Understanding the Clinical Impact of Autoimmune Hepatitis

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Thesis Abstract

Background: Autoimmune hepatitis (AIH) is a heterogenous, chronic inflammatory liver disease that remains a challenge in diagnosis and management. Failure to achieve biochemical remission has serious consequences (cirrhosis, liver failure and death). Existing immunosuppressive treatments (mainly prednisolone and azathioprine) are usually lifelong and some patients develop intolerable side effects. There is a pervading perception amongst clinicians that AIH is easy to treat and the treatments are effective. There is a gap between clinician perception, clinical data and viewpoints of patients.

Aims and Methods: To explore the mechanistic evidence behind three domains of unmet need in AIH (inadequate response to therapy, effective therapy with undesirable side-effects and quality of life) using the national UK-AIH multicentre cohort. Clinical indices and quality of life (QOL) information were analysed. A novel thiopurine metabolite (deoxythioguanosine in DNA of leukocytes, dtG DNA) as a potential biomarker for treatment optimisation was explored in a subgroup of 57 patients, divided by treatment response.

Findings: In a cohort of approximately 1000 patients, 29 different treatment regimens were reported and biochemical remission rate was 59%. Remission rates were significantly higher in transplant centres compared to non-transplant centres. 55% remain on corticosteroids. There was no significant correlation in the leukocyte metabolite (dtG DNA) levels with disease response or azathioprine dose which suggests that this biomarker is not clinically useful. There was evidence of QOL impairment compared to the general population with corticosteroid use being strongly associated with decreased QOL, independent of remission status.

Conclusions: This demonstrates suboptimal areas of care for patients with AIH with variable treatment regimens, suboptimal remission rates, high use of corticosteroids and poor QOL. This highlights the need for better corticosteroid-free therapy approaches and emphasizes the need for continued future efforts in improving treatment approaches for AIH (which encompasses better therapies combined with focus on improving QOL).

Dedication

This thesis is dedicated to a few people who were my ‘pushers’ for this pushcart. With their drive and energy constantly behind me, they edged me on, bit by bit and later the push I needed when ideas and motivation stalled. Thank you, Jennifer and Dai May for that driving force which compelled me to finish, my parents whom I shall always be grateful for, my family, my friends and colleagues who were my cheer-leading team with special mention to Sree for her persistent phone calls to motivate me in the last leg of the journey. Last but not least, my Heavenly Father who is my prime source of inspiration and wisdom.

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Professor Julia Newton[a] (Newcastle University) and Professor John Crawford[b] (University of Aberdeen) for their generosity in sharing their data. ([a]Fatigue Impact Scale (FIS) and Hospital Anxiety Depression Scale (HADS) questionnaire results in PBC patients and healthy controls, Cognitive Failure Questionnaire results in healthy controls; [b]HADS data of normal population).

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All the members of the clinical and research teams who have recruited to the UK-AIH study as well as the participants from all sites who submitted their data. This project would not have been possible without your support and hard work.

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Abbreviations

6-MP	6-Mercaptopurine
6-MMP	6-methylated mercaptopurine
6-TGN	6-thioguanine nucleotide
6-TU	6-thiouric acid
AASLD	American Association for the Study of Liver Diseases
ACTH	Adrenocorticotrophic hormone
AIH	Autoimmune hepatitis
ALP	Alkaline phosphatase
ALT	Alanine transferase
AMA	Anti-mitochondrial antibody
ANA	Antinuclear antibody
APC	Antigen presenting cell
APECED	polyendocrinopathy-candidiasis-ectodermal dystrophy
ASGPR	asia glycoprotein receptor
AST	Aspartate transferase
AZA	Azathioprine
BMI	Body mass index
BR	Biochemical remission
BSG	British Society of Gastroenterology
Bx	Biopsy
CBR	Clinical and biochemical remission
CDF	clinical data forms
CFQ	Cognitive Failure Questionnaire
CI	Confidence interval
CK7	Cytokeratin 7
CMV	Cytomegalovirus
CNI	Calcineurin inhibitors
DEXA	dual energy X-ray absorptiometry
DNA	deoxyribonucleic acid
dTG	deoxythioguanosine
Dx	Diagnosis
EASL	European Association for the Study of the Liver
EBV	Ebstein-Barr virus
EQ-5D	European Quality of Life-5 Dimension
EQ-5D-3L	European Quality of Life-5 Dimension-3 Level
EQ-5D-5L	European Quality of Life-5 Dimension-5 Level
FIS	Fatigue Impact Scale
F/up	Follow up
GGT	Gamma glutamyl transpeptidase
GMPs	guanosine monophosphate synthetase
HADS	Hospital Anxiety Depression Scale

HADS-A	Hospital Anxiety Depression Scale-Anxiety
HADS-D	Hospital Anxiety Depression Scale-Depression
HAI	Hepatitis activity index
HAV	Hepatitis A virus
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCC	Hepatocellular cancer
HCV	Hepatitic C virus
HE	Hepatic encephalopathy
HGPRT	Hypoxanthine guanine phosphoribosyl transferase
HLA	Human leukocyte antigen
HRQOL	Health-related quality of life
IAIHG	International Autoimmune Hepatitis Group
IBD	Inflammatory bowel disease
ICF	Informed consent form
IgG	Immunoglobulin G
IFN	interferon
IL	interleukin
IMPDH	inosine monophosphate dehydrogenase
ITT	Intention to treat
KIRs	Killer-cell immunoglobulin-like receptors
LC1	Liver cytosol-1
LCMS/MS	Liquid Chromatography with tandem mass spectrometry
LE	lupus erythematosus
LFTs	Liver function tests
LKM	Liver-kidney microsomal
LP	liver pancreas antigen
MA	Mycophenolic acid
MCID	Minimal clinical important difference
MeMPNs	Methyl mercaptopurine nucleotides
MMF	Mycophenolate mofetil
NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis
NICE	National Institute for Health and Care Excellence
NK	Natural Killer
ns	not significant
OR	odds ratio
OLTx	orthotopic liver transplantation
p-ANCA	perinuclear antineutrophil cytoplasmic antibody
PBC	Primary biliary cholangitis
PBMC	Peripheral blood mononuclear cells
PIS	Patient information sheet
Pred	Prednis(ol)one
PSC	Primary sclerosing cholangitis
RBC	Red blood cell

RCTs	Randomised controlled trials
Rx	Treatment
SGOT	serum glutamic oxaloacetic transaminase
SIR	Standardised incident rate
SLA	Soluble liver antigen
SLE	Systemic lupus erythematosus
SMA	Smooth muscle antibody
SMR	Standardised mortality ratio
SNPs	single nucleotide polymorphisms
TCR	T cell receptor
TG	thioguanine
TGF	transforming growth factor
TGMP	thioguanosine monophosphate
TGNs	thioguanine nucleotides
TIMP	thioinosine monophosphate
TPMT	thiopurine methyl transferase
TNF	Tumour necrosis factor
Treg	T regulator cells
TXMP	thioxanthosine monophosphate
UI	Utility Index
UDCA	Ursodeoxycholic acid
UK	United Kingdom
ULN	Upper limit of normal
VAS	Visual analogue scale
XO	Xanthine oxidase

Chapter 1. Autoimmune Hepatitis

1.1 Introduction

Autoimmune hepatitis (AIH) is a rare, progressive inflammatory liver disease that may present in either acute or chronic forms. It can progress rapidly to acute liver failure or the development of cirrhosis if not effectively treated (Heneghan *et al.*, 2013; Manns, Lohse and Vergani, 2015; Krawitt, 2006). It is rare with a prevalence of approximately 17 per 100,000 population in Northern Europe popuations, (Boberg *et al.*, 1998) affects all ages, has a female preponderance and is described in a wide range of ethnic groups (Al-Chalabi *et al.*, 2006; Werner *et al.*, 2008). AIH was one of the first liver conditions in which effective therapy was described. Studies from the 1960s to the 1980s (Cook, Mulligan and Sherlock, 1971; Kirk *et al.*, 1980; Soloway *et al.*, 1972; Murray-Lyon, Stern and Williams, 1973; Summerskill *et al.*, 1975) established combination therapy with prednisolone and azathioprine as the mainstay of induction treatment in treatment naïve patients; demonstrating improved biochemistry, liver histology and survival. The perception of the treatability of AIH, in an era when other liver conditions were largely untreatable led, however, to complacency in the area of AIH and little has changed in the decades since these landmark studies were performed.

Existing immunosuppressive treatments (mainly prednisolone and azathioprine) are usually lifelong. Not everyone responds adequately with approximately 20% not achieving remission and some developing intolerable side effects. Failure to achieve biochemical remission is associated with increased risk of liver-related death and transplantation (Gleeson, Heneghan and British Society of, 2011). AIH accounts for 6% of elective liver transplantation in the UK (NHS Blood and Transplant 2019/2020, 2020). Long-term mortality is substantial (18% at 10 years, 52% at 20 years) (Hoeroldt *et al.*, 2011). There is a pervading perception amongst clinicians that AIH is easy to treat and the treatments are effective. There is a gap between clinician perception, clinical data and viewpoints of patients.

This thesis is concerned with the concept of clinical impact of AIH and aims to exploring the clinical and mechanistic evidence behind unmet need in AIH using the UK-AIH national multi-centre cohort.

This introductory chapter provides background information on AIH, which comprises of the history of the discovery of this disease, the epidemiology, pathogenesis, clinical presentation, diagnosis/diagnostic scores, management/treatment, disease course, long-term clinical outcomes (encompassing complications of the disease and prognosis) and factors associated with disease progression and outcomes. It also elaborates on the evolution of managing this disease and the domains of unmet need.

1.2 History Of The Discovery Of AIH

Autoimmune hepatitis (AIH) is an autoimmune chronic disease of the liver. Historically before the 1940s, chronic liver disease with its dire consequences of cirrhosis or death was attributed to toxins such as alcohol, infection, pigment (iron), lardaceous (amyloid) deposition in the liver parenchyma, nutritional disorders and 'healed acute yellow atrophy' (Mallory, 1932). During the hepatitis epidemics of World War II, it was noted that not all cases of acute viral hepatitis resulted in full recovery or death; Instead, there were observations of 'non-recovery' or prolonged liver injury which led to the concept of 'chronic hepatitis'. Subsequently, the terms 'chronic *active* hepatitis' and 'chronic *inactive* hepatitis' were used, depending on the presence or absence of symptoms respectively (Reuben, 2003).

With gradual reports of cases of chronic hepatitis associated with high serum proteins affecting children and young adults with female preponderance, (Cullinan, 1936; Amberg, 1942; Wood *et al.*, 1948) a distinct disease entity was realised and first reported by Waldenström at a conference in 1950 (Waldenstrom, 1950). The disease entity was fully reported as a chronic form of hepatitis in young women with hypergammaglobulinaemia in the absence of cirrhosis, which responds well to adrenocorticotrophic therapy (ACTH) (Waldenstrom, 1952; Manns, Lohse and Vergani, 2015).

At this point, autoimmunity was not a well-known phenomenon and most of the necessary diagnostic laboratory procedures (liver biopsy, serum aminotransferases, serum autoantibodies) were not routinely available. Serum gamma globulin was the exception and increased levels have been reported in association with cases of cirrhosis reported in the early 1940s (Mackay, 2008; Stern and Reiner, 1946).

In 1948, the association between the newly termed Lupus erythematosus (LE) cells and lupus the disease was established. Two reports were then published, reporting of LE cells being found in two patients with hypergammaglobulinaemia and active chronic hepatitis (presumed to be viral in origin then) (Joske and King, 1955) and in the ascites of a patient with cirrhosis (Leoni, 1954). This led to the landmark Lancet article by Mackay and colleagues describing seven patients with hypergammaglobulinaemia, chronic hepatitis and LE cells – a new liver disorder, termed "lupoid hepatitis" was introduced. For a time, there was an incorrect distinction between lupoid and LE-cell negative chronic hepatitis. The term also introduced some confusion (for non-hepatologists particularly) that lupoid-hepatitis was a common feature of systemic lupus erythematosus (SLE). It was subsequently discovered that antinuclear antibodies (ANA) positivity was the determining criterion for the 'classic lupoid hepatitis", rather than LE cell positivity (Holborow *et al.*, 1963; Mackay, Weiden and Hasker, 1965; Reuben, 2003).

With the advent of needle liver biopsy, previously used terms for symptom activity - 'chronic active hepatitis' and 'chronic inactive hepatitis' were used to denote histological disease activity (Reuben, 2003). With increasing evidence that the disease was best accounted for by an autoimmune reaction in the liver, the name 'autoimmune hepatitis' was first suggested by MacKay and colleagues in 1965 (Mackay, Weiden and Hasker, 1965) and officially endorsed in 1993 (Johnson and McFarlane, 1993; Mackay, 2008). The term 'lupoid hepatitis' gradually died out and it is important to note that AIH and systemic lupus erythematosus (SLE) remain different autoimmune diseases, which may occur together (Manns, Lohse and Vergani, 2015).

Summary points

- AIH was first described by Waldenström in 1952 as a 'chronic form of hepatitis in young women with hypergammaglobulinaemia in the absence of cirrhosis which responds well to ACTH'.
- AIH was previously called 'lupoid hepatitis' for a period due to lupus erythematosus (LE) cells found in patients with hypergammaglobulinaemia and active chronic hepatitis.
- Previous terms used for AIH include 'chronic active hepatitis' and 'chronic inactive hepatitis' – these were initially based on presence or absence of symptoms but with the increasing availability of liver biopsy, became based on histological disease activity.

- With increasing recognition of autoimunity being the cause, the name 'autoimmune hepatitis' was first coined by MacKay and colleagues in 1965 and officially endorsed in 1993.

1.3 Epidemiology

AIH is a rare disease with a reported prevalence of 1 to 24 per 100 000 in Europe (Feld *et al.*, 2005; Boberg *et al.*, 1998; Primo *et al.*, 2004; Gronbaek, Vilstrup and Jepsen, 2014; Werner *et al.*, 2008; van Gerven *et al.*, 2014). In a prospective UK study evaluating all patients with bilirubin 120 micromol/Litre (identified by a biochemistry laboratory serving 3 general hospitals and the community), AIH accounted for two of 121 patients presenting to hospital with jaundice (Whitehead, Hainsworth and Kingham, 2001; Gleeson, Heneghan and British Society of, 2011). In Asia (Singapore, Brunei, Japan and Korea), a similar prevalence has been reported between 4 to 23.4 per 100 000 (Lee *et al.*, 2001; Jalihal, Telisinghe and Chong, 2009; Yoshizawa *et al.*, 2016; Kim *et al.*, 2017). Higher prevalence rates have been reported in areas of stable populations – 42.9 cases per 100 000 and 24.5 cases per 100 000 inhabitants for Alaska natives and New Zealand respectively (Hurlburt *et al.*, 2002; Ngu *et al.*, 2010). There has been a reported increase in incidence by nearly 50% in Spain, Denmark, Sweden and Netherlands over the past few decades (Gronbaek, Vilstrup and Jepsen, 2014; Primo *et al.*, 2009; van Gerven *et al.*, 2014). A recent population-based study (Canterbury, New Zealand) reported a significantly higher incidence of AIH in the 2014-2016 period (2.39 per 100 000, 95% CI, 1.76-3.23) compared to 2008-2010 period (1.37 per 100 000; 95% CI, 0.91-2.06) ($p<0.05$) whilst incidences of other autoimmune liver diseases (PBC and PSC) were unchanged (Lamba, Ngu and Stedman, 2021). This change in incidence cannot be entirely attributable to relative case ascertainment rates and needs further study. A meta-analysis of 22 studies reported the pooled worldwide annual incidence and prevalence of AIH to be 1.37 (95% CI: 0.95-1.80) and 17.44 (95% CI: 12.01-22.87) per 100 000 persons respectively. The pooled annual incidence for Asian, European and American population was 1.31 (95% CI: 0.42-2.20), 1.37 (95% CI: 1.10-1.64) and 1.00 (95% CI: 0.44-1.56) per 100 000 persons respectively; pooled prevalence for Asian, European and American population was 12.99 (95% CI: 2.05-23.92), 19.44 (95% CI: 15.63-23.24) and 22.80 (95% CI: -13.48 – 59.07) per 100 000 persons respectively (Lv *et al.*, 2019).

AIH has a female preponderance (3 to 4 times more frequent than men) and has been described in many ethnic groups worldwide (Zolfino *et al.*, 2002; D'Souza *et al.*, 2005; Chung *et al.*, 2007; Al-Chalabi *et al.*, 2006). There have been studies describing a bimodal age of presentation (one peak during childhood/teenage years and another between the 4th and 6th decade of life) (Werner *et al.*, 2008; van Gerven *et al.*, 2014; Gronbaek, Vilstrup and Jepsen, 2014; Abe *et al.*, 2011; Manns *et al.*, 2010a) whilst other studies have reported patients being diagnosed at older ages (above 65 years) (Schramm *et al.*, 2001; Peng *et al.*, 2014; Chen, Eslick and Weltman, 2014; Al-Chalabi *et al.*, 2006; Ngu *et al.*, 2010). Generally, AIH affects all age groups and can present at any age.

Summary points

- AIH is a rare disease with a reported prevalence of 1-24 per 100 000 in Europe and pooled worldwide prevalence and incidence of of 17.4 and 1.4 per 100 000 persons respectively.
- Incidence of AIH has increased over recent years, based on reports from Europe and New Zealand – this merits further study.
- AIH has a female preponderance (4:1), affects many ethnic groups and all age groups and can present at any age (with some studies reporting a bimodal age of presentation: childhood/teenage and between 40-60 years).

1.4 Pathogenesis

The aetiology of AIH remains unknown and not fully understood. The aetiology of AIH is currently thought to be as a result of an interaction between a trigger such as a drug or virus and the environment in a genetically susceptible individual (Donaldson, 2004). This interactions remains mostly undefined. There is loss of immunological tolerance to hepatic autoantigens. At a molecular level, it is thought to encompass the autoantigen, the major histocompatabilty complex and the T-cell receptor (Chapman *et al.*, 2019).

1.4.1 Genetic Aspects

There are several genetic associations that have been reported to interact in influencing susceptibility to AIH, its clinical features, therapy response and prognosis. The genes of the

human leukocyte antigen (HLA) region (the human major histocompatibility complex, MHC) which are located on the short arm of chromosome 6 have been found to have the strongest associations with AIH – these genes are implicated in the initiation of an adaptive immune response as these are involved in presenting antigenic peptides to T cells (Liberal *et al.*, 2011; Manns, Lohse and Vergani, 2015). Distinct MHC II alleles and specific small nucleotide polymorphisms within regulatory genes (e.g. CTLA-4 and TNF-alpha) have been associated with increased risk of developing AIH as suggested by genetic evidence (Agarwal *et al.*, 2000; Czaja *et al.*, 1993b; Hardtke-Wolenski *et al.*, 2017).

HLA-DR3 and HLA-DR4 have been found to confer susceptibility to classical (type 1) AIH in Caucasians (Europe and North America). DRB1*0301 and DRB1*0401 are the common genotypes in North America whilst DRB1*1301 is common in South America. The first genome-wide association study (GWAS) in the Netherlands confirmed this finding (Donaldson, 2004; Czaja *et al.*, 1993a; de Boer *et al.*, 2014). HLA DR3 and DR4 have been incorporated into the revised diagnostic scoring system International AIH group (IAIHG) due to their particularly strong association (Alvarez *et al.*, 1999a). In Japanese patients, HLA DRB1*0405 was seen at an increased frequency (Umemura *et al.*, 2014) whilst in Latin Americans, HLA-DR6 (DRB1*1301) was found at significantly increased frequency in patients with AIH compared to controls (Fainboim *et al.*, 1994).

HLA DR7 (DRB1*0701) and DR3 (DRB1*0301) are reported to confer a susceptibility to type 2 AIH. Those with DR7 positivity have a more aggressive disease phenotype and worse overall prognosis (Ma *et al.*, 2006). HLA-DQB1*0201 has been associated with the development of type 2 AIH but this allele is in linkage disequilibrium with DRB1*0701 and DRB1*0301 which are both linked with type 2 AIH (Djilali-Saiah *et al.*, 2004).

The variants of SH2B3 (chromosome 12) and CARD 10 (chromosome 22) have been reported to be associated with type 1 AIH in the Dutch genome-wide association study (GWAS) on AIH (de Boer *et al.*, 2014).

Natural killer (NK) cells play a vital role in the innate immune response to viruses, bacteria and tumour cells. The cell-mediated responses of NK cells have been implicated in the pathogenesis of AIH. Killer-cell immunoglobulin-like receptors (KIRs) are key regulators of NK-cell-mediated immune response. KIRs are expressed by NK cells – these bind to MHC class I

molecules, which causes an inhibitory signal that allows NK cells to transform into their full cytotoxic function. An Italian study (114 AIH patients, 221 healthy controls) found that the activating KIR gene KIR2DS1 had a predictive potential for early onset of type 1 AIH. KIR3DL1/HLA-Bw4 and KIR2DL3/HLA-C1 were found in low freqency whilst there was a high frequency of HLA-C2 high affinity ligands for KIR2DS1 which led the authors to conclude this could contribute to unwanted NK cell autoreactivity in AIH type 1 (Littera *et al.*, 2016).

A Japanese study (154 patients with AIH and 201 controls) identified KIR3DL1/HLA-B Bw4-80Ile phenotype was significantly associated with AIH and was an independent susceptibility gene along with HLA-DRB1*04:05-DQB1*04:01 haplotype. There were lower frequencies of KIR2DL1/HLA-C2 and KIR3DL1/HLA-B Bw4- 80Thr in AIH patients which suggested a protecting effect by these KIR-HLA pairs. The study found that KIR3DL1/HLA-B Bw4 and KIR3DL1/HLA-B Bw4-80Ile were protective against liver decompensation and liver-related death and the absence of KIR3DL1/HLA-B Bw4 and the presence of cirrhosis at diagnosis were strongly related to AIH progression (Umemura *et al.*, 2019).

With regards to non-MHC related associations, the substitution from A (adenine) to G (guanine) in exon 1 of the CTL-4 gene have been reported to confer susceptibility to type 1 AIH in Caucasians from North America (Agarwal *et al.*, 2000).

Approximately 20% of patients with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) have a form of AIH resembling type 2 AIH. APECED is a monogenic autosomal recessive disorder due to homozygous mutations in the AIRE1 gene. (Liston *et al.*, 2005) The AIRE1 gene has not been found to be increased in AIH or other autoimmune liver diseases such as PBC and PSC but the gene was observed in children with acute liver failure – therefore the APECED syndrome should be considered as a cause for acute liver failure (Lankisch *et al.*, 2005; Manns, Lohse and Vergani, 2015).

1.4.2 Precipitating Factors

Drugs, vaccines, herbs and viruses have been documented to be a precipitating factor for AIH (see **Table 1**) although the association may be coincidental (Gleeson, Heneghan and British Society of, 2011). Antibiotics (nitrofurantoin and minocycline), statins and anti-tumour necrosis factor (TNF) agents such as infliximab and adalimumab have been reported as drug-triggers for AIH. It is often challenging to distinguish the difference between drug-induced liver

injury and AIH due to overlapping clinical and histological features (Weiler-Normann and Schramm, 2011). In a study of 261 patients with AIH by Bjornsson and colleagues, 9.2% had 'drug-induced AIH'. The histological and serological findings as well as the therapy-response was similar between AIH cases and 'drug-induced AIH'. The only difference found was that there were no relapses in 'drug-induced AIH' after cessation of immunosuppression in contrast to the 65% relapse rate after withdrawal of immunosuppression in the AIH cases (Björnsson *et al.*, 2010).

Table 1: Precipitating factors of AIH reported in literature

Precipitating factor		Reference
Viruses	Hepatitis A	(Vento and Cainelli, 2004; Huppertz *et al.*, 1995; Singh *et al.*, 2007)
	Hepatitis C	(Dalekos *et al.*, 1999; Muratori *et al.*, 1994; Vento *et al.*, 1997)
	Hepatitis E	(Nagasaki *et al.*, 2005)
	Cytomegalovirus (CMV)	(Castellote, Guell and Porta, 2001; Kamisako, Tsubaki and Adachi, 1997)
	Epstein-Barr virus (EBV)	(Nobili *et al.*, 2003; Vento *et al.*, 1995)
	Varicella zoster	
	Human herpes 6	(Al-Hamoudi, 2009)
	Measles	(Grima *et al.*, 2008)
	Brucellosis	(Vento *et al.*, 1996)
Antibiotics	Minocycline	(Onder *et al.*, 2005; Selimoglu and Ertekin, 2003)
	Doxycycline	(Bhat *et al.*, 1998; Abe *et al.*, 2003; Goldstein *et al.*, 2000; Colmegna, Perandones and Chaves, 2000; Basude and Dhawan, 2007)
	Nitrofurantoin	(Sharp, Ishak and Zimmerman, 1980; Stricker *et al.*, 1988; Amit, Cohen and Ackerman, 2002)
	Levofloxacin	(Juarez *et al.*, 2014)
Anti-protozoal	Ornidazole	(Kosar *et al.*, 2001; Ersoz *et al.*, 2011)
Anti-tumour necrosis factor (TNF)	Infliximab	(Rodrigues *et al.*, 2015; Efe, Purnak and E., 2010; Germano *et al.*, 2005)
	Adalimumab	(Adar *et al.*, 2010; Rodrigues *et al.*, 2015)
	Natalizumab	

	Efalizumab	(Martinez-Lapiscina *et al.*, 2013; Antezana *et al.*, 2015) (Primo, Michavila and Jimenez, 2010)
Nonsteroidal Anti-Inflammatory	Indometacin Diclofenac	(Abraham *et al.*, 2008) (Scully, Clarke and Barr, 1993)
Anti-metabolite	Methotrexate	(Moreno-Otero *et al.*, 2011)
Anti-diabetic	Liraglutide	(Kern *et al.*, 2014)
Interferon	Interferon beta 1a Interferon alpha	(Duchini, 2002; Pulicken *et al.*, 2006; Villamil *et al.*, 2015) (Heathcote, 1995; Cholongitas *et al.*, 2006; Farhat, Johnson and Williams, 1994; Kontorinis *et al.*, 2006)
Anti-monoclonal antibodies	Masitinib Glivec Imatinib mesylate	(Salvado *et al.*, 2015) (Charier *et al.*, 2009) (Aliberti *et al.*, 2009)
Anticholesterol	Simvastatin Fluvastatin Atorvastatin Ezetimibe Fibrates	(Alla *et al.*, 2006) (Castiella, Fernandez and Zapata, 2007) (Castiella, Fernandez and Zapata, 2007) (van Heyningen, 2005) (Ganne-Carrie *et al.*, 1998)
Anti-reflux	Ranitidine	(Luparini *et al.*, 2000)
Other drugs	Methyldopa Terbinafine Anastrazole Herbal	(Shalev *et al.*, 1983) (Paredes and Lewis, 2007) (Islam *et al.*, 2014; Inno *et al.*, 2011) (Kamiyama *et al.*, 1997; Borum, 2001)
Vaccinations	Hepatitis A vaccination	(Berry and Smith-Laing, 2007)

1.4.3 Relevant Autoantigens

Currently, the autoantigens responsible for starting the cascade of events in AIH have yet to be identified. A few relevant autoantigens are described below.

(i) Asialoglycoprotein receptor (ASGPR): The asialoglycoprotein receptor (ASGPR) is an established liver-specific autoantigen. A multicentre study of Asian, European and North American patients found 85% (53/62) of AIH patients had anti-human ASGPR with reduction in titre with response to immunosuppression. In in-vitro studies, ASGPR has been demonstrated to be secreted by peripheral blood mononuclear cells (PBMC) from patients

with AIH or PBC but not chronic viral hepatitis. Liver-infiltrating T-cells (obtained from liver biopsies of patients with AIH and PBC) showed a proliferative response to ASGPR (Löhr *et al.*, 1992). ASGPR however is also present in other diseases (e.g. hepatitis C, PSC, PBC) which suggests it may be part of other processes not specific to AIH (Villalta *et al.*, 2015).

(ii) Anti-CYP2D6: In type 2 AIH, anti-liver kidney microsomal-1 (anti-LKM-1) is directed at an epitope on cytochrome P450 2D6. A German study found that PBMCs from AIH patients (n=8) exhibited a proliferative response on exposure to 23-amino acid LKM peptide and a shorter 18-amino acid LKM peptide. LKM-specific T cell responses reduced after immunosuppresive therapy was started. Activated LKM-specific T cells released interferon gamma (IFN-gamma) but no or little interleukin-4 (Löhr *et al.*, 1996).

(iii) Anti-Soluble liver antigen and liver-pancreas antign (SLA/LP antigen): Soluble liver antigen and liver-pancreas antigen (SLA/LP) have been shown to be specific to AIH and the high degree of specificity suggests a possible role in the pathogenesis of AIH. SLA and LP antigen were shown to be identical, hence the SLA/LP antibody term was suggested. Cloning experiments identified a 422 amino acid protein as the sole target antigen. SLA/LP antigen is unique, presumed to code for an enzyme (suggested to be a UGA-suppressor t-RNA-associated protein). The SLA/LP antibody is of the IgG1 subtype. Molecular mimicry was initially hypothesized as a driver for autoimmunity to SLA/LP however no evidence was found for this in one report. Anti-SLA/LP antibofies remain important diagnostic markers for AIH (Wies *et al.*, 2000; Herkel *et al.*, 2002).

1.4.4 Immune Regulatory Mechanisms

The immunopathogenesis of AIH is thought to be due to a loss in self-tolerance to hepatocyte autoantigens from defective immune response or imbalance in immunological regulatory mechanisms. This leads to cytotoxic T-cell mediated hepatocellular injury with important involvement of multiple T cell subsets and B cells (Mack *et al.*, 2020; Assis, 2020). The details of these multistep mechanisms can be found in several excellent review articles (Mack *et al.*, 2020; Assis, 2020; Floreani *et al.*, 2018; Wang and Zhang, 2018; Sucher *et al.*, 2019). The multi-step process is summarised simplistically as follows:

1. Thymic autoantigen-specific naïve T-regulator cells (Treg) become incapable of preventing immune responses to hepatic autoantigens during hepatic or systemic immune responses to environmental triggers (e.g. viruses, drugs)
2. Self-antigenic peptides to T-cell receptor (TCR) of naïve $CD4^+$ T helper cells (Th0) are presented by antigen-presenting cells (APC), dendrite cells and other cells capable of presenting antigens
3. Pro-inflammatory cytokines (Interleukin (IL)-12, IL-6, transforming growth factor (TGF)-β) are secreted, leading to the development of Th1, Th2 and Th17 T-cells
    i) Th1 cells secrete IL-2 and IFN (Interferon)-γ; These stimulate CD8+ cells to induce expression of HLA class I and HLA class II molecules on hepatocytes and mediates activation of macrophages which release IL-1 and TNF-α
    ii) T-regs and Th2 cells produce IL-4, IL-10 and IL-13 which stimulate the maturation of B cells and plasma cells which produces autoantibodies [involved in antibody-mediated cellular toxicity and complement activation]
    iii) Th17 cells secrete pro-inflammatory cytokines (IL-17, IL-22, TNF-α and chemokine ligand (CCL-20) which suppress T-regs and leads to initiation and perpetuation of autoimmune liver damage (via cytotoxic T-lymphocytes, Th1, Th17, macrophages, natural killer cells and complement activation)

The number of Th17 cells correlates with the degree of liver fibrosis. The numerical reduction in T-regs leads to impaired tolerance to autoantigens. Defective T-regs in AIH are thought to be associated with lower expression of forkhead box P3 (FOXP3) (Liberal, Vergani and Mieli-Vergani, 2015).

Summary points

- The aetiology of AIH remains not fully understood and is thought to be as a result of an interaction between a trigger (such as a drug or virus) and the environment in a genetically susceptible individual. The interaction remains poorly defined.
- At a molecular level, it is thought to encompass the autoantigen, the major histocompatability complex (HLA) and the T-cell receptor.
- Notable genetic associations with AIH: HLA-DR3 and HLA-DR4 confers susceptibility to type 1 AIH in Caucasians whilst HLA DR7 and DR3 confers susceptibility to type 2 AIH.

- Precipitating factors for AIH include drugs (notably, antibiotics – nitrofurantoin, minocycline; statins and anti-TNF agents) and viruses.
- Although the current autoantigens responsible for initiating the cascade of events in AIH are yet to be identified, relevant autoantigens implicated in AIH include the Asialoglycoprotein receptor (ASGPR), anti-cytochrome P450 2D6 and anti-soluble liver antigen and liver-pancreas antigen (SLA/LP Ag).
- The current immunopathogenesis of AIH is thought to be due to loss of self-tolerance to hepatocyte autoantigens from defective immune response or immunological regulatory mechanisms leading to cytotoxic T-cell mediated hepatocellular injury with important involvement of multiple T cell subset and B cells.

1.5 Clinical Spectrum

AIH is a heterogeneous disease with a wide spectrum of presentations, making its management very challenging.

1.5.1 Clinical presentation

The classical presentation for AIH is in a chronic fashion with a 3-6 months history of abnormal liver function tests (LFTs) – mainly raised serum transaminases (alanine transferase (ALT) and/or aspartate transferase (AST), usually accompanied by hyperglobulinaemia or raised immunoglobulin G (IgG) (Gleeson, Heneghan and British Society of, 2011). The reported symptoms are non-specific and include a wide range - fatigue, anorexia, nausea, amenorrhoea, arthralgia, general ill-health, right upper quadrant pain, lethargy, pruritus, and weight loss; these can pre-date the abnormal LFTs by years. Occasionally there may be rarer features such as maculopapular skin rash and unexplained fever (Werner *et al.*, 2008; van Gerven *et al.*, 2014; Gronbaek, Vilstrup and Jepsen, 2014; Wong *et al.*, 2012; Czaja, 2013b; Muratori *et al.*, 2009; Abe *et al.*, 2011; Feld *et al.*, 2005; Zachou *et al.*, 2011; European Association for the Study of the, 2015; Mack *et al.*, 2020). About a third of patients (30%) can have underlying cirrhosis on first presentation with some patients (particularly the elderly) presenting with ascites or variceal bleed, suggesting liver decompensation (Gleeson, Heneghan and British Society of, 2011; Werner *et al.*, 2008; Gronbaek, Vilstrup and Jepsen, 2014; Muratori *et al.*,

2009; Feld *et al.*, 2005). AIH can present without symptoms in 25-34% of patients even in those with cirrhosis (Feld *et al.*, 2005; Kogan *et al.*, 2002).

AIH can also present acutely – either as an acute exacerbation of previously undiagnosed chronic AIH or true acute AIH without histological findings of chronic liver disease (European Association for the Study of the, 2015). The serum ALT or AST can be in the 1000s. This acute presentation has been reported to occur in 25-75% of patients with AIH (Crapper *et al.*, 1986; Nikias, Batts and Czaja, 1994; Czaja, 2013a). Patients presenting acutely can present with acute severe fulminant hepatitis with jaundice and development of hepatic encephalopathy within 26 weeks of the disease. This can often be preceded by nausea, anorexia and influenza-like symptoms (Czaja, 2016; Herzog *et al.*, 1997; Kessler *et al.*, 2004; Miyake *et al.*, 2006b; Ichai *et al.*, 2007). The liver histology can be atypical when AIH presents acutely – lobular hepatitis and centrilobular necrosis being more common and cirrhosis being less common (Nikias, Batts and Czaja, 1994; Al-Chalabi *et al.*, 2008a; Iwai *et al.*, 2008). In addition, serum autoantibodies may be absent initially before developing at a later stage or are weakly positive in 29-39% of acute severe AIH. Serum IgG level is normal in 25-39% (Fujiwara, Fukuda and Yokosuka, 2008; Yasui *et al.*, 2011).

1.5.2 Overlap syndrome

Patients with AIH can also present or develop clinical, biochemical, serological and/or histological features of primary biliary cholangitis (PBC) or primary sclerosing cholangitis (PSC) (Boberg *et al.*, 2011). Conversely, patients with PBC or PSC can also develop features of AIH. These phenomena are described as “overlap syndrome”. Other terms used to describe this include “the hepatitic forms of PBC”, “secondary autoimmune hepatitis”, “autoimmune cholangitis”, “autoimmune sclerosing cholangitis, or “combined hepatic/cholestatic syndromes” (Boberg *et al.*, 1998; Gregorio *et al.*, 2001; Rojas *et al.*, 2014; Mieli-Vergani *et al.*, 2009). There are currently no internationally-agreed criteria to define these variant conditions. The International AIH Group (IAIHG) diagnostic scoring criteria (elaborated in Section 1.6) has low-sensitivity to pick up clinically defined “overlap” therefore use of this criteria is currently not recommended (European Association for the Study of the, 2015).

1.5.3 Recurrence or De novo development after liver transplantation

AIH can also recur or develop *de novo* after liver transplantation and should be considered in all patients with graft dysfunction. Recurrence frequencies ranges from 8-68%, depending on the liver tissue examinations by protocol or by clinical indication (Czaja, 2016; Neuberger *et al.*, 1984; Ratziu *et al.*, 1999; Molmenti *et al.*, 2002; Duclos-Vallee *et al.*, 2003; Vogel *et al.*, 2004). De novo AIH occurs at a frequency of 1-7% of patients, mainly children (Kerkar *et al.*, 1998; Hernandez *et al.*, 2001; Gupta *et al.*, 2001; Aguilera *et al.*, 2001). Both de-novo and recurrent AIH are managed by increase of immunosuppression including corticosteroids and azathioprine (Gleeson, Heneghan and British Society of, 2011; European Association for the Study of the, 2015). The use of long-term low dose corticosteroids together with additional immunosuppressant agents has been shown to reduce the risk of recurrent AIH in the transplanted graft in one UK study (n=73, recurrence rate of 11% at 10 years). The survival rate was 86% and 73% at 5 and 10 years respectively. The percentage of patients who were free from sepsis at 5 and 10 years were 80% and 63% respectively. The percentage of patients free from osteoporosis at 5 and 10 years were 82% and 58% respectively. These rates did not occur more often compared to published literature on liver transplant recipients (Krishnamoorthy *et al.*, 2016).

1.5.4 Concurrent immune diseases

AIH is associated with a variety of other autoimmune diseases in 30-50% of patients. These include thyroiditis, PBC, PSC, inflammatory bowel disease, coeliac disease, rheumatoid arthritis, mixed connective tissue disease, Sjogren's, SLE, fibrosing alveolitis, glomerulonephritis, thrombocytopenia, haemolytic anaemia, diabetes, psoriasis, vitiligo, uveitis, polymyositis, multiple sclerosis, mononeuritis multiplex, antiphospholipid syndrome (Werner *et al.*, 2008; Gronbaek, Vilstrup and Jepsen, 2014; Muratori *et al.*, 2009; Zachou *et al.*, 2011; Boberg *et al.*, 2011; Lewin *et al.*, 2009; Panetta *et al.*, 2012; Teufel *et al.*, 2010; Muratori *et al.*, 2016a; Wong *et al.*, 2017). Thyroiditis occurs at the highest prevalence of 10-23%. A recent retrospective Chinese study of autoimmune liver disease (total = 324, AIH=143) found the highest incidence of autoimmune-thyroid disease in the AIH patients (45.8%) followed by AIH-PBC overlap syndrome (39.5%) and PBC (22.6%) autoimmune liver diseases (Zeng *et al.*, 2020).

1.5.5 Variation in presentation according to ethnicity/race

AIH can also vary in presentation according to ethnicity and race. Some of this may be due to more complex socioeconomic reasons which includes differences in access to health care and delay in diagnosis. Alaskan patients tend to present with acute jaundice. African-American patients have been reported to present with higher prevalence of cirrhosis and liver failure at a younger age compared to those of Northern European descent (Verma, Torbenson and Thuluvath, 2007). A study looking into non-Caucasian patients (n=12) found more cholestatic biochemical and histological features with higher rate of non-response to therapy compared to their European-Caucasian population of AIH patients which was not explained by differences in access to medical care (Zolfino *et al.*, 2002; Gleeson, Heneghan and British Society of, 2011). A study compared a population of patients with AIH (n=115) from Brazil to a Northern American patient population (n=161) and found that patients from Brazil had earlier disease onset with higher serum levels of AST and gamma-globulin (Czaja *et al.*, 2002). Japanese patients on the other hand have later disease onset that responds to less potent immunosuppression (Nakamura *et al.*, 1998; Wong *et al.*, 2012). In a retrospective study (n=183 with AIH), Hispanics (55%) were noted to have higher prevalence of cirrhosis whilst Asians had poorer survival outcomes. Genetic differences are thought to play a contributory role in to variations in disease severity, therapy response and mortality (Wong *et al.*, 2012).

Summary points

- AIH is a heterogeneous disease with a wide spectrum of presentations:
    - Chronic abnormal LFTs with non-specific symptoms or asymptomatic
    - Decompensated cirrhosis (ascites, variceal bleeding)
    - Acute severe hepatitis which can progress to acute liver failure
- Other AIH manifestations – AIH with overlap syndrome with PBC or PSC; and recurrence or de-novo AIH after liver transplantation.
- AIH is associated with a variety of other autoimmune diseases in 30-50% of patients (thyroiditis occurs at the highest prevalence at 10-23%).
- AIH presentation can vary with ethnicity and race – African American patients have a higher prevalence of cirrhosis and liver failure at a younger age compared to Northern European descent; Brazilian patients had earlier disease onset with higher serum AST

and gamma-globulin levels compared to North Americans whilst Japanese patients have later disease onset and responds to less potent immunosuppressants.

1.6 Diagnosis/Diagnostic Scores

The diagnosis of AIH can be challenging due to its heterogeneity. At present, it relies on a combination of biochemical (raised ALT or AST enzymes), immunological (raised serum IgG, raised titres of liver autoantibodies (≥1:40 except in children where lower titres are accepted) and histological features with exclusion of other liver diseases. The International Autoimmune Hepatitis Group (IAIHG) diagnostic criteria and scoring system was developed by the IAIHG, a group of experts in the field of autoimmune liver disease in 1993 (Johnson and McFarlane, 1993). This scoring system was initially developed as a research tool to standardise practice and allow comparison between patients in trials of AIH. This criteria was widely accepted and gradually integrated into clinical practice. It was then revised in 1999 (Alvarez *et al.*, 1999a) and then simplified in 2008 to ease clinical use in routine practice (Hennes *et al.*, 2008b). **Tables 2** and **3** show the Comprehensive 1999 IAIHG criteria and the Simplified 2008 IAIHG criteria respectively. The components of the IAIHG scoring criteria are not exclusive to AIH in isolation but through the combination of clinical, biochemical and histological features together with its response to corticosteroid treatment (the latter not being included in the Simplified criteria), the patients are scored into 'definite' and 'probable' AIH.

The scores can be applied pre-treatment and post-treatment. Using the revised 1999 criteria, a pre-treatment score of 10 points or more or a post-treatment score of 12 points or more indicate "probable" AIH with a sensitivity of 100%, specificity of 73% and diagnostic accuracy of 67% (Czaja, 2008a). The simplified criteria has been reported to have a sensitivity of >80% and a specificity of >95% (Yeoman *et al.*, 2009; Gatselis *et al.*, 2010). Both systems are not fool proof and can result in exclusion of atypical cases or failure to diagnose AIH in patients with concurrent autoimmune or non-autoimmune liver diseases (particularly more so with the simplified criteria - 5% in one study) (Czaja, 2008a). The revised system may be more useful in evaluation of 'difficult' cases with atypical features of AIH (Gatselis *et al.*, 2010).

These scoring systems (revised original and simplified) have their limitations which include the lack of validation by prospective studies, insufficient accuracy in the setting of concurrent

disease (PSC, PBC, NAFLD/NASH, liver transplantation or fulminant liver failure), non-inclusion of other serological markers such as anti-SLA and dependence on autoantibody determinations by indirect immunofluorescence (titres) rather than by enzyme-linked immunoassay (units) (Vergani *et al.*, 2004; Mack *et al.*, 2020).

Diagnostic scoring systems can be used to help make the diagnosis of AIH in challenging cases but are ultimately useful in defining clinical cohorts for AIH research studies.

Table 2: Revised Diagnostic criteria for diagnosis of AIH

[Adapted from (Alvarez *et al.*, 1999a; Czaja, 2016)]

Clinical features	Points	Clinical features	Points
Female	+2	**Average alcohol intake**	
		< 25g/day	+2
		>60g/day	-2
ALP: AST (or ALT) ratio		**Histologic findings**	
<1.5	+2	Interface hepatitis	+3
1.5-3.0	0	Lymphoplasmacytic infiltrate	+1
>3.0	-2	Rosette formation	+1
		Biliary changes	-3
		Other atypical changes	-3
		None of above	-5
Serum Globulin or IgG above ULN		**Concurrent immune disease, including coeliac disease**	+2
>2.0	+3		
1.5-2.0	+2		
1.0-1.5	+1		
<1.0	0	**Other autoantibodies**	+2
ANA, SMA or anti-LKM1		**HLA DRB1*03 or DRB1*04**	+1
>1:80	+3		
1:80	+2		
1:40	+1		
<1:40	0		
AMA positive	-4	**Response to corticosteroids**	
		Complete	+2
		Relapse after drug withdrawal	+3
Hepatitis markers		Hepatotoxic drug exposure	
Positive	-3	**Positive**	-4
Negative	+3	**Negative**	+1
Aggregate score pre-treatment		**Aggregate score post treatment**	
Definite autoimmune hepatitis	>15	Definite autoimmune hepatitis	>17
Probable autoimmune hepatitis	10-15	Probable autoimmune hepatitis	12-17

ALP, alkaline phosphatase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; IgG, immunoglobulin G; ULN, upper limit of the normal range; HLA, human leukocyte antigen; ANA, antinuclear antibodies; SMA, smooth muscle antibodies; anti-LKM1, antibodies to liver kidney microsome type 1; AMA, antimitochondrial antibodies.

Table 3: Simplified diagnostic criteria for the diagnosis of AIH

[adapted from (Hennes *et al.*, 2008b)]

Feature	Score
ANA or SMA + ≥1:40	+1
ANA or SMA + ≥1:80 Or LKM+ ≥1:40 Or SLA + any titre	+2*
IgG > ULN	+1
IgG >1.1x ULN	+2
Liver histology	
Compatible with AIH	+1
Typical of AIH	+2
Absence of viral hepatitis	
No	0
Yes	+2
DIAGNOSIS:	
Probable AIH	Total score: ≥6 points
Definite AIH	Total score: ≥7 points

* Maximum of 2 points for all autoantibodies

1.6.1 Laboratory features

The increase in ALT or AST level was previously set at 3x the ULN for a diagnosis of AIH to be considered but this is no longer the case as the degree of ALT elevation does not reliably reflect severity of AIH at the histological level (Gleeson, Heneghan and British Society of, 2011; Johnson and McFarlane, 1993; Alvarez *et al.*, 1999a; European Association for the Study of the, 2015). The alkaline phosphatase (ALP) may be normal or slightly raised. A raised ALP 2x the ULN may point to the presence of a concurrent overlap syndrome. The ALT, AST, bilirubin and gamma-glutamyl transpeptidase can fluctuate in AIH (McFarlane, 2002). Two studies have shown that elevated ALT and gamma-GT levels but not ALP can be used as an independent predictor of treatment outcome (Muratori *et al.*, 2009; Zachou *et al.*, 2011).

Elevated levels of serum immunoglobulin G or gamma globulin are found in 85% of patients with AIH (Al-Chalabi *et al.*, 2006; Al-Chalabi *et al.*, 2008b; Floreani *et al.*, 2006). Up to 15% of

patients with type 1 AIH and 25% of patients with type 2 AIH have normal IgG levels (Czaja *et al.*, 1996).

1.6.2 Autoantibodies

Autoantibodies play an important role in the diagnosis and classification of AIH. These are however not specific to AIH and its use in clinical practice is fraught with challenges due to the lack of standardisation of the laboratory techniques used as well as varying quality of commercial substrates used for its detection (European Association for the Study of the, 2015).

AIH is divided into two subtypes based on the autoantibody profiles. Type 1 AIH is associated with positive antinuclear antibody (ANA) and anti-smooth muscle antibody (SMA) and makes up 75% of patients (Werner *et al.*, 2008; van Gerven *et al.*, 2014; Muratori *et al.*, 2009). Type 2 AIH is associated with positive anti-liver kidney microsomal-1 (LKM-1) antibody or anti-liver cytosolic-1 antibody (LC-1). In an Italian series comprising of 38 patients with type 2 AIH, 66% had anti-LKM-1 and 53% had anti-LC-1 levels (Muratori *et al.*, 2009). The features of these two subtypes of AIH can be summarised in **Table 4**. Antibodies to soluble liver antigen (SLA) or Liver-Pancreas antigen (LP) are found in 10-30% of patients with AIH (Wies *et al.*, 2000). Antibodies to perinuclear antineutrophil cytoplasmic antibody (p-ANCA) can also be found, and on rare occasion in isolation (Krawitt, 2006). Previously, there was a third subtype of AIH associated with anti-SLA or LP antibodies but this remains controversial as their histological features are similar to type 1 AIH and the clinical course and response to corticosteroids do not differ much from type 1.

Table 4: Classification of AIH according to autoantibody type

[adapted from (Gleeson, Heneghan and British Society of, 2011; Krawitt, 2006)

Feature	Type 1 AIH	Type 2 AIH
Characteristic autoantibodies	ANA ASMA Anti-actin antibody Anti-SLA/LP antibodies Atypical p-ANCA	Anti-LKM-1 Anti-LC1
Geographic variation	Worldwide	Worldwide
Age at presentation	Any age	Predominantly childhood and young adulthood
Sex (Female: Male)	3:1	10:1
Clinical severity	Broad range	Generally severe
Histopathological features at presentation	Broad range: mild disease to cirrhosis	Generally advanced, increased inflammation or cirrhosis
Treatment failure	Infrequent	Frequent
Relapse after drug withdrawal	Variable	Common
Need for long-term maintenance	Variable	Approximately 100%
Association with other autoimmune disorders	Common	Common†

p-ANCA – perinuclear antineutrophil cytoplasmic antibody
†autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (Obermayer–Straub *et al.*, 2001)

Autoantibody titres of 1:40 to 1:80 are generally accepted as positive, depending the assay used whereas in children, lower titres (1:20 for ANA and SMA and 1:10 for a-LKM1) are clinically significant. ANA and SMA are conventionally detected using the immunofluorescence method whilst SLA and LP antibodies are detected using enzyme-linked immunosorbent assay (ELISA). Anti-actin antibodies can also be present in type 1 AIH although tests for this antibody are rarely available In commercial laboratories (Krawitt, 2006).

