.94)	P = .634 U = 2255
	Severe	21	2.55 (0.82)	
Itch	None/mild	200	2.49 (0.92)	P = .484 U = 3238
	Severe	30	2.55 (0.92)	
Emotional	None/mild	194	2.50 (1.01)	P = .390 U = 2784
	Severe	26	2.63 (0.98)	
Social	None/mild	195	2.41 (0.86)	P = .057 U = 2119
	Severe	17	3.05 (1.13)	
General symptoms	None/mild	214	2.44 (0.90)	P = .771 U = 908
	Severe	8	2.52 (0.49)	

Table A2.2. *Serum IL-6 markers (pg/ml) by PBC-40 domain scores deemed clinically significant for study 1.*

PBC-40 Domain	Clinically significant symptoms	number	Median IL-6 ng/ml (IQR)	Significance+
Cognitive	Not c/s	228	2.49 (0.91)	P = .628 U = 7235
	c/s	61	2.55 (0.91)	
Itch	Not c/s	146	2.48 (0.96)	P = .330 U = 11131
	c/s	143	2.55 (0.88)	
Emotional	Not c/s	263	2.53 (0.91)	P = .300 U = 9372
	c/s	26	2.63 (0.97)	
Social	Not c/s	220	**2.46 (0.93)**	P = .057 U = 2119
	c/s	69	**2.61 (0.76)**	
General symptoms	Not c/s	190	2.44 (0.90)	P = .005 U = 11279
	c/s	99	264 (0.78)	

n=number of cases in each group; analysis using Mann Whitney U; $P < 0.05$ considered statistically significant

(emc), e. m. c. (2018) *OCALIVA 5mg film-coated tablets*. Available at: https://www.medicines.org.uk/emc/product/2561/smpc#gref (Accessed: 10th May 2022).

Abella, V., Scotece, M., Conde, J., Pino, J., Gonzalez-Gay, M. A., Gómez-Reino, J. J., Mera, A., Lago, F., Gómez, R. and Gualillo, O. (2017) 'Leptin in the interplay of inflammation, metabolism and immune system disorders', *Nature Reviews Rheumatology,* 13(2), pp. 100-109.

Abid, H., Ryan, Z. C., Delmotte, P., Sieck, G. C. and Lanza, I. R. (2020) 'Extramyocellular interleukin-6 influences skeletal muscle mitochondrial physiology through canonical JAK/STAT signaling pathways', *Faseb j,* 34(11), pp. 14458-14472.

Abu-Shakra, M., Zisman, D., Balbir-Gurman, A., Amital, H., Levy, Y., Langevitz, P., Tishler, M., Molad, Y., Aamar, S., Roser, I., Avshovich, N., Paran, D., Reitblat, T., Mader, R., Savin, H., Friedman, J., Lieberman, N. and Ehrlich, S. (2018) 'Effect of Tocilizumab on Fatigue and Bone Mineral Density in Patients with Rheumatoid Arthritis', *Isr Med Assoc J,* 20(4), pp. 239-244.

Abureesh, M., Alkhayyat, M., Abualnadi, I., Badran, R., Henneberry, J. D., Sadiq, W., Novakovic, V., Barkin, R., Barkin, S. and Deeb, L. S. (2022) 'Epidemiology of Depressive Disorders in Patients With Liver Cirrhosis: A Population-Based Study in the United States', *Prim Care Companion CNS Disord,* 24(1).

Acevedo Ribó, M., Moreno Planas, J. M., Sanz Moreno, C., Rubio González, E. E., Rubio González, E., Boullosa Graña, E., Sanchez-Turrión, V., Sanz Guajardo, D. and Cuervas-Mons, V. (2005) 'Therapy of intractable pruritus with MARS', *Transplant Proc,* 37(3), pp. 1480-1.

Acuña-Castroviejo, D., Escames, G., Venegas, C., Díaz-Casado, M. E., Lima-Cabello, E., López, L. C., Rosales-Corral, S., Tan, D.-X. and Reiter, R. J. (2014) 'Extrapineal melatonin: sources, regulation, and potential functions', *Cellular and Molecular Life Sciences,* 71(16), pp. 2997-3025.

Addison, O., Marcus, R. L., Lastayo, P. C. and Ryan, A. S. (2014) 'Intermuscular Fat: A Review of the Consequences and Causes', *International Journal of Endocrinology,* 2014, pp. 1-11.

Adegbola, S. O., Sahnan, K., Warusavitarne, J., Hart, A. and Tozer, P. (2018) 'Anti-TNF therapy in Crohn's disease', *International journal of molecular sciences,* 19(8), pp. 2244.

Aguilar-Vazquez, A., Chavarria-Avila, E., Salazar-Paramo, M., Armendariz-Borunda, J., Toriz-González, G., Rodríguez-Baeza, M., Sandoval-Rodriguez, A., Villanueva-Pérez, A., Godínez-Rubí, M. and Medina-Preciado, J.-D. (2022) 'Impaired muscle strength is associated with ultrastructure damage in myositis', *Scientific Reports,* 12(1), pp. 17671.

Ahboucha, S. and Butterworth, R. F. (2007) 'The neurosteroid system: an emerging therapeutic target for hepatic encephalopathy', *Metab Brain Dis,* 22(3-4), pp. 291-308.

Ahboucha, S., Butterworth, R. F., Pomier-Layrargues, G., Vincent, C., Hassoun, Z. and Baker, G. B. (2008a) 'Neuroactive steroids and fatigue severity in patients with primary biliary cirrhosis and hepatitis C', *Neurogastroenterol Motil,* 20(6), pp. 671-9.

Ahboucha, S., Jiang, W., Chatauret, N., Mamer, O., Baker, G. B. and Butterworth, R. F. (2008b) 'Indomethacin improves locomotor deficit and reduces brain concentrations of neuroinhibitory steroids in rats following portacaval anastomosis', *Neurogastroenterol Motil,* 20(8), pp. 949-57.

Ahboucha, S., Layrargues, G. P., Mamer, O. and Butterworth, R. F. (2005) 'Increased brain concentrations of a neuroinhibitory steroid in human hepatic encephalopathy', *Ann Neurol,* 58(1), pp. 169-70.

Ahboucha, S., Pomier-Layrargues, G., Mamer, O. and Butterworth, R. F. (2006) 'Increased levels of pregnenolone and its neuroactive metabolite allopregnanolone in autopsied brain tissue from cirrhotic patients who died in hepatic coma', *Neurochem Int,* 49(4), pp. 372-8.
Ahboucha, S., Pomier-Layrargues, G., Vincent, C., Hassoun, Z., Tamaz, R., Baker, G. and Butterworth, R. F. (2008c) 'Reduced plasma dehydroepiandrosterone sulfate levels are significantly correlated with fatigue severity in patients with primary biliary cirrhosis', *Neurochemistry International,* 52(4-5), pp. 569-574.
Akamizu, T., Shinomiya, T., Irako, T., Fukunaga, M., Nakai, Y., Nakai, Y. and Kangawa, K. (2005) 'Separate measurement of plasma levels of acylated and desacyl ghrelin in healthy subjects using a new direct ELISA assay', *The Journal of Clinical Endocrinology & Metabolism,* 90(1), pp. 6-9.
Akberova, D., Kiyasov, I. A., Abdulganieva, D. and Odintsova, A. (2022) 'Changes of Serum Cytokine Levels and Relation to Clinical Specificity in Patients with Primary Biliary Cholangitis', *BioNanoScience,* 12(1), pp. 267-273.
Al-Dury, S. and Marschall, H. U. (2018) 'Ileal Bile Acid Transporter Inhibition for the Treatment of Chronic Constipation, Cholestatic Pruritus, and NASH', *Front Pharmacol,* 9, pp. 931.
Al-Harthy, N., Kumagi, T., Coltescu, C. and Hirschfield, G. M. (2010) 'The specificity of fatigue in primary biliary cirrhosis: Evaluation of a large clinic practice', *Hepatology,* 52(2), pp. 562-570.
Ala, A., Stanca, C. M., Bu-Ghanim, M., Ahmado, I., Branch, A. D., Schiano, T. D., Odin, J. A. and Bach, N. (2006) 'Increased prevalence of primary biliary cirrhosis near superfund toxic waste sites', *Hepatology,* 43(3), pp. 525-531.
Alallam, A., Barth, D. and Heathcote, E. J. (2008) 'Role of Plasmapheresis in the Treatment of Severe Pruritus in Pregnant Patients with Primary Biliary Cirrhosis: Case Reports', *Canadian Journal of Gastroenterology,* 22(5), pp. 505-507.
Albayda, J., Khan, A., Casciola-Rosen, L., Corse, A. M., Paik, J. J. and Christopher-Stine, L. (2018) 'Inflammatory myopathy associated with anti-mitochondrial antibodies: A distinct phenotype with cardiac involvement', *Seminars in Arthritis and Rheumatism,* 47(4), pp. 552-556.
Ali, A. H., Carey, E. J. and Lindor, K. D. (2015) 'Recent advances in the development of farnesoid X receptor agonists', *Annals of translational medicine,* 3(1).
Aller, R., De Luis, D. A., Moreira, V., Boixeda, D., Moya, J. L., Fernandez-Rodriguez, C. M., San Román, A. L., Ávila, S. and Bárcena, R. (2001) 'The effect of liver transplantation on circulating levels of estradiol and progesterone in male patients: Parallelism with hepatopulmonary syndrome and systemic hyperdynamic circulation improvement', *Journal of Endocrinological Investigation,* 24(7), pp. 503-509.
Amodio, P. and Montagnese, S. (2015) 'Clinical neurophysiology of hepatic encephalopathy', *J Clin Exp Hepatol,* 5(Suppl 1), pp. S60-8.
Anacker, C., Zunszain, P. A., Carvalho, L. A. and Pariante, C. M. (2011) 'The glucocorticoid receptor: pivot of depression and of antidepressant treatment?', *Psychoneuroendocrinology,* 36(3), pp. 415-25.
Angermüller, S. (1989) 'Peroxisomal oxidases: cytochemical localization and biological relevance', *Progress in histochemistry and cytochemistry,* 20(1), pp. III-63.
Annual report on liver transplantation: Report for 2019/2020 (2020): NHS Blood and Transplant. Available at: https://nhsbtdbe.blob.core.windows.net/umbraco-assets-corp/19867/nhsbt-liver-transplant-report-1920.pdf (Accessed: 27th January 2021).
Antoniu, S. A. (2010) 'Pitrakinra, a dual IL-4/IL-13 antagonist for the potential treatment of asthma and eczema', *Curr Opin Investig Drugs,* 11(11), pp. 1286-94.

Armstrong, A. and Eck, S. L. (2003) 'EpCAM: A New Therapeutic Target for an Old Cancer Antigen', *Cancer Biology & Therapy,* 2(4), pp. 320-325.
Askgaard, G., Madsen, L. G., von Wowern, N., Winther-Jensen, M., Lau, C. J., Christensen, A. I., Crooks, C., West, J. and Jepsen, P. (2023) 'Social support and risk of mortality in cirrhosis: A cohort study', *JHEP Rep,* 5(1), pp. 100600.
Assarsson, E., Lundberg, M., Holmquist, G., Björkesten, J., Bucht Thorsen, S., Ekman, D., Eriksson, A., Rennel Dickens, E., Ohlsson, S., Edfeldt, G., Andersson, A.-C., Lindstedt, P., Stenvang, J., Gullberg, M. and Fredriksson, S. (2014) 'Homogenous 96-Plex PEA Immunoassay Exhibiting High Sensitivity, Specificity, and Excellent Scalability', *PLoS ONE,* 9(4), pp. e95192.
Baatar, D., Patel, K. and Taub, D. D. (2011) 'The effects of ghrelin on inflammation and the immune system', *Molecular and cellular endocrinology,* 340(1), pp. 44-58.
Bäckström, T. (2021) 'Premenstrual syndrome (PMS) due to allopregnanolone paradoxical effects but possible to treat?', *Maturitas,* 152, pp. 68-69.
Bäckström, T., Bixo, M., Johansson, M., Nyberg, S., Ossewaarde, L., Ragagnin, G., Savic, I., Strömberg, J., Timby, E., Van Broekhoven, F. and Van Wingen, G. (2014) 'Allopregnanolone and mood disorders', *Progress in Neurobiology,* 113, pp. 88-94.
Bäckström, T., Bixo, M. and Strömberg, J. (2015) 'GABAA Receptor-Modulating Steroids in Relation to Women's Behavioral Health', *Current Psychiatry Reports,* 17(11).
Bäckström, T., Das, R. and Bixo, M. (2022) 'Positive GABAA receptor modulating steroids and their antagonists: Implications for clinical treatments', *Journal of Neuroendocrinology,* 34(2), pp. e13013.
Bäckström, T., Ekberg, K., Hirschberg, A. L., Bixo, M., Epperson, C. N., Briggs, P., Panay, N. and O'Brien, S. (2021) 'A randomized, double-blind study on efficacy and safety of sepranolone in premenstrual dysphoric disorder', *Psychoneuroendocrinology,* 133, pp. 105426.
Ballantyne, J. C., Loach, A. B. and Carr, D. B. (1988) 'Itching after epidural and spinal opiates', *Pain,* 33(2), pp. 149-160.
Ballard, C., O'Brien, J., Barber, B., Scheltens, P., Shaw, F., McKeith, I. and Kenny, R. A. (2000) 'Neurocardiovascular instability, hypotensive episodes, and MRI lesions in neurodegenerative dementia', *Ann N Y Acad Sci,* 903, pp. 442-5.
Ban, A., Inaba, M., Furumitsu, Y., Okamoto, K., Yukioka, K., Goto, H. and Nishizawa, Y. (2010) 'Time-course of health status in patients with rheumatoid arthritis during the first year of treatment with infliximab', *Biomed Pharmacother,* 64(2), pp. 107-12.
Banks, W. A. (2005) 'Blood-brain barrier transport of cytokines: a mechanism for neuropathology', *Current pharmaceutical design,* 11(8), pp. 973-984.
Barak, V., Selmi, C., Schlesinger, M., Blank, M., Agmon-Levin, N., Kalickman, I., Gershwin, M. E. and Shoenfeld, Y. (2009) 'Serum inflammatory cytokines, complement components, and soluble interleukin 2 receptor in primary biliary cirrhosis', *J Autoimmun,* 33(3-4), pp. 178-82.
Barazzoni, R., Bosutti, A., Stebel, M., Cattin, M. R., Roder, E., Visintin, L., Cattin, L., Biolo, G., Zanetti, M. and Guarnieri, G. (2005) 'Ghrelin regulates mitochondrial-lipid metabolism gene expression and tissue fat distribution in liver and skeletal muscle', *American Journal of Physiology-Endocrinology and Metabolism,* 288(1), pp. E228-E235.
Barazzoni, R., Cappellari, G. G., Semolic, A., Ius, M., Dore, F., Giacca, M., Zanetti, M., Vinci, P. and Guarnieri, G. (2017) 'Intravenous lipid infusion and total plasma fatty acids positively modulate plasma acylated ghrelin in vivo', *Clinical Nutrition,* 36(3), pp. 775-781.
Barone, F., Gardner, D. H., Nayar, S., Steinthal, N., Buckley, C. D. and Luther, S. A. (2016) 'Stromal Fibroblasts in Tertiary Lymphoid Structures: A Novel Target in Chronic Inflammation', *Frontiers in Immunology,* 7.

Barrington, W. W., Angle, C. R., Willcockson, N. K., Padula, M. A. and Korn, T. (1998) 'Autonomic function in manganese alloy workers', *Environ Res,* 78(1), pp. 50-8.
Barron‐Millar, B., Ogle, L., Mells, G., Flack, S., Badrock, J., Sandford, R., Kirby, J., Palmer, J., Jopson, L., Brain, J., Smith, G. R., Rushton, S., Hegade, V. S., Jones, R., Rushbrook, S., Thorburn, D., Ryder, S., Hirschfield, G., Dyson, J. K. and Jones, D. (2021) 'The Serum Proteome and Ursodeoxycholic Acid Response in Primary Biliary Cholangitis', *Hepatology*.
Bassendine, M. F., Jones, D. E. and Yeaman, S. J. (1997) 'Biochemistry and autoimmune response to the 2-oxoacid dehydrogenase complexes in primary biliary cirrhosis', *Semin Liver Dis,* 17(1), pp. 49-60.
Bearn, J., Allain, T., Coskeran, P., Munro, N., Butler, J., McGregor, A. and Wessely, S. (1995) 'Neuroendocrine responses to d-fenfluramine and insulin-induced hypoglycemia in chronic fatigue syndrome', *Biol Psychiatry,* 37(4), pp. 245-52.
Belelli, D. and Lambert, J. J. (2005) 'Neurosteroids: endogenous regulators of the GABAA receptor', *Nature Reviews Neuroscience,* 6(7), pp. 565-575.
Bell, L. N., Wulff, J., Comerford, M., Vuppalanchi, R. and Chalasani, N. (2015) 'Serum metabolic signatures of primary biliary cirrhosis and primary sclerosing cholangitis', *Liver International,* 35(1), pp. 263-274.
Bellapart, J. and Fraser, J. F. (2009) 'Transcranial Doppler assessment of cerebral autoregulation', *Ultrasound Med Biol,* 35(6), pp. 883-93.
Ben-Ari, Z., Schafer, Z., Sulkes, J., Manhaim, V., Tur-Kaspa, R. and Fainaru, M. (2002) 'Alterations in serum leptin in chronic liver disease', *Dig Dis Sci,* 47(1), pp. 183-9.
Bengtsson, S. K., Johansson, M. and Backstrom, T. (2016) 'Long-term continuous allopregnanolone elevation causes memory decline and hippocampus shrinkage, in female wild-type B6 mice', *Horm Behav,* 78, pp. 160-7.
Bengtsson, S. K., Nyberg, S., Hedström, H., Zingmark, E., Jonsson, B., Bäckström, T. and Bixo, M. (2015) 'Isoallopregnanolone antagonize allopregnanolone-induced effects on saccadic eye velocity and self-reported sedation in humans', *Psychoneuroendocrinology,* 52, pp. 22-31.
Benjamini, Y. and Hochberg, Y. (1995) 'Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing', *Journal of the Royal Statistical Society: Series B (Methodological),* 57(1), pp. 289-300.
Berekmeri, A., Mahmood, F., Wittmann, M. and Helliwell, P. (2018) 'Tofacitinib for the treatment of psoriasis and psoriatic arthritis', *Expert review of clinical immunology,* 14(9), pp. 719-730.
Bergasa, N. V. (2008) 'Pruritus in primary biliary cirrhosis: pathogenesis and therapy', *Clin Liver Dis,* 12(2), pp. 385-406; x.
Bertholdt, L., Gudiksen, A., Schwartz, C. L., Knudsen, J. G. and Pilegaard, H. (2017) 'Lack of skeletal muscle IL-6 influences hepatic glucose metabolism in mice during prolonged exercise', *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology,* 312(4), pp. R626-R636.
Bettelli, E., Carrier, Y., Gao, W., Korn, T., Strom, T. B., Oukka, M., Weiner, H. L. and Kuchroo, V. K. (2006) 'Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells', *Nature,* 441(7090), pp. 235-238.
Beuers, U., Gerken, G. and Pusl, T. (2006) 'Biliary drainage transiently relieves intractable pruritus in primary biliary cirrhosis', *Hepatology,* 44(1), pp. 280-281.
Beuers, U., Kremer, A. E., Bolier, R. and Elferink, R. P. J. O. (2014) 'Pruritus in cholestasis: Facts and fiction', *Hepatology,* 60(1), pp. 399-407.
Bhandari, K. and Kapoor, D. (2022) 'Fatigue in Cirrhosis', *Journal of Clinical and Experimental Hepatology,* 12(2), pp. 617-624.

Biagini, M. R., Tozzi, A., Milani, S., Grippo, A., Amantini, A., Capanni, M., Galli, A. and Surrenti, C. (2008) 'Fatigue in primary biliary cirrhosis: a possible role of comorbidities', *Eur J Gastroenterol Hepatol,* 20(2), pp. 122-6.
Birke, G. and Draguhn, A. (2010) 'No simple brake--the complex functions of inhibitory synapses', *Pharmacopsychiatry,* 43 Suppl 1, pp. S21-31.
Biswal, B., Yetkin, F. Z., Haughton, V. M. and Hyde, J. S. (1995) 'Functional connectivity in the motor cortex of resting human brain using echo-planar MRI', *Magn Reson Med,* 34(4), pp. 537-41.
Bjelland, I., Dahl, A. A., Haug, T. T. and Neckelmann, D. (2002) 'The validity of the Hospital Anxiety and Depression Scale: An updated literature review', *Journal of Psychosomatic Research,* 52(2), pp. 69-77.
Blackburn, P., Freeston, M., Baker, C. R., Jones, D. E. J. and Newton, J. L. (2007) 'The role of psychological factors in the fatigue of primary biliary cirrhosis', *Liver International,* 27(5), pp. 654-661.
Bliss, T. V. and Collingridge, G. L. (1993) 'A synaptic model of memory: long-term potentiation in the hippocampus', *Nature,* 361(6407), pp. 31-9.
Bonfante, H. d. L., Almeida, C. d. S., Abramo, C., Grunewald, S. T. F., Levy, R. A. and Teixeira, H. C. (2017) 'CCL2, CXCL8, CXCL9 and CXCL10 serum levels increase with age but are not altered by treatment with hydroxychloroquine in patients with osteoarthritis of the knees', *International Journal of Rheumatic Diseases,* 20(12), pp. 1958-1964.
Bonifati, C., Carducci, M., Fei, P. C., Trento, E., Sacerdoti, G., Fazio, M. and Ameglio, F. (1994) 'Correlated increases of tumour necrosis factor‐α, interleukin‐6 and granulocyte monocyte‐colony stimulating factor levels in suction blister fluids and sera of psoriatic patients relationships with disease severity', *Clinical and experimental dermatology,* 19(5), pp. 383-387.
Bonventre, J. V. (2009) 'Kidney injury molecule-1 (KIM-1): a urinary biomarker and much more', *Nephrology Dialysis Transplantation,* 24(11), pp. 3265-3268.
Bossen, L., Rebora, P., Bernuzzi, F., Jepsen, P., Gerussi, A., Andreone, P., Galli, A., Terziroli, B., Alvaro, D., Labbadia, G., Aloise, C., Baiocchi, L., Giannini, E., Abenavoli, L., Toniutto, P., Marra, F., Marzioni, M., Niro, G., Floreani, A., Møller, H. J., Valsecchi, M. G., Carbone, M., Grønbæk, H. and Invernizzi, P. (2020) 'Soluble CD163 and mannose receptor as markers of liver disease severity and prognosis in patients with primary biliary cholangitis', *Liver International,* 40(6), pp. 1408-1414.
Bossù, P., Ciaramella, A., Salani, F., Bizzoni, F., Varsi, E., Di Iulio, F., Giubilei, F., Gianni, W., Trequattrini, A., Moro, M. L., Bernardini, S., Caltagirone, C. and Spalletta, G. (2008) 'Interleukin-18 produced by peripheral blood cells is increased in Alzheimer's disease and correlates with cognitive impairment', *Brain Behav Immun,* 22(4), pp. 487-92.
Bouassida, A., Chamari, K., Zaouali, M., Feki, Y., Zbidi, A. and Tabka, Z. (2010) 'Review on leptin and adiponectin responses and adaptations to acute and chronic exercise', *British Journal of Sports Medicine,* 44(9), pp. 620-630.
Boudes, P., Michael, G., Bowlus, C., Hirschfield, G., Jones, D., Odin, J., Sheridan, D., Gitlin, N., Harrison, S., Hassanein, T., Levy, C., Shiffman, M., Thorburn, D., Thuluvath, P. J., Vierling, J., Choi, Y. J., Varga, M., Steinberg, A., McWherter, C. and Martin, R. (2018) 'Pharmacokinetics and pharmacodynamics of seladelpar, a potent and selective PPAR-delta, in patients with primary biliary cholangitis', *Journal of Hepatology,* 68.
Bower, J. E., Ganz, P. A., Irwin, M. R., Arevalo, J. M. G. and Cole, S. W. (2011) 'Fatigue and gene expression in human leukocytes: Increased NF-κB and decreased glucocorticoid signaling in breast cancer survivors with persistent fatigue', *Brain, Behavior, and Immunity,* 25(1), pp. 147-150.
Bowlus, C. L., Galambos, M. R., Aspinall, R. J., Hirschfield, G. M., Jones, D. E. J., Dorffel, Y., Gordon, S. C., Harrison, S. A., Kremer, A. E., Mayo, M. J., Thuluvath, P. J., Levy, C., Swain, M.

