Aspects of Longer-Term Management in Autoimmune Hepatitis

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Abstract

Introduction: Autoimmune hepatitis (AIH) is a chronic, immune-mediated liver disease which, untreated, can lead to cirrhosis and death. Treatment usually includes a steroid (usually Prednisolone, sometimes Budesonide) with a steroid-sparing agent such as Azathioprine. The aims of treatment are to obtain remission whilst minimising treatment-related side effects. There are many unanswered questions regarding the longer-term management.

Aims: Analyse a large single-centre cohort of AIH patients to address: a) whether there is a role for immunosuppression withdrawal b) the role of metabolite monitoring to optimise Azathioprine dosing, c) the role of Fibroscan, a non-invasive method of fibrosis assessment to monitor fibrosis, d) the impact of AIH on bone health (osteoporosis and fracture risk), e) and f) the long-term outcomes of patients with AIH – is AIH a lifelong disease?

Results: a) Although not standard practice, on retrospective analysis, 26 patients had immunosuppression withdrawn after at least 2 years' treatment. Six of the 26 (23%) patients had relapses whilst off treatment over 1.29(0.5-9.6) (median (range)) years after stopping IST. Importantly, there were no liver-related deaths in this group.

b) After increasing the AZA dose to 2mg/kg and measuring metabolites, 15 patients subsequently needed dose reduction (raising metabolites and side-effects). However, therapeutic 6-TGN levels were obtained 322((123-482) (median(range)) with AZA

dosing 1.3 (0.59-2.14) mg/kg (median(range)). Rates of histological remission were no higher than standard dosing of 1mg/kg AZA.

c) In a small pilot study, Fibroscan proved effective at detecting significant fibrosis using a cut-off of 11kPa, sensitivity was 83%, specificity 90% and AUROC 0.9.

d) Bone health was reviewed in this proactively managed group of steroid-treated patients. Bone mineral density (BMD) remained similar to an age/gender-matched population (Z score 0.1). Total fracture rate was not obviously different from comparable data in the general population. Patient age and hip bone mineral density predicted fracture risk.

e) Despite treatment, AIH patients have inferior liver-related survival (SMR 1.59(1.28-1.90), counting transplant as liver-related death. Considering a cohort of 330 patients with complete data capture between 1987-2016, all-cause and liver-related death/transplant rates: 24% (all cause) and 11% (liver) after 10 years and 51% and 21% respectively after 20 years. Five out of 65 patients in the third and fourth decade of follow-up relapsed and five developed de novo cirrhosis. Relapse rate per decade was not significantly different in patients followed up in the second twenty years, compared with patients followed from diagnosis (0.71 relapses/decade compared with 0.93 relapses/decade P = 0.23).

Conclusion: AIH is a life-long disease, whereby patients continue to suffer relapses and progression to cirrhosis on treatment. In future, treatment regimes are likely to

evolve to minimise dose and duration of treatment. Long-term management in a specialist care setting is important to ensure remission is obtained and retained, whilst mitigating drug side-effects and optimising quality of life.

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Abbreviations

6-MMP, 6-Methyl-mercaptopurine;

6-TGN, 6-Thioguanine nucleotide;

AIH, autoimmune hepatitis;

ANA, antinuclear antibody

CBR, complete biochemical remission

BMD, bone mineral density

BMI, body mass index

CKD, chronic kidney disease

CMV, cytomegalovirus

CTLA-4, cytotoxic t lymphocyte antigen 4

DXA, dual-energy X-ray absorptiometry

ELF, Enhanced Liver Fibrosis;

FibroQ, Fibroquotient

FS, Fibroscan

GPRD, GP research database

HAI, histology activity index

HAV, hepatitis A virus

HR-pQCT, high-resolution peripheral quantitative computed tomography

HSV, herpes simplex virus

IAIHG, International Autoimmune Hepatitis Group

IBD, inflammatory bowel disease

IL-23, interleukin-23

IOF, International Osteoporosis Foundation

IQR, interquartile range

IS, immunosuppression

IST, immunosuppressive treatment

kPa, kilopascals

LKM-1, liver kidney microsomal antibody

LS, liver stiffness

MCV, mean corpuscular volume

MOF, major osteoporotic fracture

MOP, major osteoporosis score

MMF, Mycophenolate Mofetil

NODM, new-onset diabetes mellitus

NOGG, the National Osteoporosis Guidelines Group

PBC, primary biliary cholangitis

PSC, primary sclerosing cholangitis

RANKL, receptor activator of nuclear factor-k

RCTs, Randomised Controlled Trials

ROC, Receiver Operating Characteristic

SMA, smooth muscle antibody

SMR, standardised mortality ratio

TE, transient elastography

TNF alpha, tumour necrosis factor

ULN, upper limit of normal

VFA, vertebral fracture assessment

VAS, visual analogue scale

WHO, World Health Organisation

1 Introduction

Autoimmune hepatitis (AIH) is a chronic inflammatory liver disease of unknown aetiology, first described by Waldenström in 1950.[1] The annual incidence based on registry studies is 1.68 per 100,000 population in Denmark (prevalence 18-24 per 100,000 adults),[2] and 2.08 per 100,000 population in England.[3] AIH is a heterogenous disease, however it predominantly affects women at an older age.[4,5] The diagnosis is made based on clinical features, raised transaminases, globulins, autoantibodies including antinuclear (ANA), smooth muscle (SMA) or liverkidney microsomal 1 (LKM-1) antibodies and histological features. Scoring systems such as the International Autoimmune Hepatitis Group (IAHG) Score incorporate clinical and histological features to give a likelihood of the diagnosis of AIH (see section 1.1.1).

Presentation is varied and can range from insidious onset to acute liver failure and also in the post-transplantation setting *de novo* or as recurrence. Untreated, AIH often results in liver fibrosis, liver failure and death (unless transplanted). Due to the insidious nature, 30% patients are cirrhotic at first presentation.[6,7] Associations are seen with other autoimmune diseases including thyroid disease, primary biliary cholangitis (PBC), primary sclerosing cholangitis (PSC) and inflammatory bowel disease (IBD).

The pathogenesis of AIH is not fully understood. Genetic and environmental factors have been identified (figure 1.1). Susceptibility genes results in a failure of self-tolerance and a T cell mediated response. Genetic factors identified include HLA loci I, II, III and non-HLA loci, including cytotoxic T lymphocyte antigen-4 (CTLA-4),[8] TNFa[9] and interleukin 17 receptor (IL-17)[10]. There is a five-fold risk in first degree relatives although the absolute risk of developing AIH is low (1/850).[11] Environmental

triggers include: infections such as hepatitis A virus (HAV), cytomegalovirus (CMV) and herpes simplex virus (HSV), and drugs such as nitrofurantoin and minocycline activating self-reactive lymphocytes.

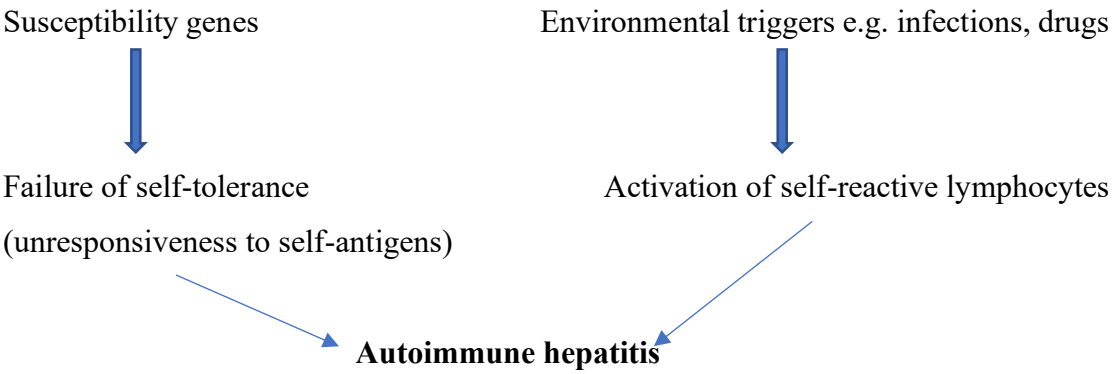


Figure 1.1: AIH pathogenesis, environmental/genetic interplay

As AIH is a rare condition there are many unanswered questions regarding management with only fifteen RCTs currently published.

1.1.1 Presenting features and diagnosis

The diagnosis of autoimmune hepatitis is based upon a combination of biochemical, serological and histological markers alongside exclusion of other liver disorders. Presentation is variable. Around 25% patients are asymptomatic at diagnosis.[6,7,12-14] However, patients may present with non-specific symptoms including fatigue, anorexia and weight loss, nausea and amenorrhoea. Other symptoms include joint pains, maculopapular rash and unexplained fever.[15] Clinical features include jaundice[15] and features of decompensation, such as ascites. Less than 5% patients present with acute liver failure.[16-20] In a large multicentre audit of patients presenting with AIH, the commonest presentation was jaundice with or without itch (42% patients).[14]

The heterogeneity in clinical presentation and lack of specific or sensitive laboratory markers has led to the use of scores to help make a diagnosis. Diagnostic criteria were compiled by the International Autoimmune Hepatitis Group in 1999 (table 1.1).[4]

Parameter/Feature	**Score**	**Parameter/Feature**	**Score**
Female sex	**+2**	Drug history	
		Positive	**-4**
		Negative	**+1**
ALP:AST (or ALT) ratio		Average alcohol intake	
<1.5	**+2**	**<25g/day**	**+2**
1.5-3.0	**0**	**>60g/day**	**-2**
>3.0	**-2**		
Serum globulins or IgG above normal		Liver histology	
		Interface hepatitis	**+3**
>2.0	**+3**	**Predominantly lymphoplasmacytic infiltrate**	**+1**
1.5-2.0	**+2**	**Rosetting of liver cells**	**+1**
1.0-1.5	**+1**	**None of the above**	**-5**
<1.0	**0**	**Biliary changes**	**-3**
		Atypical features	**-3**
ANA, SMA or LKM-1		Other autoimmune disease(s)	
>1:80	**+3**	**In either patient or first degree relative**	**+2**
1:80	**+2**		
1:40	**+1**		
<1:40	**0**		
AMA positive	**-4**	Optional additional parameters	
		Seropositivity for other defined antibodies	**+2**
		HLA DR3 or DR4	**+1**
Hepatitis viral markers		Response to therapy	
Positive	**-3**	**Remission alone**	**+2**
Negative	**+3**	**Remission with relapse**	**+3**
		Interpretation of aggregate scores	
		Pre-treatment: Definite AIH	**>15**
		Probable AIH	**10-15**
		Post-treatment: Definite AIH	**>17**
		Probable AIH	**12-17**

Table 1.1: Modified diagnostic criteria for the diagnosis of autoimmune hepatitis (AIH), modified from Alvarez et al. 1994, reproduced with permission from Prof. Gleeson

The AIHG criteria gives weight to different diagnostic criteria, resulting in a pre- and post-treatment score indicating whether a diagnosis of AIH is probable or definite. Key laboratory features include, elevated serum ALT and AST, raised immunoglobulins, exclusion of viral hepatitis and high-circulating autoantibodies. A simplified scoring system was later devised[21] and validated[22-25], but sensitivity is lacking, missing 5-10% of cases defined by 1999 criteria.

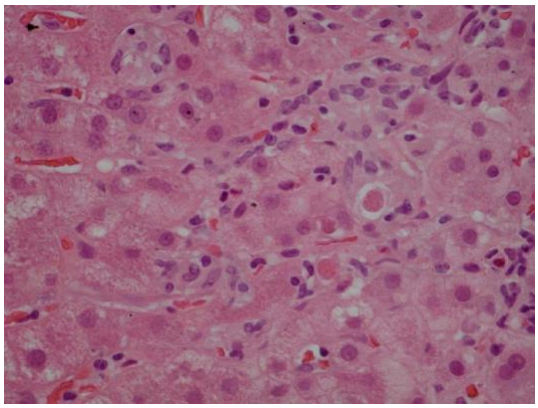


Figure 1.2: Liver histology showing interface hepatitis and plasma cell infiltrate. Reproduced with permission from Dr Dube.

Histological features associated with AIH include interface hepatitis,[4,21,26] periportal lymphoplasmacytic infiltration, hepatocyte swelling and necrosis,[27,28] rosetting of liver cells and emperipolesis. However, rosettes and emperipolesis have also been found in other conditions including primary biliary cholangitis and drug-induced liver injury.[29-31] A group of experts met to define standards for histological diagnosis of AIH. However, only 13/17 histopathologists voted that liver biopsy was mandatory for diagnosis of AIH. In addition, the panel agreed there were no pathognomonic histological features of AIH.[32]

1.1.2 Treatment

The mainstay of treatment of autoimmune hepatitis is with a glucocorticoid (usually Prednisolone, and sometimes Budesonide) and a steroid-sparing agent (usually Azathioprine). The evidence for treatment comes from controlled trials in the early 1970s, showing that Prednisolone improved survival[33,34] and from a more recent multicentre audit.[35] The primary aim of treatment is to prevent liver-related death. Despite treatment, there is evidence of frequent progression to cirrhosis and increased SMR.[36] Two important surrogates are: (a) the prevention of fibrosis progression (poor prognostic indicator), (b) the prevention of relapse (maintenance treatment effective, only multiple relapses predict poor outcome).[32,53,5] Secondary aims include minimising side effects and optimising quality of life.

Treatment is not always indicated. The benefits in older asymptomatic patients with mild interface hepatitis (necroinflammatory score 4-6) is not clear.[27] However, in a recent multicentre UK audit 8% of patients did not receive steroids and these had (correcting for predictive baseline factors) a 4-fold increased risk of all-cause and 9-fold increase in liver-related death/transplantation, compared to steroid-treated patients.[35]

Treatment should be considered when there is moderate or severe inflammation (one or more of serum ALT>5 times normal, serum globulins >2 times normal, liver biopsy showing confluent necrosis). Untreated patients have a poor prognosis with 5- and 10-years survival of 50% and 10% respectively when these criteria are met.

Treated patients have a transplant-free survival of 90%.[36-39] over 10 years. Other reasons for considering treatment include symptoms and liver cirrhosis[7,15,40,41]. Cirrhosis confers an increased risk of liver-related death or transplantation (hazard ratio 21.25).[38] However, newer data demonstrates that asymptomatic, non-cirrhotic patients also benefit from treatment.[35]

1.1.2.1 ***Induction of remission***

The aim of treatment is to induce remission. This can be defined as clinical remission (no longer symptomatic), biochemical (normalisation of serum transaminases and g-globulin) or histological (no or minimal residual inflammation on follow-up liver biopsy). [37] Other terms used to assess response include complete or partial response (See table 1.2).[5]

Term	Definition
Clinical remission	Disappearance of symptoms
Biochemical remission	AST and ALT within the normal range
Histological remission	Minimal chronic hepatitis (Hepatitis Activity Index ≤ 4/18) or no inflammation
Complete response	Normalisation of aminotransferases and IgG/gamma-globulin and histological resolution.
Incomplete response	Improvement in clinical biochemical and histological parameters without reaching complete resolution.
No response/treatment failure	No improvement or worsening of clinical, biochemical or histological features despite treatment.
Relapse	After obtaining a complete response, defined by serum ALT three times the upper limit of normal and or raised Immunoglobulin G over 2g/L following tapering of steroids or complete withdrawal of immunosuppression.