The fluorescence staining pattern for ANA in AIH is usually homogenous using Hep2 cells (similar to systemic lupus erythematosus). Speckled and nuclear work patterns can also be seen in AIH although these are not specific to AIH and can be seen in PBC (Czaja *et al.*, 1997).

15% of patients have antibodies to double-stranded DNA. There have been no specific patterns in isolation or combination for ANA that are specific to AIH (Czaja, 1999).

The SMA is detected in the arterial walls of rodent kidney, liver and stomach via immunofluorescent staining. The SMA can have three patterns (detected in the kidney):

- V (vessels)
- G (glomeruli) and
- T (tubules)

The VG and VGT patterns are frequently associated with AIH whilst the V pattern is also found in viral liver disease and extrahepatic autoimmune diseases (Bottazzo *et al.*, 1976; Manns, Lohse and Vergani, 2015; Vergani *et al.*, 2004).

Autoantibody titre levels do not always establish or exclude the diagnosis respectively as the titre levels can fluctuate in the disease course. The titre levels only correlate 'roughly' with disease activity therefore the levels do not need to be monitored regularly (Czaja, 1999; European Association for the Study of the, 2015). In the paediatric population, one small study by Gregorio et al investigated 19 patients prospectively and found positive correlation between AST and LKM-1 titres (in type 2 AIH) and SMA titres (in type 1 AIH) respectively, suggesting autoantibodies may be used to monitor disease activity in paediatric AIH (Gregorio *et al.*, 2002). A separate study (which included paediatric patients, median age 10) by Muratori et al found a correlation between anti-LC1 antibodies with disease activity (significant reduction in titre, or disappearance during remission and increase during disease relapse) (Muratori *et al.*, 1998). As these were small studies, unsurprisingly, recent American practice guidance have stated that autoantibodies are not established biomarkers of disease activity or treatment outcome at present (Mack *et al.*, 2020).

1.6.3 Liver histology

Liver histology is required for the diagnosis of AIH as biochemical and current immunological blood tests are insufficiently specific for a definite diagnosis of AIH. This was required by the IAIHG in both the revised and simplified criteria. At least 20% of patients with biopsy-proven non-alcohol fatty liver disease meet the criteria for probable diagnosis of AIH prior to liver biopsy (Adams, Lindor and Angulo, 2004) therefore liver biopsy remains a recommendation

for diagnosing AIH unless there are significant contraindications or the patient has severe co-morbidities. In addition, it plays a vital role for the differential diagnosis of AIH as it may identify alternative or concurrent aetiology for the underlying liver tissue injury. Liver histology is also useful in guiding management of AIH as it provides information on the grade of inflammatory activity and staging of fibrosis which will help guide treatment decisions, assess treatment response and provide information on prognosis (Krawitt, 2006; European Association for the Study of the, 2015; Tiniakos, Brain and Bury, 2015).

AIH is classically characterised by chronic hepatitis - portal mononuclear-cell infiltrate extending into the lobule (interface hepatitis, originally called piece-meal necrosis) is a typical feature of AIH. This occurs in 84-98% of patients with AIH (Al-Chalabi *et al.*, 2006; Al-Chalabi *et al.*, 2008b; de Boer *et al.*, 2015; Gurung *et al.*, 2018; Kumari *et al.*, 2013) but can also be seen in drug-related, viral or immune-mediated disease (Gleeson, Heneghan and British Society of, 2011; Gurung *et al.*, 2018). The three features of lymphoplasmacytic infiltrates, hepatocyte rosettes (small groups of hepatocytes arranged around a small, sometimes not visible central lumen) and emperipolesis (i.e. endocytosed lymphocytes within hepatocytes) are typically found in AIH although these are not specific to AIH. If all three features are present, this is considered “typical” for AIH and is given a score of +2 in the simplified scoring system whilst a score of +1 would be given if not all three features were present (i.e. “compatible” for AIH, see Section 1.6, Table 3) (Hennes *et al.*, 2008b). There is usually a predominance of plasma cells in the portal infiltrate but these are present only in 2/3 of biopsies and their absence does not preclude the diagnosis (Tiniakos, Brain and Bury, 2015). Emperipolesis and rosettes are features which are not consistently reported amongst histopathologists. Morphological interpretation has also not been provided in the simplified IAIHG criteria. Several studies evaluating the histological features of AIH in comparison with either acute non-immune hepatitis, cholestatic liver disease or drug-induced liver injury suggest that emperipolesis or rosettes are not particularly specific for AIH (de Boer *et al.*, 2015; Suzuki *et al.*, 2011; Balitzer *et al.*, 2017). This has led to several modified histological scoring systems being described – Balitzer et al used the inflammatory activity, extent of plasma cells and results of copper/CK7 staining and found this increased the number of cases diagnosed with probable/definite AIH by 17% (these cases would have otherwise been diagnosed as non-AIH by the simplified score). In other words, by calculating the total simplied score (>6) using the modified histologic criteria, there was an increase of AIH cases from 69% to 86%. This

modified histologic criteria also facilitated the diagnosis of AIH in those with features of acute hepatitis which is not fully covered by the simplified criteria (Balitzer *et al.*, 2017). Gurung et al developed a different modified criteria, requiring the presence of both prominent plasma cells (plasma cells comprise ≥20% of inflammatory cells or presence of plasma cell clusters) and Kupffer cell hyaline globules. Application of this scoring system in their study population increased the specifity of the simplified diagnostic criteria by 67% (from 0%) but reduced the sensitivity to 77% (from 100%). The study used a comparison group with chronic hepatitis C. The authors concluded that further studies using different control groups were needed for further validation (Gurung *et al.*, 2018).

Other findings such as eosinophils, lobular inflammation, bridging necrosis and multiacinar necrosis may be present (Manns *et al.*, 2010a; Manns, Lohse and Vergani, 2015). Biliary features such as interlobular bile duct destruction (seen in 12% of AIH biopsies) and lymphocytic infiltration of bile duct epithelium may be present and are not diagnostic for 'overlap' with PBC not does it preclude a diagnosis of AIH. Poorly defined granulomas have been seen in 9-11% of AIH cases but only 4.8% of granulomatous hepatitis cases are aetiologically related to AIH with the most common aetiology being PBC (23.8%) (Tiniakos, Brain and Bury, 2015; Gaya *et al.*, 2003). Fibrosis is usually present at time of diagnosis in varying stages and about one third of patients have established histological cirrhosis (Czaja and Carpenter, 1993; European Association for the Study of the, 2015).

In patients presenting with acute severe AIH, the centrilobular zone tends to be more affected histologically. Centrilobular necrosis may be a precursor to portal involvement and an indicator of disease severity. This finding however is not a specific finding for acute-severe AIH and can also be found in chronic AIH or acute-on-chronic disease. It may be a useful finding for diagnosis of new acute-onset AIH if it is present without portal involvement. Massive hepatic necrosis is a common finding (up to 45%) in patients with acute severe AIH and AIH-acute-liver failure. This however can be found in acute liver failure due to viral infection and other aetiologies. Central perivenulitis (lymphoplasmacytic infiltration surrounding the central vein) is an important feature in acute AIH. This can be found along with centrilobular necrosis, lymphoid aggregates and plasma cell-enriched infiltration (Rahim, Miquel and Heneghan, 2020; Yeoman *et al.*, 2011; Abe *et al.*, 2012).

Summary points

- The diagnosis of AIH can be challenging due to its heterogeneity.
- Diagnosis of AIH is based on biochemical (raised ALT or AST enzymes), immunological (raised serum IgG, raised titres of liver autoantibodies – at least 1:40 - ANA, SMA, anti-LKM-1, anti-LP, SLA) and histological features (typical features: lymphoplasmacytic infiltrates, hepatocyte rosettes and emperipolesis) with exclusion of other liver diseases.
- Two IAIHG diagnostic criteria and scoring systems are used – the Revised original IAIHG criteria (1999) and the Simplified criteria (2008) – these combine biochemical, histological features together with response to corticosteroid treatment to score 'definite' or 'probable' AIH and can be applied pre and post treatment. These can be helpful in difficult cases but are more useful in defining clinical cohorts for AIH studies.
- AIH can be divided into two subtypes based on autoantibody profile: Type 1 AIH (positive ANA and SMA) and type 2 AIH (Anti-LKM-1 or anti-LC-1).

1.7 Management Of AIH And Disease Course

Treatment of AIH consists of immunosuppressive therapy. Most patients require lifelong maintenance therapy and only a minority of patients are able to maintain remission after treatment withdrawal. The goals in managing AIH are as follows:

i) Achieve complete biochemical and histological remission
ii) Prevent or minimise the risk of disease relapse
iii) Prevent progression of liver disease to death or liver transplantation
iv) Reduce or minimise risk of side effects of treatment

In order to understand the need for treatment of AIH, elaboration on the natural history of untreated AIH is required.

1.7.1. Natural history of AIH without treatment

Information on the natural history of AIH without treatment was derived from published experiences prior to the regular use of immunosuppressive treatments and from the placebo-

arms of the early randomised controlled trials (RCTs) (Mistilis, Skyring and Blackburn, 1968; Cook, Mulligan and Sherlock, 1971; Soloway *et al.*, 1972; Murray-Lyon, Stern and Williams, 1973; De Groote, Fevery and Lepoutre, 1978). The caveat for this information is that this precedes the discovery of hepatitis C virus.

Patients who were diagnosed with 'active chronic hepatitis' (a historical term used for AIH) had a quoted average life expectancy of less than five years (Bearn, Kunkel and Slater, 1956; Mackay and Wood, 1962; MacLachlan *et al.*, 1965). In the RCTs from the 1970s evaluating prednis(ol)one with or without azathioprine in patients with moderate to severe AIH (AST >10x ULN or AST >5x ULN with gamma-globulin levels >2x ULN, confluent necrosis on liver biopsy) patients who were untreated on the placebo arm had very poor prognosis and significantly higher mortality (41-56%) compared to those who were treated (6-15%) (Cook, Mulligan and Sherlock, 1971; Soloway *et al.*, 1972; Summerskill *et al.*, 1975). If the patient survived the early active phase, he or she frequently developed cirrhosis, oesophageal varices and subsequent haemorrhage and may die from complications of cirrhosis many years later (Bartholomew *et al.*, 1960; Read, Sherlock and Harrison, 1963; Cook, Mulligan and Sherlock, 1971; Mistilis, Skyring and Blackburn, 1968; Soloway *et al.*, 1972; Murray-Lyon, Stern and Williams, 1973; Schalm *et al.*, 1977).

The natural history of untreated AIH (as derived from the first randomised controlled trial evaluating prednisolone alone versus placebo (Cook, Mulligan and Sherlock, 1971) with 27 patients in the placebo arm) was as follows:

- Non-cirrhotic patients – 5/27 (19%) mortality at two years without treatment
- Cirrhotic patients – 10/27 (37%) mortality at two years without treatment

The combined mortality was 15/27 (56%) for both non-cirrhotic and cirrhotic patients at two years without treatment. The trial was discontinued as it was considered unjustifiable on ethical grounds.

From second randomised controlled trial evaluating induction therapy: prednisone alone, prednisone with azathioprine combination, azathioprine alone and placebo (with 17 patients in the placebo arm, 8 with biopsy-proven cirrhosis) (Soloway *et al.*, 1972), the natural history of untreated AIH was as follows:

- 7/17 (41%) of patients (non-cirrhotic and cirrhotic) with untreated severe disease died within 6 months of diagnosis.

All cirrhotic patients who died were reported to die of hepatic failure. On follow-up, cirrhosis developed in 4/31 (13%) patients in the placebo or azathioprine arm.

Summerskill and colleagues compared the the effects of prednisone given on alternate days in doses titrated by a standard mechanism to achieve resolution of symptoms, abnormal LFTs and histological features of disease activity with those of treatments earlier used (Prednisone 15mg/day or Prednisone 10mg with azathioprine 50mg) (Summerskill *et al.*, 1975). The control arm was a combination of placebo and azathioprine alone and this similarly showed a 41% (12/29) mortality.

The three trials above are elaborated in further detail in *Section 1.7.2*. These early landmark trials in AIH showed corticosteroids with or without azathioprine improves survival dramatically.

Therefore, following on from these trials, patients who fit the criteria of moderate to severe hepatitis should be offered immunosuppressive therapy due to the clear survival benefits. The EASL AIH guidelines recommend that treatment should be given to all patients with active disease (with hepatitis activity index (HAI) of ≥4/18) or advanced fibrosis or cirrhosis (European Association for the Study of the, 2015).

The decision to treat patients with no or only mild symptoms who have mild disease - no or only mild laboratory and histological findings (i.e. ALT <3x ULN and mild activity: HAI <4/18, with no advanced fibrosis) can be more controversial due to the natural history being uncertain. There are no prospective randomized controlled treatment trials for these patients. One uncontrolled study reported the 10-year survival of asymptomatic patients (majority of whom had mild disease and half were not treated) being 80% and no different from the treated patients [83.8%, p = not significant (ns)] (Feld *et al.*, 2005). Conversely, another uncontrolled study by Czaja and colleagues evaluated their cohort of 282 patients with AIH of which 21 patients were untreated. All 21 patients were classed retrospectively as having non-severe disease (i.e. no features of the following: AST >10x ULN or AST >5xULN and serum gamma-globulin levels >2x ULN or histological features of bridging necrosis or multi-acinar necrosis). 8 patients had a follow-up data (mean: 77 +/- 31 months). Untreated patients had

a lower cumulative 10-year survival at 67% compared to 98% in the treated patients (p=0.01) with the survival difference occurring mainly within 2 years after presentation (Czaja, 2009a).

The decision to start treatment in patients with mild AIH disease should therefore be individualised and be based on the patient's age, co-morbidity, patient-preference and serology. The clinician also needs to weigh the benefits of achieving disease remission and preventing disease progression and symptom-development with the risks of treatment-related side-effects (European Association for the Study of the, 2015). If a patient is to be untreated, close follow-up is required as AIH is a fluctuating disease with Feld et al's study reporting 25% of asymptomatic patients developing subsequent symptoms (Feld *et al.*, 2005) and untreated patients have the potential of developing HCC or end-stage liver disease with liver failure (Czaja, 2009a).

The natural history of treated AIH is further elaborated in Section 1.8 (Long-term Outcomes).

1.7.2 Remission Induction Treatment with Prednis(ol)one and Azathioprine

The first line treatment of AIH is prednisolone (or prednisone) with or without azathioprine (AZA). The evidence for this originated from three prospective randomised controlled trials conducted between 1960 to 1980 (see **Table 5**) (Cook, Mulligan and Sherlock, 1971; Soloway *et al.*, 1972; Murray-Lyon, Stern and Williams, 1973; Kirk *et al.*, 1980). These trials showed that prednisolone alone or in combination with AZA in severe cases of AIH improved survival, symptoms, laboratory tests and histological findings. It is important to note that these trials were conducted decades before the discovery of the hepatitis C virus (HCV) therefore some of the patients in these trials can be assumed to have had HCV infection. Nevertheless, AIH became the first liver disease in which medical treatment improved survival.

In the first prospective randomised controlled trial conducted in the Royal Free Hospital, London (Cook, Mulligan and Sherlock, 1971), prednisolone 15mg was compared to no treatment/placebo (n=22 and 27 respectively) in patients with 'active chronic hepatitis' (a historical term for AIH) over a duration of 30-72 months. The follow-up duration varied from 2-6 years. The inclusion criteria included biochemical criteria ('hepatocellular jaundice' or raised transaminases, hypergammaglobulinaemia and chronicity more than 3 months) as well as histological criteria (evidence of activity, piecemeal necrosis and cellular infiltration especially with plasma cells). Other causes such as alcohol, viruses, Wilson's and drugs had to

be excluded and the patients were corticosteroid and ACTH naïve. At the end of two years, there was a significant difference in standardised mortality ($p<0.01$) of the placebo group (51.2%) compared to the prednisolone group (15.2%). In addition, prednisolone significantly improved some of the LFTs and biochemistry (bilirubin, total globulin and albumin) compared to placebo. The study concluded that prednisolone, used in the early active phase of the disease (first 2-3 years) increased life expectancy. As the life expectancy benefits were starkly clear with treatment, the trial was discontinued as it was considered unjustifiable on ethical grounds to proceed. This study became a landmark study as it was the first to show that therapy reduced mortality in chronic liver disease.

Another trial in patients with AIH (then referred to as "active chronic hepatitis") was conducted as a controlled prospective double blind trial by King's College Hospital, London (Murray-Lyon, Stern and Williams, 1973). The study compared the effect of prednisone 5mg three times a day (n=22) to AZA 75mg/day (n=25). If patients were seen soon after presentation and were steroid and AZA naïve, they would receive prednisone 30mg/day or AZA 112.5mg/day, this being reduced after four weeks to maintenance levels. At the end of two years, two patients were removed from the trial – one due to failure to control the disease and the other due to crush fractures of the thoracic vertebrae. The probability for survival was calculated for each group at 6, 12, 18 and 24 months, making allowance for the number of cases remaining in the trial at these times. Survival on prednisone was considerably greater than survival on AZA but the statistical assessment of significance was an approximation because the data was not normally distributed ($p\approx0.5$). There was 1 death in the Prednisone group as opposed to 6 deaths in the AZA group. In the first 6 months, there was significant reduction in AST, ALP ($p<0.05$) and gamma globulin ($p<0.02$) in the Prednisone group but not in the AZA group. The gamma globulin remained significantly lower ($p<0.01$) in the prednisone group compared to AZA group. 5/13 with radiologically proven oesophageal varices on admission to the trial bled during the 2 years. All 5 were in the AZA group and 3 died as a result. Varices developed in 7/13 of the AZA group as opposed to 1/12 in the Prednisone group ($p<0.05$), suggesting that the underlying liver disease continued to progress. With regards to side effects, there were 11 patients who developed facial mooning, 3 who developed diabetes mellitus and 2 who developed hypertension in the Prednisone group (approximately 32% of the patients). In the AZA group, 3 patients had to be withdrawn due to failure to control the disease and one other due to lack of compliance. The study found that prednisone 15mg/day

was more effective than AZA 75mg/day for increasing life expectancy of patients with chronic active hepatitis with or without cirrhosis. The study concluded that prednisone was superior at improving liver function, preventing the development of oesophageal varices and prolonging survival. However, the side effects with prednisone were troublesome and sometimes serious (Murray-Lyon, Stern and Williams, 1973). Azathioprine monotherapy should therefore not be used as induction therapy in new presentation of AIH.

The Mayo Clinic group (USA) conducted a prospective double-blind randomised controlled trial comparing the effects of prednisone alone, combination doses of prednisone and AZA, AZA only and placebo on the course and prognosis of severe "chronic active liver disease" (another historical term used to describe AIH), using predefined criteria for diagnosis and response. The four arms of treatment were as follows:

i. Prednisone 20mg only [Induction therapy: Prednisolong 60mg/day for 1 week, 40 mg/day for 1 week, 30mg/day for 2 weeks then 20 mg/day maintenance]
ii. Combination Prednisone and AZA [Induction: Prednisolone 30mg/day for 1 week, 20mg/day for 1 week, 15mg/day for 2 weeks then 10mg/day maintenance AZA 50mg]
iii. AZA 100mg/day alone
iv. Placebo

The study found the overall response of patients receiving prednisone alone or combination therapy were significantly superior to those receiving AZA or placebo ($p < 0.05$). Clinical and biochemical and histological resolution ($p < 0.01$) at 6 months was better in Prednisone and Combination group compared to the other 2 groups. The study found that 80% of patients on either prednisone or prednisone plus AZA achieved a serum ALT of less than twice the ULN within 6 months. Treatment failure was commoner with AZA or placebo (14/31) than with prednisone and combination, 2/32), $p < 0.05$. The mortality was 6% (prednisone group) and 7% (combination group) and was significantly different from 41% mortality in the placebo group ($p < 0.05$ and $p < 0.005$ respectively). Mortality was also high if AZA monotherapy was used as induction (36% mortality). The study concluded that prednisone alone or prednisone and AZA in combination improved life expectation, led to resolution of clinical and biochemical, immunochemical and histological abnormalities (Soloway *et al.*, 1972). Once again,

azathioprine monotherapy for induction therapy showed high mortality compared to prednisolone and should not be used in newly presenting AIH.

The same group conducted a follow-up study evaluating a fifth treatment strategy: Prednisone (Pred) given on alternate days in doses titrated by a standard mechanism compared to treatment regimens used earlier. Prednisone was given at similar induction - 60mg/day (1 week), 40mg/day (1 week), 30mg/day (2 weeks) followed by alternate day dosage after 3 months when necessary (10 mg increments of prednisone given to maintain serum transaminases at less than twice the ULN (within the limits of 10 - 50mg prednisone alternate days). The total number of patients in each arm were 30 (Pred), 30 (Pred + AZA), 31 (Pred titration) and 29 in the control group (Placebo [16] and AZA [13] combined). The mortality for the groups were as follows:

i. Pred 20mg od: 3/30, 10% mortality
ii. Pred 10mg + AZA 50mg (Combination): 2/30, 7% mortality
iii. Pred-Titrated alt days: 2/30, 7% mortality
iv. Placebo or AZA 100mg: 12/29, 41% mortality

Two-thirds of the patients in the Pred group had major side-effects attributable to steroids (gross cosmetic changes, diabetes, hypertension, cataracts, psychosis, duodenal ulcer, osteoporosis with aseptic necrosis of the hip or vertebral collapse). These complications were much less common with the Pred-Titrated or Combination ($p < 0.001$). With regards to AZA, two patients receiving combination developed severe skin rashes which resolved on cessation of AZA and reappeared on drug reintroduction. The study concluded that combination therapy (Pred + AZA) is the initial treatment of choice as it achieved similar resolution to Pred alone but with less side effects. Pred titration was less effective than Pred or Combination (Summerskill *et al.*, 1975).

A Danish study conducted a prospective, unblinded, randomised trial (over a period of 6 years) on the effect of AZA (10mg/kg/week for 2 weeks followed by 5mg/kg/week maintenance) versus prednisone (<70kg – 10mg/day; ≥70kg 15mg/day) in patients with AIH (defined as those with circulating autoantibodies and no HBV). 16% and 45% of the patients in the AZA group and prednisone group respectively achieved remission (defined biochemically as ALT and IgG <ULN), showing the superiority of prednisone monotherapy over azathioprine monotherapy

in remission induction. This study however did not find any differences in survival between the two regimens (mortality was 27% for the AZA group compared to 28% for the Prednisone group) (Tage-Jensen *et al.*, 1982).

The early trials for current established therapy are summarised in **Table 5**. The evidence has also been subject to a systematic review (Lamers *et al.*, 2010). These studies indicate that the best treatment regimens are the prednisolone/azathioprine combination regime which combines both maximum efficacy with minimal side effects (Gleeson, Heneghan and British Society of, 2011).

The British Society of Gastroenterology (BSG), European Association for the Study of the Liver (EASL) and American Association for the Study of Liver Diseases (AASLD) have produced guidelines with suggested starting doses of predniso(lo)ne, varying from 30mg/day to 60mg/day (Gleeson, Heneghan and British Society of, 2011; Mack *et al.*, 2020) or 1mg/kg (European Association for the Study of the, 2015).

A retrospective multicentre European cohort study recently found that dose of prednisolone at induction was less relevant than assumed. The study compared high-dose prednisolone ≥0.5mg/kg/day (n=281) versus low-dose prednisolone (<0.5mg/kg/day, n=170) to compare the efficacy of different dose ranges of predniso(lo)ne induction treatment. There was no significant difference in transaminase normalisation at 6 months (p=0.20) but there was significantly higher amount of prednisolone received over time in the high-dose group compared to the lower-dose group (3780mg versus 2573mg, median doses over 6 months; p<0.01). The study concluded that the initial predniso(lo)ne dose below 0.5mg/kg/day significantly decreases unnecessary exposure to predniso(lo)ne in AIH patients (Pape *et al.*, 2019).

AZA can be started (either at 50mg/day in USA, or 1mg/kg (UK) or 1-2mg/kg (European)) on induction with prednisolone, or on a more pragmatic approach, be started after a few weeks of prednisolone induction therapy. This delay in AZA institution, although not evidence-based, would help in cases where there was diagnostic uncertainty and also avoids the diagnostic dilemma of differentiating between AZA-induced hepatotoxicity and delay in primary response.

The treatment of AIH is guided by response with the treatment regimens tailored to the response of the patient and tolerance of treatment. Prednisolone is reduced gradually by 5-10mg per week according to the response. If AZA intolerance due to side-effects occur, AZA can be switched to second-line treatments such as mycophenolate mofetil (MMF) or the prednisolone dose doubled for a period. The current aim of therapy is to achieve normalisation of transaminases and IgG (within the normal limits).

The BSG guidance (2011) suggest keeping the patient on prednisolone (5-10mg/day) with azathioprine (1mg/kg) followed by a liver biopsy at 12-24 months (total of up to 30 months) to confirm histological remission before attempting steroid withdrawal. The European and American guidance however recommends a gradual taper of corticosteroids (with a trial of steroid withdrawal). Immunosuppression withdrawal can be considered after at least 24-36 months of biochemical remission, with a repeat liver biopsy being considered prior to this (Gleeson, Heneghan and British Society of, 2011; Mack *et al.*, 2020; European Association for the Study of the, 2015).

Table 5: Summary of early randomised trials for current established therapy in AIH [adapted from Lamers et al., 2010]

(Cook, Mulligan and Sherlock, 1971; Soloway *et al.*, 1972; Murray-Lyon, Stern and Williams, 1973; Summerskill *et al.*, 1975; Tage-Jensen *et al.*, 1982; Lamers *et al.*, 2010)

First author, journal, year	Inclusion	Exclusion	Intervention	Treatment duration	Patients	Remission (%)	Mortality (%)
Cook, Quarterly, Journal of Medicine, 1971	"Chronic active hepatitis" as defined by (1) & (2): (1) Biochemical: "hepatocellular jaundice" (raised transaminases) Hypergammaglobulinaemia Chronic: >3 months (2) Histological Evidence of activity; piecemeal necrosis and cellular infiltration, especially with plasma cells. (44 biopsy-proven before study; 3 during the study)	•Corticosteroid/ ACTH naïve •Excluded alcohol, viruses, drugs ***(Hep C undiscovered)**	• Prednisolone 15 mg/day • No intervention	30–72 months (2.5-6 years)	22 27	- -	14 56
Soloway, Gastroenterology, 1972	"Chronic active liver disease" as defined by clinical, biochemical, histological abnormalities: (1) Chronicity - >10 weeks (2) Activity – 10x ↑SGOT* or sustained 5x ↑SGOT with 2x ↑ Ɣ-globulin (3) Histology – moderate or severe piecemeal necrosis + associated inflammatory changes Post-pubertal patient (age 12 – 75)	Encephalopathy while taking 1g protein/kg of body weight, Alcohol liver disease, alcohol excess >2ounces/d, malignant disease (except skin)	•Prednisone 60 mg/day 1 week, 40 mg/day 1 week, 30 mg/day for 2 weeks, 20 mg/day maintenance •Prednisone 30 mg/day 1 week, 20 mg/day 1 week, 15 mg/day for 2 weeks, 10 mg/day maintenance + AZA 50 mg/day •AZA 100mg alone •Placebo	3 months to 3.5 years	18 14 14 17	44 21 7 0	6 7 36 41

First author, journal, year	Inclusion	Exclusion	Intervention	Treatment duration	Patients	Remission (%)	Mortal-ity (%)
Murray-Lyon, Lancet, 1973	"Active chronic hepatitis" as defined by: (1) At least 3 months of AST >100 (normal: 50 IU/L) and/or serum-gamma-globulin >1.5 g/ 100 ml (ULN: 1.3/100 mL) (2) Liver biopsy consistent with chronic aggressive hepatitis (De Groote *et al.*, 1968)	"Alcoholic" "Laxative abuser" Removed - PBC, extrahepatic obstruction	• Prednisone 5mg TDS • AZA 75mg/d	2 years	22 25	- -	5 24 (p≈0.05)
Summer-skill, Gut, 1975	Similar criteria used by Soloway et al (severe chronic active liver disease)	Similar criteria used by Soloway et al	• Prednisone 60 mg/day 1 week, 40 mg/day 1 week, 30 mg/day for 2 weeks, 20 mg/day maintenance • Prednisone 30 mg/day 1 week, 20 mg/day 1 week, 15 mg/day for 2 weeks, 10 mg/day maintenance + azathioprine 50 mg/day • Prednisone in titrated doses given on alternate days • Placebo/azathioprine 100 mg/day	36 months	30 30 31 29 (16/13)	37 53 10 0	10 7 7 41 (38/46)
Tage-Jensen, Liver, 1982	Biopsy-proven chronic compensated cirrhosis or chronic aggressive hepatitis	More than 50 g alcohol/day during last 5 years HBV, HAV, Wilson's, PBC, h'chrom and α1-antritypsin deficiency excluded	• Azathioprine 10 mg/kg/week, first 2 weeks 5 mg/kg/week • Prednisone <70 kg 10 mg/day, ≥70 kg 15 mg/day	38 (12-83) months	37 47	16 45	27 28

1.7.3 Budesonide as an alternative to prednisolone

Budesonide has been evaluated as an alternative to prednisolone in AIH. Budesonide is a synthetic second generation glucocorticoid with high hepatic first pass metabolism (90%). It is associated with low corticosteroid bioavailability and low steroid-specific side effects. Danielsson et al first evaluated budesonide use in a single-centre open label study in 13 AIH patients. The study found significant reduction in ALT and IgG at the end of the study period (9 months) and concluded that oral budesonide appeared to decrease liver inflammation in AIH with low frequency of steroid-associated side effects (Danielsson and Prytz, 1994). There have been 5 retrospective studies describing the use of budesonide in AIH (these are summarised in **Table 6**). There has only been one prospective randomised multicentre controlled trial to date, evaluating budesonide as a first line therapy for AIH (Manns *et al.*, 2010b). 208 patients with AIH were randomised to receive either budesonide (3mg three times/day for 2 weeks then titrated to response) or prednisone (40mg/day for 2 weeks then titrated down in a fixed-dose fashion – high-dose regimen and low-dose regimen, depending on response) over 6 months. This was in combination with AZA 1-2mg/kg/day. Patients who achieved biochemical remission at 3 months were eligible for an open-label arm of budesonide 3mg three times/day from Month 7. The patients either had a first diagnosis of acute AIH based on liver histology ≤3 months before screening or were experiencing relapse after diagnosis of AIH based on liver histology findings of ≤12 months before screening based on IAIHG criteria. The primary efficacy end point was complete biochemical remission (normal transaminase levels, AST and ALT) with absence of predefined steroid-specific side effects. Viral hepatitis was excluded rigorously, in contrast with early trials. Patients with cirrhosis were excluded. 47% of patients in the budesonide-treated group achieved the primary efficacy end-point compared to 18.4% of the prednisone-treated group. The complete biochemical remission rate for the first 6 months was 60% (budesonide) compared to 38.8% (prednisone) for intention to treat and this difference reached statistical difference with p=0.001. This significant difference remained when comparing per-protocol populations. A potential confounder for the results is that more males were enrolled in the budesonide group compared to the prednisone group ($p < 0.009$); additionally, a pre-planned subgroup analysis found that males responded better to budesonide than females ($p<0.001$). The limitations of the study include the lack of follow-up histological data to confirm remission and the relatively short blinded phase of the trial (6 months). The study was also criticized for the relatively

lower remission rates in the prednisone arm compared to earlier published case series. From the perspective of steroid-specific adverse effects, significantly more patients had no side effects in the budesonide group (72%) compared to the prednisone group (46.6%), $p<0.001$. (See *Section 1.7.5 Treatment related side effects* for more details on the budesonide and prednisone-related side effects). Budesonide with azathioprine remains an alternative therapy for non-cirrhotic patients who either are anticipated to experience or who experience significant steroid-related side effects from prednisolone.

Budesonide has been reviewed in a retrospective German study as second-line therapy in patients. 60 patients with AIH were reviewed – 30 (50%) were switched to budesonide due to prednisolone-related side-effects and the other portion were switched due to inability to reduce their prednisolone below acceptable levels. Biochemical response was 55% at 6 months, 70% at 12 months, 67% after 24 months. Budesonide-induced side-effects were mild but effectiveness was felt to be limited in a significant proportion of patients. Close follow-up was advised (Peiseler *et al.*, 2018).

Table 6: Studies on Budesonide Use as first-line therapy in AIH

(Delgado *et al.*, 2013; Efe *et al.*, 2012; Manns *et al.*, 2010b; Woynarowski *et al.*, 2013; Csepregi *et al.*, 2006; Wiegand *et al.*, 2005)

First author, journal, year	Study	Drugs	Treatment Duration, mean (range)	Patients	Treatment response	p value	Steroid specific side effects	p value
Delgado et al 2013	Multicentre, retrospective	Budesonide / AZA Prednisolone / AZA Budesonide Prednisolone	At least 24 months	11 71 10 6	64% CBR 55% 9% 17%	Not significant	Not reported specifically	NA
Efe et al, 2012	Multicentre, retrospective	Budesonide / AZA	10 months (2-24 months)	14[a]	71% BR	NA	Not reported specifically	NA
Manns et al, 2010	Multicentre, RCT	Budesonide / AZA Prednisone / AZA	6 months	102 105	60% BR 39% BR	<0.001	28% 53%	<0.001
Woynarowski et al, 2013	Multicentre RCT (Paediatric subcohort of Manns et al, 2010)	Budesonide / AZA Prednisone / AZA	6 months	19	32% 33%	Not significant	47% 63%	Not significant
Csepregi et al, 2006	Multicentre open label	Budesonide	8 months (1-12 months)	7[a] (3 with advanced fibrosis)	57% BR	NA	33% (overall)	NA
Wiegand et al, 2005	Multicentre open label	Budesonide	3 months	12	58% BR[b]	NA	Not reported specifically	NA

AIH, autoimmune hepatitis; BR, biochemical remission; CBR clinical and biochemical remission; RCT, randomised controlled trial, NA – not applicable
[a]Overlap syndromes excluded
[b]According to old AASLD definition of biochemical response: ALT <2x ULN

1.7.4 Alternative potential first-line therapies

Mycophenolate mofetile (MMF) is an ester prodrug of mycophenolic acid (MA) that inhibits inosine monophosphate – this prevents purine nucleotide synthesis leading to inhibition of T-cell and B-cell proliferation.

A study looked at MMF (1.5 - 2g/day) with prednisolone as first-line treatment for 59 treatment-naïve patients with AIH. The study was uncontrolled and non-randomised, conducted over a median duration of 26 months. 88% of patients showed initial response to therapy with normalisation of transaminases and gamma-globulins within 3 months. 37% of patients achieved complete response off prednisolone and two patients discontinued MMF due to severe side effects. The study only had follow-up histological data for 9 patients – and this showed that inflammation improved in all second and/or third biopsies; fibrosis improved in 3 patient while the remaining patients were stable (Zachou *et al.*, 2011).

This same group then reported on the long-term efficacy of MMF as first line-treatment of AIH. The group prospectively compared 109 patients on MMF and prednisolone to 22 on prednisolone and AZA. 93.6% had initial response to MMF within 2 (1-18) months with 71.6% achieving complete response (normal transaminases and IgG) on treatment and 78.2% maintaining remission off prednisolone. 75% remained in remission after 24 (2-129) months with remission maintenance being associated with longer MMF treatment ($p=0.005$), higher baseline ALT ($p<0.02$), lower IgG on 6 months ($p=0.004$) and histological improvement (Zachou *et al.*, 2016).

A meta-analysis (7 studies, 583 patients) compared MMF and prednisone to AZA and prednisone. The reported remission rates (defined as normal ALT, AST and IgG) were 33.33 - 86.67% and non-response rates were 15.15 - 66.67%. Remission rates of ALT ($p <0.05$, Cochran Q: $I^2 = 49\%$) and IgG level ($p<0.01$, $I^2 = 0$) were superior with MMF and prednisone. There was also a lower non-response rate (6.42% -33.33%, $p<0.01$, $I^2 = 90\%$) (Yu *et al.*, 2019). Recent AASLD guidance however deemed there was still insufficient data to recommend its first-line use (Mack *et al.*, 2020).

An ongoing multicentre randomised open-label intervention study is currently underway in Netherlands to compare MMF versus AZA as induction therapy together with corticosteroids in treatment-naïve AIH patients. (https://clinicaltrials.org ; NCT02900443)

Calcineurin inhibitors have been evaluated as first-line agents in AIH (Jiménez-Rivera *et al.*, 2015; Van Thiel *et al.*, 1995). The evidence for the use of ciclosporin (CsA) come mainly from studies in the paediatric population where this was used as a primary therapy in severe disease or to prevent steroid side effects. The case series report a biochemical response rate of 84-100% (Alvarez *et al.*, 1999b; Malekzadeh *et al.*, 2001; Sciveres *et al.*, 2004). Ciclosporin-A has been reported to induce biochemical remission – 75% complete remission rate in a paediatric study by Nastasio and colleagues (n=15 AIH, Follow-up: median: 8.6, range: 4-20.4 years) (Nastasio *et al.*, 2019). An older study evaluating the use of ciclosporin in AIH patients (n=84) showed a 94% remission rate (normalisation of transaminases). There were only mild, transient side-effects – hypertrichosis and moderate gingival hypertrophy occurred more frequently than the rest (Cuarterolo *et al.*, 2006). A preliminary study (n=21) in 1995 evaluated tacrolimus monotherapy as first line treatment and reported a reduction in serum AST and ALT by 70% and 80% respectively at 3 months (Van Thiel *et al.*, 1995). This was however not developed further. At present, calcineurin inhibitors are not recommended as first-line therapy in AIH.

1.7.5 Treatment-related Side Effects (corticosteroids and azathioprine)

Corticosteroid-related side effects include cosmetic changes (weight gain, Cushingoid features such as facial rounding and dorsal hump formation, striae, acne, alopecia and facial hirsutism), diabetes, psychosis, hypertension, cataracts, osteoporosis with vertebral collapse, pancreatitis, opportunistic infection and malignancy (Summerskill *et al.*, 1975; Manns *et al.*, 2010a; Wang and Czaja, 1989; Lamers *et al.*, 2010; Czaja, 2008b). In the early studies, diabetes occurred in 15-20% and osteoporotic vertebral collapse in 5-10%. Other adverse effects reported included perforated duodenal ulcer, haemetemesis, aseptic necrosis of hip, myositis and terminal bronchopneumonia. Cosmetic changes occurred in 80% of patients after two years of corticosteroid treatment. Severe complications usually occurred with prednisolone monotherapy (mainly at doses >20mg/day) and after more than 18 months duration. Treatment discontinuation due to these complications occurred in 13% of which 47% were due to intolerable cosmetic changes or obesity. Combination treatment with AZA and prednisolone is associated with lower frequency of corticosteroid-related adverse events (5%) (Cook, Mulligan and Sherlock, 1971; Soloway *et al.*, 1972; Murray-Lyon, Stern and Williams, 1973; Gleeson, Heneghan and British Society of, 2011). Subsequent studies with combination therapy report an approximate 30% prevalence of steroid-associated side-effects. After

withdrawal of prednisolone, Cushingoid facies have been reversed in 26/67 patients (39%) whilst 48% lost weight (Stellon *et al.*, 1988; Johnson and McFarlane, 1993).

A more recent report by the Dutch group evaluated 476 patients with AIH. 25% of patients experienced adverse events (cataract, diabetes or fractures) after the diagnosis of AIH. There was increased odds of fractures associated with low dose predniso(lo)ne (0.1-5.0mg/day) whilst higher doses (>5.0 mg/day) were associated with increased odds of cataracts and diabetes. Budesonide was also associated with increased odds of cataract and fractures, independent of predniso(lo)ne use in the previous 1-3 years. The study concluded that even low doses of corticosteroids were associated with significant adverse events, contrary to the widely-held perception that adverse events are prevented by low-dose administration (van den Brand *et al.*, 2019b).

Patients on long-term corticosteroids and risk factors for osteoporosis should have their bone-density measured with dual energy X-ray absorptiometry (DEXA) scans at the start of treatment and then at regular intervals (1-5 yearly depending on risk profile) (European Association for the Study of the, 2015). In a German study (n=211 AIH patients who underwent DEXA scan measurement), 15.6% had osteoporosis (with prevalence of osteoporosis in patients above 50 years being 19.2%) and 42.9% had osteopaenia. Independent risk factors for developing osteoporosis included older age (above 54 years), duration of corticosteroid use (>90 months), low body mass index (BMI) < 23 kg/m^2 and increased liver fibrosis (transient elastography >8kPa) (Schmidt *et al.*, 2020).

In a single-centred UK report (abstract form) evaluating 309 patients with AIH, 10% had diabetes mellitus at diagnosis of AIH. In the remaining 279 patients (254 (91%) on prednisolone therapy), new-onset diabetes mellitus occurred in 49 (19%). Independent risk factors for developing new onset diabetes mellitus were higher BMI at AIH diagnosis, Asian or African-Caribbean ethnicity, older age at commencing prednisolone treatment and initial dose of prednisolone >40mg/day. The authors concluded that high-dose prednisolone should be used with caution in AIH patients with risk factors for new onset diabetes melitus (Dixon *et al.*, 2019).

In the RCT of budesonide (n=102 on budesonide, n=105 on prednisone) (Manns *et al.*, 2010b), treatment-related adverse effects occurred in the following frequency in the budesonide / prednisone group:

- Weight gain (4.9% / 19.0%)
- Headache (11.8% / 7.6%)
- Mood alterations (9.8% / 7.6%)
- Muscular weakness (4.9% / 7.6%)
- Hypertension (2.9% / 6.7%)
- Insomnia (1.0% / 4.8%).

There was a significant difference between the groups at month 6 with budesonide having less steroid-specific side-effects compared to the prednisone group (intention to treat (ITT): 25.4%, lowest level of 97.5% Confidence interval (CI) at 12.3%, $p<0.001$). At month 12, there was a 40% reduction in the incidence of steroid-specific side-effects amongst those converted from prednisone to budesonide (44.8% at month 6 on entry, versus 26.4% at month 12, $p=0.002$). The incidence of pre-defined steroid-specific side-effects (in the first 6 months) were as follows:

- Moon face (10% / 42%, ITT)
- Acne (8% / 15%)
- Hirsutism (9% / 3%)
- Skin striae (2% / 4%)
- buffalo hump (1% / 4%)
- Diabetes (4% / 0%)

No patients developed increased intraocular pressure or glaucoma.

AZA-related side-effects and complications occur in about 25% of patients, leading to drug withdrawal in about 10% of patients (Johnson, McFarlane and Williams, 1995; Heneghan *et al.*, 2006). About 5% of patients develop early adverse reactions (nausea, vomiting, arthralgias, fever, skin rash or pancreatitis) (Bajaj *et al.*, 2005). Nausea and anorexia occur in about 10-20% of patients. Other side effects include bone marrow suppression, malignancy, skin rash, pancreatitis, arthralgia and opportunistic infection (Eland *et al.*, 1999; DePinho, Goldberg and Lefkowitch; Ben Ari *et al.*, 1995; Czaja and Carpenter, 2006b; Marcen *et al.*, 2003; Silman *et al.*, 1988). Further elaboration on the increased risk of malignancy and azathioprine is found in *Section 1.8.2*.

Patients on long-term azathioprine require regular blood-count monitoring (suggested frequency: 3-monthly) to monitor for bone marrow depression, although the risk for serious marrow depression reduces with time (Gleeson, Heneghan and British Society of, 2011).

1.7.6 Thiopurine metabolite measurement and thiopurine methyltransferase (TPMT)

Azathioprine breakdown products (thiopurine metabolites) in red-blood cells [6 thioguanine (6TGN) and 6-methyl mercaptopurine (6MMP)] have been used to predict drug toxicity, check drug adherence and predict disease response.

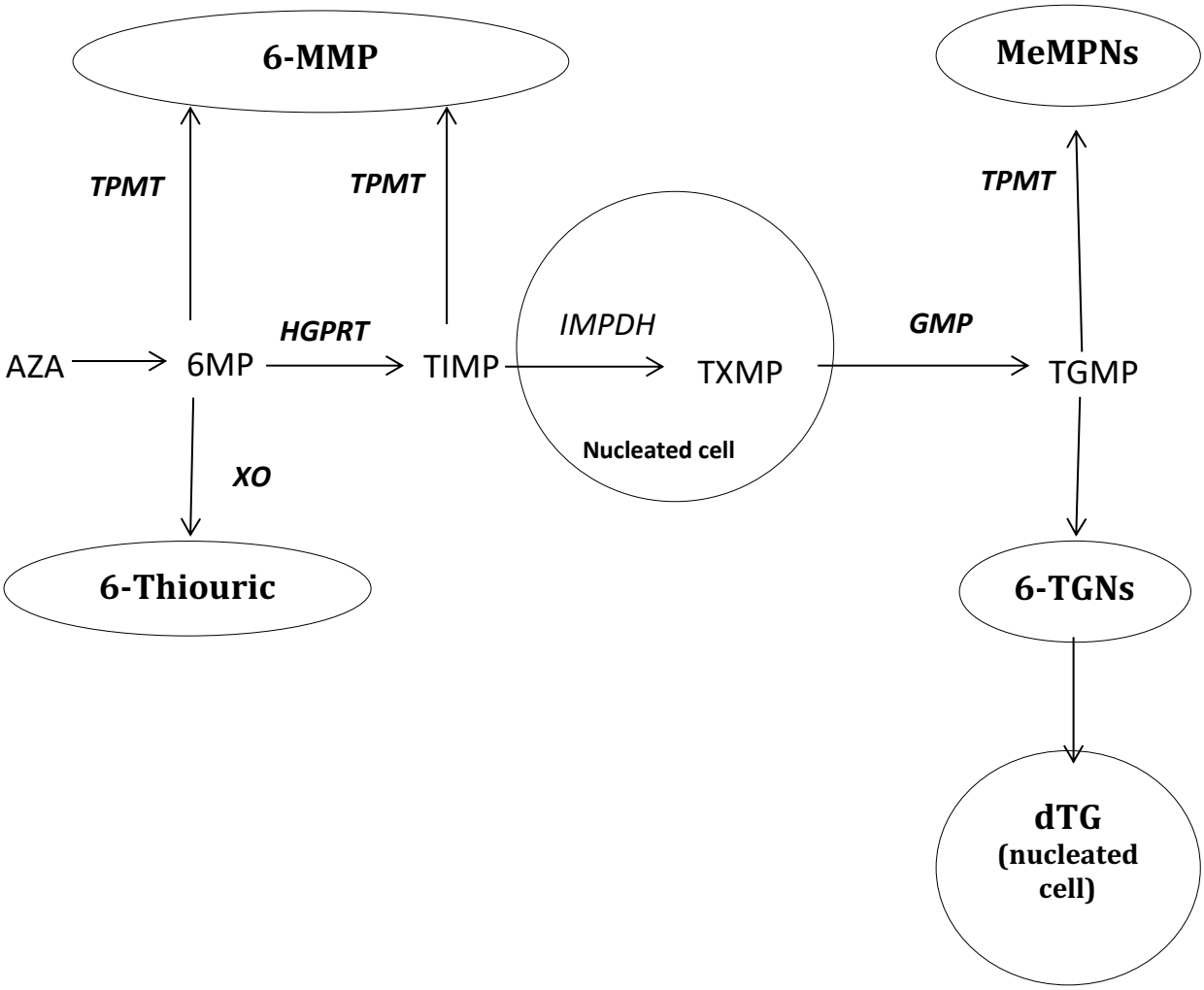
Thiopurine metabolism

Azathioprine, a pro-drug, once ingested is first converted to 6-Mercaptopurine (6MP) in a non-enzymatic manner (via conjugataion with glutathione). 6MP is then metabolised through three competing multi-enzymatic pathways via three enzymes - thiopurine methyltransferase (TPMT), hypoxanthine guanine phosphoribosyl transferase (HGPRT) and xanthine oxidase (XO) (see **Figure 1**) (Coulthard *et al.*, 2017; Haglund *et al.*, 2011; Coulthard and Hogarth, 2005; Lindqvist *et al.*, 2007; Lennard *et al.*, 1987).

i) 6MP can firstly be activated by HGPRT to thioinosine monophosphate (TIMP) followed by thioguanosine monophosphate (TGMP) in two steps - firstly thioxanthosine monophosphate (TXMP) is formed by inosine-monophosphate dehydrogenase (IMPDH). This key enzyme is only present in nucleated cells (Haglund *et al.*, 2011). TXMP is then converted to TGMP via guanosine monophosphate synthetase (GMPs). TGMP is then inactivated via TPMT to methyl mercaptopurine nucleotides (MeMPNs) (which inhibits de novo purine synthesis) or converted into 6-thioguanosine nucleotides (6-TGNs) which exerts its cytotoxic effects through incorporation into DNA of nucleated cells to cause DNA damage.
ii) Inactivation via TPMT to 6-methylated mercaptopurine (6-MMP) - a hepatotoxic metabolite in high concentrations
iii) Inactivation via XO to 6-thiouric acid (6TU)

6-thioguanine nucleotide (6-TGN) is the active metabolite, responsible for therapeutic cytotoxic and immunosuppressive effects through incorporation into DNA of nucleated cells to cause DNA damage. **Table 7** summarises the key metabolites in thiopurine metabolism.