G., Neff, G. W., Sheridan, D. A., Stanca, C. M., Berg, C. P., Goel, A., Shiffman, M. L., Vierling, J. M., Boudes, P., Steinberg, A., Choi, Y. J. and McWherter, C. A. (2022) 'A phase II, randomized, open-label, 52-week study of seladelpar in patients with primary biliary cholangitis', *J Hepatol,* 77(2), pp. 353-364.
Bowlus, C. L., Pockros, P. J., Kremer, A. E., Pares, A., Forman, L. M., Drenth, J. P. H., Ryder, S. D., Terracciano, L., Jin, Y., Liberman, A., Pencek, R., Iloeje, U., MacConell, L. and Bedossa, P. (2020) 'Long-Term Obeticholic Acid Therapy Improves Histological Endpoints in Patients With Primary Biliary Cholangitis', *Clin Gastroenterol Hepatol,* 18(5), pp. 1170-1178 e6.
Bowlus, C. L., Trauner, M., Liberman, A., Malecha, E., Macconell, L., Kremer, A. E., Nevens, F. and Floreani, A. (2021) 'Long-term efficacy and safety of obeticholic acid in patients with PBC from the POISE trial grouped biochemically by risk of disease progression', *Digestive and Liver Disease,* 53, pp. S18.
Boyer, J. L. (2013) 'Bile formation and secretion', *Compr Physiol,* 3(3), pp. 1035-78.
Bradley, J. (2008) 'TNF-mediated inflammatory disease', *The Journal of Pathology,* 214(2), pp. 149-160.
Brandt, J., Haibel, H., Cornely, D., Golder, W., Gonzalez, J., Reddig, J., Thriene, W., Sieper, J. and Braun, J. (2000) 'Successful treatment of active ankylosing spondylitis with the anti–tumor necrosis factor α monoclonal antibody infliximab', *Arthritis & Rheumatism: Official Journal of the American College of Rheumatology,* 43(6), pp. 1346-1352.
Breidert, M., Zimmermann, T. F., Schneider, R., Ehninger, G. and Brabant, G. (2004) 'Ghrelin/Leptin-imbalance in patients with primary biliary cirrhosis', *Exp Clin Endocrinol Diabetes,* 112(3), pp. 123-6.
Breuhahn, K., Baeuerle, P. A., Peters, M., Prang, N., Töx, U., Köhne-Volland, R., Dries, V., Schirmacher, P. and Leo, E. (2006) 'Expression of epithelial cellular adhesion molecule (Ep-CAM) in chronic (necro-)inflammatory liver diseases and hepatocellular carcinoma', *Hepatology Research,* 34(1), pp. 50-56.
Brevini, T., Maes, M., Webb, G. J., John, B. V., Fuchs, C. D., Buescher, G., Wang, L., Griffiths, C., Brown, M. L., Scott, W. E., Pereyra-Gerber, P., Gelson, W. T. H., Brown, S., Dillon, S., Muraro, D., Sharp, J., Neary, M., Box, H., Tatham, L., Stewart, J., Curley, P., Pertinez, H., Forrest, S., Mlcochova, P., Varankar, S. S., Darvish-Damavandi, M., Mulcahy, V. L., Kuc, R. E., Williams, T. L., Heslop, J. A., Rossetti, D., Tysoe, O. C., Galanakis, V., Vila-Gonzalez, M., Crozier, T. W. M., Bargehr, J., Sinha, S., Upponi, S. S., Fear, C., Swift, L., Saeb-Parsy, K., Davies, S. E., Wester, A., Hagström, H., Melum, E., Clements, D., Humphreys, P., Herriott, J., Kijak, E., Cox, H., Bramwell, C., Valentijn, A., Illingworth, C. J. R., Dahman, B., Bastaich, D. R., Ferreira, R. D., Marjot, T., Barnes, E., Moon, A. M., Barritt, A. S., Gupta, R. K., Baker, S., Davenport, A. P., Corbett, G., Gorgoulis, V. G., Buczacki, S. J. A., Lee, J.-H., Matheson, N. J., Trauner, M., Fisher, A. J., Gibbs, P., Butler, A. J., Watson, C. J. E., Mells, G. F., Dougan, G., Owen, A., Lohse, A. W., Vallier, L. and Sampaziotis, F. (2023) 'FXR inhibition may protect from SARS-CoV-2 infection by reducing ACE2', *Nature,* 615(7950), pp. 134-142.
Buechler, C., Ritter, M., Orsó, E., Langmann, T., Klucken, J. and Schmitz, G. (2000) 'Regulation of scavenger receptor CD163 expression in human monocytes and macrophages by pro- and antiinflammatory stimuli', *Journal of Leukocyte Biology,* 67(1), pp. 97-103.
Burak, K. W., Le, T. and Swain, M. G. (2002) 'Increased sensitivity to the locomotor-activating effects of corticotropin-releasing hormone in cholestatic rats', *Gastroenterology,* 122(3), pp. 681-8.
Burgess, N., Maguire, E. A. and O'Keefe, J. (2002) 'The human hippocampus and spatial and episodic memory', *Neuron,* 35(4), pp. 625-641.
Burmester, G. R., Feist, E., Kellner, H., Braun, J., Iking-Konert, C. and Rubbert-Roth, A. (2011) 'Effectiveness and safety of the interleukin 6-receptor antagonist tocilizumab

after 4 and 24 weeks in patients with active rheumatoid arthritis: the first phase IIIb real-life study (TAMARA)', *Annals of the rheumatic diseases,* 70(5), pp. 755-759.
Burmester, G. R., Kremer, J. M., Van den Bosch, F., Kivitz, A., Bessette, L., Li, Y., Zhou, Y., Othman, A. A., Pangan, A. L. and Camp, H. S. (2018) 'Safety and efficacy of upadacitinib in patients with rheumatoid arthritis and inadequate response to conventional synthetic disease-modifying anti-rheumatic drugs (SELECT-NEXT): a randomised, double-blind, placebo-controlled phase 3 trial', *The Lancet,* 391(10139), pp. 2503-2512.
Burns, K. and Vandenheuvel, J. (2007) 'Modulation of PPAR activity via phosphorylation', *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids,* 1771(8), pp. 952-960.
Byron, O. and Lindsay, J. G. (2017) 'The Pyruvate Dehydrogenase Complex and Related Assemblies in Health and Disease', *Subcellular Biochemistry*: Springer International Publishing, pp. 523-550.
Cagliani, R., Pozzoli, U., Forni, D., Cassinotti, A., Fumagalli, M., Giani, M., Fichera, M., Lombardini, M., Ardizzone, S., Asselta, R., de Franchis, R., Riva, S., Biasin, M., Comi, G. P., Bresolin, N., Clerici, M. and Sironi, M. (2013) 'Crohn's Disease Loci Are Common Targets of Protozoa-Driven Selection', *Molecular Biology and Evolution,* 30(5), pp. 1077-1087.
Campanella, G. S., Tager, A. M., El Khoury, J. K., Thomas, S. Y., Abrazinski, T. A., Manice, L. A., Colvin, R. A. and Luster, A. D. (2008) 'Chemokine receptor CXCR3 and its ligands CXCL9 and CXCL10 are required for the development of murine cerebral malaria', *Proceedings of the National Academy of Sciences,* 105(12), pp. 4814-4819.
Capuron, L. and Miller, A. H. (2011) 'Immune system to brain signaling: neuropsychopharmacological implications', *Pharmacol Ther,* 130(2), pp. 226-38.
Carbone, M., Bufton, S., Monaco, A., Griffiths, L., Jones, D. E. and Neuberger, J. M. (2013a) 'The effect of liver transplantation on fatigue in patients with primary biliary cirrhosis: A prospective study', *Journal of Hepatology,* 59(3), pp. 490-494.
Carbone, M., Mells, G. F., Pells, G., Dawwas, M. F., Newton, J. L., Heneghan, M. A., Neuberger, J. M., Day, D. B., Ducker, S. J., Consortium, U. P., Sandford, R. N., Alexander, G. J. and Jones, D. E. (2013b) 'Sex and age are determinants of the clinical phenotype of primary biliary cirrhosis and response to ursodeoxycholic acid', *Gastroenterology,* 144(3), pp. 560-569 e7; quiz e13-4.
Carbone, M., Sharp, S. J., Flack, S., Paximadas, D., Spiess, K., Adgey, C., Griffiths, L., Lim, R., Trembling, P., Williamson, K., Wareham, N. J., Aldersley, M., Bathgate, A., Burroughs, A. K., Heneghan, M. A., Neuberger, J. M., Thorburn, D., Hirschfield, G. M., Cordell, H. J., Alexander, G. J., Jones, D. E. J., Sandford, R. N. and Mells, G. F. (2016) 'The UK‐PBC risk scores: Derivation and validation of a scoring system for long‐term prediction of end‐stage liver disease in primary biliary cholangitis', *Hepatology,* 63(3), pp. 930-950.
Carey, E. J., Ali, A. H. and Lindor, K. D. (2015) 'Primary biliary cirrhosis', *The Lancet,* 386(10003), pp. 1565-1575.
Carlini, V. P., Monzón, M. E., Varas, M. M., Cragnolini, A. B., Schiöth, H. B., Scimonelli, T. N. and de Barioglio, S. R. (2002) 'Ghrelin increases anxiety-like behavior and memory retention in rats', *Biochem Biophys Res Commun,* 299(5), pp. 739-43.
Carlini, V. P., Varas, M. M., Cragnolini, A. B., Schiöth, H. B., Scimonelli, T. N. and de Barioglio, S. R. (2004) 'Differential role of the hippocampus, amygdala, and dorsal raphe nucleus in regulating feeding, memory, and anxiety-like behavioral responses to ghrelin', *Biochem Biophys Res Commun,* 313(3), pp. 635-41.
Castle, A. and Castle, D. (2005) 'Ubiquitously expressed secretory carrier membrane proteins (SCAMPs) 1-4 mark different pathways and exhibit limited constitutive trafficking to and from the cell surface', *Journal of Cell Science,* 118(16), pp. 3769-3780.
Castro, M., Corren, J., Pavord, I. D., Maspero, J., Wenzel, S., Rabe, K. F., Busse, W. W., Ford, L., Sher, L., FitzGerald, J. M., Katelaris, C., Tohda, Y., Zhang, B., Staudinger, H., Pirozzi, G.,

Amin, N., Ruddy, M., Akinlade, B., Khan, A., Chao, J., Martincova, R., Graham, N. M. H., Hamilton, J. D., Swanson, B. N., Stahl, N., Yancopoulos, G. D. and Teper, A. (2018) 'Dupilumab Efficacy and Safety in Moderate-to-Severe Uncontrolled Asthma', *New England Journal of Medicine,* 378(26), pp. 2486-2496.
Catalán, D., Mansilla, M. A., Ferrier, A., Soto, L., Oleinika, K., Aguillón, J. C. and Aravena, O. (2021) 'Immunosuppressive Mechanisms of Regulatory B Cells', *Frontiers in Immunology,* 12.
Cauch-Dudek, K., Abbey, S., Stewart, D. E. and Heathcote, E. J. (1998) 'Fatigue in primary biliary cirrhosis', *Gut,* 43(5), pp. 705-710.
Cauli, O., Mansouri, M. T., Agusti, A. and Felipo, V. (2009) 'Hyperammonemia Increases GABAergic Tone in the Cerebellum but Decreases It in the Rat Cortex', *Gastroenterology,* 136(4), pp. 1359-1367.e2.
Cava, A. L. and Matarese, G. (2004) 'The weight of leptin in immunity', *Nature Reviews Immunology,* 4(5), pp. 371-379.
Ceddia, R. B., William Jr, W. N. and Curi, R. (2001) 'The response of skeletal muscle to leptin', *Frontiers in Bioscience-Landmark,* 6(3), pp. 90-97.
Cerqueira, N. M. F. S. A., Oliveira, E. F., Gesto, D. S., Santos-Martins, D., Moreira, C., Moorthy, H. N., Ramos, M. J. and Fernandes, P. A. (2016) 'Cholesterol Biosynthesis: A Mechanistic Overview', *Biochemistry,* 55(39), pp. 5483-5506.
Cerri, G., Cocchi, C. A., Montagna, M., Zuin, M., Podda, M., Cavallari, P. and Selmi, C. (2010) 'Patients with primary biliary cirrhosis do not show post-exercise depression of cortical excitability', *Clinical Neurophysiology,* 121(8), pp. 1321-1328.
Cesari, M., Penninx, B. W. J. H., Pahor, M., Lauretani, F., Corsi, A. M., Williams, G. R., Guralnik, J. M. and Ferrucci, L. (2004) 'Inflammatory Markers and Physical Performance in Older Persons: The InCHIANTI Study', *The Journals of Gerontology: Series A,* 59(3), pp. M242-M248.
Chae, S.-C., Park, Y.-R., Song, J.-H., Shim, S.-C., Yoon, K.-S. and Chung, H.-T. (2005) 'The polymorphisms of Tim-1 promoter region are associated with rheumatoid arthritis in a Korean population', *Immunogenetics,* 56, pp. 696-701.
Chae, S.-C., Song, J.-H., Heo, J.-C., Lee, Y.-C., Kim, J.-W. and Chung, H.-T. (2003) 'Molecular variations in the promoter and coding regions of human Tim-1 gene and their association in Koreans with asthma', *Human immunology,* 64(12), pp. 1177-1182.
Chang, J. C., Go, S., de Waart, D. R., Munoz‐Garrido, P., Beuers, U., Paulusma, C. C. and Oude Elferink, R. (2016) 'Soluble adenylyl cyclase regulates bile salt‐induced apoptosis in human cholangiocytes', *Hepatology,* 64(2), pp. 522-534.
Chappell, M. C. (2012) 'Nonclassical renin-angiotensin system and renal function', *Compr Physiol,* 2(4), pp. 2733-52.
Chen, J., Chen, Y., Liu, D., Lin, Y., Zhu, L., Song, S., Hu, Y., Liang, T., Liu, Y., Liu, W., Weng, L., Li, Q., Ge, S. and Ascherman, D. P. (2022) 'Predictors of long-term prognosis in rheumatoid arthritis-related interstitial lung disease', *Scientific Reports,* 12(1), pp. 9469.
Chen, L., Zhou, T., Wu, N., O'Brien, A., Venter, J., Ceci, L., Kyritsi, K., Onori, P., Gaudio, E., Sybenga, A., Xie, L., Wu, C., Fabris, L., Invernizzi, P., Zawieja, D., Liangpunsakul, S., Meng, F., Francis, H., Alpini, G., Huang, Q. and Glaser, S. (2019) 'Pinealectomy or light exposure exacerbates biliary damage and liver fibrosis in cholestatic rats through decreased melatonin synthesis', *Biochim Biophys Acta Mol Basis Dis,* 1865(6), pp. 1525-1539.
Chen, M., Quan, C., Diao, L., Xue, F., Xue, K., Wang, B., Li, X., Zhu, X., Zheng, J. and Cao, H. (2018) 'Measurement of cytokines and chemokines and association with clinical severity of dermatomyositis and clinically amyopathic dermatomyositis', *British Journal of Dermatology,* 179(6), pp. 1334-1341.
Chen, Q., Lai, L., Chi, X., Lu, X., Wu, H., Sun, J., Wu, W., Cai, L., Zeng, X., Wang, C., Chen, W. and Peng, A. (2020) 'CD19⁺CD24^{hi}CD38^{hi} B

Cell Dysfunction in Primary Biliary Cholangitis', *Mediators of Inflammation,* 2020, pp. 3019378.
Cheng, L., Dong, R., Song, C., Li, X., Zhang, L., Shi, M., Lv, C., Wang, L., Kou, J., Xie, H., Feng, W. and Zhao, H. (2022) 'Mediation Effects of IL-1β and IL-18 on the Association Between Vitamin D Levels and Mild Cognitive Impairment Among Chinese Older Adults: A Case-Control Study in Taiyuan, China', *Front Aging Neurosci,* 14, pp. 836311.
Chiricozzi, A., Maurelli, M., Peris, K. and Girolomoni, G. (2020) 'Targeting IL-4 for the Treatment of Atopic Dermatitis', *Immunotargets Ther,* 9, pp. 151-156.
Choi, K., Roh, S.-G., Hong, Y.-H., Shrestha, Y. B., Hishikawa, D., Chen, C., Kojima, M., Kangawa, K. and Sasaki, S.-I. (2003) 'The role of ghrelin and growth hormone secretagogues receptor on rat adipogenesis', *Endocrinology,* 144(3), pp. 754-759.
Choy, E. H., De Benedetti, F., Takeuchi, T., Hashizume, M., John, M. R. and Kishimoto, T. (2020) 'Translating IL-6 biology into effective treatments', *Nature Reviews Rheumatology,* 16(6), pp. 335-345.
Choy, E. H. S. and Panayi, G. S. (2001) 'Cytokine Pathways and Joint Inflammation in Rheumatoid Arthritis', *New England Journal of Medicine,* 344(12), pp. 907-916.
Chuang, Y. H., Lian, Z. X., Cheng, C. M., Lan, R. Y., Yang, G. X., Moritoki, Y., Chiang, B. L., Ansari, A. A., Tsuneyama, K., Coppel, R. L. and Gershwin, M. E. (2005) 'Increased levels of chemokine receptor CXCR3 and chemokines IP-10 and MIG in patients with primary biliary cirrhosis and their first degree relatives', *J Autoimmun,* 25(2), pp. 126-32.
Coelho, F. M., Narciso, F. M. S., Oliveira, D. M. G., Pereira, D. S., Teixeira, A. L., Teixeira, M. M., Souza, D. G. and Pereira, L. S. M. (2010) 'sTNFR-1 is an early inflammatory marker in community versus institutionalized elderly women', *Inflammation Research,* 59(2), pp. 129-134.
Colquhoun, D. (2014) 'An investigation of the false discovery rate and the misinterpretation of
p
-values', *Royal Society Open Science,* 1(3), pp. 140216.
Columba-Cabezas, S., Elena, B. S. and Aloisi, A., Francesca (2003) 'Lymphoid Chemokines CCL19 and CCL21 are Expressed in the Central Nervous System During Experimental Autoimmune Encephalomyelitis: Implications for the Maintenance of Chronic Neuroinflammation', *Brain Pathology,* 13(1), pp. 38-51.
Comerford, I., Harata-Lee, Y., Bunting, M. D., Gregor, C., Kara, E. E. and McColl, S. R. (2013) 'A myriad of functions and complex regulation of the CCR7/CCL19/CCL21 chemokine axis in the adaptive immune system', *Cytokine & Growth Factor Reviews,* 24(3), pp. 269-283.
consortium, U.-P. *About UK-PBC*. Available at: http://www.uk-pbc.com/about/aboutuk-pbc/ (Accessed: 29 July 2022 2022).
Cordell, H. J., Han, Y., Mells, G. F., Li, Y., Hirschfield, G. M., Greene, C. S., Xie, G., Juran, B. D., Zhu, D. and Qian, D. C. (2015a) 'International genome-wide meta-analysis identifies new primary biliary cirrhosis risk loci and targetable pathogenic pathways', *Nature communications,* 6(1), pp. 8019.
Cordell, H. J., Han, Y., Mells, G. F., Li, Y., Hirschfield, G. M., Greene, C. S., Xie, G., Juran, B. D., Zhu, D., Qian, D. C., Floyd, J. A., Morley, K. I., Prati, D., Lleo, A., Cusi, D., Canadian, U. S. P. B. C. C., Italian, P. B. C. G. S. G., Consortium, U.-P., Gershwin, M. E., Anderson, C. A., Lazaridis, K. N., Invernizzi, P., Seldin, M. F., Sandford, R. N., Amos, C. I. and Siminovitch, K. A. (2015b) 'International genome-wide meta-analysis identifies new primary biliary cirrhosis risk loci and targetable pathogenic pathways', *Nat Commun,* 6, pp. 8019.
Corominas, H., Alegre, C., Narváez, J., Fernández-Cid, C. M., Torrente-Segarra, V., Gómez, M. R., Pan, F. M., Morlà, R. M., Martínez, F. J. R., Gómez-Centeno, A., Ares, L. L., Molina, R. G., González-Albo, S. P., Dalmau-Carolà, J., Pérez-García, C., Álvarez, C. B., Ercole, L. and

Terrancle, M. (2019) 'Correlation of fatigue with other disease related and psychosocial factors in patients with rheumatoid arthritis treated with tocilizumab: ACT-AXIS study', *Medicine (Baltimore),* 98(26), pp. e15947.
Corpechot, C., Abenavoli, L., Rabahi, N., Chretien, Y., Andreani, T., Johanet, C., Chazouilleres, O. and Poupon, R. (2008) 'Biochemical response to ursodeoxycholic acid and long-term prognosis in primary biliary cirrhosis', *Hepatology,* 48(3), pp. 871-7.
Corpechot, C., Carrat, F., Gaouar, F., Chau, F., Hirschfield, G., Gulamhusein, A., Montano-Loza, A. J., Lytvyak, E., Schramm, C., Pares, A., Olivas, I., Eaton, J. E., Osman, K. T., Dalekos, G., Gatselis, N., Nevens, F., Cazzagon, N., Zago, A., Russo, F. P., Abbas, N., Trivedi, P., Thorburn, D., Saffioti, F., Barkai, L., Roccarina, D., Calvaruso, V., Fichera, A., Delamarre, A., Medina-Morales, E., Bonder, A., Patwardhan, V., Rigamonti, C., Carbone, M., Invernizzi, P., Cristoferi, L., Van Der Meer, A., De Veer, R., Zigmond, E., Yehezkel, E., Kremer, A. E., Deibel, A., Dumortier, J., Bruns, T., Große, K., Pageaux, G.-P., Wetten, A., Dyson, J., Jones, D., Chazouillères, O., Hansen, B. and De Lédinghen, V. (2022) 'Liver stiffness measurement by vibration-controlled transient elastography improves outcome prediction in primary biliary cholangitis', *Journal of Hepatology*.
Corpechot, C., Carrat, F., Poujol-Robert, A., Gaouar, F., Wendum, D., Chazouillères, O. and Poupon, R. (2012) 'Noninvasive elastography-based assessment of liver fibrosis progression and prognosis in primary biliary cirrhosis', *Hepatology,* 56(1), pp. 198-208.
Corpechot, C., Chazouilleres, O. and Poupon, R. (2011) 'Early primary biliary cirrhosis: biochemical response to treatment and prediction of long-term outcome', *J Hepatol,* 55(6), pp. 1361-7.
Corpechot, C., Chazouillères, O., Rousseau, A., Le Gruyer, A., Habersetzer, F., Mathurin, P., Goria, O., Potier, P., Minello, A., Silvain, C., Abergel, A., Debette-Gratien, M., Larrey, D., Roux, O., Bronowicki, J.-P., Boursier, J., De Ledinghen, V., Heurgue-Berlot, A., Nguyen-Khac, E., Zoulim, F., Ollivier-Hourmand, I., Zarski, J.-P., Nkontchou, G., Lemoinne, S., Humbert, L., Rainteau, D., Lefèvre, G., De Chaisemartin, L., Chollet-Martin, S., Gaouar, F., Admane, F.-H., Simon, T. and Poupon, R. (2018) 'A Placebo-Controlled Trial of Bezafibrate in Primary Biliary Cholangitis', *New England Journal of Medicine,* 378(23), pp. 2171-2181.
Corpechot, C., Chrétien, Y., Chazouillères, O. and Poupon, R. (2010) 'Demographic, lifestyle, medical and familial factors associated with primary biliary cirrhosis', *J Hepatol,* 53(1), pp. 162-9.
Costenbader, K. H., Gay, S., Alarcón-Riquelme, M. E., Iaccarino, L. and Doria, A. (2012) 'Genes, epigenetic regulation and environmental factors: Which is the most relevant in developing autoimmune diseases?', *Autoimmunity Reviews,* 11(8), pp. 604-609.
Cummings, D. E. (2006) 'Ghrelin and the short-and long-term regulation of appetite and body weight', *Physiology & behavior,* 89(1), pp. 71-84.
Cummings, D. E., Purnell, J. Q., Frayo, R. S., Schmidova, K., Wisse, B. E. and Weigle, D. S. (2001) 'A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans', *Diabetes,* 50(8), pp. 1714-1719.
Curtiss, M. and Colgan, J. (2007) 'The role of the T-cell costimulatory molecule Tim-1 in the immune response', *Immunol Res,* 39(1-3), pp. 52-61.
Cytochrome Oxidase. Available at: https://chem.libretexts.org/@go/page/97882.
D'Mello, C., Le, T. and Swain, M. G. (2009) 'Cerebral Microglia Recruit Monocytes into the Brain in Response to Tumor Necrosis Factor Signaling during Peripheral Organ Inflammation', *Journal of Neuroscience,* 29(7), pp. 2089-2102.
D'Mello, C., Riazi, K., Le, T., Stevens, K. M., Wang, A., McKay, D. M., Pittman, Q. J. and Swain, M. G. (2013) 'P-selectin-mediated monocyte-cerebral endothelium adhesive interactions link peripheral organ inflammation to sickness behaviors', *J Neurosci,* 33(37), pp. 14878-88.

D'Mello, C. and Swain, M. G. (2014) 'Liver-brain interactions in inflammatory liver diseases: implications for fatigue and mood disorders', *Brain Behav Immun,* 35, pp. 9-20.
D'Souza, A. M., Neumann, U. H., Glavas, M. M. and Kieffer, T. J. (2017) 'The glucoregulatory actions of leptin', *Molecular Metabolism,* 6(9), pp. 1052-1065.
D'Amato, D., De Vincentis, A., Malinverno, F., Viganò, M., Alvaro, D., Pompili, M., Picciotto, A., Palitti, V. P., Russello, M., Storato, S., Pigozzi, M. G., Calvaruso, V., De Gasperi, E., Lleo, A., Castellaneta, A., Pellicelli, A., Cazzagon, N., Floreani, A., Muratori, L., Fagiuoli, S., Niro, G. A., Feletti, V., Cozzolongo, R., Terreni, N., Marzioni, M., Pellicano, R., Pozzoni, P., Baiocchi, L., Chessa, L., Rosina, F., Bertino, G., Vinci, M., Morgando, A., Vanni, E., Scifo, G., Sacco, R., D'Antò, M., Bellia, V., Boldizzoni, R., Casella, S., Omazzi, B., Poggi, G., Cristoferi, L., Gerussi, A., Ronca, V., Venere, R., Ponziani, F., Cannavò, M., Mussetto, A., Fontana, R., Losito, F., Frazzetto, E., Distefano, M., Colapietro, F., Labanca, S., Marconi, G., Grassi, G., Galati, G., O'Donnell, S. E., Mancuso, C., Mulinacci, G., Palermo, A., Claar, E., Izzi, A., Picardi, A., Invernizzi, P., Carbone, M. and Vespasiani-Gentilucci, U. (2021) 'Real-world experience with obeticholic acid in patients with primary biliary cholangitis', *JHEP Reports,* 3(2), pp. 100248.
D'Mello, C. and Swain, M. G. (2016) 'Immune-to-Brain Communication Pathways in Inflammation-Associated Sickness and Depression', *Inflammation-Associated Depression: Evidence, Mechanisms and Implications*: Springer International Publishing, pp. 73-94.
Dadar, M., Fereshtehnejad, S. M., Zeighami, Y., Dagher, A., Postuma, R. B. and Collins, D. L. (2020) 'White Matter Hyperintensities Mediate Impact of Dysautonomia on Cognition in Parkinson's Disease', *Mov Disord Clin Pract,* 7(6), pp. 639-647.
Dalakas, M. C. (2004) 'Inflammatory disorders of muscle: progress in polymyositis, dermatomyositis and inclusion body myositis', *Current Opinion in Neurology,* 17(5).
Dalal, J. J. and Mishra, S. (2017) 'Modulation of myocardial energetics: An important category of agents in the multimodal treatment of coronary artery disease and heart failure', *Indian Heart Journal,* 69(3), pp. 393-401.
Dantzer, R., O'Connor, J. C., Freund, G. G., Johnson, R. W. and Kelley, K. W. (2008) 'From inflammation to sickness and depression: when the immune system subjugates the brain', *Nature reviews. Neuroscience,* 9(1), pp. 46-56.
Dash, A., Figler, R. A., Blackman, B. R., Marukian, S., Collado, M. S., Lawson, M. J., Hoang, S. A., Mackey, A. J., Manka, D., Cole, B. K., Feaver, R. E., Sanyal, A. J. and Wamhoff, B. R. (2017) 'Pharmacotoxicology of clinically-relevant concentrations of obeticholic acid in an organotypic human hepatocyte system', *Toxicology in Vitro,* 39, pp. 93-103.
Davidsen, P. K., Herbert, J. M., Antczak, P., Clarke, K., Ferrer, E., Peinado, V. I., Gonzalez, C., Roca, J., Egginton, S., Barberá, J. A. and Falciani, F. (2014) 'A systems biology approach reveals a link between systemic cytokines and skeletal muscle energy metabolism in a rodent smoking model and human COPD', *Genome Medicine,* 6(8), pp. 59.
de Araújo, F. F., Lima Torres, K. C., Viana Peixoto, S., Pinho Ribeiro, A. L., Vaz Melo Mambrini, J., Bortolo Rezende, V., Lima Silva, M. L., Loyola Filho, A. I., Teixeira-Carvalho, A., Lima-Costa, M. F. and Martins-Filho, O. A. (2020) 'CXCL9 and CXCL10 display an age-dependent profile in Chagas patients: a cohort study of aging in Bambui, Brazil', *Infectious Diseases of Poverty,* 9(1), pp. 51.
de Boer, C. J., van Krieken, J. H. J. M., Janssen-van Rhijn, C. M. and Litvinov, S. V. (1999) 'Expression of Ep-CAM in normal, regenerating, metaplastic, and neoplastic liver', *The Journal of Pathology,* 188(2), pp. 201-206.
De Graaf, K. L., Lapeyre, G., Guilhot, F., Ferlin, W., Curbishley, S. M., Carbone, M., Richardson, P., Moreea, S., Mccune, C. A., Ryder, S. D., Chapman, R. W., Floreani, A., Jones, D. E., De Min, C., Adams, D. H. and Invernizzi, P. (2018) 'NI‐0801, an anti‐chemokine (C‐X‐C motif) ligand 10 antibody, in patients with primary biliary cholangitis and an