Table 1.2: Definitions of remission and relapse commonly used in AIH[42,43]

Previously Prednisolone has been used as monotherapy (60mg/day, titrating to 20mg/day maintenance. Evidence comes from clinical trials from the Mayo Clinic showing outcomes with titrated Prednisolone or Azathioprine (AZA) monotherapy were superior to placebo.[44,45] Azathioprine monotherapy is not effective in inducing remission.[34] Corticosteroids act rapidly inhibiting T-lymphocyte activation and subsequent cytokine production. AZA blocks the process of lymphocyte precursors from the bone marrow differentiating into mature cells and hence can take at least three months to take its effect.[46]

However, combination therapy with Prednisolone and Azathioprine (AZA) (30mg/day titrating down to 10mg/day maintenance with AZA 1mg/kg) has demonstrated superior outcomes compared with Prednisolone monotherapy (8% relapse at one year compared to 32% when Azathioprine was discontinued.)[47]

National and International guidelines vary in the initial starting doses, however all agree on the recommendation for a corticosteroid plus Azathioprine.[5,37,48] Recent American guidelines included Budesonide as an alternative to Prednisolone in patients without cirrhosis or an acute severe presentation.[48] Budesonide is a second generation corticosteroid and has a 90% first-pass metabolism in the liver. It is contraindicated in cirrhotic patients, because portal shunting and altered hepatic metabolism and reduce therapeutic efficacy.[49] A randomised trial of Budesonide and weight-based AZA (1-2mg/kg daily) in newly-diagnosed patients demonstrated biochemical remission at 6 months (60% versus 39% in Prednisolone treated) with fewer steroid-related side effects (28% versus 53%; $p<0.001$, in Prednisolone treated patients).[50] There is limited evidence suggesting preservation of bone mineral density (see chapter 6).[51,52] A recent large multi-centre audit also supports a role for Budesonide and outcomes over 5 years were not inferior to the use of prednisolone.[35] Percentage fall in ALT after 1, 3, 6 and 12 months was similar in Budesonide and Prednisolone treatment with significantly fewer side effects (13% vs. 3%; $p=0.02$). Budesonide use in a single centre cohort of 60 patients with longer follow-up (31 months on average) resulted in long-term remission in 40-50% patients. However 23% patients were switched back to other therapies due to insufficient disease control.[53] A recent Spanish multicentre retrospective study demonstrated that Budesonide was less effective at inducing remission compared to Prednisolone (biochemical remission rate was to 49% compared

to 87% in prednisolone treated patients p<0.001) however there were fewer side effects with Budesonide.[54]

Traditionally, recommended Prednisolone dose at initiation has been as high as 0.5-1mg/kg.[5] Increasingly, there is evidence that a lower initiation dose of Prednisolone (20mg daily) may be adequate as no statistical difference was seen between high and low dose Prednisolone used as induction therapy.[55] High doses of Prednisolone may be harmful. Over 35mg Prednisolone or 0.5mg/kg dosing was independently associated with worse mortality in a multicentre audit.[35]

Approximately 90% patients with standard treatment will obtain biochemical remission usually within 2 years. [37] Histological remission lags behind biochemical remission. [44] At follow-up liver biopsy, around 60% patients achieve histological remission.[39] Failure to achieve histological remission is associated with less fibrosis regression on biopsy and excess mortality (standardised mortality ratio 1.4 vs. 0.7 P=<0.05).[39]

Another goal of therapy is to prevent relapse. Relapse is defined by serum ALT three times the upper limit of normal and or raised Immunoglobulin G over 2g/L following tapering of steroids or complete withdrawal of immunosuppression. [42] Multiple, but not single, relapses are associated with fibrosis progression and poor outcome.[36,56,57]

1.1.2.2 *Maintenance treatment*

1.1.2.2.1 Maintenance of remission with Azathioprine monotherapy: metabolite monitoring

The aim of long-term management is to maintain remission whilst minimising drug-related side effects. Side-effects are described below. Evidence supporting steroid withdrawal comes from Stellon *et al.* from King's College. A randomised controlled trial was carried out in 47 patients in biochemical and histological remission for at least one year with standard treatment, comparing higher dose Azathioprine (2mg/kg/day) with withdrawal of Prednisolone versus continuing Prednisolone and a lower dose of Azathioprine (1mg/kg/day). After 1 year follow-up there was no difference in liver biochemistry or histology between the two groups.[58] One longer-term study of patients treated with AZA 2mg/kg/day over a median follow-up of 67 months, reported 83% maintenance of remission. A reduction in steroid-related side effects was seen with only four patients developing myelosuppression related to a higher dose of Azathioprine.[59] Higher all-cause and liver-related death was seen in patients who did not receive a steroid sparing agent was also demonstrated in a large multicentre audit ($p<0.001$).[35]

1.1.2.3 *Side-effects of standard treatment*

Long-term immunosuppressive treatment is necessary to retain remission, prevent flare-ups and progression of liver fibrosis to cirrhosis. However, there are possible unwanted effects. Patients are committed to taking life-long treatment. Recent quality of life (QOL) studies have shown that patients with AIH have lower scores compared with controls and high anxiety and depression scores.[60,61] There are also risks of side effects as described below.

1.1.2.3.1 Prednisolone and Budesonide

Prednisolone	**%**	**Azathioprine**	**%**
Weight gain	20	'Gastric flu'	15
Diabetes	6-20	Severe neutropenia	2-12
Hypertension	10	Pancreatitis	1.5-7
Moon facies	15-20	Liver injury	1-4
Acne	15-20		
Cataract	10		
Psychosis	1.5-6		
Low trauma fracture	5-10		

Table 1.3: Prednisolone and Azathioprine side effects reported in AIH: copied with permission from Professor Gleeson[62]

Prednisolone, has predominantly glucocorticoid activity and is most commonly used for long-term disease suppression. Prednisolone use is associated with many side effects summarised in the table (table 1.3).[62]

Prednisolone-related side effects can limit adherence to treatment. Weight gain and diabetes are some of the most significant side-effects. Others include hypertension, dyslipidaemia and cardiovascular disease. Side effects are related to higher dose regimens than lower dose Prednisolone.[44] A study involving collection of retrospective data in 476 patients in a Dutch registry demonstrated that cataracts, diabetes and/or fractures occurred in 25% patients.[63] In addition, they demonstrated dose thresholds for

side effects with new onset fractures occurring at ≤5mg Prednisolone, whereas diabetes and cataracts occurred with doses over 5mg.[63] A higher initial Prednisolone dose (≥ 40mg) has been associated with new-onset diabetes mellitus (NODM).[64] Stopping Prednisolone reverses many side-effects[59] but not diabetes. One group reported NODM only resolved in only 1 out of 20 patients upon Prednisolone discontinuation.[64]

Budesonide is an alternative corticosteroid with a high first-pass metabolism in the liver resulting in reduced systemic bioavailability. However, side effects still occur. Switching from Prednisolone to Budesonide led to a 40% reduction in incidence of steroid-specific side effects in a randomised controlled trial.[50] Data from a multicentre audit, showed Budesonide from the start of treatment (n = 58) resulted in fewer side effects, no development of NODM and no difference in all-cause or liver-related death/transplantation between Budesonide and Prednisolone treated patients.[35] However, a Dutch registry study demonstrated that Budesonide increased the odds of cataracts and fractures, independent of previous Prednisolone use.[63] Bone health in AIH patients is further addressed in detail in Chapter 6.

1.1.2.3.2 Azathioprine

Azathioprine is a steroid-sparing agent, used in combination with Prednisolone (enabling lower steroid dose and limiting side effects) and also as monotherapy in maintaining remission. Side effects related to Azathioprine include bone marrow suppression, gastrointestinal side effects (most commonly nausea or vomiting), flu-like symptoms, and joint pain (see table 1.3).[62] Mild leucopenia due to bone marrow suppression is common, however the need to discontinue due to severe leucopenia is less common. Myelotoxicity resulted in discontinuation of AZA in 3 (1.7%) out of 32

patients who discontinued treatment, out of 173 AZA treated patients.[65] Dose reduction due to leucopaenia was necessary in 3 (out of 70 patients) with average TGN concentrations of 236, 294 and 469 pmol/8 X 10^8 RBCs.[66] Overall, 15.1.% patients discontinued Azathioprine in the first year in a retrospective cohort study from 12 European centres of 631 patients. Cytopenia was the reason for discontinuation in 11 (1.7%) patients.[67] Regular monitoring of the full blood count is therefore recommended on treatment.[59,68] Side effects can result in discontinuation in up 17% patients.[65,68,69] Pancreatitis[70] and cholestasis[71] occur less commonly. Azathioprine is a purine analogue and blocks DNA replication and the de novo pathway of purine synthesis. It is metabolised by thiopurine methyltransferase (TPMT) to mercaptopurine-derived thioguanine nucleotide (TGN) cytotoxic metabolites (see figure 4.1). Around 0.3% of the population lack the TPMT enzyme, resulting in severe myelosuppression. Many doctors, choose to check levels of TPMT prior to starting treatment. However, in a Swedish study, adverse effects were not predicted by TPMT activity, 16% patients with normal TPMT activity experience side effects compared with 20% with intermediate TPMT activity.[65]

Therapeutic effects of Azathioprine are exerted through the metabolite 6-TGN, whereas hepatotoxic effects occur with 6-MMP levels above 5700 pmol/8 X 10^8 RBC. In Chapter 4 I give further details of my study addressing whether patient outcomes can be improved by monitoring metabolites and increasing Azathioprine dose from 1mg/kg to 2mg/kg in patients with subtherapeutic 6-TGN levels. Heneghan *et al.* demonstrated metabolite monitoring did not always prevent Azathioprine toxicity and advanced fibrosis was more predictive of toxicity. TPMT activity, but not metabolites, was lower

in patients with stage 3-4 fibrosis compared to patients with stage 1-2 fibrosis (30 ± 1.92 v 25.2 ± 1.93 P= 0.044).[72] This finding has also been replicated by others.[65,66]

Long-term immunosuppression has been associated with malignancy in organ transplantation including renal transplantation,[73] myasthenia gravis[74] and rheumatoid arthritis.[75] The risk of developing cancer in renal transplant patients at 5, 10 and 15 years was 8, 17 and 30% respectively. Age, duration of follow-up and immunosuppression with Ciclosporin were significantly associated with cancer risk.[73] A dose response relationship was seen in a case control study based on a Danish population-based registry. Patients with a high cumulative dose of Azathioprine (defined as over 150g) had an OR for non-melanoma skin cancer (NMSC) 4.6 (95% CI 1.7–12.5) or long-term use OR 4.8 (95% CI 1.7–13.6) versus OR 3.3 (95% CI 1.5–7.3) for those who had ever used Azathioprine.[74] The overall increase of malignancy with Azathioprine use in organ transplantation is 2-4 fold, whereby the risk of non-melanoma skin cancer is increased 30-40 fold and lymphoproliferative cancer 6-20 fold.[76-79] In IBD patients, an increased risk of malignancy has been related to duration of thiopurine immunosuppression.[80]

Fewer studies have assessed the cancer risk in autoimmune hepatitis. Two studies in AIH have demonstrated an increased risk of malignancy: 31 cancers in 130 patients over 1156 patient years and 92 cancers in 634 patients over 8036 patient years respectively.[81,82] A standardised mortality ratio (SMR) of 1.4 for extrahepatic malignancy was observed, although this was not statistically significant.[81] Five haematological malignancies were observed in a New Zealand cohort of AIH patients

when 0.9 were expected, SIR 5.2 (95% CI 1.7-12.2, P= <0.001).[81] An increased risk of non-melanoma skin cancer has also been demonstrated.[81-84] A Danish registry study demonstrated a dose/duration relationship with a 10-year cancer risk of 13.6% with a relative risk of 1.5 (95% CI 1.3-1.7) compared to controls. The 10-year risk of hepatocellular carcinoma was 0.5% (95% CI 0.2-1.1).[85]

It is unlikely that the increased cancer rate is related to surveillance bias, as the rate of some cancers is not increased (e.g. breast cancer). In addition, the lifetime risk of cancer in an AIH cohort was no higher than the background population in a large Swedish study. The SIR increased to 1.51 when the risk was calculated from the date of AIH diagnosis.[86] One study demonstrated an association between immune disorders such as Hashimoto's thyroiditis, Sjogren's disease, Coeliac disease and Crohn's disease and an increased risk of malignancy.[87] A 1.75-fold risk of lymphoma was seen in rheumatoid arthritis, systemic lupus erythematosus and psoriasis. However, it is difficult to ascertain whether this risk is related to the disease or immunosuppression. [87,88] A meta-analysis of PBC studies (2000-16,000 patients) suggests an overall increased risk of extra-hepatic cancer (RR 1.55 (1.28-1.83)) but not in any individual cancer.

A possible mechanism through which Azathioprine increases the risk of skin cancer is through incorporation of the Azathioprine metabolite, 6-thioguanine (6-TG) into cellular DNA. 6-TG has been demonstrated to interact with ultraviolet (UV) A radiation and photosensitise skin to UV radiation.[89]

1.1.3 Optimising use of Azathioprine

1.1.3.1 *Using an increased dose of Azathioprine*

National guidelines recommend increasing the Azathioprine dose from 1mg/kg to 2mg/kg where inadequate response has been achieved.[37] International guidelines recommend dosing of 1-2mg/kg[5] Azathioprine or 50-150mg.[48] In a study of 72 patients maintained on Azathioprine 2mg/kg/day who had been in histological remission for at least 12 months, 83% patients remained in clinical and biochemical remission over 67 months median follow-up.[59] However, long-term data on efficacy of a 2mg/kg dosing regime are lacking, including the risk of side effects such as cancer. I carry out work addressing the outcome of increasing Azathioprine to 2mg/kg with metabolite monitoring later in this thesis (see Chapter 4).

1.1.3.2 *Metabolite monitoring to optimise therapeutic effect*

Monitoring of AZA metabolites is proposed in the American guidelines[48] to help adjustment of AZA dose to achieve a therapeutic range and avoid toxicity. The basis of these recommendations comes from research in the paediatric population.[90,91] A Swedish group study of 238 patients, with TPMT genotyping demonstrated 207 wild-type and 22 heterozygous patients. They found that patients with normal TPMT activity had lower 6-TGN levels than patients with intermediate TPMT activity patients. Patients with a partial (rather than complete) response had higher thiopurine doses (1.64 vs 1.1.9mg/kg; p=0.012) and TPMT activity resulting in similar TGN levels but higher 6-methylthioinosine diphosphate (meTIMP) levels. They concluded that thiopurine metabolite measurement was useful when patients do not respond to standard thiopurine treatment and was useful in identifying patients with shifted metabolism.[65] However, heterozygosity for the low-activity allele (in about 10% patients) has not been

shown to be a reliable predictor of Azathioprine efficacy or toxicity and cytopenia can occur more commonly in cirrhotic patients irrespective of TPMT activity.[68,72]

In patients with treatment failure where lack of compliance or altered metabolism is suspected, metabolite measurement may be helpful. A target range has not been fully established in AIH, however work from Dhaliwal *et al.* demonstrated that patients who were able to maintain remission (n= 53) had a significantly higher than average TGN level than patients who did not (n=17). Average TGN levels were 237 versus 177 pmol/8X10^8 RBCs; P= 0.025.[66]

1.1.4 Second- and third-line immunosuppressive agents

1.1.4.1 ***Mycophenolate mofetil***

Mycophenolate mofetil (MMF) is the pro-drug of mycophenolate acid (MPA), an inhibitor of inosine-5'-monophosphate dehydrogenase. MPA acts by depleting guanosine nucleotides in lymphocytes, suppressing cell-mediated immune responses and antibody formation.[92] It is widely used in organ transplantation to prevent rejection. In patients with homozygous TPMT deficiency, Mycophenolate is the second-line drug of choice. In addition, evidence supports a role for MMF in patients intolerant to Azathioprine. Rates of biochemical remission range from 43-88%[93,94] in patients intolerant of AZA. A recent metanalysis supports use of MMF in patients intolerant of Azathioprine, with a pooled response rate of 82% (95% CI 88-87%).[95] On the other hand, MMF is less effective in Azathioprine non-responders with remission rates of 0-

25% reported.[93,94] Despite this evidence, MMF is recommended as a second-line treatment in AZA non-responders in the American guidelines.[85]

One prospective study evaluated the role of MMF as a first-line agent in AIH with Prednisolone.[96] 78 of 109 (71.6%) patients had complete response on treatment, however 61 of 78 (78.2%) maintained remission off Prednisolone. However, this group considered patients with mild hepatitis on biopsy as having a complete response. Maintenance of remission was associated with longer MMF treatment ($p=0.005$), higher baseline ALT ($p<0.02$) and lower IgG at 6 months ($p = 0.004$) and histological improvement. A recently published propensity score comparison with AZA demonstrated that overall efficacy was significantly higher in the MMF compared to the AZA group ($p<0.001$). MMF use was associated with complete biochemical response whereas discontinuation of AZA due to intolerance/insufficient response limited its efficacy.[97]

Further evidence is needed to support a role for MMF as a first-line agent. There is currently a Dutch trial in progress. Caveats to its use include, expense and contraindication in pregnancy[98]. In Chapter 7 I further evaluate its role in Azathioprine intolerant patients.

1.1.4.2 ***Calcineurin inhibitors***

Evidence supports a role for calcineurin inhibitors such as Ciclosporin or Tacrolimus where unsatisfactory responses are obtained to first-line treatments. Their major benefit is of potent immunosuppressive effect and rapid onset of action. However, side effects

can limit use in some patient groups (hypertension, renal dysfunction, diabetes, hyperlipidaemia, and neurological effects).[99] Evidence in AIH is limited.[100] A Spanish multicentre study included 23 patients who received Tacrolimus as second-line therapy (13% due to drug toxicity and remainder due to ineffectiveness). They reported 78% patients responded to treatment with a significant improvement in liver enzymes and IgG.[101] In the context of severe AIH, one study reported that 7 out of 9 patients responded to Tacrolimus as salvage therapy.[102]

1.1.4.3 ***Other immunosuppressive agents***

There is much interest in the role of salvage therapies in treatment resistant patients. Data is limited however, anti-TNF-a drugs such as Infliximab[103,104] and monoclonal antibodies against the B-cell surface receptor, Ritixumab[105,106] have been used with therapeutic effect.