Figure 1: Simplified azathioprine metabolism



Abbreviations:
AZA - azathioprine, 6MP - 6-mercaptopurine, 6-MMP - 6-methylated mercaptopurine, 6-TGN - 6 thioguanine nucleotide, dTG-deoxythioguanosine in DNA, GMPS - guanosine monophosphate synthetase, HGPRT - hypoxanthine guanine phosphoribosyl transferase, IMPDH - inosine monophosphate dehydrogenase, meTGMP - methylated thioguanosine monophosphate, MeMPN - methyl mercaptopurine nucleotides, TIMP - thioinosine monophosphate, TXMP - thioxanthosine monophosphate, TGMP - thioguanosine monophosphate, TPMT – thiopurine methyltransferase, XO - xanthine oxidase

Table 7: Key metabolites in Thiopurine Metabolism

	Metabolite	Effect	Pathway
1.	**6-thioguanine nucleotides (6-TGNs)**	Metabolite responsible for therapeutic effect and can cause myelosuppression at high levels	Activation pathway
2.	**6-methylated mercaptopurine (6-MMP)**	Possible hepatotoxic metabolite in high concentration	Inactivation
3.	**6-Thiouric acid (6-TU)**	Inactive metabolite	Inactivation
4.	**6-TGN-methylated metabolite (methyl mercaptopurine nucleotides [MeMPNs])**	Provides some inhibition of de-novo purine synthesis	6MP is converted to thioinosine monophosphate [TIMP] which can be inactivated by methylation

Thiopurine methyltransferase (TPMT) is a key enzyme in azathioprine metabolism. Azathioprine is converted to 6-mercaptopurine (6MP) which is then metabolised to either the active product, 6TGN [responsible for drug therapeutic effect] or 6-thiouric acid (6TU) and 6MMP [both the latter being inactivated products]. TPMT catalyses the conversion of 6MP to inactive products which includes 6MMP. Low TPMT activity leads to the risk of high-levels of 6TGN being produced which is associated with severe cytopaenia and bone marrow suppression. Low TPMT enzyme activity occurs in 1/300 of the general population (homozygosity for the low-activity gene allele). These patients experience serious bone marrow toxicity due to increased production of active 6-merceptopurine metabolites. 11% of people have intermediate TPMT activity as they are heterozygous for the low-activity allete. Measurement of TPMT enzyme activity (genotyping and phenotyping) is advised to exclude homozygous TPMT deficiency (Lennard *et al.*, 1990).

Studies in AIH patients on the utility of checking TPMT have shown variable results as neither intermediate TPMT enzyme activity or 6-mercaptopurine levels are reliable in predicting AZA response or toxicity. AZA intolerance or toxicity still occurred in patients who have normal or near normal TPMT levels (Heneghan *et al.*, 2006; Ferucci *et al.*, 2011; Dhaliwal *et al.*, 2012;

Nguyen *et al.*, 2010). This is thought to be due to variable enzyme activity, variable gene penetrance and alternative metabolism pathways (Mack *et al.*, 2020).

If treated with AZA doses of between 1-2.5mg/kg or 75mg/m^2, patients lacking TPMT activity can develop high 6TGN levels that can lead to life-threatening leukopenia or cytotoxicity (Lennard *et al.*, 1987; Lennard *et al.*, 1990; Lennard, Van Loon and Weinshilboum, 1989). The cytotoxicity is mediated by a variety of mechanisms, including inhibition of de-novo purine synthesis, disruption of G-protein signalling (Tiede *et al.*, 2003) and incorporation of thioguanine nucleotides (TGNs) into DNA with subsequent mismatching to thymidine, causing cell death by post-replicative mismatch repair (Karran and Attard, 2008; Lennard *et al.*, 1997; Waters and Swann, 1997).

Therapeutic drug monitoring using RBC metabolites (6-TGNs and 6-MMPs) was first used in childhood leukaemia (Lennard *et al.*, 1987) followed by inflammatory bowel disease and autoimmune hepatitis. Optimal thiopurine dose proved challenging due to drug intolerance from adverse reactions and treatment-resistant disease. This leads to up to a third of IBD patients discontinuing thiopurines and a quarter of AIH patients. This is thought to be due to the variable activity of key enzymes in thiopurine metabolism as well as the variable formation of metabolites (Moon and Loftus, 2016). However, thiopurine metabolites do not always correlate to biological or clinical response as seen from conflicting results in several IBD studies (Moon and Loftus, 2016; Konidari *et al.*, 2014; Lee *et al.*, 2015; Osterman *et al.*, 2006) and in AIH (Heneghan *et al.*, 2006; Ferucci *et al.*, 2011; Dhaliwal *et al.*, 2012; Nguyen *et al.*, 2010).

In IBD management, meta-analyses suggest that the therapeutic range of 6-TGNs is between 235 - 450 pmol/8 × 10^8 RBCs (Dubinsky *et al.*, 2000; Warner *et al.*, 2018). Conversely, there have been several studies that have been unable to confirm the threshold values for clinical response (Cuffari *et al.*, 1996; Moon and Loftus, 2016). Additionally, in the same meta-analysis of 12 studies by Osterman et al, 36% of patients in clinical remission had 6-GTNs below the threshold level (Osterman *et al.*, 2006). 6-TGN-guided IBD-treatments improved clinical outcomes in 90% of patients compared with 33% (not guided by 6-TGNs) (Smith *et al.*, 2013) and informed clinical decision making in 2/3 of patients (Wilson *et al.*, 2020). 6-MMP levels above 5700 pmol/8 × 10^8 RBCs has been associated with a threefold increased risk of hepatotoxicity (Dubinsky *et al.*, 2000). In IBD, the use of RBC thiopurine metabolite monitoring

is to maximise therapeutic response rather than avoid adverse events (Moon and Loftus, 2016).

There is however only limited evidence that the RBC thiopurine metabolite levels are good indicators of biological response in AIH. The lack of concordance between metabolite levels and biological effect may be due to RBC not being nucleated or the cells responsible for immunosuppression. A single-centred study in the UK (Heneghan *et al.*, 2006) found no threshold level for RBC 6-TGNs to maintain clinical remission in AIH, which was similar to the findings of Ferruci and colleagues' study (Ferucci *et al.*, 2011) which also reported no correlation between RBC metabolite levels and remission or toxicity. In contrast, Dhaliwal et al reported that AIH patients who maintained clinical remission had higher 6-TGNs (average 237 pmol/8 × 10^8 RBCs; n=57) compared to those who did not maintain remission (average 177 pmol/8 × 10^8 RBCs; n=17,p = 0.025), with no difference in methylated metabolite levels between the two groups. An increased risk of hepatotoxicity was, however, associated with 6-MMP levels of >5700 pmol/8 × 10^8 RBCs but in only two patients. The group concluded that a 6-TGN >220 pmol/8 × 10^8 RBCs associated with remission (OR 7.7) with no association found between 6-TGN, 6-MMP or TPMT activity and the development of leukopenia (Dhaliwal *et al.*, 2012). In a paediatric study assessing 28 patients with AIH, this found that in the 61% achieving remission, 33% had 6-TGN values within the target range proposed for patients with IBD: 250-450 pmol/8 × 10^8 RBCs. Additionally, there was no difference in metabolite concentrations between those in remission and those with active disease (Nguyen *et al.*, 2010).

In a UK retrospective matched cohort study (214 patients with AIH), a group of 109 patients with measured thiopurine metabolite levels was compared with a control group (n=105) who had weight-based thiopurine dosing with no metabolite monitoring. Patients with metabolite monitoring were found to have more occurrence of biochemical response (at 6-month follow-up) compared to those on weight-based regimen (77% vs 60%, p=0.008). Those with biochemical response had TGN levels within the therapeutic range (225-450 pmol/8 × 10^8 RBCs) significantly more often than patients who failed to achieve or lost biochemical response (40% vs. 13%, p<0.0001). The group also found that a high proportion of patients (75%) with TGN levels in the subtherapeutic range (75-225 pmol/8 × 10^8 RBCs) remained in biochemical response compared to 81% (p=0.589); there were significantly fewer adverse drug reactions in this group (44%) compared to 86% of patients with therapeutic TGN levels (86%), p=0.0002. The group concluded that a personalised approach strategy in using

metabolite levels can optimise treatment regimens with less occurrence of adverse drug reactions whilst maintaining biochemical response (Candels *et al.*, 2021).

Allopurinol with low-dose thiopurine has been used to redirect the thiopurine metabolism towards 6-TGN formation instead of 6-MMPs. A Dutch study evaluated the use of allopurinol with low dose thiopurine combination in 8 patients with AIH with a skewed thiopurine metabolism. The reasons for switching were dose-limiting intolerance (n=3), non-response (n=3) or loss of response (n=2). The study showed all 8 patients had biochemical improvement in their ALT levels which were sustained in 7 patients. The study concluded allopurinol could be used safely and was effective in optimising thiopurine therapy in patients with intolerance and/or non-response due to unfavourable thiopurine metabolism (de Boer *et al.*, 2013).

1.7.7 Relapse and Maintenance therapy

Currently, there is no clear evidence of the optimal duration of treatment. The ultimate goal would be to achieve histological remission before consideration of treatment withdrawal. The European guidelines recommend that treatment be continued for at least three years and for at least 24 months after achieving biochemical remission (normalisation of transaminases and IgG level) (European Association for the Study of the, 2015). Histological remission lags behind biochemical remission by several months (Soloway *et al.*, 1972). The decision to withdraw treatment should be individualised depending on the patient's course of disease, initial presentation and response to treatment.

Disease relapse occurs in 50-90% of patients within 12 months of stopping treatment and 70% within three years (Hegarty *et al.*, 1983; van Gerven *et al.*, 2013b; Czaja, 2002; Johnson, McFarlane and Williams, 1995; Manns and Strassburg, 2001). Relapse can also occur later on in life. A relapse is formally defined by the IAIHG criteria as an ALT elevation of >3x ULN however it can also present with milder increase in ALT and/or increase in IgG. Generally, a relapse is more likely to occur if a patient has required a longer duration in achieving biochemical remission or has persistent inflammation prior to withdrawal of treatment (as evidenced by persistently raised transaminases and/or IgG) (Gleeson, Heneghan and British Society of, 2011). From retrospective studies, the factors that have been associated with relapse are as follows:

a. At presentation:
    - long symptom duration (McFarlane, 1998)
    - high serum globulin (Verma *et al.*, 2004)
    - LKM antibody positivity (McFarlane, 1998)
    - SLA/Liver Pancreas antigen (LP) positivity or no immune markers(Baeres *et al.*, 2002)
b. On treatment
    - short treatment duration (Kanzler *et al.*, 2001a)
    - long time to remission (Verma *et al.*, 2004)
c. Pre-treatment withdrawal
    - raised serum ALT or AST (Verma *et al.*, 2004; Montano-Loza, Carpenter and Czaja, 2007c)
    - raised serum globulin IgG (Verma *et al.*, 2004)
    - any persisting inflammation on liver histology (Verma *et al.*, 2004; Czaja and Carpenter, 2003)
    - presence of portal tract plasma cells on liver histology (Verma *et al.*, 2004)

A relapse is treated similarly with predniso(lo)ne and azathioprine and is as effective as inducing remission as the primary induction treatment. 80% of patients will achieve biochemical remission following relapse. Patients with multiple relapses have been shown to be more likely to experience treatment-related side effects, develop progressive fibrosis or cirrhosis and progress to death or liver transplantation (Montano-Loza, Carpenter and Czaja, 2007a; Hoeroldt *et al.*, 2011; Czaja, 2002).

AZA should be increased to 2mg/kg/day as maintenance therapy following a relapse. This should then be continued indefinitely. The remission rate for AZA monotherapy at this dose over a median follow-up of 67 months has been quoted at 83% (Stellon *et al.*, 1988; Lamers *et al.*, 2010; Johnson, McFarlane and Williams, 1995; European Association for the Study of the, 2015).

An alternative regime is a low-dose prednisolone maintenance regime (with or without AZA). The study (Czaja, 1990) which used this regimen however aimed for a serum AST <5x the normal value which is still now classed as not achieving biochemical remission. The main

advantage of low dose corticosteroids would be the reduction in severity of side effects to steroids (Gleeson, Heneghan and British Society of, 2011).

1.7.8 Second-line therapies in AIH

For patients who are intolerant to or have unsatisfactory response to AZA, second-line immunsuppressive therapies include mycophenolate mofetil (MMF) or calcineurin inhibitors (CNI) – ciclosporin or tacrolimus. None of these have been tested in randomised controlled trials and the evidence for the use of these medications are based on case-series, open-label and retrospective studies.

i. Mycophenolate mofetil (MMF)

MMF is the most widely used second-line agent in AIH, based on a recent survey of expert hepatologists (n=450, 28 centres) (Liberal *et al.*, 2017).

A meta-analysis of MMF use as second-line therapy in AIH (12 studies, n=397) showed a pooled overall response rate of 58% (5 studies specific the reason for MMF use – 82% for AZA intolerance and 32% for treatment failure). MMF was generally well-tolerated with pooled adverse event rate of 14% and resultant discontinuation rate of 8% (Santiago *et al.*, 2019).

Another meta-analysis evaluating second line therapies in AIH (15 eligible studies out of 1532 - 8 evaluating MMF and prednisone, n=162) - MMF was the most evaluated second-line therapy. The mean reduction of aminotransferases was 79% whilst histological remission was 89% for the MMF regimen (De Lemos-Bonotto *et al.*, 2018).

An Australian retrospective observational cohort study of AIH patients (17 major liver centres) reported a 60% (63/105) biochemical remission rate for patients receiving MMF rescue therapy. MMF therapy showed better response rates when used in patients intolerant to primary therapy compared to those with treatment failure to primary therapy (62% versus 38%). Older age, lower pre-treatment IgG levels and higher INR were identified as predictors for a favourable disease response. Serious adverse events occurred in 3 patients (2.7%) including 1 death; 10 (9.2%) patients stopped MMF due to adverse events (Nicoll *et al.*, 2019; Roberts *et al.*, 2018).

A multicentre retrospective study (19 centres in Europe, United States, Canada and China) described data from patients with AIH who had received second line immunosuppression. Out

of the 201 patients, 121 patients received MMF for a median of 62 months. 92% of these patients were switched to MMF due to side effects from predniso(lo)ne or AZA achieved biochemical remission. The remission rate for patients who had been switched to MMF due to non-response to standard therapy was 34%. 8.3% of patients receiving MMF experienced side effects necessitating treatment cessation. The study concluded that long-term MMF was generally well-tolerated by patients and was effective in previous complete responders who were intolerant to standard-of-care therapy (Efe *et al.*, 2017).

A UK retrospective study (Plymouth) looked at 20 patients with AIH (18 were intolerant to AZA and 2 had refractory disease). 74% (14 patients) achieved complete biochemical remission (normalisation of transaminase levels) after a median of 47 (5-83) months. 3 patients were intolerant to MMF and 2 were refractory to MMF. The latter two were the same patients who had initially been unresponsive to AZA therapy (Jothimani, Cramp and Cross, 2014).

A retrospective study by Hlivko and colleagues looked at 29 patients with AIH who received MMF (dose: 500mg - 2000mg/day) - 12 due to intolerance or non-response to prednisone with or without AZA whilst 17 as initial therapy with or without prednisone. 10/29 (34%) discontinued MMF due to side effects whilst 16 (55%) achieved remission. It is important to note that remission in this study was defined as resolution of symptoms, reduction in serum aminotransferase levels to <2x ULN, normalisation of serum bilirubin and gamma globulin levels and improvement in liver histology to normal or only mild portal hepatitis. On comparing histological and clinical data of the 29 patients on MMF with those of the patients on standard therapy, absence of cirrhosis on initial liver biopsy was identified as the sole independent variable significantly associated with eventual need for MMF (Hlivko *et al.*, 2008).

Another retrospective study examined 36 patients with AIH who were given MMF as second-line therapy after failing standard therapy with steroids and AZA. A large proportion of the patients (28/36) had experienced side effects from AZA leading to its cessation. 39% experienced remission on MMF (defined as AST <2x ULN). Of the 8 patients with prior non-response to AZA, only 25% achieved biochemical remission compared to 43% of the 28 patients with AZA intolerance (p=non-significant). The study concluded that MMF seemed to be an alternative for patients who were intolerant to AZA but suggested that a majority of patients fail MMF if they were switched because of insufficient response to AZA (Hennes *et al.*, 2008a).

Sharzehi and colleagues examined 90 patients with AIH retrospectively – 21 patients were identified to have been given MMF – 12(57%) for refractory disease and nine (43%) for medication intolerance. None of the 12 patients with refractory disease had complete response on MMF although there was biochemical improvement. 88% of those converted due to intolerance achieved complete remission. There was also a mean reduction in steroid dose from 18.9mg/day to 7.8mg/day (p=0.01) for all patients converted to MMF (Sharzehi *et al.*, 2010).

Another retrospective study in California (USA) looked at 15 patients with AIH who received MMF (monotherapy or combination with prednisone after failure or intolerance of initial regimen). 12 patients received combination therapy with MMF whilst 3 patients were on MMF monotherapy. The ALT levels reduced significantly from 91.73 +/- 88.69 to 60.87 +/- 71.2 (p=0.03) on MMF treatment. Both inflammatory scores (2.59 +/- 0.97 to 1.14 +/- 1.21, p=0.02) and Ishak fibrosis scores (4.1 +/- 1.37 to 2.5 +/- 1.51, p=0.02) reduced (Inductivo–Yu *et al.*, 2007). Other case-series have reported MMF being able to induce and maintain remission safely when given to patients either refractory or intolerant to AZA and steroids (Richardson, James and Ryder, 2000; Devlin *et al.*, 2004; Brunt and Di Bisceglie, 2004; Wolf *et al.*, 2009).

A multicentre retrospective study on 16 patients who received MMF (having failed previous standard therapy) reported a 50% complete response with 25% non-response and 12.5% relapse rate after 26.5 months (Chatur *et al.*, 2005).

In summary, MMF as second-line therapy in AIH has a pooled response rate of 58% with higher rates of remission for those switched due to prednisolone/AZA intolerance (up to 92% biochemical remission rate) compared to those switched for non-response (34%). About 8% of patients experience side-effects requiring treatment cessation.

ii. 6-Mercaptopurine

A case-series of three patients with AIH (one of which was a paediatric case , age 13) used 6MP as rescue therapy in two patients and alternative therapy for side-effects to AZA. In the two adult patients (one who had rescue therapy for incomplete response to AZA and the other with intolerance to AZA) - both achieved biochemical remission and one (who had liver biopsy) showed significant histological improvement (Pratt, Flavin and Kaplan, 1996).

6-mercaptopurine was used as second-line therapy in a retrospective study of 22 patients with AIH. 15/20 (75%) of the patients with AZA intolerance who were switched to 6-MP responded biochemically to therapy (8 (53%) – complete response, 7 (47%) – partial remission). In the other 5 patients, these had to be switched to an alternative therapy due to 6MP intolerance. 2 patients with insufficient response to AZA did not respond to 6MP as well (Hubener *et al.*, 2016).

Taken together, 6-MP is a potential effective treatment for those who have AZA intolerance.

iii. Calcineurin inhibitors (CNIs)

a. Ciclosporin

The use of ciclosporin as second-line therapy is based on case series. In non-response AIH (adults), the biochemical response reported from two case-series (n=6) was ≥80% (Sherman, Narkewicz and Pinto, 1994; Fernandes *et al.*, 1999).

b. Tacrolimus

Tacrolimus has been evaluated in the setting of treatment failure, incomplete response and AZA intolerance in several studies. The efficacy has been reported as moderate to high. Two small studies (single-centre) reported normalisation of serum aminotransferases to tacrolimus in (10/11) 91% and (12/13) 92% respectively. Tacrolimus trough levels ranged from 1 – 10 ng/mL. Adverse effects included headache (4), nausea/vomiting (1), hair loss (1) – all of which did not require treatment cessation. 2 patients had to have tacrolimus discontinued due to development of tremors, hypertension, and generalised oedema (1) and haemolytic uraemic syndrome (1) respectively (Aqel *et al.*, 2004; Tannous *et al.*, 2011).

Larsen and colleagues reported 7/9 (78%) remission rate for patients on tacrolimus (in combination with prednisolone) for AIH refractory to standard therapy. There was significant improvement in ALT, IgG and histological inflammation and fibrosis with no significant increase in creatinine obesrved (Larsen *et al.*, 2007).

A two-centre retrospective study (UK, Germany) reported on 17 patients with “difficult to treat AIH” (defined as ALT >2x ULN and elevated IgG for 6 months despite therapy compliance) who were given tacrolimus (median duration 24 months). This series has the longest follow-up duration (median 60 months) compared to other case series. 1 patient had been intolerant

to AZA and 16 had incomplete remission to standard immunosuppression. 9/17 (52%) patients remained on tacrolimus therapy at the end of follow-up. These patients also achieved prednisolone dose reduction from median dose of 10 to 5mg (p=0.18). The 1-year normalisation of either serum ALT or IgG level at 29% and 50% respectively with significant improvement of AST at the end of follow-up compared to pre-tacrolimus therapy (p<0.001). None of the patients developed renal insufficiency. 48% stopped treatment for various reasons (2 – non-compliance, 1 – liver transplantation, 2 – developed PSC overlap, 3 – side-effects of abdominal pain, headache, tremor and vomiting). The study concluded that tacrolimus could be used in compliant patients with difficult to treat AIH in experienced centres (Than *et al.*, 2016b).

A retrospective multi-centre study (19 centres – Europe, United States, Canada and China) on second-line therapies in AIH described a 94% biochemical remission rate to tacrolimus. 34 (42.5%) had been switched to tacrolimus due to intolerance to azathioprine/predniso(lo)ne therapy whilst 46 (57.5%) had incomplete response/treatment failure to standard of care. 10 (12.5%) patients developed side effects leading to treatment withdrawal. The side effects reported were: neurological (4), hypertension and generalised oedema (2), gastrointestinal (2), hair loss (1) and renal failure (1) (Efe *et al.*, 2017).

Two meta analyses on use of tacrolimus as second-line therapy reported an improvement or normalisation of serum aminotransferases in 75-94%. Side-effects leading to reduced dose or discontinuation of tacrolimus occurred in 17-25% (De Lemos-Bonotto *et al.*, 2018; Hanouneh *et al.*, 2019).

Taken together, tacrolimus has a 58-94% rate of biochemical remission (is a potential second or third-line therapy for patients who fail to respond to first line standard therapy.

iv. Other immunomodulatory agents

The use of other immunomodulatory agents are mostly anecdotal as only small case series or case reports have been published.

Cyclophosphamide (1 - 1.5mg/kg/day) with corticosteroids (1mg/kg) has been used to induce remission in one report comprising of three patients with severe AIH. Histological remission was also maintained with low doses of corticosteroids (2.5 - 10mg/day) with 50mg of cyclophosphamide every other day. There was no relapse of AIH or severe side effects over

the cumulative observation period of 12 years. The report proposed cyclophosphamide as an alternative to AZA in cases of AIH with severe side effects or intolerance to standard therapy (Kanzler *et al.*, 1997). There has however not been any further reports of long-term outcomes nor other experiences described.

In a case report, methotrexate was used in (7.5mg orally per week) in a patient with AIH due to flare of disease on addition of AZA (and patient refusal to ciclosporin). Methotrexate treatment resulted in successful normalisation of liver enzymes, improved liver histology and remission maintenance with a corticosteroid-sparing effect (Burak, Urbanski and Swain, 1998). However, as methotrexate is teratogenic in women of childbearing age and has been associated with causing liver fibrosis (Parker, Oo and Adams, 2012), this option has not been used widely.

Rituximab has been used in various autoimmune conditions such as rheumatoid arthritis, SLE and vasculitides. Rituximab is a anti-CD-20 monoclonal antibody that depletes B cells. In one case report, rituximab was given to a patient with corticosteroid-resistant idiopathic thrombocytopaenic purpura and concomitant AIH. In this case report, the patient achieved rapid increase in her platelet count and unexpected normalisation of serum transaminases after 4 weeks of treatment with rituximab (375mg/m^2 weekly for 4 weeks). The effect on her liver enzymes was sustained for 5 months (Santos *et al.*, 2006). Another case report of a patient with B-cell lymphoma and corticosteroid-resistant PBC/AIH overlap syndrome was given rituximab weekly (375mg/m^2) for 12 weeks (with a few months' after the initial 8 weeks of therapy) for both conditions. This resulted in improvement in symptoms, laboratory and histological findings (Barth and Clawson, 2010).

In an open-label, single-centre pilot study, Burak and colleagues used rituximab (1000mg two weeks apart on days 1 and 15) in six patients with definite biopsy-proven AIH who had failed standard therapy (prednisone and AZA). 3 were intolerant to first-line therapy (one was intolerant to MMF following AZA) and 3 were refractory to first line therapy (including one who who tried AZA and MMF). Follow-up duration was 72 weeks. By week 24, there was significant biochemical improvement (AST and IgG) and successful weaning of prednisone in 3 out of the 4 subjects (one flaring after steroid withdrawal). Two patients had been intolerant to prednisolone (headaches) prior to study entry. There was also improvement in inflammation grade in all 4 subjects who had repeat liver histology at week 48. The study

concluded that rituximab was safe, well-tolerated and resulted in biochemical improvement in patients with refractory AIH (Burak *et al.*, 2013).

A retrospective study from the International AIH Group (IAIHG) evaluated 22 patients with type 1 AIH (UK, Germany, Canada) who received rituximab for difficult-to-treat AIH. There was significant improvement in transaminases and albumin following rituximab therapy ($p<0.001$) and 62% (13/21) being able to have dose reduction of prednisolone. Median post-treatment follow-up period was 10 years (1-10) with 71% having flare-free AIH disease at 2 years. 5 patients had disease flare, 4 of which received a second course of rituximab. There were no documented serious adverse events from rituximab. The study concluded that rituximab was associated with improvement in liver biochemistry, absence of clinical disease flares and a redution in prednisolone dose. The group advocated for more controlled trials evaluating B-cell targeting therapies in AIH (Than *et al.*, 2019).

Infliximab, a biological anti-tumour necrosis factor agent (TNF) has been used as rescue therapy in difficult-to-treat AIH. In a case series of 11 patients, off-label infliximab therapy (over 6 months) resulted in improvement in biochemical markers of inflammation (transaminases and IgG). Seven out of eleven patients had infectious complications (Weiler-Normann *et al.*, 2013). There have also been anecdotal reports of patients with AIH and inflammatory bowel disease (IBD) who had an improvement in hepatitis on treatment of the IBD with anti-TNF-alpha therapy (Parker, Oo and Adams, 2012). However, these reports need to be balanced with other reports on AIH triggered by anti-TNF agents (Rodrigues *et al.*, 2015; Cravo, Silva and Serrano, 2010; Germano *et al.*, 2005; Efe, Purnak and E., 2010). Therefore, further evidence on its efficacy and toxicity in AIH is required before any recommendations on its use can be given.

Sirolimus has been used for post-liver transplant AIH in a single report (n=6) with good response although one had the drug withdrawn for suspected post-transplant lymphoproliferative disorder (but did not develop this subsequently) (Kerkar *et al.*, 2005).

Thioguanine (TG) is an alternative thiopurine drug which is directly metabolised to 6-thioguanine (6-TGN). 6-TGN is an active metabolite of azathioprine and responsible for the immunosuppressive effect. Azathioprine and 6-MP are metabolised by a complex enzymatic pathway via thiopurine-S-methyltransferase (TPMT), producing 6TGN as well as methylated metabolites. The methylated metabolites have been associated with AZA intolerance. In TPMT

deficiency, toxic amounts of 6TGN are produced which leads to bone marrow suppression. However, there is reduced burden of methylated products with the use of TG compared to AZA or 6-MP. This has led to its use in patients with AIH and inflammatory bowel disease (IBD). There were initial concerns around drug-safety due to reports of nodular regenerative hyperplasia (Dubinsky *et al.*, 2003) however, subsequent studies in IBD and AIH have not found this to be the case (Meijer *et al.*, 2016; Legué *et al.*, 2018). A French study evaluated 17 patients with AIH who were treated with TG (16 had been intolerant to AZA, 1 was non-responsive to AZA). TG therapy led to normalisation of serum aminotransferases in 64% of patients. 1 patient had NRH on follow-up biopsy with no portal hypertension however there were subtle vascular changes prior to TG therapy, suggesting other factors were involved (Legué *et al.*, 2018). A retrospective national study over a 17-year period in Netherlands reported TG-therapy experience in 52 patients with AIH and AIH variant syndromes. 73% had intolerable side-effects on AZA or 6MP, leading to switch to TG. No serious adverse events were found in the whole study cohort (median follow-up of 18 months) and TG was well-tolerated in 79% of patients with 13% having intolerable side-effects. 24/29 (83%) patients who continued TG therapy achieved biochemical remission (normalisation of ALT and IgG) (van den Brand *et al.*, 2018). Thioguanine is potentially a promising second-line treatment for AIH patients – its use is currently being evaluated in a multicentre collaborative trial (Mack *et al.*, 2020).

Summary points

- The natural history of AIH without treatment (derived from placebo-arms of early randomised controlled trials) showed an average life expectancy of less than five years with significantly higher mortality (41-56%) compared to those who were treated (6-15%).
- Treatment of AIH consists of immunosuppressants (usually lifelong) with the goals of treatment: i) achieve complete biochemical and histological remission,ii) prevent/minimise risk of disease relapse, iii) prevent progression of liver disease to death or liver transplantation and iv) reduce/minimise risk of side-effects of treatment.
- Treatment should be given to all patients with active disease (with hepatitis activity index (HAI) of ≥4/18) or advanced fibrosis or cirrhosis.
- Predniso(lo)ne alone or in combination with azathioprine is the established first line remission-induction therapy in AIH based on the results of three prospective

randomised controlled trials in the 1970s. These trials showed that prednisolone alone or in combination with azathioprine improved survival, symptoms, biochemistry and histological findings.

- Budesonide has been evaluated in one prospective randomised controlled trial in patients with AIH and found a 60% biochemical remission rate (compared with 39% with prednisone); 72% having no side effects with budesonide compared to the prednisone group (47%); limitations of the study include – lower biochemical remission rates in the prednisone group, short follow-up, lack of histology and there being more males in the treatment arm.
- Budesonide is currently recommended as an alternative therapy (used in combination with azathioprine) for non-cirrhotic patients who either are anticipated to experience or experience steroid-related side-effects from prednisolone.
- Mycophenolate mofetile (MMF) and calcineurin inhibitors (ciclosporin) have been evaluated as alternative first-line therapies in either uncontrolled studies or case-series with some promising results however at present, both are not recommended in international guidelines.
- Corticosteroid-related side-effects include cosmetic changes (weight gain, Cushingoid features), diabetes, psychosis, hypertension, cataracts, osteoporosis with vertebral collapse, pancreatitis, opportunistic infection and malignancy. Studies with combination therapy (AZA and prednisolone) report and approximate 30% prevalence of steroid-associated side-effects.
- AZA-related side-effects and complications occur in about 25% of patients, leading to drug withdrawal in about 10% of patients.
- Azathioprine metabolism is complex and genetic polymorphisms in AZA metabolism can lead to variable levels in azathioprine breakdown products (thiopurine metabolites) in red-blood cells [6 thioguanine (6TGN) and 6-methyl mercaptopurine (6MMP)]. Both of these can lead to adverse drug effects.
- Studies in AIH patients on the utility of checking Thiopurine methyltransferase (TPMT) (a key enzyme in AZA metabolism) have shown variable results as neither intermediate TPMT enzyme activity or TGN levels are reliable in predicting AZA response or toxicity.
- There is limited evidence that the RBC thiopurine metabolite levels are good indicators of biological response in AIH. The lack of concordance between metabolite levels and

biological effect may be due to RBC not being nucleated or the cells responsible for immunosuppression.

- Studies evaluating RBC thiopurine metabolite levels in AIH patients have reported contradictory results with two studies not identifying a threshold associated maintained remission or toxicity whilst one other study reported a therapeutic lower limit of 220 pmol/8 × 10^8 RBCs being associated with remission with no association found between 6-TGN, 6MMP or TPMT activity and the development of leukopenia. A personalised approach in optimising treatment using metabolite levels has been reported to be associated with less adverse drug reactions whilst maintaining biochemical response.
- Allopurinol with low-dose thiopurine has been used to redirect the thiopurine metabolism towards 6-TGN formation instead of 6-MMPs.
- The study concluded allopurinol could be used safely and was effective in optimising thiopurine therapy in patients with intolerance and/or non-response due to unfavourable thiopurine metabolism.
- Disease relapse occurs in 50-90% of patients within 12 months of stopping treatment and 70% within three years.
- Factors associated with relapse are:

  At presentation: long symptom duration, high serum globulin, LKM antibody positivity, SLA/Liver Pancreas antigen (LP) positivity or no immune markers

  i) On treatment: short treatment duration, long time to remission
  ii) Pre-treatment withdrawal: raised serum ALT or AST, raised serum globulin IgG, any persisting inflammation on liver histology, presence of portal tract plasma cells on liver histology
- A relapse is treated similarly with predniso(lo)ne and azathioprine; 80% of patients will achieve biochemical remission following relapse. and is as effective as inducing remission as the primary induction treatment.
- AZA should be increased to 2mg/kg/day as maintenance therapy following a relapse and continued indefinitely. Alternatively, a low-dose prednisolone maintenace regime can be used.
- Second-line immunosuppressive therapies are used for patients who are intolerant or have unsatisfactory response to AZA; none of these have been tested in randomised controlled trials and are based on case-series, open-label and retrospective studies.

- These include:
  i) MMF - pooled response rate of 58% with higher rates of remission for those switched due to prednisolone/AZA intolerance (92%) compared to those switched for non-response (34%); 8% experience side-effects requiring treatment cessation
  ii) 6-MP – 53% (complete response) and 47% (partial response) in a small retrospective study [in patients switched for AZA intolerance]
  iii) Ciclosporin – 80% biochemical response in non-response AIH
  iv) Tacrolimus – 58-94% biochemical remission in patients with AIH refractory to standard therapy
  v) Other immunomodulatory agents include cyclophosphamide, methotrexate, rituximab, infliximab, sirolimus and thioguanine

1.8 Long-Term Outcomes

1.8.1 Development of cirrhosis, prognosis and liver-related outcomes (death and transplantation)

AIH is a chronic relapsing disease which can progress to cirrhosis and liver failure requiring transplantation despite successful induction therapy. About 30% of patients with AIH present with cirrhosis. In general, cirrhosis at presentation is associated with a poorer outcome compared to those without cirrhosis though not all studies have shown this. About a median figure of 14% (range 6-40%) develop de-novo cirrhosis despite treatment (12% and 24% over 10 and 20 years respectively) (Gleeson, 2019; van den Brand *et al.*, 2019a; Feld *et al.*, 2005; Czaja and Carpenter, 2006a). A summary of AIH centre experiences (which includes outcomes and mortality) is detailed in **Table 8** at the end of section 1.9.

Initial studies of treated AIH had suggested optimistic prognosis with 10-year survival of 90% which was not different from the general population (Roberts, Therneau and Czaja, 1996; Kanzler *et al.*, 2001b; Kirstein *et al.*, 2015).

However, this has been superceded by the landmark report by Hoeroldt and colleagues which published follow-up data of patients in their second decade. This revealed a dismal 20-year mortality rate (death or transplantation) of 52%. This study (n=245) reported that 36% of

patients presented with cirrhosis with 10% developing cirrhosis during follow-up. 70 patients died (30 from liver-disease) and 11 required liver transplantation. This was despite 93% of patients achieving biochemical response after 12 months of treatment. Transplant-free survival was 82% at 10 years and 48% at 20 years after diagnosis with liver-related death or transplantation at 91% and 70% respectively. The standardised mortality ratio (SMR) for all cause death was 1.63 (95% confidence interval [CI], 1.25-2.02), 1.86 when considering liver transplant as 'death' (95% CI, 1.49-2.26) and 0.91 for non-liver related death (95% CI, 0.62-1.19). On Cox-regression analysis, the factors significantly associated with liver-related death or transplant were liver decompensation, cirrhosis at any time, failure to normalise ALT levels within 12 months and 4 relapses per 10 years (Hoeroldt *et al.*, 2011).

Subsequent studies concur, with AIH patients having a two-fold higher mortality than the general population (Ngu *et al.*, 2012; Gronbaek, Vilstrup and Jepsen, 2014; Sharma *et al.*, 2020).

The study by Ngu and colleagues (n=133, 45 (34%) cirrhotics with median follow-up: 9 years (1-30)) reported a mortality rate of 32%, 2% requiring liver transplantation and 13% had liver-related death. Kaplan-Meier estimates showed that 10-year adverse liver event-free survival was 80% (Ngu *et al.*, 2013).

A population-based Danish study (n=1721) found that 371(22%) died during a total of 11,832 years of follow up (median: 5.9 years per patient). 129 (39%) of patients died from liver-related causes. The 1 and 10 year mortality risks of AIH were 6% (95% CI 4.9-7.20) and 26.4% (95% CI: 23.7-29.1) respectively. AIH patients had higher mortality risk than the population controls particularly in the first year after AIH diagnosis during which mortality hazard ratio was 5.76 (95% CI 4.66-7.11) (Gronbaek, Vilstrup and Jepsen, 2014).

More recently, a Dutch study (n=385 with AIH) with 10-year follow-up data reported a 14% death rate, 40% of which were liver-related. The percentage of non-liver related causes of death were as follows: Circulatory system diseases (21.8%), respiratory system diseases (3.6%), malignant diseases (21.8%), other causes of death (9.1%) and unknown (3.6%). AIH patients with cirrhosis had significantly higher mortality than the general population (SMR 1.9; 95% CI, 1.2-3.4) whilst those without cirrhosis did not (SMR, 1.2; 95% CI, 0.8-1.8). Survival of AIH patients did not differ from those of the general population in Netherlands unless patients had cirrhosis (van den Brand *et al.*, 2019a).

In general, a median figure of 49% (0%-73%) of all deaths or transplants in AIH are due to liver disease. Liver-related deaths are three times the number of transplants (Gleeson, 2019). Studies report about 2-9% of patients with AIH requiring liver transplantation (see **Table 8**).

A population-based study in Sweden (n = 6016 AIH cases) reported a two-fold increased risk of death in patients with AIH. This was especially high in those with cirrhosis, portal hypertension and overlap with cholestatic disease. The 10-year cumulative incidence of death for AIH patients was 32.3% (95% CI, 31.3 – 33.6) compared to 14.1% (95% CI, 13.7-14.5) in the general population. AIH was associated with an increased risk of death from cardiovascular disease (hazard ratio (HR) 1.27, 95% CI, 1.15-1.40), liver disease (HR 66.24, 95% CI, 48.19-91.03) and extrahepatic malignancy (hazard ratio 1.69, 95% CI, 1.51-1.89) (Sharma *et al.*, 2020).

1.8.2 Hepatocellular cancer and extrahepatic malignancy

Development of hepatocellular cancer (HCC) occurs in 1-4% of AIH cases (Montano-Loza, Carpenter and Czaja, 2008; Teufel *et al.*, 2009; Werner *et al.*, 2009).

In the Swedish study by Werner et al (n = 473 AIH patients), there was an overall increased risk for malignancies with a standardised incident rate (SIR) of 1.51 (95% CI 1.10-2.03) when calculated from the time of diagnosis of AIH. This was mainly attributed to HCC which had a SIR of 23.28 (95% CI 7.5-54.34). 4 (1%) patients developed HCC and all four had cirrhosis. There was an increased risk for lymphomas with a SIR of 13.09 (95% CI 4.22 – 30.56). There was no clear relation to azathioprine therapy but an association could not be ruled out. There was also increased risk of skin cancers in the AIH cohort (SIR of 6.17) with 7 patients having 15 skin cancers; 5 of these had been taking azathioprine for years whilst two had never been exposed to azathioprine (Werner *et al.*, 2009). A further Swedish registry study (n=634 AIH patients) found an increased risk for malignancies compared to the general Swedish population with the SIR for hepatobiliary cancer: 54.55 (95% CI 19.92-99.99) and non-melanoma skin cancer with SIR 9.87 (95% CI 6.26-14.81). The HCC incidence rate was 0.3% (Danielsson Borssén *et al.*, 2017).

In Yeoman et al's study in the UK, the rate of HCC development was 9.3% in patients who had cirrhosis at presentation. This occurred more frequently in patients with cirrhosis than without (3.4%, p=0.048) or who had a variceal bleed as the index presentation of AIH (20% vs 5.3%,

p=0.003). The median duration of time of confirmed cirrhosis to a diagnosis of HCC was 102.5 months (12-195) (Yeoman *et al.*, 2008).

A German study evaluated their cohort of 278 patients with AIH of which 32% had cirrhosis. No patients developed hepatocellular cancer during their follow-up (average of 4.8 years per patient) (Teufel *et al.*, 2009).

In Wong et al's study (USA) (n=322 AIH cases), 6 patients developed HCC, all of which had cirrhosis. The risk of HCC was calculated as 1.9% per year (Wong *et al.*, 2011).

The New Zealand population-based study (130 AIH patients) reported a significantly increased risk for hepatic and extrahepatic malignancy in the AIH cohort with a SMR for all-cause mortality at 2.1 (95% CI 1.4-3.1), SMR for hepatobiliary mortality at 42.3 (95% CI 20.3-77.9) and SIR for extra-hepatic malignancy at 2.7 (95% CI 1.8-3.9) (Ngu *et al.*, 2012).

A more recent systematic review (25 studies, n=6528) showed a pooled incidence rate for HCC was 3.06 per 1000 patients years (95% CI 2.22-4.23, p=0.002). In patients with cirrhosis at diagnosis of AIH, the pooled incidence of HCC was one per 100 patients years (less frequent than hepatitis B-related cirhrosis, hepatitis C-related cirrhosis or primary biliary cholangitis). 92/93 (99%) of the patients who developed HCC had cirrhosis at the time of HCC diagnosis or prior to HCC diagnosis (Tansel *et al.*, 2017).

In a Japanese study of 256 AIH patients (16% with histological cirrhosis) with mean follow-up of 8.8 (+/- 6.7 years), 27 (10.5%) of patients developed malignancy. 85% had been treated with prednisolone and 30% with azathioprine. Hepatocellular cancer occurred in 10 patients whilst cholangiocarcinoma in one person. 73% of those with hepatobiliary cancer had cirrhosis. Other malignancies included: gastric cancer (3), pancreatic cancer (2), oral/pharyngeal cancer (2), colorectal cancer (20), breast cancer (2), lung cancer (1), cervical uterine cancer (1), acute myeloid leukaemia (1), malignant lymphoma (1) and malignant melanoma (1). The SIR for malignancies in AIH was significantly high at 2.04 (95% CI, 1.34 – 2.96), particularly in women (2.49, 95% CI, 1.60-3.71). Hepatobiliary cancer and oral/pharyngeal cancer had high SIR at 14.15 (95% CI, 7.05-25.30) and 14.61 (95% CI, 1.64-52.77) respectively. The study identified the risk factors for hepatobiliary cancer in AIH were low levels of ALT ($p = 0.0226$), low platelet count ($p < 0.0001$) and cirrhosis ($p = 0.0004$) at diagnosis of AIH. The risk factor for extrahepatic malignancy was only relapse of AIH (p=0.0485). The probability of developing malignancy (on

Kaplan Meier analysis) was 14% and 46% at 10 and 20 years respectively for patients with cirrhosis. In contrast, the probability for non-cirrhotics was 0.6% and 5.6% at 10 and 20 years respectively. There was a two-fold increase in incidence of malignancy in AIH patients (2.5 times in females). The main risk factor identified for extrahepatic malignancy in AIH is disease relapse (Arinaga-Hino *et al.*, 2018).

Azathioprine use has been reported to be associated with increased risk in lymphoma and non-melanoma skin cancer, particularly in patients who have had solid-organ transplantation (Na *et al.*, 2016; Jiyad *et al.*, 2016), those with rheumatoid arthritis (RA) (van den Reek *et al.*, 2014) and inflammatory bowel disease (IBD) (Beigel *et al.*, 2014; Kotlyar *et al.*, 2015). Disease activity in RA has been linked with the increased risk of lymphomas (Baecklund *et al.*, 2006) whilst use of other immunosuppressants, particularly anti-TNF therapy in IBD have been contributory factors in IBD studies evaluating cancer-risk with thiopurines (Subramaniam and Pavli, 2012; Lemaitre *et al.*, 2017). There has however not been an increased trend in non-Hodgkin's lymphoma amongst AIH patients compared to IBD patients – whether this is due to lower AZA doses used in AIH (1-1.5mg/kg/day) compared to the higher AZA doses (2-2.5mg/kg/day) used in IBD, or other factors is not known (Czaja, 2013c).

In the Swedish study (mentioned above), there was increased incidence of skin cancer and lymphoma in the AIH cohort, but no clear cause/effect link could be established with azathioprine (Werner *et al.*, 2009).

Non-melanoma skin cancers may be the most common extrahepatic malignacies which develop in AIH (Czaja, 2013c). A single-centred American study (n=45 AIH patients) evaluated their cohort, comparing it to an age and sex-matched general population and found the SIR was increased by 28.5 for squamous cell cancer and 5.0 for basal cell cancer. Those who developed non-melanoma skin cancer were older on an average of 24 years and had late onset AIH (mean age of onset 66.0 versus 45.4 years, p=0.0003) (Leung *et al.*, 2010).

In a single-centre UK study (n=241) reported in abstract form (median follow-up 12 (0.5-43 years), there was an increased SIR of 41 (95% CI 20-75) for HCC, 2.94 (95% CI 1.08-6.4) for lymphoproliferative cancer and 2.95 (95% CI 1.65-4.87) for non-melanoma skin cancer (Hoeroldt *et al.*, 2016).

Although not clearly shown in AIH patients, due to the known increased risk of skin cancer associated with AZA, AIH patients on long-term AZA should be advised to exercise caution with regards to excessive sun exposure (European Association for the Study of the, 2015).

Other extrahepatic malignancies which have been described as case reports include Hodgkin's lymphoma following mycophenolate mofetil treatment (Adams *et al.*, 2010), Merkel cell carcinoma (primary neuroendocrine carcinoma of the skin) after three decades of immunosuppressive treatment for AIH (Lillis, Ceilley and Nelson, 2005) and large granular lymphocytic leukaemia after three years of therapy for AIH, ulcerative colitis and lymphocytosis (Kondo, Watanabe and Iwasaki, 2001).

Summary points

- AIH is a chronic relapsing disease which can progress to cirrhosis and liver failure requiring transplantation despite successful induction therapy.
- About 30% of patients with AIH present with cirrhosis whilst 14% (6-40%) of patients develop cirrhosis with follow-up.
- Initial studies of treated AIH were from tertiary centres and suggested optimistic prognosis with 10-year survival of 90% however this has been superceded by a subsequent landmark report from a non-tertiary centre (by Hoeroldt et al) revealing a poorer long-term outlook with a 20-year mortality rate (death or transplantation) of 52%. Subsequent studies concur with AIH patients having a two-fold higher mortality than the general population, particularly in the first year following diagnosis (mortality hazard ratio 5.76).
- Hepatocellular cancer develops in 1-4% of AIH cases with one study reporting the rate of HCC development being 9.3% in patients who were cirrhotic at presentation.
- There is an increased risk of extrahepatic malignancy with AIH with one study quoting a SIR of 28.5 and 5.0 for squamous cell cancer and basal cancer respectively.
- Immunosuppressant therapy (particularly azathioprine) may play a contributory role to the increased risk in malignancy with AIH though this has not been definitively shown in studies.

1.9 Factors Associated With Progression Of Disease And Poor Outcomes (Liver-Related Death Or Transplantation)

1.9.1 Cirrhosis (at presentation or at any time) and liver decompensation at presentation

Cirrhosis at presentation or at any time is generally associated with poor liver-related outcomes. Failure to attain normalisation of transaminases as well as repeated relapses have been identified as predictors for development of cirrhosis (Hoeroldt *et al.*, 2011; Gleeson, 2019; van den Brand *et al.*, 2019a).

In the study by Roberts and colleagues (n=128 with mean follow-up 129 ± 92 months), 29% had cirrhosis at presentation and 41% developed cirrhosis. The study reported 8% mortality for cirrhotics at 10 years. 78% of those with cirrhosis achieved biochemical and histological remission but 76% of these relapsed. 14% had treatment failure. 4/37 (11%) went on to have liver transplantation. The 10-year survival expectation for cirrhotics was excellent at 89% and was similar to non-cirrhotics (90%). The overall 10-year survival (cirrhotics and non-cirrhotics) was 93% and this was similar to that of an age- and sex-matched cohort from the population at large (94%) (Roberts, Therneau and Czaja, 1996).

Conversely, the study by Feld and colleagues (n=125 patients, mean follow-up of 8 years) found that 16/42 (38%) of the cirrhotic patients at presentation reached an end-point for liver disease (9 undergoing liver transplantation, 5 dying of end-stage liver disease, 2 dying of heart disease). In contrast, only 4/83 (5%) of patients without cirrhosis at presentation reached the endpoint (liver transplantation, death), $p<0.001$. 25 (81%) had complete response, 4 (13%) had partial response and 14 (45%) had a relapse (Feld *et al.*, 2005).

A study by Seela and colleagues (n=42) evaluated 42 patients, 5 had cirrhosis on biopsy at presentation. In the follow-up period (mean 16 yrs), 18 (42%) patients developed complications from cirrhosis (hepatic encephalopathy, oesophageal varices, ascites, hepatic hydrothorax), 1(2%) received liver transplantation and 1 (2%) died (Seela, Sheela and Boyer, 2005).

Histological evidence of cirrhosis is associated with a poorer survival and higher requirement for liver transplantation (Hoeroldt *et al.*, 2011; van den Brand *et al.*, 2019a; Feld *et al.*, 2005; Kirstein *et al.*, 2015). This however differs from the New Zealand study (Ngu *et al.*, 2013) and Japanese study (Yoshizawa *et al.*, 2012) which reported that neither advanced fibrosis nor

cirrhosis at first diagnosis were predictors of poor liver-related outcome. This difference may be accounted for by ethnic differences.

The Sheffield group reported presence of cirrhosis at any time (either at diagnosis, subsequent development or time of onset unknown) as well as liver decompensation being factors strongly associated with liver-related death or transplantation ($\chi^2 = 12$, $p = 0.01$) (Hoeroldt *et al.*, 2011).

A Swedish national study of AIH patients (n=473, median follow-up 7.2 years (0-45)] found that cirrhosis at diagnosis significantly correlated with later death or liver transplantation (p=0.014). In addition, having decompensated liver cirrhosis (defined as the presence of oesophageal varices and/or ascites) was significantly correlated with death or liver transplantation (p=0.02). The transplantation-free survival at 5 and 10 years was 93% and 84% respectively with the corresponding 5-year and 10-year survival for a subcohort of cirrhotic patients (n=42) calculated at 75.6% and 59.2% respectively (Werner *et al.*, 2010).

1.9.2 Multiple relapses/ Poor response / Long duration to achieve remission / elevated ALT

Patients who have multiple relapses are more likely to progress to cirrhosis, liver transplantation and death from liver failure (Czaja *et al.*, 1984; Czaja, 2002; Montano-Loza, Carpenter and Czaja, 2007a).