incomplete response to ursodeoxycholic acid', *Hepatology Communications,* 2(5), pp. 492-503.
De Vries, E., Bolier, R., Goet, J., Parés, A., Verbeek, J., De Vree, M., Drenth, J., Van Erpecum, K., Van Nieuwkerk, K., Van Der Heide, F., Mostafavi, N., Helder, J., Ponsioen, C., Oude Elferink, R., Van Buuren, H. and Beuers, U. (2020) 'Fibrates for Itch (FITCH) in Fibrosing Cholangiopathies: A Double-Blind, Randomized, Placebo-Controlled Trial', *Gastroenterology*.
Deisseroth, A., Ko, C.-W., Nie, L., Zirkelbach, J. F., Zhao, L., Bullock, J., Mehrotra, N., Del Valle, P., Saber, H., Sheth, C., Gehrke, B., Justice, R., Farrell, A. and Pazdur, R. (2015) 'FDA Approval: Siltuximab for the Treatment of Patients with Multicentric Castleman Disease', *Clinical Cancer Research,* 21(5), pp. 950-954.
Del Brutto, O. H., Mera, R. M., Costa, A. F., Rumbea, D. A., Recalde, B. Y., Peñaherrera, E. and Del Brutto, V. J. (2022) 'Decreased Nighttime Heart Rate Variability and Progression of White Matter Hyperintensities of Presumed Vascular Origin. A Prospective Study in Community-Dwelling Older Adults', *Journal of Stroke and Cerebrovascular Diseases,* 31(6), pp. 106479.
Deligiannidis, K. M., Fales, C. L., Kroll-Desrosiers, A. R., Shaffer, S. A., Villamarin, V., Tan, Y., Hall, J. E., Frederick, B. B., Sikoglu, E. M., Edden, R. A., Rothschild, A. J. and Moore, C. M. (2019) 'Resting-state functional connectivity, cortical GABA, and neuroactive steroids in peripartum and peripartum depressed women: a functional magnetic resonance imaging and spectroscopy study', *Neuropsychopharmacology,* 44(3), pp. 546-554.
DEMITRACK, M. A., DALE, J. K., STRAUS, S. E., LAUE, L., LISTWAK, S. J., KRUESI, M. J. P., CHROUSOS, G. P. and GOLD, P. W. (1991) 'Evidence for Impaired Activation of the Hypothalamic-Pituitary-Adrenal Axis in Patients with Chronic Fatigue Syndrome', *The Journal of Clinical Endocrinology & Metabolism,* 73(6), pp. 1224-1234.
Dhillon, S. (2017) 'Tofacitinib: A Review in Rheumatoid Arthritis', *Drugs,* 77(18), pp. 1987-2001.
Di Lazzaro, V., Rothwell, J. and Capogna, M. (2018) 'Noninvasive Stimulation of the Human Brain: Activation of Multiple Cortical Circuits', *The Neuroscientist,* 24(3), pp. 246-260.
Di Padova, C., Tritapepe, R., Rovagnati, P. and Rossetti, S. (1984) 'Double-blind placebo-controlled clinical trial of microporous cholestyramine in the treatment of intra-and extra-hepatic cholestasis: relationship between itching and serum bile acids', *Methods and findings in experimental and clinical pharmacology,* 6(12), pp. 773-776.
Diano, S., Farr, S. A., Benoit, S. C., McNay, E. C., da Silva, I., Horvath, B., Gaskin, F. S., Nonaka, N., Jaeger, L. B., Banks, W. A., Morley, J. E., Pinto, S., Sherwin, R. S., Xu, L., Yamada, K. A., Sleeman, M. W., Tschöp, M. H. and Horvath, T. L. (2006) 'Ghrelin controls hippocampal spine synapse density and memory performance', *Nat Neurosci,* 9(3), pp. 381-8.
digital, N. (2019) *Adults' average BMI*. Available at: http://healthsurvey.hscic.gov.uk/data-visualisation/data-visualisation/explore-the-trends/weight/adult/bmi.aspx (Accessed: 11 May 2023.
Dinarello, C., Novick, D., Kim, S. and Kaplanski, G. (2013) 'Interleukin-18 and IL-18 Binding Protein', *Frontiers in Immunology,* 4.
Dixit, V. D., Schaffer, E. M., Pyle, R. S., Collins, G. D., Sakthivel, S. K., Palaniappan, R., Lillard, J. W. and Taub, D. D. (2004) 'Ghrelin inhibits leptin-and activation-induced proinflammatory cytokine expression by human monocytes and T cells', *The Journal of clinical investigation,* 114(1), pp. 57-66.
Doberer, K., Duerr, M., Halloran, P. F., Eskandary, F., Budde, K., Regele, H., Reeve, J., Borski, A., Kozakowski, N. and Reindl-Schwaighofer, R. (2021) 'A randomized clinical trial of anti–IL-6 antibody clazakizumab in late antibody-mediated kidney transplant rejection', *Journal of the American Society of Nephrology,* 32(3), pp. 708-722.

Dodgson, S. J. (1991) *The carbonic anhydrases: overview of their importance in cellular physiology and in molecular genetics.* Springer.
Donner, M. G., Schumacher, S., Warskulat, U., Heinemann, J. and Häussinger, D. (2007) 'Obstructive cholestasis induces TNF-α- and IL-1β-mediated periportal downregulation of Bsep and zonal regulation of Ntcp, Oatp1a4, and Oatp1b2', *American Journal of Physiology-Gastrointestinal and Liver Physiology,* 293(6), pp. G1134-G1146.
Duan, R.-S., Yang, X., Chen, Z.-G., Lu, M.-O., Morris, C., Winblad, B. and Zhu, J. (2008) 'Decreased Fractalkine and Increased IP-10 Expression in Aged Brain of APP swe Transgenic Mice', *Neurochemical research,* 33, pp. 1085-1089.
Dubin, C., Del Duca, E. and Guttman-Yassky, E. (2021) 'The IL-4, IL-13 and IL-31 pathways in atopic dermatitis', *Expert Review of Clinical Immunology,* 17(8), pp. 835-852.
Dubocovich, M. L. (2007) 'Melatonin receptors: Role on sleep and circadian rhythm regulation', *Sleep Medicine,* 8, pp. 34-42.
Dunn, S. M., Bateson, A. N. and Martin, I. L. (1994) 'Molecular neurobiology of the GABAA receptor', *International review of neurobiology,* 36, pp. 51-96.
Dyson, J. K., Blain, A., Foster Shirley, M. D., Hudson, M., Rushton, S. and Jeffreys Jones, D. E. (2021) 'Geo-epidemiology and environmental co-variate mapping of primary biliary cholangitis and primary sclerosing cholangitis', *JHEP Reports,* 3(1), pp. 100202.
Dyson, J. K., Wilkinson, N., Jopson, L., Mells, G., Bathgate, A., Heneghan, M. A., Neuberger, J., Hirschfield, G. M., Ducker, S. J., Sandford, R., Alexander, G., Stocken, D. and Jones, D. E. J. (2016) 'The inter-relationship of symptom severity and quality of life in 2055 patients with primary biliary cholangitis', *Alimentary Pharmacology & Therapeutics,* 44(10), pp. 1039-1050.
Dzaja, A., Dalal, M. A., Himmerich, H., Uhr, M., Pollmacher, T. and Schuld, A. (2004) 'Sleep enhances nocturnal plasma ghrelin levels in healthy subjects', *American Journal of Physiology-Endocrinology and Metabolism,* 286(6), pp. E963-E967.
Egholm, C., Heeb, L. E. M., Impellizzieri, D. and Boyman, O. (2019) 'The Regulatory Effects of Interleukin-4 Receptor Signaling on Neutrophils in Type 2 Immune Responses', *Frontiers in Immunology,* 10.
El-Gindy, E. M., Ali-Eldin, F. A. and Meguid, M. A. (2012) 'Serum leptin level and its association with fatigue in patients with chronic hepatitis C virus infection', *Arab J Gastroenterol,* 13(2), pp. 54-7.
El-Zayat, S. R., Sibaii, H. and Mannaa, F. A. (2019) 'Toll-like receptors activation, signaling, and targeting: an overview', *Bulletin of the National Research Centre,* 43(1), pp. 187.
ELIAS, E. and BURRA, P. (1991) 'Primary biliary cirrhosis: symptomatic treatment', *Journal of gastroenterology and hepatology,* 6(6), pp. 570-573.
Elkind, M. S. V., Moon, M., Rundek, T., Wright, C. B., Cheung, K., Sacco, R. L. and Hornig, M. (2021) 'Immune markers are associated with cognitive performance in a multiethnic cohort: The Northern Manhattan Study', *Brain, Behavior, and Immunity,* 97, pp. 186-192.
Elmquist, J. K., Maratos-Flier, E., Saper, C. B. and Flier, J. S. (1998) 'Unraveling the central nervous system pathways underlying responses to leptin', *Nature neuroscience,* 1(6), pp. 445-450.
'ENHANCE: Safety and Efficacy of Seladelpar in Patients With Primary Biliary Cholangitis-A Phase 3, International, Randomized, Placebo-Controlled Study', (2021) *Gastroenterol Hepatol (N Y),* 17(2 Suppl 3), pp. 5-6.
Evgeniy, N., Saeed, F., Eugen, F., Mariana, I., Elena, K., Diana, G. K., Aleksey, L. M., Mikhail, S., Rumen, S., Elena, V. Z. and Mark, G. (2022) 'Olokizumab, a monoclonal antibody against interleukin 6, in combination with methotrexate in patients with rheumatoid arthritis inadequately controlled by methotrexate: efficacy and safety results of a randomised controlled phase III study', *Annals of the Rheumatic Diseases,* 81(4), pp. 469.

Fabriek, B. O., Dijkstra, C. D. and van den Berg, T. K. (2005) 'The macrophage scavenger receptor CD163', *Immunobiology,* 210(2), pp. 153-160.
Falguières, T., Castle, D. and Gruenberg, J. (2012) 'Regulation of the MVB Pathway by SCAMP3', *Traffic,* 13(1), pp. 131-142.
Fang, S.-C., Wu, Y.-L. and Tsai, P.-S. (2020) 'Heart Rate Variability and Risk of All-Cause Death and Cardiovascular Events in Patients With Cardiovascular Disease: A Meta-Analysis of Cohort Studies', *Biological Research For Nursing,* 22(1), pp. 45-56.
Febbraio, M. A., Hiscock, N., Sacchetti, M., Fischer, C. P. and Pedersen, B. K. (2004) 'Interleukin-6 is a novel factor mediating glucose homeostasis during skeletal muscle contraction', *Diabetes,* 53(7), pp. 1643-1648.
Febbraio, M. A. and Pedersen, B. K. (2002) 'Muscle‐derived interleukin‐6: mechanisms for activation and possible biological roles', *The FASEB journal,* 16(11), pp. 1335-1347.
Feigelstock, D., Thompson, P., Mattoo, P., Zhang, Y. and Kaplan, G. G. (1998) 'The human homolog of HAVcr-1 codes for a hepatitis A virus cellular receptor', *J Virol,* 72(8), pp. 6621-8.
Feldmann, M. (2002) 'Development of anti-TNF therapy for rheumatoid arthritis', *Nature Reviews Immunology,* 2(5), pp. 364-371.
Felger, J. C., Haroon, E., Patel, T. A., Goldsmith, D. R., Wommack, E. C., Woolwine, B. J., Le, N.-A., Feinberg, R., Tansey, M. G. and Miller, A. H. (2020) 'What does plasma CRP tell us about peripheral and central inflammation in depression?', *Molecular Psychiatry,* 25(6), pp. 1301-1311.
Fernández-de-las-Peñas, C., Arendt-Nielsen, L., Díaz-Gil, G., Gómez-Esquer, F., Gil-Crujera, A., Gómez-Sánchez, S. M., Ambite-Quesada, S., Palomar-Gallego, M. A., Pellicer-Valero, O. J. and Giordano, R. (2022) 'Genetic Association between ACE2 (rs2285666 and rs2074192) and TMPRSS2 (rs12329760 and rs2070788) Polymorphisms with Post-COVID Symptoms in Previously Hospitalized COVID-19 Survivors', *Genes,* 13(11), pp. 1935.
Ferrario, C. M., Chappell, M. C., Tallant, E. A., Brosnihan, K. B. and Diz, D. I. (1997) 'Counterregulatory actions of angiotensin-(1-7)', *Hypertension,* 30(3 Pt 2), pp. 535-41.
Ferraz, H. B., Bertolucci, P. H., Pereira, J. S., Lima, J. G. and Andrade, L. A. (1988) 'Chronic exposure to the fungicide maneb may produce symptoms and signs of CNS manganese intoxication', *Neurology,* 38(4), pp. 550-3.
Ferrucci, L., Penninx, B. W. J. H., Volpato, S., Harris, T. B., Bandeen-Roche, K., Balfour, J., Leveille, S. G., Fried, L. P. and Md, J. M. G. (2002) 'Change in Muscle Strength Explains Accelerated Decline of Physical Function in Older Women With High Interleukin-6 Serum Levels', *Journal of the American Geriatrics Society,* 50(12), pp. 1947-1954.
Fischer, C. P. (2006) 'Interleukin-6 in acute exercise and training: what is the biological relevance?', *Exerc Immunol Rev,* 12, pp. 6-33.
Fishbein, A. B., Vitaterna, O., Haugh, I. M., Bavishi, A. A., Zee, P. C., Turek, F. W., Sheldon, S. H., Silverberg, J. I. and Paller, A. S. (2015) 'Nocturnal eczema: Review of sleep and circadian rhythms in children with atopic dermatitis and future research directions', *Journal of Allergy and Clinical Immunology,* 136(5), pp. 1170-1177.
Fisk, J. D., Ritvo, P. G., Ross, L., Haase, D. A., Marrie, T. J. and Schlech, W. F. (1994) 'Measuring the functional impact of fatigue: initial validation of the fatigue impact scale', *Clin Infect Dis,* 18 Suppl 1, pp. S79-83.
Floreani, A., Marchiori, M., Bonato, S., Zucchetto, M., Naccarato, R. and Chiaramonte, M. (1995) 'Cognitive assessment in primary biliary cirrhosis: a case-control study', *Am J Gastroenterol,* 90(2), pp. 250-3.
Forton, D. M. (2004) 'Fatigue and primary biliary cirrhosis: association of globus pallidus magnetisation transfer ratio measurements with fatigue severity and blood manganese levels', *Gut,* 53(4), pp. 587-592.

Fragiadaki, K., Tektonidou, M. G., Konsta, M., Chrousos, G. P. and Sfikakis, P. P. (2012) 'Sleep disturbances and interleukin 6 receptor inhibition in rheumatoid arthritis', *The Journal of rheumatology,* 39(1), pp. 60-62.
Freedman, M. R., Holzbach, R. T. and Ferguson, D. R. (1981) 'Pruritus in cholestasis: no direct causative role for bile acid retention', *The American journal of medicine,* 70(5), pp. 1011-1016.
Friedman, J. M. and Halaas, J. L. (1998) 'Leptin and the regulation of body weight in mammals', *Nature,* 395(6704), pp. 763-770.
Fuchs, E. and Weber, K. (1994) 'Intermediate filaments: structure, dynamics, function and disease', *Annual review of biochemistry,* 63(1), pp. 345-382.
Fujii, M., Ohgami, S., Asano, E., Nakayama, T., Toda, T., Nabe, T. and Ohya, S. (2019) 'Brain allopregnanolone induces marked scratching behaviour in diet-induced atopic dermatitis mouse model', *Scientific Reports,* 9(1).
Fujino, H., Tanaka, M., Imamura, M., Morio, K., Ono, A., Nakahara, T., Murakami, E., Kawaoka, T., Takahashi, S., Miki, D., Tsuge, M., Hiramatsu, A., Aikata, H., Hayes, C. N. and Chayama, K. (2019) 'Pruritus in patients with chronic liver disease and serum autotaxin levels in patients with primary biliary cholangitis', *BMC Gastroenterology,* 19(1), pp. 169.
Furst, D., Thenkondar and Townes, S. (2012) 'The impact of tocilizumab on physical function and quality of life in patients with rheumatoid arthritis: a systematic literature review and interpretation', *Open Access Rheumatology: Research and Reviews*, pp. 87.
Fussey, S. P., Guest, J. R., James, O. F., Bassendine, M. F. and Yeaman, S. J. (1988) 'Identification and analysis of the major M2 autoantigens in primary biliary cirrhosis', *Proc Natl Acad Sci U S A,* 85(22), pp. 8654-8.
Gabay, C., Fautrel, B., Rech, J., Spertini, F., Feist, E., Kötter, I., Hachulla, E., Morel, J., Schaeverbeke, T., Hamidou, M. A., Martin, T., Hellmich, B., Lamprecht, P., Schulze-Koops, H., Courvoisier, D. S., Sleight, A. and Schiffrin, E. J. (2018) 'Open-label, multicentre, dose-escalating phase II clinical trial on the safety and efficacy of tadekinig alfa (IL-18BP) in adult-onset Still's disease', *Ann Rheum Dis,* 77(6), pp. 840-847.
Galluzzi, S., Nicosia, F., Geroldi, C., Alicandri, A., Bonetti, M., Romanelli, G., Zulli, R. and Frisoni, G. B. (2009) 'Cardiac Autonomic Dysfunction Is Associated With White Matter Lesions in Patients With Mild Cognitive Impairment', *The Journals of Gerontology: Series A,* 64A(12), pp. 1312-1315.
García-Suárez, C., Crespo, J., Luis Fernández-Gil, P., Amado, J. A., García-Unzueta, M. T. and Pons Romero, F. (2004) 'Plasma leptin levels in patients with primary biliary cirrhosis and their relationship with degree of fibrosis', *Gastroenterología y Hepatología,* 27(2), pp. 47-50.
Garin, M. C., Burns, C. M., Kaul, S. and Cappola, A. R. (2013) 'Clinical review: The human experience with ghrelin administration', *J Clin Endocrinol Metab,* 98(5), pp. 1826-37.
Gauldie, J., Richards, C., Harnish, D., Lansdorp, P. and Baumann, H. (1987) 'Interferon beta 2/B-cell stimulatory factor type 2 shares identity with monocyte-derived hepatocyte-stimulating factor and regulates the major acute phase protein response in liver cells.', *Proceedings of the National Academy of Sciences,* 84(20), pp. 7251-7255.
Gauna, C., Delhanty, P. J., Hofland, L. J., Janssen, J. A., Broglio, F., Ross, R. J., Ghigo, E. and van der Lely, A. J. (2005) 'Ghrelin stimulates, whereas des-octanoyl ghrelin inhibits, glucose output by primary hepatocytes', *The Journal of Clinical Endocrinology & Metabolism,* 90(2), pp. 1055-1060.
Gee, L. M. V., Barron-Millar, B., Leslie, J., Richardson, C., Zaki, M. Y. W., Luli, S., Burgoyne, R. A., Cameron, R. I. T., Smith, G. R., Brain, J. G., Innes, B., Jopson, L., Dyson, J. K., Mckay, K. R. C., Pechlivanis, A., Holmes, E., Berlinguer-Palmini, R., Victorelli, S., Mells, G. F., Sandford, R. N., Palmer, J., Kirby, J. A., Kiourtis, C., Mokochinski, J., Hall, Z., Bird, T. G., Borthwick, L. A., Morris, C. M., Hanson, P. S., Jurk, D., Stoll, E. A., Lebeau, F. E. N., Jones, D. E. J. and Oakley,

F. (2022) 'Anti–Cholestatic Therapy with Obeticholic Acid Improves Short-Term Memory in Bile Duct Ligated Mice', *The American Journal of Pathology*.
Gee, L. M. V., Barron-Millar, B., Leslie, J., Richardson, C., Zaki, M. Y. W., Luli, S., Burgoyne, R. A., Cameron, R. I. T., Smith, G. R., Brain, J. G., Innes, B., Jopson, L., Dyson, J. K., Mckay, K. R. C., Pechlivanis, A., Holmes, E., Berlinguer-Palmini, R., Victorelli, S., Mells, G. F., Sandford, R. N., Palmer, J., Kirby, J. A., Kiourtis, C., Mokochinski, J., Hall, Z., Bird, T. G., Borthwick, L. A., Morris, C. M., Hanson, P. S., Jurk, D., Stoll, E. A., Lebeau, F. E. N., Jones, D. E. J. and Oakley, F. (2023) 'Anti–Cholestatic Therapy with Obeticholic Acid Improves Short-Term Memory in Bile Duct–Ligated Mice', *The American Journal of Pathology,* 193(1), pp. 11-26.
Genazzani, A. R., Petraglia, F., Bernardi, F., Casarosa, E., Salvestroni, C., Tonetti, A., Nappi, R. E., Luisi, S., Palumbo, M., Purdy, R. H. and Luisi, M. (1998) 'Circulating levels of allopregnanolone in humans: gender, age, and endocrine influences', *J Clin Endocrinol Metab,* 83(6), pp. 2099-103.
Gershwin, M. E., Ansari, A. A., Mackay, I. R., Nakanuma, Y., Nishio, A., Rowley, M. J. and Coppel, R. L. (2000) 'Primary biliary cirrhosis: an orchestrated immune response against epithelial cells', *Immunol Rev,* 174, pp. 210-25.
Gershwin, M. E. and Mackay, I. R. (2008) 'The causes of primary biliary cirrhosis: Convenient and inconvenient truths', *Hepatology,* 47(2), pp. 737-45.
Gershwin, M. E., Selmi, C., Worman, H. J., Gold, E. B., Watnik, M., Utts, J., Lindor, K. D., Kaplan, M. M. and Vierling, J. M. (2005) 'Risk factors and comorbidities in primary biliary cirrhosis: A controlled interview-based study of 1032 patients', *Hepatology,* 42(5), pp. 1194-1202.
Gerussi, A., Carbone, M., Corpechot, C., Schramm, C., Asselta, R. and Invernizzi, P. (2021) 'The genetic architecture of primary biliary cholangitis', *Eur J Med Genet,* 64(9), pp. 104292.
Gervois, P., Torra, I. P., Fruchart, J. C. and Staels, B. (2000) 'Regulation of lipid and lipoprotein metabolism by PPAR activators', *Clin Chem Lab Med,* 38(1), pp. 3-11.
Gieseck, L., Richard, Ramalingam, R., Thirumalai, Hart, M., Kevin, Vannella, M., Kevin, Cantu, A., David, Lu, W.-Y., Ferreira-González, S., Forbes, J., Stuart, Vallier, L. and Wynn, A., Thomas (2016) 'Interleukin-13 Activates Distinct Cellular Pathways Leading to Ductular Reaction, Steatosis, and Fibrosis', *Immunity,* 45(1), pp. 145-158.
Gieseck, R. L., Wilson, M. S. and Wynn, T. A. (2018) 'Type 2 immunity in tissue repair and fibrosis', *Nature Reviews Immunology,* 18(1), pp. 62-76.
Gil-Campos, M., Aguilera, C. M., Canete, R. and Gil, A. (2006) 'Ghrelin: a hormone regulating food intake and energy homeostasis', *British Journal of Nutrition,* 96(2), pp. 201-226.
Goldblatt, J. (2001) 'Grip Strength and Subjective Fatigue in Patients With Primary Biliary Cirrhosis', *JAMA,* 285(17), pp. 2196.
Goldblatt, J., Taylor, P. J. S., Lipman, T., Prince, M. I., Baragiotta, A., Bassendine, M. F., James, O. F. W. and Jones, D. E. J. (2002) 'The true impact of fatigue in primary biliary cirrhosis: A population study', *Gastroenterology,* 122(5), pp. 1235-1241.
Gonzalez-Polo, V., Pucci-Molineris, M., Cervera, V., Gambaro, S., Yantorno, S. E., Descalzi, V., Tiribelli, C., Gondolesi, G. E. and Meier, D. (2019) 'Group 2 innate lymphoid cells exhibit progressively higher levels of activation during worsening of liver fibrosis', *Annals of Hepatology,* 18(2), pp. 366-372.
Goodpaster, B. H., Park, S. W., Harris, T. B., Kritchevsky, S. B., Nevitt, M., Schwartz, A. V., Simonsick, E. M., Tylavsky, F. A., Visser, M., Newman, A. B. and Study, f. t. H. A. (2006) 'The Loss of Skeletal Muscle Strength, Mass, and Quality in Older Adults: The Health, Aging and Body Composition Study', *The Journals of Gerontology: Series A,* 61(10), pp. 1059-1064.

Gortan Cappellari, G. and Barazzoni, R. (2019) 'Ghrelin forms in the modulation of energy balance and metabolism', *Eating and Weight Disorders - Studies on Anorexia, Bulimia and Obesity,* 24(6), pp. 997-1013.
Gossec, L., Steinberg, G., Rouanet, S. and Combe, B. (2015) 'Fatigue in rheumatoid arthritis: quantitative findings on the efficacy of tocilizumab and on factors associated with fatigue. The French multicentre prospective PEPS Study', *Clin Exp Rheumatol,* 33(5), pp. 664-670.
Gouras, G. K., Olsson, T. T. and Hansson, O. (2015) 'β-amyloid Peptides and Amyloid Plaques in Alzheimer's Disease', *Neurotherapeutics,* 12(1), pp. 3-11.
Graves, P. R. and Haystead, T. A. (2002) 'Molecular biologist's guide to proteomics', *Microbiol Mol Biol Rev,* 66(1), pp. 39-63; table of contents.
Griffiths, L., Dyson, J. K. and Jones, D. E. (2014) 'The new epidemiology of primary biliary cirrhosis', *Semin Liver Dis,* 34(3), pp. 318-28.
Griffiths, L. and Jones, D. E. (2014) 'Pathogenesis of Primary Biliary Cirrhosis and Its Fatigue', *Digestive Diseases,* 32(5), pp. 615-625.
Grønbaek, H., Kreutzfeldt, M., Kazankov, K., Jessen, N., Sandahl, T., Hamilton-Dutoit, S., Vilstrup, H. and J. Møller, H. (2016) 'Single-centre experience of the macrophage activation marker soluble (s)CD163 - associations with disease activity and treatment response in patients with autoimmune hepatitis', *Alimentary Pharmacology & Therapeutics,* 44(10), pp. 1062-1070.
Grønbæk, H., Sandahl, T. D., Mortensen, C., Vilstrup, H., Møller, H. J. and Møller, S. (2012) 'Soluble CD163, a marker of Kupffer cell activation, is related to portal hypertension in patients with liver cirrhosis', *Alimentary Pharmacology & Therapeutics,* 36(2), pp. 173-180.
Grover, V. P. B., Southern, L., Dyson, J. K., Kim, J. U., Crossey, M. M. E., Wylezinska-Arridge, M., Patel, N., Fitzpatrick, J. A., Bak-Bol, A., Waldman, A. D., Alexander, G. J., Mells, G. F., Chapman, R. W., Jones, D. E. J. and Taylor-Robinson, S. D. (2016) 'Early primary biliary cholangitis is characterised by brain abnormalities on cerebral magnetic resonance imaging', *Alimentary Pharmacology & Therapeutics,* 44(9), pp. 936-945.
Gualberto Cardoso, P. R., Diniz Lopes Marques, C., de Melo Vilar, K., Dantas, A. T., Branco Pinto Duarte, A. L., Pitta, I. d. R., Galdino da Rocha Pitta, M. and Barreto de Melo Rêgo, M. J. (2021) 'Interleukin-18 in Brazilian Rheumatoid Arthritis Patients: Can Leflunomide Reduce It?', *Autoimmune Diseases,* 2021, pp. 1-6.
Gyermek, L., Iriarte, J. and Crabbe, P. (1968) 'Steroids. CCCX. Structure-activity relation of some steroidal hypnotic agents', *Journal of medicinal chemistry,* 11(1), pp. 117-125.
Hale, M., Newton, J. L. and Jones, D. E. J. (2012) 'Fatigue in primary biliary cirrhosis', *BMJ,* 345(oct22 1), pp. e7004-e7004.
Hamaguchi, Y., Kaido, T., Okumura, S., Kobayashi, A., Shirai, H., Yagi, S., Kamo, N., Okajima, H. and Uemoto, S. (2017) 'Impact of Skeletal Muscle Mass Index, Intramuscular Adipose Tissue Content, and Visceral to Subcutaneous Adipose Tissue Area Ratio on Early Mortality of Living Donor Liver Transplantation', *Transplantation,* 101(3), pp. 565-574.
Hammer, H. B., Agular, B. and Terslev, L. (2022) 'Fatigue Is Not Associated With Objective Assessments of Inflammation During Tocilizumab Treatment of Patients With Rheumatoid Arthritis', *ACR Open Rheumatology,* 4(3), pp. 202-208.
Han, S. S., Feng, Z. Q., Liu, R., Ye, J., Cheng, W. W. and Bao, J. B. (2020) 'Bioinformatics Analysis and RNA-Sequencing of SCAMP3 Expression and Correlated Gene Regulation in Hepatocellular Carcinoma', *Onco Targets Ther,* 13, pp. 1047-1057.
Han, W. K., Bailly, V., Abichandani, R., Thadhani, R. and Bonventre, J. V. (2002) 'Kidney Injury Molecule-1 (KIM-1): a novel biomarker for human renal proximal tubule injury', *Kidney Int,* 62(1), pp. 237-44.