1.1.5 Non-invasive Fibrosis Assessment

One of the key objectives in management of autoimmune hepatitis is to prevent progression of fibrosis. Historically, liver biopsy has been the gold standard for liver fibrosis assessment and is needed for IAHG scoring at diagnosis. However, liver biopsy is associated with risk of bleeding (major bleeding <2%) and death (<1 in 1000).[107] There has therefore been much interest in the use of non-invasive techniques. Serum biomarker panels have been used including Fibrosis-4 index (FIB-4)[108] and the enhanced liver fibrosis (ELF) test[109]. Their role in assessing dynamic changes in liver

fibrosis and outcome needs further evaluation and is not recommended in standard practice.[48]

Fibrosis can also be assessed via assessment of liver stiffness. Most centres including our own, have access to Vibration-Controlled Transient Elastography (Fibroscan) and to a lesser extent Acoustic Radiation Force Impulse scanning. Magnetic Resonance Elastography is currently only accessible in larger centres and for research purposes.

1.1.5.1 *Vibration-Controlled Transient Elastography (VCTE) (Fibroscan)*

This technology has only been widely available in the UK in the last 10 years. A strong association with histological stage of fibrosis has been demonstrated in AIH studies.[110-112] However, liver stiffness estimation is affected by both inflammation and fibrosis, which should be considered when interpreting results.[110] Hartl *et al.* demonstrated that at presentation, the Fibroscan result correlated with inflammation rather than stage of fibrosis. After six months, it is possible to differentiate between F0-2 and F3-4 fibrosis. Later work, demonstrated follow-up data, showing improvements in liver stiffness associated with biochemical remission and regression of fibrosis (-7.5%/year; 95% CI -11%to -2.0%; p=0.003), after 6 months treatment.[113] I further evaluate the role of Fibroscan in AIH in Chapter 5.

1.1.5.2 *Acoustic Radiation Force Impulse (ARFI) Scanning*

ARFI measures liver stiffness by measuring changes in shear wave propagation speed. Displacement of short duration bursts of radiated sound waves are interpreted as

changes in liver stiffness.[114] Accuracy of diagnosing of cirrhosis exceeded 93% (sensitivity 93%, specificity 85%) in one study.[115] There is limited published data in the AIH population. Goertz *et al.* included 85 patients with autoimmune liver diseases (31 with AIH). They proposed a cut-off of 2.04m/s for detecting cirrhosis with a sensitivity of 90% and specificity of 74.7% (AUROC 89.2%).[116]

1.1.5.3 ***Magnetic Resonance Elastography (MRE)***

MRE is a promising new technique for fibrosis staging in AIH. Accuracy of 97% with high sensitivity (90%) and specificity (100%) for advanced fibrosis was reported in one study.[117] MRE outperformed fibrosis scoring systems for the diagnosis of cirrhosis (FIB-4, APRI).

1.1.6 Long-term outcome

Cohort studies[7,15,35,36,38,69,118-129] and registry studies[2,3,130,131] have previously reported on long-term outcome. Our own centre previously reported survival rates of 82% ± 3% and 48% ± 5% for all-cause death at 10 and 20 years respectively and 91 ± 2% and 70 ± 5% for liver-related death or transplantation.[36] However, these studies were limited by lack of complete data capture which could result in skewed survival curves. I attempt to overcome this limitation by reporting on a cohort of 330 patients with complete data capture from 1987-2016.

1.1.7 Immunosuppression withdrawal

The importance of obtaining and maintaining remission has already been established.[44,45] Treatment is associated with the risk of side effects and adverse health-

related quality of life.[61] In some circumstances, including treatment side effects, new malignancy and patient choice, treatment withdrawal may be desirable. Reported relapse rates off treatment vary between 25%[132] to 100%.[13] Increased success of IST withdrawal has been reported with longer duration of treatment and sustained normal AST, ALT and IgG for at least 2 years before attempting withdrawal.[5,133] 67% patients treated for more than four years, 17% treated for 2-4 years and 10% treated for 1-2 years maintained remission after drug withdrawal.[134] Risk factors for relapse off treatment include lack of precipitant,[135]coincident autoimmune disease, [136] high IAIHG diagnostic score, [137] and longer time to biochemical remission.[133,138]

Safety of treatment withdrawal is an important consideration. Two liver-related deaths have been reported; one in a cirrhotic patient, following and attributable to immunosuppressive treatment (IST) withdrawal.[56,138] Six patients (five with cirrhosis) required hospitalisation, suffering decompensation of liver disease[138,139]. However, other groups displayed no obvious consequences of relapse.[139,140] Patients who sustained remission, compared to those who relapsed, had similar rates of progression to cirrhosis and of liver-related death or transplantation. However, remission was defined as ALT <2XULN, so this comparison may not be valid based on current definitions of remission.

Another factor when deciding whether to withdraw IST is whether re-treatment with corticosteroids would be acceptable to the patient. This should be discussed with the patient before IST is withdrawn.

Both the European and American guidelines propose consideration of treatment withdrawal. Treatment withdrawal after at least three years treatment and after at least 24 months of biochemical remission is suggested by EASL.[5] Fibroscan is proposed as a possible surrogate marker of biochemical remission. Patients not obtaining biochemical remission had a higher average liver stiffness than those obtaining remission (6.4 ± 3.2kPa compared to 9.2±9.1 kPa not obtaining remission).[113] Use of Fibroscan in the context of treatment withdrawal requires prospective evaluation and is not examined as part of my work.

1.1.8 Conclusion

AIH is a chronic immune-mediated disease. Biochemical remission should be the goal of treatment as persisting transaminases are predictive of relapse after withdrawal of treatment,[133,138] activity on liver biopsy[141], progression to cirrhosis[138] and poor outcome.[118] Corticosteroid (usually Prednisolone) and Azathioprine remain the mainstay of treatment. Recommended dosing of Azathioprine by international bodies is variable. There is a risk of toxicity and side effects. My work attempts to address the safety and efficacy of a regime of increasing Azathioprine dose from 1 to 2mg/kg as well as monitoring Azathioprine metabolites, 6-TGN and 6-MMP. I will report on final dose of Azathioprine achieved with corresponding 6-TGN levels and the relationship to histological remission.

Consideration of immunosuppression withdrawal in AIH has only recently been recommended in International AIH guidelines.[48] Published relapse rates off treatment are variable but, in many reports, high (25-100%). I review data and propose criteria

which can help clinicians decide which patients may be suitable for consideration of IST withdrawal.

New non-invasive techniques are available for monitoring progression of fibrosis (Fibroscan). Fibroscan results needs to be interpreted with caution in patients with active disease.[110] However, Fibroscan offers several benefits over liver biopsy including being safe and accessible. In this thesis, I will assess the accuracy of Fibroscan for diagnosing significant fibrosis in AIH patients.

Corticosteroid treatment is associated with many side-effects, particularly risk to bone health. On withdrawal of Prednisolone, bone mineral density recovers.[142] We have a large cohort of patients, many followed up for over 20 years. I report of outcome in this proactively managed group of patients.

Azathioprine intolerance due to GI side effects is reported in about 10% patients. There is a role for MMF as a second-line agent. I review the outcome in our patients who have switched to MMF for intolerance to IST.

There is limited data on long-term outcome of AIH patients. I report on the long-term outcome of a large cohort of patients with complete data capture, including a comparison of outcome in patients followed for over 20 years compared to those in the first 20 years. Despite treatment, patients continue to develop cirrhosis and suffer relapses.

2 Aims and objectives

In this thesis, I consider some important management issues in a patient's journey. The aims and objectives are as follows:

Firstly: I discuss if there is a role for withdrawal of immunosuppressive treatment by systematically reviewing the literature

Secondly, I perform a retrospective analysis of a large single centre cohort to:

i) carry out a prospective study analysing the role of metabolite monitoring to optimise Azathioprine treatment

ii) examine the role of Fibroscan to predict liver fibrosis

iii) look at the course of bone health (as assessed by DEXA scanning) over several years in steroid treated patients and assess fracture risk

iv) assess long-term outcome in AIH, in particular,

   a. factors associated with survival

   b. outcome over the second twenty years of follow-up

   c. outcome of switching to Mycophenolate Mofetil in patients intolerant of Azathioprine

   d. outcome in patients withdrawing from immunosuppression

3 Systematic Review: stopping immunosuppressive treatment in autoimmune hepatitis (AIH): is it justified (and in whom and when)?

The contents of this chapter have been published and adapted for the thesis.[143]

3.1 Introduction

Notwithstanding the high initial remission rate, longer-term management of AIH remains suboptimal. Despite treatment, de-novo cirrhosis develops in 18(6-54) (median (range))% patients[15,36,56,128,138,144-147] and also premature death with standardised mortality ratios of 2.0-4.0.[36,81,148,149] In part, this is because AIH frequently relapses after stopping treatment and sometimes despite continuing treatment. Multiple relapses are associated with fibrosis progression and a poor outcome.[36,56,57] However, even independently of clinical and biochemical relapse, fibrosis may still progress and cirrhosis develop. This is probably because of incomplete suppression of liver inflammation. Even in patients achieving biochemical remission, failure to obtain histological remission was associated in one study with failure of fibrosis to regress and with poorer long-term survival.[39]

One important question relates to how long should immunosuppressive treatment (IST) of AIH be continued. Recent management guidelines have not resulted in a consensus.

The recent EASL guidelines recommend consideration of IST withdrawal[5] after at least three years of treatment and at least 24 months after biochemical remission is attained. The BSG Guidelines recommend an "individualised" approach to treatment. Demonstration of biochemical and histological remission and treatment duration of at least 24 months are the minimal criteria set out by the AASLD guidelines for consideration of IST withdrawal.[42]

In this systematic review we explore the available published evidence on withdrawal of IST in AIH in an attempt to address:

(a) The relative benefits and risks of withdrawing versus its long-term continuation

(b) Which patients are suitable for IST withdrawal

(c) When is the optimal time to stop treatment?

3.2 Methods

An electronic search of publications in English on PubMed and the University of Sheffield online catalogue, Starplus up to December 2018 using the following keywords: 'autoimmune hepatitis', 'immunosuppression withdrawal', 'treatment', 'side effects', 'bone health', 'osteoporosis', 'fracture', 'malignancy', 'cancer', and 'relapse'. Clinical trials status was checked on http://www.clinicaltrial.gov and http://www.clinicaltrialsregister.eu (European Medicines Agency, 1995-2018; National Institues of Health). In total, 93 studies/articles were included for review from the literature search.

3.3 Results

3.3.1 Immunosuppression treatment withdrawal in AIH:

3.3.1.1 *(a) Overview*

Studies of IST withdrawal are summarised in Table 3.1. Most involve fewer than 100 patients. The main focus has been on AIH relapse and its response to re-treatment, with few studies including data on the long-term outcomes of liver-death/transplantation.

Relapse is usually defined according to the IAIHG criteria as serum ALT three times the upper limit of normal (ULN) and or raised Immunoglobulin G over 2g/L.[42] However, the definition can vary (see Table 1.2). Definition of disease remission prior to treatment withdrawal also varies. Thus, older studies defined biochemical remission as serum ALT of less than twice normal. In more recent studies biochemical remission required achieving a serum ALT within the normal range. Some studies required normal serum globulin/IgG as well as normal ALT. Histological remission was not always demonstrated. Finally, duration of treatment and follow up after IST withdrawal have varied. It is therefore unsurprising that the reported relapse rates range from 25%[132] to 100%[13] (table 3.1).

Citation (number of patients withdrawing from IST (n))	Mean age at diagnosis (D) or withdrawal (W) (yrs)	Biochemical remission (definition and/or duration)	Histological remission at withdrawal of IST	Follow-up after IST withdrawal	Relapse definition	Percentage relapse and outcome	Timing of relapse from IST withdrawal
Czaja *et al.*[140] (52)	36+/-2 (13-75) (W)	AST <2XULN	100% biopsied	Mean 54 +/- 4 mths (6-101 mths)	AST >3XULN or ↑ γ-globulins	46% (24), 33% (17) loss of remission (LOR), no deaths*	7 ± 1 mth (1.5-25 mths)
Hegarty *et. al.*[139] (30)	16-67 (W)	Normal biochemical indices 18 mths (1.5-9 years)	100% histological remission	9 (5-52) weeks	Symptoms ± AST >5XULN	87% (26 patients), 1 death†	Median 9 (5-52) weeks
Kanzler *et al.*[134] (28)	45.8 (9-77) (D)	Normal transaminases ‡	No data	Mean 98 (12-405) mths		75% (21 patients) no adverse outcomes	19 within 15 mths (2 at 4 and 5 yrs)
Muratori [13] (12)	36 (SD 21) (D)	Normal transaminases and γ-globulins §	92% (11) biopsied, 83%(10) remission			100% (12), successful retreatment**	5-10 mths
Verma *et al.*[138] (40)	38 (10-71) (D)	AST/ALT < 2 ULN 44 (2-192) mths relapse group, 65 (26-156) remission group	No data	No data	ALT/AST>2XULN	75% (30 patients), 1 death	2(0.5-23) mths (median (range))
Montano-Loza *et al.*[56] (132)	46±1 (mean) 48, (13-83) (median (range)) (D)	AST<2XULN	No interface hepatitis. Portal hepatitis 82 (63%).	No data	AST>3XULN	77% (102) patients, one liver related death or transplant††	Mean 10 ±2, 3(1-120) mths (median (range))

Table 3.1: Studies of Immunosuppression Withdrawal

* 20 out of 24 patients successfully retreated

† 25 satisfactory response to retreatment, 6 (3-10 weeks)

‡ Treated mean 32.2 (12-81) months

§ Median treatment course of 36 months (range 24–43)

** All relapses retreated successfully

†† Three adverse outcomes

Citation (number of patients withdrawing from IST (n))	Mean age at diagnosis (D) or withdrawal (W) (yrs)	Biochemical remission (definition and/or duration)	Histological remission at withdrawal of IST	Follow-up after IST withdrawal	Relapse definition	Percentage relapse and outcome	Timing of relapse from IST withdrawal
Van Gerven *et al.*[136] (131)	Relapse / LOR group 36 (4-83) (median (range)) (W)	Normal ALT +/- IgG 6.8 years (2-16 years)	18% (24) patients*	Median 8.8 years (2-30 years)	ALT>3XULN and/or increase in IgG to > 2g/L	47% (61 patients), loss of remission 42% (56 patients), no adverse liver outcomes	
Hartl *et al.*[133] (28)	37 (11-76) (W)	Repeatedly normal ALT and IgG. 45 mths (24-111)	11 out of 28 patients‡‡	28 (17-57) mths	ALT +/-IgG > ULN or clinical symptoms	46% (13), no adverse liver outcomes§§	
Zachou[96] (40)	47 +/16 remission, 40 +/-14 relapse (D)	Normal serum transaminases +/-IgG Treated for 60 (24-132) mths prior to withdrawal	95% (38) patients, 78% (31) in remission		AST/ ALT >3XULN and/or IgG >2 g/L	25% (10), no adverse outcomes reported	
Guirguis *et al.*[132] (32)	Unknown. Juvenile onset excluded. (W)	ALT or AST persistently normal or near normal for 6/12 > 6 mths	28% (9) biopsied	8.4(0.13-17) mths (median (percentile))		25% (8), no adverse outcomes reported	Within 12 mths***

Table 3.1: Studies of Immunosuppression Withdrawal Continued

‡‡ 17 opted not to have biopsy, 78 others excluded as biopsy did not confirm remission

§§ 1 patient did not achieve biochemical remission within 17 months

*** No change in fibrosis in 7 patients, decline in fibrosis in 3 patients

Even in patients meeting the AASLD criteria of biochemical remission (normal transaminases and globulin/IgG), relapse rates (defined as serum ALT >3XULN) after stopping IST remain 25-100%. There is no statistically significant association between the risk of relapse and ALT prior to stopping IST, comparing studies which defined remission as a normal ALT versus those that did not p= 0.648 (see table 3.1). Most relapses occur within 6-12 months.[133,138,140] However, the Mayo Clinic has described later relapses in 8 patients (10%) 49-265 months after drug withdrawal.[150]

Transient elevations in transaminases can occur after withdrawal of IST and it is worthwhile repeating LFTs in the first instance to check for normalisation. It is not common practice to re-biopsy patients to confirm relapse. There is some justification for this: in patients with established AIH and a serum ALT rise to more the 2XULN, biopsy confirmed AIH in nearly all cases[141]. However, this study was performed almost 40 years before the discovery of Hepatitis C and the development of virological testing. Apart from viral hepatitis, other conditions which could simulate relapse include drug-induced liver injury, biliary diseases and portal or hepatic vein thrombosis. These conditions should thus be excluded, firstly by appropriate non-invasive testing, and when doubt persists, re-biopsy.

3.3.1.2 *Factors Associated with Relapse (Table 3.2)*

The balance of available evidence (at least one report and no dissenting reports) suggests that several factors are associated with an increased risk of relapse after withdrawal of IST (table 3.2 section A).