In the population-based study by Ngu and colleagues (n=133, median follow-up: 9 years (1-30), incomplete normalisation of ALT at 6 months ($p<0.01$), serum albumin, age at presentation of ≤20 years or > 60 years (p=0.01) were found to be independent predictors of poor liver-related outcomes. There were 32(24%) deaths, 13 (10%) liver-related deaths and 3(2%) requiring liver transplantations. 35% of the patients failed to normalise ALT at 6 months after diagnosis (Ngu *et al.*, 2013).

In the landmark report by the Sheffield group (n=245) with a median follow-up of 9.4 years (0.01 - 36), biochemical response rate was 93% after one year (Hoeroldt *et al.*, 2011). Failure to achieve normal serum ALT levels within 12 months of commencing treatment emerged as a significant adverse prognostic factor for liver-related death or transplantation ($\chi^2 = 20$, $p = 0.001$). De novo cirrhosis developed at a rate of 12% after 10 years and 34% after 20 years. There was a positive association on Cox regression analysis between de novo cirrhosis development and the increasing time to initial normalisation of serum ALT level ($p = 0.02$) and

number of relapses per decade (p = 0.005). The former (time to achieve normal serum ALT level) however did not emerge as a significant factor for poor liver-related outcomes (hazard ratio 0.79, CI, 0.59-1.08). On the other hand, a relapse rate of 4 or more episodes per decade was significantly associated with liver-related death or transplantation ($\chi^2 = 26$, $p < 0.01$).

In the study by Montano-Loza and colleagues (n=132), they found that patients who had relapsed repeatedly after initial treatment withdrawal developed cirrhosis more commonly than patients who sustained remission (18/48 [38%] versus 1/22 [5%], p=0.004) and those who relapsed once (18/48 [38%] versus 2/21 [10%], p=0.02). Relapse in this study was defined as increase in serum AST level to more than 3x the ULN. Hepatic death or need for liver transplantation was also more frequent in patients who had multiple relapses than those who sustained remission (13/64 [20%] versus 0/30 [0%], p=0.008) and those who relapsed once (13/64 [20%] vs 1/38 [3%], p=0.003). Therefore, the study concluded that multiple relapses are associated with a poorer prognosis than sustained remission or single relapse episodes (Montano-Loza, Carpenter and Czaja, 2007a). Using the same population, the authors also found that serum AST levels at the end of treatment were higher in patients who subsequently relapsed than in those who sustained remission (p=-0.03) and IgG levels were also higher in these patients prior to termination of therapy. They concluded that patients who are treated to normal AST, gamma-globulin and IgG levels have a lower frequency of relapse than others despite comparable histological findings (Montano-Loza, Carpenter and Czaja, 2007c).

Werner and colleagues evaluated the outcomes of a sub cohort of 163 patients with AIH – on comparing the initial response to therapy, a tendency for longer transplantation-free survival was noted for those who achieved complete compared to partial remission (p=0.067). The patients who did not achieve remission had significantly worse outcome with higher number of deaths and liver transplantations compared to patients with complete and partial remission (p<0.001). Further, those who developed cirrhosis at 12 and 24 months had significantly higher ALT levels than those who did not (p=0.031). The study evaluated the factors present at baseline which could predict the outcome (particularly death or liver transplantation) and found that only decompensated liver disease and/or cirrhosis (albumin, INR) at the time of diagnosis could predict death or liver transplantation (Werner *et al.*, 2010).

A Canadian study reviewed 163 patients with AIH (108 non-transplant, 55 who required liver transplantation). Complete remission (CR) was defined as improvement to 2x ULN whilst

incomplete response was some response but no CR in 3 years and no response was no improvement after 3 years. The study found that 87% of patients who achieved an early treatment response (ER) (defined as 50% improvement at 6 months of therapy) did not require liver transplantation compared to 16% who failed early treatment response who required liver transplantation. Multiple regression analysis revealed that patients who failed ER were more likely to proceed to liver transplantation compared to a patient who achieved ER (OR 16.8, 95% CI: 7.5-37.7) (Tan *et al.*, 2005).

Rapidity of treatment response has also been identified as a prognostic factor. The study by Czaja and colleagues (n=146 AIH patients) found that rapid responders (responded within or ≤ 6 months) had a lower frequency of progression to cirrhosis (18% versus 54%, p=0.03) and liver transplantation (2% versus 15%, p<0.05) compared to late responders (Czaja, 2009b).

In another study by Czaja and colleagues (n=107), patients who sustained remission after first treatment (n=22) were compared to those who relapsed after their first treatment. Remission in this study was defined as AST <3x ULN. The frequency of progression to cirrhosis during treatment were similar in both groups. Interestingly, none of those with sustained remission after the first treatment had developed cirrhosis during initial therapy but this was not statistically significant in comparison to those who relapsed (4/46, 9%). Death from hepatic failure or those who required liver transplantation was 4% in those with sustained remission versus 10% in those who continued to relapse (p=0.5) (Czaja, 2002).

In Miyake's study (n=84), 11 (13%) developed decompensated liver disease. The study's novel finding (using a time-dependent multivariate model) was that elevated serum ALT during the follow-up period (>40 IU/L) was significantly associated with progression of the disease. Other factors that showed a significant association with progression of AIH included the starting dose of corticosteroid (<20mg prednisolone/day) and relapse within 3 months after normalisation of ALT with initial treatment (Miyake *et al.*, 2005).

In another Japanese study by Yoshizawa and colleagues (n=203), repeated relapses was a significant risk factor associated with liver-related death on multivariate analysis (hazard ratio, 12.8, 95% CI, 1.5-109.9, p=0.02) (Yoshizawa *et al.*, 2012).

In Muratori's study (n=163) which included children, there was a statistically significant difference in outcomes between those who had complete remission (n=23) and those in

partial or no remission (n=66). In the complete remission group, there were no transplants or deaths with only one patient progressing from mild to severe histology as opposed to three requiring orthotopic liver transplantation (OLT), 3 listed for OLT, 5 deaths from hepatic failure, 14 progressing from mild to severe histology, 9 with severe histology progressing to cirrhosis, 1 with clinical cirrhosis (ascites) and 1 developing HCC ($p<0.0001$) (Muratori *et al.*, 2009).

1.9.3 Confluent necrosis and persistent inflammation on histology

In a study by Roberts and colleagues (n=128 AIH patients), patients with confluent necrosis on diagnostic histology were found to progress more commonly than patients with only periportal hepatitis (53% versus 32%, $p=0.05$). Additional Cox-model time-dependent univariate analysis found that patients with confluent necrosis (periportal hepatitis and bridging necrosis or multilobular necrosis) at diagnosis had 1.9-fold relative risk to develop cirrhosis ($p<0.05$) compared with patients with only periportal hepatitis at diagnosis (Roberts, Therneau and Czaja, 1996). Persistent inflammation on liver histology while on treatment have been associated with progressive fibrosis or development of cirrhosis (Czaja and Carpenter, 2004).

Czaja et al's study in 2003 found that elevation of AST 2x above ULN was associated with histological signs of disease activity in half of the patients. Even in patients with a normal transaminase level, 19% had histological features of active AIH (Czaja and Carpenter, 2003). This may be due to the fact that histological improvement lags behind clinical and biochemical improvement by 3-8 months (Czaja, Wolf and Baggenstoss, 1981; Luth *et al.*, 2008).

The study by Verma et al which aimed to determine predictors of relapse in patients with AIH evaluated 14 AIH patients' histology, comparing relapsers versus non-relapsers. They found that histologically, those who relapsed had a higher fibrosis, portal and total plasma cell score compared to the non-relapsers. There was however no significant different in the Histology Activity Index (HAI) scores between the groups. Logistic regression analysis revealed that portal plasma cell score was the only independent histological predictor of relapse: OR 10.6 (95% CI 1.0-107), $p=0.04$) (Verma *et al.*, 2004).

Luth et al assessed 132 biopsies from 82 patients with AIH. A histologic activity index (HAI) score of ≥4/18 has been defined as more than minimal histologic activity and is considered to be a risk factor for disease progression. This study found that presence of both elevated ALT

and IgG were associated with high inflammatory activity (histologic activity scores ≥6) with 99% sensitivity. Histologic remission is reliably indicated by normalisation of both serum parameters but about half of the patients with normal serum parametres still showed residual histologic activity of HAI 4 or 5. However, the patients with HAI scores 4 or 5 were at significantly lower risk of fibrosis progression than patients with scores ≥6 ($p<0.02$, OR 14.2). The study concluded that histologic activity seemed to be reliably indicated by elevated serum parametres. Normalisation of serum parametres is not a reliable marker for complete histologic remission (HAI 1-3) however normalised serum parametres identified patients at low risk of fibrosis progression (Luth *et al.*, 2008).

Dhaliwal et al studied 120 patients who received immunosuppressive treatment and underwent a follow-up liver biopsy after at least 6 months of sustained biochemical remission (normal ALT and globulin). Persisting histological activity (Ishak HAI ≥4) was found in 55 (46%) of patients. On comparing biochemistry, this group had a higher serum ALT (24 vs 18 IU/L, $p=0.003$) and AST (27 vs 23 IU/L, $p=0.03$) concurrent to the follow-up biopsy compared with patients in histological remission (HAI ≤3). There was less frequent regression of fibrosis on follow-up biopsy in this group compared with those who achieved histological remission (32 vs 60%, $p=0.004$). Mortality was increased in this group as well with SMR 1.4 vs 0.7, $p<0.05$. The excess mortality was due to liver disease. Persisting histological activity was independently associated with all cause death/transplantation (HR 3.1, 95% CI 1.2-8.1, $p=0.02$) on multivariate analysis. The variables of liver-related death and transplantation fell short of significance on this analysis (HR 9.7, 95% CI 0.84-111.6 $p=0.07$) (Dhaliwal *et al.*, 2015).

Conversely, in Putra et al (2016)'s study (40 liver biopsies from 20 consecutive AIH patients who underwent repeat biopsy), 70% of patients showed improved aminotransferase levels and there was no significant correlation found with the inflammatory and fibrosis progression ($p=1.00$, $p=0.116$ respectively). It has to be noted that there were 3 (15%) patients with atypical initial biopsies (one with overlap AIH/PBC and another suspicious of AIH/PBC and another with mild portal lymphocytic infiltrate). On repeat biopsies, 40% of patients showed pathology other than AIH (3 steatohepatitis; 5 cholangiopathy features). 7 (35%) patients showed progression of fibrosis, all showing newly diagnosed cirrhosis (Putra, Toor and Suriawinata, 2016).

1.9.4 Age of diagnosis

The Sheffield study identified age at presentation as a risk factor for all-cause mortality (hazard ratio 1.05, 95% CI: 1.02-1.07, p<0.001) but not for liver-related death or transplantation (hazard ratio 1.022, 95% CI: 0.997-1.05, p = 0.061). Patients diagnosed with AIH before the age of 45 had a higher mortality risk with standardised mortality ratio of 5.16 for all-cause mortality (Hoeroldt *et al.*, 2011).

1.9.5 Female Gender

The impact of gender has been explored in several studies. Notably, one study by Al-Chalabi and colleagues (238 patients with AIH (187 women)) found that women fared worse than men. The study was aimed at looking at outcomes of men. Male patients had a higher relapse rate (71% (men) versus 55% (women) experiencing at least one relapse, p=0.0591) and younger median age of disease onset (39 years versus 49 years in women, p=0.0589). This was postulated to be related to the increased prevalence of HLA A1-B8-DR3 in men. The study found that men had a significantly better cumulative overall survival (Log Rank test p=0.024, hazard ratio 0.467) compared to women although the cumulative liver-related survival in men and women with AIH did not differ significantly (Log rank test, p=0.109) (Al-Chalabi *et al.*, 2008b). In a later study by Ngu and colleagues looking at 133 AIH patients (26% males), male patients were found to be significantly more likely to have cirrhosis at diagnosis compared to female patients (OR 2.78, 95% CI: 1.23-6.18, p=0.01). Gender did not however emerge as a significant factor associated with poor outcomes (Ngu *et al.*, 2012). Similarly, in a study by Kirstein and colleagues (Hannover, Germany, n=354), gender did not emerge as a significant factor associated with remission or poor outcomes (Kirstein *et al.*, 2015). In the study by Hoeroldt and colleagues, male gender was not significantly associated with liver-related death or transplantation (hazard ratio 0.80 [C.I. 0.32-2.38]) (Hoeroldt *et al.*, 2011). In a recent Swedish study, men were diagnosed earlier (p<0.001) and died younger than women (p = 0.002) though no gender differences were found when evaluating transplant-free, overall survival and liver-related death (Danielsson Borssén *et al.*, 2017).

1.9.6 Ethnicity – African-American men

A retrospective study covering a 10-year period (1996-2006) looked at 101 patients with AIH (36.6% were black in ethnicity) and found that black patients had higher proportions with

cirrhosis (56.7% versus non-blacks, 37.5%, p=0.061), liver failure at first presentation (37.8% versus 9.3%, p=0.001) and referral for liver transplantation (51.3% versus 23.4%, p=0.004). Black patients were less likely to achieve remission (75.8% versus 90%, p=0.016) and had a four-fold increased mortality compared to non-black patients (24.3% versus 6.2%, p=0.009). On Kaplan Meier analysis, the probability of development of poor outcome was significantly higher in blacks (p=0.003). Three independent predictors of poor outcome were identified – black ethnicity, presence of cirrhosis and fibrosis stage at presentation. 85.7% of black males had poorer outcomes (85.7%) compared to 50% of black females with poor outcomes (p=0.002). Non-black females (27%) were more likely to have poorer outcome compared to non-black males(12.5%) (p=0.001). It was noted that non-compliance was higher in blacks (20.6% versus 8.3%, p=0.019) which could partially explain the result. However even on excluding the black patients with non-compliance and poor outcomes, a poor outcome was still more likely in blacks (51.3% versus 23.4%, p=0.004). Black patients had a younger age of presentation, indicating a more aggressive disease. Access to healthcare did not appear to be a contributory factor (Verma *et al.*, 2004).

In the Sheffield study, non-white ethnicity did not emerge as a factor associated with liver-related death or transplantation (hazard ratio 0.0.5 [C.I. 0.00 – 74.0]) (Hoeroldt *et al.*, 2011).

More recently, a retrospective multicentre study (Netherlands and UK) described their findings on 88 black patients with AIH compared to 897 white patients with AIH. Black patients presented at a younger age (0.007), had higher IgG levels (mean 31.0 mg/dL vs 27.5 mg/dL, p=0.04), higher proportion with SLE (10% versus 2%, p≤0.001) and had increased risk for liver transplantation and liver-related death (hazard ratio 2.4, 95% CI 1.4-4.0, p<0.001). Overall mortality was similar between both groups. There was there were no significant differences between autoantibody profiles, IAIHG group scores, sex distribution of disease nor any difference in proportions of patients with response to therapy (86% vs 91%, p=0.20) or rate in relapse (57% vs 50%, p=0.3) (de Boer *et al.*, 2019).

1.9.7 Type 2 AIH

Type 2 AIH have an increased risk for relapse compared to type 1 patients. A retrospective analysis on outcomes of orthotopic liver transplantation (OLT) (n=9 type 1 AIH, 7 type 2 AIH) found that Type 2 AIH patients had a significantly higher incidence of cirrhosis at the time of diagnosis, more corticosteroid-resistance and higher Child-Pugh score at time of liver

transplantation. Three of the type 2 AIH patients required emergency OLT whilst all of the type 1 AIH patients had elective OLT (Cattan *et al.*, 2005).

1.9.8 SLA positive AIH

Patients with SLA positive AIH have been reported to relapse more frequently (Kanzler *et al.*, 1999; Baeres *et al.*, 2002). In addition, a study that contained mostly paediatric patients with AIH (33 type 1 AIH and 31 type 2 AIH) found SLA positivity in 58% of patients with type 1 and type 2 AIH. SLA positivity conferred a worse prognosis as the disease course was more severe with worse histological findings, longer duration to achieve remission, more relapses and poorer outcomes (liver transplantation or death) compared to SLA negative patients (Ma *et al.*, 2006). The German study (n=354) also identified SLA antibodies as a factor significantly associated with reduced overall and liver-transplant-free survival (p=0.037) (Kirstein *et al.*, 2015).

The experiences by AIH centres with emphasis on outcomes are summarised in **Table 8**.

Summary points

- In patients with AIH, cirrhotic patients (either at presentation or during follow-up) and those presenting with decompensated disease had a higher rate of liver transplantation, development of complications from cirrhosis and death from end-stage liver disease (approximately 40% of patients reaching liver-related end-points).
- Patients who have multiple relapses, have poor response to therapy/failed therapy, require long duration to achieve remission or have elevated ALT are more likely to progress to cirrhosis, liver transplantation and death from liver failure.
- Confluent necrosis (periportal hepatitis and bridging necrosis or multilobular necrosis) and persistent inflammation on histology are associated with AIH disease progression (progressive fibrosis or development of cirrhosis).
- Portal plasma cell score is the only independent histological predictor of relapse.
- Age at diagnosis (<45 years) has been shown to be associated with all-cause mortality in AIH patients but not with liver-related death or transplantation.
- Female gender has been shown in one study to be associated with poorer cumulative liver-related survival in patients with AIH however three other studies did not find gender emerging as a significant factor associated with poor outcomes.

- Black ethnicity is associated with reduced rates of disease remission, higher proportions of cirrhosis, liver failure at first presentation, referral for liver transplantation and 4-fold increased mortality, even after excluding non-compliance (which is noted to be higher in blacks) in one study (Verma and colleagues). A subsequent study (de Boer and colleagues) did not find any difference in mortality.
- Black patients have a younger age of presentation indicating a more aggressive disease. Access to healthcare does not appear to play a role.
- Type 2 AIH has an increased risk for relapse compared to type 1 patients with higher incidence of cirrhosis at time of diagnosis, more corticosteroid-resistance and higher Child-Pugh-score at time of liver transplantation.
- SLA positive AIH have increased frequency of relapses, conferring a worse prognosis in disease course with more severe histological findings, longer duration to achieve disease remission and poorer outcomes.

Table 8: AIH Centre(s) Experiences / Population-based studies

First author, journal, year, country	Patients (n), Follow-up Duration	Number (%) presenting with cirrhosis	Number (%) treated, Treatment	Definition of remission, Remission (%)	Definition of relapse, Relapse (n(%))	Number (%) of patients which developed cirrhosis	OLTx	Survival/Mortality/ Poor outcome n (%)
Schvarcz, J of Hepatol, 1993, Sweden	33 Median F/up: 86 months, ~7 yrs (22-148)	16 (48%) had nodular fibrosis or cirrhosis on histology	32 (97%) received Pred +/- AZA (No Rx: 1)	Normal ALT, AST, IgG 28/32 (87.5%)	Relapse after complete withdrawal of Rx 4 (12.5%)	None	None	94% 5 year survival 90% 10 year survival (No deaths due to liver failure or portal HT)
Johnson, NEJM, 1995, UK	72 Median F/up: 12 yrs (3-25)	27 (38%)	All patients received Pred + AZA	Absence of symptoms suggestive of relapse and normal AST + ɣ-globulin +/- liver Bx showing minimal inflammation 60 (83%) on AZA alone	AST ≥3x ULN or ↑in AST + ↑ ɣ-globulin AND re-emergence of symptoms 12 (17%)	Not reported	Not reported	13% mortality (1 liver failure)
Newton, Age & Ageing, 1997, UK	54 Mean F/up: 6.5 yrs Range: 1-14 yrs	30% (<65 yrs) 36% (≥65 yrs)	88% of patients age <65 and 58% of patients (≥65 yrs) received Pred +/- AZA	Not reported	Not reported	26% in both groups		6 (11%) overall mortality 3(6%) died from complications of chronic-liver disease
Parker, Q J Med 1997, UK	41 (11 year period)	14 (34%)	39 (95%) received Pred +/- AZA	**Complete remission:** Normal LFTs 28 (68%) **Partial remission:** Improvement in LFTs: 7 (17%)	Relapse (IAIHG 1993) 7 (17%)	1 (2%)	Not reported	5 year survival: 64%

First author, journal, year, country	Patients (n), Follow-up Duration	Number (%) presenting with cirrhosis	Number (%) treated, Treatment	Definition of remission, Remission (%)	Definition of relapse, Relapse (n(%))	Number (%) of patients which developed cirrhosis	OLTx	Survival/Mortality/ Poor outcome n (%)
Roberts, Gastro-enterology, 1996, USA	128 Mean F/up: 129 ± 92 m [~11 yrs] Range: 2-320 m 18% 20 yrs f/up	37 (29%)	78 (61%) received Prednisone + AZA 50 (39%) – higher doses of pred alone	IAIHG 1993* (normal LFTs at 1 yr or <2xULN at 6/12 and improvement of symptoms) **CIRRHOSIS:** (Remission) 29 (78%) **NO CIRRHOSIS:** (Remission) 69(76%)	↑AST or ALT >2xULN of histological evidence of activity +/- symptoms **CIRRHOSIS:** Relapse: 22/29 (76%) **NO CIRRHOSIS:** Relapse: 51/69 (74%)	36/91 (41%)	9 (7%) (all had cirrhosis)	**CIRRHOSIS:** 97% (5 yr survival) 89% (10 yr survival) 8% mortality for those with cirrhosis at entry 6% mortality for those who developed cirrhosis **NON-CIRRHOSIS:** 94% (5 yr) 90% (10 yr) 7% mortality for non-cirrhotics
Kanzler, Z Gastro, 2001, Germany	103 Mean F/up: 95 (12-405 months) [8 yrs] [5 excluded as presented with liver failure requiring OLTx]	30 (29%)	103 (100%) 7 – pred only 91 (pred+AZA, 1.5mg/kg) 4 (cyclophos)	CR: normal LFTs, IgG & minimal inflam. on liver Bx at 1 yr 91% CR Sustained remission after Rx withdrawal: 25% (within 1st yr) 6.8% long term	↑AST or ALT >2xULN of histological evidence of activity +/- symptoms Relapse during maintenance: 36% Relapse after Rx withdrawal: 75% :	Not reported	3 (3%)	5 – cardiac disease 1 – HCC (but beyond F/up period) 1 suicide Survival did not differ from norm population: 99% (5 yrs) 98% (10 yrs)

First author, journal, year, country	Patients (n), Follow-up Duration	Number (%) presenting with cirrhosis	Number (%) treated, Treatment	Definition of remission, Remission (%)	Definition of relapse, Relapse (n(%))	Number (%) of patients which developed cirrhosis	OLTx	Survival/Mortality/ Poor outcome n (%)
Czaja, Hepatology, 2002, USA	107 Mean F/up: 127 ± 25 m [~10 yrs]	27/105 (26%) – cirrhosis on Bx	All patients received Prednisone +/- AZA	Sustained remission: Absence of symptoms & AST <3x ULN. 46 (43%) [Remission after 1st Rx (22) + Remission after re-treatment (24)]	↑AST >3x ULN Relapse after 1st treatment: 85(79%)	14 (13%)	7 (7%)	4% mortality* (in those with sustained remission) versus 10%* (in those who continued to relapse) (p=0.5) *death fr hepatic failure/required OLTx in those with sustained remission
Verma, Am. J of Gastro, 2004, USA	71 Median F/up: 52 m (18-336) [~4 yrs]	20 (28%)	All patients received Pred +/- AZA - 10 required additional ciclosporin (8 achieved remission)	Sustained remission: AST/ALT<2xULN 67/69 (97%)	AST/ALT >2x ULN 30 (42%) **i) Developed cirrhosis on Rx:** Relapse: 11/12 (92%) **ii) No Cirrh on Rx:** Relapse: 11/19 (58%) *p=0.04 (i. vs ii.)* **iii) Cirrhosis at Dx:** Relapse: 8/9 (89%)	14(20%)	None	Deaths: 3 (4%) 11 (15%) poor outcomes: 4 – liver failures (2 deaths) 2 variceal bleeds 3 ascites 1 decompensated cirrhosis 1 HCC (died)

First author, journal, year, country	Patients (n), Follow-up Duration	Number (%) presenting with cirrhosis	Number (%) treated, Treatment	Definition of remission, Remission (%)	Definition of relapse, Relapse (n(%))	Number (%) of patients which developed cirrhosis	OLTx	Survival/Mortality/ Poor outcome n (%)
Feld, Hepatology, 2005, Canada	126 (2 also were HCV RNA +ve) Mean F/up: 8+/-6.86 yrs (0.6-29.3) for symptomatic	42 (33%)	98 (78%) were treated -33(26%) with steroids only -65 (52%) steroids + AZA	CR: symptomatic improvement a/w normalised AST/ALT,bili,IgG within 1 yr of Rx OR at least 50% improvement of all LFTs during Mont 1 of Rx with AST/ALT falling to <2x ULN within 6 months of starting Rx Complete Response: 83/125 (66%)	Relapse defined by IAIHG 1999 criteria Relapse 34/125 (27%) Partial response: 13/125	Not reported	10 (8%)	8 (7%) died fr complications of liver disease 2 (2%) died of MI 91% 5-year survival to liver related death or OLT 85% 5-year survival to liver related death or OLT
Granito, APT, 2005, Italy	76 Median: 5 yrs 1-16) and 8 yrs (1-14) for Grp A and B respectively	Grp A: 20% Grp B: 20% **Grp A:** Age ≥65 yrs: 20 patients; **Grp B:** Age <65 yrs: 56 patients)	All received methylpred +/- AZA	IAIHG 1999 Complete remission: 64 (84%) Older grp: 18 (90%) Younger grp: 46 (82%), p=ns	IAIHG 1999 Relapse: 33 (43%) Older grp: 7 (35%) Younger grp: 26 (56%), p=ns		1 (Grp A)	Deaths: 3 (4%) from end-stage liver disease (Grp B) - 2 sepsis - 1 hepatorenal Sx p=ns
Miyake, J of Hep, 2005, Japan	84 Median F/up: 70.5 m (16.2-163) [~6 yrs]	7(8%) on biopsy	76 (90%) had either Pred or Pred + AZA or Pred +UDCA 8 – UDCA only	Not formally defined; Normal ALT for 2 years was followed by pred withdrawal	2x ULN following normalisation of ALT 33 (39%)	Decompensated liver cirrhosis (progression)@ 11(13%)	None	11 (13%) with varices & splenomeg developed decompensated liver cirrhosis (Child's B or C)

First author, journal, year, country	Patients (n), Follow-up Duration	Number (%) presenting with cirrhosis	Number (%) treated, Treatment	Definition of remission, Remission (%)	Definition of relapse, Relapse (n(%))	Number (%) of patients which developed cirrhosis	OLTx	Survival/Mortality/ Poor outcome n (%)
Seela, Liv Int, 2005, USA	42 Mean F/up: 16 yrs (7-43) Median:13.5 yrs	5 (12%) (1 overlap PBC, 1 overlap PSC)	All received Prednisone + AZA	Normalisation of LFTs 36 (86%)	ALT >ULN +/- symptoms after both Pred and AZA were withdrawn 36 (86%)	20(48%) progressed to cirrhosis based on biopsy, liver spleen scans or development of oesophageal varices)	1 (2%)	Death: 1 (2%) 4 hepatic encephalopathy 8 – varices (1 bleeding oesophageal varices + TIPS) 5 Ascites 1 hep. Hydrothorax 6 evaluated for OLTx
Al-Chalabi, J of Hep, 2006, UK	164 (43 vs 121) Median F/up: 9 yrs (1-28) for Grp 1 (age > 60yrs) & 14 yrs (1-33) for Grp 2 (age 6-59 yrs)	Grp1: 13/43(30%) Grp 2: 33/119 (28%)	All received Pred +/- AZA	IAIHG 1999 Complete response: Grp 1: 95% Grp 2: 92% (p=ns)	IAIHG 1999 Any relapse: Grp 1: 42% Grp 2: 70% (p=0.002)	Not defined	See next column	Total deaths/OLTx: Grp 1: 13(30%) Grp 2: 27 (22%) Liver-related deaths or OLTx: Grp 1: 5(12%) Grp 2: 18(15%) (p=ns)
Czaja & Carpenter, Hepatology, 2006, USA	205 50% had 5-yr F/up	53 (26%)	87% had steroid-based Rx	Absence of symptoms, Normal ALT or near norm <2xULN + histological improvement to no/minimal inflam. Activity. 104 (51%) 22% had sustained remission (no symptoms, normal ALT)	82 (40%)	Not reported	19 (9%) hepatic death or need for OLTx	19 (9%) hepatic death or need for OLTx

First author, journal, year, country	Patients (n), Follow-up Duration	Number (%) presenting with cirrhosis	Number (%) treated, Treatment	Definition of remission, Remission (%)	Definition of relapse, Relapse (n(%))	Number (%) of patients which developed cirrhosis	OLTx	Survival/Mortality/ Poor outcome n (%)
Floreani, APT, 2006, Italy [3 centres]	73 (3 centres) Mean F/up: 91 ± 61m [~8 yrs]	24 (38%) [2 went on to have OLTx]	All received Pred + AZA	Remission not defined 60 (82%) (*BUT on Rx)	Not reported	Not reported	7 (9.5%)	Cumulative Transplant-free probability of survival: 73.5% (at 280 months) 9 varices; 4 ascites 1 HCC; no deaths recorded
Montano-Loza, Hepatology, 2007, USA	214 F/up not stated	49 (23%)	All received Pred +/- AZA	Absence of symptoms, normal AST or near-normal AST and histology showing minimal or no inflammatory activity 200 (93%)	No definition of relapse; Treatment failure – progressive ↑AST or Bili Rx failure: 14(7%) – 10 had resolution with higher dose AZA & Pred	9 (4%) 9/11 of Rx failures developed cirrhosis	3 (1%) [3/14 Rx failures (21%) required OLT]	Deaths: 4 (2%) 2-liver failure 1 variceal bleed 1 HCC [29% of Rx failures died]
Montano-Loza, Liver Int and American J of Gastro, 2007, USA	132 F/up: 133 ± 19 m	Not reported	All patients had pred+AZA or pred in higher doses alone	Sustained remission: Absence of symptoms and AST <3xULN 30 (23%) sustained remission	AST 3x > ULN 102(77%)	21 (16%)	See next column	Death from hep failure/OLT: 13 (20%) in those with multiple relapse vs 0% with sustained remission (p=0.008) or 1(3%) with single relapse P=significant

First author, journal, year, country	Patients (n), Follow-up Duration	Number (%) presenting with cirrhosis	Number (%) treated, Treatment	Definition of remission, Remission (%)	Definition of relapse, Relapse (n(%))	Number (%) of patients which developed cirrhosis	OLTx	Survival/Mortality/ Poor outcome n (%)
Werner, Scand J Gastro, 2008, Sweden, National survey (11 centres)	473 (2 had HCV, 47 AIH/PBC, 10 AIH/PSC, 2 AIH-autoimmune cholangitis) Median F/up: 8.8 yrs (1-45)	33% of 398 (with Bx)	See study 2010	See study 2010	See study 2010	See study 2010	See study 2010	31 (7%) Ascites 17 (4%) Oesophageal Varices 8 (2%) Variceal Bleeding 6 (1%) HE
Al-Chalabi, Clin Gast & Hep, 2008, UK	213 F/up: Not specified	Grp 1: AST<2xULN (AST <100) (n=26): **65%** cirrhosis at Dx Grp 2: AST 2-10xULN (AST 100-500) (n=71): **56%** cirrhosis Grp 3: AST>10x (AST>500) (n=116): **19%** cirrhosis	All patients received Pred +/- AZA	Complete response: IAIHG criteria (1993) 95% in all grps had complete response at 1 yr	AST/ALT >2x ULN or liver Bx showing active disease +/- symptoms % with >1 relapse: Grp 1: 52% Grp 2: 60% Grp 3: 63% No difference in relapse/rate of relapse between groups	Not reported	See next column	Grp 1 & 2 have significantly worse outcome (risk of OLT /death) than grp 3 (60% survival vs 82%, p=0.01, OR 2.1) **Grp 1 & 2** - More likely to present with ascites (p<0.001), haemetemesis (p=0.009) and cirrhosis (p<0.001) % Liver related deaths/transplant: Grp 1: 78% Grp 2: 61% Grp 3: 47%
Muratori, J of Hep, 2009, Italy	163 F/up: 67(10-180)	35 (23%)	All patients received Pred +/- AZA	Complete remission (CR): Disappearance of symptoms and normal ALT and gamma-globulin CR: 42 (26%)	Partial remission (PR): ULN < ALT< 2x ULN: 77 (47%) No remission: 44 (27%)	9 (severe histology to cirrhosis)	3 (in those who had partial remission)	Deaths: 5 (3%) (hep failure) - 3 listed for OLT - 1 ascites - 1 HCC

First author, journal, year, country	Patients (n), Follow-up Duration	Number (%) presenting with cirrhosis	Number (%) treated, Treatment	Definition of remission, Remission (%)	Definition of relapse, Relapse (n(%))	Number (%) of patients which developed cirrhosis	OLTx	Survival/Mortality/ Poor outcome n (%)
Werner, Scand J Gastro, 2010, Sweden [National survey (11 centres)]	473 (subcohort of 163 for survival plots and progression) Median F/up: 7.2 yrs (0-45)	140 (30%)	93% Rx with steroids at any time; 66% with thiopurine and steroids at 10 yrs	Normal ALT at 1st yr: 282 (60%)	Partial remission (ALT <5x ULN): 133 (28%) No remission: 11(2%)	15/163 (9%)	24 (5%)	OLTx-free survival: 93% at 5 yrs 84% at 10 yrs 61 (13%) died Ascites 15% of 304 Oes varices 18% of 222 Var. bleeding: 10% HE 7% of 300
Hoeroldt, Gastroente-rology, 2011, UK	245 Median F/up: 9.4 yrs (0.01-36)	89(36%) Decompensa-tion#: 47 (19%) at presentation #variceal bleeding or ≥2 of the following: varices at endoscopy, visible ascites, marked coagulopathy (>4s) and HE	224 (91%) treated with steroids at any stage AZA >4 weeks (188/208) - 90%	Biochemical remission: Normal ALT (≤55 IU/L) within 1 yr of Dx 229 (93%)	IAIHG 1999: 1) ↑ ALT 2x above nadir or ULN with symptoms or 2) ALT >3x ULN 31% - 5 years 41% - 10 years 51% - 20 years	24/146 (16%) De-novo cirrhosis dev: 12% after 10 yrs 34% after 20 yrs	11 (4%)	Mortality: 18% at 10 yrs 52% at 20 yrs Liver-related deaths: 30 (12%) - 8 HCC - 21 decompensation - 1 AZA-related bone-marrow failure & sepsis

First author, journal, year, country	Patients (n), Follow-up Duration	Number (%) presenting with cirrhosis	Number (%) treated, Treatment	Definition of remission, Remission (%)	Definition of relapse, Relapse (n(%))	Number (%) of patients which developed cirrhosis	OLTx	Survival/Mortality/ Poor outcome n (%)
Yeoman, Hepatology, 2011, UK	72 F/up: Not available (10 lost to follow-up after median 91 days)	34 (47%)	All patients received Pred + AZA	Biochemical remission (normal AST and globulin) 62 (89%)	Treatment failure: Requirement of 2nd-line Rx within 90days, progression to subacute liver failure or death during index presentation: 13/72 (18%) Biochemical relapse: 11% of surviving non-responders 22% responders	Not reported	3 (4%)	Deaths*: 5 (7%) - 2 from sepsis post OLTx - 1 tac responder but died later - 1 sepsis, multiorgan failure - 1 unrelated death (haemorrhage fr C-section 7 yrs later) *(4 were Rx failures)
Malikzadeh, Hepat. Mon, 2012, Iran	102 Mean F/up: 60 months (sd 38.4) [5 yrs]	42 (41%)	Pred (30-50) and AZA 50, then 2mg/kg Cyclosporine if non-responsive	CR: Normal LFTs, IgG 80 (79%) at 1 yr 53 (52%) at 5 yrs	↑ ALT 3x Rx failure (clinical decompensation): 22 - 6 OLT, 3 died - 13 - decomp [note- 16 non-compliant to Rx]	27 (34%)	6 (6%)	6 OLT 3 deaths whilst waiting for OLT 10 yr survival: 96%
Yoshizawa, Hepatology 2012, Japan	203 Mean F/up: 131 (13-431 months) [10.9 yrs]	26 (12.8%)	Pred 50-60 followed by AZA 50 18 (severe hep)– methylpred 0.5-1g (3 days)	Normal ALT, IgG, Bili for >=6 months 203 (100%)	↑ ALT 2x (>90) 48 (24%)	Not reported	Those requiring OLTx were excluded	Death: 22 (11%) Liver-related death: 7 (3%) Kaplan Meir-curve: Survival similar to gen population

First author, journal, year, country	Patients (n), Follow-up Duration	Number (%) presenting with cirrhosis	Number (%) treated, Treatment	Definition of remission, Remission (%)	Definition of relapse, Relapse (n(%))	Number (%) of patients which developed cirrhosis	OLTx	Survival/Mortality/ Poor outcome n (%)
Ngu, Hepatology, 2012, New-Zealand, Population-based study	130 (also had 70 PBC, 81 PSC) Median F/up: 7 yrs (1-29)	Not reported	Not reported	Not reported	Not reported	Not reported	2 (3%)	Deaths = 31(24%) Malignancy = 31(24%) SMR: Hepatobiliary cause of death: 42.3 & all cause: 2.1. (compared to gen. population)
Ngu, Hepatology, 2013, New Zealand, Population-based study	133 Median F/up: 9 yrs (1-30)	45(34%)	All received Pred +/- AZA	65% had complete normalisation of ALT at 6/12 (no IgG)	Not reported	Not reported	3 (2%)	Deaths: 32 (24%) Liver related deaths: 13 (liver failure 11, HCC 2)
Delgado, J of Dig Diseases, 2013, Israel, Multi-centre	100 F/up: 15 yr period	21/94 (22%) with cirrhosis on Bx - Includes 15 PBC/overlap	98% treated with steroids +/- AZA (Other Rx: budesonide & UDCA)	Complete remission: Normal ALT, AST, bili, gamma-glob +/- normal pathological findings or minimal liver inflammation 56/98 (57%)	Relapse: clinical and biochemical flare off Rx 11 (20%) relapsed 42 partial response/failure of Rx (AASLD 2010 definition)	Not reported	Not reported	Deaths: 7 (7%) (liver-related) 97% estimated 1-yr survival 90% estimated 10-yr survival

First author, journal, year, country	Patients (n), Follow-up Duration	Number (%) presenting with cirrhosis	Number (%) treated, Treatment	Definition of remission, Remission (%)	Definition of relapse, Relapse (n(%))	Number (%) of patients which developed cirrhosis	OLTx	Survival/Mortality/ Poor outcome n (%)
van Gerven, J of Hep, 2013, Netherlands, Multi-centre	131 Median F/up after drug withdrawal: 9 yrs (2-30)	18 (14%)	All received either Pred alone or Pred + AZA	Sustained remission: absence of symptoms & normal ALT for at least 2 yrs (And no relapse after drug withdrawal) Sustained remission 14 (11%)	Relapse: ALT >3x ULN and/or IgG > 2g/dL (AASLD 2010) Relapse & loss of remission after discontinuation of Rx 2 yrs later: 59% at 1 yr 73% at 2yrs 81% at 3yrs, 88% at 5 yrs	10 (8%) progressed in fibrosis or cirrhosis	None	No liver related deaths, 1 cardiac death
van Gerven Scand J Gastro, 2014, Netherlands, Epidemiolo-gical study	1313 (1110 pure AIH, 193 overlaps) F/up: 10 (0-44)	From pure AIH: 108 (12%) cirrhosis at presentation	627 (49%) used COMB; 34% used pred or AZA mono-Rx	Not reported	Not reported	Not reported	19/1110 (2%)	59 (9%) with decompensated liver cirrhosis 7(1%) HCC
Gronbaek, J of Hep, 2014, Denmark (Population-based)	1721 (11,832 years of follow-up, median 5.9 years per patient)	28% (of 1318 who had Bx)	Not reported	Not reported	Not reported	Not reported	27 (2%)	Deaths: 129/334 (39%) - liver related including 4% fr HCC 1st year of Dx: 6x higher mortality than gen. population

First author, journal, year, country	Patients (n), Follow-up Duration	Number (%) presenting with cirrhosis	Number (%) treated, Treatment	Definition of remission, Remission (%)	Definition of relapse, Relapse (n(%))	Number (%) of patients which developed cirrhosis	OLTx	Survival/Mortality/ Poor outcome n (%)
Kirstein, Hepatology, 2015, Germany	354 (6 had overlap with PSC, 33 overlap with PBC) Median F/up: 10 yrs (1m-40yrs)	25%	255/259 (98%) with available Rx data had Rx: Pred or Budesonide +/- AZA	Complete biochemical remission (CBR): normalisation of ALT 77% of 319 (CBR)	56% relapsed	7/56 (13%)	Not clear	Dx <18 yrs, histological cirrhosis at 1st Dx & SLA/LP Abs are major risk factors for poor short and long-term outcome.
Hartl, J of Hep, 2015, Germany	28 (1 AIH/PBC and 1 AIH/PSC) Median duration of Rx: 48.5 m (35-179)	7%	28 (100%)	Repeatedly normal ALT and IgG 15(54%) remained in long-term remission after 28m (17-57) after Rx withdrawal	Relapse: Rise in ALT and IgG > ULN requiring retreatment after drug-withdrawal 13(46%) required re-treatment	Not reported	Not reported	Not reported
Muratori, Digestive & Liver Dis, 2016, Italy	282 Median f/up: 48 m (3-288) for complete response; 70 m (3-372) for incomplete/absent response	37 (13%)	All patients received methylpred +/- AZA	Responder: Normal ALT 166 (59%)	Relapse during Rx: 147/206(52%) Incomplete response (abnormal ALT <2xULN); No response (abnormal ALT >2xULN); 116 (41%) had incomplete/absent response	Not reported	Not reported	Not reported

First author, journal, year, country	Patients (n), Follow-up Duration	Number (%) presenting with cirrhosis	Number (%) treated, Treatment	Definition of remission, Remission (%)	Definition of relapse, Relapse (n(%))	Number (%) of patients which developed cirrhosis	OLTx	Survival/Mortality/ Poor outcome n (%)
Than, Hepatol Int, 2016, UK & Singapore	159 (**C**aucasian) 40 (**A**sian) 57 (**S**'porean Asian) Median F/up: 4 yrs (1 m – 18 yrs) (UK) Median F/up: 4 yrs (1 m- 11 yrs) (S'pore)	C: 81 (51%) A: 16 (40%) S: 12 (23%)	98% had steroids (S'pore), 93% (UK) 75% & 63% had AZA (S vs UK)	Not reported	0.33 flares per patient-year (UK) 0.22 flares per patient year (S'pore)	Not reported	Not reported	Transplant-free survival (5 years): 84% (UK Asians) 93% (Singapore) **HCC** C: 3 (2%) A: 0 S: 1 (2%)
Danielsson Borssen, Scand J of Gastro 2017, Sweden	634 Median F/up: 11 yrs (0-50.5)	178 (28%)	90% treated	Not reported	Not reported	70 (11%)	33 (5%)	150 (24%) deaths 49 (33%) liver-related deaths
Gordon, Liver Int 2018, UK	1267 No follow-up data	254 (23%) (histological cirrhosis)	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported
van den Brand, Clin Gastro & Hep, 2019, Netherlands	385 AIH Median F/up: 10 yrs	52 (18%)	449 (100%)	375/385 (97%)	ALT >3xULN or IgG >20g/L 200 (45%) relapse – 150 (87%) of these whilst on Rx	20 (6%)	4 (1%)	AIH - 55 (14%) died (10 yrs) 26(43%) – liver-related death
Sharma, Clin Gastro Hepatol 2020, Sweden	6016 AIH Median F/up: 9.5 yrs (4 – 21)	823 (13.7%)	All	Not reported	Not reported	Not reported	233 (3.9%)	Liver related death: 681 (11.4%) All-cause mortality: 2952 (49.1%) Risk of death: Hazard ratio: 2.29

(Schvarcz, Glaumann and Weiland, 1993; Roberts, Therneau and Czaja, 1996; Johnson, McFarlane and Williams, 1995; Newton *et al.*, 1997; Czaja, 2002; Kanzler *et al.*, 2001b; Verma *et al.*, 2004; Feld *et al.*, 2005; Granito *et al.*, 2005; Miyake *et al.*, 2005; Seela, Sheela and Boyer, 2005; Floreani *et al.*, 2006; Czaja and Carpenter, 2006a; Al-Chalabi *et al.*, 2006; Montano-Loza, Carpenter and Czaja, 2007a; Montano-Loza, Carpenter and Czaja, 2007c; Montano-Loza, Carpenter and Czaja, 2007b; Al-Chalabi *et al.*, 2008a; Werner *et al.*, 2008; Muratori *et al.*, 2009; Werner *et al.*, 2010; Yoshizawa *et al.*, 2012; Ngu *et al.*, 2012; Ngu *et al.*, 2013; Delgado *et al.*, 2013; Gronbaek, Vilstrup and Jepsen, 2014; Muratori *et al.*, 2016b; Yeoman *et al.*, 2011; Hoeroldt *et al.*, 2011; van Gerven *et al.*, 2013b; van Gerven *et al.*, 2014; Kirstein *et al.*, 2015; Hartl *et al.*, 2015; Than *et al.*, 2016a; Danielsson Borssén *et al.*, 2017; van den Brand *et al.*, 2019a; Gordon *et al.*, 2018; Sharma *et al.*, 2020)

1.10 Quality of Life in Autoimmune Hepatitis

Up to 50% of patients with AIH are symptomatic with fatigue, general ill health, abdominal pain and joint pain despite treatment (van Gerven *et al.*, 2014; Kogan *et al.*, 2002).

A survey amongst members of the Dutch liver patient association evaluated HRQOL in liver disease of different aetiologies using the Dutch Short Form Survey-36 (SF-36), the Liver Disease Symptom Index (LDSI) 2.0 and the Multidimensional Fatigue Index-20. The subset of patients with AIH (n=142) had significantly lower scores (and thus worse HRQOL) in all SF-36 scales (particularly physical problems or general health scales), as well as significantly worse fatigue scores (reported via the Multidimensional Fatigue Index-20 questionnaire) compared to Dutch healthy controls (van der Plas, Hansen and de Boer, 2007).

A study of 24 children with AIH or with Primary Sclerosing Cholangitis/AIH overlap using the Pediatric Quality of Life (PedsQL) 4.0 questionnaire showed significant impairment of HRQOL. This was associated with the presence of frequent liver disease related symptoms (particularly abdominal pain, fatigue and mood symptoms) (Gulati *et al.*, 2013).

A German single-center study (n=103) using patient-reported HRQOL data found higher rates of depression and anxiety in AIH patients compared to the general population. A major associated factor was concern regarding the risk and implications of progressive liver disease. There was also a correlation found between prednisolone use and depression (Schramm *et al.*, 2014).

A Canadian study (n=52) described an association between psychosocial distress, non-adherence to treatment and incomplete response to therapy in AIH (Sockalingam *et al.*, 2012).

More recently (following the publication of this thesis' UK-AIH QOL data), a Polish single-centered study evaluated their population of patients (140 with AIH) using the Short-Form Survey-36 (SF-36), Modified Fatigue Impact Scale (MFIS) and Patient Health Questionnaire-9 (PHQ-9) compared to a matched healthy-control group (n=170). There was significant impairment of HRQOL in the AIH patients with association with physical fatigue, anxiety and depression ($p<0.001$) (Janik *et al.*, 2019). A subsequent Japanese study (n=62 AIH patients) also reported impaired HRQOL in their cohort of patients in remission (Takahashi *et al.*, 2020).

1.11 Unmet Need In Autoimmune Hepatitis

Unmet need persists in AIH despite well-established evidence-based treatments. Unmet need can be divided into three domains:

a. Inadequate response to therapy
b. Effective therapy with inappropriate side effects
c. Quality of life

1.11.1 Inadequate response to therapy

As elaborated in the above sections of this chapter, AIH remains a diagnostic and therapeutic challenge with one third of patients (~30%) presenting with cirrhosis and about a median figure of 14% (range: 6-40%) of patients developing de-novo cirrhosis with despite treatment (Gleeson, 2019; van den Brand *et al.*, 2019a; Feld *et al.*, 2005; Czaja and Carpenter, 2006a). Patients usually remain on lifelong immunosuppressive therapy (predominantly prednisolone and azathioprine). Treatment-related side-effects can be significant resulting in 10-25% therapy withdrawal. Up to 50-90% relapse within a year of stopping therapy and 70% within three years. Up to 20% of patients do not achieve disease remission. In some cases, failure to achieve biochemical remission (normal ALT and IgG) is associated with an increased risk of liver-related death and transplantation. In other cases, despite achieving complete biochemical response to treatment (normalisation of ALT and IgG), serious liver-related end-points are reached. This is particularly noted in the landmark report by Hoeroldt et al (n=245), 93% of patients achieved a biochemical response to therapy after 12 months. In spite of this, transplant-free survival at 20 years was poor at 48% with the excess mortality due to liver-related death (Hoeroldt *et al.*, 2011).

This raises the question of whether current established treatments are being utilised optimally or whether more patient-customised fine-tuning of azathioprine dose based on pharmacodynamic markers of drug-effect would improve biochemical response rates to treatment.

A highly attractive potential approach to optimising the balance between efficacy and tolerability of azathioprine therapy would be a biomarker of actual drug exposure and efficacy. Much research has focused on azathioprine red-blood-cell (RBC) metabolites as a predictor of adverse effects and therapeutic response. The RBC assay measuring thiopurine metabolites

(6-TGN and 6-MMP) was first developed to monitor thiopurine drug compliance and titrate dose in childhood leukaemia (Lennard *et al.*, 1987). Since then, its role as a predictor of adverse effects, therapeutic response and for monitoring drug compliance has been extensively explored in inflammatory bowel disease (IBD) and extended to AIH. However, thiopurine metabolites do not always correlate to biological or clinical response as seen by conflicting results in several studies in IBD (Moon and Loftus, 2016; Konidari *et al.*, 2014; Lee *et al.*, 2015; Osterman *et al.*, 2006) and AIH (Heneghan *et al.*, 2006; Ferucci *et al.*, 2011; Dhaliwal *et al.*, 2012; Nguyen *et al.*, 2010). This may be due to the current measured metabolite levels being in the red blood cells (erythrocytes) whilst leukocytes are considered the main target cells of azathioprine.