Harb, H. and Chatila, T. A. (2020) 'Mechanisms of Dupilumab', *Clinical & Experimental Allergy,* 50(1), pp. 5-14.
Harmer, D., Gilbert, M., Borman, R. and Clark, K. L. (2002) 'Quantitative mRNA expression profiling of ACE 2, a novel homologue of angiotensin converting enzyme', *FEBS Lett,* 532(1-2), pp. 107-10.
Hassler, E. M., Deutschmann, H., Almer, G., Renner, W., Mangge, H., Herrmann, M., Leber, S., Michenthaler, M., Staszewski, A., Gunzer, F., Partl, R. and Reishofer, G. (2021) 'Distribution of subcutaneous and intermuscular fatty tissue of the mid-thigh measured by MRI—A putative indicator of serum adiponectin level and individual factors of cardio-metabolic risk', *PLOS ONE,* 16(11), pp. e0259952.
Hauser, M. A. and Legler, D. F. (2016) 'Common and biased signaling pathways of the chemokine receptor CCR7 elicited by its ligands CCL19 and CCL21 in leukocytes', *Journal of Leukocyte Biology,* 99(6), pp. 869-882.
He, A., Feldman, S. R. and Fleischer, A. B. (2018) 'An assessment of the use of antihistamines in the management of atopic dermatitis', *Journal of the American Academy of Dermatology,* 79(1), pp. 92-96.
'Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology', (1996) *Circulation,* 93(5), pp. 1043-65.
Hedrick, M. N., Lonsdorf, A. S., Shirakawa, A.-K., Lee, C.-C. R., Liao, F., Singh, S. P., Zhang, H. H., Grinberg, A., Love, P. E. and Hwang, S. T. (2009) 'CCR6 is required for IL-23–induced psoriasis-like inflammation in mice', *The Journal of clinical investigation,* 119(8), pp. 2317-2329.
Heerkens, M., Dedden, S., Scheepers, H., Van Paassen, P., Masclee, A., De Die-Smulders, C., Olde Damink, S. W. M., Schaap, F. G., Jansen, P., Koek, G., Beuers, U. and Verbeek, J. (2019) 'Effect of Plasmapheresis on Cholestatic Pruritus and Autotaxin Activity During Pregnancy', *Hepatology,* 69(6), pp. 2707-2710.
Hegade, V. S., Bolier, R., Oude Elferink, R. P., Beuers, U., Kendrick, S. and Jones, D. E. (2016a) 'A systematic approach to the management of cholestatic pruritus in primary biliary cirrhosis', *Frontline Gastroenterology,* 7(3), pp. 158-166.
Hegade, V. S., Kendrick, S. F. W., Dobbins, R. L., Miller, S. R., Thompson, D., Richards, D., Storey, J., Dukes, G. E., Corrigan, M., Oude Elferink, R. P. J., Beuers, U., Hirschfield, G. M. and Jones, D. E. (2017) 'Effect of ileal bile acid transporter inhibitor GSK2330672 on pruritus in primary biliary cholangitis: a double-blind, randomised, placebo-controlled, crossover, phase 2a study', *The Lancet,* 389(10074), pp. 1114-1123.
Hegade, V. S., Khanna, A., Walker, L. J., Wong, L.-L., Dyson, J. K. and Jones, D. E. J. (2016b) 'Long-Term Fenofibrate Treatment in Primary Biliary Cholangitis Improves Biochemistry but Not the UK-PBC Risk Score', *Digestive Diseases and Sciences,* 61(10), pp. 3037-3044.
Hegade, V. S., Mells, G. F., Beuers, U., Kremer, A. E., Invernizzi, P., Karlsen, T. H., Hirschfield, G., Sandford, R. N., Kendrick, S. and Jones, D. E. 'Patient experience and characteristics of cholestatic pruritus in the UK-PBC research cohort'. *65th Annual Meeting of the American Association for the Study of Liver Diseases: The Liver Meeting 2014*: Newcastle University.
Hegade, V. S., Mells, G. F., Fisher, H., Kendrick, S., DiBello, J., Gilchrist, K., Alexander, G. J., Hirschfield, G. M., Sandford, R. N. and Jones, D. E. J. (2019) 'Pruritus Is Common and Undertreated in Patients With Primary Biliary Cholangitis in the United Kingdom', *Clin Gastroenterol Hepatol,* 17(7), pp. 1379-1387.e3.
Hegade, V. S., Mells, G. F., Lammert, C., Juran, B., Lleo, A., Carbone, M., Lazaridis, K., Invernizzi, P., Kendrick, S., Sandford, R., Hirschfield, G. and Jones, D. E. (2015) 'P1152 : A Comparative study of pruritus in PBC cohorts from UK, USA and Italy', *Journal of Hepatology,* 62.

Henriksen, J. H., Holst, J. J., Møller, S., Brinch, K. and Bendtsen, F. (1999) 'Increased circulating leptin in alcoholic cirrhosis: Relation to release and disposal', *Hepatology,* 29(6), pp. 1818-1824.
Herd, M. B., Belelli, D. and Lambert, J. J. (2007) 'Neurosteroid modulation of synaptic and extrasynaptic GABAA receptors', *Pharmacology & Therapeutics,* 116(1), pp. 20-34.
Herman, J. P., Mcklveen, J. M., Ghosal, S., Kopp, B., Wulsin, A., Makinson, R., Scheimann, J. and Myers, B. (2016) 'Regulation of the Hypothalamic-Pituitary-Adrenocortical Stress Response', *Comprehensive Physiology*, pp. 603-621.
Hirano, T. (2020) 'IL-6 in inflammation, autoimmunity and cancer', *International Immunology,* 33(3), pp. 127-148.
Hirota, K., Yoshitomi, H., Hashimoto, M., Maeda, S., Teradaira, S., Sugimoto, N., Yamaguchi, T., Nomura, T., Ito, H. and Nakamura, T. (2007) 'Preferential recruitment of CCR6-expressing Th17 cells to inflamed joints via CCL20 in rheumatoid arthritis and its animal model', *The Journal of experimental medicine,* 204(12), pp. 2803-2812.
Hirschfield, G. M., Beuers, U., Corpechot, C., Invernizzi, P., Jones, D., Marzioni, M. and Schramm, C. (2017) 'EASL Clinical Practice Guidelines: The diagnosis and management of patients with primary biliary cholangitis', *Journal of Hepatology,* 67(1), pp. 145-172.
Hirschfield, G. M., Dyson, J. K., Alexander, G. J. M., Chapman, M. H., Collier, J., Hübscher, S., Patanwala, I., Pereira, S. P., Thain, C., Thorburn, D., Tiniakos, D., Walmsley, M., Webster, G. and Jones, D. E. J. (2018) 'The British Society of Gastroenterology/UK-PBC primary biliary cholangitis treatment and management guidelines', *Gut,* 67(9), pp. 1568-1594.
Hirschfield, G. M., Gershwin, M. E., Strauss, R., Mayo, M. J., Levy, C., Zou, B., Johanns, J., Nnane, I. P., Dasgupta, B., Li, K., Selmi, C., Marschall, H. U., Jones, D. and Lindor, K. (2016) 'Ustekinumab for patients with primary biliary cholangitis who have an inadequate response to ursodeoxycholic acid: A proof-of-concept study', *Hepatology,* 64(1), pp. 189-99.
Hirschfield, G. M., Heathcote, E. J. and Gershwin, M. E. (2010) 'Pathogenesis of cholestatic liver disease and therapeutic approaches', *Gastroenterology,* 139(5), pp. 1481-96.
Hirschfield, G. M., Liu, X., Xu, C., Lu, Y., Xie, G., Lu, Y., Gu, X., Walker, E. J., Jing, K., Juran, B. D., Mason, A. L., Myers, R. P., Peltekian, K. M., Ghent, C. N., Coltescu, C., Atkinson, E. J., Heathcote, E. J., Lazaridis, K. N., Amos, C. I. and Siminovitch, K. A. (2009) 'Primary Biliary Cirrhosis Associated withHLA, IL12A,andIL12RB2Variants', *New England Journal of Medicine,* 360(24), pp. 2544-2555.
Hirschfield, G. M., Mason, A., Luketic, V., Lindor, K., Gordon, S. C., Mayo, M., Kowdley, K. V., Vincent, C., Bodhenheimer, H. C., Parés, A., Trauner, M., Marschall, H.-U., Adorini, L., Sciacca, C., Beecher-Jones, T., Castelloe, E., Böhm, O. and Shapiro, D. (2015) 'Efficacy of Obeticholic Acid in Patients With Primary Biliary Cirrhosis and Inadequate Response to Ursodeoxycholic Acid', *Gastroenterology,* 148(4), pp. 751-761.e8.
Hisamoto, S., Shimoda, S., Harada, K., Iwasaka, S., Onohara, S., Chong, Y., Nakamura, M., Bekki, Y., Yoshizumi, T. and Ikegami, T. (2016) 'Hydrophobic bile acids suppress expression of AE2 in biliary epithelial cells and induce bile duct inflammation in primary biliary cholangitis', *Journal of Autoimmunity,* 75, pp. 150-160.
Hollingsworth, K. G., Jones, D. E., Aribisala, B. S., Thelwall, P. E., Taylor, R., Newton, J. L. and Blamire, A. M. (2009) 'Globus pallidus magnetization transfer ratio, T₁and T₂in primary biliary cirrhosis: Relationship with disease stage and age', *Journal of Magnetic Resonance Imaging,* 29(4), pp. 780-784.
Hollingsworth, K. G., Jones, D. E. J., Taylor, R., Frith, J., Blamire, A. M. and Newton, J. L. (2010a) 'Impaired cerebral autoregulation in primary biliary cirrhosis: implications for the pathogenesis of cognitive decline', *Liver International,* 30(6), pp. 878-885.
Hollingsworth, K. G., Macgowan, G. A., Morris, L., Bates, M. G., Taylor, R., Jones, D. E., Newton, J. L. and Blamire, A. M. (2012) 'Cardiac torsion-strain relationships in fatigued

primary biliary cirrhosis patients show accelerated aging: a pilot cross-sectional study', *J Appl Physiol (1985),* 112(12), pp. 2043-8.
Hollingsworth, K. G., Newton, J. L., Robinson, L., Taylor, R., Blamire, A. M. and Jones, D. E. (2010b) 'Loss of capacity to recover from acidosis in repeat exercise is strongly associated with fatigue in primary biliary cirrhosis', *J Hepatol,* 53(1), pp. 155-61.
Hollingsworth, K. G., Newton, J. L., Taylor, R., McDonald, C., Palmer, J. M., Blamire, A. M. and Jones, D. E. (2008) 'Pilot study of peripheral muscle function in primary biliary cirrhosis: potential implications for fatigue pathogenesis', *Clin Gastroenterol Hepatol,* 6(9), pp. 1041-8.
Honda, A., Tanaka, A., Kaneko, T., Komori, A., Abe, M., Inao, M., Namisaki, T., Hashimoto, N., Kawata, K., Takahashi, A., Ninomiya, M., Kang, J. H., Arakawa, M., Yamagiwa, S., Joshita, S., Umemura, T., Sato, K., Kaneko, A., Kikuchi, K., Itakura, J., Nomura, T., Kakisaka, K., Fujii, H., Kawada, N., Takikawa, Y., Masaki, T., Ohira, H., Mochida, S., Yoshiji, H., Iimuro, S., Matsuzaki, Y. and Takikawa, H. (2019) 'Bezafibrate Improves GLOBE and UK‐PBC Scores and Long‐Term Outcomes in Patients With Primary Biliary Cholangitis', *Hepatology,* 70(6), pp. 2035-2046.
Hoogland, I., Houbolt, C., van Westerloo, D. J., van Gool, W. A. and van de Beek, D. (2015) 'Systemic inflammation and microglial activation: systematic review of animal experiments', *Journal of neuroinflammation,* 12(1), pp. 1-13.
Horiuchi, T., Mitoma, H., Harashima, S.-i., Tsukamoto, H. and Shimoda, T. (2010) 'Transmembrane TNF-α: structure, function and interaction with anti-TNF agents', *Rheumatology,* 49(7), pp. 1215-1228.
Hornig, M., Gottschalk, C. G., Eddy, M. L., Che, X., Ukaigwe, J. E., Peterson, D. L. and Lipkin, W. I. (2017) 'Immune network analysis of cerebrospinal fluid in myalgic encephalomyelitis/chronic fatigue syndrome with atypical and classical presentations', *Translational Psychiatry,* 7(4), pp. e1080-e1080.
Horsfield, M. A. (2005) 'Magnetization Transfer Imaging in Multiple Sclerosis', *Journal of Neuroimaging,* 15, pp. 58S-67S.
Hosoda, H., Kojima, M., Matsuo, H. and Kangawa, K. (2000) 'Ghrelin and des-acyl ghrelin: two major forms of rat ghrelin peptide in gastrointestinal tissue', *Biochemical and biophysical research communications,* 279(3), pp. 909-913.
Hosonuma, K., Sato, K., Yamazaki, Y., Yanagisawa, M., Hashizume, H., Horiguchi, N., Kakizaki, S., Kusano, M. and Yamada, M. (2015) 'A prospective randomized controlled study of long-term combination therapy using ursodeoxycholic acid and bezafibrate in patients with primary biliary cirrhosis and dyslipidemia', *Am J Gastroenterol,* 110(3), pp. 423-31.
Houssiau, F. A., Devogelaer, J. P., Van Damme, J., de Deuxchaisnes, C. N. and Van Snick, J. (1988) 'Interleukin-6 in synovial fluid and serum of patients with rheumatoid arthritis and other inflammatory arthritides', *Arthritis Rheum,* 31(6), pp. 784-8.
Howel, D., Fischbacher, C. M., Bhopal, R. S., Gray, J., Metcalf, J. V. and James, O. F. (2000) 'An exploratory population-based case-control study of primary biliary cirrhosis', *Hepatology,* 31(5), pp. 1055-1060.
Howland, R. H. (2014) 'Vagus nerve stimulation', *Current behavioral neuroscience reports,* 1, pp. 64-73.
Huet, P.-M., Deslauriers, J., Tran, A., Faucher, C. and Charbonneau, J. (2000) 'Impact of fatigue on the quality of life of patients with primary biliary cirrhosis', *The American Journal of Gastroenterology,* 95(3), pp. 760-767.
Ikoma, A., Steinhoff, M., Ständer, S., Yosipovitch, G. and Schmelz, M. (2006) 'The neurobiology of itch', *Nature reviews neuroscience,* 7(7), pp. 535-547.
Intercept (2017) *Nice Recommends Ocalivar (Obeticholic Acid) for the treatment of patients with Primary Biliary Cholangitis in England, Wales and Northern Ireland*.

Available at: https://ir.interceptpharma.com/news-releases/news-release-details/nice-recommends-ocalivar-obeticholic-acid-treatment-patients (Accessed: January 4, 2021.
Inui, A. (2001) 'Ghrelin: An orexigenic and somatotrophic signal from the stomach', *Nature Reviews Neuroscience,* 2(8), pp. 551-560.
Irwin, M. R., Olmstead, R. and Carroll, J. E. (2016) 'Sleep Disturbance, Sleep Duration, and Inflammation: A Systematic Review and Meta-Analysis of Cohort Studies and Experimental Sleep Deprivation', *Biological Psychiatry,* 80(1), pp. 40-52.
Ivaska, J., Pallari, H.-M., Nevo, J. and Eriksson, J. E. (2007) 'Novel functions of vimentin in cell adhesion, migration, and signaling', *Experimental Cell Research,* 313(10), pp. 2050-2062.
Iwamoto, S., Kido, M., Aoki, N., Nishiura, H., Maruoka, R., Ikeda, A., Okazaki, T., Chiba, T. and Watanabe, N. (2013) 'TNF-α is essential in the induction of fatal autoimmune hepatitis in mice through upregulation of hepatic CCL20 expression', *Clinical Immunology,* 146(1), pp. 15-25.
Iwaszko, M., Biały, S. and Bogunia-Kubik, K. (2021) 'Significance of Interleukin (IL)-4 and IL-13 in Inflammatory Arthritis', *Cells,* 10(11), pp. 3000.
Jacobs, A. H. and Tavitian, B. (2012) 'Noninvasive Molecular Imaging of Neuroinflammation', *Journal of Cerebral Blood Flow & Metabolism,* 32(7), pp. 1393-1415.
Jacoby, A., Rannard, A., Buck, D., Bhala, N., Newton, J. L., James, O. F. and Jones, D. E. (2005) 'Development, validation, and evaluation of the PBC-40, a disease specific health related quality of life measure for primary biliary cirrhosis', *Gut,* 54(11), pp. 1622-9.
Jahn, C. E., Schaefer, E. J., Taam, L. A., Hoofnagle, J. H., Lindgren, F. T., Albers, J. J., Jones, E. A. and Brewer, H. B. (1985) 'Lipoprotein abnormalities in primary biliary cirrhosis', *Gastroenterology,* 89(6), pp. 1266-1278.
James, O. F., Bhopal, R., Howel, D., Gray, J., Burt, A. D. and Metcalf, J. V. (1999) 'Primary biliary cirrhosis once rare, now common in the United Kingdom?', *Hepatology,* 30(2), pp. 390-394.
Jang, D.-I., Lee, A.-H., Shin, H.-Y., Song, H.-R., Park, J.-H., Kang, T.-B., Lee, S.-R. and Yang, S.-H. (2021) 'The Role of Tumor Necrosis Factor Alpha (TNF-α) in Autoimmune Disease and Current TNF-α Inhibitors in Therapeutics', *International Journal of Molecular Sciences,* 22(5), pp. 2719.
Jensen, K. J., Alpini, G. and Glaser, S. (2013) 'Hepatic nervous system and neurobiology of the liver', *Comprehensive Physiology,* 3(2), pp. 655-665.
Jewett, B. E. and Sharma, S. (2022) 'Physiology, GABA', *StatPearls*. Treasure Island (FL): StatPearls Publishing
Copyright © 2022, StatPearls Publishing LLC.
Jin, X. Y. and Khan, T. M. (2016) 'Quality of life among patients suffering from cholestatic liver disease-induced pruritus: A systematic review', *Journal of the Formosan Medical Association,* 115(9), pp. 689-702.
Johansson, I.-M., Birzniece, V., Lindblad, C., Olsson, T. and Bäckström, T. (2002) 'Allopregnanolone inhibits learning in the Morris water maze', *Brain Research,* 934(2), pp. 125-131.
Johansson, M., Agusti, A., Llansola, M., Montoliu, C., Stromberg, J., Malinina, E., Ragagnin, G., Doverskog, M., Backstrom, T. and Felipo, V. (2015) 'GR3027 antagonizes GABAA receptor-potentiating neurosteroids and restores spatial learning and motor coordination in rats with chronic hyperammonemia and hepatic encephalopathy', *Am J Physiol Gastrointest Liver Physiol,* 309(5), pp. G400-9.
Johansson, M., Mansson, M., Lins, L. E., Scharschmidt, B., Doverskog, M. and Backstrom, T. (2018) 'GR3027 reversal of neurosteroid-induced, GABA-A receptor-mediated inhibition of human brain function: an allopregnanolone challenge study', *Psychopharmacology (Berl),* 235(5), pp. 1533-1543.

Johns, M. W. (1991) 'A New Method for Measuring Daytime Sleepiness: The Epworth Sleepiness Scale', *Sleep,* 14(6), pp. 540-545.
Johnston, C. I. (1994) 'Tissue angiotensin converting enzyme in cardiac and vascular hypertrophy, repair, and remodeling', *Hypertension,* 23(2), pp. 258-268.
Jones, D. E., Al-Rifai, A., Frith, J., Patanwala, I. and Newton, J. L. (2010a) 'The independent effects of fatigue and UDCA therapy on mortality in primary biliary cirrhosis: results of a 9 year follow-up', *J Hepatol,* 53(5), pp. 911-7.
Jones, D. E., Hollingsworth, K., Fattakhova, G., MacGowan, G., Taylor, R., Blamire, A. and Newton, J. L. (2010b) 'Impaired cardiovascular function in primary biliary cirrhosis', *Am J Physiol Gastrointest Liver Physiol,* 298(5), pp. G764-73.
Jones, D. E., Watt, F. E., Grove, J., Newton, J. L., Daly, A. K., Gregory, W. L., Day, C. P., James, O. F. and Bassendine, M. F. (1999a) 'Tumour necrosis factor-alpha promoter polymorphisms in primary biliary cirrhosis', *J Hepatol,* 30(2), pp. 232-6.
Jones, D. E., Watt, F. E., Metcalf, J. V., Bassendine, M. F. and James, O. F. (1999b) 'Familial primary biliary cirrhosis reassessed: a geographically-based population study', *J Hepatol,* 30(3), pp. 402-7.
Jones, D. E. J. (2006) 'Four year follow up of fatigue in a geographically defined primary biliary cirrhosis patient cohort', *Gut,* 55(4), pp. 536-541.
Jones, D. E. J. (2008) 'Pathogenesis of primary biliary cirrhosis', *Postgraduate Medical Journal,* 84(987), pp. 23-33.
Jones, D. E. J., Wetten, A., Barron-Millar, B., Ogle, L., Mells, G., Flack, S., Sandford, R., Kirby, J., Palmer, J., Brotherston, S., Jopson, L., Brain, J., Smith, G. R., Rushton, S., Jones, R., Rushbrook, S., Thorburn, D., Ryder, S. D., Hirschfield, G. and Dyson, J. K. (2022) 'The relationship between disease activity and UDCA response criteria in primary biliary cholangitis: A cohort study', *eBioMedicine,* 80, pp. 104068.
Jones, E. A. (2002) *Metabolic Brain Disease,* 17(4), pp. 275-281.
Jones, J. M., Morrell, J. C. and Gould, S. J. (2000) 'Identification and Characterization of HAOX1, HAOX2, and HAOX3, Three Human Peroxisomal 2-Hydroxy Acid Oxidases *', *Journal of Biological Chemistry,* 275(17), pp. 12590-12597.
Jopson, L. and Jones, D. E. (2015) 'Fatigue in Primary Biliary Cirrhosis: Prevalence, Pathogenesis and Management', *Dig Dis,* 33 Suppl 2, pp. 109-14.
Jopson, L., Newton, J. L., Palmer, J., Floudas, A., Isaacs, J., Qian, J., Wilkinson, J., Trenell, M., Blamire, A., Howel, D. and Jones, D. E. (2015) 'RITPBC: B-cell depleting therapy (rituximab) as a treatment for fatigue in primary biliary cirrhosis: study protocol for a randomised controlled trial: Figure 1', *BMJ Open,* 5(8), pp. e007985.
Kaibori, M., Ishizaki, M., Iida, H., Matsui, K., Sakaguchi, T., Inoue, K., Mizuta, T., Ide, Y., Iwasaka, J., Kimura, Y., Hayashi, F., Habu, D. and Kon, M. (2015) 'Effect of Intramuscular Adipose Tissue Content on Prognosis in Patients Undergoing Hepatocellular Carcinoma Resection', *Journal of Gastrointestinal Surgery,* 19(7), pp. 1315-1323.
Kalaitzakis, E., Josefsson, A., Castedal, M., Henfridsson, P., Bengtsson, M., Hugosson, I., Andersson, B. and Björnsson, E. (2012) 'Factors Related to Fatigue in Patients With Cirrhosis Before and After Liver Transplantation', *Clinical Gastroenterology and Hepatology,* 10(2), pp. 174-181.e1.
Kaplan, G., Totsuka, A., Thompson, P., Akatsuka, T., Moritsugu, Y. and Feinstone, S. (1996) 'Identification of a surface glycoprotein on African green monkey kidney cells as a receptor for hepatitis A virus', *The EMBO journal,* 15(16), pp. 4282-4296.
Kasaian, M. T., Page, K. M., Fish, S., Brennan, A., Cook, T. A., Moreira, K., Zhang, M., Jesson, M., Marquette, K., Agostinelli, R., Lee, J., Williams, C. M., Tchistiakova, L. and Thakker, P. (2014) 'Therapeutic activity of an interleukin-4/interleukin-13 dual antagonist on oxazolone-induced colitis in mice', *Immunology,* 143(3), pp. 416-27.