Table 3.2: Factors Associated with Relapse on IST Withdrawal

A. Associated with relapse	Comments
Lack of identifiable trigger (drug)	No relapses in patients withdrawing from IS with likely drug precipitant [135]
Coincident autoimmune disease	[136] (Univariate analysis only)
Psychological stress	[151]
Raised ALT at discontinuation of treatment	[133,138,152] Lower globulin or ALT at discontinuation associated with lower risk of relapse
Longer time to biochemical remission	[133,138] +
High AIH diagnostic score (1999 IAIHG criteria [4])	[137]
Previous combination therapy with Prednisolone	[136]
B. Not associated with relapse	
Gender	[134,136,139]
HLA haplotype	[38,153]
Activity on histology ++	[56,137,154]
Advanced fibrosis stage or cirrhosis	[56,136,139,155]

C Conflicting reports	Association	No association
Younger age	[12,38,136,137]	[134,139,144]
Soluble liver, smooth muscle, asialoglycoprotein and liver kidney microsomal antibodies	[156-159]	[136]
Low RBC concentrations TGN+++	[66]	[160]
Serum IgG prior to IS withdrawal	[56,133]	[136]
Duration treated prior to IS withdrawal ++++	[96,134,153]	[56,136,139,152]

+ No significant association between time to biochemical remission in patients remaining in remission versus those relapsing (2.7 vs. 5.3 months, HR 2.18; CI 0.69-6.84 p= 0.18)[133]

++ Association with presence of plasma cells and failure of complete resolution but provided no statistical analysis [155].

+++ Mean of several measurements[66], single measurements only[160]

++++ No apparent explanation for discrepant results

Relapse is rare when the initial episode of AIH has been linked to a drug[135]. In most studies, longer time to achieve biochemical remission has been associated with relapse.[96,133] For example, inability to achieve remission within five months of treatment was associated with >90% probability of relapse (PPV=100%, NPV=32%).[138] Patients with higher levels or ALT or gamma globulin prior to stopping treatment (even if within the normal range) were more likely to relapse. We can infer from Hartl's data we should be aiming for ALT levels less than half the ULN, because patients remained in remission after treatment withdrawal [133,138,152] In another study, predictors of relapse included prior use of dual IST.[136] The reason for this association is unclear. The need for steroids as well as a steroid-sparing agent might indicate more severe or resistant disease.[136] In one case control study psychological stress was positively associated with relapse.[151] This may be related to increased levels of proinflammatory cytokines and immune dysregulation related to actibation of the hpothalamic-pituitary axis and the sympathetic nervous system.

Other factors appear not to be associated with AIH relapse (table 3.2 section B). These include gender,[134,136,139] initial severity of hepatitis[56,137,154] (on diagnostic biopsy) and severity of liver fibrosis.[56,136,139,155]

The evidence linking other factors to relapse risk is contradictory (table 3.2 section C). These include age at diagnosis, although the studies suggesting an association with younger age have tended to be larger than those suggesting no association.[12,38,69,136,137,139,144] Also, serum IgG prior to treatment withdrawal[56,133,136] and histological activity (or lack thereof) on follow-up biopsy, may predict a low relapse rate.[155] However, the attainment of normal histology is relatively rare. Finally, it is unclear if duration of IST prior to withdrawal affects the risk of

relapse, although comparisons between mean duration of treatment in different studies do suggest some association.[96,133]

The role of Azathioprine metabolite monitoring in predicting risk of relapse is not established. In a prospective study, involving several assays per patient, Dhaliwal *et al.* found higher average thioguanine nucleotide (TGN) levels (237 v 177 ($p = 0.025$)) in patients who remained in remission compared to those not in remission.[66] In an earlier study, quartile analysis did not demonstrate a threshold for 6-TGN levels at which remission would be maintained.[72]

Most studies have assessed relapse rate after withdrawal of Azathioprine. In a Greek study of patients who had received Mycophenolate for 60 (24-132) (median (range)) months[96] an "off treatment" relapse rate of 25% over 5(2-24 months) was reported.

3.3.1.3 ***Consequences of Relapse***

In studies from the Mayo Clinic and King's College Hospital, London[139,140] there were no obvious consequences of relapse. Indeed, patients who sustained remission, compared to those who relapsed, had similar rates of progression to cirrhosis and of liver-related death or transplantation. However, remission was defined as ALT <2XULN, so this comparison may not be valid. Two deaths out of 70 patients have been reported, one in a cirrhotic patient, following and attributable to IST withdrawal.[56,138] Six additional patients (five with cirrhosis) required hospitalisation, suffering decompensation of liver disease.[138,139]

In other studies, a single relapse did not appear to influence the longer-term outcome of AIH.[36] However multiple relapses do have a harmful effect. In one study the number of prior relapses in patients who developed cirrhosis (3.1 ± 0.4) and liver decompensation (3.5 ± 0.5) was higher than in patients not developing these complications (1.8 ± 0.2 and 1.7 ± 0.2 respectively).[56] In another long-term study, more than four relapses per decade of follow up was associated with an adverse outcome.[36] In a third study, more than three relapses predicted a poorer outcome.[38]

3.3.1.4 *Response to retreatment and to second withdrawal*

Reassuringly, 80-90% patients re-achieve biochemical remission on reinstitution of Prednisolone.[140] Standard (Prednisolone and Azathioprine) initial treatment regimes were more successful in obtaining remission on retreatment than single maintenance regimes (low dose Prednisolone or Azathioprine). Relapses were successfully retreated with Prednisolone 30mg daily and Azathioprine 75mg daily within 10 (6(3-10)) (median (range)) weeks in 25 out of 26 patients.[139] In a Dutch study, all patients who relapsed were successfully retreated within 3 (2-53) months[136]. Subsequent attempts to withdraw treatment in 32 patients who had previously relapsed resulted in a further relapse after 10 (3-60) months.[136]

3.3.2 Options for continuing IST in AIH

Strategies for continuing treatment after remission is achieved have been evaluated primarily with regard to prevention of relapse. Details of relapse rates on different regimes are as follows:

(a) Prednisolone monotherapy. In a one-year RCT involving 50 patients in whom Azathioprine was stopped after attaining biochemical and histological remission, but who continued low-dose (5-10mg) Prednisolone, relapse rate was 32%, compared to only 8% in those who continued on standard treatment (Azathioprine (1mg/kg) plus Prednisolone[47]. These rates are lower than most reported relapse rates off treatment (Table 3.1), suggesting

that Prednisolone alone is of some value in preventing relapse. Prednisolone was also shown to maintain serum ALT at <3XULN in patients subject to recurrent relapse, although this is hardly an optimal endpoint and these patients had a high rate of disease progression.[161]

(b) Azathioprine. In another 12-month RCT, patients who phased out Prednisolone after biochemical and histological remission but who continued Azathioprine at the higher dose of 2 mg/kg/day, had a relapse rate of zero, similar to those continuing standard treatment. As expected, steroid-related side effects improved only in the Prednisolone withdrawal group, although less than half of patients lost weight.[58]

In a follow-up observational study of 72 patients treated with Prednisolone and Azathioprine 1mg/kg/day, Prednisolone was withdrawn and Azathioprine increased to 2mg/kg/day. Patients were in complete biochemical and histological remission for at least one-year. 83% patients maintained remission over a median follow-up of 67 months.[59] The dose of Azathioprine was subsequently reduced to 1mg/kg in 26 patients and 5 of these then had a disease relapse.

Based on these few studies, Azathioprine has become the standard maintenance regime for AIH. In the UK[162] and the Netherlands[136] most patients receive this treatment, often for decades. In addition, many patients receive long-term Prednisolone as additional maintenance therapy, usually in doses <10 mg/day. It has not been established definitively whether Prednisolone improves the efficacy of 2mg/kg Azathioprine per day in preventing relapse. However, given its partial efficacy as monotherapy and in combination with the lower dose of Azathioprine, this seems plausible.

However, despite its efficacy in preventing disease relapse, it remains unclear whether routinely continuing IST (Azathioprine +/- Prednisolone) prevents development of cirrhosis or liver related death or need for transplantation. This strategy has never been (and is unlikely to be) compared (with regard to these "hard" long-term endpoints), to a strategy of stopping IST and reinstituting it in the event of a relapse.

(c) Other immunosuppressive drugs

There are no data on relapse with use of other steroid-sparing agents such as maintenance MMF monotherapy, calcineurin inhibitors or budesonide in preventing relapse.

3.3.3 Adverse effects of long-term immunosuppression treatment

It is not within the remit of this review to provide comprehensive description of all side effects of IST, but it is important to understand why IST withdrawal may be desirable.

(a) Corticosteroids

Short-term side effects of steroids are well recognised (see section 1.1.2.3). In a multicentre study by the UK-AIH consortium, 55% patients were taking "long-term" corticosteroids (at least 12 months after diagnosis of AIH).[147] Long-term consequences of steroid therapy are not well documented in patients with AIH.[163]

(i) Mental Health and Quality of Life

In a recent study of consecutive AIH patients (77% of whom were in biochemical remission), higher anxiety and depression scores were documented compared to controls; depression was

associated with on-going steroid therapy.[60] Further work from the UK-AIH consortium has also shown that corticosteroid use was associated with a reduction in health-related quality of life, including reduced mobility, anxiety and depression and increased fatigue.[61]

(ii) Metabolic syndrome and cardiovascular disease

New onset of diabetes is reported in 6-20% of steroid treated patients with AIH.[62] Prednisolone induces hepatic insulin resistance.[164] The risk of diabetes development is dose-dependent with an Odds Ratio of 1.77 for doses of 1-39 mg/day, 3.02 for 40 to 79 mg/d, 5.82 for 80 to 119 mg/d, and 10.34 for 120 mg/d or more.[165] Patients with diabetes require additional monitoring and often insulin dose adjustment during steroid treatment.[166]

Weight gain is reported in around 20% of patients receiving steroids for AIH,[62] but has not been adequately quantified. Johnson *et al.* demonstrated that weight gain is not always reversible on stopping treatment. Only 32 (out of 72) patients lost 6.4 (1.5-22.3) (median (range)) kilograms body weight in a study of corticosteroid withdrawal.[59] Unsurprisingly, hepatic steatosis worsens in about 25% Prednisolone treated patients with AIH.[167] It then may be difficult to identify whether raised LFTs are related to an AIH flare or steatosis. Research is needed to determine if steatosis results in fibrosis progression. Corticosteroids may also be associated with an increased risk of cardiovascular disease. Whilst cohort studies have been inconclusive, support for such an association has come from two large database studies.[168-170]

(iii) Bone health

There are few data on bone health in AIH. Low trauma (fragility) fractures have been reported in 5-10% Prednisolone treated AIH patients.[62] In a study of steroid-treated patients (with a variety of conditions) in a GP research database (GPRD) the relative risk of fractures, in particular vertebral fracture and proximal femur, was positively associated with steroid dose. Compared to controls, the risk (95% CI) of vertebral fracture was 5.2 (4.2-6.3) fold increased in patients receiving high doses (greater than 7.5mg) and 1.5 (1.2-2.0) fold increased in those receiving low-dose (less than 2.5mg) Prednisolone. The relative risk of proximal femur fractures was 2.3 (1.9-2.7) and 0.9 (0.8-1.2) fold increased in patients receiving high and low steroid doses respectively.[171] In a study of a GP registry an increased risk of osteoporotic fracture was associated with intermittent high dose Prednisolone or a cumulative dose of over 5 grams. The relative risk of osteoporotic fracture was 3.6 (2.5-5.2).[172]

Corticosteroid therapy is associated with decreased intestinal absorption and increased renal excretion of calcium. Two meta-analyses including steroid-treated patients have demonstrated a beneficial effect of calcium and vitamin D supplements on BMD but not fracture.[173,174] There is a lack of long-term outcome data on use of bisphosphonates in steroid-treated patients with chronic liver disease. A multicentre study of postmenopausal women (without chronic liver disease) showed less bone loss and fewer vertebral and non-vertebral fractures in patients receiving alendronate compared to placebo. New vertebral fractures were found in 6.2 % of women in the placebo group, as compared with 3.2 % in the pooled alendronate groups (P = 0.03).[175] Recent data supports a role for budesonide in patients at risk of osteoporosis. Fifteen patients who had osteopenia at index bone density scan had repeat scans after a median 2 years, with bone density improved in 6 patients, stable in 8 and worse in only one patient.[53]

(b) Azathioprine

Blood count monitoring is important, as there is a risk of marrow suppression. Leucopenia was reported in 6% and 12% of patients in two studies investigating the role of metabolite monitoring in AIH. The risk of Azathioprine induced hepatotoxicity and/or pancreatitis is 1-3%.[66,160] The risks of marrow toxicity and hepatotoxicity are higher in patients with cirrhosis.[65,160] In patients given azathioprine for other conditions, marrow toxicity is associated with high levels of 6-thioguanine nucleotide (6-TGN) metabolites.[176,177] This has not been demonstrated in patients with AIH,[65,160] however here there may be a relationship between Azathioprine hepatotoxicity and 6-methyl-mercaptopurine (6-MMP) metabolite levels.[66]

(c) Mycophenolate

Side effects associated with mycophenolate usage include headache, diarrhoea, nausea, dizziness, hair loss and neutropenia.[178] This drug has been associated with adverse foetal outcomes[179] and should not be given to women of childbearing age.

(d) Cancer risk on immunosuppressive therapy

Long-term use of non-steroidal IST has been associated with malignancy in organ transplantation including renal transplantation[73] and inflammatory bowel disease (IBD).[180] In patients with AIH, the overall risk of extra-hepatic malignancy is increased compared to the general population.[81,82,84,149,181] There are specific increases in risk of non-melanotic skin cancer and lymphoproliferative cancers. Fatal hepatosplenic T cell lymphoma (HSTCL) has been reported in patients taking Azathioprine for over 10 years.[182,183] In IBD patients the absolute risk of developing HSTCL on thiopurine monotherapy is 1:45000 in patients with IBD (and was higher in in those under 35).

We have recently observed an association with malignancy and duration of non-steroid IST in AIH. 241 patients were followed up for a total of 3154 patient years and cancers were identified from clinical records and confirmed by data from the regional cancer registry. For patients on non-steroidal IST (usually Azathioprine) for >10 years and for 4 weeks -10 years hazard ratio was 8.7 and 2.3 compared to those on IST for < 4 weeks.[149]

A recent large study of cardiac and renal transplant patients demonstrated a lower risk of squamous cell carcinoma in patients receiving mycophenolate (OR 0.45, 95% CI 0.29-0.69) compared with those treated with Azathioprine (OR 2.67 95% CI 1.23-5.76).[184]

3.3.4 Implications for management

In theory, long-term IST might reduce progression of liver disease by preventing relapses and perhaps also by suppressing on-going low-grade inflammation. On the other hand, long-term IST might also increase the risk of cancer. It is not known whether routine use of maintenance therapy is, overall, a better strategy than selective use in patients who have had one or more relapses or in those who are deemed at high risk of relapse. These two strategies have not been directly compared (and are unlikely to be). In a long-term observational study[36] no association was found between outcome and percentage of follow-up time on Azathioprine. Thus, the decision whether to stop or to continue must be individualised.

(a) Factors influencing a decision to stop IST

Factors which might favour stopping (as opposed to continuing) IST include:

(i) Factors predicting a low risk of relapse. These include presence of a likely (drug or viral) precipitant of AIH, a short time to achieve and subsequent maintenance of normal serum transaminases. The relative weighting of these factors is unknown. It is not possible to conclude whether age and duration of treatment are predictive factors.

(ii) Absence of cirrhosis or decompensation. The presence of these features increases the chances that a relapse would be harmful – indeed nearly all of the few reported instances of liver decompensation/death during relapse were in patients with cirrhosis.

(iii) Good tolerance of initial steroid regime and absence of contraindications to further steroid therapy – suggesting that a further course needed to treat a relapse might be acceptable to the patients. However, in patients without cirrhosis, Budesonide is an alternative re-treatment option in those previously experiencing Prednisolone-related side effects.

(iv) Development of malignancy. The relationship between duration of non-steroidal IST and development of cancer in patients with organ transplants,[73] inflammatory bowel disease[180] and AIH[82,149] is consistent with a cause and effect relationship. Thus, recurrent skin cancers might justify IST withdrawal. However, it is a separate matter whether the course of a given malignancy is altered by continuation of IS therapy. The CESAME cohort followed 405 IBD patients with a prior cancer for 2.9 years. Rates of new and recurrent cancer were compared between patients who were either exposed or not exposed to a thiopurine. Thiopurine exposure did not affect the crude incidence rate of new or recurrent cancer. This study suggests it may be safe to restart a thiopurine in an IBD patient with a prior cancer.[185]

(b) IST withdrawal strategy

The optimal timing of IST withdrawal is not adequately defined, although there are a few guiding principles. The patient should be in clinical and biochemical remission (normal transaminases and serum IgG/globulin) as this is associated with lower relapse rates following IST withdrawal.[133] The optimal duration of remission is inadequately defined but should probably be at least 12 months.