Biologically, the lack of a tight correlation between RBC metabolites and clinical response is due to cellular enzyme differences as RBCs lack inosine-monophosphate dehydrogenase (IMPDH), the enzyme critical for the formation of active thiopurine metabolites. In addition, these active metabolites are very labile, posing a significant challenge in accurate concentration measurements. Attempts to measure thiopurine metabolites in nucleated blood cells still have technical and methodological flaws and have not replaced the RBC assay as a routine test (Erdmann *et al.*, 1991; Hofmann *et al.*, 2012).

To address the difficulties in using metabolite measurements for predicting clinical response and measuring drug efficacy, assays to measure the final end products of thiopurine metabolism have been developed in leukaemia (Jacobsen, Schmiegelow and Nersting, 2012) and IBD (Coulthard *et al.*, 2016a). These measure deoxythioguanosine in DNA (dTG-DNA), 6-TGNs that are incorporated into patient DNA. Coulthard et al published their method to measure dTG-DNA (using blood samples from 20 AZA-treated IBD patients (10 with Ulcerative Colitis [UC] and 10 with Crohn's Disease [CD]) (Coulthard *et al.*, 2016b). They also determined the levels of dTG-DNA in a cohort of patients with IBD (9 treated with AZA and 9 with low dose AZA with allopurinol (LDAA) and showed that dTG-DNA levels correlated to drug dose but not RBC 6TGN levels (Mann-Whitney test, $z = 2.2$, $p = 0.003$). The study did not examine correlation of dTG-DNA with clinical response (Coulthard *et al.*, 2017).

This novel thiopurine metabolite (incorporated deoxythioguanosine into DNA of white blood cells) should be explored as a potential marker of disease response, compliance and AZA dose in patients with AIH for thiopurine treatment optimisation.

1.11.2 Effective therapy with inappropriate side effects

AIH treatment remains reliant on old-fashioned staple combined therapies of corticosteroids and azathioprine – this is in comparison with the majority of other autoimmune conditions which have moved on to corticosteroid-free therapies, mainly due to the associated significant side-effect burden. Patients who require long-term corticosteroids are at risk of developing adverse effects (e.g. cosmetic changes, sleep disturbance, weight gain, diabetes, osteoporosis, pancreatitis, opportunistic infection, malignancy etc). There is an approximate 30% prevalence of unwanted corticosteroid-associated side-effects. Azathioprine-related adverse effects and complications include nausea, arthralgia, bone marrow suppression, malignancy, pancreatitis, opportunistic infection etc. These occur in about 25% of patients leading to drug withdrawal in about 10% of patients (Johnson, McFarlane and Williams, 1995; Heneghan *et al.*, 2006). There is a need to elucidate the benefits and risks of reducing corticosteroid exposure and identify ways to reduce or stop drugs such as azathioprine to reduce the long-term exposure to toxic medications.

1.11.3 Quality of life

The nature and extent of impact of AIH and its treatments on health-related quality of life has not been widely reported. Up to 50% of patients with AIH are symptomatic with fatigue, general ill health, abdominal pain and joint pain despite treatment (van Gerven et al., 2014, Kogan et al., 2002). Reports from patients with AIH suggest there are significant physical and psychological effects. These have not been completely characterised (European Association for the Study of the, 2015). Additionally, differentiating symptoms attributable to the disease itself versus treatment-related side-effects can be challenging. Studies on health-related quality of life (HRQOL) in AIH have been mainly single-center with small numbers but these suggest that there is a significant issue with quality of life (QOL) in AIH. There is therefore a need to evaluate the impact of AIH and its therapies on health-related quality of life.

Thesis aims

This thesis aims to elaborate on the clinical impact of AIH by elucidating the clinical and mechanistic evidence behind unmet need in AIH using the UK-AIH national multi-centre cohort.

This thesis aims to explore the clinical characteristics of the UK-AIH cohort to identify present-day gaps in management, potential factors/trends/predictors of inadequate response to

treatment and disease progression. Clinical indices, adherence and health-related quality of life data were analysed. A novel thiopurine metabolite (deoxythioguanosine in DNA) was also explored as a potential marker for thiopurine treatment optimisation.

This thesis will elaborate on the above further.

Chapter 2. Methodology – the United Kingdom AIH (UKAIH) Cohort

2.1 Introduction

The United Kingdom Autoimmune Hepatitis (UKAIH) study is a national cross-sectional multi-centre cohort developed to evaluate the management and outcome of adult patients with AIH in the UK and facilitate the development, evaluation and implementation of improved therapy. A key aim is to determine the unmet needs of patients with AIH.

The protocol was approved by the National Health Service (NHS) Health Research Authority (IRAS ID: 144806, REC reference: 14/LO/0303, URL: https://www.hra.nhs.uk/planning-and-improving-research/application-summaries/research-summaries/the-uk-aih-cohort/) and was conducted in accordance with the International Council for Harmonisation (ICH) Good Clinical Practice (GCP) guidelines and the Declaration of Helsinki. Written informed consent regarding the use of data was obtained. A substantial amendment in November 2015 enabled additional data on variables at diagnosis, complications from progressive liver disease and treatment effects to be collected.

2.2 Study Design

UKAIH is an observational study. This thesis covers data from the cohort of prevalent cases of people with a clinical diagnosis of AIH.

2.3 Place of study

Patients were recruited from secondary and tertiary hospital settings from March 2014 until Feb 2017. The study started out initially recruiting participants under the care of Newcastle upon Tyne Hospitals NHS Foundation Trust and University Hospitals Birmingham NHS Foundation Trust. Once the study procedures were established at these two sites, additional sites were opened through NIHR invitation. Additionally, a launch event of the UK-AIH study was held and clinicians/potential principal investigators (PIs) at hospitals were notified to

contact the Newcastle research team to express their interest in being a site for the study. The list of hospitals who participated are found in **Appendix A**.

2.4 Subjects

The UK-AIH patient cohort is comprised of people aged 16 years old and above who carry a clinical diagnosis of AIH and are able to provide informed consent. The participants had to be living in the United Kingdom and are all people under medical care for a diagnosis of AIH in the UK.

For prevalent cases, patients with a current clinical diagnosis of AIH (as evaluated a priori by their treating clinician) were recruited. See **Table 9 and 10** for inclusion and exclusion criteria. Since patients were recruited based on this retrospective clinical diagnosis, no attempt was made to calculate the International Autoimmune Hepatitis Group (Alvarez *et al.*, 1999a) or the simplified IAIHG diagnostic criteria from 2008 (Hennes *et al.*, 2008b). However, a subsequent amendment to the protocol allowed retrospective retrieval of this data.

For the analysis of the novel thiopurine metabolite (deoxyguanosine in DNA), the patients were divided into two groups: A (complete responder) and B (incomplete responder) based on set inclusion and exclusion criteria. See **Appendix B: Tables 1-4**. Patients in this analysis had to fulfill the simplified IAIHG diagnostic criteria from 2008 (Hennes *et al.*, 2008b).

The scoring system is adapted from simplified IAIHG criteria (Hennes et al 2008). There is no single diagnostic test for AIH and these criteria have been agreed to ensure consistency in clinical studies. A score of 5 was used in the inclusion criteria (see **Appendix B: Table 1, 3**) as equivalent to the criteria for 'definite' AIH. The additional 2 points (for absence of viral hepatitis) would be gained when evaluating the exclusion criteria in **Appendix B: Table 2** and **4** (which would bring the total score to 7, in keeping with 'definite' AIH under the simplified IAIHG criteria – see Chapter 1, Section 1.6 – Table 3).

Table 9: Main UK-AIH cohort inclusion criteria

	Criterion	Reason
1	Under medical care (primary or secondary care) in the UK for a diagnosis of autoimmune hepatitis	This is the whole patient population likely to benefit from any study outputs
2	Able to give informed consent	The small minority who cannot give informed consent are not otherwise different from the rest of the study population, so have the potential to benefit from study outputs without the need to participate

Table 10: Main UK-AIH cohort exclusion criteria

	Criterion	Reason
1	Age under 16 years	The systems and safeguards of the study will be established in consenting adult patients. If the findings of this study are encouraging, we intend to move towards recruiting a paediatric cohort.

2.5 Study procedures

2.5.1 Recruitment

Potential participants were identified by the clinical team that normally looks after their AIH and invited to participate by the consultant responsible for the care of their AIH or by one of his/her clinical team, or by a trained research nurse. Each site had its own methods in identifying their AIH patients (these included having a registry of patients with AIH diagnosis, screening clinic notes to identify AIH patients and etc). Invitations would take place during scheduled clinical outpatient appointments or during inpatient care. Some potential participants may be invited by post using a standard letter enclosing the patient information sheet (PIS), informed consent form (ICF) and a copy of the participant questionnaire for

information. These people would then arrange to attend hospital specifically for study recruitment or may be recruited at the time of their next scheduled outpatient review.

A unique subject number was allocated to partipants upon providing informed consent to join the study. This was a 6-digit number in the form SSPPPP where SS is the two-digit site number (e.g. 01 for Newcastle, 02 for Birmingham and so on) allocated when a site if initiated and a PPPP is a four-digit number allocated sequentially to patients recruited at that site (so 01-0001 is the first patient to consent at Newcastle, 02-0104 is the 104th patient to consent at Birmingham, and so on).

2.5.2 Data Collection

i. Demographic data

Demographic data was collected using clinical data forms (age, gender, weight) and self-reported participant questionnaires (height, ethnicity).

ii. Clinical data

Clinical data evaluating risk factors for progressive liver disease, variables at diagnosis, biochemical results, immunosuppressive therapies, therapy response, presence of cirrhosis, complications of chronic liver disease and treatment complications were collected from patient records by study-team members as follows:

- Year of diagnosis
- Biochemical status: ALT, AST, IgG levels (on recruitment or latest values)
- Variables at diagnosis: ANA, SMA, LKM, SLA, AMA, IgG, Hepatitis B surface Antigen (HBsAg), Hepatitis C virus (HCV), Hepatitis A IgM, Hepatitis E IgM, CMV IgM, EBV IgM*
- Current therapy for AIH
- Past treatment history related to steroid dose use in the 12 months prior to inclusion in the study
- Information on whether an attempt at corticosteroid reduction to less than <Prednisolone 10mg/day or <budesonide 6mg/day was attempted*
- Number of disease flares in the past 12 months requiring increase in corticosteroid dose or introduction of corticosteroids

- Information on whether ALT normalises with corticosteroid therapy for a flare in the past 12 months*
- Treatment response in the past 12 months
- Presence of cirrhosis on diagnosis and whether biopsy-confirmed
- Development of cirrhosis since diagnosis (where applicable) and whether biopsy-confirmed
- Development of complications of cirrhosis (ascites, hepatic encephalopathy, varices)*
- Need for liver transplantation
- Treatment effects - osteoporosis, diabetes mellitus type 2 and/or hypertension at diagnosis and since diagnosis*
- Smoking status*
- Alcohol intake*

*A protocol amendment to the study in November 2015 allowed for these additional data to be collected retrospectively

For the interrogation of clinical data in Chapter 3 and 4, biochemical remission status was assessed using ALT and IgG values at the time of recruitment to the study.

In Chapter 4, in order to assess factors associated with incomplete treatment response, two clearly defined patient groups from the UK-AIH study were used:

1. Group A: 'Responders to therapy': Patients on azathioprine in biochemical remission as defined by normal alanine transaminase (ALT) and immunoglobulin G (IgG) with no corticosteroid therapy in the past 12 months before recruitment

2. Group B: 'Incomplete responders to therapy' – Patients on azathioprine who were not in biochemical remission* or requiring high-dose corticosteroids (defined as prednisolone ≥10mg or budesonide ≥6mg) to maintain normal biochemistry[#]

**ALT remains higher than the upper reference limit (ULN) for the analysing laboratory after initial 12 months of treatment OR at least two ALT values >2x ULN at least 4 weeks apart during the past year despite low dose corticosteroid (prednisolone <10mg per day or equivalent) with or without other immunosuppressive therapy*

[#]*Requirement for ≥10mg a day of prednisolone or equivalent corticosteroid plus another immunosuppressive agent to keep ALT in the normal range, with a record that ALT rises if corticosteroid dose is reduced below this level.*

The full inclusion and exclusion criteria for Group A and B are detailed in the **Appendix B: Tables 1-4.**

For the interrogation of clinical data together with health utility in Chapter 5, the following definition of biochemical remission status was used: Normal ALT and IgG at the time of recruitment and within the preceding 12 months with no documented flares in the last 12 months. The upper limit of normal (ULN) levels of ALT and IgG were based on each site's pathology laboratory ULN. A disease flare was defined as an increase in ALT level above the upper limit of normal (ULN) in the previous 12 months requiring treatment with higher corticosteroid dose than the patient's maintenance dose or through introduction of corticosteroid therapy in patients with corticosteroid-free maintenance. The upper limit of normal (ULN) used for ALT and IgG levels were based on each site's pathology laboratory ULN.

The presence of cirrhosis at presentation within the cohort was defined histologically or clinically by radiological evidence or transient elastography. Development of decompensated liver disease and need for liver transplantation were collected as surrogate markers for disease severity and treatment failure.

The prevalence of autoimmune disorders and other specific co-morbidities (in patients, first-degree relatives and other relatives) were collected via self-reported participant questionnaires. Other medications (by patient free-text) (see **Appendix C: Data Forms – Participant Questionnaire 1 and 2**). The list of disorders (which included some autoimmune disorders) were as follows:

- AIH
- PBC
- PSC
- Ulcerative colitis (UC)
- Coeliac disease
- Thyroid disease
- Rheumatoid arthritis (RA)

- Systemic lupus erythematosus (SLE)
- Mixed connective tissue disease
- Sjogren's syndrome
- Diabetes mellitus (DM) type 1
- DM type 2
- Liver cancer
- Any other cancer
- Osteoporosis
- Fractured (broken hip)

iii. Adherence to treatment data

Adherence to treatment was collected using **Participant Questionnaire 1** and **Clinical Data Form (CDF)-A** (see **Appendix C: Data Forms**). The questions and various responses are also described in **Chapter 4: Table 21.** As the CDF also had an identical adherence question (a) with identical responses, any missing data from the patient questionnaire: question (a) was completed using the adherence response from the CDF. In the setting of differing responses, the higher numerical value response was used.

iv. Thiopurine metabolite deoxythioguanosine in DNA (dTG DNA) data

Sample Preparation and Liquid Chromatography with tandem mass spectrometry (LC-MS/MS) Analysis of Thioguanine Incorporated into DNA (dTG DNA)

Using previously published methods (Daly *et al.*, 1996), DNA was isolated from whole blood sample collected in EDTA tubes. Using a recently-developed high performance liquid chromatography (HPLC)-mass spectrometry method (Coulthard *et al.*, 2016b), the ratio of deoxythioguanosine (dTG) relative to deoxyadenosine (dA) in DNA (expressed as the number of moles of dTG/1 × 10^6 moles of dA) was analysed. Chromatographic separation of dTG and dA was with a XSelect HSS T3 3.5 µm 4.6 × 100mm (Waters) column with a VanGuard 3.5 µm 3.9 × 5 guard column maintained at 30°C. Analytes were eluted with mobile phases of 0.01 M aqueous 0.05 % formic acid (A) and 0.05% formic acid in acetonitrile (B). The flow rate was 0.5 mL/min and the mobile phase system consisted of a starting condition of 1% buffer B increasing to 3% at 1.1 min, 8% at 2.4 min and increasing to a maximum of 30% at 4.1 min then decreasing to 5% at 4.5 min, maintained until 5.5 min then decreasing to 1% for an

equilibration period of 2.5 min. Standards ranged from 100 µg/mL dA and 2000 ng/mL dTG and were serially diluted in water to 0.39 µg/mL dA and 3.9 ng/mL dTG, respectively. High (40,000 ng/mL dA:400 ng/mL dTG), medium (4,000 ng/mL dA:40 ng/mL dTG) and low (400 ng/mL dA:4 ng/mL dTG) matrix controls were prepared in triplicate. Standards, matrix controls and samples were injected in a volume of 50 µL; for sample injections this is equivalent to 0.2 µg patient DNA per injection.

Samples were genotyped by DNA analysis of single nucleotide polymorphisms (SNPs) for TPMT status using Taqman PCR. The SNPs were identified using Taqman® predesigned and custom SNP genotyping assays (ThermoFisher Scientific, Waltham, MA) in accordance with the manufacturer's recommendations (Nicoletti *et al.*, 2017).

v. Quality of life data

Health-related quality of life (HRQOL) and symptom impact information were collected using patient-reported European Quality-of-life 5-Dimension 5-Level (EQ-5D-5L), Fatigue Impact Scale (FIS), Cognitive Failure Questionnaire (CFQ) and Hospital Anxiety Depression (HADS) tools.

The HRQOL tools are elaborated further in Chapter 5.

Refer to **Appendix C: Data Forms** for the clinical data form and participant questionnaires:

- Clinical Data Form A
- Participant Questionnaire 1
- Participant Questionnaire 2 – contains CFQ and FIS questionnaires
- Hospital Anxiety Depression Scale (HADS) Questionnaire
- Health-Related Quality of Life (HRQOL) Questionnaire – contains EQ-5D-5L tool

2.5.3 Statistical Analysis

This is elaborated separately in Chapter 3 (Clinical Data), Chapter 4 (Thiopurine deoxythioguanosine in DNA metabolites) and Chapter 5 (Quality of Life).

Chapter 3. Clinical Data from the UK-AIH Cohort

Refer to **Appendix D: Publications**: Inequity of care provision and outcome disparity in autoimmune hepatitis in the United Kingdom.

Reference: Aliment Pharmacol Ther 2018 Nov; 48(9): 951-960.

URL: https://onlinelibrary.wiley.com/doi/full/10.1111/apt.14968

DOI: 10.1111/apt.14968

3.1 Introduction

Autoimmune hepatitis (AIH) is a rare, progressive inflammatory condition of the liver that may present in either acute or chronic forms (Heneghan *et al.*, 2013; Manns, Lohse and Vergani, 2015; Krawitt, 2006). If not effectively treated it can progress rapidly to acute liver failure or the development of cirrhosis. Treatment paradigms established in the 1960s and 70s utilising corticosteroids and azathioprine to achieve and sustain remission have remained largely unchanged over several decades (Heneghan *et al.*, 2013). Although case series of patients treated in specialist centres suggest that disease remission can be achieved in up to 80% of patients, there is concern that real world disease outcomes in patients treated across the spectrum of health care settings may be substantially worse (Hoeroldt *et al.*, 2011). This leaves patients at risk of progression to end-stage disease for which liver transplantation is the only effective therapy (Gleeson, Heneghan and British Society of, 2011; European Association for the Study of the, 2015; Mack *et al.*, 2020).

Current international treatment guidelines have defined initial management and treatment models in AIH (Gleeson, Heneghan and British Society of, 2011; European Association for the Study of the, 2015; Mack *et al.*, 2020). The goals of treatment are: Biochemical and histological

remission with effective control of symptoms, followed by long-term maintenance of the remission state. This should be achieved ideally with minimisation of the dose of corticosteroid with full withdrawal being the ultimate aim. Corticosteroids in the form of prednis(ol)one or budesonide with azathioprine are recommended by all guidelines. Where possible, azathioprine monotherapy as maintenance is advised to minimise steroid side effects and their impact on quality of life. The majority of patients need long-term treatment to prevent relapse (Muratori *et al.*, 2010; van Gerven *et al.*, 2013a). There are increasing numbers of patients who suffer from unpleasant side-effects, poorly controlled disease and a life-long immunosuppression burden (Johnson, McFarlane and Williams, 1995; McFarlane *et al.*, 2004; Schramm *et al.*, 2014; Sockalingam *et al.*, 2012). Induction (and often maintenance) therapy is still largely based around corticosteroids, an approach that has been superseded in most other autoimmune conditions due to the morbidity associated with these agents. Long-term steroids are associated with risk of the metabolic syndrome and its sequelae, osteoporosis, weight gain and disturbance of sleep and mood. Even in patients with established remission, unwanted effects of corticosteroids and immunosuppressants can contribute to adverse outcomes in 30-40% (Johnson, McFarlane and Williams, 1995).

There is limited evidence base for the management of patients who are non-responders to conventional immunosuppression. Second and third-line alternative immunotherapies in patients intolerant of azathioprine, whilst recommended, can be variable in efficacy and tolerability (Hubener *et al.*, 2016; Manns *et al.*, 2010b; Sharzehi *et al.*, 2010; Than *et al.*, 2016b). The majority of data relating to treatment outcomes in AIH is derived from large referral centres (Johnson, McFarlane and Williams, 1995; Kirk *et al.*, 1980). Even amongst these expert centres, there are significant differences in relation to approach to treatment (Liberal *et al.*, 2017).

There has been a growing awareness in recent years of the inadequacies in service provision for many common liver diseases and attention focused on the public health issues pertaining to the burden of liver disease in the UK (Williams *et al.*, 2014; Williams *et al.*, 2017; Williams *et al.*, 2015). There has however been little attention, to date, given to rarer liver diseases such as primary biliary cholangitis (PBC), primary sclerosing cholangitis (PSC) and AIH in these documents. The unmet needs and requirements of these patient groups, whilst known, have never been properly quantified (Dyson *et al.*, 2015; Sebode *et al.*, 2017).

As the majority of data on outcomes and treatments of AIH are derived from tertiary centres, which may not accurately reflect the full spectrum of care delivery, the nationwide, United Kingdom Autoimmune Hepatitis (UK-AIH) cohort was established to quantify this, using "real world" data and represent multiple hospital practice settings in the UK. The United Kingdom AIH (UK-AIH) group's intention is to use this platform to define current practice in the management of AIH within the UK, and to develop, evaluate and implement improved approaches to treatment. The goals of the current study are to evaluate current treatment practice, assess real-life treatment regimens, evaluate remission rates and determine the real-life unmet clinical needs of patients with AIH.

3.2 Methods

Adult patients (≥16 years) from the UK-AIH patient cohort (recruited from March 2014 to March 2017) were included (see **Chapter 2, Section 2.4**). Eligible patients carried a clinical diagnosis of AIH for more than a year (as evaluated a priori by their treating clinician).

Demographic and clinical data, including risk factors for progressive liver disease, were collected on standardised data collection forms completed by the local managing clinicians (see **Chapter 2: Section 2.5.2: i) and ii)** for more details). The presence of cirrhosis at the time of diagnosis of disease and development of cirrhosis since diagnosis (where applicable) were collected. Cirrhosis was based on either liver histology or clinically by radiological evidence or transient elastography. Surrogate markers for disease severity and treatment failure included the development of decompensated liver disease (ascites, hepatic encephalopathy), varices and need for liver transplantation. Data on ethnicity, height and weight and prevalence of additional autoimmune disorders were collected using additional completed questionnaires.

As the study's initial protocol was based on retrospective clinical diagnosis, data on serum autoantibodies was not collected initially and no attempt was made to calculate the International Autoimmune Hepatitis Group (Alvarez *et al.*, 1999a) or the simplified IAIHG diagnostic criteria from 2008 (Hennes *et al.*, 2008b). A subsequent protocol amendment in November 2015 allowed retrospective retrieval of this data. This included variables at diagnosis: Serum autoantibody titres, IgG levels and viral serology including hepatitis A, B, C, E viruses, Cytomegalovirus, and Epstein-Barr virus.

Biochemical remission status was assessed using contemporaneous ALT and IgG values at study recruitment (rather than at the time of original diagnosis). The upper limits of normal of ALT and IgG for each recruiting centre were utilised. Disease flare was defined as the need to treat an increased ALT level in the previous 12 months with a higher corticosteroid dose than their maintenance dose or through introduction of corticosteroid therapy in patients on corticosteroid-free maintenance.

Data were analysed using SPSS version 22, GraphPad Prism 7 and SAS 9.4. Non-parametric data are presented as median and range. Continuous variables were described as median, minimum and maximum. Difference between proportions were analysed using the z test. Logistic regression was performed to assess risk factors for cirrhosis development. All p values reported are two-sided, and $p < 0.05$ was considered statistically significant.

Dr Jessica Dyson contributed to the statistical analysis and interpretation of data.

3.3 Results

3.3.1 Demographics and baseline features

A total of 1249 patients were recruited into the study cohort from 44 centres (seven liver transplant centres and 37 non-transplant centres). 635 (51%) patients were under the care of transplant units and 614 (49%) under nontransplant centres. There was a strong female preponderance (n=1006, 81%). 93% of the 1201 patients with available data were white Caucasian in ethnicity (see **Table 11**). The median age at inclusion into the study for the whole cohort was 58 years (17-95 years) and median age at diagnosis of AIH in this cohort was 50 years (range 2-86 years).

There were 79 (6%) patients who were diagnosed below the age of 16. 65/79 (82%) were under the care of transplant units. Patients managed in transplant centres were diagnosed at a younger age than those managed in non-transplant centres (median 42 years [range 2-86 years]) with 116 (18%) of patients diagnosed at 20 years or younger compared with median age 55 years (range 4- 86 years) and 30 (5%) diagnosed at 20 years or younger ($p < 0.0001$). The median duration of follow-up for the whole cohort was 7 years (1-57). The duration of

follow-up between disease diagnosis and study enrolment was longer in transplant centres with a median of 8 (1-57) years versus 6 (1-41) years.

Table 11: Distribution of patients according to ethnicity

Ethnicity	Number	Percentage
White	1115	92.8%
Indian / Pakistani / Bangladeshi	39	3.2%
Black / African / Caribbean	22	1.8%
Mixed / multiple ethnic groups	14	1.2%
SouthEast Asian / Chinese	4	0.3%
Other	7	0.6%

The baseline characteristics (divided into transplant and nontransplant units) at time of study entry are summarised in **Table 12.** The median body mass index (BMI) of the whole cohort at study recruitment was 28.4 kg/m^2 (range 15.1-64.0). The proportion of patients according to age at diagnosis divided into five categories of 20-year age brackets are shown in **Figure 2**.

The majority of patients (39%) were diagnosed between the age of 41 and 60 years which is in keeping with other studies (Hoeroldt *et al.*, 2011; Danielsson Borssen *et al.*, 2017; Gronbaek, Vilstrup and Jepsen, 2014; van Gerven *et al.*, 2014; Werner *et al.*, 2008).

Almost all patients [734/ 742 (99%)] with available data were abstinent from alcohol or consumed alcohol within recommended national limits in accordance with UK guidelines prior to 2014 (National Institute for Health and Care, 2012) (below 15 units per week for women and 22 units per week for men).

There were 69/739 (9%) (with available data) who were current smokers (this is less than the 14% of the total adult UK population who are reported to be current smokers by Cancer Research UK (Cancer Research UK, 2021). 221/739 (30%) were previous smokers.

There were 51 (4%) patients who had undergone liver transplantation at time of recruitment into the study. These were excluded from further analyses (apart from age at diagnosis and the development of cirrhosis) with the denominators reflecting this.

685 (77%) patients with available data had either positive anti-nuclear (ANA) and/or anti-smooth muscle antibodies (ASMA) consistent with Type 1 AIH with only 2% having anti-liver kidney microsomal antibodies (LKM) consistent with Type 2 AIH. 14% of patients had negative ANA, ASMA and anti-LKM positive at diagnosis however, complete antibody data was not available for 29% with many missing or not having had LKM or anti-soluble liver antigen (SLA) antibodies checked.

Blood parametres to assess biochemical remission status (defined as normal ALT and IgG at study recruiment) were available for 1114 patients. 460 (41%) of these were not in biochemical remission at study entry.

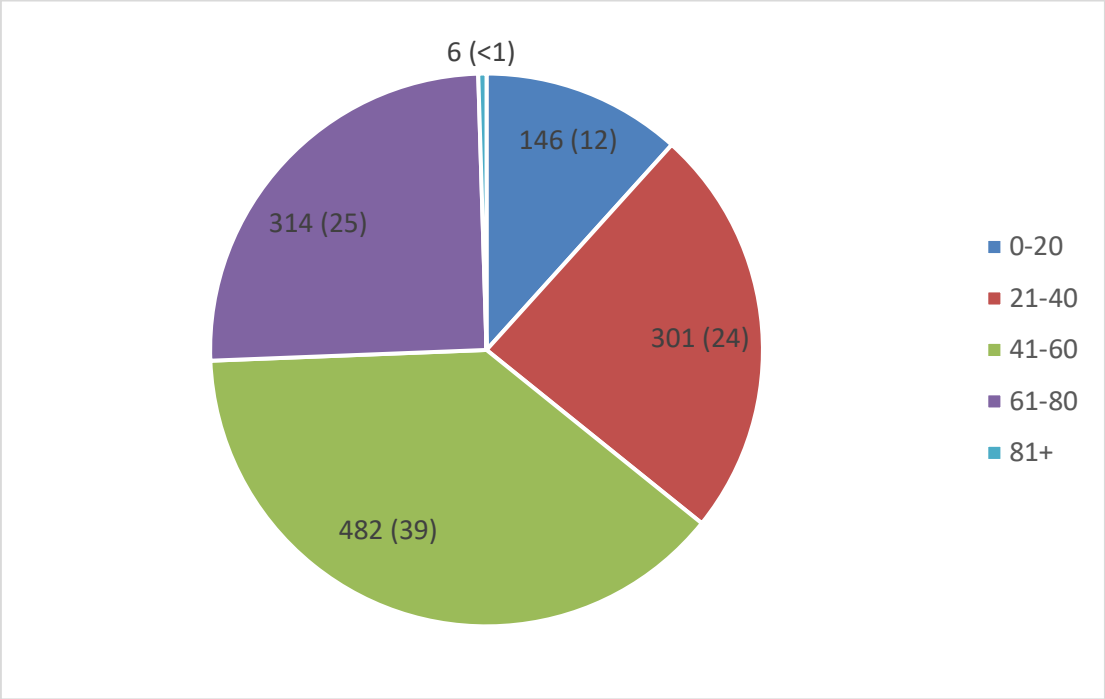
Table 12: Baseline characteristics of patients at time of study entry, self-reported (n=1249) and patient-reported presence of other autoimmune conditions (n=1192, 596 in both types of units)

	Transplant units	Non-transplant units
Age at study entry (years), median (range)	52 (17-91)	63 (18-95)
Weight (kg), median (range)	75 (40-169)	76 (38-165)
Height (cm), median (range)	162.5 (132.5-192.5)	162.5 (132.4-192.5)
BMI (kg/m^2), median (range)	28 (15-64)	29 (16-62)

Other autoimmune conditions	Transplant units n (%)	Non-transplant units, n (%)	p value
Thyroid disease	67 (11)	110 (18.5)	0.0004
Rheumatoid arthritis	29 (4.9)	52 (8.7)	0.008
PBC	33 (6)	39 (7)	0.47
Ulcerative colitis or Crohn's disease	33 (5.5)	25 (4.2)	0.281
Coeliac disease	17 (2.9)	24 (4)	0.266
SLE	21 (4)	19 (3.2)	0.748
Sjogren's syndrome	15 (2.5)	24 (4)	0.143

Type 1 Diabetes mellitus	15 (2.5)	12 (2)	0.559
PSC	20 (3.4)	7 (1.2)	0.011
Mixed connective tissue disorder	7 (1.2)	4 (0.7)	0.363
Other autoimmune condition	79 (13.3)	100 (16.8)	0.088

Figure 2: Age at diagnosis in 20 year age brackets for whole cohort (n=1249), %. Patients were categorised according to age at presentation; 0 to 20 years, 21 to 40 years, 41 to 60 years, 61 to 80 years, over 81 years.



3.3.2 Immunosuppressive Therapies and Remission rates

Data on drug regimens for patients without previous liver transplantation (n=1198) were collected. The treatment regimens, biochemical remission rates and the number of patients with cirrhosis at diagnosis are summarised in **Table 13**. Patients were stratified according to the treatment regimen.

Table 13: Summary of treatment regimens, biochemical remission rates (normal ALT and IgG) and number of patients cirrhotic at diagnosis according to treatment regimen (n=1198, excluding liver transplant patients)[a]

Simplified drug regimen	Number of patients (%)	Number in biochemical remission (%)	Number cirrhotic at diagnosis (%)
Azathioprine/6MP alone	392 (33)	252/362 (70)	82/385 (21)
Azathioprine/6MP + prednisolone	316 (26)	175/299 (59)	78/309 (25)
Prednisolone alone	103 (9)	43/85 (51)	28/98 (29)
Budesonide alone	19 (2)	5/19 (26)	2/19 (11)
No immunosuppression	85 (7)	51/79 (65)	15/84 (18)
MMF/MA alone	58 (5)	35/54 (65)	11/57 (19)
MMF/MA + prednisolone	112 (9)	53/107 (50)	34/112 (30)
CNI-containing regimen[b]	56 (5)	16/54 (29)	19/56 (34)
Triple immunosuppression	28 (2)	5/27 (19)	9/27 (33)
Other immunosuppression regimen	53 (4)	24/51 (47)	3/53 (6)

6MP = 6-mercaptopurine; MMF = mycophenolate mofetil; MA = mycophenolic acid; CNI = calcineurin inhibitor

[a]all available data presented but some missing data resulting in denominators for the calculation of biochemical remission and numbers cirrhotic at diagnosis being smaller than the total number of included patients

[b]some patients on a CNI-containing regimen were receiving triple immunosuppression so appear in both groups resulting in n>1198

219/1158 (19%) with available data had a flare in ALT in the previous 12 months requiring an increase (or addition) in corticosteroid treatment. The maximum number of flares suffered by an individual was 11 (range 0-11).

In the 1114 (93%) on immnosuppressive therapy, 29 different treatment regimens were reported (this does not include dose variations or transplant patients). The different combinations are listed in **Table 14.** The dose ranges of the major immunosuppressants used for AIH treatment are summarised in **Table 15**.

Table 14: Treatment combinations (n=1198)

	Treatment regimens	Number (%)		Treatment regimens	Number (%)
1.	6MP + AZA	1 (0.08)	16.	MMF + AZA + Pred	1 (0.08)
2.	6MP + Budesonide	3 (0.25)	17.	MMF + BUD	9 (0.75)
3.	6MP + Pred	25 (2.09)	18	MMF + PRED	106 (8.85)
4.	6MP ONLY	26 (2.17)	19.	MMF ONLY	56 (4.67)
5.	AZA + Budesonide	38 (3.17)	20.	Pred only	103 (8.60)
6.	AZA + Pred	291 (24.29)	21.	Tac + 6MP + PRED	1 (0.08)
7.	AZA + Pred + budesonide	2 (0.17)	22.	Tac + AZA	2 (0.17)
8.	AZA ONLY	367 (30.63)	23.	Tac + AZA + budesonide	1 (0.08)
9.	Budesonide + Pred	2 (0.17)	24.	Tac + AZA + Pred	7 (0.58)
10.	Budesonide only	19 (1.59)	25.	Tac + MA + Pred	1 (0.08)
11.	Ciclosporin + AZA + Pred	3 (0.25)	26.	Tac + MMF + budesonide	1 (0.08)
12.	Ciclosporin + Pred	3 (0.25)	27.	Tac + MMF + Pred	11 (0.92)
13.	Ciclosporin ONLY	1 (0.08)	28.	Tac + Pred	20 (1.67)
14.	MA + Pred	6 (0.50)	29.	Tac only	5 (0.42)
15.	MA ONLY	2 (0.17)		No treatment	85 (7.1)

6MP = 6-mercaptopurine; AZA = Azathioprine, Pred = prednisolone, MMF = mycophenolate mofetil; MA = mycophenolic acid; Tac = tacrolimus

Table 15: Dose ranges of major immunosuppressants

Drug regimen	Daily dose median and range
Corticosteroids	
Prednisolone	5mg (0.3 - 60.0)
Budesonide	3mg (0.4 – 9.0)
Thiopurines	
Azathioprine	100mg (25-250)
▪ **Dose per mg/kg**	1.1mg/kg (0.2-2.8)
6-Mercaptopurine (6MP)	150mg (12-150)
▪ **Dose per mg/kg**	0.7mg/kg (0.1-2.0)
Mycophenolate	
Mycophenolate mofetil (MMF)	1000mg [200 -3000]
Mycophenolic acid (MA)	1010mg [720 - 2000]
Calcineurin inhibitors (CNIs)	
Tacrolimus	2mg [0.5-9.0]
Ciclosporin	100mg [75-300]

There were 653/1198 patients (55%) who were on long-term corticosteroid therapy as part of their treatment regimen. This is despite treatment guidelines recommending that maintenance corticosteroids not be used. 124/1198 (10%) patients were on corticosteroid monotherapy (103 [9%] in the form of prednisolone). Of these, 57/106 (54%) patients were not in biochemical remission. Patients who required lower doses of corticosteroids or who were on no corticosteroids at all were more likely to be in biochemical remission. These data are summarised in **Table 16**.

Table 16: Details regarding long-term corticosteroid dose and remission rates (n=1198, excludes transplant patients)[c]

Corticosteroid dose	Number of patients (% of cohort)	Number of patients in biochemical remission (%)
≥ Prednisolone 10mg per day ("high dose")	172 (14.4%)	57/162 (35.2%)
≥ Budesonide 6mg per day ("high dose")	30 (2.5%)	7/29 (24%)
< Prednisolone 10mg per day	410 (34%)	229/378 (61%)
Prednisolone ≥7.5mg per day	249 (21%)	97/234 (41%)
Prednisolone ≤5mg per day	313 (26%)	177/287 (62%)
≤ Budesonide 3mg per day	45 (4%)	21/44 (48%)
No corticosteroid	545 (45%)	342/506 (68%)

[c]all available data presented but some missing data resulting in denominators for the calculation of biochemical remission being smaller than the total number of included patients

Azathioprine monotherapy was the commonest treatment (367/1198, 31%) followed by prednisolone combined with azathioprine (291/1198, 24%). Prednisolone with mycophenolate mofetil (MMF) or myphenolic acid (MA) combination therapy was the third most used combination (112, 9.3%) followed by prednisolone monotherapy (103, 8.6%). These combinations account for 72% of patients. There were 85 patients (7%) on no AIH treatment. 34 were receiving ursodeoxycholic acid (UDCA) whilst 3 were on rituximab (2 were on combination azathioprine and prednisolone whilst the other was on prednisolone monotherapy).

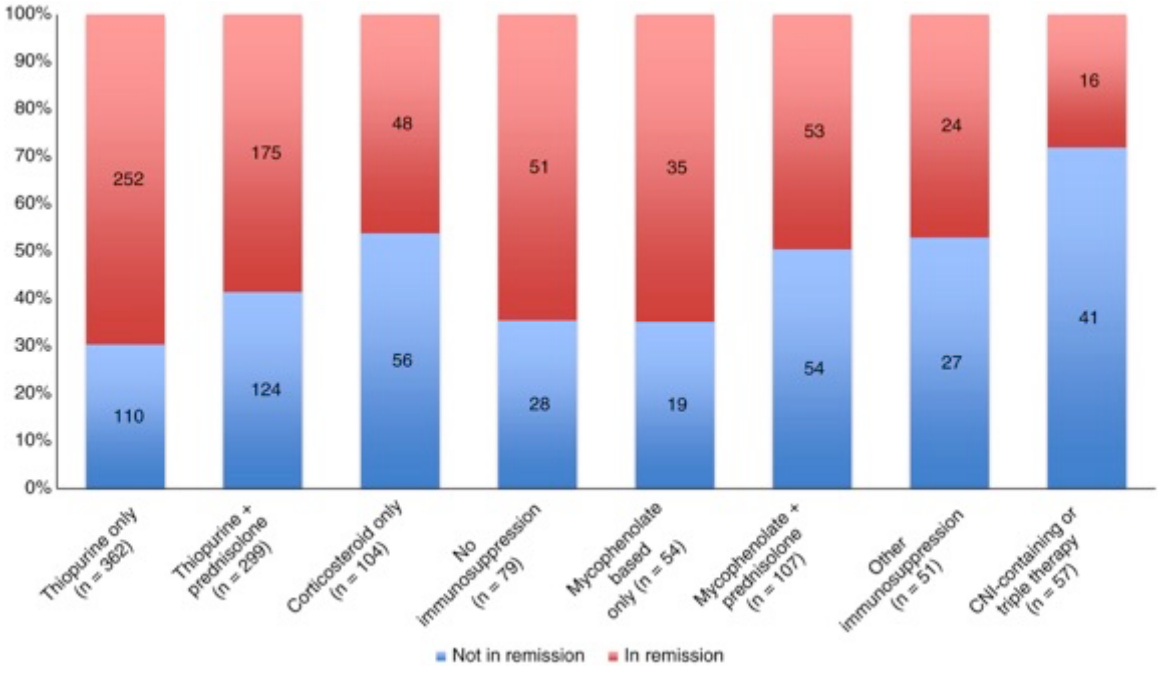
There were 658/1198 (55%) patients taking azathioprine +/- prednisolone. The median dose of azathioprine was 1.1 mg/kg/d (range 0.2-2.8 mg/kg/d). Biochemical data (ALT and IgG) were available for 614 patients on azathioprine +/- prednisolone. Of these, 398 (65%) were in biochemical remission. The rates of biochemical remission were lower (256/500 patients, 51% overall) for patients on other treatment regimens. There were 714 patients taking azathioprine with the doses being available for 696 patients. 232 (33%) were taking less than the 1 mg/kg/d minimum recommended dose. Of these, 66/220 patients with available biochemical data (30%) were not in biochemical remission.

There were 708 (59%) patients who were treated with thiopurines (either azathioprine or 6-mercaptopurine alone - 392 patients) or in combination with prednisolone (316 patients, 26% of whole cohort). For those taking thiopurine therapy alone, 70% (252/362) were in biochemical remission. In contrast, the rate of biochemical remission for patients receiving thiopurine therapy together with prednisolone was significantly lower at 59% (175/299), p = 0.003.

170 (14%) patients were on mycophenolate-based treatment with or without prednisolone (typically used in patients with either intolerance or are non-responsiven to thiopurines). Of the 54 patients who took either mycophenolate mofetil [MMF] or myphenolic acid [MA]) in isolation, 35 (65%) were in biochemical remission. However, in those who took MMF or MA with prednisolone [112 (9%)], only 50% were in biochemical remission, p = 0.066).

There were 59 (5%) patients taking calcineurin inhibitor (CNI) or three drug regimen suggesting difficult to control disease. Of these, only 16/57 (28%) were in biochemical remission and 19/58 (39%) had established cirrhosis at diagnosis. The complexity of treatment regimen and likelihood of treatment response is summarised by **Figure 3.** There were 85 (7%) patients on no immunosuppression. Of these, 65% were in remission with 15 (18%) patients being cirrhotic at diagnosis.

Figure 3: Breakdown of patients by remission status according to simplified drug combinations (excluding transplant patients)



CNI: Calcineurin inhibitor

3.3.3 Location of care

586 of the non-transplanted patients (n= 1198) were cared for in transplant units (49%) and 612 in non-transplant units. Treatment regimens differed significantly between transplant centres and non-transplant centres (**Table 17**). The total number of combinations of therapy were similar between transplant and non-transplant centres (26 vs 27) but patients being cared for in transplant centres were more likely to be on either a triple immunosuppression regimen (p = 0.006) including CNI, anti-CD20 or anti-Tumour Necrosis Factor (TNF) therapy or a regimen including a CNI (p = 0.002).

405 patients in non-transplant centres were looked after by a dedicated hepatologist whereas, 207 patients were looked after by a gastroenterologist. There were fewer combinations of immunosuppression used by gastroenterologists compared to those working as dedicated hepatologists (22 vs 18). In comparing the use of a CNI or triple immunosuppression between hepatologists and gastroenterologists (in non-transplant units), no statistical difference was found.

There was a significantly higher proportion of patients who were in remission in transplant centres compared to non-transplant centres, 62% versus 55% (p = 0.028). No significant difference was found in disease flares, the proportion of patients maintained on higher doses of corticosteroids (defined in the study protocol as ≥10 mg of prednisolone per day or budesonide ≥6 mg/d) or the number who had an attempt to reduce their corticosteroid dose in the previous 12 months between transplant and non-transplant units or care under a hepatologist or gastroenterologist.

Table 17: Comparisons between patients cared for in transplant units and non-transplant centres and, non-transplant centres, in centres with and without specialist hepatologist (n=1198, transplant patients excluded)

	Transplant unit (n=586)	Non-transplant centres (n=612)	p value	Hepatologist (n=405)	No hepatologist (n=207)	p value
Age of patients at diagnosis, median (range)	42 (2-86)	55 (4-86)	<0.0001	55 (2-86)	55 (7-79)	0.564
Number of treatment combinations used	26	27		22	18	
Number of patients on regimen including a CNI	39 (6%)	17 (2.9%)	0.002	8 (1.98%)	9 (4.4%)	0.133
Number of patients on triple immunosuppression	21 (3.6%)	7 (1.14%)	0.006	5 (1.2%)	2 (0.97%)	0.759
Not in biochemical remission (ALT and IgG)	214/562 (38%)	246/552 (44.6%)	0.028	156/366 (42.6%)	90/186 (48.4%)	0.199
Maintenance high-dose corticosteroids during past 12 months	107/586 (18.3%)	93/612 (15.2%)	0.156	54 (13.3%)	39 (18.8%)	0.085
Attempt to reduce corticosteroid dose during last 12 months	25/42 (60%)	55/80 (69%)	0.308	35/46 (76.1%)	20/34 (58.8%)	0.101
Previous Corticosteroid treatment for disease flare	118/572 (21%)	101/586 (17%)	0.142	67/393 (17%)	34/193 (18%)	0.865
Cirrhotic at diagnosis	145/573 (25.3%)	127/603 (21.1%)	0.085	88/402 (21.9%)	39/201 (19.4%)	0.473

3.3.4 Development of cirrhosis (includes transplanted patients)

One of the major goals of therapy in AIH is prevention of cirrhosis. There were however 289/1223 (24%) patients who were already cirrhotic at the time of diagnosis. Those who were diagnosed at 20 years old or younger were significantly more likely to be cirrhotic at diagnosis than patients presenting over the age of 20 (48/141[34%] vs 241/1082[22%], p = 0.002) although it is unclear whether this represents a more aggressive disease course in children and young adults or a higher likelihood of delayed diagnosis.

On excluding the cirrhotics, 131/934 (14%) developed cirrhosis following diagnosis. The risk factors for developing cirrhosis are summarised in **Table 18**. Patients aged 20 years or younger at diagnosis (including transplant patients) were more likely to develop histological or radiological evidence of cirrhosis during follow-up than patients aged more than 20 years (29% vs 14%, p = 0.0007). Patients in biochemical remission at study entry were less likely to develop cirrhosis however this fell short of statistical significance. The total daily azathioprine and corticosteroid dose were not associated statistically with the development of cirrhosis during follow-up. Whether or not patients were under the care of a hepatologist or gastroenterologist was not associated with disease progression, suggesting that patients with more stable disease were looked after appropriately.

Table 18: Risk factors for developing cirrhosis (either histological and/or radiological) during follow-up from diagnosis to point of study entry (excluding patients who were cirrhotic at diagnsis)

Risk Factor		Number developing cirrhosis (%)		Number developing cirrhosis (%)	p value
Age at diagnosis[d]	≤20 y	27/94 (29)	>20 y	104/836 (14)	0.0007
Biochemical remission at study entry	In remission	49/514 (10%)	Not in remission	44/331 (13%)	0.092
Azathioprine dose	<1 mg/kg/d	17/176 (10)	≥1 mg/kg/d	33/344 (10)	0.984
Corticosteroid dose	≥prednisolone 10 mg and/or budesonide 6 mg/d	18/140 (13)	Prednisolone ≤5 mg and/or budesonide 3 mg/d[e]	80/901 (9)	0.134
Specialist care (for non transplant unit)	Hepatologist	28/311 (9)	No hepatologist	7/160 (4)	0.070

[d]Taking the event of liver transplantation as evidence of the development of cirrhosis.
[e]Includes patients on no corticosteroid.

The uni- and multi-variate analyses for non-transplanted patients are summarised in **Table 19**. For these analyses, years since diagnosis has been used instead of age at diagnosis to enable exclusion of the transplanted patients, that is, ensuring that the same patient group is used for each variable and that each patient is only included once in the analyses. Increasing years since diagnosis remained significant as a predictor for development of cirrhosis on uni- and multi-variate analysis. Patients in biochemical remission at study entry were less likely to develop cirrhosis, however, this fell short of statistical significance. On examining treatment regimens (compared to standard therapy with azathioprine +/- prednisolone), the use of triple immunosuppression or a CNI-containing regimen was associated with a higher probability of developing cirrhosis (p = 0.011) but this effect became just non-significant (p = 0.053) on multi-variate analysis when other risk factors were included in the model. Place of care was associated with a more aggressive disease phenotype with transplant units having a greater proportion of patients developing cirrhosis following their initial diagnosis in both the univariate and multiple logistic regression models.

Table 19: Univariate and multiple logistic regression model of risk factors for developing cirrhosis (either histological and/or radiological) during follow‐up from diagnosis to point of study entry (excluding patients who were cirrhotic at diagnosis

Risk factor	Univariate		Multiple	
	Odds ratio	p value	Odds ratio	p value
Biochemical remission at entry (yes vs no)	0.7033 [0.455, 1.087]	0.1129	0.7514 [0.469, 1.203]	0.2335
Treatment regimen				
Corticosteroid only vs standard[f]	1.8688 [0.955, 3.655]	0.0678	1.5573 [0.76, 3.191]	0.2262
None vs standard	0.9356 [0.383, 2.288]	0.884	0.8025 [0.303, 2.123]	0.6575
Other vs standard	0.9398 [0.535, 1.651]	0.829	0.9743 [0.536, 1.772]	0.9321
Triple or CNI vs standard	2.8558 [1.272, 6.411]	0.011	2.3512 [0.99, 5.586]	0.0528

Transplant unit (yes vs no)	2.3485 [1.484, 3.716]	0.0003	2.0458 [1.258, 3.327]	0.0039
Years since diagnosis	1.1029 [1.075, 1.131]	0.0001	1.0972 [1.069, 1.126]	0.0001

CNI: calcineurin inhibitor.

[f]Standard therapy = azathioprine +/-prednisolone.

3.4 Discussion

This large nationwide multicentre study of "real world" clinical practice in AIH clearly demonstrates the unmet need in this complex, heterogeneous disease. There are significant limitations in the effectiveness of care for AIH and a high degree of variability in practice and quality between unit types.