Kask, K., Bäckström, T., Nilsson, L.-G. and Sundström-Poromaa, I. (2008) 'Allopregnanolone impairs episodic memory in healthy women', *Psychopharmacology,* 199(2), pp. 161-168.
Katz, P. (2017) 'Fatigue in Rheumatoid Arthritis', *Current Rheumatology Reports,* 19(5), pp. 25.
Kawano, M., Hirano, T., Matsuda, T., Taga, T., Horii, Y., Iwato, K., Asaoku, H., Tang, B., Tanabe, O. and Tanaka, H. (1988) 'Autocrine generation and requirement of BSF-2/IL-6 for human multiple myelomas', *Nature,* 332(6159), pp. 83-85.
Kawata, K., Kobayashi, Y., Gershwin, M. E. and Bowlus, C. L. (2012) 'The Immunophysiology and Apoptosis of Biliary Epithelial Cells: Primary Biliary Cirrhosis and Primary Sclerosing Cholangitis', *Clinical Reviews in Allergy & Immunology,* 43(3), pp. 230-241.
Kazankov, K., Barrera, F., Møller, H. J., Bibby, B. M., Vilstrup, H., George, J. and Grønbæk, H. (2014) 'Soluble CD163, a macrophage activation marker, is independently associated with fibrosis in patients with chronic viral hepatitis B and C', *Hepatology,* 60(2), pp. 521-530.
Kazankov, K., Møller, H., Lange, A., Birkebaek, N., Holland‐Fischer, P., Solvig, J., Hørlyck, A., Kristensen, K., Rittig, S. and Handberg, A. (2015) 'The macrophage activation marker s CD 163 is associated with changes in NAFLD and metabolic profile during lifestyle intervention in obese children', *Pediatric Obesity,* 10(3), pp. 226-233.
Kelesidis, T., Kelesidis, I., Chou, S. and Mantzoros, C. S. (2010) 'Narrative review: the role of leptin in human physiology: emerging clinical applications', *Ann Intern Med,* 152(2), pp. 93-100.
Kempler, P., Váradi, A., Kádár, É. and Szalay, F. (1994) 'Autonomic and peripheral neuropathy in primary biliary cirrhosis: evidence of small sensory fibre damage and prolongation of the QT interval', *Journal of Hepatology,* 21(6), pp. 1150-1151.
Kendzerska, T. B., Smith, P. M., Brignardello-Petersen, R., Leung, R. S. and Tomlinson, G. A. (2014) 'Evaluation of the measurement properties of the Epworth sleepiness scale: A systematic review', *Sleep Medicine Reviews,* 18(4), pp. 321-331.
Kenny, R. A., Kalaria, R. and Ballard, C. (2002) 'Neurocardiovascular instability in cognitive impairment and dementia', *Ann N Y Acad Sci,* 977, pp. 183-95.
Kenyon, A. P., Tribe, R. M., Nelson-Piercy, C., Girling, J. C., Williamson, C., Seed, P. T., Vaughan-Jones, S. and Shennan, A. H. (2010) 'Pruritus in pregnancy: a study of anatomical distribution and prevalence in relation to the development of obstetric cholestasis', *Obstet Med,* 3(1), pp. 25-9.
Keresztes, K. (2004) 'Autonomic and sensory nerve dysfunction in primary biliary cirrhosis', *World Journal of Gastroenterology,* 10(20), pp. 3039.
Kerfoot, S. M., D’Mello, C., Nguyen, H. H., Ajuebor, M. N., Kubes, P., Le, T. and Swain, M. G. (2006) 'TNF‐α‐secreting monocytes are recruited into the brain of cholestatic mice', *Hepatology,* 43.
Keski-Rahkonen, P., Huhtinen, K., Poutanen, M. and Auriola, S. (2011) 'Fast and sensitive liquid chromatography–mass spectrometry assay for seven androgenic and progestagenic steroids in human serum', *The Journal of Steroid Biochemistry and Molecular Biology,* 127(3-5), pp. 396-404.
Khanna, A., Jopson, L., Howel, D., Bryant, A., Blamire, A., Newton, J. L. and Jones, D. E. (2019) 'Rituximab Is Ineffective for Treatment of Fatigue in Primary Biliary Cholangitis: A Phase 2 Randomized Controlled Trial', *Hepatology,* 70(5), pp. 1646-1657.
Khanna, A., Jopson, L., Howel, D., Bryant, A., Blamire, A., Newton, J. L., Wilkinson, J., Steel, A. J., Bainbridge, J., Stefanetti, R., Cassidy, S., Houghton, D. and Jones, D. E. (2018) 'Rituximab for the treatment of fatigue in primary biliary cholangitis (formerly primary

biliary cirrhosis): a randomised controlled trial', *Efficacy and Mechanism Evaluation,* 5(2), pp. 1-78.
Khodarahmi, R., Aghelan, Z., Kiani, S., Vaisi-Raygani, A., Sadeghi, M. and Nasiri, A. (2020) 'Emerging regulatory roles of mitochondrial sirtuins on pyruvate dehydrogenase complex and the related metabolic diseases: Review', *Biomedical Research and Therapy,* 7(2), pp. 3645-3658.
Khurana, S. and Singh, P. (2006) 'Rifampin is safe for treatment of pruritus due to chronic cholestasis: a meta‐analysis of prospective randomized‐controlled trials', *Liver International,* 26(8), pp. 943-948.
Kim, J., Nakajima, K., Oomura, Y., Wayner, M. J. and Sasaki, K. (2009) 'Electrophysiological effects of ghrelin on pedunculopontine tegmental neurons in rats: An in vitro study', *Peptides,* 30(4), pp. 745-757.
Kimura, A. and Kishimoto, T. (2010) 'IL-6: Regulator of Treg/Th17 balance', *European Journal of Immunology,* 40(7), pp. 1830-1835.
Kita, H., Matsumura, S., He, X. S., Ansari, A. A., Lian, Z. X., Van de Water, J., Coppel, R. L., Kaplan, M. M. and Gershwin, M. E. (2002) 'Quantitative and functional analysis of PDC-E2-specific autoreactive cytotoxic T lymphocytes in primary biliary cirrhosis', *J Clin Invest,* 109(9), pp. 1231-40.
Kitajima, Y., Hyogo, H., Sumida, Y., Eguchi, Y., Ono, N., Kuwashiro, T., Tanaka, K., Takahashi, H., Mizuta, T., Ozaki, I., Eguchi, T., Kimura, Y., Fujimoto, K. and Anzai, K. (2013) 'Severity of non-alcoholic steatohepatitis is associated with substitution of adipose tissue in skeletal muscle', *Journal of Gastroenterology and Hepatology,* 28(9), pp. 1507-1514.
Kjøbsted, R., Hingst, J. R., Fentz, J., Foretz, M., Sanz, M.-N., Pehmøller, C., Shum, M., Marette, A., Mounier, R. and Treebak, J. T. (2018) 'AMPK in skeletal muscle function and metabolism', *The FASEB journal,* 32(4), pp. 1741.
Klein, R. S., Lin, E., Zhang, B., Luster, A. D., Tollett, J., Samuel, M. A., Engle, M. and Diamond, M. S. (2005) 'Neuronal CXCL10 directs CD8+ T-cell recruitment and control of West Nile virus encephalitis', *Journal of virology,* 79(17), pp. 11457-11466.
Koivuranta, K. T., Hakkola, E. H. and Hiltunen, J. K. (1994) 'Isolation and characterization of cDNA for human 120 kDa mitochondrial 2,4-dienoyl-coenzyme A reductase', *Biochem J,* 304 (Pt 3)(Pt 3), pp. 787-92.
Kojima, M., Hosoda, H., Date, Y., Nakazato, M., Matsuo, H. and Kangawa, K. (1999) 'Ghrelin is a growth-hormone-releasing acylated peptide from stomach', *Nature,* 402(6762), pp. 656-660.
Koob, G. F. (1999) 'Corticotropin-releasing factor, norepinephrine, and stress', *Biol Psychiatry,* 46(9), pp. 1167-80.
Koper, O. M., Kamińska, J., Sawicki, K. and Kemona, H. (2018) 'CXCL9, CXCL10, CXCL11, and their receptor (CXCR3) in neuroinflammation and neurodegeneration', *Advances in clinical and experimental medicine : official organ Wroclaw Medical University,* 27(6), pp. 849-856.
Koronowski, K. B., Kinouchi, K., Welz, P.-S., Smith, J. G., Zinna, V. M., Shi, J., Samad, M., Chen, S., Magnan, C. N., Kinchen, J. M., Li, W., Baldi, P., Benitah, S. A. and Sassone-Corsi, P. (2019) 'Defining the Independence of the Liver Circadian Clock', *Cell,* 177(6), pp. 1448-1462.e14.
Kouwenhoven, T. A., van de Kerkhof, P. C. M. and Kamsteeg, M. (2017) 'Use of oral antidepressants in patients with chronic pruritus: A systematic review', *J Am Acad Dermatol,* 77(6), pp. 1068-1073.e7.
Kowdley, K. V., Luketic, V., Chapman, R., Hirschfield, G. M., Poupon, R., Schramm, C., Vincent, C., Rust, C., Parés, A., Mason, A., Marschall, H.-U., Shapiro, D., Adorini, L., Sciacca, C., Beecher-Jones, T., Böhm, O., Pencek, R. and Jones, D. (2018) 'A randomized trial of obeticholic acid monotherapy in patients with primary biliary cholangitis', *Hepatology,* 67(5), pp. 1890-1902.

Krauthausen, M., Kummer, M. P., Zimmermann, J., Reyes-Irisarri, E., Terwel, D., Bulic, B., Heneka, M. T. and Müller, M. (2015) 'CXCR3 promotes plaque formation and behavioral deficits in an Alzheimer's disease model', *The Journal of clinical investigation,* 125(1), pp. 365-378.
Kremer, A. E., Beuers, U., Oude-Elferink, R. P. and Pusl, T. (2008a) 'Pathogenesis and treatment of pruritus in cholestasis', *Drugs,* 68, pp. 2163-2182.
Kremer, A. E., Beuers, U., Oude-Elferink, R. P. J. and Pusl, T. (2008b) 'Pathogenesis and Treatment of Pruritus in Cholestasis', *Drugs,* 68(15), pp. 2163-2182.
Kremer, A. E., Le Cleac'h, A., Lemoinne, S., Wolf, K., De Chaisemartin, L., Chollet-Martin, S., Humbert, L., Rainteau, D., Poupon, R., Rousseau, A., Chazouillères, O. and Corpechot, C. (2019) 'Antipruritic effect of bezafibrate and serum autotaxin measures in patients with primary biliary cholangitis', *Gut,* 68(10), pp. 1902-1903.
Kremer, A. E., Martens, J. J., Kulik, W., Ruëff, F., Kuiper, E. M., van Buuren, H. R., van Erpecum, K. J., Kondrackiene, J., Prieto, J., Rust, C., Geenes, V. L., Williamson, C., Moolenaar, W. H., Beuers, U. and Oude Elferink, R. P. (2010) 'Lysophosphatidic acid is a potential mediator of cholestatic pruritus', *Gastroenterology,* 139(3), pp. 1008-18, 1018.e1.
Kremer, A. E., Mayo, M. J., Hirschfield, G., Levy, C., Bowlus, C. L., Jones, D. E., Steinberg, A., McWherter, C. A. and Choi, Y. J. (2022) 'Seladelpar improved measures of pruritus, sleep, and fatigue and decreased serum bile acids in patients with primary biliary cholangitis', *Liver Int,* 42(1), pp. 112-123.
Kremer, A. E., Van Dijk, R., Leckie, P., Schaap, F. G., Kuiper, E. M. M., Mettang, T., Reiners, K. S., Raap, U., Van Buuren, H. R., Van Erpecum, K. J., Davies, N. A., Rust, C., Engert, A., Jalan, R., Oude Elferink, R. P. J. and Beuers, U. (2012) 'Serum autotaxin is increased in pruritus of cholestasis, but not of other origin, and responds to therapeutic interventions', *Hepatology,* 56(4), pp. 1391-1400.
Krieger, D., Krieger, S., Jansen, O., Gass, P., Theilmann, L. and Lichtnecker, H. (1995) 'Manganese and chronic hepatic encephalopathy', *Lancet,* 346(8970), pp. 270-4.
Kuhl, U., Ulrich, G. and Schultheiss, H.-P. (1987) 'Cross-reactivity of antibodies to the ADP/ATP translocator of the inner mitochondrial membrane with the cell surface of cardiac myocytes', *European Heart Journal,* 8(suppl J), pp. 219-222.
Kuilman, T., Michaloglou, C., Vredeveld, L. C., Douma, S., van Doorn, R., Desmet, C. J., Aarden, L. A., Mooi, W. J. and Peeper, D. S. (2008) 'Oncogene-induced senescence relayed by an interleukin-dependent inflammatory network', *Cell,* 133(6), pp. 1019-1031.
Kuiper, E. M., Hansen, B. E., de Vries, R. A., den Ouden-Muller, J. W., van Ditzhuijsen, T. J., Haagsma, E. B., Houben, M. H., Witteman, B. J., van Erpecum, K. J., van Buuren, H. R. and Dutch, P. B. C. S. G. (2009) 'Improved prognosis of patients with primary biliary cirrhosis that have a biochemical response to ursodeoxycholic acid', *Gastroenterology,* 136(4), pp. 1281-7.
Kulkarni, N., Meitei, H. T., Sonar, S. A., Sharma, P. K., Mujeeb, V. R., Srivastava, S., Boppana, R. and Lal, G. (2018) 'CCR6 signaling inhibits suppressor function of induced-Treg during gut inflammation', *Journal of Autoimmunity,* 88, pp. 121-130.
Kulkarni, N., Pathak, M. and Lal, G. (2017) 'Role of chemokine receptors and intestinal epithelial cells in the mucosal inflammation and tolerance', *Journal of Leucocyte Biology,* 101(2), pp. 377-394.
Kumagi, T., Guindi, M., Fischer, S. E., Arenovich, T., Abdalian, R., Coltescu, C., Heathcote, E. J. and Hirschfield, G. M. (2010a) 'Baseline ductopenia and treatment response predict long-term histological progression in primary biliary cirrhosis', *Am J Gastroenterol,* 105(10), pp. 2186-94.
Kumagi, T., Guindi, M., Fischer, S. E., Arenovich, T., Abdalian, R., Coltescu, C., Heathcote, J. E. and Hirschfield, G. M. (2010b) 'Baseline Ductopenia and Treatment Response Predict

Long-Term Histological Progression in Primary Biliary Cirrhosis', *Official journal of the American College of Gastroenterology | ACG,* 105(10), pp. 2186-2194.
Kushnir-Sukhov, N. M., Brown, J. M., Wu, Y., Kirshenbaum, A. and Metcalfe, D. D. (2007) 'Human mast cells are capable of serotonin synthesis and release', *J Allergy Clin Immunol,* 119(2), pp. 498-9.
Kwok, T., Medovich, S. C., Silva-Junior, I. A., Brown, E. M., Haug, J. C., Barrios, M. R., Morris, K. A. and Lancaster, J. N. (2022) 'Age-Associated Changes to Lymph Node Fibroblastic Reticular Cells', *Frontiers in Aging,* 3.
Labenz, C., Prochaska, J. H., Huber, Y., Nagel, M., Straub, B. K., Wild, P., Galle, P. R. and Schattenberg, J. M. (2019) 'Cardiovascular Risk Categories in Patients With Nonalcoholic Fatty Liver Disease and the Role of Low‐Density Lipoprotein Cholesterol', *Hepatology Communications,* 3(11), pp. 1472-1481.
Laharie, D., Bourreille, A., Branche, J., Allez, M., Bouhnik, Y., Filippi, J., Zerbib, F., Savoye, G., Nachury, M. and Moreau, J. (2012) 'Ciclosporin versus infliximab in patients with severe ulcerative colitis refractory to intravenous steroids: a parallel, open-label randomised controlled trial', *The Lancet,* 380(9857), pp. 1909-1915.
Lal, D., Thakur, M. and Bihari, C. (2020) 'Serum Leptin Serves as an Inflammatory Activity Marker and Predicts Steroid Response in Autoimmune Hepatitis', *Journal of Clinical and Experimental Hepatology,* 10(6), pp. 574-580.
Lamireau, T., Zoltowska, M., Levy, E., Yousef, I., Rosenbaum, J., Tuchweber, B. and Desmoulière, A. (2003) 'Effects of bile acids on biliary epithelial cells: proliferation, cytotoxicity, and cytokine secretion', *Life Sci,* 72(12), pp. 1401-11.
Lan, J., Ge, J., Yu, J., Shan, S., Zhou, H., Fan, S., Zhang, Q., Shi, X., Wang, Q., Zhang, L. and Wang, X. (2020) 'Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor', *Nature,* 581(7807), pp. 215-220.
Landi, A., Broadhurst, D., Vernon, S. D., Tyrrell, D. L. J. and Houghton, M. (2016) 'Reductions in circulating levels of IL-16, IL-7 and VEGF-A in myalgic encephalomyelitis/chronic fatigue syndrome', *Cytokine,* 78, pp. 27-36.
Landi, A., Weismuller, T. J., Lankisch, T. O., Santer, D. M., Tyrrell, D. L. J., Manns, M. P. and Houghton, M. (2014) 'Differential Serum Levels of Eosinophilic Eotaxins in Primary Sclerosing Cholangitis, Primary Biliary Cirrhosis, and Autoimmune Hepatitis', *Journal of Interferon & Cytokine Research,* 34(3), pp. 204-214.
Langedijk, J., Beuers, U. H. and Oude Elferink, R. P. J. (2021) 'Cholestasis-Associated Pruritus and Its Pruritogens', *Front Med (Lausanne),* 8, pp. 639674.
LaPorte, S. L., Juo, Z. S., Vaclavikova, J., Colf, L. A., Qi, X., Heller, N. M., Keegan, A. D. and Garcia, K. C. (2008) 'Molecular and structural basis of cytokine receptor pleiotropy in the interleukin-4/13 system', *Cell,* 132(2), pp. 259-72.
Lattie, E. G., Antoni, M. H., Fletcher, M. A., Penedo, F., Czaja, S., Lopez, C., Perdomo, D., Sala, A., Nair, S., Fu, S. H. and Klimas, N. (2012) 'Stress management skills, neuroimmune processes and fatigue levels in persons with chronic fatigue syndrome', *Brain, Behavior, and Immunity,* 26(6), pp. 849-858.
Leckie, P., Tritto, G., Mookerjee, R., Davies, N., Jones, D. and Jalan, R. (2012) ''Out-patient' albumin dialysis for cholestatic patients with intractable pruritus', *Aliment Pharmacol Ther,* 35(6), pp. 696-704.
Lessard, C. J., Ice, J. A., Adrianto, I., Wiley, G. B., Kelly, J. A., Gaffney, P. M., Montgomery, C. G. and Moser, K. L. (2012) 'The genomics of autoimmune disease in the era of genome-wide association studies and beyond', *Autoimmunity Reviews,* 11(4), pp. 267-275.
Levy, C., Bowlus, C., Neff, G., Swain, M. G., Michael, G., Mayo, M. J., Goel, A., Trivedi, P., Hirschfield, G., Aspinall, R., Kremer, A. E., Gordon, S. C., Borg, B., Harrison, S., Jones, D., Thuluvath, P., Doerffel, Y., Stanca, C., Sheridan, D., Bacon, B., Berg, C., Thorburn, D., Vierling, J., Shiffman, M., Odin, J., Hassanein, T., Peyton, A., Gulamhusein, A., Landis, C.,

Bernstein, D., Corless, L., Woerns, M.-A., Buggisch, P., Bergheanu, S., Chen, W., Seput-Dingle, F., Mao, L., Li, Y., Yang, K., Muslimova, E., Choi, Y.-J., Varga, M., Dickinson, K., Boudes, P., McWherter, C. and Steinberg, A. (2020) 'Durability of treatment response after 1 year of therapy with seladelpar in patients with primary biliary cholangitis (PBC): final results of an international phase 2 study', *Journal of Hepatology,* 73, pp. S464-S465.
Levy, C., Peter, J. A., Nelson, D. R., Keach, J., Petz, J., Cabrera, R., Clark, V., Firpi, R. J., Morelli, G., Soldevila-Pico, C. and Lindor, K. (2011) 'Pilot study: fenofibrate for patients with primary biliary cirrhosis and an incomplete response to ursodeoxycholic acid', *Alimentary Pharmacology & Therapeutics,* 33(2), pp. 235-242.
Lewis, J. (2018) 'Histopathology of granulomatous liver disease', *Clin Liver Dis (Hoboken),* 11(3), pp. 77-80.
Li, T. and Chiang, J. Y. (2009) 'Regulation of bile acid and cholesterol metabolism by PPARs', *PPAR Res,* 2009, pp. 501739.
Li, X., Li, Y., Xiao, J., Wang, H., Guo, Y., Mao, X., Shi, P., Hou, Y., Zhang, X., Zhao, N., Zheng, M., He, Y., Ding, J., Tan, Y., Liao, M., Li, L., Peng, Y., Li, X., Pan, Q., Xie, Q., Li, Q., Li, J., Li, Y., Chen, Z., Huang, Y., Assis, D. N., Cai, S. Y., Boyer, J. L., Huang, X., Tang, C. E., Liu, X., Peng, S. and Chai, J. (2023) 'Unique DUOX2(+)ACE2(+) small cholangiocytes are pathogenic targets for primary biliary cholangitis', *Nat Commun,* 14(1), pp. 29.
Liao, F., Rabin, R. L., Yannelli, J. R., Koniaris, L. G., Vanguri, P. and Farber, J. M. (1995) 'Human Mig chemokine: biochemical and functional characterization', *J Exp Med,* 182(5), pp. 1301-14.
Liaskou, E., Patel, S. R., Webb, G., Bagkou Dimakou, D., Akiror, S., Krishna, M., Mells, G., Jones, D. E., Bowman, S. J., Barone, F., Fisher, B. A. and Hirschfield, G. M. (2018) 'Increased sensitivity of Treg cells from patients with PBC to low dose IL-12 drives their differentiation into IFN-γ secreting cells', *Journal of Autoimmunity,* 94, pp. 143-155.
Licinio, J., Mantzoros, C., Negrão, A. B., Cizza, G., Wong, M.-L., Bongiorno, P. B., Chrousos, G. P., Karp, B., Allen, C. and Flier, J. S. (1997) 'Human leptin levels are pulsatile and inversely related to pituitary–ardenal function', *Nature medicine,* 3(5), pp. 575-579.
Lindegaard, B., Matthews, V. B., Brandt, C., Hojman, P., Allen, T. L., Estevez, E., Watt, M. J., Bruce, C. R., Mortensen, O. H. and Syberg, S. (2013) 'Interleukin-18 activates skeletal muscle AMPK and reduces weight gain and insulin resistance in mice', *Diabetes,* 62(9), pp. 3064-3074.
Lindor, K. D., Bowlus, C. L., Boyer, J., Levy, C. and Mayo, M. (2018) 'Primary Biliary Cholangitis: 2018 Practice Guidance from the American Association for the Study of Liver Diseases', *Hepatology*.
Liu, J. Z., Almarri, M. A., Gaffney, D. J., Mells, G. F., Jostins, L., Cordell, H. J., Ducker, S. J., Day, D. B., Heneghan, M. A., Neuberger, J. M., Donaldson, P. T., Bathgate, A. J., Burroughs, A., Davies, M. H., Jones, D. E., Alexander, G. J., Barrett, J. C., Sandford, R. N. and Anderson, C. A. (2012) 'Dense fine-mapping study identifies new susceptibility loci for primary biliary cirrhosis', *Nature Genetics,* 44(10), pp. 1137-1141.
Liu, Z., Li, F., Pan, A., Xue, H., Jiang, S., Zhu, C., Jin, M., Fang, J., Zhu, X., Brown, M. A. and Wang, X. (2019) 'Elevated CCL19/CCR7 Expression During the Disease Process of Primary Sjögren's Syndrome', *Frontiers in Immunology,* 10.
Lleo, A. (2022) 'A change of paradigm in PBC: Pursuing normal alkaline phosphatase', *eBioMedicine,* 82, pp. 104150.
Lubel, J. S., Herath, C. B., Tchongue, J., Grace, J., Jia, Z., Spencer, K., Casley, D., Crowley, P., Sievert, W. and Burrell, L. M. (2009a) 'Angiotensin-(1–7), an alternative metabolite of the renin–angiotensin system, is up-regulated in human liver disease and has antifibrotic activity in the bile-duct-ligated rat', *Clinical science,* 117(11), pp. 375-386.
Lubel, John S., Herath, Chandana B., Tchongue, J., Grace, J., Jia, Z., Spencer, K., Casley, D., Crowley, P., Sievert, W., Burrell, Louise M. and Angus, Peter W. (2009b) 'Angiotensin-(1–

7), an alternative metabolite of the renin–angiotensin system, is up-regulated in human liver disease and has antifibrotic activity in the bile-duct-ligated rat', *Clinical Science,* 117(11), pp. 375-386.
Ludwig, J., Dickson, E. R. and Mcdonald, G. S. A. (1978) 'Staging of chronic nonsuppurative destructive cholangitis (syndrome of primary biliary cirrhosis)', *Virchows Archiv A Pathological Anatomy and Histology,* 379(2), pp. 103-112.
Luisi, S., Petraglia, F., Benedetto, C., Nappi, R. E., Bernardi, F., Fadalti, M., Reis, F. M., Luisi, M. and Genazzani, A. R. (2000) 'Serum Allopregnanolone Levels in Pregnant Women: Changes during Pregnancy, at Delivery, and in Hypertensive Patients', *The Journal of Clinical Endocrinology & Metabolism,* 85(7), pp. 2429-2433.
MacKenzie, G. and Maguire, J. (2013) 'Neurosteroids and GABAergic signaling in health and disease', *Biomol Concepts,* 4(1), pp. 29-42.
Maeda, M. H., Tsuji, S. and Shimizu, J. (2012) 'Inflammatory myopathies associated with anti-mitochondrial antibodies', *Brain,* 135(6), pp. 1767-1777.
Maes, M., Scharpé, S., Meltzer, H. Y., Bosmans, E., Suy, E., Calabrese, J. and Cosyns, P. (1993) 'Relationships between interleukin-6 activity, acute phase proteins, and function of the hypothalamic-pituitary-adrenal axis in severe depression', *Psychiatry Research,* 49(1), pp. 11-27.
Mainardi, M., Fusco, S. and Grassi, C. (2015) 'Modulation of Hippocampal Neural Plasticity by Glucose-Related Signaling', *Neural Plasticity,* 2015, pp. 657928.
Malato, J., Sotzny, F., Bauer, S., Freitag, H., Fonseca, A., Grabowska, A. D., Graça, L., Cordeiro, C., Nacul, L., Lacerda, E. M., Castro-Marrero, J., Scheibenbogen, C., Westermeier, F. and Sepúlveda, N. (2021a) 'The SARS-CoV-2 receptor angiotensin-converting enzyme 2 (ACE2) in myalgic encephalomyelitis/chronic fatigue syndrome: A meta-analysis of public DNA methylation and gene expression data', *Heliyon,* 7(8), pp. e07665.
Malato, J., Sotzny, F., Bauer, S., Freitag, H., Fonseca, A., Grabowska, A. D., Graça, L., Cordeiro, C., Nacul, L., Lacerda, E. M., Castro-Marrero, J., Scheibenbogen, C., Westermeier, F. and Sepúlveda, N. (2021b) 'The SARS-CoV-2 receptor angiotensin-converting enzyme 2 (ACE2) in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome: analysis of high-throughput epigenetic and gene expression studies', *medRxiv*.
Manousou, P., Kolios, G., Drygiannakis, I., Koulentaki, M., Pyrovolaki, K., Voumvouraki, A., Notas, G., Bourikas, L., Papadaki, H. A. and Kouroumalis, E. (2013) 'CXCR3 axis in patients with primary biliary cirrhosis: a possible novel mechanism of the effect of ursodeoxycholic acid', *Clinical and Experimental Immunology,* 172(1), pp. 9-15.
Marjot, T., Ray, D. W., Williams, F. R., Tomlinson, J. W. and Armstrong, M. J. (2021) 'Sleep and liver disease: a bidirectional relationship', *The Lancet Gastroenterology & Hepatology*.
Marvie, P., Lisbonne, M., L'Helgoualc'H, A., Rauch, M., Turlin, B., Preisser, L., Bourd-Boittin, K., Théret, N., Gascan, H., Piquet-Pellorce, C. and Samson, M. (2009) 'Interleukin-33 overexpression is associated with liver fibrosis in mice and humans', *Journal of Cellular and Molecular Medicine,* 14(6b), pp. 1726-1739.
Mastorakos, G., Chrousos, G. P. and Weber, J. S. (1993) 'Recombinant interleukin-6 activates the hypothalamic-pituitary-adrenal axis in humans', *The Journal of Clinical Endocrinology & Metabolism,* 77(6), pp. 1690-1694.
Matikainen, S., Paananen, A., Miettinen, M., Kurimoto, M., Timonen, T., Julkunen, I. and Sareneva, T. (2001) 'IFN‐α and IL‐18 synergistically enhance IFN‐γ production in human NK cells: differential regulation of Stat4 activation and IFN‐γ gene expression by IFN‐α and IL‐12', *European journal of immunology,* 31(7), pp. 2236-2245.
Mattalia, A., Quaranta, S., Leung, P. S. C., Bauducci, M., Van De Water, J., Calvo, P. L., Danielle, F., Rizzetto, M., Ansari, A., Coppel, R. L., Rosina, F. and Gershwin, M. E. (1998) 'Characterization of antimitochondrial antibodies in healthy adults', *Hepatology,* 27(3), pp. 656-661.