Should patients undergo repeat liver biopsy prior to IS withdrawal? This is recommended in the EASL guidelines but is not based on hard evidence. Histology on follow-up biopsy does not consistently predict likelihood of relapse (see above). However, a biopsy can sometimes be justified because it might demonstrate progression of liver disease to cirrhosis. Such patients should probably not stop immunosuppression because of the potential harmful effects of a relapse (see above). Furthermore, it may detect on-going inflammation, even in the presence of normal serum transaminases. Such patients, irrespective of whether or not they relapse, had, in one study, a worse long-term outcome than those in whom inflammation resolves. Therefore, there is a case for continued IST, but it would be logical to change to another drug. However, potentially harmful effects of alternative IST would need to be considered.

There is no proven optimal IST withdrawal regime. In practice, treatment is tapered prior to stopping. Maintenance of remission on a tapered dose is reassuring prior to full IST withdrawal. Suggested duration of steroid tapering regimes vary from 6-8 weeks[42] to 3-4 month intervals.[5]

In one study, when steroids were tapered by 2.5mg every 2 weeks 75% of patients suffered withdrawal arthralgia and myalgia, which persisted for up to twelve months in over half.[58]

Therefore, our suggested tapering duration is 3 months, between the above two suggested regimes (6 weeks recommended by AASLD,[42] 6 months by a Dutch group.[136]) With regard to Azathioprine there is a lack of evidence for tapering.

3.3.5 Long-term monitoring and assessment of fibrosis

Following IST withdrawal all patients should be monitored, especially over the first year, as this is when there is the highest risk of relapse.[138,140] All patients should receive long-term follow-up, as relapses can occur over 10 years after treatment withdrawal.[150] Fibroscan (transient elastography) offers a safe and probably effective method for surveying patients and identifying those with fibrosis progression. In a pilot study, liver fibrosis could be accurately estimated in patients who had been treated for more than six months.[110] In a subsequent study, overall liver stiffness improved in patients in biochemical remission but did not improve in those who were not in biochemical remission.[113] Like the authors, we propose annual Fibroscan should be incorporated into the assessment of AIH patients.[113]

3.4 Summary and conclusions

This review, whilst highlighting the paucity of evidence and the difficulties in predicting which patients with AIH can stop immunosuppression, attempts to develop a strategy for rational decision-making in this respect. Further prospective multicentre studies are needed to define more clearly:

(a) Demographic, laboratory, histological and treatment-related factors predicting disease relapse

(b) Consequences of stopping IST and of its continuation, on (i) disease progression, ideally utilising serial non-invasive measures such as Transient elastography and

Enhanced Liver Fibrosis (ELF) testing (ii) patient reported measures of physical and mental well-being

(c) The effect of continued IST (and its discontinuation) on the incidence and the course of malignancy[186]

Such information will help to refine further the information used to decide whether, when and in whom to stop IST in AIH. We need other (and better) interventions to lower the risk of relapse. Rituximab is an anti-CD20 monoclonal antibody that depletes B cells and case reports in AIH demonstrated improvement in biochemistry and histology.[106,187]A preliminary report from Kings reports on a role for IL-2 infusion (in 2 patients).[188]

4 Role of metabolite monitoring in AIH

4.1 Introduction

Initial standard management of AIH includes Prednisolone and Azathioprine. The British guidelines recommend an initial dose of 1mg/kg[37] Azathioprine (AZA) whereas the EASL guidelines[163] recommend 1-2mg/kg dosing. The aim is to obtain remission; however, published rates of biochemical remission at 12 months vary between 16-100%[44,137] and histological remission is achieved by 2 years in only 54% of patients with 1mg/kg dosing.[39] Failure to achieve remission is associated with worsening fibrosis and increased mortality.[39,189] Azathioprine monotherapy (at a dose of 2mg/kg/day) is associated with no relapses over 12 months in an RCT[58] and 17% at 5 years in another study.[59]

AZA is metabolised into pharmacologically active 6-Thioguanine nucleotides (6-TGN) responsible for the immunosuppressive effect and inactive 6-Methymercaptopurine (6-MMP) (see figure 4.1). Raised 6-TGN levels may result in a cytotoxic effect, whereas raised 6-MMP results in hepatotoxicity. AZA is non-enzymatically converted to Mercaptopurine (6MP). 6MP is then metabolised by three different pathways. Two pathways result in the formation of inactive metabolites. The first pathway converts 6-MP to thiouric acid (6-TU) converted by xanthine oxidase (XO). Thiopurine methyltransferase (TPMT) converts 6-MP to inactive 6-methylmercaptopurine in the second pathway. TPMT also metabolises 6-thioinosine monophosphate(6-TIMP) into 6-methylmercaptopurine riboside (6-MMPR). The active metabolites are produced via the third pathway; conversion of 6MP by hypoxanthine phosphoribosyl transferase (HPRT) to mercaptopurine nucleotide, which is then

metabolised into TGN metabolites. 6-TGN is incorporated into nucleic acid instead of guanine nucleotides. It inhibits purine and protein synthesis in lymphocytes, exerting its immunosuppressive effect.

TPMT activity is variable between individuals due to genetic polymorphisms. 89% Caucasians are homozygous for the wild-type allele, 11% heterozygous and 0.3% homozygous for the mutant allele. This results in normal, intermediate and negligible TPMT function respectively.[190] A heterozygous TPMT genotype typically occurs in a patient with a single non-functional allele, resulting in intermediate activity. Absent TPMT activity precludes use and lower starting doses should be used in patients with low activity. In addition, some patients preferentially produce 6-MMP rather than 6-TGN, known as hypermethylation. 6-MMP levels of over 5700 pmol/8 X 10^8 RBC are associated with a three-fold risk of hepatotoxicity.[191] Previous studies suggest increased AZA toxicity in cirrhotic patients which is not predicted by TPMT activity.[72]

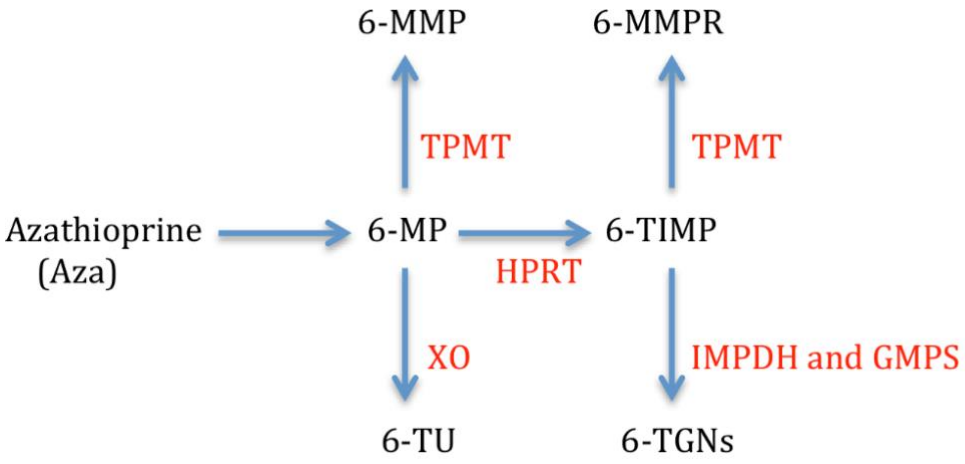


Figure 4.1 Metabolism of Azathioprine: after non-enzymatic cleavage to mercaptopurine, there are three competing pathways. Two pathways result in formation of inactive metabolites: firstly, conversion in 6-TU by xanthine oxidase and secondly by TPMT into 6-MMPR. The active metabolite, 6-TGN is results from a series of steps involving HPRT converting 6-MP to 6-TIMP. 6-TU = 6-thiouric acid, XO = xanthine oxidase, TPMT = thiopurine methyltransferase, 6-MP = mercaptopurine, 6MMP = 6 methylmercaptopurine, HPRT = hypoxanthine phosporibosyl transferase, 6-TIMP = 6-thioinosine monophosphate, 6-TGNs = 6-thioguanine monophosphate, 6-

MMP = 6-methylmercaptopurine, 6-MMPR = 6-methylmercaptopurine riboside, IMPDH = Inosine-5'-monophosphate dehydrogenase, GMPS = guanosine monophosphate synthetase

Azathioprine side effects occur in approximately 10-20% of patients and include hepatotoxicity, acute cholestatic hepatitis, pancreatitis, nausea and vomiting, rash, bone marrow suppression, veno-occlusive disease, opportunistic infections and malignancy.[42]

Previous work has demonstrated that higher 6-TGN levels are associated with therapeutic effect in both IBD[192-194] and AIH.[66] Metanalyses involving the IBD cohort demonstrate that clinical response is best observed when 6-TGN levels are between 235pmol/8X10^8-450pmol/8X10^8. [191,194]Our group demonstrated that 6-TGN levels >220 predicted remission (OR 7.7, P=0.007).[66] Monitoring of 6-TGN levels has been associated with improved clinical response and safety profiles in the IBD setting.[195,196] However, one IBD study showed that weight-based thiopurine dose is weakly correlated with 6-TGN blood concentrations.[195] This was also demonstrated in a non-IBD study, including rheumatology patients treated with Azathioprine.[197]

There are few published prospective studies on Azathioprine metabolism in patients with AIH from initiation of treatment. In a recent retrospective matched cohort study comparing weight-based dosing of Azathioprine with metabolite monitoring, biochemical response was higher in the metabolite monitoring group. 214 patients seen between 1999-2019 were split into two groups, 109 patients had dose adjusted to metabolites, and 105 patients had weight-based dosing. Although patients were managed in a similar way with Prednisolone and Azathioprine, metabolite levels were checked adhoc. Median time from presentation to metabolite testing was 8 (0-31) years. Reasons for metabolite testing were diverse, including guiding IS reduction, failure to achieve or loss of biochemical remission and assessing compliance and toxicity.

TPMT levels were checked in 48% of the metabolite group and only 15% of the non-metabolite group. More patients were on Prednisolone and Azathioprine in the metabolite group (68% compared to 45% in weight-based group). Rates of biochemical response were 71-80% in patients with subtherapeutic metabolite levels compared to 81% in patients with 6-TGN levels within the normal range (225-450 pmol/8X10^8 erythrocytes). Patients with 6-TGN levels <75 pmol/8X10^8 erythrocytes had higher transaminases than patients with 6-TGN levels within the therapeutic range. Although the group reported higher rates of biochemical response in the patients having metabolite monitoring compared with weight-based dosing at 6 months (77% v*s*. 60% p=0.008), it is difficult to draw comparisons due to the nature of data collection and disparities in the patient groups.[198]

The present study is prospective, in a population of newly diagnosed AIH patients. We assess the outcome of Azathioprine introduction and of then increasing dose from 1 to 2mg/kg after three months with metabolite monitoring. We assess the percentage of patients able to achieve, tolerate and maintain higher dose, rates of biochemical remission (12 months) and histological remission (2 years). For the purposes of the study, biochemical remission was defined as normalisation of ALT and AST. Immunoglobulin G levels were not always checked in this cohort as it was not standard practice. However, data on IgG where available is presented.

4.2 Patients and Methods

4.2.1 Study population and treatment

This prospective study included patients with AIH (based on 1999 International Group Criteria) presenting between 2013-2017 at the Liver Unit, Sheffield Teaching Hospitals NHS Foundation Trust. Patients were followed until 31/8/17. The metabolites (6-TGN and 6-MMP)

and TPMT were assayed at City Hospital Birmingham. Not all patients were taking AZA at end of follow-up (reasons explained in results). Patients were monitored by consultants and hepatology clinical nurse specialists with expertise in AIH management. Prior to the study, Azathioprine metabolites were checked to monitor initiation and dose adjustment of Azathioprine to ensure metabolites were within the therapeutic range. It is also our unit's standard of care to arrange a liver biopsy after 2 years to ensure histological remission is obtained before withdrawing corticosteroids. This is because regimes to prevent relapse have been evaluated in patients with histological remission and fibrosis progression correlates with the degree of residual inflammation of biopsy.[189,199,200]

As part of the new regime (figure 4.1), patients were initially treated with Prednisolone and TPMT levels were checked. TPMT displays genetic polymorphism resulting in null or decreased enzyme activity, increasing the risk of myelosuppression. Azathioprine was started at a dose of 1mg/kg, usually after a few weeks of Prednisolone treatment, when the TPMT levels were available. We aimed to increase AZA dose from 1 to 2 mg/kg after three months, with metabolite monitoring, to achieve 6-TGN levels between 250-450 pmol/8×10^8 RBCs and 6-MMP levels <5000 pmol/8×10^8 RBCs.

The AZA dose would be reduced if:

(i) any clinical side effects

(ii) cytopenia develops

(iii) liver enzymes worsen (doubling of serum ALT or any rise in bilirubin from pre-escalation value

(iv) Raised blood TGN (active AZA metabolite) levels or 6-MMP levels

We continued Prednisolone(10mg/day) and AZA in above dose until serum ALT has remained normal for a further 2 years and then repeat liver biopsy (as per unit's standard practice) to assess for histological remission. Rates of histological remission will be compared to patients on standard therapy (1mg/kg).[39]

Twenty-six patients were managed as such. Fourteen patients underwent a liver biopsy after two years of immunosuppression (IS). Reasons for the biopsy not being done in other 12 patients include: awaited at end of follow-up (5), done but inadequate for analysis (1), patient choice (1), old age (2), lost to active follow-up (1), liver transplant (1) and on liver transplant list (1). In patients with histological activity (Necroinflammatory score ≥4) on follow-up liver biopsy, Tacrolimus therapy was considered (see results for details).

4.2.2 Histological assessments

Liver histology was reviewed by a single histopathologist (AD) who completed a detailed proforma including pertinent histological features of AIH. Histological remission is defined as a histological activity index (HAI) of ≤3 and persisting histological activity as HAI≥4. Fibrosis was staged using the Ishak fibrosis staging system from 0-6 (0= no fibrosis, 6= cirrhosis). 25/26 patients had an initial biopsy and 14/26 had a second biopsy by the end of the study period.

4.2.3 Statistical analysis

Statistical analysis was performed using Predictive Analytics Software (PASW) 25.0 for Windows (IBM SPSS Inc. Chicago). Categorical data are summarised as frequencies and percentages and continuous data as median (range). Statistical comparisons were performed

using the Mann-Whitney test for two unpaired continuous variables and Fisher's exact test or chi-squared test for dichotomous variables.

4.2.4 Ethics

The study of metabolite monitoring in AIH was approved by the Sheffield Research Ethics Committee, reference number 014036 and also the Sheffield Teaching Hospitals Foundation Trust (STHFT) Clinical Effectiveness Unit – reference 7822.

4.3 Results:

4.3.1 Summary of patient characteristics

A summary of patient characteristics is demonstrated in table 4.1.

Patients (n)	26
Gender (female(male))	21(5)
Age at diagnosis (median(range))	54(19-74)
Definite AIHG score (probable)	17(9)
Cirrhosis at presentation (n(%))	3(12)
Low TPMT activity (heterozygotes)	3(12)
2mg/kg dosing (n(%))	21 (86)
Follow-up time from diagnosis (months (median(range))	35 (8-53)

Table 4.1: Patient characteristics

4.3.2 Effect of Azathioprine dose increase

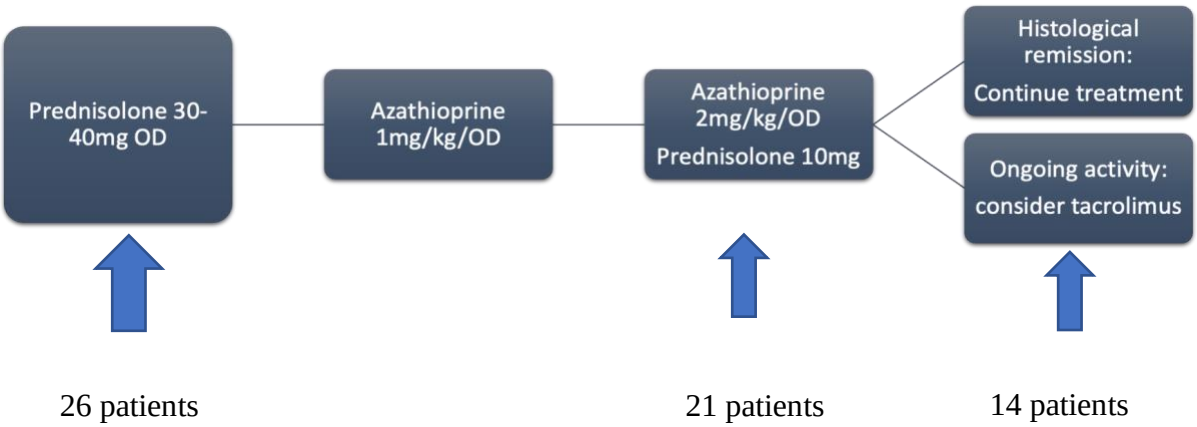


Figure 4.2: Patient management flow chart

Figure 4.2 demonstrates the number of patients who were managed as per protocol. 26 patients commenced Prednisolone 30-40mg and were commenced on Azathioprine 1mg/kg. After three months, Azathioprine dose was increased to 2mg/kg. This escalation to 2mg/kg dosing was achieved in 21/26 (81%) patients. In the other five patients, 6-TGN levels were already either within (n=1) or above (n=3) the therapeutic range of 250-450 pmol/8×10^8 RBCs and one patient developed nausea.