1. Biochemical remission rate is lower than benchmark figure

The first key observation is that the biochemical remission rate using standard criteria is only 59%; this figure falls far short of the benchmark figure of 80% - demonstrated to be achievable in specialist centres with a specific interest in disease management and structures in place to deliver optimised care (Hoeroldt *et al.*, 2011).

2. There are multiple treatment regimens used with corticosteroid therapy being used in more than 50% of patients with AIH in this study

The second observation is that there were over 50% of patients with AIH in this national study receiving ongoing corticosteroid therapy, in spite of multiple clinical practice guidelines suggesting that maintenance should be with thiopurine monotherapy (Gleeson, Heneghan and British Society of, 2011; European Association for the Study of the, 2015; Mack *et al.*, 2020). There was apparent confusion and inconsistency around appropriate treatment with 29 individual treatment regimens described even before allowing for dose variations.

3. Achieving and maintaining biochemical remission is more likely in transplant centres although there is a bias towards a more severe disease phenotype

The third key observation is that patients receiving care in transplant centres in the UK were more likely to achieve and maintain remission than those looked after in other care locations, despite an apparent bias towards a more severe disease phenotype.

This nation-wide cross-section of AIH patients includes approximately 1 in 10 with this rare disease in the UK. This study population's demographic characteristics and disease were in keeping with previous published series although concurrent PSC was more common in transplant units (Wong *et al.*, 2017; van Gerven *et al.*, 2014; Al-Chalabi *et al.*, 2006). This suggests that differences in the behaviour of the cohort long term are unlikely to be attributable to the characteristics of the population, but rather, the care received. There was variation in risk seen within this cohort's patients with those presenting at 20 years or younger having the highest rate of cirrhosis at diagnosis and progression to cirrhosis during follow-up, similar to other studies (Ngu *et al.*, 2013; Montano-Loza, Carpenter and Czaja, 2007b). The profile of disease phenotype in this cohort is in keeping with previously published studies of children with AIH, particularly, Type 2 AIH which is associated with detectable anti-Liver Kidney Microsomal (LKM) antibodies in serum (Gregorio *et al.*, 1997).

Current treatment regimens are clearly not adequate for a considerable number of patients with 41% not achieving biochemical remission, 19% suffering a disease flare in the preceding 12 months requiring increase or addition in corticosteroid treatment and 14% developing cirrhosis despite treatment. This is comparable to a smaller Swedish registry-based study of 473 patients (Werner *et al.*, 2010) that found 40% of patients did not achieve remission in the first year. As failure to maintain biochemical remission and normalise ALT are surrogate markers for progression of liver disease and poor outcome (Hoeroldt *et al.*, 2011; Miyake *et al.*, 2006a; Montano-Loza, Carpenter and Czaja, 2007c; Ngu *et al.*, 2013), this emphasizes the ongoing need for better therapies.

Corticosteroid use was high in this cohort with 55% of patients using prednisolone or budesonide as current therapy. Corticosteroid use has been shown to be strongly associated with decreased health-related quality of life that is independent of biochemical remission status (see **Chapter 5** and reference (Wong *et al.*, 2018)). 38% (421/1111) of patients were classified as obese with a BMI > 30 kg/m^2. This is higher than the quoted national England Obesity adult rates of 28% (Balogun, 2022). BMI was generally high in this UK-AIH cohort, with the median being 28.4 kg/m^2 and mean BMI 29.4 kg/ m^2. The latter is again higher than the mean BMI of 27.6 in adults in England (according to the 2019 England Health Survey) (Balogun,

2022). The high median BMI may reflect the excessive high use of corticosteroids. Corticosteroid therapy in combination with weight gain, is likely to result in the development of secondary disease such as metabolic syndrome, hypertension and hyperlipidaemia in this patient cohort. In older data reports, patients who successfully withdraw completely from corticosteroids lose an average of 6 kg of weight per patient (Johnson, McFarlane and Williams, 1995).

Thiopurine therapy use, either azathioprine or mercaptopurine (+/- corticosteroid) was 63% (753/1198 patients) of the whole patient cohort with 359/753 (48%) taking corticosteroid therapy. This suggests that adherence to established treatment guidelines recommending thiopurine monotherapy for AIH patients in the UK (European Association for the Study of the Liver and British Society of Gastroenterology guidelines) is poor. The median azathioprine dose in this cohort was only 1.1 mg/kg/d. This study's protocol did not include the checking of thiopurine drug metabolite levels nor details of side effects, adverse events or reasons for poor treatment tolerance. However, published data shows that an azathioprine dose of up to 2 mg/kg/d can result in enhanced long-term remission rates in AIH with the consequent ability to withdraw corticosteroids succesfully from the treatment regimen for the majority of patients (Stellon *et al.*, 1988).

There was a difference in the approach to management of AIH between transplant and non-transplant centres. Although the number of treatment regimens used were similar in transplant compared to non-transplant units (26 vs 27), patients were more likely to be exposed to a wider range of novel treatment options in AIH management (eg, CNIs, three-drug regimens or biological agents such as infliximab or rituximab). This individualised and more nuanced approach to care in transplant centres appears to translate to less disease fluctuation with higher rates of biochemical remission in patients which is likely to have a consequent protective element in relation to hepatic outcomes (Hoeroldt *et al.*, 2011).

This study of patients with AIH demonstrates "real world" data and reveals the therapeutic challenges that have been discussed in the literature (Liberal *et al.*, 2017; Zachou *et al.*, 2013; Czaja, 2013d). The poor remission rates reported in this study suggest there are significant unmet needs in treatments for patients with AIH. There has been significant progress for the majority of other autoimmune disorders which has facilitated corticosteroid-free regimens. These include the use of disease-modifying agents and novel antibodies used in diseases such as Multiple Sclerosis, Inflammatory Bowel Disease and Rheumatoid Arthritis (Burmester and

Pope, 2017; Ontaneda *et al.*, 2017). The change in treatment paradigms for these conditions demonstrate the significant benefits gained from the explosion and expansion of novel therapeutics. There is a pressing need for novel therapeutic approaches and targets in AIH, being an orphan disease with potential hard outcomes such as cirrhosis development, death and liver transplantation. There have been some exploration of potential targets for AIH treatment but none have been realised in clinical practice as yet (Czaja, 2013d). Patients with liver disease, particularly AIH (with its rarity) are disadvantaged by inertia from the medical community and a reluctance from the pharmaceutical industry to offer the use of potentially useful therapeutic agents in clinical trials. For example, antibody therapies such as anti-CD20 or anti-TNF therapy have only been utilised in less than 50 patients worldwide with AIH; and even then, only in the context of late disease (Burak *et al.*, 2013; Weiler-Normann *et al.*, 2013). The alternative treatment paradigm in contrast should be one of targeting potential disease modifiers or stoppers aggressively at the onset or initial stage of early disease to avoid life-long therapy with corticosteroids and other drugs that have been become outdated for the a large number of other inflammatory autoimmune disorders.

Although this study reveals important findings on the reality of care for AIH in the UK, there are study limitation to take note of. Firstly, this is an observational cohort study describing outcomes in practice and looks at prevalent cases. This limits the breadth of data capture and will miss very high-risk patients who died from the disease early in the disease-course; these would thus feature in an incident but not a prevalent cohort. However, this approach does avoid the potential for a concentrated prospective study which focuses on and potentially leads to artificial improvement in the delivered quality of the care.

Secondly, this study relates only to practice in the UK. It would be of great interest to replicate this approach in other health-care settings to see if there are similar limitations in care. Limited and anecdotal data suggest that there are.

Thirdly, the categorisation into transplant units and nontransplant units was a robust but slightly blunt approach. This study has tried to account for centres with a specialist interest in AIH (where excellent results are reported (Hoeroldt *et al.*, 2011)) by sub-dividing nontransplant units into those with and without dedicated hepatologists (data courtesy of Jessica Dyson and Mark Hudson from national survey of liver services). It may be that these centres improve the apparent outcomes in the nontransplant centre group as a whole (as seen

with the significantly lower rate of disease flares in centres with a hepatologist), masking the true scale of the care quality divide.

Fourthly, there may be a degree of selection bias as only interested centres participated in the study which could potentially underestimate the extent of the realities of care for AIH in the UK.

Concluding remarks

In conclusion, despite its limitations the UK-AIH cohort demonstrates significant discrepancies in care delivery for patients with AIH. Where there is failure to achieve biochemical remission, one must ask whether existing evidence-based therapies are being optimally deployed, if more individualised dose adjustment based on markers of pharmacodynamic effect would increase the proportion achieving response or whether new therapeutic options are required. This data emphasises the urgent need for the development of better therapies with less side effects and optimised utilisation of current therapies.

Chapter 4. Exploring incorporating deoxythioguanosine into DNA (dTG) in azathioprine-treated patients with autoimmune hepatitis

4.1 Introduction

Azathioprine (AZA), a thiopurine drug is the first-line steroid-sparing immunosuppressant used for AIH (Soloway *et al.*, 1972; Summerskill *et al.*, 1975; Lamers *et al.*, 2010). Studies in the 1970s demonstrated that azathioprine in combination with prednisolone is an effective therapy in inducing clinical, biochemical and histological remission with significant survival improvement compared to placebo (Soloway *et al.*, 1972; Summerskill *et al.*, 1975). However, azathioprine is associated with side effects such as arthralgia, skin rash, nausea, bone marrow suppression, pancreatitis, skin cancer, solid organ malignancy and opportunistic infection (Johnson, McFarlane and Williams, 1995; Bajaj *et al.*, 2005; Czaja and Carpenter, 2006b). Approximately 25% of patients develop side effects and 10% require cessation of therapy (Al-Chalabi *et al.*, 2008b). Guidelines state that azathioprine monotherapy should be the goal of treatment (European Association for the Study of the, 2015; Mack *et al.*, 2020). Older data suggests that only 18% are unable to maintain remission on AZA monotherapy (Johnson, McFarlane and Williams, 1995) suggesting that it is not an effective approach in clinical practice.

More recently, "real world" data from the UK-AIH study showed that as many as 30% of the patients on thiopurine monotherapy (33% of the population) were not in biochemical remission, suggesting a more complex picture. [See Chapter 3 and (Dyson *et al.*, 2018)] Balancing optimal azathioprine dosing, adverse effects and clinical efficacy in AIH patients can be a challenge. AZA intolerance is principally attributed to the complex metabolism of the drug (Heneghan *et al.*, 2006).

As elaborated in **Chapter 1: Section 1.11.1**, azathioprine red-blood-cell (RBC) metabolites as a predictor of adverse effects and therapeutic response have been evaluated in AIH and IBD with conflicting results with regards to clinical response.

The aim of this study was to evaluate a novel leukocyte thiopurine metabolite: incorporated dTG into the DNA of white blood cells as a potential marker of disease response, compliance and AZA dose in patients with AIH, using two subsets of patients from the UK-AIH cohort.

4.2 Methods

Patients (≥16 years old) from the UK-AIH study were utilised. The diagnosis of AIH was based on the simplified 2008 IAIHG criteria (scoring at least 7 points for definite AIH) (Hennes *et al.*, 2008b). Samples from patients recruited from Mar 2014 to Mar 2016 were analysed. Patients with other concurrent liver diseases (including PBC, PSC and overlap) and previous liver transplantation were excluded. Biochemical remission was defined as normal alanine transaminase (ALT) and immunoglobulin G (IgG). In order to assess factors associated with incomplete treatment response, two clearly defined patient groups from the UK-AIH study were used: Group A (Responders to therapy) and Group B (Incomplete responders to therapy). Further information can be found in **Chapter 2: Section 2.5.2 (ii-iv)**. The full inclusion and exclusion criteria for Group A and B are detailed in the **Appendix B: Tables 1-4.**

Demographic, clinical data and adherence data were collected as detailed in **Chapter 2**.

The sample preparation and LC-MS/MS method is elaborated in **Chapter 2**: **Section 2.5.2: iv**.

Statistical analysis

Descriptive statistics are presented as frequencies [n(%)], or median (range), as appropriate. Exploratory analyses were conducted to investigate the relationship between disease response and dTG DNA. Logistic regression was performed with response status (responder or incomplete responder) as the binary dependent variable and dTG DNA as a covariate. An equivalent model was also considered where only the subset of patients with ALT levels higher than the ULN on recruitment were included in the incomplete responder group. Models were adjusted for age and sex. Spearman's correlation coefficient was used to assess the relationship between azathioprine dose (mg/kg) and dTG DNA. Data were analysed using SPSS and R v4.0.0.

4.3 Results

A total of 57 autoimmune hepatitis patient samples from three UK transplant centres (Newcastle, King's College Hospital London and Cambridge) were analysed. There were 27 in the Responder Group (A) and 30 in the Incomplete responder group (B). Six were found to be

heterozygous TPMT *1/*3A (n=3 in Group A and n=3 in Group B) and the remaining were TPMT wild-type.

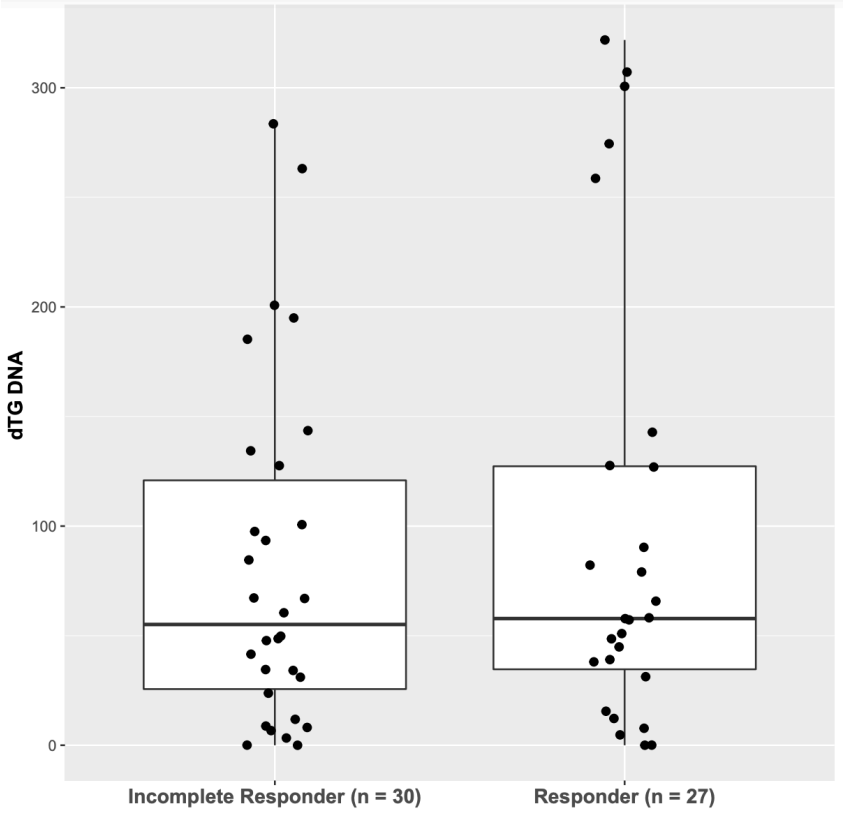
The demographic, biochemical and clinical data are summarised in **Table 20**. Patients were found to be significantly older in the responder group with a median age of 59 years versus 41.5 years in the incomplete responder group (p = 0.009). Additionally, median dose of azathioprine dose by weight (mg/kg) was noted to be lower than the recommended 1mg/kg in the Responder group. The median AZA doses between both groups differed significantly (p = 0.009). The percentage of patients with cirrhosis in both groups were similar.

Table 20: Demographic, biochemical and clinical data [figures are expressed as median, range or number (%)]

Data	Responder (A) [n=27]	Incomplete responder (B) [n=30]	p value
Females [n (%)]	20 (74%)	21 (70%)	0.776
Age (years)	59 (29-86)	41.5 (22-82)	0.009
Azathioprine dose (mg/day)	75 (25-150)	100 (25-150)	0.073
Weight (kg)	76 (50-144)	71 (50-118)	0.146
Azathioprine dose by weight (mg/kg/day)	0.97 (0.33-1.7)	1.19 (0.21 – 2.38)	0.009
BMI (kg/m^2)	27.4 (17.8 - 46.1)	26.3 (19.5 - 35.3)	0.213
BMI ≥ 30 [n (%)]	10 (37%)	4 (13%)	0.063
Patients on prednisolone [n (%)]	0	23 (77%)	N/A
Prednisolone dose (mg)	N/A	5 (2-60)	N/A
Patients on budesonide [n (%)]	0	3 (10%)	N/A
Budesonide dose (mg)	0	9 (6-9)	N/A
Additional immunosuppressant	0	MMF = 1 (3%) Tacrolimus = 2 (7%)	N/A
ALT (IU/L), median (range)	17 (10-33)	35 (9-381)	<0.0001
IgG (g/L), median (range)	11.8 (7.9-17.8)	12.4 (7.2-35.4)	0.197
Duration of diagnosis, median (range) in years	9.5 (1-30)	9 (1-33)	0.603
Cirrhosis **Present at diagnosis** **Developed since diagnosis**	10 (37%) 7 (26%) 3 (11%)	8 (27%) 8 (27%) 0	0.569 >0.999 0.099
Ratio nmol dTG/10^6 nmol dA (dTG DNA)	57.8 (0 – 321.8)	55.1 (0-283.6)	0.748

The distribution of dTG DNA values are displayed graphically in **Figure 4**. The median dTG DNA was 57.8 (0 – 321.8) in the responder group and 55.1 (0 – 283.6) in the incomplete responder group (p=0.748). No evidence of an association between response status and dTG DNA was found after controlling for age and gender ($\hat{\beta}_{\text{dTG DNA}}$ = 0.002 [95% CI: -0.004, 0.009], p = 0.45). Similar conclusions were obtained when only patients with ALT levels higher than the ULN on recruitment were included in the incomplete responder group (n=13) while the responder group was defined as previously ($\hat{\beta}_{\text{dTG DNA}}$ = 0.006 [95% CI: -0.003, 0.017], p = 0.23). No evidence of significant correlation was observed between azathioprine dose (mg/kg) and dTG DNA (Spearman's rank correlation coefficient: r_s = 0.04 [-0.23, 0.30]).

Figure 4: Distribution of dTG DNA values according to response



Adherence data was then reviewed to explore whether this may play a role in clinical response, particularly in the 4 patients with absent dTG DNA (see **Figure 5**). The questions and various responses are summarised in **Table 21**. Both the clinical data form (CDF) and participant questionnaire had an identical adherence question (a); any missing data from the patient questionnaire was completed using the response from the CDF and if there were differing responses, the higher numerical value response was used. Adherence data for question (a)

was available for 56/57 patients whilst there were 51/57 responses for question (b) (see **Table 21**).

Table 21: Adherence questions and responses in both groups

Adherence questions	Response	Responder (A) (n=27)	Incomplete Responder (B) (n=30)
(a) Which of these best describes how often you miss a dose of your prescribed AIH medicines?	0 = Once or twice a year	21 (78%)	17 (57%)
	1 = Once or twice a month	4 (15%)	6 (20%)
	2 = Once or twice a week	0	2 (3.6%)
	3 = Several times a week	2 (7%)	4 (13%)
	4 = Most days of the week	0	0 (0%)
			[1 blank]
(b) How many days in the last week have you taken all your prescribed AIH medicine?	▪ 7 days	22 (81.5%)	23 (77%)
	▪ 6 days	0	2 (7%)
	▪ 5 days	2 (7.4%)	1 (3%)
	▪ 4 days	0	0
	▪ 3 days	0	0
	▪ 2 days	0	0
	▪ 1 day	0	0
	▪ 0 day	0	1 (3%)
		[3 blanks]	[3 blanks]

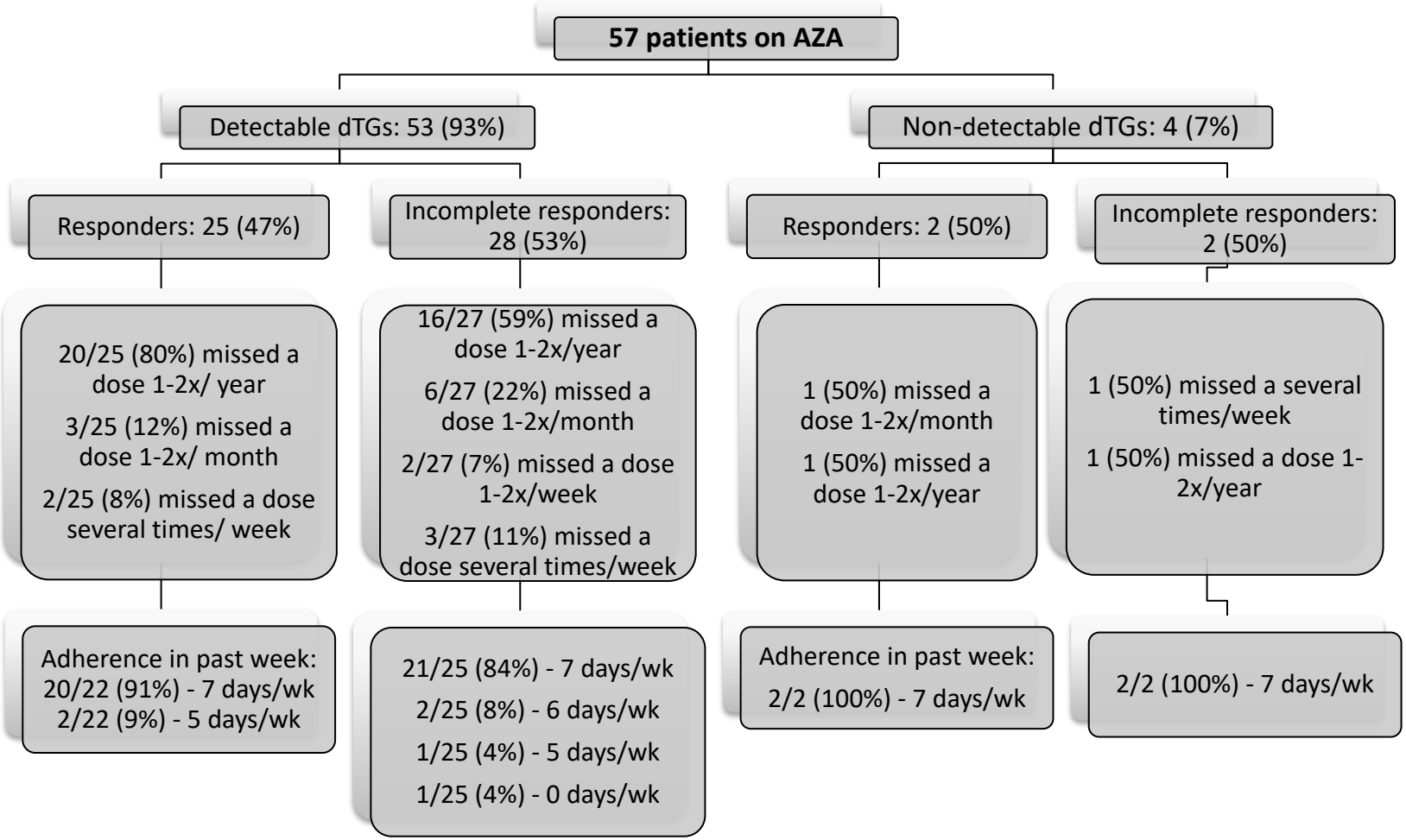
Out of the whole cohort (excluding 6 patients with blank answers), 47/51 (92%) of patients reported taking their AIH medications at least 6 or more days of the week. The number of patients missing their medications several times a week was 4/29 (14%) (with available data) in the Incomplete Responder (B) group which is about twice the number compared to 2/27 (7%) in the Responder group although the comparison did not reach statistical significance (p=0.671).

Out of the 57 patients on azathioprine, 53 (93%) had detectable dTG DNA (see **Figure 5**). In these 53 patients, 25 (47%) were in the Responder group (A) whilst 28 (53%) were in the Incomplete Responder (B) group. Adherence data for question (a) was available in all of Group

A and 27/28 of Group B participants. More patients reported missing their medication dose several times per week (3/27, 11%) in Group B (Incomplete Responder) compared to Group A (2/25, 8%) ($p>0.99$).

There were 2 patients from Group A and 2 from Group B with no detectable dTG DNA. In the Incomplete Responder (B) group, 1 patient (50%) missed their dose of AIH medications several times per week and the other missed their dose 1-2x/month. Adherence was slightly better in the Responder (A) group where 1 patient (50%) missed their dose of AIH medications 1-2x/month whilst the other missed their medications 1-2x/year. All 4 patients reported full 7-day compliance during the week prior to study recruitment.

Figure 5: Adherence data



4.5 Discussion

This study did not identify any correlation between levels of dTG in DNA with disease-response or azathioprine dosage. Adherence was not a major issue amongst the majority of patients but there were more than twice the number of patients missing their medications several times a week in the Incomplete Responder group compared to the Responder group.

In the 4 patients with no measurable dTG DNA, only 2 patients reported missing their medication 1-2x/month. Although the numbers are too small to make any concrete conclusions, one could postulate that the 2 patients can be classed as pseudo-non-responders due to their adherence being less than 100% compliance. On a similar note, those who have detectable dTGs who remain incomplete responders (n=28) and report 100% treatment compliance could be classed as true non-responders to therapy. This subset could be targeted for second line therapies or future novel therapies.

TPMT is the key enzyme involved in regulating 6-TGNs. Pre-treatment screening for TPMT enzyme activity has been recommended to reduce the risk of adverse events and improve drug tolerability. TPMT activity is determined by genes which are highly polymorphic, with at least 10 variant alleles being associated with low enzyme activity (Dubinsky *et al.*, 2000; Czaja, 2020). TPMT has a trimodal distribution in Caucasians, with around 89% being homozygous for the wild-type allele with normal enzyme activity, 11% being heterozygous with intermediate enzyme activity and 0.3% being homozygous for the deficiency-alleles with no functional activity (Lennard *et al.*, 1990). Patients with low TPMT activity are at risk of producing high levels of 6-TGNs which is associated with increased risk of myelosuppression. High TPMT activity is associated with a skewed drug metabolism (shunting or hypermethylation). 6MP is preferentially metabolised to methylated metabolites (6-MMP and MeMPNs), resulting in reduced 6-TGN production, which is associated with reduced therapeutic effect or azathioprine non-response. The increased methylated by-products are associated with hepatotoxicity (Warner *et al.*, 2018; Dubinsky *et al.*, 2000).

Studies in paediatric acute lymphoblastic leukaemia (ALL) have shown that patients with lower TPMT activities and/or higher 6-TGN levels have a lower risk of relapse but a higher incidence of neutropaenia (Lennard *et al.*, 2015). Studies in IBD have been conflicting - high TPMT activity has been reported to be significantly associated with AZA non-response and intermediate activity with AZA intolerance (GI upset, pancreatitis, neutropaenia) (Ansari *et al.*,

2002). However, 50-75% of patients developing leukopaenia have a normal TPMT and only 3% of hypermethylators have very high TPMT (Colombel *et al.*, 2000; Warner *et al.*, 2018). A meta-analysis evaluated the associations between TPMT polymorphism and AZA-induced adverse events in patients (n=645) with autoimmune diseases (this included 146 patients with AIH). There was an association of TPMT polymorphisms with overall AZA-induced adverse events, bone marrow toxicity and gastric intolerance but not with hepatotoxicity. In the sub-group analysis however, there was a significant association between TPMT polymorphisms and AZA-induced bone-marrow toxicity in Asian populations but not in Caucasian populations. TPMT polymorphisms can explain a variable proportion but not all incidences of AZA-related adverse events. Additionally, a normal TPMT genotype does not exclude the development of side effects (Liu *et al.*, 2015). Checking the TPMT phenotype (via enzyme assay) is the preferred option compared to TPMT genotype as the assay will pick up completely deficient patients irrespective of the TPMT genotype. Various factors (such as red cell age) can influence TPMT activity and this contributes to discordance between TPMT phenotype and genotype. Thioxanthine, produced during 6MP metabolism can inhibit TPMT activity. Co-administration of 5-ASA drugs can also increase production of 6-TGNs and increase leukopaenia incidence (Warner *et al.*, 2018).

Studies in AIH have not found TPMT testing (whether genotype or phenotype) predictive for AZA-related toxicity. Langley and colleagues evaluated the TPMT activity in 72 patients with AIH and found TPMT activities were significantly lower in patients with AZA intolerance compared to those with sustained remission on AZA alone (p=0.003) and those on combined therapy (p=0.0001) A 25% discordance was noted though between phenotype and/or genotype and response to AZA (Langley *et al.*, 2002). Two following studies of AIH patients (n=86 in each) did not find any association between TPMT phenotype or genotype in predicting AZA toxicity (Heneghan *et al.*, 2006; Czaja and Carpenter, 2006b). This may possibly be due to the lower thiopurine doses used to treat patients with AIH which usually does not cause bone marrow suppression in patients with intermediate TPMT activity. TPMT determination before commencing thiopurines are generally recommended to avoid the risk of severe bone marrow toxicity in the rare 1 in 300 patients with complete TPMT deficiency (Hindorf *et al.*, 2010).

In this study, 3/27 (11%) and 3/30 (10%) in Group A and B respectively were heterozygote in TPMT genotype with no significant difference between these 2 proporportions (p=1.00) whilst

the rest of the patients had the wild-type TPMT genotype. Data on historical phenotype TPMT levels were collected but as there was a lot of missing data, this could not be explored further for comparison.

The use of allopurinol with low-dose thiopurine to redirect thiopurine metabolism towards formation of 6-TGN instead of 6-MMPS has been evaluated by de Boer and colleagues. They concluded that allopurinol use was safe and effective in thiopurine therapy optimisation in patients with AIH who were intolerant or had failed to respond due to the underlying unfavourable thiopurine metabolism (de Boer *et al.*, 2013). In our study, none of our patients were on allopurinol. Coulthard and colleagues have previously correlated dTG-DNA levels with drug dose in their cohort of patients with IBD (9 on AZA and 9 with low dose AZA with allopurinol) (Coulthard *et al.*, 2017). The role of allopurinol combined with low dose AZA and its effect on thiopurine metabolite levels and clinical response is an avenue that should be explored further in AIH.

Fibrosis stage has been reported to be of possible influence on AZA metabolism. In the study by Heneghan et al, drug toxicity related to AZA was significantly more likely to occur in patients who had bridging fibrosis or cirrhosis (stage III/IV) on liver biopsy. RBC TPMT enzyme activity was also significantly lower in patients with stage III/IV compared to patients with lesser degrees of fibrosis (Heneghan *et al.*, 2006). In this UK-AIH study, cirrhosis was present in 10 (37%) of the responder group and 8 (27%) of the incomplete responder. There were equal numbers of patients with cirrhosis amongst the 4 patients with no measurable dTG DNA (one in each group). In this study, cirrhosis is unlikely to have been an influencing factor on dTG DNA.

Obesity has been associated with lower RBC 6-TGN levels. A study in patients with IBD (n=132) found that obese patients (BMI ≥ 30) were more likely to have subtherapeutic 6-TGN levels (<230 pmol/8 × 10^8 RBCs) compared to normal BMI (65% versus 35%, p=0.02) (Poon *et al.*, 2015). In our study, there were more obese patients in the responder group (37%) versus incomplete responder (13%) with mean BMI between groups differing by 2 points (28.6 vs 26.3 kg/m^2). Although dTG-DNA levels did not differ between the groups, obesity as an influencing factor in AZA response and adverse events in AIH should be explored further.

This study is the first study to evaluate a novel thiopurine metabolite involving nucleated cells in AIH and deserves merit for its exploratory nature. The study is multi-centre, utilising

patients from 3 liver transplant centres, making the findings more generalizable. The study has limitations – It was a cross-sectional study, measuring dTG DNA at a single point in the patient's disease course. Duration of disease did not differ between both groups in this study (~9 years). The study could be improved with prospective serial measurements of dTG DNA as well as the measurement of RBC-thiopurine metabolite and TPMT phenotype for comparison.

Although the concept behind this study was a good one, there was unfortunately no significant correlation found between levels of dTG in DNA with disease response or azathioprine dose. This suggests that the biomarker is not clinically useful in AIH. This study's findings imply that DNA incorporation of thioguanine nucleotides is not the sole reason for thiopurine toxicity and therefore adds to our knowledge of how these safe efficacious drugs exert their effect. Research is ongoing in the context of inflammatory bowel disease and the possible implications of dTG in DNA on incidence of secondary cancers in patients treated with thiopurines based on data generated from studies on a cohort of patients with IBD (Coulthard *et al.*, 2017). This study therefore is informative for other studies with respect to thiopurine biology.

Chapter 5. The Impact of AIH on Quality of Life in patients

The content in this chapter has been published in 2018 (see reference below) and adapted accordingly for the purposes of this thesis. The article is an Open Access article and the Publisher permits non-commercial use of the work as published, without adaptation or alteration provided the work is fully attributed.

Refer to **Appendix D: Publications**: The Impact of AIH and its treatment on Health Utility

Reference: Hepatology 2018; 68(4): 1487-1497.

URL: https://aasldpubs.onlinelibrary.wiley.com/doi/full/10.1002/hep.30031

DOI: 10.1002/hep.30031

5.1 Introduction

AIH remains a diagnostic and therapeutic challenge with at least a third of patients presenting with cirrhosis, a fifth having relapsing disease and 30-50% developing cirrhosis despite treatment (Gleeson, Heneghan and British Society of, 2011). AIH therefore has the potential to cause significant medical and economic burdens on affected patients and health care delivery systems, respectively. Current management of AIH largely comprises corticosteroids alone (prednisolone mainly) or in combination with azathioprine with 38-93% of patients achieving remission (Czaja, 2002; Hoeroldt *et al.*, 2011) but up to 90% having a disease relapse after withdrawal of therapy (European Association for the Study of the, 2015). Other second-line immunosuppressants include mycophenolate mofetil (MMF), cyclosporine and tacrolimus but these lack evidence based on randomized controlled trials. All these agents, particularly corticosteroids, can be associated with important side effects such as the metabolic syndrome with its sequelae, osteoporosis, weight gain and disturbance in sleep and mood (Cook, Mulligan and Sherlock, 1971; Murray-Lyon, Stern and Williams, 1973; Soloway *et al.*, 1972; Summerskill *et al.*, 1975; Manns *et al.*, 2010b).

Studies on health-related quality of life (HRQOL) in AIH have been mainly single-center with small numbers but these suggest that there is a significant issue with quality of life (QOL) in

AIH and that further formal exploration of its impact is warranted (van der Plas, Hansen and de Boer, 2007; Gulati *et al.*, 2013; Schramm *et al.*, 2014; Janik *et al.*, 2019; Sockalingam *et al.*, 2012). [See **Chapter 1: Section 1.10** for elaboration on studies in HRQOL in AIH.]

Quality of life is a critically important issue for patients, and one that is increasingly prioritised by regulatory bodies when evaluating the benefits of new drugs. A deep understanding of HRQOL is therefore essential if we are to make progress with therapy in AIH.

In this study, the unique cohort of prevalent cases in the United Kingdom-AIH (UK-AIH) study was utilised to explore the impact of AIH and its treatments on patient life quality using the European Quality-of-Life 5-Dimension 5-Level (EQ-5D-5L) tool to evaluate HRQOL (European Quality of Life, 1990). With this tool, health state utilities were calculated. Health state utilities are values which represents an individual's preferred value for specific health states relative to full health. These are fundamental in assessing cost-effectiveness and cost utility of the management of disease (McLernon, Dillon and Donnan, 2008). As fatigue, cognitive impairment, anxiety and depression are important symptoms that have been reported in chronic liver diseases and have significant impact on QOL (van der Plas, Hansen and de Boer, 2007; Schramm *et al.*, 2014; Mells *et al.*, 2013; Newton *et al.*, 2008b; Newton *et al.*, 2008a), three other qualitative tools were used to explore the impact of specific symptom sets on HRQOL: Fatigue Impact Score (FIS) (Fisk *et al.*, 1994), Cognitive Failure Questionnaire (CFQ) (Broadbent, 1982) and Hospital Anxiety Depression Scale (HADS)] (Zigmond and Snaith, 1983).

5.2 Patients and Methods

Study population: Adult patients (≥16 years) from the UK-AIH study with a current clinical diagnosis of AIH (as evaluated by their treating clinician) were recruited from a secondary hospital care setting between March 2014 until Jan 2017. (See Chapter 2 for further details)

Quality of life and symptom impact measures:

The **EQ-5D-5L tool** is a simple, generic HRQOL instrument comprising five health dimensions which generates a health utility index (UI) and a visual analogue scale (VAS). The European Quality-of-life 5-Dimension (EQ-5D) tool was first introduced as a three level version (EQ-5D-3L) in 1990 with three levels of severity (no problems, some problems, extreme problems) and was subsequently revised in 2009 to include five levels of severity (EQ-5D-5L) in order to

improve the instrument's sensitivity and reduce ceiling effects compared to EQ-5D-3L (European Quality of Life, 1990; Janssen *et al.*, 2008). The EQ-5D tool has been used to evaluate HRQOL in a wide spectrum of diseases including liver disease, notably hepatitis C (Siebert *et al.*, 2001), hepatitis B (Kim *et al.*, 2012), liver transplantation (Bryan *et al.*, 1998) and other chronic liver diseases. The EQ-5D-5L is widely used internationally as a patient-reported outcome measure and is the preferred tool of England's health authority, the National Institute for Health and Care Excellence (NICE) for use in cost-effectiveness analysis (National Institute for Health and Care, 2013a). The EQ-5D-5L tool is comprised of the EQ descriptive system of five health dimensions (see below) and the EQ-visual analogue scale (EQ-VAS):

- Mobility
- Self-care
- Usual activities
- Pain/discomfort
- Anxiety/depression

Each health dimension has five levels (scored from 1 to 5):

- No problems (Score 1)
- Slight problems (Score 2)
- Moderate problems (Score 3)
- Severe problems (Score 4)
- Extreme problems. (Score 5)

The respondent indicates his/her health state by ticking the box against the most appropriate statement in each of the five health dimensions. Utility Index (UI) is then calculated from these five health dimensions using the EQ-5D-5L Value Set for England (Devlin *et al.*, 2016). The UI ranges from -0.28 ('worst possible health') to 1.00 ('best possible health'). The EQ-VAS records the participants' self-rated health on a vertical VAS with end-points labelled 'the worst health you can imagine' ('0') at the bottom of the scale and 'the best health you can imagine' ('100') at the top of the scale respectively. A higher UI and VAS denotes a higher HRQOL. For the purpose of this thesis, the term UI is used to represent HRQOL. (See **Appendix C: Data Forms: Health-related Quality of Life Questionnaire**)

The **Fatigue impact scale (FIS) tool** is an assessment tool developed to evaluate the impact of fatigue on the activities and quality of daily life. It has been validated in chronic fatigue syndrome, multiple sclerosis (Fisk *et al.*, 1994), and PBC (Prince *et al.*, 2000). It consists of 40 items addressing the impact of fatigue on aspects of daily life (maximum score 160), containing three intermixed domains addressing physical, cognitive (maximum score 40 each) and psychosocial (maximum score 80) elements of fatigue. The FIS is scored on a 5-point Likert scale (0 = no problem to 4 = extreme problem). The higher the total score, the higher the impact of fatigue. (See **Appendix C: Data Forms: Patient Questionnaire 2 (PQ2) section C: Fatigue)**

The **Cognitive Failure Questionnaire (CFQ)** is a tool that assesses the prevalence of cognitive symptoms by measuring the frequency of cognitive slips or failures occurring in everyday life. These include memory, attention, concentration, forgetfulness, word-finding abilities and confusion. The questionnaire consists of 25 questions encompassing failures in perception, memory and motor function. The patient rates how often these failures occur on a five-point Likert scale of 0-4 (0 = never, 4 = very often). The responses are summed to obtain a total CFQ score. The higher the score, the greater the cognitive impairment (overall range 0-100) (Broadbent, 1982). (See **Appendix C: Data Forms: Patient Questionnaire 2 (PQ2) section B: Memory and Concentration)**

The **Hospital Anxiety Depression Scale (HADS) tool** is a validated 14-item measure of current anxiety (HADS-A) and depression (HADS-D) optimized for use in patients with chronic disease. (Broadbent, 1982). The HADS was developed as a screening instrument for use in a hospital outpatient setting and is aimed at detecting the presence and severity of depression and anxiety in non-psychiatric settings (Bjelland *et al.*, 2002). Anxiety and depression subscales (comprising of 7 items) are scored separately (range 0-21 for each subscale). For each subscale, a score of 0-7 indicates no anxiety or depression, 8-10 borderline “caseness” and a score of ≥11 is clinically significant, indicating “caseness” for depression or anxiety (Mells *et al.*, 2013; Zigmond and Snaith, 1983). (See **Appendix C: Data Forms: Hospital Anxiety and Depression scale (HADS) questionnaire)**

Clinical data collection, group comparison and group subtypes: (See **Chapter 2: Data Collection**) Demographic and clinical data were collected from patient records by study team members using clinical data forms. Patients with previous liver transplantation were excluded from this analysis. Clinical data was collected on gender, age at inclusion of study, year of

diagnosis, biochemical results on recruitment date (Serum ALT and IgG) or within 12 months of recruitment date, number of flares in the past 12 months, immunosuppressive treatment, presence of cirrhosis (defined histologically or clinically by radiological evidence or transient elastography) and diagnosis of osteoporosis (on diagnosis of AIH and since diagnosis).

Biochemical remission was defined as normal ALT and IgG at the time of recruitment (and within the preceding 12 months) with no documented flares in the last 12 months. A flare was defined as an abnormal ALT above the upper limit of normal (ULN) requiring an increase or addition of corticosteroid treatment. The ULN used for ALT and IgG levels were based on each site's pathology laboratory ULN. Ethnicity and data on other medical conditions including PBC, PSC, ulcerative colitis or Crohn's disease and rheumatoid arthritis (RA), were collected using patient-reported "tick-box" questionnaires.

A cohort of PBC patients obtained from the UK-PBC cohort (n=1665) with known age, gender, EQ-5D-5L UI and EQ-VAS scores were used for comparison (Rice *et al.*, 2016).

Statistical analysis: To compare the EQ-5D-5L HRQOL scores with UK population norms, the EQ-5D-5L dimension scores in our cohort were converted to EQ-5D-3L index values using the crosswalk calculator as there is currently no published population norms available using the EQ-5D-5L value set. The crosswalk calculator determines a UI value for each EQ-5D-5L state by firstly predicting the likelihood of being in each EQ-5D-3L state and, secondly, calculating the weighted sum of the UI values across the EQ-5D-3L states. This is based on an analysis of data from co-administering both tools to 3691 respondents (van Hout *et al.*, 2012). These values were then compared to EQ-5D-3L values of UK population norms (Kind, Hardman and Macran, 1999) using the two sample t-test after standardisation for age and gender and reported as mean (standard deviation). A cohort of PBC patients obtained from the UK-PBC cohort (n=1665) with known age, gender, EQ-5D-5L UI were used to compare the EQ-5D-5L UI in AIH patients with those with PBC (Rice *et al.*, 2016).

Regression analysis was used to explore the association of various demographic and clinical covariates with HRQOL in the UKAIH cohort. The primary outcome measure of interest was UI (utility index), a continuous measure of HRQOL. Due to the highly skewed nature of this variable, quantile regression with the median as the chosen quantile. Univariate regression models were developed for each covariate of interest and all models were adjusted for age, gender and biochemical remission status as appropriate.

Further regression analysis was used to explore the relationship between various other HRQOL outcome measures and corticosteroid use. These outcome measures included the five individual health dimensions of the EQ-5D-5L (analysed using ordinal regression), continuous measures FIS and CFQ (analysed using quantile regression) and the three-level classification of HADS-A and HADS-D (analysed using ordinal regression) using STATA version 14.1 and R version 3.3.0.

The UK population norms for HADS was kindly provided by Prof John Crawford (Aberdeen University) (Crawford *et al.*, 2009). To compare the HADS scores and HADS categories with UK population norms, the Mann-Whitney test, Pearson's chi-squared test and linear regression test were utilised as approprate (using IBM SPSS version 24 and MiniTab 24).

FIS scores from a healthy control cohort and PBC cohort as well as CFQ scores for healthy controls were kindly provided by Prof Julia Newton (Newcastle University). FIS and CFQ comparison between cohorts were done using the Kruskall-Wallis test and Pearson's chi-squared test respectively was utilised (using IBM SPSS version 24 and MiniTab 24).

Patients with previous liver transplantation were excluded from HRQOL analysis.

5.3 Results

5.3.1 Demographic, clinical and HRQOL characteristics:

Data from 990 patients were analyzed from 39 hospitals (32 non-transplant centers and 7 transplant centers). **Table 22** shows the clinical characteristics of the study population and self-reported comorbidities. **Table 23** shows further details on therapy regimens and **Table 24** summarizes the HRQOL and symptom severity characteristics.

Of the study participants, 795 (80%) were female, 92% of Caucasian ethnicity, median age at inclusion to study was 58 years (17-95) and median duration of disease was 7 years (0-57). Median BMI was 27.4 (16.7-60.5) kg/m^2. 79% of those with available data had positive anti-nuclear antibody (ANA) or smooth muscle antibody (SMA) or soluble liver antigen (SLA) antibodies and 1% had anti-Liver-Kidney Microsomal (LKM) positivity. 558 (56%) were in biochemical remission, 545 (55%) of patients were on corticosteroids and 330 (33%) had

cirrhosis. There were 25 (3%) patients on triple immunosuppression (i.e. taking two immunosuppressants and corticosteroids).

In the 521 (52.6%) patients with available extended data, 27 (5.2%), 7 (1.3%) and 48 (9.2%) had developed ascites, hepatic encephalopathy and varices respectively. 29 (3%) had developed osteoporosis since diagnosis of AIH.

Table 22: Clinical Characteristics of the Study Population

Biochemical tests	**Median (range)**
ALT (IU/L) (n=965)	25.0 (4.0-1315.0)
ALT:ULN ratio	0.6 (0.1-35.0)
IgG (g/L) (n=742)	12.5 (2.1-51.1)
IgG:ULN ratio	0.8 (0.2-3.2)
Biochemical Remission	**n (%)**
Normal ALT only [according to each site's ULN, n=965]	758 (78.5%)
Normal ALT and IgG [n=736]	449 (61.0%)
Normal ALT and IgG with no documented flares last 12 months [used in regression analysis, n=990]	558 (56.4%)
Therapy and Dose per day	**n = 990**
Prednisolone	499 (50.4%)
Dose (mg)	5 (0.2-60)
Budesonide	47 (4.7%)
Dose (mg)	3 (0.4-9)
Azathioprine	581 (58.7%)
Dose (mg)	100 (25-250)
Dose/weight (mg/kg)	1.2 (0.2-2.8)
6-Mercaptopurine	48 (4.8%)
Dose (mg)	50 (12-150)
Dose/weight (mg/kg)	0.7 (0.1-2.0)
Mycophenolate (MMF/MA)	166 (16.8%)
Dose (mg)	1000 (200-3000)
Tacrolimus	41 (4.1%)

Dose (mg)	2 (0.5-9)
Ciclosporin	6 (0.6%)
Dose (mg)	125 (50-200)
Self-reported co-morbidities	**n = 956**
Primary biliary cholangitis	64 (6.7%)
Primary sclerosing cholangitis	22 (2.3%)
Rheumatoid arthritis	60 (6.3%)
Osteoporosis	147 (15.3%)
Ulcerative colitis/Crohn's	48 (5.0%)

Table 23: Therapy regimens

Therapy	n	%	Median dose (range) (mg)
Prednisolone monotherapy	90	9.1%	7.3 (0.5-50)
Prednisolone combination	409	41.3%	5.0 (0.2-60)
Budesonide monotherapy	13	1.3%	6 (1.5-9.0)
Budesonide combination	34	3.4%	3 (0.4-9.0)
Azathioprine monotherapy	299	30.2%	100 (25-250)
Dose/weight (mg/kg)			1.1 (0.2-2.6)
Azathioprine combination	282	28.5%	100 (25-250)
Dose/weight (mg/kg)			1.2 (0.2-2.8)
6-Mercaptopurine monotherapy	22	2.2%	50 (25-100)
6-Mercaptopurine combination	26	2.6%	50 (12-150)
Mycophenolate mofetil/ mycophenolic acid monotherapy	50	5.1%	1000 (500 – 3000)
Mycophenolate mofetil/ mycophenolic acid combination	116	11.7%	1000 (200 – 3000)
Tacrolimus monotherapy	3	0.3%	1.5 (1.0-2.0)
Tacrolimus combination	38	3.8%	2 (0.5-9.0)
Cyclosporine monotherapy	1	0.1%	75 (NA)
Cyclosporine combination	5	0.5%	150 (50-300)

Table 24: HRQOL and symptom impact characteristics

HRQOL measures		Median (range)
EQ-5D-5L UI	(n=986)	0.89 (-0.19 – 1.00)
EQ-VAS	(n=985)	80 (10-100)
FIS	(n=950)	27 (0-160)
CFQ	(n=950)	32 (0-100)
HADS-A score	(n=966)	5 (0-21)
HADS-D score	(n=965)	3 (0-18)
HADS-A score classifications		**Number (%)**
No anxiety (0 – 7)		637 (66.0%)
Borderline (8 – 10)		181 (18.7%)
Clinically significant (≥11)		148 (15.3%)
HADS-D score classifications		**Number (%)**
No depression (0 – 7)		816 (84.6%)
Borderline (8 – 10)		96 (10.0%)
Clinically significant (≥11)		53 (5.5%)

5.3.2 HRQOL and Utility Abnormality in AIH

The median EQ-5D-5L utility index (UI) value was 0.89 (-0.19 - 1.00) and the median EQ-VAS was 80 (10-100) with a mean EQ-VAS of 74 (IQR 30). There were 255 (26%) patients with UI = 1 ("perfect health state") and 6 (0.6%) patients with negative UI ("health state worse than death"). The mean EQ-VAS scores split by age groups and gender can be seen in **Figure 6** whilst the EQ-VAS distribution according to age groups can be seen in **Table 25**. The median VAS of males and females were similar. 83(8%) of patients had a VAS of 100.

The distribution of EQ-5D-5L responses can be seen in **Figure 7**, split by health domain. Of the five domains, the pain/discomfort domain had the highest proportion of patients reporting problems (58%) followed by anxiety (43%) whilst the self-care domain had the lowest (11%) (see **Table 26** which details the percentage of patients according to EQ-5D-5L response levels, split by health domain and age groups).