Mcdonald, C., Newton, J., Lai, H. M., Baker, S. N. and Jones, D. E. (2010) 'Central nervous system dysfunction in primary biliary cirrhosis and its relationship to symptoms', *Journal of Hepatology,* 53(6), pp. 1095-1100.
McDonald, R. J. and White, N. M. (1993) 'A triple dissociation of memory systems: hippocampus, amygdala, and dorsal striatum', *Behav Neurosci,* 107(1), pp. 3-22.
McHedlidze, T., Waldner, M., Zopf, S., Walker, J., Rankin, A. L., Schuchmann, M., Voehringer, D., McKenzie, A. N., Neurath, M. F., Pflanz, S. and Wirtz, S. (2013) 'Interleukin-33-dependent innate lymphoid cells mediate hepatic fibrosis', *Immunity,* 39(2), pp. 357-71.
Mcintire, J. J., Umetsu, S. E., Macaubas, C., Hoyte, E. G., Cinnioglu, C., Cavalli-Sforza, L. L., Barsh, G. S., Hallmayer, J. F., Underhill, P. A., Risch, N. J., Freeman, G. J., Dekruyff, R. H. and Umetsu, D. T. (2003) 'Hepatitis A virus link to atopic disease', *Nature,* 425(6958), pp. 576-576.
Mcnally, R. J. Q., Ducker, S. and James, O. F. W. (2009) 'Are transient environmental agents involved in the cause of primary biliary cirrhosis? Evidence from space-time clustering analysis', *Hepatology,* 50(4), pp. 1169-1174.
Mease, P. J. (2002) 'Tumour necrosis factor (TNF) in psoriatic arthritis: pathophysiology and treatment with TNF inhibitors', *Annals of the Rheumatic Diseases,* 61(4), pp. 298-304.
Meda Spaccamela, V., Valencia, R. G., Pastukhov, O., Duppenthaler, A., Dettmer, M. S., Erb, J., Steiner, U. C., Hillinger, S., Speckmann, C., Ehl, S., Reichenbach, J. and Siler, U. (2019) 'High Levels of IL-18 and IFN-γ in Chronically Inflamed Tissue in Chronic Granulomatous Disease', *Front Immunol,* 10, pp. 2236.
Mehrabani, S., Askari, G., Miraghajani, M., Tavakoly, R. and Arab, A. (2019) 'Effect of coenzyme Q10 supplementation on fatigue: A systematic review of interventional studies', *Complement Ther Med,* 43, pp. 181-187.
Meitei, H. T., Jadhav, N. and Lal, G. (2021) 'CCR6-CCL20 axis as a therapeutic target for autoimmune diseases', *Autoimmunity Reviews,* 20(7), pp. 102846.
Mellergård, J., Edström, M., Vrethem, M., Ernerudh, J. and Dahle, C. (2010) 'Natalizumab treatment in multiple sclerosis: marked decline of chemokines and cytokines in cerebrospinal fluid', *Multiple Sclerosis Journal,* 16(2), pp. 208-217.
Mells, G. F., Floyd, J. A. B., Morley, K. I., Cordell, H. J., Franklin, C. S., Shin, S.-Y., Heneghan, M. A., Neuberger, J. M., Donaldson, P. T., Day, D. B., Ducker, S. J., Muriithi, A. W., Wheater, E. F., Hammond, C. J., Dawwas, M. F., Jones, D. E., Peltonen, L., Alexander, G. J., Sandford, R. N. and Anderson, C. A. (2011) 'Genome-wide association study identifies 12 new susceptibility loci for primary biliary cirrhosis', *Nature Genetics,* 43(4), pp. 329-332.
Mells, G. F., Pells, G., Newton, J. L., Bathgate, A. J., Burroughs, A. K., Heneghan, M. A., Neuberger, J. M., Day, D. B., Ducker, S. J., Sandford, R. N., Alexander, G. J. and Jones, D. E. J. (2013) 'Impact of primary biliary cirrhosis on perceived quality of life: The UK-PBC national study', *Hepatology,* 58(1), pp. 273-283.
Meng, F. and Lowell, C. A. (1997) 'Lipopolysaccharide (LPS)-induced macrophage activation and signal transduction in the absence of Src-family kinases Hck, Fgr, and Lyn', *J Exp Med,* 185(9), pp. 1661-70.
Meng, J., Moriyama, M., Feld, M., Buddenkotte, J., Buhl, T., Szöllösi, A., Zhang, J., Miller, P., Ghetti, A. and Fischer, M. (2018) 'New mechanism underlying IL-31–induced atopic dermatitis', *Journal of Allergy and Clinical Immunology,* 141(5), pp. 1677-1689. e8.
Metcalf, J., Bhopal, R., Gray, J., Howel, D. and James, O. (1997) 'Incidence and prevalence of primary biliary cirrhosis in the city of Newcastle upon Tyne, England', *International Journal of Epidemiology,* 26(4), pp. 830-836.
Metcalf, J. V., Mitchison, H. C., Palmer, J. M., Jones, D. E., Bassendine, M. F. and James, O. F. (1996) 'Natural history of early primary biliary cirrhosis', *Lancet,* 348(9039), pp. 1399-402.

Milliner, D. S., Harris, P. C., Sas, D. J., Cogal, A. G. and Lieske, J. C. (2022) 'Primary hyperoxaluria type 1', *GeneReviews®[Internet]*.
Mills, G. B. and Moolenaar, W. H. (2003) 'The emerging role of lysophosphatidic acid in cancer', *Nature Reviews Cancer,* 3(8), pp. 582-591.
Mitchison, H. C., Bassendine, M. F., Hendrick, A., Bennett, M. K., Bird, G., Watson, A. J. and James, O. F. W. (1986) 'Positive antimitochondrial antibody but normal alkaline phosphatase: Is this primary biliary cirrhosis?', *Hepatology,* 6(6), pp. 1279-1284.
Moestrup, S. and Møller, H. (2004) 'CD163: a regulated hemoglobin scavenger receptor with a role in the anti‐inflammatory response', *Annals of medicine,* 36(5), pp. 347-354.
Momah, N., Silveira, M. G., Jorgensen, R., Sinakos, E. and Lindor, K. D. (2012) 'Optimizing biochemical markers as endpoints for clinical trials in primary biliary cirrhosis', *Liver International,* 32(5), pp. 790-795.
Montagnese, S., De Pittà, C., De Rui, M., Corrias, M., Turco, M., Merkel, C., Amodio, P., Costa, R., Skene, D. J. and Gatta, A. (2014) 'Sleep-wake abnormalities in patients with cirrhosis', *Hepatology,* 59(2), pp. 705-712.
Montagnese, S., Lauridsen, M., Vilstrup, H., Zarantonello, L., Lakner, G., Fitilev, S., Zupanets, I., Kozlova, I., Bunkova, E., Tomasiewicz, K., Berglund, J. E., Rorsman, F., Hagström, H., Kechagias, S., Ocklind, C. E., Mauney, J., Thunarf, F., Mokhatarani, M., Bäckström, T., Doverskog, M., Lins, L.-E., Månsson, M., Samuelson, P., Nilsson, D., Schalling, M., Johansson, M., Arlander, E. and Scharschmidt, B. F. (2021) 'A pilot study of golexanolone, a new GABA-A receptor-modulating steroid antagonist, in patients with covert hepatic encephalopathy', *Journal of Hepatology,* 75(1), pp. 98-107.
Montagnese, S., Middleton, B., Mani, A. R., Skene, D. J. and Morgan, M. Y. (2010) 'On the Origin and the Consequences of Circadian Abnormalities in Patients With Cirrhosis', *American Journal of Gastroenterology,* 105(8), pp. 1773-1781.
Montagnese, S., Nsemi, L. M., Cazzagon, N., Facchini, S., Costa, L., Bergasa, N. V., Amodio, P. and Floreani, A. (2013) 'Sleep-Wake profiles in patients with primary biliary cirrhosis', *Liver International,* 33(2), pp. 203-209.
Montero, J. L., Pozo, J. C., Barrera, P., Fraga, E., Costán, G., Domínguez, J. L., Muntané, J., Rodriguez-Ariza, A., Pleguezuelo, M., Rufián, S., López-Cillero, P. and de la Mata, M. (2006) 'Treatment of refractory cholestatic pruritus with molecular adsorbent recirculating system (MARS)', *Transplant Proc,* 38(8), pp. 2511-3.
Mosher, V. A. L., Swain, M. G., Pang, J. X. Q., Kaplan, G. G., Sharkey, K. A., MacQueen, G. M. and Goodyear, B. G. (2017) 'Primary Biliary Cholangitis Alters Functional Connections of the Brain's Deep Gray Matter', *Clin Transl Gastroenterol,* 8(7), pp. e107.
Mosher, V. A. L., Swain, M. G., Pang, J. X. Q., Kaplan, G. G., Sharkey, K. A., MacQueen, G. M. and Goodyear, B. G. (2018) 'Magnetic resonance imaging evidence of hippocampal structural changes in patients with primary biliary cholangitis', *Clin Transl Gastroenterol,* 9(7), pp. 169.
Motivala, S. J., Tomiyama, A. J., Ziegler, M., Khandrika, S. and Irwin, M. R. (2009) 'Nocturnal levels of ghrelin and leptin and sleep in chronic insomnia', *Psychoneuroendocrinology,* 34(4), pp. 540-5.
Mousa, H. S., Lleo, A., Invernizzi, P., Bowlus, C. L. and Gershwin, M. E. (2015) 'Advances in pharmacotherapy for primary biliary cirrhosis', *Expert Opin Pharmacother,* 16(5), pp. 633-43.
Mu, N., Lin, F., Jiang, Z., Liang, Y. and Yang, Z. (2020) 'Characteristics of serum chemokine profile in primary biliary cholangitis', *Cytokine,* 136, pp. 155291.
Mukherji, A., Bailey, S. M., Staels, B. and Baumert, T. F. (2019) 'The circadian clock and liver function in health and disease', *Journal of Hepatology,* 71(1), pp. 200-211.
Murillo Perez, C. F., Harms, M. H., Lindor, K. D., van Buuren, H. R., Hirschfield, G. M., Corpechot, C., van der Meer, A. J., Feld, J. J., Gulamhusein, A., Lammers, W. J., Ponsioen, C.

Y., Carbone, M., Mason, A. L., Mayo, M. J., Invernizzi, P., Battezzati, P. M., Floreani, A., Lleo, A., Nevens, F., Kowdley, K. V., Bruns, T., Dalekos, G. N., Gatselis, N. K., Thorburn, D., Trivedi, P. J., Verhelst, X., Parés, A., Janssen, H. L. A., Hansen, B. E. and Group, o. b. o. t. G. P. S. (2020) 'Goals of Treatment for Improved Survival in Primary Biliary Cholangitis: Treatment Target Should Be Bilirubin Within the Normal Range and Normalization of Alkaline Phosphatase', *Official journal of the American College of Gastroenterology | ACG,* 115(7), pp. 1066-1074.
Murphy, B. (2004) 'Elevated levels of some neuroactive progesterone metabolites, particularly isopregnanolone, in women with chronic fatigue syndrome', *Psychoneuroendocrinology,* 29(2), pp. 245-268.
Murray-Brown, F. L. (2021) 'Naltrexone for cholestatic itch: a systematic review', *BMJ Supportive & Palliative Care,* 11(2), pp. 217-225.
Naftali, T., Novick, D., Gabay, G., Rubinstein, M. and Novis, B. (2007) 'Interleukin-18 and its binding protein in patients with inflammatory bowel disease during remission and exacerbation', *IMAJ-RAMAT GAN-,* 9(7), pp. 504.
Nagano, T., Yamamoto, K., Matsumoto, S., Okamoto, R., Tagashira, M., Ibuki, N., Matsumura, S., Yabushita, K., Okano, N. and Tsuji, T. (1999) *Journal of Clinical Immunology,* 19(6), pp. 422-427.
Nagaraju, K. and Morales, M. (2022) 'Targeting necroptosis for the treatment of myositis', *Nature Reviews Rheumatology,* 18(6), pp. 307-308.
Nakanishi, K. (2018) 'Unique Action of Interleukin-18 on T Cells and Other Immune Cells', *Frontiers in Immunology,* 9.
Nakanishi, K., Yoshimoto, T., Tsutsui, H. and Okamura, H. (2001) 'Interleukin-18 regulates both Th1 and Th2 responses', *Annu Rev Immunol,* 19(1), pp. 423-74.
Nakanuma, Y. and Kono, N. (1992) 'Expression of vimentin in proliferating and damaged bile ductules and interlobular bile ducts in nonneoplastic hepatobiliary diseases', *Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc,* 5(5), pp. 550-554.
Nas, K., Cevik, R., Batum, S., Sarac, A. J., Acar, S. and Kalkanli, S. (2011) 'Immunologic and psychosocial status in chronic fatigue syndrome', *Bratisl Lek Listy,* 112(4), pp. 208-12.
Neuberger, J. H., Gideon M. (2020) *Autoimmune Liver Disease: Management and Clinical Practice.* Wiley Blackwell.
Neuman, M., Angulo, P., Malkiewicz, I., Jorgensen, R., Shear, N., Dickson, E. R., Haber, J., Katz, G. and Lindor, K. (2002) 'Tumor necrosis factor-alpha and transforming growth factor-beta reflect severity of liver damage in primary biliary cirrhosis', *J Gastroenterol Hepatol,* 17(2), pp. 196-202.
Nevens, F., Andreone, P., Mazzella, G., Strasser, S. I., Bowlus, C., Invernizzi, P., Drenth, J. P. H., Pockros, P. J., Regula, J., Beuers, U., Trauner, M., Jones, D. E., Floreani, A., Hohenester, S., Luketic, V., Shiffman, M., Van Erpecum, K. J., Vargas, V., Vincent, C., Hirschfield, G. M., Shah, H., Hansen, B., Lindor, K. D., Marschall, H.-U., Kowdley, K. V., Hooshmand-Rad, R., Marmon, T., Sheeron, S., Pencek, R., Macconell, L., Pruzanski, M. and Shapiro, D. (2016) 'A Placebo-Controlled Trial of Obeticholic Acid in Primary Biliary Cholangitis', *New England Journal of Medicine,* 375(7), pp. 631-643.
Newton, J., Poyner, E. and Jones, D. E. J. (2009) 'Serum anti-mitochondrial antibodies and fatigue in primary biliary cirrhosis', *Liver International,* 29(8), pp. 1285-1286.
Newton, J. L., Allen, J., Kerr, S. and Jones, D. E. J. (2006a) 'Reduced heart rate variability and baroreflex sensitivity in primary biliary cirrhosis', *Liver International,* 26(2), pp. 197-202.
Newton, J. L., Bhala, N., Burt, J. and Jones, D. E. J. (2006b) 'Characterisation of the associations and impact of symptoms in primary biliary cirrhosis using a disease specific quality of life measure', *Journal of Hepatology,* 44(4), pp. 776-783.

Newton, J. L., Davidson, A., Kerr, S., Bhala, N., Pairman, J., Burt, J. and Jones, D. E. (2007a) 'Autonomic dysfunction in primary biliary cirrhosis correlates with fatigue severity', *Eur J Gastroenterol Hepatol,* 19(2), pp. 125-32.
Newton, J. L., Gibson, G. J., Tomlinson, M., Wilton, K. and Jones, D. (2006c) 'Fatigue in primary biliary cirrhosis is associated with excessive daytime somnolence', *Hepatology,* 44(1), pp. 91-8.
Newton, J. L., Hollingsworth, K. G., Taylor, R., El-Sharkawy, A. M., Khan, Z. U., Pearce, R., Sutcliffe, K., Okonkwo, O., Davidson, A., Burt, J., Blamire, A. M. and Jones, D. (2008a) 'Cognitive impairment in primary biliary cirrhosis: symptom impact and potential etiology', *Hepatology,* 48(2), pp. 541-9.
Newton, J. L., Hudson, M., Tachtatzis, P., Sutcliffe, K., Pairman, J., Burt, J. A. and Jones, D. E. J. (2007b) 'Population prevalence and symptom associations of autonomic dysfunction in primary biliary cirrhosis', *Hepatology,* 45(6), pp. 1496-1505.
Newton, J. L., Pairman, J., Sutcliffe, K., Wilton, K. and Jones, D. E. (2008b) 'A predictive model for fatigue and its etiologic associations in primary biliary cirrhosis', *Clin Gastroenterol Hepatol,* 6(2), pp. 228-33.
Nicklin, J., Cramp, F., Kirwan, J., Urban, M. and Hewlett, S. (2010) 'Collaboration with patients in the design of patient-reported outcome measures: capturing the experience of fatigue in rheumatoid arthritis', *Arthritis Care Res (Hoboken),* 62(11), pp. 1552-8.
Niemelä, M., Kangastupa, P., Niemelä, O., Bloigu, R. and Juvonen, T. (2016) 'Acute Changes in Inflammatory Biomarker Levels in Recreational Runners Participating in a Marathon or Half-Marathon', *Sports Med Open,* 2(1), pp. 21.
Nikolaus, S., Bode, C., Taal, E. and van de Laar, M. A. F. J. (2013) 'Fatigue and Factors Related to Fatigue in Rheumatoid Arthritis: A Systematic Review', *Arthritis Care & Research,* 65(7), pp. 1128-1146.
Nishi, Y., Hiejima, H., Hosoda, H., Kaiya, H., Mori, K., Fukue, Y., Yanase, T., Nawata, H., Kangawa, K. and Kojima, M. (2005) 'Ingested medium-chain fatty acids are directly utilized for the acyl modification of ghrelin', *Endocrinology,* 146(5), pp. 2255-2264.
O'Carroll, R. E., Hayes, P. C., Ebmeier, K. P., Dougall, N., Murray, C., Best, J. J., Bouchier, I. A. and Goodwin, G. M. (1991) 'Regional cerebral blood flow and cognitive function in patients with chronic liver disease', *Lancet,* 337(8752), pp. 1250-3.
Odle, A. K., Haney, A., Allensworth-James, M., Akhter, N. and Childs, G. V. (2014) 'Adipocyte versus pituitary leptin in the regulation of pituitary hormones: somatotropes develop normally in the absence of circulating leptin', *Endocrinology,* 155(11), pp. 4316-4328.
Oertelt, S., Rieger, R., Selmi, C., Invernizzi, P., Ansari, A. A., Coppel, R. L., Podda, M., Leung, P. S. C. and Gershwin, M. E. (2007) 'A sensitive bead assay for antimitochondrial antibodies: Chipping away at AMA-negative primary biliary cirrhosis', *Hepatology,* 45(3), pp. 659-665.
Ohta, Y., Hamada, Y. and Katsuoka, K. (2001) 'Expression of IL-18 in psoriasis', *Archives of Dermatological Research,* 293(7), pp. 334-342.
Oliveira, D., Narciso, F., Santos, M., Pereira, D., Coelho, F., Dias, J. and Pereira, L. (2008) 'Muscle strength but not functional capacity is associated with plasma interleukin-6 levels of community-dwelling elderly women', *Brazilian Journal of Medical and Biological Research,* 41, pp. 1148-1153.
Oo, Y. H., Banz, V., Kavanagh, D., Liaskou, E., Withers, D. R., Humphreys, E., Reynolds, G. M., Lee-Turner, L., Kalia, N., Hubscher, S. G., Klenerman, P., Eksteen, B. and Adams, D. H. (2012) 'CXCR3-dependent recruitment and CCR6-mediated positioning of Th-17 cells in the inflamed liver', *Journal of Hepatology,* 57(5), pp. 1044-1051.
Ostrowski, K., Rohde, T., Zacho, M., Asp, S. and Pedersen, B. K. (1998) 'Evidence that interleukin-6 is produced in human skeletal muscle during prolonged running', *J Physiol,* 508 (Pt 3)(Pt 3), pp. 949-53.

Packard, M. G. and Teather, L. A. (1998) 'Amygdala modulation of multiple memory systems: hippocampus and caudate-putamen', *Neurobiol Learn Mem,* 69(2), pp. 163-203.
Paizis, G. (2005) 'Chronic liver injury in rats and humans upregulates the novel enzyme angiotensin converting enzyme 2', *Gut,* 54(12), pp. 1790-1796.
Paizis, G., Cooper, M. E., Schembri, J. M., Tikellis, C., Burrell, L. M. and Angus, P. W. (2002) 'Up-regulation of components of the renin-angiotensin system in the bile duct–ligated rat liver', *Gastroenterology,* 123(5), pp. 1667-1676.
Paizis, G., Gilbert, R. E., Cooper, M. E., Murthi, P., Schembri, J. M., Wu, L. L., Rumble, J. R., Kelly, D. J., Tikellis, C., Cox, A., Smallwood, R. A. and Angus, P. W. (2001) 'Effect of angiotensin II type 1 receptor blockade on experimental hepatic fibrogenesis', *Journal of Hepatology,* 35(3), pp. 376-385.
Palmer, J. M., Jones, D. E., Quinn, J., Mchugh, A. and Yeaman, S. J. (1999) 'Characterization of the autoantibody responses to recombinant E3 binding protein (protein X) of pyruvate dehydrogenase in primary biliary cirrhosis', *Hepatology,* 30(1), pp. 21-26.
Panerai, R. B. (2009) 'Transcranial Doppler for evaluation of cerebral autoregulation', *Clin Auton Res,* 19(4), pp. 197-211.
Papadopoulos, V., Baraldi, M., Guilarte, T. R., Knudsen, T. B., Lacapère, J.-J., Lindemann, P., Norenberg, M. D., Nutt, D., Weizman, A., Zhang, M.-R. and Gavish, M. (2006) 'Translocator protein (18kDa): new nomenclature for the peripheral-type benzodiazepine receptor based on its structure and molecular function', *Trends in Pharmacological Sciences,* 27(8), pp. 402-409.
Pares, A., Caballeria, L. and Rodes, J. (2006) 'Excellent long-term survival in patients with primary biliary cirrhosis and biochemical response to ursodeoxycholic Acid', *Gastroenterology,* 130(3), pp. 715-20.
Park, S., Jeon, J.-H., Min, B.-K., Ha, C.-M., Thoudam, T., Park, B.-Y. and Lee, I.-K. (2018) 'Role of the Pyruvate Dehydrogenase Complex in Metabolic Remodeling: Differential Pyruvate Dehydrogenase Complex Functions in Metabolism', *Diabetes & Metabolism Journal,* 42(4), pp. 270.
Paul, S. M., Pinna, G. and Guidotti, A. (2020) 'Allopregnanolone: From molecular pathophysiology to therapeutics. A historical perspective', *Neurobiol Stress,* 12, pp. 100215.
Paumgartner, G. and Beuers, U. (2004) 'Mechanisms of action and therapeutic efficacy of ursodeoxycholic acid in cholestatic liver disease', *Clin Liver Dis,* 8(1), pp. 67-81, vi.
Pedersen, B. K. and Febbraio, M. A. (2008) 'Muscle as an Endocrine Organ: Focus on Muscle-Derived Interleukin-6', *Physiological Reviews,* 88(4), pp. 1379-1406.
Pedersen, B. K., Steensberg, A., Fischer, C., Keller, C., Keller, P., Plomgaard, P., Wolsk-Petersen, E. and Febbraio, M. (2004) 'The metabolic role of IL-6 produced during exercise: is IL-6 an exercise factor?', *Proceedings of the Nutrition Society,* 63(2), pp. 263-267.
Pedersen, B. K., Steensberg, A. and Schjerling, P. (2001) 'Exercise and interleukin-6', *Curr Opin Hematol,* 8(3), pp. 137-41.
Pells, G., Ducker, S., Palmer, J., Newton, J. and Jones, D. (2013a) 'PTU-106 Patient Impact of Inflammation in Primary Biliary Cirrhosis (Pbc): Inflammatory Cytokine Levels are Elevated but Unrelated to Fatigue Severity', *Gut,* 62(Suppl 1), pp. A89.2-A89.
Pells, G., Mells, G. F., Carbone, M., Newton, J. L., Bathgate, A. J., Burroughs, A. K., Heneghan, M. A., Neuberger, J. M., Day, D. B., Ducker, S. J., Sandford, R. N., Alexander, G. J. and Jones, D. E. J. (2013b) 'The impact of liver transplantation on the phenotype of primary biliary cirrhosis patients in the UK-PBC cohort', *Journal of Hepatology,* 59(1), pp. 67-73.
Pereira, L. S. M., Narciso, F. M. S., Oliveira, D. M. G., Coelho, F. M., Souza, D. d. G. d. and Dias, R. C. (2009) 'Correlation between manual muscle strength and interleukin-6 (IL-6)

plasma levels in elderly community-dwelling women', *Archives of Gerontology and Geriatrics,* 48(3), pp. 313-316.
Perez-Tilve, D., Heppner, K., Kirchner, H., Lockie, S. H., Woods, S. C., Smiley, D. L., Tschöp, M. and Pfluger, P. (2011) 'Ghrelin-induced adiposity is independent of orexigenic effects', *The FASEB Journal,* 25(8), pp. 2814.
Phaw, N. A., Dyson, J. K., Mells, G. and Jones, D. (2020) 'Understanding Fatigue in Primary Biliary Cholangitis', *Dig Dis Sci*.
Phaw, N. A., Leighton, J., Dyson, J. K. and Jones, D. E. (2021) 'Managing cognitive symptoms and fatigue in cholestatic liver disease', *Expert Rev Gastroenterol Hepatol,* 15(3), pp. 235-241.
Piche, T. (2002) 'Fatigue is associated with high circulating leptin levels in chronic hepatitis C', *Gut,* 51(3), pp. 434-439.
Piche, T., Schneider, S. M., Tran, A., Benzaken, S., Rampal, P. and Hébuterne, X. (2000) 'Resting energy expenditure in chronic hepatitis C', *J Hepatol,* 33(4), pp. 623-7.
Plotogea, O. M., Ilie, M., Bungau, S., Chiotoroiu, A. L., Stanescu, A. M. A. and Diaconu, C. C. (2021) 'Comprehensive Overview of Sleep Disorders in Patients with Chronic Liver Disease', *Brain Sci,* 11(2).
Poldrack, R. A. and Packard, M. G. (2003) 'Competition among multiple memory systems: converging evidence from animal and human brain studies', *Neuropsychologia,* 41(3), pp. 245-251.
Porcu, P., Mostallino, M. C., Sogliano, C., Santoru, F., Berretti, R. and Concas, A. (2012) 'Long-term administration with levonorgestrel decreases allopregnanolone levels and alters GABAA receptor subunit expression and anxiety-like behavior', *Pharmacology Biochemistry and Behavior,* 102(2), pp. 366-372.
Poupon, R. E., Chrétien, Y., Chazouillères, O., Poupon, R. and Chwalow, J. (2004) 'Quality of life in patients with primary biliary cirrhosis', *Hepatology,* 40(2), pp. 489-494.
Poykko, S. M., Kellokoski, E., Horkko, S., Kauma, H., Kesaniemi, Y. A. and Ukkola, O. (2003) 'Low plasma ghrelin is associated with insulin resistance, hypertension, and the prevalence of type 2 diabetes', *Diabetes,* 52(10), pp. 2546-2553.
Preininger, M. K. and Kaufer, D. (2022) 'Blood-Brain Barrier Dysfunction and Astrocyte Senescence as Reciprocal Drivers of Neuropathology in Aging', *Int J Mol Sci,* 23(11).
Prince, M. (2001) 'The geographical distribution of primary biliary cirrhosis in a well-defined cohort', *Hepatology,* 34(6), pp. 1083-1088.
Prince, M. I. (2002) 'Hepatitis and liver dysfunction with rifampicin therapy for pruritus in primary biliary cirrhosis', *Gut,* 50(3), pp. 436-439.
Prince, M. I., Ducker, S. J. and James, O. F. (2010) 'Case-control studies of risk factors for primary biliary cirrhosis in two United Kingdom populations', *Gut,* 59(4), pp. 508-12.
Probert, C., Hearing, S., Schreiber, S., Kühbacher, T., Ghosh, S., Arnott, I. and Forbes, A. (2003) 'Infliximab in moderately severe glucocorticoid resistant ulcerative colitis: a randomised controlled trial', *Gut,* 52(7), pp. 998-1002.
Procaccini, C., Jirillo, E. and Matarese, G. (2012) 'Leptin as an immunomodulator', *Molecular Aspects of Medicine,* 33(1), pp. 35-45.
Pryde, D. C., Dalvie, D., Hu, Q., Jones, P., Obach, R. S. and Tran, T.-D. (2010) 'Aldehyde oxidase: an enzyme of emerging importance in drug discovery', *Journal of medicinal chemistry,* 53(24), pp. 8441-8460.
Ptaszynska, M. M., Pendrak, M. L., Stracke, M. L. and Roberts, D. D. (2010) 'Autotaxin signaling via lysophosphatidic acid receptors contributes to vascular endothelial growth factor-induced endothelial cell migration', *Mol Cancer Res,* 8(3), pp. 309-21.
Qi, F., Li, D. and Zhang, Z. (2023) 'The kinetics of chemokine autoantibodies in COVID-19', *Nature Immunology,* 24(4), pp. 567-569.