Three (out of 26) patients were TPMT heterozygotes with low TPMT activity. They had significantly higher 6-TGN levels on the initial 1mg/kg dosing ($p<0.005$). 2mg/kg dosing was achieved in one of the three patients but was not maintained at end of follow-up due to raised 6-TGNs. It was not possible to increase the AZA dose due to raised 6-TGN levels (two patients).

Azathioprine dosing	Dose AZA mg/kg	6-TGN pmol/8X10^8 RBCs	6-MMP pmol/ 8X10^8 RBCs	MCV fL (80-98)
Initial dose (1mg/kg) (n=26)	0.95 (0.62-1.13)	223 (119-785)	641 (100-5588)	91.8 (65.8-105.8)
After dose increase (=26)	1.02 (0.62-2.26)	329.5 (141-802)†††	1864 (144-15960)‡‡‡	93.6 (66.4-105.8)§§§
End of follow-up (n=20)	1.24 (1.24-2.14)	294.5 (123-482)****	783 (88-4398)††††	95.8 (66.3-109)‡‡‡‡

††† N.s vs. 6-TGN at initial dose
‡‡‡ P=N.s v.s 6MMP at initial dose
§§§ P= N.s v.s. MCV at initial dose
**** P= 0.013 vs. 6-TGN after dose increase, p=N.S vs. 6-TGN at initial dose
†††† p= N.s vs.6-MMP after dose increase, P= 0.023 vs. 6-MMP at initial dose,
‡‡‡‡ P=N.s vs 6-MMP after dose increase or 6-MMP at initial dose

Table 4.2: Effect of Azathioprine dose increase on metabolite levels and MCV

Despite the above limitations, overall 6-TGN levels rose after the Azathioprine dose was increased, however this rise was not statistically significant (Table 4.2). There was a corresponding rise in 6-MMP levels and MCV. There was a significant association between TPMT levels and 6-TGN and 6-MMP levels at baseline and after a dose increase. By linear regression analysis, the 6-TGN level at baseline was negatively associated with TPMT activity ($p<0.05$). There was a positive association with baseline MMP levels and TPMT levels which did not reach significance ($p<0.87$). The negative association between 6-TGN and TPMT levels could be predicted because patients with low TPMT activity, metabolise Azathioprine preferentially to 6-TGNs.

Reason for dose reduction	Number
Nausea	3
Raised 6-TGN	7
Raised 6-MMP	4
Raised 6-TGN and 6-MMP	1
Leucopenia	1
Malignancy	1

Table 4.3: Reasons for dose reduction in 15 patients previously obtaining 2mg/kg dosing of Azathioprine

Table 4.3 demonstrates a breakdown of reasons for dose reduction in patients who obtained 2mg/kg dosing of Azathioprine. A combination of reasons including raised metabolites and clinical reasons including nausea were important.

4.3.3 Reason for Azathioprine dose reductions

21/26 patients initially achieved dosing of 2mg/kg Azathioprine. However, only 6 of these 21 patients were still receiving AZA 2mg/kg at end of follow-up (1.89(1.81-2.14) mg/kg(median(range)). In the remaining 15 (71%) patients the dose was 1.1.(0.53-1.58) mg/kg(median(range)). Reasons for dose reductions are shown in table 4.3. The most common reasons for dose reduction were raised metabolites (12/15) and nausea (3/15). One patient had AZA discontinued due to the development of squamous cell carcinoma and another had leucopenia along with raised 6-TGN levels.

In 4/26 patients who developed nausea on AZA, 6-TGN levels were (601, 434, 528 and 434). Initial 6-TGN levels were not significantly higher in patients who developed nausea (p= 0.75). No patients developed hepatotoxicity.

4.3.4 Biochemical and histological remission rates

Biochemical remission (normal ALT) was achieved in 21/26 (81%) at 6 months and 23/26 (88.5%) at 12 months. We do not have data on IgG to comment on complete biochemical response, however there is no evidence it predicts outcome. Further details can be found in supplementary table 9.1.

At the end of follow-up, 14 patients had a 2-year follow-up biopsy. Reasons for biopsies not being done are detailed in supplementary table 9.2. 7 (50%) patients were in histological remission. Overall, the necroinflammatory score was 3.5 (2-12) (median(range)), having improved from 13 (4-17) (median(range)) at diagnosis. 6-TGN levels (although higher) were not statistically different in patients obtaining remission versus those who do not (median 345 v 275; p=0.295). If more patients were included in the study, statistical significance may have been obtained (see supplementary table 9.2).

Supplementary Table 9.2 demonstrates initial biopsy data, TPMT levels, initial AZA dose and corresponding metabolite levels and follow-up histology (where available) with AZA dosing and metabolite levels.

At end of follow-up 6/26 (23%) patients were no longer taking Azathioprine. One patient was switched to Mycophenolate mofetil because of nausea. Four patients were switched to Tacrolimus due to AZA ineffectiveness, however one patient subsequently stopped Tacrolimus due to frailty. The sixth patient was on the liver transplant waiting list and not treated with Azathioprine.

4.4 Conclusions

Increasing AZA dose from 1 to 2mg/kg/day after 3 months with metabolite monitoring does not result in higher rates of histological remission compared to standard therapy. Histological remission was achieved in 50% patients compared to 54% using standard 1mg/kg/day Azathioprine dosing protocol in previous work from our unit (metabolite levels not monitored in this study).[39] Higher 6-TGN levels were not significantly associated with biochemical remission at 6/12 or 12/12.

In this cohort, attaining and maintaining 2mg/kg dosing was limited by high levels of 6-TGN and 6-MMP metabolites. Three patients suffered nausea resulting in dose reductions. Thiopurines such as Azathioprine have established associations with certain cancers: EBV lymphomas, non-melanoma skin cancer and other cancers such as urinary tract cancers.[74,76,81,82] One patient developed squamous cell carcinoma and Azathioprine was subsequently stopped. At a final dose of 1.3mg/kg, 6-TGN levels were within the therapeutic range (mean 322 pmol/8X10^8 RBC). 6-TGN levels lower than observed in IBD have been associated with biochemical remission.[65,66,201] 6-TGN levels of >220 pmol/8X10^8 were associated with biochemical remission in one AIH study.[66] AZA dose was lower in the remission group

compared to patients not in remission (1.7(0.4-2.7) versus 2.0(0.9-3.2) (mg/kg/day(median(range))). A pooled analysis of IBD patients demonstrated 6-TGN levels above a threshold of 230-260 pmol/8X10^8 were significantly more likely to be in clinical remission with an OR of 3.27.[192] Typically dosing of 1.5-2.5mg/kg is required to maintain remission in IBD.[202]

The study is limited by a small sample size. In addition, not all patients had a follow-up biopsy within the study period, mostly due to the follow-up ending before the biopsy was due or to advanced patient age precluding biopsy. Strengths include that the study was prospective, the patients were all managed as set out in methods allowing conclusions to be drawn from the results.

Measuring TPMT levels was important, as patients with low TPMT levels (TPMT heterozygosity) had higher metabolite levels than patients with TPMT levels within range at 1mg/kg dosing and therefore risk of hepatotoxicy or myelotoxicity. No patients with TPMT heterozygosity obtained 2mg/kg dosing.

Increasing dose to achieve a given TGN therapeutic range was not associated with improved biochemical or histological response. This is not what was found by Candels *et al.*[198] The group reported higher rates of biochemical response in the patients having metabolite monitoring compared to weight-based dosing at 6 months (77% v*s*. 60% p=0.008). Furthermore, rates of biochemical response were similar (71-80%) in patients with subtherapeutic metabolite levels compared to 81% in patients with 6-TGN levels within the normal range (225-450 pmol/8X10^8 erythrocytes). The two groups were distinct, median time from diagnosis to metabolite testing

was long (8(0-31) years) and the reasons for metabolite testing were diverse. Overall, this work does not support increasing the Azathioprine dose to improve rates of biochemical and histological remission.

5 Role of Fibroscan in AIH: a Pilot Study

5.1 Introduction

Fibrosis staging is very important in the management of AIH. At diagnosis, fibrosis stage influences treatment options. Initial treatment of AIH usually involves a corticosteroid such a Prednisolone or Budesonide in addition to a steroid sparing agent. Budesonide is not as effective in patients with advanced fibrosis.[203,204] In a small study of 18 AIH or overlap patients, more patients with liver fibrosis failed to respond to treatment with Budesonide compared to patients without fibrosis (60% vs. 12.5%; p=0.066). Speculated reasons include reduced hepatic glucocorticoid receptors[205]. In addition, intrahepatic shunts in patients with cirrhosis, result in more side effects[206]. Determining fibrosis stage is also important as it is an independent predictor of outcome.[39,138 36,56]

The historical gold standard for staging of liver disease is liver biopsy. In AIH, a baseline liver biopsy is also important for fulfilling the IAIHG diagnostic criteria. It is our unit's policy to repeat a liver biopsy after treatment for two years to ensure histological remission. This is also the recommendation from the BSG guidelines.[37] Fibrosis stage may progress despite treatment.[39,189] Regression of fibrosis can be seen after treatment of AIH[207]. Ongoing histological activity despite biochemical remission is associated with less fibrosis regression on follow-up biopsies.[39] Development of de- novo cirrhosis is reported in 15(0-47)% (median(range)) of patients with AIH followed up for several years, most of them receiving treatment and many with no evidence of relapses. Like cirrhosis at baseline, de novo cirrhosis is an independent predictor of poor outcome.[36,128,129]

Despite the important role of the liver histology in AIH, serial biopsies are not feasible in many patients. Firstly, there is a risk of complications from liver biopsies. In a metanalysis, bleeding occurred in up to 10.9% of image-guided liver biopsies, major bleeding episodes ranging from 0.1% to 4.6% and minor bleeding in up to 10.9% of biopsies[208]. Risk factors for bleeding included patient age >50, comorbidities and coagulation status (weak evidence). 1/10000 risk of mortality from liver biopsies has been reported.[209]. In addition, liver biopsies are invasive and can be painful.[210,211] In a French prospective nationwide survey, pain was assessed using a visual analogue scale (VAS) and was more likely in women (OR 1.65) and those with chronic HCV infection (OR 1.21).[162] In another report, 20% patients experienced severe pain assessed by VAS.[163]

Secondly, patients may not be willing to have repeat biopsies, having to make arrangements for a day case admission including time off work. Thirdly, liver biopsies take up a lot of hospital resources, including inpatient beds and expertise of a radiologist and histopathologist. The cost of histopathology processing alone is ~£155. As a result, non-invasive methods of fibrosis assessment are desirable.

Furthermore, accuracy of liver biopsy to assess staging is questionable. Sampling errors, inter and intra-observer variability may result in under- or over-staging of fibrosis.[212,213]

Transient elastography (Fibroscan; EchoSens, Paris, France) is a rapid, reproducible, bedside test to assess liver fibrosis by measuring liver stiffness.[214] The technology involves using an ultrasound transducer probe, mounted on the axis of a vibration. Vibrations of mild amplitude and low frequency are transmitted by the transducer, inducing an elastic shear wave that propagates through the liver. Pulse echo ultrasound acquisitions are used to follow the

propagation of the shear wave and measure its velocity. The stiffer the liver tissue, the faster the shear wave propagates. The test can be learned easily with high intraobserver (96-98%) and interobserver (88-98%) agreement.[215] 10 valid measurements are needed with accuracy markers including a success rate of over 60%, and interquartile range of the median IQR/median ≤ 30%. The liver stiffness is measured in kilopascals (kPa).

Fibroscan has been validated against liver biopsy for use in a variety of liver diseases, predominantly in hepatitis C virus and also HBV, NAFLD, ALD [216-218]. Reports of Fibroscan in cholestatic autoimmune liver disease demonstrate accuracy at predicting cirrhosis in primary biliary cholangitis (PBC)[219-221] and primary sclerosing cholangitis (PSC)[222]. Preliminary studies in AIH suggest reasonable accuracy of Fibroscan in detecting fibrosis compared to liver biopsy in patients who have had at least 6 months of treatment.[223-226] [227] In these, most patients had recently started treatment and thus, still had active disease. Hartl *et al.* reported on the impact of active inflammation reducing the ability of the Fibroscan to accurately predict fibrosis.[227] Minimal data on transaminases was provided. In a follow-up study, this group demonstrated a reduction in fibrosis stage by fibroscan in patients who were initially diagnosed with stage 3 or 4 fibrosis.[113] In another study, an improvement in liver stiffness was demonstrated using Fibroscan in patients who had obtained complete biochemical remission (CBR). However, the Fibroscans were carried out 8.6 ± 0.8 years after the liver biopsy without paired biopsy data.[228]

In an American cohort of 53 AIH patients, use of the M probe but not the XL probe was associated with a significant correlation with histological fibrosis. This group proposed this difference was due to reduced penetration of shear waves into intrahepatic tissue.[229] In another

study, gender and BMI correlated with LS measurements. A 1.4% increase in LS was demonstrated in patients per extra unit of BMI (not quite statistically significant).[230]

We aimed to assess the accuracy of Fibroscan in predicting histological fibrosis severity in patients who had achieved biochemical remission (normal transaminases) and were undergoing follow-up liver biopsy to confirm histological remission as per our Unit's clinical policy.

5.2 Methods

5.2.1 Patients

In this pilot study, all patients were regularly followed up at Sheffield Teaching Hospitals NHS Foundation Trust. Between 1/12/13 and 31/12/15 36 same-day Fibroscan and liver biopsy were performed in 32 patients with AIH (1999 International Group criteria). Data on clinical, biochemical and histological features were analysed retrospectively. No patient had ascites, extrahepatic cholestasis or congestive cardiac failure based on clinical and laboratory evaluation.

5.2.2 Diagnostic criteria

Autoimmune hepatitis was diagnosed by clinical, biochemical, serological and histological findings as per the BSG[37] and EASL[163] guidelines. Patients fulfilled the IAIHG criteria. Hepatitis B and C virus were excluded.

5.2.3 Stable biochemical remission

Biochemical remission was defined at ALT within the normal range.[163] IgG was not measured routinely in these patients and therefore not included in the assessment for biochemical remission.

5.2.4 Transient elastography

Liver stiffness measurement by TE was performed using Fibroscan (EchoSens, Paris, France) by trained operators. The M or XL probe were selected as necessary. A distinction was made between valid and invalid scans. A valid scan is defined as at least 10 valid measurements, an interquartile range (IQR)/median ratio of less than 30% and a success rate of at least 60%. The median value of liver stiffness (LS) was recorded in kPa.

5.2.5 Liver histology

Liver biopsies were performed as part of standard of care management and assessed independently by one expert histopathologist (AD). No patients experienced clinically relevant biopsy complications. Necroinflammatory score was graded as per the histology activity index (HAI): 0-3 minimal, 4-8 mild, 9-12 moderate and 13-18 severe inflammation. Where inflammation was graded by class rather than number, a mid-point number was used for analysis. Fibrosis was staged using Ishak fibrosis staging on a scale of 0-4 (F0 = no fibrosis, F1 = portal fibrosis without septa, F2 = portal fibrosis and few septa, F3 = numerous septa without cirrhosis, F4 = cirrhosis). The length of a biopsy should be at least 20mm.[107]

5.2.6 Data analysis

Statistical analyses were carried out using IBM® SPSS® statistics version 25. Continuous variables were expressed as a median and range as not normally distributed. We carried out Receiver Operating Characteristic (ROC) curve analysis to define the best cut-off point for

Fibroscan to distinguish between fibrosis stages 3-6 and 4-6. Optimal cut-off values between fibrosis stages were determined at the maximum sum of sensitivity and specificity. Bivariate Spearman's rank correlation (r_s) was used to analyse the correlation between models and degree of fibrosis.

5.2.7 Ethics

The study of Fibroscan in AIH was approved by the Sheffield Research Ethics Committee reference number 014034.