Following conversion to EQ-5D-3L index values using the crosswalk calculator (van Hout *et al.*, 2012), the mean UI (0.77, sd = 0.23, standardized for gender and age) was significantly lower in our cohort ($t = 11.4$, $p < 0.001$) compared to UK population norms (0.86, sd = 0.23).

When comparing our cohort of AIH patients to a large cohort of PBC patients [n=1665, median age: 67 years (29-95), 91% female], there was no statistically significant difference in UI between the two groups, after controlling for age and gender ($\hat{\beta} = 0.008$, $p = 0.41$). On graphical comparison of the EQ-5D-5L responses according to health domains between AIH and PBC, the proportion of patients experiencing problems in each of the subdomains appear similar between the two groups (see **Figure 8**).

Figure 6: Mean VAS scores according to age groups and gender

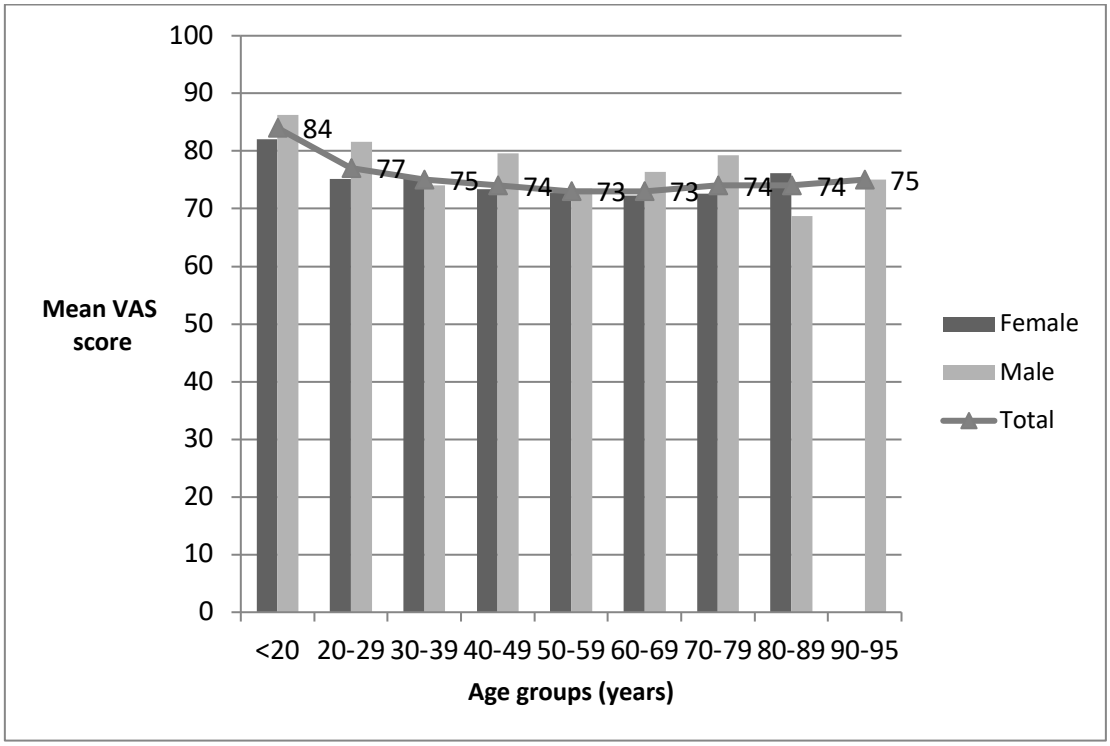


Figure 7: Distribution of EQ5D5L responses levels, split by health domain (whole cohort).

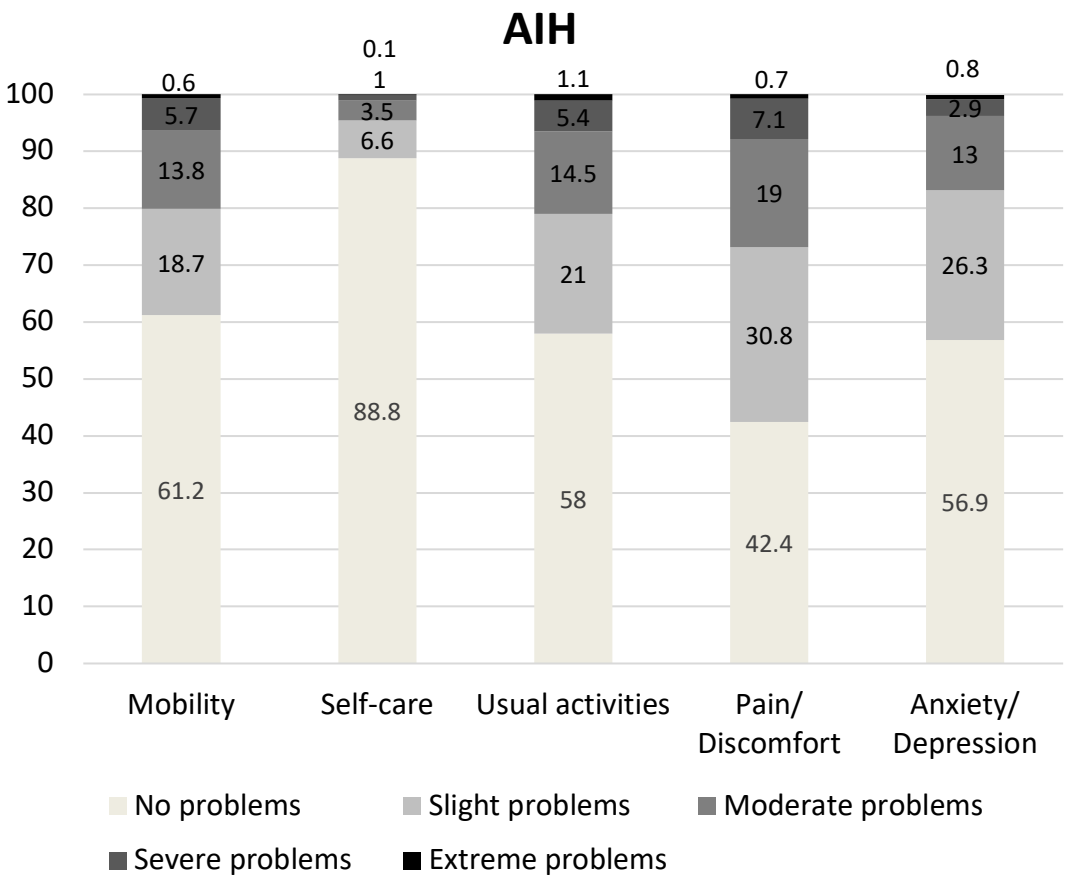


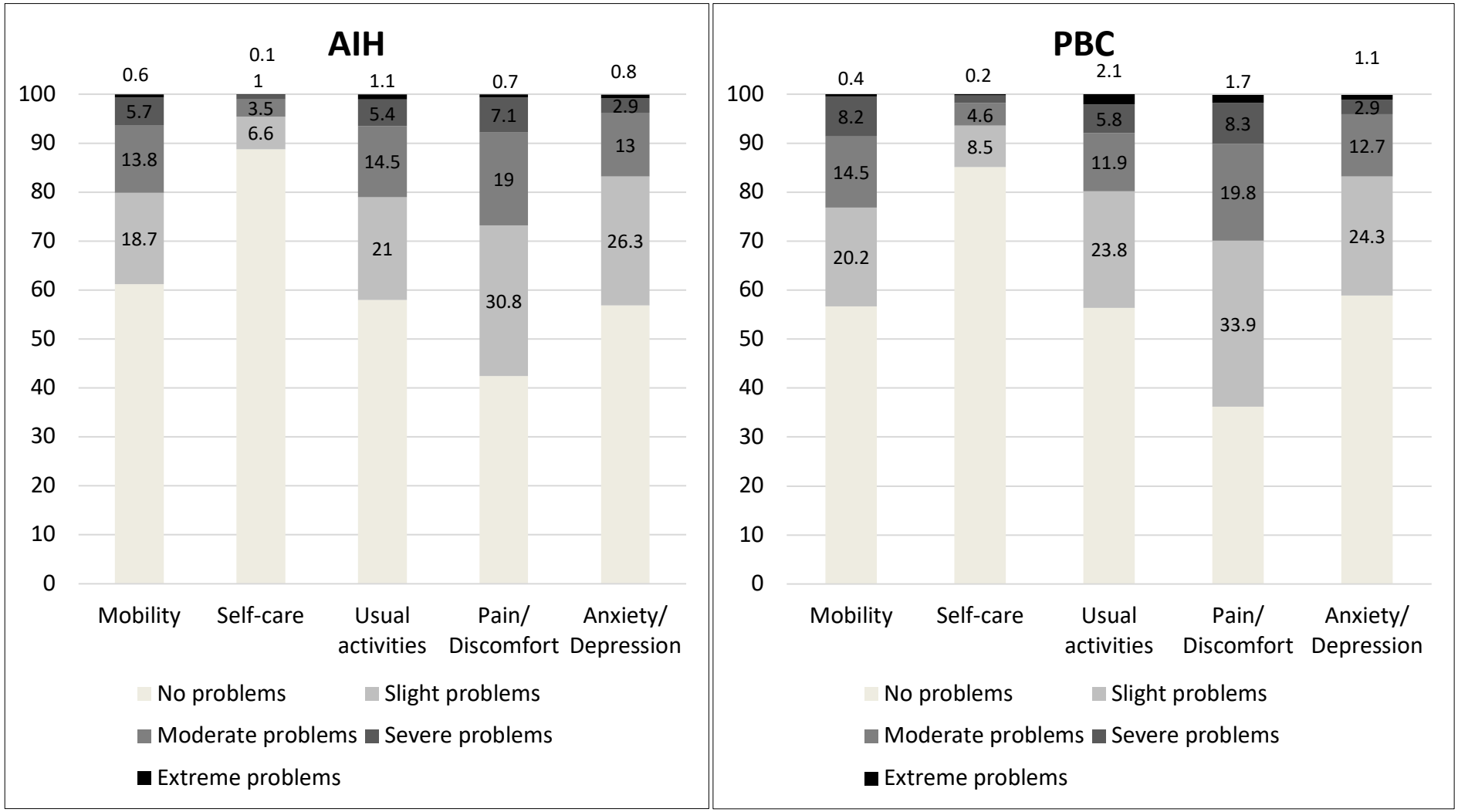
Table 25: EQ-Visual Analogue Scale (EQ-VAS) according to age groups

Age groups EQ5D5L VAS	<20 years	20-29 years	30-39 years	40-49 years	50-59 years	60-69 years	70-79 years	80-89 years	90-95 years
Median	90	80	80	75	80	75	75	75	75
25% Percentile	73	70	65	60	60	60	60	70	70
75% Percentile	95	90	90	90	90	90	90	80	80
Mean	84	77	75	74	73	73	74	74	75
Std. Deviation	16.2	17.5	18.8	18.2	19.4	19.1	18.4	13.1	7.1

Table 26: Percentage of patients according to response levels, split by health domain and age groups

EQ5D5L dimension	Age groups (years)									
	<20	20-29	30-39	40-49	50-59	60-69	70-79	80-89	90-99	Grand Total
Mobility										
▪ No problems	1%	8%	7%	11%	11%	14%	8%	1%	0%	61%
▪ Problems	0%	1%	2%	4%	8%	13%	10%	2%	0.2%	39%
Self care										
▪ No problems	0.9%	9.0%	8.5%	12.8%	15.7%	23.7%	15.3%	2.7%	0.2%	89%
▪ Problems	0%	0.3%	0.5%	1.6%	2.9%	3.0%	2.6%	0.2%	0.0%	11%
Usual activities										
▪ No problems	1%	7%	6%	8%	10%	15%	9%	2%	0%	58%
▪ Problems	0.2%	3%	3%	6%	8%	12%	9%	1%	0.2%	42%
Pain										
▪ No problems	0.6%	5%	5%	6%	8%	9%	7%	1%	0.1%	42%
▪ Problems	0.3%	4%	4%	8%	11%	18%	11%	2%	0.1%	**58%**
Anxiety / Depression										
▪ No problems	1%	5%	5%	8%	10%	16%	10%	1%	0.1%	57%
▪ Problems	0.2%	4%	4%	6%	8%	11%	8%	2%	0.1%	**43%**

Figure 8: Comparison of EQ-5D-5L response levels, split by health domains between AIH and PBC



5.3.3 Association between demographic and clinical factors and HRQOL/Utility

The results of univariable quantile regression are presented in **Table 27**. In the unadjusted regression analysis, we observed that increasing age and body mass index (BMI) are both associated with lower UI ($\hat{\beta}$ = -0.001, p < 0.001 and $\hat{\beta}$ = -0.006, p < 0.001 respectively) and female gender is associated with higher UI ($\hat{\beta}$ = 0.034, p = 0.027). On exploring place of care (transplant centers versus non-transplant centers), there was no evidence that this is associated with UI ($\hat{\beta}$ = 0.000, p = 1.000).

Patients who are in biochemical remission appear to have significantly higher UI ($\hat{\beta}$ = 0.024, p = 0.020) than those not in biochemical remission, after controlling for age and gender. In all subsequent models discussed, we controlled for age, gender and remission status. Lower cut-offs for normality for ALT, adopting the hepatitis B virus cut-offs (<30 IU/L for males and <19 for females) (National Institute for Health and Care, 2013b) was also explored but no association was found between UI and these lower cut-off levels.

There was no association found between UI and having cirrhosis or duration of disease after controlling for age, gender and remission status.

When considering the association between patient reported co-morbidities and HRQOL, patients who reported having either PBC ($\hat{\beta}$ = -0.046, p = 0.033), PSC ($\hat{\beta}$ = -0.090, p = 0.005) or RA ($\hat{\beta}$ = -0.053, p = 0.008) were found to have impaired UI compared to AIH patients not reporting these comorbidities. Self-reported osteoporosis or inflammatory bowel disease was not found to have any association with UI in this cohort.

5.3.4 Association between AIH therapies and HRQOL/Utility

Corticosteroid use was found to be significantly associated with lower UI ($\hat{\beta}$ = -0.030, p = 0.006) and this was irrespective of being on low dose corticosteroids (<10mg prednisolone or <6mg budesonide per day) or high-dose corticosteroids (≥prednisolone 10mg or ≥6mg budesonide per day) (see **Table 27**). Further, within the group on corticosteroids, there was no evidence found that dose was associated with material difference in HRQOL impairment, with impact still being seen in patients on low dose corticosteroid (**Table 27**). There was no difference in the reduced UI seen in corticosteroid users when comparing those in biochemical remission to those not in biochemical remission (this was explored via the addition of an interaction term to the model resulting in a non-significant coefficient, $\widehat{\beta}$ = -0.013, p=0.486). Additionally,

calcineurin inhibitor (CNI) use emerged as a factor showing significant association with a lower UI ($\hat{\beta}$ = -0.077, p = 0.002). Even after controlling for corticosteroid use, CNI use remained significantly associated with lower UI ($\hat{\beta}$ = -0.067, p = 0.008). There was no association found between azathioprine/6-mercaptopurine (6MP) or Mycophenolate mofetil (MMF)/ Mycophenolic acid (MA) use and impaired QOL. Further, there was also no difference between corticosteroid monotherapy and combination therapy with azathioprine (explored via the addition of an interaction term to the model resulting in a non-significant coefficient, $\hat{\beta}$=-0.024, p=0.177). Patients who take two immunosuppressants appear to have significantly lower UI values than patients not taking any immunosupressants ($\hat{\beta}$ = -0.085, p = 0.02). However, this association seems to be entirely driven by the role of calcineurin as a second choice of immunosuppressant.

Further investigation of individual health states within the EQ-5D-5L (**Table 28**) showed that corticosteroid use was associated with increased problems in the mobility ($\hat{\beta}$=0.307, p=0.026) and usual activities ($\hat{\beta}$=0.426, p=0.002) domains even after controlling for age, gender and biochemical remission. There was evidence of a borderline association between corticosteroid use and increased problems in the anxiety/depression domain ($\hat{\beta}$=0.267, p=0.043). However, there was no association found between corticosteroid use and the self-care or pain/discomfort domains.

Additional HRQOL measures (FIS, CFQ, HADS-A and HADS-D) are considered in **Table 28**, where their associations with corticosteroid use are explored. An impaired HRQOL was observed for corticosteroid users with respect to all HRQOL measures, with the association between corticosteroid use and Fatigue Impact Scale (FIS) ($\hat{\beta}$ =9.50, p<0.001) reaching statistical significance.

Table 27: Regression analysis investigating factors associated with EQ5D5L utility index value

Outcome variable	Predictor	Coefficient ($\hat{\beta}$)	95% CI	p-value
EQ-5D-5L UI value	**Demographic**			
	Age at inclusion	-0.001	(-0.002, -0.001)	<0.001
	BMI	-0.006	(-0.008, -0.005)	<0.001
	Gender (female)	0.034	(0.004, 0.064)	0.027
	Transplant centre	0.000	(-0.021, 0.021)	1.000
	Clinical covariates*			
	In biochemical remission**	0.024	(0.004, 0.043)	0.020
	Has cirrhosis	0.013	(-0.009, 0.035)	0.245
	Duration of disease	0.001	(-0.000, 0.002)	0.202
	'Normal ALT' using lower cut-offs ALT (ALT < 30 for males, ALT < 19 for females)	0.006	(-0.016, 0.029)	0.587
	Treatment*			
	Prednisolone/ Budesonide	-0.030	(-0.051, -0.009)	0.006
	▪ Low dose steroid (defined as either <10mg prednisolone/day or <6mg budesonide/day)	-0.027	(-0.050, -0.004)	0.015
	▪ High dose steroid (≥10mg prednisolone/day or ≥6mg budesonide/day)	-0.035	(-0.067, 0.004)	0.028
	▪ Corticosteroid dose (log-transformed) (corticosteroid users only, n=545)	-0.006	(-0.028, 0.016)	0.582
	Azathioprine / 6-mercaptopurine	0.009	(-0.011, 0.030)	0.356
	MMF/MA	-0.007	(-0.035, 0.020)	0.594
	Tacrolimus / Ciclosporin (CNIs)	-0.077	(-0.124, -0.030)	0.002
	Patient reported comorbidities*			
	PBC	-0.046	(-0.088, -0.004)	0.033
	PSC	-0.090	(-0.154, -0.027)	0.005
	Rheumatoid arthritis	-0.053	(-0.092, -0.014)	0.008
	Osteoporosis	-0.023	(-0.052, 0.007)	0.132
	Ulcerative colitis/Crohn's	-0.031	(-0.076, 0.014)	0.177

*Controlling for age, gender & remission status
**Controlling for age & gender

Table 28: Univariable regression analysis investigating the relationship between corticosteroid use and further HRQOL outcome measures

Outcome variable	Predictor	Coefficient	95% CI	p-value
EQ-5D-5L health states:	Corticosteroid use*			
• ***Mobility***		0.307	(0.037, 0.578)	0.026
• ***Self-care***		0.373	(-0.054, 0.800)	0.087
• ***Usual activities***		0.426	(0.162, 0.690)	0.002
• ***Pain/discomfort***		0.203	(-0.040, 0.447)	0.102
• ***Anxiety/depression***		0.267	(0.009, 0.525)	0.043
Total FIS score		9.50	(4.12, 14.9)	<0.001
CFQ score		2.46	(-0.562, 5.48)	0.111
HADS-Anxiety score		0.162	(-0.118, 0.443)	0.256
HADS-Depression score		0.327	(-0.059, 0.713)	0.096

*Controlling for age, gender & remission status

5.3.5 Hospital Anxiety and Depression Scale (HADS)

The Hospital Anxiety and Depression Scale (HADS) data for this AIH cohort and that of a normal UK population is described in **Table 29**.

Table 29: Hospital Anxiety and Depression Scale (HADS) data in AIH cohort versus normal population (Crawford *et al.*, 2009)

	AIH cohort (n=966 with available HADS)	Normal population (n=3822)
Female (number, %)	773 (80%)	2205 (57.7%)
Age (number, %)	58 years (17-95)	41 years (15-92)
HADS-A score (median, range)	5 (0-21)	6 (0-21)
HADS-D score (median, range)	3 (0-18)	3 (0-20)
HADS-A score classifications	**AIH cohort n (%)**	**Normal population n (%)**
No anxiety (0-7)	637 (66.0%)	2469 (64.6%)
Borderline (8-10)	181 (18.7%)	771 (20.2%)
Clinically significant (≥11)	148 (15.3%)	582 (15.2%)

HADS-D score classifications	AIH cohort n (%)	Normal population
No anxiety (0-7)	816 (84.6%)	3347 (87.6%)
Borderline (8-10)	96 (10.0%)	325 (8.5%)
Clinically significant (≥11)	53 (5.5%)	150 (3.9%)

i. HADS-Anxiety (HADS-A)

The HADS-A scores (continuous scale) was first compared between AIH and normal population using the Mann-Whitney test – this showed significant p value of 0.001. However, after applying the chi-square test (using the variables of AIH and normal population as 'Group'), it was established that there was no significant association between HADS-A and Group (p=0.630 when HADS-A was considered as having 3 categories - no anxiety, borderline, clinically significant/ anxiety present; p=0.994 when HADS-A was considered as having 2 categories - no anxiety/borderline or anxiety present.

On performing linear regression to explore the relationship between HADS-A scores and Group, there was a significant negative association with AIH group associated with lower HADS-A score (coefficient: −0.393, p=0.007). After controlling for age and gender, the association between HADS-A and Group was less strong and non significant (coefficient: −0.190, p=0.215).

When the pool of candidate predictors for HADS-A consisted of Group, age and gender, a stepwise variable selection procedure established age and gender as being a good predictor set. Controlling for gender, older age was associated with lower HADS-A score (coefficient: −0.036, p<0.0005); controlling for age, the male sex was associated with lower HADS-A score (coefficient: −1.228, p<0.0005). However, this model is for a mix of normal and AIH individuals and does not take into consideration which group an individual is from.

This relationship (HADS-A with age and gender as predictors) was further explored within the AIH group. Similarly, there was a significant association with age (coefficient -0.034, p<0.0005) with older age having lower HADS-A scores (similar to above) and males having significantly lower HADS-A scores (co-efficient: −1.262, p<0.0005).

ii. HADS-Depression (HADS-D)

The HADS-D scores (continuous scale) was compared between AIH and normal population using the Mann-Whitney test – this showed a non-significant p value of 0.566. Using the chi-square test however, there was evidence of an association between HADS-D and Group (p=0.033) with (i) the percentage of normal population participants who were classified as "not depressed" (87.6%) being significantly different from the percentage of AIH participants who were classified as "not depressed" (84.7%) and (ii) the percentage of normal participants who were classified as "depressed" (3.9%) being significantly different from the percentage of AIH participants who were classified as "depressed" (5.5%). The percentage of normal participants who were classified as "borderline" was not significantly different from the percentage of AIH participants who were classified as "borderline". The significant association between HADS-D and Group was also present when HADS-D was considered as having 2 categories - no depression and borderline (HADS-D score 0-10) versus depression present (HADS-D score ≥11), p=0.031.

On linear regression analysis, there was no significant correlation relationship between HADS-D scores and Group (coefficient: 0.212, p=0.07). Interestingly, although non- significant, AIH is associated with higher HADS-D score. This mirrors the chi-square analysis. After controlling for age and gender, the association between HADS-D and Group was even weaker and non significant (coefficient: 0.051, p=0.682).

When the pool of candidate predictors for HADS-D consisted of Group, age and gender, a stepwise variable selection procedure established age and gender as being a good predictor set. Controlling for gender, older age was associated with higher HADS-D score (coefficient: 0.008, p=0.004); controlling for age, the male sex was associated with lower HADS-D score (coefficient −0.257, p=0.008)." However, again, this model is for a mix of normal and AIH individuals and does not take into consideration which group an individual is from.

On evaluating HADS-D scores of the AIH group only in regression analysis with age and gender as predictors, the association did not reach statistical significance (age, p=0.889 and gender, p=0.195).

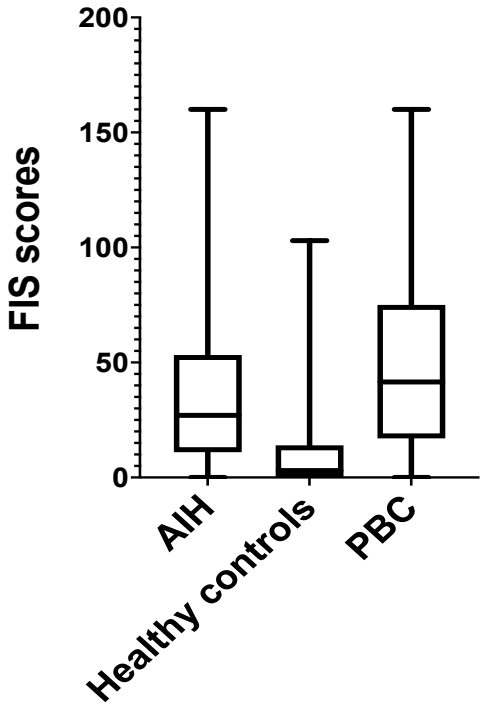
5.3.6 Fatigue Impact Scale (FIS)

The FIS scores for this AIH cohort was compared with the FIS scores of a small healthy control cohort and a PBC cohort (both cohorts' data was courtesy of Prof Julia Newman) (see **Table 30 and Figure 9**).

Table 30: Fatigue Impact Scale (FIS) scores, gender and age distribution of three groups: AIH, PBC and healthy controls

	AIH cohort (n=950)	PBC cohort (n=68)	Healthy controls (n=67)
Female	763 (80%)	68 (100%)	42 (63%)
Age (median, range)	58 (17-95)	58 (35-78) years	47 (21-77) years
FIS (median, range)	27 (0-160)	41.5 (0-160)	3 (0-103)

Figure 9: Box-plot of FIS score distribution across groups



There was a statistically significant difference between the mean-rank FIS scores of the group ($p<0.0005$) using Kruskal-Wallis test. Post-hoc test showed a statistical signicantly difference between AIH and healthy controls ($p<0.0005$), healthy controls and PBC ($p<0.0005$) and AIH and PBC ($p=0.012$) [All p values adjusted by the Bonferonni correction].

Controlling for age and gender, the expected FIS value for a participant with AIH is 24.20 higher than a person in the healthy control cohort ($p<0.0005$) and the expected FIS value for a participant with PBC is 36.31 higher than a person in the healthy control cohort ($p<0.0005$). Controlling for age and Group, the expected FIS value for someone who is female is 7.61 higher than someone who is male ($p=0.003$). Controlling for gender and Group, each year increment in age translates to decrease in expected FIS of 0.142 ($p=0.024$).

Within the AIH cohort, two groups were further compared: those on steroids (n=523) with those off steroids (n=427); the median FIS scores of those on steroids (32) was significantly different from those off steroids (22), $p<0.0001$. FIS scores were also compared between those not in biochemical remission (median FIS = 31, n=415) versus those in biochemical remission (median FIS = 24, n=535) and this also differed significantly ($p=0.0008$).

5.3.7 Cognitive Failure Questionnaire (CFQ)

The CFQ scores for this AIH cohort was compared with that of a small healthy control cohort (data courtesy of Prof Julia Newman) (see **Table 31**).

Table 31: Cognitive Failure Questionnaire (CFQ) scores, gender and age distribution of AIH cohort and healthy controls

	AIH cohort (n=950)	Healthy controls (n=191)
Female	763 (80%)	144 (75%)
Age (median, range)	58 (17-95)	57 (17-86) years
CFQ (median, range)	32 (0-100)	36 (0-68)

Although there was significant difference between the medians of the group ($p=0.005$) with the AIH cohort having lower CFQ scores than healthy controls, this is not relevant as the area of interest is higher CFQ scores which is associated with the presence of cognitive impairment.

When the pool of candidate predictors for CFQ consisted of Group, age and gender, a stepwise variable selection procedure established age and gender as being a good predictor set. Controlling for gender, older age was associated with lower CFQ score (coefficient: −0.077, $p=0.014$). Controlling for age, the female sex was associated with higher CFQ scores (co-

efficient: 4.29, p=0.001). This model is for a mix of healthy controls and and AIH individuals and does not take into consideration which group an individual is from.

5.4 Discussion

This study is unique as it is the first comprehensive study to report the HRQOL with health utility and it was undertaken in the largest cohort of patients with AIH to date. The multi-centre nature of the patient population (a combination of liver transplant centres, tertiary hospitals and district general hospitals) ensures a comprehensive spectrum of patients and provides important real-world data.

5.4.1 HRQOL and health utility in AIH patients is significantly impaired compared to the normal population

The first key finding is that HRQOL and health utility was significantly impaired in this cohort of AIH patients when compared to the normal population. Patients with chronic liver disease have impaired HRQOL compared to the normal population regardless of aetiology (Younossi *et al.*, 2001; Afendy *et al.*, 2009). The different studies evaluating HRQOL in AIH have already been touched on in Chapter 1 (Section 1.10) and are limited by the relatively smaller numbers and being single-centred.

Health utility is a critical parameter which plays an integral role in the assessment of the value of current and emerging therapies in disease (McLernon, Dillon and Donnan, 2008). Health state values or 'utilities' are HRQOL measures that produces a single derived value representing the individual's preferences to a health state on a scale from 0 (dead) to 1 (perfect health). The advantage of using health utility measures is that this was designed to provide interval measures. This enables comparisons to be made across studies, patient populations, interventions and calculation of quality adjusted life years (QALYs) for evaluation of cost-effectiveness and decisions around heath resource allocation policies (Fisk *et al.*, 2005). This study adds to current portfolio of HRQOL studies in AIH which will help with future studies, particularly in evaluation of cost-effectiveness of current and future treatments for patients with AIH and development of AIH-specific HRQOL measures.

5.4.2 HRQOL impairment in AIH patients are at a level similar to patients with PBC

This cohort's patients with AIH were found to have a level of HRQOL impairment similar to that seen in PBC, a disease where impairment of QOL is well described d (with fatigue and pruritus playing a major role) (Mells *et al.*, 2013). As shown in **Figure 8** the proportion of patients experiencing problems in each of the EQ-5D-5L health domains appeared similar between the AIH and PBC groups and there was no statistically significant difference in UI between the two groups after controlling for age and gender.

5.4.3 Biochemical remission is associated with better HRQOL in AIH patients

Biochemical remission (defined as normal ALT and IgG, surrogate markers of disease activity) was significantly associated with higher (improved) health utility compared to those not in biochemical remission after controlling for age and gender. In the German study by Schramm and colleagues, which found reduced mental wellbeing in their patients with AIH, the majority (77%) were in biochemical remission (Schramm *et al.*, 2014). This compares with the 56% remission rate in this "real world" nationwide patient cohort. Our relatively lower proportion of patients in remission may be due to the stricter definition for biochemical remission applied in our study which takes into account the reported trend of the biochemical parameters in the previous 12 months. Incomplete biochemical remission (failure of transaminases and IgG levels to normalise) is predictive of relapse after treatment withdrawal, histological activity, progression to cirrhosis and poor outcome (European Association for the Study of the, 2015). One would therefore expect patients with incomplete biochemical remission (with ongoing inflammation) to have poorer HRQOL. Patients who have not achieved complete biochemical remission are more likely to be on dual or triple immunosuppression (potentially at higher doses), to have had more courses of corticosteroids and are at increased risk of complications of progressive disease. All these factors could also impact on quality of life and utility. In this cohort of patients in incomplete biochemical remission, 72% were on corticosteroids as opposed to 42% of those in biochemical remission and 8% were on CNIs as opposed to 2% respectively.

a. Exploring lower cut-off values of ALT

ALT, a liver transaminase enzyme is a marker of liver inflammation and used as a surrogate in monitoring for disease activity in AIH. In chronic hepatitis B viral (HBV) infection, lower cut-offs for normal ALT values (<30 IU/L for males and <19 IU/L for females) have been suggested

(National Institute for Health and Care, 2013b) to enable early detection of liver damage and better differentiate active chronic HBV from inactive chronic carriers (Ijaz *et al.*, 2011). This current UK-AIH study also explored lower cut-off ALT values (<30 IU/L for males and <19 IU/L for females) but no association was found between utility index and these lower ALT values.

5.4.4. Corticosteroids are associated with reduced HRQOL, independent of age, gender and biochemical remission status

The most striking finding in this study was that corticosteroids were associated with significantly lower levels of utility, even after controlling for age, gender and, crucially, biochemical remission status. On further analysis of the five EQ-5D-5L health domains, corticosteroid use was associated with increased problems with mobility and usual activities. One of the well known side-effects of corticosteroids is weight gain. Almost a third (31%) of the patients on corticosteroids were obese with BMI ≥30 which may contribute to problems in mobility and usual activities.

Corticosteroids were the first therapy shown to improve the outcome of patients with AIH by significantly reducing mortality compared to placebo in the early controlled trials. However, these trials reported a high proportion of corticosteroid-related side effects including Cushingoid features in 20-50% of patients, diabetes in 15-20%, hypertension, cataracts, psychosis and osteoporotic vertebral collapses in 5-10% (Gleeson, Heneghan and British Society of, 2011; Cook, Mulligan and Sherlock, 1971; Murray-Lyon, Stern and Williams, 1973; Soloway *et al.*, 1972; Summerskill *et al.*, 1975). Subsequent studies reported a 30-53% prevalence of corticosteroid-related side-effects (Manns *et al.*, 2010b; Stellon *et al.*, 1988; Johnson, McFarlane and Williams, 1995). Despite the adverse effects associated with corticosteroids, a significant proportion (38%-85%) of patients remain on corticosteroids long-term (Werner *et al.*, 2008; Landeira *et al.*, 2012). The impact of corticosteroids on QOL has been studied in other diseases dependent on long-term corticosteroids such as systemic lupus erythematosus (Kuriya *et al.*, 2008), sarcoidosis (Judson *et al.*, 2015), liver transplantation (Zaydfudim *et al.*, 2012) and historically, rheumatoid arthritis (McDougall *et al.*, 1994). The recognition of the impact of oral corticosteroids in systemic lupus erythematosus has even catalysed the development of a Systemic Lupus Erythematosus-Specific Steroid Questionnaire (Mathias *et al.*, 2017). This is the first study in AIH that has explored the specific impact of corticosteroids on QOL. Following the publication of this UK-AIH HRQOL data, a Japanese study evaluated and compared the QOL in patients with AIH (n=265) with chronic hep C (n=88) and

healthy volunteers (n=97) using the Chronic Liver Disease questionnaire (CLDQ) and the 36-Item Short Form Survey (SF-36). The adjusted odds ratio of the worry domain in the CLDQ was significantly higher in patients with AIH treated with prednisolone (OR 1.79, p=0.038) than in those who were not on prednisolone. There was a negative correlation between the dose of prednisolone with the worry domain score (rs=-0.13, p=0.026) The study found treatment with prednisolone and dosage of prednisolone were factors associated with HRQOL impairment in AIH patients (along with other factors: disease progression and co-morbidities) (Takahashi *et al.*, 2018).

The study by Schramm and colleagues found a correlation between rates of depression with prednisolone use in their AIH cohort (Schramm *et al.*, 2014). In comparison, no correlation was found between corticosteroid use and HADS-A or HADS-D in this UKAIH study. Although a significant association ($p<0.002$) was found between corticosteroid use with HADS-D scores in this cohort (when treating HADS as a continuous scale), this association became non-significant (p=0.1) from a clinical context on treating HADS-D as a categorical variable (i.e. clinically significant scores ≥11 versus borderline scores (8-10) and not clinically significant scores (0-7)).

A key conclusion of this study is that clinicians should be more aware of the potential for impaired life quality and health utility in patients with AIH who are on corticosteroids. This should be factored into decisions regarding the appropriateness of long-term corticosteroid therapy with clinicians being aware that steroid minimisation, as opposed to discontinuation, may not confer benefit.

5.4.5 Calcineurin inhibitors (CNIs)

The use of CNIs (tacrolimus or ciclosporin) in AIH appears to be growing although the evidence base is limited (case-series in refractory patients). CNIs are well known to have associated side-effects such as renal impairment, neurotoxicity, hypertension and gum hypertrophy (European Association for the Study of the, 2015; Sciveres *et al.*, 2004; Tannous *et al.*, 2011). CNIs are often second or third-line therapies for azathioprine or mycophenolate mofetil intolerant patients or those who have failed to respond to conventional therapies. Although only 4% of this study population were on CNIs, it is striking to note that this still emerged as a covariate significantly associated with lower UI even after controlling for corticosteroid use. This highlights the need for therapies that are better tolerated with better efficacy.

5.4.6 Fatigue does not appear to play a major role in HRQOL impairment in AIH

Fatigue was evaluated as a potential factor which might underpin poor quality of life and health utility in AIH. The overall median fatigue impact scale (FIS) score for this AIH cohort was 27 (maximum possible score of 160) [see **Table 24** and **Table 30**]. Although direct comparisons could not be made, this score was not dissimilar to the median FIS scores of a normal control population [score 28(0-128)] reported in a study by Goldblatt and colleagues which explored the impact of fatigue in PBC which compared FIS scores in PBC (n=136), normal controls (n=136) and AIH (n=38). As a reference point, the median FIS scores for the PBC and AIH population in this study were 40 and 20.5, respectively, and FIS scores in the PBC population were noted to be significantly worse when compared to the matched controls and AIH population (Goldblatt *et al.*, 2002).

An attempt at comparing the FIS scores of this UK-AIH cohort (n=950 available data) with FIS scores of 67 healthy controls and 68 PBC patients was done (see section 5.3.6) which produced a significant p-value ($p<0.0005$). This result however was rejected as the comparison was felt have a high likelihood of type 1 error due to the very small numbers of healthy controls and PBC patients used (more than 10 times less than the UK-AIH cohort). Ideally, the FIS scores for a similar-sized matched healthy-control group and PBC group would be used for comparison.

Fatigue scores were higher in patients not in remission and in patients taking corticosteroids although fatigue did not appear to be the major driving force between corticosteroid-associated impairment of health utility, as seen by the overall median numerical FIS score not being dissimilar to the Goldblatt study's FIS score of a normal control population. This was not directly compared though and therefore deserves further interrogation for future work.

Patients on corticosteroids were noted to have significantly worse fatigue scores than those not on corticosteroids as evidenced by the significant association ($p<0.001$) between corticosteroid use and FIS. The regression co-efficient for the FIS comparison analysis was 9.5 (see **Table 28**), implying that on average, patients taking corticosteroids scored an additional 9.5 points on the FIS scale. Although no data exists for minimal clinical important difference (MCID) in FIS for liver diseases, as a reference point, the reported minimal important difference for FIS in MS patients ranged from 9-24 points for different patient and clinician rating anchors (Rendas-Baum *et al.*, 2010; Nordin *et al.*, 2016). Corticosteroids are known to

cause sleep disturbance or insomnia (Idzikowski and Shapiro, 1993) which could contribute to fatigue.

In contrast to this UK-AIH study's findings, the Dutch study (mentioned in Section 4.1 – Introduction) had found significantly worse fatigue scores (MDF Index-20) in the 142 AIH patients compared to healthy controls (van der Plas, Hansen and de Boer, 2007). The Polish study (n=140) found that their AIH patients had significantly higher Modified Fatigue Impact Scale scores compared to the general population ($p<0.001$) (Janik *et al.*, 2019) whilst the Japanese study (62 with AIH in remission, 39 with chronic hep C and 66 with PBC), used the Chronic Liver Disease Questionnaire (CLDQ) and found significantly lower scores for the fatigue score for AIH patients in remission compared to those with eradicated Hep C ($p=0.01$) and similar to scores in patients with PBC (Takahashi *et al.*, 2020).

5.4.7 The proportion of patients with clinically significant anxiety and depression scores (as evaluated by HADS) did not differ from normal population

The HADS tool was designed to identify patients with greater probability of depression who should then have further evaluation with a clinical interview or a more extended measuring tool. It is not a gold-standad tool to measure severity of depression. (Kendrick *et al.*, 2009) The authors of the HADS tool suggested a cut-off score of ≥11 for probable 'caseness' of the disorder and ≥8 for possible disorder (Zigmond and Snaith, 1983). Further studies found that a score of ≥8 to define 'caseness' was better in getting the optimal balance between sensitivity and specificity of HADS cut-off scores (Bjelland *et al.*, 2002). Additionally, there are numerous publications using the cut-off scores of 8-10 to describe mild anxiety/depression respectively and a score of 11 or more to describe moderate to severe anxiety/depression (for which active intervention is recommended) (Kendrick *et al.*, 2009).

The overall median HADS-anxiety (HADS-A) and HADS-depression (HADS-D) score for the cohort was 5 (no anxiety) and 3 (no depression) respectively. There was no significant difference in proportions of patients with or without anxiety/depression when compared with a normal UK population sample (Crawford *et al.*, 2009) (**see *Section 5.3.5* and Table 29**). These scores are also comparable to another normal UK population sample published in 2001 (Crawford *et al.*, 2001) which reported a median score of 6 (HADS-A) and 3 (HADS-D) respectively. A more recent UK cohort of normative HADS data with a more narrow age range (25-65 years) found a similar median depression score of 3 (IQR 1-6). The overall median

anxiety score was not reported but median anxiety score for women (6, IQR 4-9) was higher than in men (5, IQR 2-8) and increased with age in both groups (Breeman *et al.*, 2015). This was similar to findings in this AIH cohort with male gender being significantly associated with lower HADS-A score though older age conversely had lower anxiety scores. Although direct data comparison has not been made, the reason for this difference may be due to this AIH cohort having a wider spread of ages (17-95 years) compared to this 2015 cohort by Breeman and colleagues.

On initial analysis, there was a significantly different proportion of patients with clinically significant depression scores (5.5%) in the AIH cohort compared to the normal population (3.9%), p=0.031 however this association became non-significant on controlling for gender and age (p=0.682). Older age and male gender was associated with lower HADS-D score when both cohorts were evaluated together however this association did not reach statistical significance when evaluating the AIH group on its own.

The German study evaluating QOL in AIH patients (using PHQ-9 tool) found the frequency of other depressive syndrome was twice as high as in the general population (5.9% versus 2.6%, p=0.046); the scoring for a major depressive syndrome was 5 times as freqent compared to the general population with the rate of a major depressive syndrome in AIH patients (10.8%) as high as in patients with inflammatory rheumatic diseases (11.0%) (Schramm *et al.*, 2014). The Polish study (following this UK-AIH's published HRQOL data) found that their cohort of patients with AIH had significantly impaired scores for anxiety and depression (p<0.001 respectively) with 19% having moderate (PHQ-9 >10) and 10% having severe (PHG-9>15) depression. There was a strong correlation between depression and chronic fatigue (r=0.068, p<0.001) (Janik *et al.*, 2019).

5.4.8 Cognitive impairment does not appear to play a major role in impaired HRQOL in AIH

The median score for the Cognitive Failure Questionnaire (CFQ) tool was 32 (0-100) with a mean of 34 (sd 18). For comparison, a study evaluating QOL in a cohort of 103 patients with liver transplantation (median time since transplantation of 40 months [range 2-155]) reported a mean CFQ score of 38 (sd 25.2) (Elliott *et al.*, 2011). Comparisons with a small group of healthy controls (courtesy of Prof Julia Newton) showed significant difference between the median scores of the CFQ tool with AIH having lower CFQ scores than healthy controls

indicating cognitive impairment is not a problem in AIH. There was no association between corticosteroid use and worse CFQ scores in this study. These observations suggest that in AIH, unlike in PBC, cognitive impairment symptoms do not appear to be major factors in impaired health utility.

5.4.9 Demographic factors

In the **unadjusted** regression analysis, increasing age and body mass index (BMI) had a negative correlation to UI whilst female gender had higher UI values.

i. Age

The association between increased age and reduced HRQOL has been noted in a study looking at HRQOL in chronic liver diseases of various aetiologies (n=1103, 13(1%) had AIH) where age correlated significantly with every scale of SF-36 (Afendy 2009). It has to be noted however that this specific population is quite different from this UK-AIH cohort (40% female with 69% had cirrhosis compared to this UK-AIH cohort - 80% female and 33% cirrhosis). In contrast, a study evaluating 2055 patients with PBC found that age at presentation is associated with impact on perceived QOL with younger patients being more affected - every 10-year increase in age at presentation was associated with a 14% decrease in risk of poor perceived QOL (Dyson *et al.*, 2016b).

ii. BMI

About a third (32%) of this cohort with known BMI were obese (BMI≥30) which is likely to be contributing to the impairment of UI as obesity is known to significantly impair HRQOL in patients (Taylor *et al.*, 2013). However, approximately more than half of our population (55%) were on corticosteroids which could be a contributory factor due to its known side effect of weight gain.

iii. Female gender

Female gender (in the unadjusted regression analysis) was found to be associated with higher UI values in our cohort compared to males. The impact of gender on AIH outcomes have been studied in other studies with conflicting results. One study (n= 238) reported men with AIH having a higher relapse rate, younger age of disease onset but significantly better long-term survival and outcomes than women (Al-Chalabi *et al.*, 2008b) whilst another study reported

no difference in long-term outcomes between men and women (Kirstein *et al.*, 2015). A study (New Zealand, n=133 AIH patients, 26% males) found male patients were significantly more likely to have cirrhosis at diagnosis compared to female patients (OR 2.78, 95% CI: 1.23-6.18, p=0.01) but gender did not emerge as a significant factor associated with poor outcomes. (Ngu *et al.*, 2012) The recent Polish study with 140 AIH patients (which follows after the publication of this HRQOL data) conversely found that female patients had a greater impairment of physical aspects of the Short Form (36) Health Survey and Modified Fatigue Impact Score.

5.4.10 Cirrhosis

Cirrhosis did not emerge as a co-factor associated with poorer utility, even after controlling for age, gender and remission status. Schramm and colleagues also found that the presence of cirrhosis in AIH patients was not significantly associated with depressive symptoms and the physical component score of the 12-Item Short-Form Health Survey (SF-12) in the AIH patients did not differ between those with and without cirrhosis (Schramm *et al.*, 2014). The impact of complications of cirrhosis (hepatic encephalopathy, ascites and varices) was not explored as the numbers were very low (n=82) and information was only available in 52% of the whole cohort (due to incomplete retrospective collection of data from centres). Janik and colleagues' study (n=140) similarly did not find any association between presence of cirrhosis and impaired HRQOL in their study (Janik *et al.*, 2019).

5.4.11 Duration of disease

Duration of disease did not emerge as a significant factor in HRQOL impairment in this cohort. This concurred with the Polish and Japanese QOL studies in AIH patients (Janik *et al.*, 2019; Takahashi *et al.*, 2020).

5.4.12 Co-Morbidities

A small proportion of this cohort's AIH patients that reported concurrent PBC (6.7%), PSC (2.3%) and RA (6.3%) had significantly impaired UI compared to those not reporting these comorbidities. However, even if these patients (n=146) were excluded from the analysis, the overall result of significant impairment of UI compared to the general population remains ($t = 9.18$, $p<0.001$). The Pain/Discomfort health domain had the highest proportion (57%) of patients reporting problems (ranging from slight to extreme). Having RA as a co-morbidity may play a role as the 6% of patients reporting RA were found to have significantly impaired UI

compared to those not reporting this. There was no association found between corticosteroid use with Pain/Discomfort domain. Reassuringly, most of the cohort did not report problems in the self-care domain (89%).

5.4.13 Study limitations

One of the limitations of this study is that it is cross-sectional in nature, therefore any associations found between HRQOL and other factors must be interpreted cautiously, especially with respect to causality. There was an association between corticosteroid use and reduced utility index, independent of remission status and other known confounders (age, gender, co-morbidity etc). However, there may be other unknown and unmeasured confounders that was unable to be adjusted for.

The population norms used for comparison were based on data from the UK's most recent survey in 1993. It is possible that average health has improved over time across the age groups, with the published population norms therefore likely to be conservative.

It would have been ideal to have longitudinal data on the patients in this cohort which would allow the effect of treatment to be studied over time. Socioeconomic data would have been desirable for more detailed health economic analysis. Another limitation of the study would be that the data on co-morbidities are self-reported data by patients rather than data based on more accurate diagnostic criteria which would be highly ideal, particularly for those with overlap or concurrent PBC or PSC.

5.4.14 Concluding remarks

There is increasing recognition that quality of life management should be a priority in managing patients with AIH as it can have an impact on compliance to medication as well as outcomes. Despite established therapies, little information has been published on HRQOL and utilities for health states resulting from AIH. This current study is the start of bridging this gap and highlights the impaired HRQOL in patients with AIH. In addition, it can be used to inform on cost-effectiveness of current treatment regiments in the UK. There may also a role for the development of AIH-specific HRQOL measures, similar to the PBC-40 (a PBC-specific HRQOL tool) (Jacoby, 2005).

In summary, this data show evidence of HRQOL impairment in a large cohort of AIH patients compared to the general population as well as impairment similar to that seen in patients with

PBC. Furthermore, corticosteroid use shows an association with decreased HRQOL which is independent of remission status. This highlights the need for better, and ideally corticosteroid-free, future therapy approaches and emphasizes the need for future novel therapeutic trials in AIH.

Chapter 6. Summary and Conclusions

Autoimmune hepatitis (AIH) is a heterogenous, chronic inflammatory liver disease that remains a challenge in diagnosis and management. Failure to achieve biochemical remission has serious consequences (cirrhosis, liver failure and death). Existing immunosuppressive treatments (mainly prednisolone and azathioprine) are usually lifelong and some patients develop intolerable side effects. Treatment paradigms in autoimmune hepatitis (AIH) have remained largely unchanged for decades. Balancing treatment efficacy with adverse side effects remains a therapeutic challenge for AIH. There is a widespread perception amongst clinicians that treatments for AIH are effective and the disease is easy to treat.

This thesis set out to explore the concept of clinical impact of AIH and the clinical and mechanistic evidence behind unmet need in AIH using the UK-AIH national multi-centre cohort. Unmet need was divided into three broad categories - inadequate response to treatment, effective therapy with inappropriate side-effects and quality of life. This thesis sets out to identify the gaps between clinician perception, clinical data and viewpoints of patients.

This thesis describes two large detailed sets on how we deliver care and treatments and it describes quality of life and health utility in patients with AIH. It identified major gaps in treatment of AIH, disease outcomes and quality of life in AIH. In essence, we are not as good as we think we are in our approach in managing and treating AIH.