Quarneti, C., Muratori, P., Lalanne, C., Fabbri, A., Menichella, R., Granito, A., Masi, C., Lenzi, M., Cassani, F., Pappas, G. and Muratori, L. (2015) 'Fatigue and pruritus at onset identify a more aggressive subset of primary biliary cirrhosis', *Liver International,* 35(2), pp. 636-641.
Quinn, J., Diamond, A. G., Palmer, J. M., Bassendine, M. F., James, O. F. W. and Yeaman, S. J. (1993) 'Lipoylated and unlipoylated domains of human PDC-E2 as autoantigens in primary biliary cirrhosis: Significance of lipoate attachment', *Hepatology,* 18(6), pp. 1384-1391.
Rajapaksha, I. G., Angus, P. W. and Herath, C. B. (2019) 'Current therapies and novel approaches for biliary diseases', *World J Gastrointest Pathophysiol,* 10(1), pp. 1-10.
Ramírez-Vélez, R., Sáez De Asteasu, M. L., Martínez-Velilla, N., Zambom-Ferraresi, F., García-Hermoso, A., Recarey, A. E., Fernández-Irigoyen, J., Santamaría, E., Palomino-Echeverría, S. and Izquierdo, M. (2020) 'Circulating Cytokines and Lower Body Muscle Performance in Older Adults at Hospital Admission', *The journal of nutrition, health & aging,* 24(10), pp. 1131-1139.
Raszeja-Wyszomirska, J. (2017) 'Brain Perfusion Scintigraphy in Evaluation of Pathogenesis of Fatigue in Patients with Primary Biliary Cholangitis (PBC) – A Pilot Study', *International Journal of Clinical and Experimental Medical Sciences,* 3(4).
Rathinam, V. A., Vanaja, S. K. and Fitzgerald, K. A. (2012) 'Regulation of inflammasome signaling', *Nature immunology,* 13(4), pp. 333-342.
Ratziu, V. (2021) 'Obeticholic Acid for the Treatment of Nonalcoholic Steatohepatitis', *Clinical Liver Disease,* 17(6), pp. 398-400.
Reddy, D. S. (2003) 'Pharmacology of endogenous neuroactive steroids', *Crit Rev Neurobiol,* 15(3-4), pp. 197-234.
Reddy, D. S. (2010) 'Neurosteroids', *Sex Differences in the Human Brain, their Underpinnings and Implications*: Elsevier, pp. 113-137.
Reig, A., Sese, P. and Pares, A. (2018) 'Effects of Bezafibrate on Outcome and Pruritus in Primary Biliary Cholangitis With Suboptimal Ursodeoxycholic Acid Response', *Am J Gastroenterol,* 113(1), pp. 49-55.
Rennert, P. D. (2011) 'Novel roles for TIM-1 in immunity and infection', *Immunology letters,* 141(1), pp. 28-35.
Renzi, A., Glaser, S., Demorrow, S., Mancinelli, R., Meng, F., Franchitto, A., Venter, J., White, M., Francis, H., Han, Y., Alvaro, D., Gaudio, E., Carpino, G., Ueno, Y., Onori, P. and Alpini, G. (2011) 'Melatonin inhibits cholangiocyte hyperplasia in cholestatic rats by interaction with MT1 but not MT2 melatonin receptors', *Am J Physiol Gastrointest Liver Physiol,* 301(4), pp. G634-43.
Ribeiro, L. F., Catarino, T., Santos, S. D., Benoist, M., van Leeuwen, J. F., Esteban, J. A. and Carvalho, A. L. (2014) 'Ghrelin triggers the synaptic incorporation of AMPA receptors in the hippocampus', *Proceedings of the National Academy of Sciences,* 111(1), pp. E149-E158.
Rice, S., Albani, V., Minos, D., Fattakhova, G., Mells, G. F., Carbone, M., Flack, S., Varvaropoulou, N., Badrock, J., Spicer, A., Sandford, R. N., Shirley, M. D. F., Coughlan, D., Hirschfield, G., Taylor-Robinson, S. D., Consortium, U.-P., Vale, L. and Jones, D. E. J. (2020) 'Effects of Primary Biliary Cholangitis on Quality of Life and Health Care Costs in the United Kingdom', *Clin Gastroenterol Hepatol*.
Richardson, C., Cain, N., Bartel, K., Micic, G., Maddock, B. and Gradisar, M. (2018) 'A randomised controlled trial of bright light therapy and morning activity for adolescents and young adults with Delayed Sleep-Wake Phase Disorder', *Sleep Medicine,* 45, pp. 114-123.

Rieger, R., Oertelt, S., Selmi, C., Invernizzi, P., Podda, M. U. and Gershwin, M. E. (2005) 'Decreased Serum Leptin Levels in Primary Biliary Cirrhosis: A Link between Metabolism and Autoimmunity?', *Annals of the New York Academy of Sciences,* 1051(1), pp. 211-217.
Robel, P. and Baulieu, E.-E. (1994) 'Neurosteroids: biosynthesis and function', *Trends in Endocrinology & Metabolism,* 5(1), pp. 1-8.
Roberts, S. B., Ismail, M., Kanagalingam, G., Mason, A. L., Swain, M. G., Vincent, C., Yoshida, E. M., Tsien, C., Flemming, J. A., Janssen, H. L. A., Hirschfield, G. M., Hansen, B. E. and Gulamhusein, A. F. (2020) 'Real‐World Effectiveness of Obeticholic Acid in Patients with Primary Biliary Cholangitis', *Hepatology Communications,* 4(9), pp. 1332-1345.
Roda, G., Marocchi, M., Sartini, A. and Roda, E. (2011) 'Cytokine Networks in Ulcerative Colitis', *Ulcers,* 2011, pp. 391787.
Rodriguez-Manzanet, R., Dekruyff, R., Kuchroo, V. K. and Umetsu, D. T. (2009) 'The costimulatory role of TIM molecules', *Immunological Reviews,* 229(1), pp. 259-270.
Roels, H., Lauwerys, R., Buchet, J. P., Genet, P., Sarhan, M. J., Hanotiau, I., de Fays, M., Bernard, A. and Stanescu, D. (1987) 'Epidemiological survey among workers exposed to manganese: effects on lung, central nervous system, and some biological indices', *Am J Ind Med,* 11(3), pp. 307-27.
Rong, G., Zhong, R., Lleo, A., Leung, P. S. C., Bowlus, C. L., Yang, G.-X., Yang, C.-Y., Coppel, R. L., Ansari, A. A., Cuebas, D. A., Worman, H. J., Invernizzi, P., Gores, G. J., Norman, G., He, X.-S. and Gershwin, M. E. (2011) 'Epithelial cell specificity and apotope recognition by serum autoantibodies in primary biliary cirrhosis', *Hepatology,* 54(1), pp. 196-203.
Rose, C., Butterworth, R. F., Zayed, J., Normandin, L., Todd, K., Michalak, A., Spahr, L., Huet, P. M. and Pomier–Layrargues, G. (1999) 'Manganese deposition in basal ganglia structures results from both portal-systemic shunting and liver dysfunction', *Gastroenterology,* 117(3), pp. 640-644.
Rosenthal, N. E., Joseph-Vanderpool, J. R., Levendosky, A. A., Johnston, S. H., Allen, R., Kelly, K. A., Souetre, E., Schultz, P. M. and Starz, K. E. (1990) 'Phase-shifting effects of bright morning light as treatment for delayed sleep phase syndrome', *Sleep,* 13(4), pp. 354-61.
Rossi, S., Hallett, M., Rossini, P. M. and Pascual-Leone, A. (2009) 'Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research', *Clinical Neurophysiology,* 120(12), pp. 2008-2039.
Rőszer, T. (2015) 'Understanding the Mysterious M2 Macrophage through Activation Markers and Effector Mechanisms', *Mediators of Inflammation,* 2015, pp. 1-16.
Roth, G. A., Zimmermann, M., Lubsczyk, B. A., Pilz, J., Faybik, P., Hetz, H., Hacker, S., Mangold, A., Bacher, A., Krenn, C. G. and Ankersmit, H. J. (2010) 'Up-Regulation of Interleukin 33 and Soluble ST2 Serum Levels in Liver Failure', *Journal of Surgical Research,* 163(2), pp. e79-e83.
Rubin, E., Schaffner, F. and Popper, H. (1965) 'PRIMARY BILIARY CIRRHOSIS. CHRONIC NON-SUPPURATIVE DESTRUCTIVE CHOLANGITIS', *Am J Pathol,* 46(3), pp. 387-407.
Rudic, J. S., Poropat, G., Krstic, M. N., Bjelakovic, G. and Gluud, C. (2012) 'Ursodeoxycholic acid for primary biliary cirrhosis', *Cochrane Database of Systematic Reviews*.
Rupprecht, R. (2003) 'Neuroactive steroids: mechanisms of action and neuropsychopharmacological properties', *Psychoneuroendocrinology,* 28(2), pp. 139-168.
Sahu, A. (2003) 'Leptin signaling in the hypothalamus: emphasis on energy homeostasis and leptin resistance', *Front Neuroendocrinol,* 24(4), pp. 225-53.
Salmaggi, A., Gelati, M., Dufour, A., Corsini, E., Pagano, S., Baccalini, R., Ferrero, E., Scabini, S., Silei, V., Ciusani, E. and De Rossi, M. (2002) 'Expression and Modulation of IFN-γ-Inducible Chemokines (IP-10, Mig, and I-TAC) in Human Brain Endothelium and Astrocytes: Possible Relevance for the Immune Invasion of the Central Nervous System

and the Pathogenesis of Multiple Sclerosis', *Journal of Interferon & Cytokine Research,* 22(6), pp. 631-640.
Samson, L. D., Buisman, A.-M., Ferreira, J. A., Picavet, H. S. J., Verschuren, W. M. M., Boots, A. M. and Engelfriet, P. (2022) 'Inflammatory marker trajectories associated with frailty and ageing in a 20-year longitudinal study', *Clinical & Translational Immunology,* 11(2), pp. e1374.
Samur, S., Klebanoff, M., Banken, R., Pratt, D. S., Chapman, R., Ollendorf, D. A., Loos, A. M., Corey, K., Hur, C. and Chhatwal, J. (2017) 'Long-term clinical impact and cost-effectiveness of obeticholic acid for the treatment of primary biliary cholangitis', *Hepatology,* 65(3), pp. 920-928.
Sandahl, T. D., Grønbæk, H., Møller, H. J., Støy, S., Thomsen, K. L., Dige, A. K., Agnholt, J., Hamilton-Dutoit, S., Thiel, S. and Vilstrup, H. (2014) 'Hepatic macrophage activation and the LPS pathway in patients with alcoholic hepatitis: a prospective cohort study', *Official journal of the American College of Gastroenterology| ACG,* 109(11), pp. 1749-1756.
Sandborn, W. J., Su, C., Sands, B. E., D'Haens, G. R., Vermeire, S., Schreiber, S., Danese, S., Feagan, B. G., Reinisch, W. and Niezychowski, W. (2017) 'Tofacitinib as induction and maintenance therapy for ulcerative colitis', *New England Journal of Medicine,* 376(18), pp. 1723-1736.
Sasaki, M., Ikeda, H., Haga, H., Manabe, T. and Nakanuma, Y. (2005) 'Frequent cellular senescence in small bile ducts in primary biliary cirrhosis: a possible role in bile duct loss', *The Journal of Pathology,* 205(4), pp. 451-459.
Sasaki, M., Miyakoshi, M., Sato, Y. and Nakanuma, Y. (2010a) 'Autophagy mediates the process of cellular senescence characterizing bile duct damages in primary biliary cirrhosis', *Laboratory Investigation,* 90(6), pp. 835-843.
Sasaki, M., Miyakoshi, M., Sato, Y. and Nakanuma, Y. (2010b) 'Modulation of the microenvironment by senescent biliary epithelial cells may be involved in the pathogenesis of primary biliary cirrhosis', *Journal of Hepatology,* 53(2), pp. 318-325.
Sasaki, M. and Nakanuma, Y. (2010) 'Biliary epithelial apoptosis, autophagy, and senescence in primary biliary cirrhosis', *Hepat Res Treat,* 2010, pp. 205128.
Sasaki, M. and Nakanuma, Y. (2012) 'Novel approach to bile duct damage in primary biliary cirrhosis: participation of cellular senescence and autophagy', *Int J Hepatol,* 2012, pp. 452143.
Sasaki, M., Sato, Y. and Nakanuma, Y. (2020) 'Increased p16(INK4a)-expressing senescent bile ductular cells are associated with inadequate response to ursodeoxycholic acid in primary biliary cholangitis', *J Autoimmun,* 107, pp. 102377.
Sato, K., Glaser, S., Kennedy, L., Liangpunsakul, S., Meng, F., Francis, H. and Alpini, G. (2019) 'Preclinical insights into cholangiopathies: disease modeling and emerging therapeutic targets', *Expert Opinion on Therapeutic Targets,* 23(6), pp. 461-472.
Sato, K., Meng, F., Francis, H., Wu, N., Chen, L., Kennedy, L., Zhou, T., Franchitto, A., Onori, P., Gaudio, E., Glaser, S. and Alpini, G. (2020) 'Melatonin and circadian rhythms in liver diseases: Functional roles and potential therapies', *Journal of Pineal Research,* 68(3), pp. e12639.
Sato, T., Nakamura, Y., Shiimura, Y., Ohgusu, H., Kangawa, K. and Kojima, M. (2012) 'Structure, regulation and function of ghrelin', *J Biochem,* 151(2), pp. 119-28.
Satoh, M., Haruta-Satoh, E., Yamada, M., Kado, S. and Nomura, F. (2013) 'Overexpression of hydroxymethylglutaryl CoA synthase 2 and 2, 4-dienoyl-CoA reductase in rat pancreas following chronic alcohol consumption', *Pancreas,* 42(3), pp. 475-482.
Schaap, F. G., Trauner, M. and Jansen, P. L. (2014) 'Bile acid receptors as targets for drug development', *Nature reviews Gastroenterology & hepatology,* 11(1), pp. 55-67.
Schattenberg, J. M., Pares, A., Kowdley, K. V., Heneghan, M. A., Caldwell, S., Pratt, D., Bonder, A., Hirschfield, G. M., Levy, C., Vierling, J., Jones, D., Tailleux, A., Staels, B., Megnien,

S., Hanf, R., Magrez, D., Birman, P. and Luketic, V. (2021) 'A randomized placebo-controlled trial of elafibranor in patients with primary biliary cholangitis and incomplete response to UDCA', *J Hepatol,* 74(6), pp. 1344-1354.
Schmidt, M., Scheulen, M. E., Dittrich, C., Obrist, P., Marschner, N., Dirix, L., Schmidt, M., Rüttinger, D., Schuler, M., Reinhardt, C. and Awada, A. (2010) 'An open-label, randomized phase II study of adecatumumab, a fully human anti-EpCAM antibody, as monotherapy in patients with metastatic breast cancer', *Annals of Oncology,* 21(2), pp. 275-282.
Schmierer, K., Scaravilli, F., Altmann, D. R., Barker, G. J. and Miller, D. H. (2004) 'Magnetization transfer ratio and myelin in postmortem multiple sclerosis brain', *Annals of neurology,* 56(3), pp. 407-415.
Schrezenmaier, C., Gehrking, J. A., Hines, S. M., Low, P. A., Benrud-Larson, L. M. and Sandroni, P. (2005) 'Evaluation of orthostatic hypotension: relationship of a new self-report instrument to laboratory-based measures', *Mayo Clin Proc,* 80(3), pp. 330-4.
Schulze, K., Becker, B. F., Schauer, R. and Schultheiss, H. P. (1990) 'Antibodies to ADP-ATP carrier--an autoantigen in myocarditis and dilated cardiomyopathy--impair cardiac function.', *Circulation,* 81(3), pp. 959-969.
Schutyser, E., Struyf, S. and Van Damme, J. (2003) 'The CC chemokine CCL20 and its receptor CCR6', *Cytokine Growth Factor Rev,* 14(5), pp. 409-26.
Schweitzer, S. C. and Evans, R. M. (1998) 'Vimentin and lipid metabolism', *Sub-cellular biochemistry,* 31, pp. 437-462.
Schwörer, H., Hartmann, H. and Ramadori, G. (1995) 'Relief of cholestatic pruritus by a novel class of drugs: 5-hydroxytryptamine type 3 (5-HT3) receptor antagonists: effectiveness of ondansetron', *Pain,* 61(1), pp. 33-37.
Selmi, C., Coppel, R. L. and Gershwin, M. E. (2006) 'Primary biliary cirrhosis', *The Autoimmune Diseases*: Elsevier, pp. 749-765.
Selmi, C., Mayo, M. J., Bach, N., Ishibashi, H., Invernizzi, P., Gish, R. G., Gordon, S. C., Wright, H. I., Zweiban, B., Podda, M. and Gershwin, M. E. (2004) 'Primary biliary cirrhosis in monozygotic and dizygotic twins: Genetics, epigenetics, and environment', *Gastroenterology,* 127(2), pp. 485-492.
Shaffer, F. and Ginsberg, J. P. (2017) 'An Overview of Heart Rate Variability Metrics and Norms', *Front Public Health,* 5, pp. 258.
Shaheen, A.-A., Kaplan, G. G., Almishri, W., Vallerand, I., Frolkis, A. D., Patten, S. and Swain, M. G. (2018) 'The impact of depression and antidepressant usage on primary biliary cholangitis clinical outcomes', *PLOS ONE,* 13(4), pp. e0194839.
She, S., Zhang, Q., Shi, J., Yang, F. and Dai, K. (2022) 'Roles of Autotaxin/Autotaxin-Lysophosphatidic Acid Axis in the Initiation and Progression of Liver Cancer', *Frontiers in Oncology,* 12.
Shi, T., Zhang, T., Zhang, L., Yang, Y., Zhang, H. and Zhang, F. (2015) 'The Distribution and the Fibrotic Role of Elevated Inflammatory Th17 Cells in Patients With Primary Biliary Cirrhosis', *Medicine (Baltimore),* 94(44), pp. e1888.
Shields, P. L., Morland, C. M., Salmon, M., Qin, S., Hubscher, S. G. and Adams, D. H. (1999) 'Chemokine and chemokine receptor interactions provide a mechanism for selective T cell recruitment to specific liver compartments within hepatitis C-infected liver', *J Immunol,* 163(11), pp. 6236-43.
Shigehara, K., Shijubo, N., Ohmichi, M., Yamada, G., Takahashi, R., Okamura, H., Kurimoto, M., Hiraga, Y., Tatsuno, T., Abe, S. and Sato, N. (2000) 'Increased levels of interleukin-18 in patients with pulmonary sarcoidosis', *Am J Respir Crit Care Med,* 162(5), pp. 1979-82.
Shimizu, H., Kakizaki, S., Tsuchiya, T., Nagamine, T., Takagi, H., Takayama, H., Kobayashi, I. and Mori, M. (1998) 'An increase of circulating leptin in patients with liver cirrhosis', *Int J Obes Relat Metab Disord,* 22(12), pp. 1234-8.

Shimoda, S., Nakamura, M., Ishibashi, H., Kawano, A., Kamihira, T., Sakamoto, N., Matsushita, S., Tanaka, A., Worman, H. J., Gershwin, M. E. and Harada, M. (2003) 'Molecular mimicry of mitochondrial and nuclear autoantigens in primary biliary cirrhosis', *Gastroenterology,* 124(7), pp. 1915-25.
Shirakawa, T., Deichmann, K. A., Izuhara, K., Mao, X.-Q., Adra, C. N. and Hopkin, J. M. (2000) 'Atopy and asthma: genetic variants of IL-4 and IL-13 signalling', *Immunology Today,* 21(2), pp. 60-64.
Siddiqui, M. S., Van Natta, M. L., Connelly, M. A., Vuppalanchi, R., Neuschwander-Tetri, B. A., Tonascia, J., Guy, C., Loomba, R., Dasarathy, S., Wattacheril, J., Chalasani, N. and Sanyal, A. J. (2020) 'Impact of obeticholic acid on the lipoprotein profile in patients with non-alcoholic steatohepatitis', *Journal of Hepatology,* 72(1), pp. 25-33.
Siennicki-Lantz, A., Lilja, B., Rosén, I. and Elmståhl, S. (1998) 'Cerebral blood flow in white matter is correlated with systolic blood pressure and EEG in senile dementia of the Alzheimer type', *Dement Geriatr Cogn Disord,* 9(1), pp. 29-38.
Siever, L. J. and Davis, K. L. (1985) 'Overview: Toward a dysregulation hypothesis of depression', *The American Journal of Psychiatry,* 142, pp. 1017-1031.
Silveira, M. G., Gossard, A. A., Stahler, A. C., Jorgensen, R. A., Petz, J. L., Ali, A. H. and Lindor, K. D. (2017) 'A Randomized, Placebo-Controlled Clinical Trial of Efficacy and Safety: Modafinil in the Treatment of Fatigue in Patients With Primary Biliary Cirrhosis', *Am J Ther,* 24(2), pp. e167-e176.
Silverberg, J. I., Guttman-Yassky, E., Thaçi, D., Irvine, A. D., Stein Gold, L., Blauvelt, A., Simpson, E. L., Chu, C.-Y., Liu, Z., Gontijo Lima, R., Pillai, S. G. and Seneschal, J. (2023) 'Two Phase 3 Trials of Lebrikizumab for Moderate-to-Severe Atopic Dermatitis', *New England Journal of Medicine,* 388(12), pp. 1080-1091.
Simmonds, M. and Gough, S. (2007) 'The HLA Region and Autoimmune Disease: Associations and Mechanisms of Action', *Current Genomics,* 8(7), pp. 453-465.
Simpson, E. L., Bieber, T., Guttman-Yassky, E., Beck, L. A., Blauvelt, A., Cork, M. J., Silverberg, J. I., Deleuran, M., Kataoka, Y., Lacour, J.-P., Kingo, K., Worm, M., Poulin, Y., Wollenberg, A., Soo, Y., Graham, N. M. H., Pirozzi, G., Akinlade, B., Staudinger, H., Mastey, V., Eckert, L., Gadkari, A., Stahl, N., Yancopoulos, G. D. and Ardeleanu, M. (2016) 'Two Phase 3 Trials of Dupilumab versus Placebo in Atopic Dermatitis', *New England Journal of Medicine,* 375(24), pp. 2335-2348.
Sinha, L., Liston, R., Testa, H. J. and Moriarty, K. J. (1998) 'Idiopathic bile acid malabsorption: qualitative and quantitative clinical features and response to cholestyramine', *Alimentary Pharmacology & Therapeutics,* 12(9), pp. 839-844.
Sly, W. S. and Hu, P. Y. (1995) 'Human carbonic anhydrases and carbonic anhydrase deficiencies', *Annual review of biochemistry,* 64(1), pp. 375-401.
Smolen, J. S., Beaulieu, A., Rubbert-Roth, A., Ramos-Remus, C., Rovensky, J., Alecock, E., Woodworth, T. and Alten, R. (2008) 'Effect of interleukin-6 receptor inhibition with tocilizumab in patients with rheumatoid arthritis (OPTION study): a double-blind, placebo-controlled, randomised trial', *Lancet,* 371(9617), pp. 987-97.
Soehnlein, O. and Lindbom, L. (2008) 'Neutrophil-derived azurocidin alarms the immune system', *Journal of Leukocyte Biology,* 85(3), pp. 344-351.
Solmonson, A. and Deberardinis, R. J. (2018) 'Lipoic acid metabolism and mitochondrial redox regulation', *Journal of Biological Chemistry,* 293(20), pp. 7522-7530.
Song, Y., Liu, C., Liu, X., Trottier, J., Beaudoin, M., Zhang, L., Pope, C., Peng, G., Barbier, O., Zhong, X., Li, L. and Wang, L. (2017) 'H19 promotes cholestatic liver fibrosis by preventing ZEB1‐mediated inhibition of epithelial cell adhesion molecule', *Hepatology,* 66(4), pp. 1183-1196.
Soret, P. A., Lam, L., Carrat, F., Smets, L., Berg, T., Carbone, M., Invernizzi, P., Leroy, V., Trivedi, P., Cazzagon, N., Weiler-Normann, C., Alric, L., Rosa-Hezode, I., Heurgue, A.,