5.3 Results

Characteristic	
Female (male)	21(6)
Age (median(range)) (years)	54 (17-78)
Duration of treatment before follow-up biopsy and **F**ibroscan (years)	3.1 (2.1-24.9)
Biochemical/serological markers (median(range)): ALT (<33 iU/L) Globulins (18-36/L IgG (6-16g/L)	 20 (9-75) 24 (20-32) 9.3 (6.7-19)
Treatment: Prednisolone Budesonide Azathioprine MMF Tacrolimus	 25 2 23 3 1
Fibroscan indices: LSM((Median(range))kPa) IQR/med ((median(range)%) Success rate ((median(range))%) Valid scans	 6.1 (3.3-21.1) 16 (1-25) 100 (77-100) 27
Biopsy data: Ishak necroinflammatory score (median(range)) Ishak fibrosis score (median(range)) Biopsy length ((median(range))mm)	 4(2-10) 3(0-6) 18(9-32)

Table 5.1: Patient characteristics

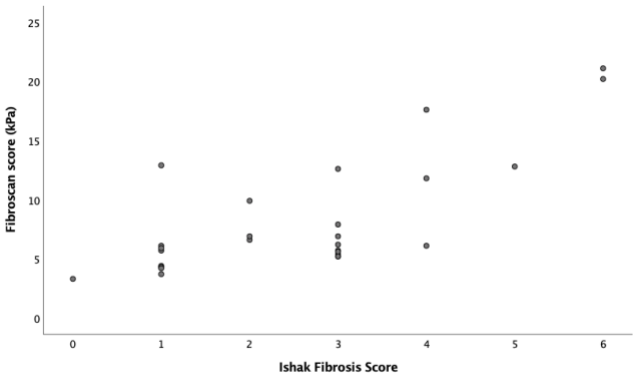
27 patients (21 female, aged 54(17-78) (median(range))) underwent same day Fibroscans and liver biopsies after 3.1(2.1-24.9) years of treatment (table 5.1). 6 other patients did not have valid scans and have been excluded from the analysis. Most patients were treated with Prednisolone (25/27) and Azathioprine (23/27). ALT was 20 (9-75) (median(range)) considering a normal value <33iU/L). 89% patients had normal serum ALT (biochemical

remission) on the day. All 27 patients had a valid scan. A valid scan is defined as at least 10 valid measurements, an interquartile range (IQR)/median ratio of less than 30% and a success rate of at least 60%. The median LSM was 6.75kPa. Ishak necroinflammatory score ≤3 (minimal inflammation or histological remission) was present in 12 biopsies (33%).

Patients	Ishak Fibrosis Stage (n)	AUROC	Fibroscan cut-off score (kPa)	Sens. (%)	Spec. (%)	PPV (%)	NPV (%)
Valid scans (n=27)	5-6 (n=3)	0.97	11.0	100	83	43	00
	4-6 (n=6)	0.91	11.0	83	90	71	95
	3-6 (n=15)	0.72	7.0	47	83	78	55

Table 5.2: Accuracy of Fibroscan in Prediction of Liver Fibrosis

A receiver operating characteristic curve was constructed to determine the cut-off values which best discriminated between fibrosis stages 3-6, 4-6 and 5-6. Using a cut off value of 11kPa for predicting Ishak stage 4-6 or 5-6 fibrosis, considering valid scans (n=27), the AUROC was 0.97 with sensitivity and specificity of 100 and 83% respectively (table 5.2). With a cut off Fibroscan score of 7kPa for predicting Ishak stage 3-6 fibrosis the AUROC was 0.72 and sensitivity and specificity were lower (46 and 86% respectively).



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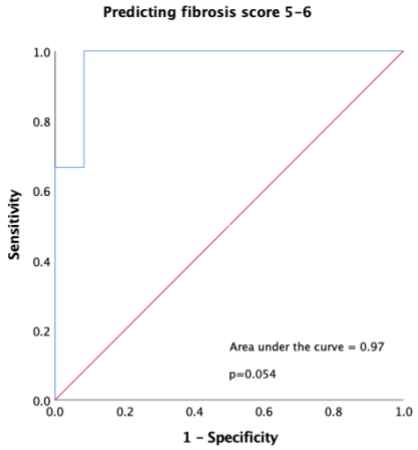

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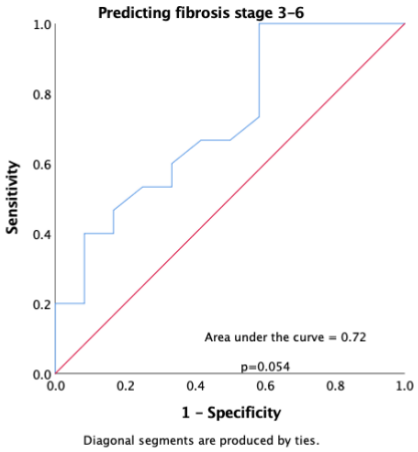

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Figure 5.1: Considering all valid scans (n=27) a) scatter plot showing correlation between Ishak fibrosis stage and Fibroscan score (kPa). There was a positive correlation between fibrosis score and Fibroscan score (rs =0.73; p= <0.05); b) Receiver operating curve for predicting Ishak fibrosis stage 5-6. A cut off Fibroscan score of 11kPa gives a 100% sensitivity and 83% specificity for detecting advanced fibrosis c) Receiver operating curve for predicting Ishak fibrosis stage 3-6. A cut off Fibroscan score of >7kPa gives a sensitivity of 47% and specificity of 83% for moderate to severe fibrosis.

There was a positive correlation between Ishak fibrosis score and Fibroscan score which was more robust for valid scans (Figure 5.1a $r_s = 0.73$; $p = <0.05$) than all scans (Figure 5.1a $r_s = 0.53$; $p<0.05$). Sensitivity and specificity were high when considering valid scans, in particular a cut off of 11kPa had a sensitivity and specificity of 100 and 83% respectively. Sensitivity of Fibroscan was low for predicting lower Ishak fibrosis scores (47% for Ishak fibrosis stage 3-6 for valid scans).

5.4 Discussion

In this study Fibroscan showed good accuracy in excluding, but lower accuracy in predicting Ishak fibrosis stage of 4 or more. Accuracy was improved if a valid scan was obtained (at least 10 valid measurements, an interquartile range (IQR)/median ratio of less than 30% and a success rate of at least 60%). Fibroscan was less accurate in predicting lower fibrosis stages.

Strengths of this study include, patients were well characterised, receiving similar treatment (usually Prednisolone or Azathioprine). Our unit's policy is to arrange a liver biopsy after two years of treatment to confirm histological remission in patients in biochemical remission. Pairing a Fibroscan with this follow-up biopsy means that there should be less of an impact of active inflammation, reducing the ability of the Fibroscan to accurately predict fibrosis.[227] This work would have been strengthened by a bigger sample size.

Hartl et *al.* [113] demonstrated a reduction in liver stiffness of 7.5%/year in patients who were in complete biochemical remission and had another Fibroscan. In another single centre retrospective study, non-invasive markers of fibrosis were assessed in the AIH population. Liver biopsy was carried out within 6 months of Fibroscan. In agreement with this work, they found that Fibroscan did not adequately differentiate mild to moderate from severe fibrosis. Optimal cut-off values for liver stiffness were higher than in this study (19kPa for fibrosis stage 4 and higher). There was a statistically significant correlation between Fibroscan score and Ishak fibrosis score ($r= 0.531$; $p<0.001$).[231]

What other non-invasive methods of fibrosis assessment have been evaluated in AIH? In addition to Fibroscan, Anastasiou *et al.* assessed the following surrogate markers of fibrosis:

APRI, FIB-4, FibroQuotient (FibroQ), and AST/ALT ratio. FIB4, NAFLD fibrosis score and FibroQ-Index showed statistically significant positive correlations with Ishak fibrosis score. No significant correlations were found between fibrosis stage and APRI, AST/ALT ratio or fibrosis stage by Gutkowski.[232] The overall performance of NAFLD fibrosis score was better than other laboratory markers.[231] FIB-4[108] and ELF[109] have been shown to be useful at predicting significant fibrosis. In terms of non-invasive imaging techniques, ARFI showed a good correlation with histological fibrosis in AIH patients $r=0.653$ $p<0.001$. This is despite 20/31 patients not yet receiving immunosuppressive treatment.[116] Magnetic resonance elastography (MRE) is a promising new technique for fibrosis staging in AIH. Accuracy of 97% with high sensitivity (90%) and specificity (100%) for predicting advanced fibrosis was reported in one study.[117] MRE outperformed fibrosis scoring systems for the diagnosis of cirrhosis (FIB-4, APRI).

A proper evaluation of all methods of fibrosis assessment against a liver biopsy is necessary in adequately sized cohorts. A combination of methods may be necessary, especially in patients with lower stages of fibrosis where Fibroscan seems to perform less well.

6 Bone Health in AIH

6.1 Introduction

Low trauma (fragility) fractures are reported in 5-10% patients with autoimmune hepatitis (AIH) receiving Prednisolone.[34,44,45,233] Studies are short term and pre-date interventions to maintain bone health. There is a paucity of information on long-term risk of fracture. There are few data evaluating the role of DEXA and of predictive scores in assessing fragility fracture risk. Here we set out to evaluate osteoporosis prevalence and fracture rate in a large cohort of patients with a long duration of follow-up.

6.1.1 Osteoporosis definition and mechanism

The definition of osteoporosis was made by the World Health Organization (WHO) in 1994 as a "progressive systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture'.[234] It is estimated to affect 200 million people worldwide with resultant increased risk of fractures and reduced quality of life, morbidity and mortality.[235] Osteoporosis is diagnosed clinically based on a bone mineral density (BMD) of ≤ -2.5 standard deviations below young adult mean. The fracture risk is increased in patients with osteoporosis compared to the standard population. The combined lifetime risk for osteoporosis related fractures (hip, forearm and vertebral fractures) coming to clinical attention is around 40%, equivalent to the risk for cardiovascular disease.[236] Roughly 1 in 2 adult women and 1 in 5 men will sustain one or more fragility fractures (a low trauma fracture sustained from a fall from standing height or less) in their lifetime.[237] Fragility fracture tends to occur in the vertebral bodies, hip, distal radius, proximal humerus and

pelvis. Hip fracture is the commonest reason for emergency surgery in older people and the commonest cause of death following a fall.[238] It also results in a significant use of health resources including hospital bed days.[239]

6.1.2 Fracture risk assessment tools: FRAX

Consideration of clinical risk factors which operate independently of BMD can improve the accuracy of BMD assessment. Age contributes to risk independently of BMD.[240,241] Risk factors independent of age and BMD are summarised in the table below (table 6.1).

Risk Factor	Significance	Reference
Low body mass index (BMI)	Predictor of hip fracture. Less useful predictor for other fractures when adjusted for BMD.	[242]
History of prior fracture	Specifically, from low trauma at a site characteristic of an osteoporotic fracture. Multiple vertebral fractures are associated with high fracture risk. One third of subsequent fractures over ten-year time frame occur in first year	[243-246]
Parental history of a hip fracture	Mostly independent of BMD.	[247]
Smoking	In part dependent on BMD.	[248]
Oral glucocorticoid therapy	Increases fracture risk in dose dependent manner, not just related to bone loss.	[171,249]
Alcohol	Dose-dependent relationship with fracture risk where 3 or more units/day are consumed.	[250]
Secondary causes of osteoporosis	Chronic conditions such as liver disease, inflammatory bowel disease, endocrine disorders (uncertain contribution from BMD and glucocorticoids). Rheumatoid arthritis increases fracture risk independently of BMD and glucocorticoid use.	[249]
Diabetes mellitus (type I and II)	Increased risk of hip and non-vertebral fracture. Insulin use and longer duration of disease in type II associated with increased risk (partly independent of BMD).	[251-253]

Table 6.1: Clinical risk factors for assessing fracture risk

FRAX score calculation is recommended by the International Osteoporosis Foundation (IOF) and WHO to express risk of fracture as an absolute risk (ten-year probability). The FRAX tool was created by the then WHO Collaborating Centre for Metabolic Bone Diseases at Sheffield. The tool uses algorithms that integrate the weight of clinical risk factors for fracture risk, with or without BMD data. The FRAX tool (www.shef.ac.uk/FRAX) calculates the 10-year

probability of hip fracture and/or of major osteoporotic fracture. A major osteoporotic fracture is defined as the clinical spine, hip, forearm or humerus fracture. The tool has been validated in independent cohorts.[241,254] QFracture is another fracture risk assessment tool based on data routinely collected in general practice, estimating 1- to 10-year cumulative incidence of hip and/or major osteoporotic fracture (see recent NOGG guidelines (URL attached in appendices) for discussion of FRAX vs QFracture). In this chapter I have not evaluated this tool.[255]

Inputs into FRAX include: age, sex, BMD, BMI, previous fragility fracture (including morphometric vertebral fracture), parental history of hip fracture, current glucocorticoid treatment (any dose by month for 3 months or more), current smoking, alcohol intake of 3 or more units daily, rheumatoid arthritis, secondary causes of osteoporosis (type I diabetes, long-standing untreated hyperthyroidism, untreated hypogonadism/premature menopause (<45 years), chronic malnutrition/malabsorption, chronic liver disease, chronic renal failure (CKD 3a-5). Femoral neck BMD can be included. There are limitations to the use of FRAX. It does not take into account, previous osteoporosis treatment or provide a weighting for the number of previous fractures. The accuracy of FRAX is reduced in patients under 40 years old. It also doesn't consider the dose response relationship of fracture risk to steroid burden. The UK National Osteoporosis Guidelines Group (NOGG) guidelines address this in part.[239] This guideline, accredited by NICE, sets out guidelines for the assessment and management of osteoporosis and prevention of fragility fractures in postmenopausal women and men aged 50 and over. FRAX adjustments at doses over 7.5mg Prednisolone daily are suggested as follows: major osteoporotic fracture risk increase by 15% and hip fracture risk increase by 20%.[256]

FRAX outputs include a 10-year probability of major osteoporotic fracture (MOF). FRAX without including BMD has a similar performance as BMD without FRAX with regard to prediction of fragility fracture and can be used to help identify patients who will respond to pharmacological interventions.[257]

10 -year probability of MOF (%) is broken down in risk categories which factor in age (see table 6.2). Within the intermediate risk category is an intervention threshold above which treatment should be considered.

Category	Action
Low risk	Lifestyle advice
Intermediate risk	Assess with BMD. If then below intervention threshold, treat as low risk. If above intervention threshold but can't calculate BMD (frailty) consider treatment.
High risk	Measure BMD, consider treatment
Very high risk	Treat and consider specialist referral

Table 6.2: FRAX assessment thresholds for 10-year probability of MOF

When BMD is included in FRAX using the web-based algorithm (www.sheffield.ac.uk/FRAX), 10-year probability of MOF or hip fracture (%) is categorised as low, high or very high risk. Consideration of other clinical risk factors (frequent falls, very low spine BMD) can reassign patients from high risk to very high risk of fracture.

Vertebral fracture assessment is also important as part of fracture risk assessment. They are not always clinically apparent and therefore not diagnosed.[258] In addition, vertebral fractures are a

strong risk factor for subsequent fracture at the spine and other skeletal sites.[259] Vertebral fracture assessment (VFA) is therefore recommended in high-risk patients.

6.1.3 Glucocorticoid induced osteoporosis mechanism:

Glucocorticoids act via the cytosolic or membrane-bound glucocorticoid receptor in a dose-dependent manner.[260] Glucocorticoids have direct and indirect effects on bone remodelling (figure 6.1). Glucocorticoid excess results in decreased muscle mass and mechanosensing, having a direct effect on osteocytes. Resultant decrease in sex steroids and reduced calcium absorption along with increased parathyroid hormone stimulate receptor activator of nuclear factor-k (RANKL) and decreased osteoprotegerin (osteoclastogenesis inhibitory factor). As a result, bone resorption occurs by osteoclasts. Decreased osteoblastogenesis results in reduced synthetic ability and bone formation. Osteocyte and osteoblast apoptosis prevent effective mechanosensing and new bone formation.