Clinician viewpoint: The first set (1249 patients including 51 with liver transplantation) features 'real world' clinical practice in AIH with significant discrepancies in the demographics and care received by patients with AIH in the UK. There is significant variability in treatment strategy with 29 individual regimens being identified. This reflects confusion and disarray in the current treatment approaches. A high proportion of patients remain on corticosteroids despite guidelines advising thiopurine monotherapy should be the goal. Biochemical remission rate using standard criteria was 59% which falls short of the benchmark figure of 80%. This overall poor remission rate suggests that there are significant unmet needs therapeutically for patients with AIH. Patients receiving care in transplant centres in the UK were more likely to achieve and maintain remission than those looked after in other care locations, despite an apparent bias towards a more severe disease phenotype.

Exploring potential for treatment optimisation with a novel metabolite: Azathioprine, an established therapy comes with some significant side-effects which can result in treatment withdrawal. Azathioprine red blood cell metabolites have been used as predictors of adverse effects and adherence. These metabolites do not, however, always correlate to clinical response as leukocytes are considered the main target cells for drug action. This thesis explored a novel thiopurine metabolite, incorporated deoxythioguanosine into the DNA of leukocytes (dTG DNA), as a potential biomarker for azathioprine treatment optimisation. The study found no significant correlation in the leukocyte metabolite (dtG DNA) levels with disease response or azathioprine dose which suggests that this biomarker is not clinically useful. Although the idea of evaluating a new leukocyte thioguanine metabolite for disease optimisation was promising, this unfortunately did not yield the desired outcome.

Patient view point: In the set of 990 patients, health-related quality of life (HRQOL), measured by the EQ-5D-5L utility index, is shown to be significantly impaired in this cohort of AIH patients compared to population norms. Corticosteroid use was strongly associated with decreased quality of life, independent of biochemical remission status. This is the first study in AIH that has explored the specific impact of corticosteroids on QOL. It was also noted that QOL impairment in AIH patients are at a level similar to patients with PBC. Biochemical remission was significantly associated with higher (improved) health utility compared to those not in biochemical remission after controlling for age and gender. Calcineurin inhibitors emerged as a covariate significantly associated with lower health utility and QOL, highlighting the need for better corticosteroid-free therapies that are better tolerated with better efficacy. This emphasizes the need for future novel therapeutic trials in AIH.

The main take home messages are that there are significant gaps in our current approaches to managing AIH with its a high degree variability in treatments used, suboptimal disease remission rates, ongoing reliance on corticosteroids with its associated side effects and reduced quality of life. Failure to achieve biochemical remission is associated with increased risk of liver-related death and transplantation.

The implications of the data in this thesis is that there seems to be a relative complacency amongst clinicians in accepting suboptimal patient outcomes as well as outmoded corticosteroid-based therapies for this disease. This cohort provides evidence of the need for increased adherence to optimal treatment approaches (described in clinical practice guidelines) through education of both clinicians and patients as well as for more potent and

patient-acceptable treatments for AIH. Azathioprine, being the most widely-used corticosteroid sparing agent is not often optimised, based on a recent survey amongst expert hepatologists (Liberal *et al.*, 2017). This thesis cohort's data reflects this with a median AZA dose of 1.1mg/kg (which can still be optimised to 2mg/kg). Patients who are intolerant or have inadequate response to azathioprine should be considered for optimisation by combining its use with allopurinol (de Boer *et al.*, 2013), switching to mercaptopurine (Hubener *et al.*, 2016) or using mycophenolate mofetil, which has recently been reported to have successful efficacy, particularly in those who are intolerant to thiopurines (Efe *et al.*, 2017). These strategies should be used to improve steroid-sparing approaches and prevent patients from having steroids escalated or being left on steroids (van den Brand *et al.*, 2019b).

Clinicians should make it a priority to listen to patients more in order to ascertain the impact of the disease and the treatments on the patients' quality of life. This is often neglected due to the single-minded focus on achieving physical disease remission. Quality of life management is increasingly recognised as a vital aspect of disease management as it can have impact on compliance to medications as well as disease outcomes. Clinicians should be more aware of the potential for impaired life quality in patients with AIH who are on corticosteroids. This should be factored into decisions regarding the appropriateness of long-term corticosteroid therapy with clinicians being aware that steroid minimisation, as opposed to discontinuation, may not confer benefit.

These two areas, better treatments (preferably corticosteroid-free) with minimal/acceptable side-effects and improving quality of life should be research priorities moving forward and we need to continue to innovate and push for ways to improve treatment approaches in AIH.

Using the analogy of the history of liver transplant management, prior to the introduction of anti-rejection immunosuppressants, mortality was high due to graft rejection with only 9% survival at one year (in the 1960s). With the breakthrough discovery of ciclosporin in 1972, a 'watershed moment' in transplantation followed by tacrolimus in 1989, the use of more efficient immunosuppression was pivotal in reducing rejection rates and greatly improving post transplantation survival (Sang and Esquivel, 2019). The pendulum swung to the other extreme as late complications of immunosuppression became a major problem and threat to liver transplantation survival. These complications included opportunistic viral, bacterial and fungal infections, bone disease, chronic kidney disease, metabolic diseases as well as de novo malignancies (Song *et al.*, 2014). Achieving optimal immunosuppression is a balancing act

between the benefit of preventing rejection versus the burden of adverse side-effects of immunosuppression. In a similar vein, in the history of AIH management, mortality was high in the untreated placebo-arm of the early therapeutic trials in AIH of the 1970s. With the introduction of corticosteroid therapy with or without azathioprine, there was substantial improvement in survival. Patients however still have to contend with substantial side-effect profile of the current range of immunosuppressants. The current therapeutic approach in AIH requires balancing the priority of achieving disease remission versus the adverse effects of long-term immunosuppression and the demonstrated impact of treatments on quality of life in AIH. There is a need for a change in our general approach to managing this disease.

Hepatology has undergone a revolution in many therapies over the decades, particularly with the advent of direct-acting anti-virals (DAAs) with nearly 99% sustained viral response rate for hepatitis C (Pawlotsky *et al.*, 2020) as well as the older hepatitis B vaccine which has been a key factor in driving down liver-related complications and mortality worldwide (Meireles, Marinho and Van Damme, 2015). Autoimmune hepatitis needs a revolution in its management with:

i) better therapies (with better remission rates and acceptable side-effect profile)
ii) better markers of response and targets of treatment
iii) better delivery systems without trading off on impact on quality of life.

There is a need to identify new targets for treatment and evaluate better ways of optimising exisitng immunosuppressive medicines as treatments for AIH. More work is required to discover better prognostic markers and predictors of inadequate response, relapse, and progression to cirrhosis. This is because the present marker of ALT and IgG are but blunt surrogates as shown by with histological remission lagging behind biochemical remission.

Using different subgroups in the UK-AIH cohort, experimental studies have evaluated the role of serum B-cell activating factor (BAFF), serum cytokine levels, natural killer and regulatory T-lymphocytes (Tregs) subsets and liver transcriptome (mRNA using Nanostring nCounter) in AIH and reported promising findings (Dyson *et al.*, 2016a; Jeffery *et al.*, 2018; Millar *et al.*, 2017). Further exploratory work is ongoing.

One Japanese study has evaluated specific micro-RNA levels in AIH patients and found positive correlation with fibrosis stage and significant reduction in cirrhotics (with AIH). The group postulated that aberrantly expressed serum micro-RNAs could be potential biomarkers in AIH

and may mediate inflammatory processes in AIH but further work is required (Migita *et al.*, 2015).

More recently, intestinal microbiome has been evaluated in AIH patients. Disease-specific faecal microbiota alteration with disease activity was seen which highlights the role of intestinal microbiota in the pathogenesis of AIH and will be a potential therapeutic target in the future (Liwinski *et al.*, 2020).

There have been some preliminary work in identifying which patients may benefit from withdrawal of immunosuppression (Harrison and Gleeson, 2019) however further studies are required.

The data from the quality of life and health utility study in this thesis can be utilised in cost-effectiveness evaluation and calculation of Quality-Adjusted-Life-Years (QALY) for current and future experimental AIH therapies. A disease-specific patient-reported outcome measure (similar to PBC-40) would be desirable for AIH in order to facilitate focused improvements in specific domains of quality of life. This should hopefully also go in parallel in the development of strategies to improve treatment adherence.

There have been some novel therapeutic trial activity in recent years. A randomised placebo-controlled phase II/III trial using a novel therapeutic anti-BAFF agent (ianalumab) in AIH is currently ongoing (Clinicaltrials.gov: NCT03217422). A novel peptide (pre-implantation factor, secreted by mammalian embryos) is being considered for future phase 1 trial in AIH. T-regulatory cell therapy (adoptive T-reg transfer) has been evaluated in other autoimmune conditions and is being considered for feasibility studies in AIH. Small studies using low-dose interleukin-2 (IL-2) in type 1 AIH patients with inadequate response to conventional second-line therapy showed a short-term expansion of circulating T-regs with no adverse events (Lim *et al.*, 2018; Rosenzwajg *et al.*, 2019). T-reg stimulators are also a potential therapeutic agent as these have been evaluated in early phase trials in other autoimmune conditions (type 1 diabetes, rheumatoid arthritis, inflammatory bowel disease) but not tested in AIH yet. Other potential future therapeutic targets in AIH include anti-interleukin 1 (IL-1), anti-interleukin-6 (IL6) and janus-kinase inhibitors (Halliday *et al.*, 2020).

AIH study populations have historically had heterogenous populations (with different definitions of remission, relapse, overlap disease etc) and this is also a limitation of this current UK-AIH study. Nevertheless, the UK-AIH platform is the start in forming a national multi-centre

research cohort, emulating other national and international platforms (e.g. UK-PBC, Global PBC Study Group and the European Reference Network for Rare Liver Disease). As AIH is a rare, heterogeneous and complex orphan disease, the formation of better networks for collaborative work, national and international disease platforms enables disease stratification and identification of target sub-groups for clinical trials (e.g. immunosuppression withdrawal in the subgroup which have a more benign disease course versus novel immunotherapies for those with a more severe aggressive phenotype). Patient-selection is key in developing a better tolerated and expanded therapeutic armoury for AIH.

In conclusion, this thesis contributes to the growing recognition that better, more consistent corticosteroid-free therapy approaches are needed in patients with autoimmune hepatitis. This should be combined with a focus on improving quality of life for patients. Future approaches should encompass individualised or tailored precision medicine as well as stratified medicine.

Appendices

Appendix A: List of Centres (UK-AIH study)

Aberdeen Royal Infirmary	Queen Elizabeth Hospital, Birmingham
Addenbrookes Hospital	Queen Elizabeth Hospital, Gateshead
St Peters Hospital	Queen Elizabeth Hospital, King's Lynn
Barnsley Hospital	Queen's Medical Centre, Nottingham
Basingstoke & North Hampshire Hospital	Royal Albert Edward Infirmary
Blackpool Victoria Hospital	Royal Bournemouth Hospital
Buckinghamshire Hospital	Royal Cornwall Hospital
Chesterfield Royal Hospital	Royal Derby Hospital
Cumberland Infirmary	Royal Hallamshire Hospital
Derriford Hospital, Plymouth	Royal Infirmary of Edinburgh
Doncaster Royal Infirmary	Royal Liverpool University Hospital
Dorset County Hospital	Royal Stoke University Hospital
Freeman Hospital	Russells Hall Hospital
Gloucestershire Royal Hospital	Salisbury District Hospital
Heartlands Hospital	Sandwell General Hospital
Hull Royal Infirmary	Singleton Hospital
James Cook University Hospital	South Tyneside District Hospital
John Radcliffe Hospital, Oxford	Southampton General Hospital
King's College Hospital, London	Southmead Hospital
King's Mill Hospital	St James' University Hospital, Leeds
New Cross Hospital	Sunderland Royal Hospital
Ninewells Hospital	The Royal Free Hospital
Norfolk and Norwich University Hospital	The Royal Surrey County Hospital
North Tyneside General Hospital	University Hospital of North Durham
Poole Hospital	University Hospital of North Tees
Princess Royal Hospital	University Hospitals Coventry & Warwick
Queen Alexandra Hospital, Portsmouth	Warwick Hospital, South Warwickshire NHS Foundation Trust

Appendix B: Inclusion and Exclusion criteria for Complete response group (A) and Incomplete Response Group (B)

Table 1. Complete response group inclusion criteria (Group A)

	Criterion	Reason
1	All criteria from Table 8 (Chapter 1)	
2	No systemic corticosteroid treatment for at least 12 months (includes oral budesonide, but nasal, inhaled and topical steroids are acceptable),	Defines a group who have clear biochemical remission
3	ALT no higher than the upper reference limit for the analysing laboratory at 12 months after starting treatment AND for the last 12 months before recruitment; there must have been at least 3 ALT measurements during those 12 months	Working definition of biochemical response to treatment
4	Hepatitis B S antigen negative, hepatitis C antibody negative (or, if HCV antibody positive, HCV RNA undetectable by PCR) at time of diagnosis	Chronic viral hepatitis can confound immune phenotype and ALT
5	No clinical suspicion of other viral hepatitis at time of diagnosis (including exclusion of acute hepatitis A and E, EBV and CMV by serology if clinically appropriate)	Acute viral hepatitis can confound diagnosis as well as immune phenotype and ALT
6	AMA titre <1:40	Associated with PBC, which may confound clinical and immunological variables
7	Definitive diagnosis of AIH as determined by a score of at least 5 on the following points system at the time of diagnosis.	The scoring system is adapted from IAIHG criteria (Hennes et al 2008). There is no single diagnostic test for AIH and these criteria have been agreed to

	ANA OR ASMA ≥1:40 (1 point) ANA AND ASMA ≥1:40 OR ANA OR ASMA ≥1:80 OR Anti-LKM ≥1:40 OR SLA+ (2 points) IgG >upper limit normal (1 point) IgG > 1.1 x upper limit normal (2 points) Liver histology compatible with AIH (1 point) Liver histology typical of AIH (2 points) Typical histology is all 3 of lymphocytic interface hepatitis, emperipolesis and rosette formation; compatible histology is chronic lymphocytic hepatitis without all three typical features.	ensure consistency in clinical studies. A score of 5 is used here as equivalent to the criteria for 'definite' AIH.

Table 2. Complete response group exclusion criteria (Group A)

	Criterion	Reason
1	All criteria from Table 9 (Chapter 1)	
2	Any of the following absent at the time of diagnosis and developed subsequently: Biopsy evidence of cirrhosis, or evidence of portal hypertension (ascites or varices on imaging, varices on gastroscopy, hepatic encephalopathy), or liver synthetic dysfunction (elevated prothrombin time unless on Warfarin, elevated bilirubin unless diagnosed Gilbert syndrome) A feature above which was present at the time of diagnosis and has <u>not</u> progressed since is <u>not</u> an exclusion.	Patients with advanced disease remain at high risk of clinical events and changes in ALT and IgG may not purely reflect AIH activity. Therefore patients who have developed features of advanced disease since diagnosis cannot be said to have had a complete response to therapy even if serum parameters have normalised.
3	Diagnosis of another liver disease, including PBC, PSC and their overlap syndromes with AIH, also chronic viral hepatitis B or C, haemochromatosis, Wilson disease, alcohol-related liver disease, alpha-1-antitrypsin deficiency, granulomatous hepatitis, hepatic sarcoidosis, Budd-Chiari syndrome, non-alcoholic steatohepatitis. The presence of liver fat on imaging is not an exclusion. Moderate or severe steatosis on biopsy is acceptable only if steatohepatitis is confidently excluded on clinical / histological evaluation.	These conditions can elevate ALT and confound the results

4	Diagnosis of lymphoma, IgG4 disease, myeloma or monoclonal gammopathy of uncertain significance (MGUS)	These conditions can elevate immunoglobulins and confound the results
5	Metastatic cancer	These conditions have a relatively high probability of influencing clinical variables or outcome during the period of study
6	Primary hepatocellular cancer, cholangiocarcinoma or pancreatic cancer	These conditions have a relatively high probability of elevating ALT or influencing clinical variables during the period of the study
7	Alcohol intake greater than 14 units a week for females, 21 units a week for males, on average over the previous 12 weeks	Higher alcohol intakes can elevate ALT and confound the results
8	HIV positive	HIV, even on antiretroviral therapy, may alter the immune phenotype and confound the results
9	Previous liver transplant	The allo-immune response of immune hepatitis after liver transplantation may differ from AIH and confound the results

Table 3. Incomplete response group inclusion criteria (Group B)

	Criterion	Reason
1	All criteria from Table 8 (Chapter 1)	
2	ALT remains higher than the upper reference limit for the analysing laboratory after initial 12 months of treatment OR At least two ALT values >2xULN at least 4 weeks apart during the past year despite low dose corticosteroid (prednisolone <10mg per day or equivalent) with or without other immunosuppressive therapy OR Requirement for ≥10mg a day of prednisolone or equivalent corticosteroid plus another immunosuppressive agent to keep ALT in the normal range, with a record that ALT rises if corticosteroid dose is reduced below this level.	Identify patients with incomplete biochemical response to treatment or progressive disease despite treatment
3	On-going treatment for AIH in the form of immunosuppressants and/or corticosteroids	Required for accurate assessment of treatment non-response rather than untreated patient
4	Hepatitis B S antigen negative, hepatitis C antibody negative (or, if HCV antibody positive, HCV RNA undetectable by PCR) at time of diagnosis	Chronic viral hepatitis can confound immune phenotype and ALT
5	No clinical suspicion of other viral hepatitis at time of diagnosis (including exclusion of acute hepatitis A and E, EBV	Acute viral hepatitis can confound diagnosis as well as immune phenotype and ALT

	and CMV by serology if clinically appropriate)	
6	AMA titre <1:40	Associated with PBC, which may confound clinical and immunological variables
7	Definitive diagnosis of AIH as determined by a score of at least 5 on the following points system at the time of diagnosis. ANA OR ASMA ≥1:40 (1 point) ANA AND ASMA ≥1:40 OR ANA OR ASMA ≥1:80 OR Anti-LKM ≥1:40 OR SLA+ (2 points) IgG >upper limit normal (1 point) IgG > 1.1 x upper limit normal (2 points) Liver histology compatible with AIH (1 point) Liver histology typical of AIH (2 points) Typical histology is all 3 of lymphocytic interface hepatitis, emperipolesis and rosette formation; compatible histology is chronic lymphocytic hepatitis without all three typical features.	The scoring system is adapted from IAIHG criteria (Hennes et al 2008). There is no single diagnostic test for AIH and these criteria have been agreed to ensure consistency in clinical studies. A score of 5 is used here as equivalent to the criteria for 'definite' AIH.

Table 4. Incomplete response group exclusion criteria (Group B)

	Criterion	Reason
1	All criteria from Table 9 (Chapter 1)	
2	Clinical suspicion that something other than AIH is responsible for a persistently elevated ALT (eg frequent non-steroidal anti-inflammatory drug use, new or worsening fatty liver disease, non-adherence to treatment)	Particularly when IgG is normal but ALT remains elevated, these factors could lead to a patient being considered to have an incomplete response incorrectly and confound the results
3	Patients whose corticosteroid dose is dictated by the requirements of another disease (eg polymyalgia rheumatica)	If the ALT and IgG are normal, the high steroid dose could lead to a patient being considered to have an incomplete response incorrectly and confound the results. Patients should only be included on the basis of on-going high-dose steroid requirements when there is record that lower doses are associated with elevation of the ALT.
4	Diagnosis of another liver disease, including PBC, PSC and their overlap syndromes with AIH, also chronic viral hepatitis B or C, haemochromatosis, Wilson disease, alcohol-related liver disease, alpha-1-antitrypsin deficiency, granulomatous hepatitis, hepatic sarcoidosis, Budd-Chiari syndrome, non-alcoholic steatohepatitis. The presence of liver fat on imaging is not an exclusion. Moderate or severe steatosis on biopsy is acceptable only if steatohepatitis is confidently excluded on clinical / histological evaluation.	These conditions can elevate ALT and confound the results

	PSC overlap/crossover is well described as a cause of persistent LFT abnormality, particularly in younger patients with AIH, and should be excluded by biopsy, MRCP or clinical assessment as part of routine clinical care.	
5	Diagnosis of lymphoma, IgG4 disease, myeloma or monoclonal gammopathy of uncertain significance (MGUS)	These conditions can elevate immunoglobulins and confound the results
6	Metastatic cancer	These conditions have a relatively high probability of influencing clinical variables or outcome during the period of study
7	Primary hepatocellular cancer, cholangiocarcinoma or pancreatic cancer	These conditions have a relatively high probability of elevating ALT or influencing clinical variables during the period of the study
8	Alcohol intake greater than 14 units a week for females, 21 units a week for males, on average over the previous 12 weeks	Higher alcohol intakes can elevate ALT and confound the results
9	HIV positive	HIV, even on antiretroviral therapy, may alter the immune phenotype and confound the results
10	Previous liver transplant	The allo-immune response of immune hepatitis after liver transplantation may differ from AIH and confound the results

Appendix C: Data Forms

Clinical Data Form A

Clinical data form A Version No: 4
Date: 02-11-2015

UK-AIH

Subject Number:

Date: D D - M M - Y Y Y Y

THE UNITED KINGDOM AUTOIMMUNE HEPATITIS COHORT
CLINICAL DATA FORM A *Groups 2a, 2b, main cohort (group 1 use forms B-D & F)*

Q1 Age **Q2 Gender** Male Female **Q3 Year of diagnosis**

Q4 Most recent weight to nearest kilogram kg

Q5 Variables at diagnosis *(ULN=upper limit of normal in that laboratory)*

Variable				Value	Year		
ANA Titre	Pos	Neg			Y Y Y Y	Not Done	Not Available
ASMA Titre	Pos	Neg			Y Y Y Y	Not Done	Not Available
LKM Titre	Pos	Neg			Y Y Y Y	Not Done	Not Available
SLA	Pos	Neg			Y Y Y Y	Not Done	Not Available
AMA Titre	Pos	Neg			Y Y Y Y	Not Done	Not Available
IgG					Y Y Y Y	Not Done	Not Available
IgG ULN					Y Y Y Y	Not Done	Not Available
HBsAg	Pos	Neg	Not Done	Not Available			
HCV Ab	Pos	Neg	Not Done	Not Available			
HAV IgM	Pos	Neg	Not Done	Not Available			
HEV IgM	Pos	Neg	Not Done	Not Available			
EBV IgM	Pos	Neg	Not Done	Not Available			
CMV IgM	Pos	Neg	Not Done	Not Available			

Q6 Current treatment doses - *please give TOTAL DAILY dose*

Prednisolone	. mg	Budesonide	mg
Azathioprine	mg	Mercaptopurine	mg
Mycophenolate mofetil	mg	Mycophenolic acid	mg
Tacrolimus	. mg	Ciclosporin	mg

Other treatments for AIH and dose:

UK-AIH

Subject Number: | | | | | |

Date: D D - M M - Y Y Y Y

Q7 Latest ALT | | | ALT upper limit normal | | Date D D - M M - Y Y

Q8 Latest IgG | | . | | IgG upper limit normal | | . | | Date D D - M M - Y Y

If IgG not measured, total globulins | | upper limit normal | |

Q9 Cirrhosis at presentation? Yes ☐ No ☐ **Q10 Confirmed on biopsy?** Yes ☐ No ☐

Q11 Cirrhosis developed since? Yes ☐ No ☐ **Q12 Confirmed on biopsy?** Yes ☐ No ☐

Q13 Has the patient been diagnosed with:

Ascites ☐ Encephalopathy ☐ Varices ☐

Q14 Has this person had a liver transplant? Yes ☐ No ☐

Q15 Year of transplant Y Y Y Y

Q16 Treatment history. In the last 12 months treatment has included:

Corticosteroid alone ☐ Immunosuppression alone ☐ Both ☐ Neither ☐

Dose has been less than 10mg prednisolone / 6mg budesonide for 12 months Yes ☐ No ☐

Q17 Has there been an attempt to reduce corticosteroids to less than 10mg prednisolone / 6mg budesonide in the past?

Yes ☐ No ☐

Subject Number: ☐☐ ☐☐☐☐

Date: D D - M M - Y Y Y Y

Q18 Treatment response. Please tick all statements that apply (Tick at least one option):

a. ALT persistently >ULN for last 12 months despite treatment ☐

b. At least 2 ALT values > 1.5xULN at least 4 weeks apart in the last 12 months ☐

c. At least 10mg pred / 6mg bud plus immunosuppression needed to keep ALT≤ULN ☐

d. Less than 10mg pred / 6mg bud plus immunosuppression needed to keep ALT≤ULN ☐

e. All ALT values ≤ULN for the last 12 months ☐

f. Other (Please provide details in box below) ☐

Q19 How many times has the participant had ALT>ULN treated with an increase in corticosteroid in the last 12 months? ☐☐

Q20 Does ALT normalise with corticosteroid treatment for a flare? Yes ☐ No ☐

Q21 Adherence to treatment. Which of these do you think best describes how often this patient misses a dose of prescribed autoimmune hepatitis medicines?
(If a dose is never missed please select "0")

Once or twice a year	Once or twice a month	Once or twice a week	Several times a week	Most days of the week
0	1	2	3	4
☐	☐	☐	☐	☐

Q22 Treatment effects. Please tick all statements that apply:

	At Diagnosis	Since Diagnosis
a. Osteoporosis	☐	☐
b. Type 2 Diabetes Mellitus	☐	☐
c. Hypertension	☐	☐

Q23 Current Alcohol intake

No Alcohol ☐ < 15 units/week ☐ < 22 units/week ☐

22-52 units/week ☐ > 52 units/week ☐

Q24 Smoking Never ☐ Ex-smoker ☐ Current Smoker ☐

Q25 Fibroscan value if available ☐☐☐.☐ kPa Date ☐☐/☐☐/☐☐

Participant Questionnaire 1

Participant Questionnaire Part 1 Version No: 3.1
Date: 02/11/2015

UK-AIH

Subject Number: ☐☐ ☐☐☐☐

Date: DD-MM-YYYY

THE UNITED KINGDOM AUTOIMMUNE HEPATITIS COHORT

PARTICIPANT QUESTIONNAIRE PART 1

Q1 How old are you? ☐☐☐ years

Q2 What is your gender? (please tick) male ☐ female ☐

Q3 What is your ethnic origin? (please tick)

- ☐ White
- ☐ Black / African / Caribbean
- ☐ South-East Asian / Chinese
- ☐ Indian / Pakistani / Bangladeshi
- ☐ Mixed / Multiple ethic groups: _____
- ☐ Other: _____

Q4 How tall are you? ☐ feet ☐☐ inches OR ☐☐☐ centimeters

Q5 Have you ever been pregnant? (please tick) ☐ yes ☐ no ☐ not applicable

Q6 Do you have any other medical problems? (please list, **or** if "no" please type N/A)

UK-AIH

Subject Number: | | | | | | |

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Q7 Do you have any of the following medical conditions yourself, or do you know if anyone who is related to you by blood has them? Please only answer about people who are related to you by blood and not people who are only related to you by marriage or adoption. A first degree relative is your brother, sister, parent or child. Other relatives include aunts, uncles, grandparents, cousins, half-brothers and half-sisters.

(Please tick all that apply; do not worry if you have not heard of all the conditions)

	Myself	First degree relatives	Other relatives
Autoimmune hepatitis	☑	☐	☐
Primary biliary cirrhosis (PBC)	☐	☐	☐
Primary sclerosing cholangitis (PSC)	☐	☐	☐
Ulcerative colitis or Crohn's disease	☐	☐	☐
Coeliac disease	☐	☐	☐
Thyroid disease	☐	☐	☐
Rheumatoid arthritis	☐	☐	☐
Systemic lupus erythematosus	☐	☐	☐
Mixed connective tissue disease	☐	☐	☐
Sjogren's syndrome	☐	☐	☐
Diabetes type 1	☐	☐	☐
Diabetes type 2	☐	☐	☐
Liver cancer	☐	☐	☐
Any other cancer	☐	☐	☐
Osteoporosis	☐	☐	☐
Fractured (broken) hip	☐	☐	☐

Participant Questionnaire 2 (contains CFQ and FIS questionnaires)

Participant Questionnaire Part 2 Version No: 5
Date: 02/11/2015

UK-AIH

Subject Number: | | | | | | |

Date: D D - M M - Y Y Y Y

THE UNITED KINGDOM AUTOIMMUNE HEPATITIS COHORT

PARTICIPANT QUESTIONNAIRE PART 2

A. Medication

Q1 What medications are you taking at the moment?

(Please include over-the-counter medications, supplements such as vitamins and herbal remedies
Or if no medications please type "nil")

Q2 Which of these best describes how often you miss a dose of your prescribed autoimmune hepatitis medicines?

(Please tick. If you do not know which medicines we mean, please ask the person who gave you this questionnaire) (Please tick "0" if you never miss a dose)

Once or twice a year	Once or twice a month	Once or twice a week	Several times a week	Most days of the week
0 ☐	1 ☐	2 ☐	3 ☐	4 ☐

Q3 How many days in the last week have you taken all your prescribed autoimmune hepatitis medicine?

(Please tick)

7	6	5	4	3	2	1	0
☐	☐	☐	☐	☐	☐	☐	☐

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Date: D D - M M - Y Y Y Y

In the following questions please select the response that best describes how you feel TODAY.

Q4 I feel my autoimmune hepatitis is under good control.

(Please tick)

Yes, definitely	Yes, probably	Not sure	Probably not	Definitely not
0 ☐	1 ☐	2 ☐	3 ☐	4 ☐

Q5 I worry about the effect my autoimmune hepatitis has on me.
(Please tick)

Never	Very rarely	Occasionally	Quite often	Very often
0 ☐	1 ☐	2 ☐	3 ☐	4 ☐

Q6 I worry about the effect my autoimmune hepatitis medicines have on me.

(Please tick)

Never	Very rarely	Occasionally	Quite often	Very often
0 ☐	1 ☐	2 ☐	3 ☐	4 ☐

Q7 My autoimmune hepatitis reduces my ability to enjoy life.

(Please tick)

Never	Very rarely	Occasionally	Quite often	Very often
0 ☐	1 ☐	2 ☐	3 ☐	4 ☐

Q8 My autoimmune hepatitis medicines reduce my ability to enjoy life.

(Please tick)

Never	Very rarely	Occasionally	Quite often	Very often
0 ☐	1 ☐	2 ☐	3 ☐	4 ☐

UK-AIH

Subject Number: | | | | | | |

Date: D D - M M - Y Y Y Y

B. Memory and concentration

The following questions are about minor mistakes which everyone makes from time to time, but some of which happen more often than others. We want to know how often these things have happened to you IN THE PAST 6 MONTHS. Please tick the appropriate box.

	Very often	Quite often	Occasionally	Very rarely	Never
1 Do you read something and find you haven't been thinking about it and must read it again?	☐	☐	☐	☐	☐
2 Do you find you forget why you went from one part of the house to the other?	☐	☐	☐	☐	☐
3 Do you fail to notice signposts on the road?	☐	☐	☐	☐	☐
4 Do you find you confuse right and left when giving directions?	☐	☐	☐	☐	☐
5 Do you bump into people?	☐	☐	☐	☐	☐
6 Do you find you forget whether you've turned off a light or a fire or locked the door?	☐	☐	☐	☐	☐
7 Do you fail to listen to people's names when you are meeting them?	☐	☐	☐	☐	☐
8 Do you say something and realise afterwards that it might be taken as insulting?	☐	☐	☐	☐	☐

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	Very often	Quite often	Occasionally	Very rarely	Never
9 Do you fail to hear people speaking to you when you are doing something else?	☐	☐	☐	☐	☐
10 Do you lose your temper and regret it?	☐	☐	☐	☐	☐
11 Do you leave important letters unanswered for days?	☐	☐	☐	☐	☐
12 Do you find you forget which way to turn on a road you know well but rarely use?	☐	☐	☐	☐	☐
13 Do you fail to see what you want in a supermarket (although it's there)?	☐	☐	☐	☐	☐
14 Do you find yourself suddenly wondering whether you've used a word correctly?	☐	☐	☐	☐	☐
15 Do you have trouble making up your mind?	☐	☐	☐	☐	☐
16 Do you find you forget appointments?	☐	☐	☐	☐	☐
17 Do you forget where you put something like a newspaper or a book?	☐	☐	☐	☐	☐

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	Very often	Quite often	Occasionally	Very rarely	Never
18 Do you find you accidentally throw away the thing you want and keep what you meant to throw away - as in the example of throwing away the matchbox and putting the used match in your pocket?	☐	☐	☐	☐	☐
19 Do you daydream when you ought to be listening to something?	☐	☐	☐	☐	☐
20 Do you find you forget people's names?	☐	☐	☐	☐	☐
21 Do you start doing one thing at home and get distracted into doing something else (unintentionally)?	☐	☐	☐	☐	☐
22 Do you find you can't quite remember something although it's "on the tip of your tongue"?	☐	☐	☐	☐	☐
23 Do you find you forget what you came to the shops to buy?	☐	☐	☐	☐	☐
24 Do you drop things?	☐	☐	☐	☐	☐
25 Do you find you can't think of anything to say?	☐	☐	☐	☐	☐

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Subject Number: | | | | | | |

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C. Fatigue (low energy)

Below is a list of statements that describe how fatigue may cause problems in people's lives. Please read each statement carefully. Tick one box that best indicates how much of a problem fatigue has been for you these **past four (4) weeks**, including today. Please tick one box for each statement and do not skip any statements.

	no problem	small problem	moderate problem	big problem	extreme problem	not applicable
1 I feel less alert	☐	☐	☐	☐	☐	☐
2 I feel that I am more isolated from social contact	☐	☐	☐	☐	☐	☐
3 I have reduced my workload or responsibilities	☐	☐	☐	☐	☐	☐
4 I am more moody	☐	☐	☐	☐	☐	☐
5 I have difficulty paying attention for a long period	☐	☐	☐	☐	☐	☐
6 I feel I cannot think clearly	☐	☐	☐	☐	☐	☐
7 I work less effectively (this applies to work inside or outside the home)	☐	☐	☐	☐	☐	☐
8 I have to rely more on others to help me or do things for me	☐	☐	☐	☐	☐	☐
9 I have difficulty planning activities ahead of time	☐	☐	☐	☐	☐	☐
10 I am more clumsy and uncoordinated	☐	☐	☐	☐	☐	☐
11 I find that I am more forgetful	☐	☐	☐	☐	☐	☐
12 I am more irritable and more easily angered	☐	☐	☐	☐	☐	☐
13 I have to be careful about pacing my physical activities	☐	☐	☐	☐	☐	☐

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	no problem	small problem	moderate problem	big problem	extreme problem	not applicable
14 I am less motivated to do anything that requires physical effort	☐	☐	☐	☐	☐	☐
15 I am less motivated to engage in social activities	☐	☐	☐	☐	☐	☐
16 My ability to travel outside my home is limited	☐	☐	☐	☐	☐	☐
17 I have trouble maintaining physical effort for long periods	☐	☐	☐	☐	☐	☐
18 I find it difficult to make decisions	☐	☐	☐	☐	☐	☐
19 I have few social contacts outside my own home	☐	☐	☐	☐	☐	☐
20 Normal day to day events are stressful for me	☐	☐	☐	☐	☐	☐
21 I am less motivated to do anything which requires thinking	☐	☐	☐	☐	☐	☐
22 I avoid situations that are stressful for me	☐	☐	☐	☐	☐	☐
23 My muscles feel much weaker than they should do	☐	☐	☐	☐	☐	☐
24 My physical discomfort is increased	☐	☐	☐	☐	☐	☐
25 I have difficulty dealing with anything new	☐	☐	☐	☐	☐	☐
26 I am less able to finish tasks that require thinking	☐	☐	☐	☐	☐	☐
27 I feel unable to meet the demands that people place on me	☐	☐	☐	☐	☐	☐
28 I am less able to provide financial support for myself and my family	☐	☐	☐	☐	☐	☐
29 I engage in less sexual activity	☐	☐	☐	☐	☐	☐

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	no problem	small problem	moderate problem	big problem	extreme problem	not applicable
30 I find it difficult to organise my thoughts when I am doing things at home or at work	☐	☐	☐	☐	☐	☐
31 I am less able to complete tasks that require physical effort	☐	☐	☐	☐	☐	☐
32 I worry about how I look to other people	☐	☐	☐	☐	☐	☐
33 I am less able to deal with emotional issues	☐	☐	☐	☐	☐	☐
34 I feel slowed down in my thinking	☐	☐	☐	☐	☐	☐
35 I feel it is hard to concentrate	☐	☐	☐	☐	☐	☐
36 I have difficulty participating fully in family activities	☐	☐	☐	☐	☐	☐
37 I have to limit my physical activities	☐	☐	☐	☐	☐	☐
38 I require more frequent or longer periods of rest	☐	☐	☐	☐	☐	☐
39 I am not able to provide as much emotional support to my family as I should	☐	☐	☐	☐	☐	☐
40 Minor difficulties seem like major difficulties	☐	☐	☐	☐	☐	☐

UK-AIH

Subject Number: | | | | | | |

Date: D D - M M - Y Y Y Y

D. Thank you

We really appreciate the time you have taken to fill out this questionnaire.

Your answers will be stored securely and combined with answers from other participants to help us understand more about autoimmune hepatitis and the effects it has on people. The people working with your answers will not know who you are or any of your personal details.

If there is anything about the way your liver problem affects you that we have not covered in the questions, please feel free to let us know about it in the box below.

Hospital Anxiety and Depression Scale No: 1.1
Date: 02-11-2015

UK-AIH

Subject Number: ☐☐ ☐☐☐☐

Date: D D - M M - Y Y Y Y

THE UNITED KINGDOM AUTOIMMUNE HEPATITIS COHORT

HOSPITAL ANXIETY AND DEPRESSION SCALE (HADS)

Under each heading, please mark the ONE box that best describes how you have been feeling DURING THE PAST WEEK.

Q1 I feel tense or 'wound up':

Most of the time	A lot of the time	From time to time, occasionally	Not at all
3	2	1	0
☐	☐	☐	☐

Q2 I still enjoy the things I used to enjoy:

Definitely as much	Not quite so much	Only a little	Hardly at all
0	1	2	3
☐	☐	☐	☐

Q3 I get a sort of frightened feeling as if something awful is about to happen:

Very definitely and quite badly	Yes, but not too badly	A little, but it doesn't worry me	Not at all
3	2	1	0
☐	☐	☐	☐

Q4 I can laugh and see the funny side of things:

As much as I always could	Not quite so much now	Definitely not so much now	Not at all
0	1	2	3
☐	☐	☐	☐

Q5 Worrying thoughts go through my mind:

A great deal of the time	A lot of the time	From time to time, but not too often	Only occasionally
3	2	1	0
☐	☐	☐	☐

Hospital Anxiety and Depression Scale No: 1.1
Date: 02-11-2015

UK-AIH

Subject Number: | | | | | | |

Date: D D - M M - Y Y Y Y

Q6 I feel cheerful:

Not at all	Not often	Sometimes	Most of the time
3	2	1	0
☐	☐	☐	☐

Q7 I can sit at ease and feel relaxed:

Definitely	Usually	Not Often	Not at all
0	1	2	3
☐	☐	☐	☐

Q8 I feel as if I am slowed down:

Nearly all the time	Very Often	Sometimes	Not at all
3	2	1	0
☐	☐	☐	☐

Q9 I get a sort of frightened feeling like 'butterflies' in the stomach:

Not at all	Occasionally	Quite Often	Very Often
0	1	2	3
☐	☐	☐	☐

Q10 I have lost interest in my appearance:

Definitely	I don't take as much care as I should	I may not take quite as much care	I take just as much care as ever
3	2	1	0
☐	☐	☐	☐

Q11 I feel restless as if I have to be on the move:

Very much indeed	Quite a lot	Not very much	Not at all
3	2	1	0
☐	☐	☐	☐

Hospital Anxiety and Depression Scale No: 1.1
Date: 02-11-2015

UK-AIH

Subject Number: ☐☐ ☐☐☐☐

Date: D D - M M - Y Y Y Y

Q12 I look forward with enjoyment to things:

As much as I ever did	Rather less than I used to	Definitely less than I used to	Hardly at all
0 ☐	1 ☐	2 ☐	3 ☐

Q13 I get sudden feelings of panic:

Very often indeed	Quite often	Not very often	Not at all
3 ☐	2 ☐	1 ☐	0 ☐

Q14 I can enjoy a good book or radio or TV program:

Often	Sometimes	Not often	Very seldom
0 ☐	1 ☐	2 ☐	3 ☐

Health Related Quality of Life Version No: 1.1
Date: 02/11/2015

UK-AIH

Subject Number: | | | | | | |

Date: D D - M M - Y Y Y Y

THE UNITED KINGDOM AUTOIMMUNE HEPATITIS COHORT

HEALTH RELATED QUALITY OF LIFE

Under each heading, please tick the ONE box that best describes your health TODAY.

1 MOBILITY:

- I have no problems in walking about ☐
- I have slight problems in walking about ☐
- I have moderate problems in walking about ☐
- I have severe problems in walking about ☐
- I am unable to walk about ☐

2 SELF-CARE:

- I have no problems washing or dressing myself ☐
- I have slight problems washing or dressing myself ☐
- I have moderate problems washing or dressing myself ☐
- I have severe problems washing or dressing myself ☐
- I am unable to wash or dress myself ☐

3 USUAL ACTIVITIES *(e.g. work, study, housework, family or leisure activities)*:

- I have no problems doing my usual activities ☐
- I have slight problems doing my usual activities ☐
- I have moderate problems doing my usual activities ☐
- I have severe problems doing my usual activities ☐
- I am unable to do my usual activities ☐

4 PAIN / DISCOMFORT:

- I have no pain or discomfort ☐
- I have slight pain or discomfort ☐
- I have moderate pain or discomfort ☐
- I have severe pain or discomfort ☐
- I have extreme pain or discomfort ☐

5 ANXIETY / DEPRESSION:

- I am not anxious or depressed ☐
- I am slightly anxious or depressed ☐
- I am moderately anxious or depressed ☐
- I am severely anxious or depressed ☐
- I am extremely anxious or depressed ☐

Health Related Quality of Life Version No: 1.1
Date: 02/11/2015

UK-AIH

Subject Number: | | | | | |

Date: D D - M M - Y Y Y Y

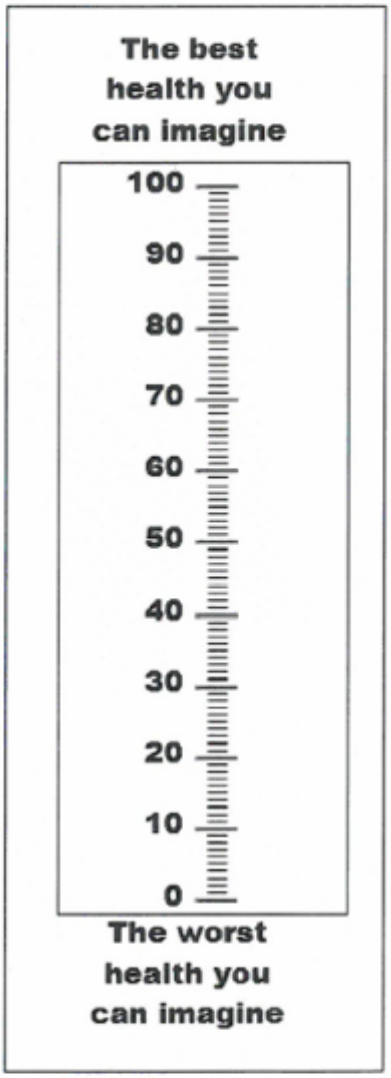
We would like to know how good or bad your health is TODAY.

This scale is numbered from 0 to 100.
100 means the best health you can imagine.
0 means the worst health you can imagine.

Mark an X on the scale to indicate how your health is TODAY.

Now, please write the number you marked on the scale in the box below.

6 YOUR HEALTH TODAY | | | |



Appendix D: Publications

1.	**Inequity of care provision and outcome disparity in autoimmune hepatitis in the United Kingdom** Authors: J Dyson[α], L L Wong [α], T Bigirumurame, G Hirschfield, S Kendrick, Y H Oo, A W Lohse, M A Heneghan, D E J Jones on behalf of the UK-AIH Consortium Reference: Aliment Pharmacol Ther 2018 Nov; 48(9): 951-960. URL: https://onlinelibrary.wiley.com/doi/full/10.1111/apt.14968 DOI: 10.1111/apt.14968 [α] *joint first authors*
2.	**The Impact of AIH and its treatment on Health Utility** Authors: L L Wong [β], H F Fisher [β], D D Stocken, S Rice, A Khanna, M Heneghan, Y H Oo, G Mells, S Kendrick J K Dyson, D E J Jones on behalf of the UK-AIH Consortium Reference: Hepatology 2018; 68(4): 1487-1497. URL: https://aasldpubs.onlinelibrary.wiley.com/doi/full/10.1002/hep.30031 DOI: 10.1002/hep.30031. [β] *joint first authors*
3.	**Exploring the Impact of Autoimmune Hepatitis on Quality of Life*** Authors: L L Wong, H Fisher, D Stocken, S Rice, Y Oo, M Heneghan, S Kendrick, J Dyson, D Jones on behalf of UKAIH Consortium Reference: Hepatology 2017; 66: Posters (Abstracts 264-2239); Abstract 332; page 187A URL: http://onlinelibrary.wiley.com/doi/10.1002/hep.29501/full#hep29501-sec-0273 DOI: http://dx.doi.org/10.1002/hep.29501 - Presented at the American Association for the Study of Liver Diseases (AASLD) Meeting (Washington DC, USA, Oct 2017) ***Presidential Poster of Distinction*** URL: https://www.baslannualmeeting.org.uk/uploads/2017%20Conference/Abstracts/P105%20-%20Exploring%20The%20Impact%20Of%20Autoimmune%20Hepatitis%20On%20Health-Related%20Quality%20Of%20Life.pdf - Presented at the British Association for the Study of Liver Disease (BASL) meeting (Coventry, UK, Sept 2017)
4.	**Changes in natural killer cells and exhausted memory regulatory T Cells with corticosteroid therapy in acute autoimmune hepatitis** Authors: H C Jeffery, M J Braitch, C Bagnall, J Hodson, L E Jeffery, R E Wawman, L L Wong, J Birtwistle, H Bartlett, A W Lohse, G M Hirschfield, J Dyson, D Jones, S G Hubscher, P Klenerman, D H Adams and Y H Oo Reference: Hepatology Communications 2018; 2: 421-436 URL: https://aasldpubs.onlinelibrary.wiley.com/doi/full/10.1002/hep4.1163 DOI: 10.1002/hep4.1163
5.	**Autoimmune Hepatitis patients with poor treatment response have a distinct liver transcriptome: implications for personalised therapy** Authors: B Millar, L L Wong, K Green, A Resteu, S Kendrick, D Jones, J Dyson, the UK-AIH Consortium. Reference: Journal of Hepatology 2017; 66(1): S364 URL: http://www.journal-of-hepatology.eu/article/S0168-8278(17)31069-3/abstract

	DOI: http://dx.doi.org/10.1016/S0168-8278(17)31069-3 - Presented at the International Liver Congress (ILC) European Association for the Study of Liver Diseases (EASL) meeting 2016, Amsterdam, Netherlands
6.	**Understanding the unmet need in Autoimmune Hepatitis – The United Kingdom Autoimmune Hepatitis Study (UKAIH)[#]** Authors: L L Wong, S Kendrick, D Jones, J Dyson. Reference: Hepatology 2016; 64: Poster Session IV: Abstract 1647; page 815A URL/DOI: http://onlinelibrary.wiley.com/doi/10.1002/hep.28800/full - Presented at the American Association for the Study of the Liver (AASLD) meeting 2016, Boston, USA ***([#]Presidential Poster of Distinction)*** URL: http://www.baslannualmeeting.org.uk/uploads/Abstracts/Hepatitis/P102.pdf - Presented at the British Association for the Study of Liver (BASL) meeting, 2016, Manchester, UK
7.	**UK-AIH - Highlighting the need for standardization of treatment in autoimmune hepatitis** Authors: L L Wong, S Kendrick, D Jones, J Dyson Reference: Hepatology 2016; 64: Poster Session IV: Abstract 1663: page 823A URL/DOI: http://onlinelibrary.wiley.com/doi/10.1002/hep.28800/full#hep28799-sec-1879 - Presented at the American Association for the Study of the Liver (AASLD) meeting 2016, Boston,USA URL: http://www.baslannualmeeting.org.uk/uploads/Abstracts/Hepatitis/P102.pdf - Presented at the British Association for the Study of Liver (BASL) meeting, 2016, Manchester, UK
8.	**Understanding the importance of pro- and anti-inflammatory cytokines in autoimmune hepatitis** Authors: J Dyson, J Palmer, L L Wong, S Kendrick, D Jones Reference: Hepatology 2016; 64: Poster Session IV: Abstract 1651: page 817A URL/DOI: http://onlinelibrary.wiley.com/doi/10.1002/hep.28800/full#hep28799-sec-1879 - Presented at the American Association for the Study of the Liver (AASLD) meeting 2016, Boston, USA URL: http://www.baslannualmeeting.org.uk/uploads/Abstracts/Hepatitis/P29.pdf - Presented at the British Association for the Study of Liver (BASL) meeting, 2016, Manchester, UK
9.	**Balance between exhausted regulatory T cells and CD161+ NK cells is crucial in acute AIH pathogenesis** Authors: H Jeffery, M Braitch, S Ubscher, L L Wong, J Hodgson, A Lohse, G Hirschfield, D Adam, D Jones, J Dyson, Y Oo Reference: Hepatology 2016; 64: Poster Session IV: Abstract 1658. Page 812A URL/ DOI: http://onlinelibrary.wiley.com/doi/10.1002/hep.28800/full#hep28799-sec-1879 - Presented at the American Association for the Study of the Liver (AASLD) meeting 2016, Boston, USA

10.	**BAFF is elevated in Autoimmune Hepatitis but does not determine disease phenotype** Authors: J Dyson, J Palmer, L L Wong, S Kendrick, D Jones Reference: Journal of Hepatology 2016 ; 64(2): S440 URL: http://www.journal-of-hepatology.eu/article/S0168-8278(16)00725-X/abstract DOI: http://dx.doi.org/10.1016/S0168-8278(16)00725-X Presented at International Liver Congress (ILC) European Association for the Study of Liver Diseases (EASL) meeting, Barcelona, Spain, 2016

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