Cervoni, J. P., Dumortier, J., Potier, P., Roux, O., Silvain, C., Bureau, C., Anty, R., Larrey, D., Levy, C., Pares, A., Schramm, C., Nevens, F., Chazouilleres, O. and Corpechot, C. (2021) 'Combination of fibrates with obeticholic acid is able to normalise biochemical liver tests in patients with difficult-to-treat primary biliary cholangitis', *Aliment Pharmacol Ther,* 53(10), pp. 1138-1146.
Stamm, T. A., Cieza, A., Coenen, M., Machold, K. P., Nell, V. P., Smolen, J. S. and Stucki, G. (2005) 'Validating the International Classification of Functioning, Disability and Health Comprehensive Core Set for Rheumatoid Arthritis from the patient perspective: a qualitative study', *Arthritis Rheum,* 53(3), pp. 431-9.
Stamova, B. S., Apperson, M., Walker, W. L., Tian, Y., Xu, H., Adamczy, P., Zhan, X., Liu, D.-Z., Ander, B. P., Liao, I. H., Gregg, J. P., Turner, R. J., Jickling, G., Lit, L. and Sharp, F. R. (2009) 'Identification and validation of suitable endogenous reference genes for gene expression studies in human peripheral blood', *BMC Medical Genomics,* 2(1), pp. 49.
Steensberg, A., van Hall, G., Osada, T., Sacchetti, M., Saltin, B. and Klarlund Pedersen, B. (2000) 'Production of interleukin-6 in contracting human skeletal muscles can account for the exercise-induced increase in plasma interleukin-6', *J Physiol,* 529 Pt 1(Pt 1), pp. 237-42.
Stone, J. H., Tuckwell, K., Dimonaco, S., Klearman, M., Aringer, M., Blockmans, D., Brouwer, E., Cid, M. C., Dasgupta, B. and Rech, J. (2017) 'Trial of tocilizumab in giant-cell arteritis', *New England Journal of Medicine,* 377(4), pp. 317-328.
Stott, B., Lavender, P., Lehmann, S., Pennino, D., Durham, S. and Schmidt-Weber, C. B. (2013) 'Human IL-31 is induced by IL-4 and promotes TH2-driven inflammation', *J Allergy Clin Immunol,* 132(2), pp. 446-54.e5.
Sugawara, I., Yamada, H., Kaneko, H., Mizuno, S., Takeda, K. and Akira, S. (1999) 'Role of Interleukin-18 (IL-18) in Mycobacterial Infection in IL-18-Gene-Disrupted Mice', *Infection and Immunity,* 67(5), pp. 2585-2589.
Sumida, H., Nakamura, K., Yanagida, K., Ohkawa, R., Asano, Y., Kadono, T., Tamaki, K., Igarashi, K., Aoki, J., Sato, S., Ishii, S., Shimizu, T. and Yatomi, Y. (2013) 'Decrease in circulating autotaxin by oral administration of prednisolone', *Clinica Chimica Acta,* 415, pp. 74-80.
Sun, Y., Koyama, Y. and Shimada, S. (2022) 'Inflammation From Peripheral Organs to the Brain: How Does Systemic Inflammation Cause Neuroinflammation?', *Frontiers in aging neuroscience,* 14, pp. 903455-903455.
Sun, Y., Zhang, J.-Y., Lv, S., Wang, H., Gong, M., Du, N., Liu, H., Zhang, N., Jing, J., Zhou, C., Zhang, F. and Wang, Z. (2014) 'Interleukin-33 Promotes Disease Progression in Patients with Primary Biliary Cirrhosis', *The Tohoku Journal of Experimental Medicine,* 234(4), pp. 255-261.
Sutton, R. E., Koob, G. F., Le Moal, M., Rivier, J. and Vale, W. (1982) 'Corticotropin releasing factor produces behavioural activation in rats', *Nature,* 297(5864), pp. 331-333.
Swain, M. G. (2006) 'Fatigue in Liver Disease: Pathophysiology and Clinical Management', *Canadian Journal of Gastroenterology,* 20(3), pp. 181-188.
Swain, M. G. and Jones, D. E. J. (2019) 'Fatigue in chronic liver disease: New insights and therapeutic approaches', *Liver Int,* 39(1), pp. 6-19.
Swain, M. G. and Le, T. (1998) 'Chronic cholestasis in rats induces anhedonia and a loss of social interest', *Hepatology,* 28(1), pp. 6-10.
Swain, M. G. and Maric, M. (1995) 'Defective corticotropin-releasing hormone mediated neuroendocrine and behavioral responses in cholestatic rats: implications for cholestatic liver disease-related sickness behaviors', *Hepatology,* 22(5), pp. 1560-4.
Swain, M. G., Patchev, V., Vergalla, J., Chrousos, G. and Jones, E. A. (1993) 'Suppression of hypothalamic-pituitary-adrenal axis responsiveness to stress in a rat model of acute cholestasis', *J Clin Invest,* 91(5), pp. 1903-8.

Takahashi, J. S. (2017) 'Transcriptional architecture of the mammalian circadian clock', *Nat Rev Genet,* 18(3), pp. 164-179.
Talwalkar, J. A., Donlinger, J. J., Gossard, A. A., Keach, J. C., Jorgensen, R. A., Petz, J. C. and Lindor, K. D. (2006) 'Fluoxetine for the Treatment of Fatigue in Primary Biliary Cirrhosis: A Randomized, Double-Blind Controlled Trial', *Digestive Diseases and Sciences,* 51(11), pp. 1985-1991.
Tanaka, A., Hirohara, J., Nakano, T., Matsumoto, K., Chazouilleres, O., Takikawa, H., Hansen, B. E., Carrat, F. and Corpechot, C. (2021) 'Association of bezafibrate with transplant-free survival in patients with primary biliary cholangitis', *J Hepatol*.
Tanaka, T., Narazaki, M. and Kishimoto, T. (2014) 'IL-6 in Inflammation, Immunity, and Disease', *Cold Spring Harbor Perspectives in Biology,* 6(10), pp. a016295-a016295.
Tanaka, T., Tsutsui, H., Yoshimoto, T., Kotani, M., Matsumoto, M., Fujita, A., Wang, W., Higa, S., Koshimoto, T. and Nakanishi, K. (2001) 'Interleukin-18 is elevated in the sera from patients with atopic dermatitis and from atopic dermatitis model mice, NC/Nga', *International archives of allergy and immunology,* 125(3), pp. 236-240.
Tazawa, R., Uchida, K., Fujimaki, H., Miyagi, M., Inoue, G., Sekiguchi, H., Murata, K., Takata, K., Kawakubo, A. and Takaso, M. (2019) 'Elevated leptin levels induce inflammation through IL-6 in skeletal muscle of aged female rats', *BMC Musculoskeletal Disorders,* 20(1).
Ter Borg, P. C., Fekkes, D., Vrolijk, J. M. and Van Buuren, H. R. (2005) 'The relation between plasma tyrosine concentration and fatigue in primary biliary cirrhosis and primary sclerosing cholangitis', *BMC Gastroenterology,* 5(1).
ter Borg, P. C., van Os, E., van den Broek, W. W., Hansen, B. E. and van Buuren, H. R. (2004) 'Fluvoxamine for fatigue in primary biliary cirrhosis and primary sclerosing cholangitis: a randomised controlled trial [ISRCTN88246634]', *BMC Gastroenterol,* 4, pp. 13.
Terasaki, S., Nakanuma, Y., Yamazaki, M. and Unoura, M. (1993) 'Eosinophilic infiltration of the liver in primary biliary cirrhosis: a morphological study', *Hepatology,* 17(2), pp. 206-12.
Tian, B., Wang, X.-L., Huang, Y., Chen, L.-H., Cheng, R.-X., Zhou, F.-M., Guo, R., Li, J.-C. and Liu, T. (2016) 'Peripheral and spinal 5-HT receptors participate in cholestatic itch and antinociception induced by bile duct ligation in rats', *Scientific Reports,* 6(1), pp. 36286.
To, T. H. M., Clark, K., Lam, L., Shelby-James, T. and Currow, D. C. (2012) 'The Role of Ondansetron in the Management of Cholestatic or Uremic Pruritus—A Systematic Review', *Journal of Pain and Symptom Management,* 44(5), pp. 725-730.
Tokunaga, R., Zhang, W., Naseem, M., Puccini, A., Berger, M. D., Soni, S., McSkane, M., Baba, H. and Lenz, H. J. (2018) 'CXCL9, CXCL10, CXCL11/CXCR3 axis for immune activation - A target for novel cancer therapy', *Cancer Treat Rev,* 63, pp. 40-47.
Toubal, A. and Lehuen, A. (2016) 'Lights on MAIT cells, a new immune player in liver diseases', *J Hepatol,* 64(5), pp. 1008-1010.
Trauner, M., Nevens, F., Shiffman, M. L., Drenth, J. P. H., Bowlus, C. L., Vargas, V., Andreone, P., Hirschfield, G. M., Pencek, R., Malecha, E. S., Macconell, L. and Shapiro, D. (2019) 'Long-term efficacy and safety of obeticholic acid for patients with primary biliary cholangitis: 3-year results of an international open-label extension study', *The Lancet Gastroenterology & Hepatology,* 4(6), pp. 445-453.
Triger, D. R., Berg, P. A. and Rodes, J. (2008) 'Epidemiology of primary biliary cirrhosis', *Liver,* 4(3), pp. 195-200.
Trottier, J., Białek, A., Caron, P., Straka, R. J., Heathcote, J., Milkiewicz, P. and Barbier, O. (2012) 'Metabolomic profiling of 17 bile acids in serum from patients with primary biliary cirrhosis and primary sclerosing cholangitis: a pilot study', *Digestive and Liver Disease,* 44(4), pp. 303-310.
Trzpis, M., Mclaughlin, P. M. J., De Leij, L. M. F. H. and Harmsen, M. C. (2007) 'Epithelial Cell Adhesion Molecule', *The American Journal of Pathology,* 171(2), pp. 386-395.

Tsuneyama, K., Van de Water, J., Leung, P. S., Cha, S., Nakanuma, Y., Kaplan, M., De Lellis, R., Coppel, R., Ansari, A. and Gershwin, M. E. (1995) 'Abnormal expression of the E2 component of the pyruvate dehydrogenase complex on the luminal surface of biliary epithelium occurs before major histocompatibility complex class II and BB1/B7 expression', *Hepatology,* 21(4), pp. 1031-7.
Tsutsumi, N., Kimura, T., Arita, K., Ariyoshi, M., Ohnishi, H., Yamamoto, T., Zuo, X., Maenaka, K., Park, E. Y., Kondo, N., Shirakawa, M., Tochio, H. and Kato, Z. (2014) 'The structural basis for receptor recognition of human interleukin-18', *Nature Communications,* 5(1), pp. 5340.
Turco, M., Cazzagon, N., Franceschet, I., Formentin, C., Frighetto, G., Giordani, F., Cellini, N., Mazzotta, G., Costa, R., Middleton, B., Skene, D. J., Floreani, A. and Montagnese, S. (2018) 'Morning Bright Light Treatment for Sleep-Wake Disturbances in Primary Biliary Cholangitis: A Pilot Study', *Frontiers in Physiology,* 9.
Turkmen, S., Backstrom, T., Wahlstrom, G., Andreen, L. and Johansson, I.-M. (2011a) 'Tolerance to allopregnanolone with focus on the GABA-A receptor', *British Journal of Pharmacology,* 162(2), pp. 311-327.
Turkmen, S., Backstrom, T., Wahlstrom, G., Andreen, L. and Johansson, I. M. (2011b) 'Tolerance to allopregnanolone with focus on the GABA-A receptor', *Br J Pharmacol,* 162(2), pp. 311-27.
Tuttle, L. J., Sinacore, D. R. and Mueller, M. J. (2012) 'Intermuscular Adipose Tissue Is Muscle Specific and Associated with Poor Functional Performance', *Journal of Aging Research,* 2012, pp. 1-7.
Tverring, J., Nielsen, N., Dankiewicz, J., Linder, A., Kahn, F. and Åkesson, P. (2020) 'Repeated measures of Heparin-binding protein (HBP) and procalcitonin during septic shock: biomarker kinetics and association with cardiovascular organ dysfunction', *Intensive Care Medicine Experimental,* 8(1), pp. 1-16.
Uenaka, T., Kowa, H., Sekiguchi, K., Nagata, K., Ohtsuka, Y., Kanda, F. and Toda, T. (2013) 'Myositis with antimitochondrial antibodies diagnosed by rectus abdominis muscle biopsy', *Muscle & Nerve,* 47(5), pp. 766-768.
Ueno, Y., Moritoki, Y., Shimosegawa, T. and Gershwin, M. E. (2007) 'Primary biliary cirrhosis: what we know and what we want to know about human PBC and spontaneous PBC mouse models', *Journal of Gastroenterology,* 42(3), pp. 189-195.
Unger, M. M., Möller, J. C., Mankel, K., Eggert, K. M., Bohne, K., Bodden, M., Stiasny-Kolster, K., Kann, P. H., Mayer, G. and Tebbe, J. J. (2011) 'Postprandial ghrelin response is reduced in patients with Parkinson's disease and idiopathic REM sleep behaviour disorder: a peripheral biomarker for early Parkinson's disease?', *Journal of neurology,* 258, pp. 982-990.
Van Deventer, S. J. (1997) 'Tumour necrosis factor and Crohn's disease', *Gut,* 40(4), pp. 443-8.
Van Dyken, S. J. and Locksley, R. M. (2013) 'Interleukin-4- and Interleukin-13-Mediated Alternatively Activated Macrophages: Roles in Homeostasis and Disease', *Annual Review of Immunology,* 31(1), pp. 317-343.
van Os, E., van den Broek, W. W., Mulder, P. G., ter Borg, P. C., Bruijn, J. A. and van Buuren, H. R. (2007) 'Depression in patients with primary biliary cirrhosis and primary sclerosing cholangitis', *J Hepatol,* 46(6), pp. 1099-103.
VanderVeen, B. N., Fix, D. K., Montalvo, R. N., Counts, B. R., Smuder, A. J., Murphy, E. A., Koh, H.-j. and Carson, J. A. (2019) 'The regulation of skeletal muscle fatigability and mitochondrial function by chronically elevated interleukin-6', *Experimental Physiology,* 104(3), pp. 385-397.

Varga, J., Heiman-Patterson, T., Muñoz, S. and Love, L. A. (1993) 'Myopathy with mitochondrial alterations in patients with primary biliary cirrhosis and antimitochondrial antibodies', *Arthritis Rheum,* 36(10), pp. 1468-75.
Vaughn-Sandler, V., Sherman, C., Aronsohn, A. and Volk, M. L. (2014) 'Consequences of Perceived Stigma Among Patients with Cirrhosis', *Digestive Diseases and Sciences,* 59(3), pp. 681-686.
Vella, C. A. and Allison, M. A. (2018) 'Associations of abdominal intermuscular adipose tissue and inflammation: The Multi-Ethnic Study of Atherosclerosis', *Obesity Research & Clinical Practice,* 12(6), pp. 534-540.
Vgontzas, A. N., Papanicolaou, D. A., Bixler, E. O., Kales, A., Tyson, K. and Chrousos, G. P. (1997) 'Elevation of Plasma Cytokines in Disorders of Excessive Daytime Sleepiness: Role of Sleep Disturbance and Obesity', *The Journal of Clinical Endocrinology & Metabolism,* 82(5), pp. 1313-1316.
Vgontzas, A. N., Papanicolaou, D. A., Bixler, E. O., Lotsikas, A., Zachman, K., Kales, A., Prolo, P., Wong, M.-L., Licinio, J. and Gold, P. W. (1999) 'Circadian interleukin-6 secretion and quantity and depth of sleep', *The Journal of Clinical Endocrinology & Metabolism,* 84(8), pp. 2603-2607.
Vleggaar, F. P., Van Buuren, H. R., Zondervan, P. E., Ten Kate, F. J. W. and Hop, W. C. J. (2001) 'Jaundice in non-cirrhotic primary biliary cirrhosis: the premature ductopenic variant', *Gut,* 49(2), pp. 276-281.
Walkery, A., Leader, L. D., Cooke, E. and VandenBerg, A. (2021) 'Review of Allopregnanolone Agonist Therapy for the Treatment of Depressive Disorders', *Drug Des Devel Ther,* 15, pp. 3017-3026.
Walton, N. and Maguire, J. (2019) 'Allopregnanolone-based treatments for postpartum depression: Why/how do they work?', *Neurobiology of Stress,* 11, pp. 100198.
Wan, W., Janz, L., Vriend, C. Y., Sorensen, C. M., Greenberg, A. H. and Nance, D. M. (1993) 'Differential induction of c-Fos immunoreactivity in hypothalamus and brain stem nuclei following central and peripheral administration of endotoxin', *Brain Res Bull,* 32(6), pp. 581-7.
Wang, D., Drenker, M., Eiz‐Vesper, B., Werfel, T. and Wittmann, M. (2008a) 'Evidence for a pathogenetic role of interleukin‐18 in cutaneous lupus erythematosus', *Arthritis & Rheumatism: Official Journal of the American College of Rheumatology,* 58(10), pp. 3205-3215.
Wang, J., Cai, Y., Ji, H., Feng, J., Ayana, D. A., Niu, J. and Jiang, Y. (2012) 'Serum IL-33 Levels Are Associated with Liver Damage in Patients with Chronic Hepatitis B', *Journal of Interferon & Cytokine Research,* 32(6), pp. 248-253.
Wang, M. (2013) 'Neurosteroids and brain aging', *Minerva ginecologica,* 65(6), pp. 587-605.
Wang, Y., Meng, J., Wang, X., Liu, S., Shu, Q., Gao, L., Ju, Y., Zhang, L., Sun, W. and Ma, C. (2008b) 'Expression of human TIM‐1 and TIM‐3 on lymphocytes from systemic lupus erythematosus patients', *Scandinavian journal of immunology,* 67(1), pp. 63-70.
Weaver, L. K., Hintz-Goldstein, K. A., Pioli, P. A., Wardwell, K., Qureshi, N., Vogel, S. N. and Guyre, P. M. (2006) 'Pivotal Advance: Activation of cell surface Toll-like receptors causes shedding of the hemoglobin scavenger receptor CD163', *Journal of Leukocyte Biology,* 80(1), pp. 26-35.
Webb, G. J. and Hirschfield, G. M. (2017) 'High-definition PBC: biology, models and therapeutic advances', *Nature Reviews Gastroenterology & Hepatology,* 14(2), pp. 76-78.
Wedell-Neergaard, A.-S., Lehrskov, L. L., Christensen, R. H., Legaard, G. E., Dorph, E., Larsen, M. K., Launbo, N., Fagerlind, S. R., Seide, S. K. and Nymand, S. (2019) 'Exercise-induced changes in visceral adipose tissue mass are regulated by IL-6 signaling: a randomized controlled trial', *Cell metabolism,* 29(4), pp. 844-855. e3.

Wein, S., Ukropec, J., Gašperíková, D., Klimeš, I. and Šeböková, E. (2007) 'Concerted Action of Leptin in Regulation of Fatty Acid Oxidation in Skeletal Muscle and Liver', *Experimental and Clinical Endocrinology & Diabetes,* 115(04), pp. 244-251.
Wetten, A., Jones, D. E. J. and Dyson, J. K. (2021) 'Specific considerations for the management of primary biliary cholangitis: are the drug treatment options good enough?', *Expert Opinion on Pharmacotherapy*, pp. 1-5.
Wetten, A., Jones, D. E. J. and Dyson, J. K. (2022) 'Seladelpar: an investigational drug for the treatment of early-stage primary biliary cholangitis (PBC)', *Expert Opinion on Investigational Drugs,* 31(10), pp. 1101-1107.
Wetten, A., Ogle, L., Mells, G., Hegade, V. S., Jopson, L., Corrigan, M., Palmer, J., Johansson, M., Bäckström, T., Doverskog, M., Jones, D. E. J. and Dyson, J. K. (2022) 'Neurosteroid Activation of GABA-A Receptors: A Potential Treatment Target for Symptoms in Primary Biliary Cholangitis?', *Canadian Journal of Gastroenterology and Hepatology,* 2022, pp. 1-13.
Wood, R. P., Ozaki, C. F., Katz, S. M., Johnston, T. D., Monsour Jr, H. P. and Dyer, C. H. (1994) 'Liver transplantation: the last ten years', *Surgical Clinics of North America,* 74(5), pp. 1133-1154.
Woods, S. C., Seeley, R. J., Porte Jr, D. and Schwartz, M. W. (1998) 'Signals that regulate food intake and energy homeostasis', *Science,* 280(5368), pp. 1378-1383.
Xia, M. Q., Bacskai, B. J., Knowles, R. B., Qin, S. X. and Hyman, B. T. (2000) 'Expression of the chemokine receptor CXCR3 on neurons and the elevated expression of its ligand IP-10 in reactive astrocytes: in vitro ERK1/2 activation and role in Alzheimer's disease', *Journal of Neuroimmunology,* 108(1), pp. 227-235.
Xiang, M., Feng, Y., Wang, Y., Wang, J., Zhang, Z., Liang, J. and Xu, J. (2021) 'Correlation between circulating interleukin-18 level and systemic lupus erythematosus: A meta-analysis', *Scientific reports,* 11(1), pp. 1-9.
Xiao, S., Brooks, C. R., Sobel, R. A. and Kuchroo, V. K. (2015) 'Tim-1 Is Essential for Induction and Maintenance of IL-10 in Regulatory B Cells and Their Regulation of Tissue Inflammation', *The Journal of Immunology,* 194(4), pp. 1602-1608.
Xu, J., Wang, Y., Khoshdeli, M., Peach, M., Chuang, J.-C., Lin, J., Tsai, W.-W., Mahadevan, S., Minto, W., Diehl, L., Gupta, R., Trauner, M., Patel, K., Noureddin, M., Kowdley, K. V., Gulamhusein, A., Bowlus, C. L., Huss, R. S., Myers, R. P., Chung, C. and Billin, A. N. (2022a) 'IL-31 levels correlate with pruritus in patients with cholestatic and metabolic liver diseases and is farnesoid X receptor responsive in NASH', *Hepatology,* n/a(n/a).
Xu, Y. F., Yao, Y., Ma, M., Yang, S. H., Jiang, P., Wang, J., Tsuneyama, K., Wang, C., Liu, X., Li, L. and Lian, Z. X. (2022b) 'The Proinflammatory Cytokines IL-18, IL-21, and IFN-γ Differentially Regulate Liver Inflammation and Anti-Mitochondrial Antibody Level in a Murine Model of Primary Biliary Cholangitis', *J Immunol Res,* 2022, pp. 7111445.
Yamano, T., Higashi, T., Nouso, K., Nakatsukasa, H., Kariyama, K., Yumoto, E., Kobayashi, Y., Yamamoto, K., Iwagaki, H., Yagi, T., Tanimoto, T., Kurimoto, M., Tanaka, N. and Tsuji, T. (2000) 'Serum interferon-gamma-inducing factor/IL-18 levels in primary biliary cirrhosis', *Clin Exp Immunol,* 122(2), pp. 227-31.
Yang, C.-Y., Ma, X., Tsuneyama, K., Huang, S., Takahashi, T., Chalasani, N. P., Bowlus, C. L., Yang, G.-X., Leung, P. S. C., Ansari, A. A., Wu, L., Coppel, R. L. and Gershwin, M. E. (2014) 'IL-12/Th1 and IL-23/Th17 biliary microenvironment in primary biliary cirrhosis: Implications for therapy', *Hepatology,* 59(5), pp. 1944-1953.
Yang, W.-H., Liu, S.-C., Tsai, C.-H., Fong, Y.-C., Wang, S.-J., Chang, Y.-S. and Tang, C.-H. (2013) 'Leptin Induces IL-6 Expression through OBRl Receptor Signaling Pathway in Human Synovial Fibroblasts', *PLoS ONE,* 8(9), pp. e75551.

Yasoshima, M., Kono, N., Sugawara, H., Katayanagi, K., Harada, K. and Nakanuma, Y. (1998) 'Increased expression of interleukin-6 and tumor necrosis factor-alpha in pathologic biliary epithelial cells: in situ and culture study', *Lab Invest,* 78(1), pp. 89-100.
Yasuda, K., Nakanishi, K. and Tsutsui, H. (2019) 'Interleukin-18 in Health and Disease', *Int J Mol Sci,* 20(3).
Yeaman, S. J., Fussey, S. P., Danner, D. J., James, O. F., Mutimer, D. J. and Bassendine, M. F. (1988) 'Primary biliary cirrhosis: identification of two major M2 mitochondrial autoantigens', *Lancet,* 1(8594), pp. 1067-70.
Yoshizaki, K., Matsuda, T., Nishimoto, N., Kuritani, T., Taeho, L., Aozasa, K., Nakahata, T., Kawai, H., Tagoh, H., Komori, T. and et al. (1989) 'Pathogenic significance of interleukin-6 (IL-6/BSF-2) in Castleman's disease', *Blood,* 74(4), pp. 1360-7.
Yurtcu, N., Caliskan, C. S., Guvey, H., Celik, S., Hatirnaz, S. and Tinelli, A. (2022) 'Predictive and Diagnostic Value of Serum Adipokines in Pregnant Women with Intrahepatic Cholestasis', *International Journal of Environmental Research and Public Health,* 19(4), pp. 2254.
Zhang, X., Sheng, J., Zhang, Y., Tian, Y., Zhu, J., Luo, N., Xiao, C. and Li, R. (2017) 'Overexpression of SCAMP3 is an indicator of poor prognosis in hepatocellular carcinoma', *Oncotarget,* 8(65), pp. 109247-109257.
Zhang, Y., Kong, D. and Wang, H. (2020) 'Mucosal-Associated Invariant T cell in liver diseases', *Int J Biol Sci,* 16(3), pp. 460-470.
Zhou, H., Lapointe, B. M., Clark, S. R., Zbytnuik, L. and Kubes, P. (2006) 'A requirement for microglial TLR4 in leukocyte recruitment into brain in response to lipopolysaccharide', *The Journal of Immunology,* 177(11), pp. 8103-8110.
Zhou, T., Kyritsi, K., Wu, N., Francis, H., Yang, Z., Chen, L., O'Brien, A., Kennedy, L., Ceci, L., Meadows, V., Kusumanchi, P., Wu, C., Baiocchi, L., Skill, N. J., Saxena, R., Sybenga, A., Xie, L., Liangpunsakul, S., Meng, F., Alpini, G. and Glaser, S. (2019) 'Knockdown of vimentin reduces mesenchymal phenotype of cholangiocytes in the Mdr2−/− mouse model of primary sclerosing cholangitis (PSC)', *EBioMedicine,* 48, pp. 130-142.
Zigmond, A. S. and Snaith, R. P. (1983) 'The Hospital Anxiety and Depression Scale', *Acta Psychiatrica Scandinavica,* 67(6), pp. 361-370.
Zorrilla, E. P., Sanchez-Alavez, M., Sugama, S., Brennan, M., Fernandez, R., Bartfai, T. and Conti, B. (2007) 'Interleukin-18 controls energy homeostasis by suppressing appetite and feed efficiency', *Proceedings of the National Academy of Sciences,* 104(26), pp. 11097-11102.
Zwarts, M. J., Bleijenberg, G. and Van Engelen, B. G. M. (2008) 'Clinical neurophysiology of fatigue', *Clinical Neurophysiology,* 119(1), pp. 2-10.