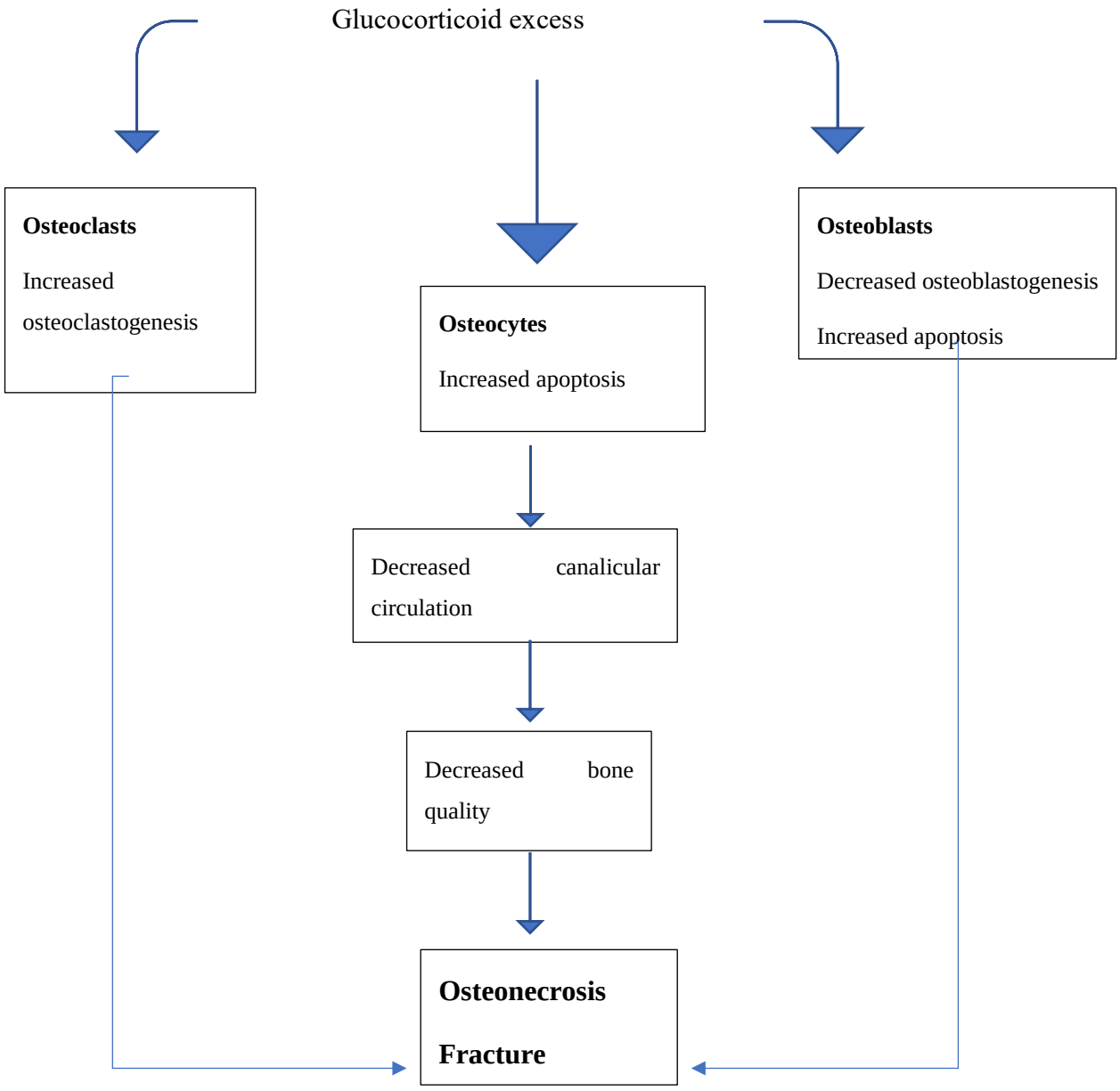


Figure 6.1: Mechanisms of Glucocorticoid-Induced Bone Loss

Vertebral fractures are most commonly associated with glucocorticoid use. The risk increases within 3 months and peaks at 12 months of glucocorticoids treatment.[172,261] Even low doses of Prednisolone (≤5mg) are associated with increased fracture risk in AIH patients.[262] Van den Brand *et al.* suggest Budesonide might be implicated in increasing fracture risk in a dose dependent manner. Correcting for Prednisolone use in the year prior to fracture, Budesonide

use was associated with an increased odds ratio in fracture. However, they state it is difficult to correct for previous Prednisolone use.[63] On the other hand, in a small study of 10 patients, there was no significant change in BMD in Budesonide treated patients however, only 3 of the cohort were postmenopausal.[51] In another study a possible role for Budesonide in patients at risk of osteoporosis was shown. Fifteen patients who had osteopenia at index bone density scan had repeat scans after a median 2 years, with bone density improved in 6 patients, stable in 8 and worse in only one patient.[53]

In a retrospective cohort study using data from the General Practice Research Database (GPRD) relative risk of vertebral fractures increased in a dose dependent manner but even at 2.5mg daily the relative risk was 1.55 (1.20-2.01). They demonstrated that fracture risk declined towards baseline after stopping corticosteroids.[171] This finding was confirmed in a large retrospective study where major osteoporotic fracture risk was not increased in patients with intermittent or past use of glucocorticoids.[263] Fracture risk related to glucocorticoid use is especially associated with high daily dose (>7.5mg/day Prednisolone), cumulative glucocorticoid >5g, current or recent (<3 months) use of glucocorticoid, glucocorticoid-associated myopathy increasing fall risk and glucocorticoid-induced hypogonadism.[171,172,261]

6.1.4 Fracture risk in autoimmune hepatitis

Osteoporosis is a severe extrahepatic complication of autoimmune liver diseases (PBC[264,265], PSC[266] and AIH[63,267]). The prevalence of osteoporosis in patients with cirrhosis is between 12-55%.[268-276] Schmidt *et al.* identified an osteoporosis prevalence of 19.2% in patients over 50 years in a cohort of 211 AIH patients.[267] This author describes limitations in BMD assessment as it does not allow for differentiation between poor bone mineralisation (i.e. osteomalacia)

and loss of bone mass and/or bone microarchitecture. Using High-resolution peripheral quantitative computed tomography (HR-pQCT) they found that AIH patients had primarily cortical bone loss at the distal radius and tibia compared with health controls. Total BMD was also significantly lower in AIH patients, at the distal radius compared to controls. Z scores were low suggesting lower than normal BMD in AIH patients. Cortical BMD was significantly lower at the distal radius but not tibia. Interestingly, Fibroscan values, cumulative Prednisolone dose and serum levels of ALT and IgG were not associated with bone microarchitecture. Cortical thickness was associated with age but not disease stage or severity.[277] A reduction in cortical bone thickness is also seen in other inflammatory diseases such as rheumatoid arthritis[278] This association could be related to increased inflammatory cytokines. A reduction in cortical bone mass has been linked to T helper 17 cell frequency in PSC.[266]

Fracture risk in AIH patients has been investigated by a Dutch group.[63] They reviewed the Dutch AIH Study Group registry data to find patients with incident fractures. 102 patients (15% patients) had a fracture. They found that Prednisolone and Budesonide use were predictive of one or more fractures, however in a sub-analysis, Prednisolone but not Budesonide was associated with nonvertebral fractures. They demonstrated a dose response relationship with every additional milligram of Prednisolone resulting in an Odds ratio of 1.05 for bone fracture (1.01-1.1.10 (95% CI) p=0.01) and Budesonide resulting in an Odds ratio of 1.14 (1.03-1.27 (95% CI) p=0.02).

6.1.5 Prevention and treatment of osteoporosis

Lifestyle measures should be encouraged to prevent bone loss. Weight-bearing exercise can help control weight and reduce immobility as a cause of bone loss.[279] Smoking cessation, limitation in alcohol consumption and the assessment and management of falls risk should also be considered.

Vitamin D is essential for calcium absorption and bone mineralisation. Deficiency is common in AIH patients. Levels ≤29ng/ml occur in 68-81%[280,281] AIH patients and severe deficiency (<20ng/ml) in 20%. However, variation in results may occur depending on the time of year the samples are taken. Vitamin D levels should be checked at diagnosis. Glucocorticoids also increase urinary calcium excretion. Calcium (1000-1200mg daily) and vitamin D (at least 400-800 IU daily) are recommended for all patients receiving glucocorticoids.[279,282] Significant improvements in BMD are seen in patients treated with calcium and vitamin D, even in patients treated with low-dose Prednisolone (5mg OD).[283,284] However they do not completely prevent bone loss in patients receiving high doses of steroid.[285]

There is extensive data from randomised controlled trials support a role for bisphosphonates increasing bone mineral density in glucocorticoid treated patients.[286-288] Data supports a role for bisphosphonates in the management of osteoporosis in liver disease patients. In an RCT comparing monthly ibandronate and weekly alendronate, increases in BMD were seen and only one patient with alendronate developed a new vertebral fracture. [289-291] A systemic review of patients in the post-transplant setting demonstrated a role for bisphosphonates in improving lumbar spine and hip bone mineral density and reducing fracture incidence. Total fracture incidence was 6.6.% (CI: 3.4-12.4%) in bisphosphonate treated patients compared to 19.1% (CI: 14.3-25.1%) in patients receiving calcium and vitamin D.[292]

National and International guidelines recognise the importance of proactive management to prevent fractures in AIH patients. National AIH guidelines recommend a DEXA scan at the start of steroid treatment and repeating at 1-2 yearly intervals whilst on Prednisolone treatment. Patients should receive calcium and vitamin D.[37] Recent American guidelines recommend a baseline DEXA in patients with risk factors for osteoporosis and every 2-3 years if risk factors persist.[48,279,293] The NOGG guidelines recommend that patients treated with high dose glucocorticoids (≥7.5mg/day prednisolone or equivalent over 3 months) should be referred urgently for BMD assessment as there is a risk of rapid bone loss on starting treatment. Oral bisphosphonates should be started if a delay in assessment is anticipated.[239]

6.2 Patients and Methods

6.2.1 Study population and treatments

I carried out a retrospective audit of bone health in steroid-treated AIH patients, to assess:

a. Bone mineral density and whether it changes with time
b. Rate and factors associated with new fragility fractures
c. Utility of fracture risk (FRAX) scores and bone mineral density (BMD) in predicting actual fracture risk

I included 232 AIH patients meeting IAIHG 1999 criteria presenting between 1971-2016 who had all undergone at least one DEXA scan at Sheffield Teaching Hospitals since 1994. Patients who were investigated elsewhere were not included as important clinical information at the time of scanning would not be available. See table 5.1 for demographic, clinical and treatment

characteristics. First DEXA scan was performed 4(-13-406) months (median(range)) after diagnosis.

6.2.2 DEXA scan and FRAX calculation

Patients attended the metabolic bone clinic at Sheffield Teaching Hospitals and underwent a clinical assessment in addition to having a DEXA scan. The DEXA scan determines the bone mineral density (BMD). The BMD is compared to healthy young adults (T score) and age-matched controls (Z score). As part of their assessment from 2007 patients completed a questionnaire providing important clinical details including fracture history, date of menarche, menopause status, calcium intake and medication (see S 9.3).

I calculated the FRAX score at the time of index DEXA scan using the web-based algorithm (www.sheffield.ac.uk/FRAX**).** I did not adjust the FRAX score for steroid dosage as this is not included on the web-based algorithm. All patients had chronic liver disease which is a risk factor for secondary osteoporosis and this box was selected in all post-diagnosis patients on the online FRAX calculator. Most patients had height and weight measured at time of first DEXA scan. Where this information was not available, height and weight information was gathered from the notes. A small number of patients had a DEXA scan within the year before diagnosis of AIH and it was therefore not repeated at diagnosis. The FRAX score was calculated based on the data at the time of DEXA rather than the later AIH diagnosis.

Fracture data was collected from (a) patient questionnaire and vertebral fracture assessment at time of DEXA scans (b) medical notes and (c) IMPAX (digital radiology imaging system). Fractures were categorised into fragility and non-fragility fractures.

6.2.3 Statistical analysis

Statistical analysis was performed using SPSS for windows version 25 (SPSS Inc, Chicago, IL). Categorical data was summarised as frequencies and percentages and continuous data as medians and ranges. We identified variables with significant associations with BMD and fractures using Cox regression analysis. Variables significantly associated on univariate analysis were assessed further by backward stepwise Cox multiple regression analysis. Fracture free survival was calculated by life table analysis (Kaplan-Meier) and sub-groups comparisons analysed using the log-rank test.

6.2.4 Ethics

The study of Osteoporosis in Autoimmune Hepatitis was approved by the Sheffield Research Ethics Committee, reference number 014033.

6.3 Results

6.3.1 Baseline characteristics

Characteristic	Number
Patients presenting 1971-2016	232
Sex n (Female/male) %	190/42 (82/18)
Age at diagnosis (median(range))	56(2-94)
ALT U/L (median(range))	492 (42-2214)
AST U/L (median(range))	419 (39-1825)
Bilirubin (median(range))	31 (4-507)
Albumin gm/L (median(range))	36 (17-49)
Globulin (gm/L) (median(range))	44 (22-167)
PBC or PSC overlap at diagnosis (%)	12 (5)
Definite/probable AIH	149/81
AIH score (median(range))	17 (8-26)
Cirrhosis (n (%))	22 (10%)
Decompensation (n (%))	57 (25%)
AIH Treatment at time of first DEXA scan: Prednisolone n (%) Prednisolone cumulative dose (mg) (median/range)) Azathioprine (n) Prednisolone and Azathioprine (n) Budesonide Second- and third-line agents: Mycophenolate mofetil (MMF) Tacrolimus	 196 (89%) 2100 (0-166065) 142 124 5 12 2

Table 6.3: Baseline characteristics; demographic, clinical and treatment details

Number of DEXA scans (median(range))	2 (1-9)
Bone protection treatment (during follow-up): Calcium and vitamin D (n (%)) Bisphosphonates (n (%))	 206 (89%) 128 (56%)
Follow-up (years)	9(0.4-36)

Table 6.4: Follow-up data and bone health therapy.

6.3.2 Trends in DEXA results

	DEXA 1	**DEXA 2**	**DEXA 3**	**DEXA 4**	**DEXA 5**
N	232	164	105	60	24
Years post Dx (median(range))	0.8 (-1.1-33.8)	3.0 (0-37.0)	7.0 (1.4-39.2)	10.5 (4.2-41.3)	13.0 (7.3-43.4)
Age	62	63	64.5	69	68
Hip					
BMD	0.87+0.15	0.86+0.16	0.83+0.16	0.79+0.17	0.79+0.15
T score	-0.7+1.15	-0.85+1.15	01.01+1.16	-1.33+1.23	-1.30+1.12
Z score	0.10+1.21	0.15+1.08	0.02+1.06	-0.04+1.13	0.32+0.86
Osteopenia	41 (18%)	43 (26%)	47 (45%)	38 (63%)	10 (42%)
Osteoporosis	8 (3%)	9 (5%)	12 (11%)	9 (15%)	4 (4%)
Spine					
BMD	0.93+0.17	0.92+0.17	0.92+0.15	0.92+0.16	0.93+0.17
T score	-1.14+1.5	-1.12+1.33	-1.13+1.16	-1.15+1.44	-1.06+1.52
Z score	--.1.1+1.53	0.29+1.67	0.18+1.58	0.55+1.69	0.83+1.57
Osteopenia	81 (35%)	54 (33%)	31 (30%)	19 (32%)	7 (29%)
Osteoporosis	46 (20%)	24 (15%)	20 (19%)	11 (18%)	4 (17%)

Table 6.5: Hip and lumbar BMD values on first and subsequent DEXA scans

Overall, 232 patients had 2 (1-9) (median(range)) DEXA scans during 9 (0.4-36) years (median(range)) follow-up (table 6.3, table 6.4).

Results of first and of subsequent DEXA scans are shown in table 6.5. Based on hip T scores, on the first scan 8 (3%) of patients, had osteoporosis and 41 (18%) had osteopenia. However, Z score was close to zero, suggesting that, overall, these patients with AIH had “normal” bone mineral density for age.

On subsequent DEXA scanning (table 6.3), hip BMD and T-score showed slight progressive overall falls, however Z score rose slightly. There was a significant fall in hip BMD between DEXA 1 and 5 ($p = 0.02$) and decrease in spine BMD between DEXA 1 and 5 approaching significance ($p=0.057$). The percentage change in BMD score between the first and second DEXA scans showed no correlation with interval or cumulative Prednisolone dose between the scans.

Spinal BMD and T score remained stable on repeated DEXA scanning and Z score also rose slightly.

	Hip BMD		Spine BMD	
Parameter (no. of patients)	Univariate P value	Multivariate P value (HR(CI))	Univariate P Value	Multivariate P Value (HR(CI))
Excluding Major Osteoporosis Score (MOP) score				
Sex (240)	<0.001	0.001(1.24(0.65-1.95))	Ns.	Ns.
Age at scan (233)	<0.001	0.001 (0.998(0.997-1.000)	0.023	Ns.
Body Mass Index	<0.001	0.001 (1.01(1.00-1.01))	<0.001	<0.001 (1.00(1.00-1.01))
Fibrosis score (diagnostic biopsy)	<0.001	0.016 (0.98(0.97-1.00)	<0.001	<0.001 (0.97(0.96-0.99))
Cumulative pred. dose before DEXA	0.059	Ns.	NS.	Ns.
Including Major Osteoporosis (MOP) score				
Sex	<0.001	<0.001 (1.08(1.02-1.14))	NS.	Ns.
MOP score (229)	<0.001	<0.001 (0.993(0.0991-0.995))	<0.001	<0.001 (0.996(0.994-0.998))
Age at scan	<0.001	NS.	0.023	NS.
Body Mass Index	<0.001	0.001(1.01(1.00-1.01))	<0.001	0.001 (1.01(1.00-1.01))
Fibrosis score	<0.001	0.012 (0.98(0.97-1.00))	<0.001	0.004 (0.970.97-1.00))
Cumulative pred. dose before DEXA	0.059	Ns.	NS.	Ns.

Table 6.6: Parameters associated with hip bone mineral density on first post-diagnosis DEXA scan

Factors negatively associated with lower BMD on univariate regression analysis (table 6.6) were female sex, lower BMI, Ishak fibrosis score on diagnostic liver biopsy and also time of, age at and cumulative Prednisolone dose (almost reached significance) up to first DEXA scan.

On multivariate analysis, all these factors apart from cumulative Prednisolone dose showed independent associations with hip BMD.

When the Major Osteoporosis (MOP) Score (derived from multiple osteoporosis risk factors) was included, it showed a strong association with hip BMD, which persisted in multivariate analysis, along with BMI, fibrosis score, and time from diagnosis to DEXA but no longer, with age or with cumulative Prednisolone dose.

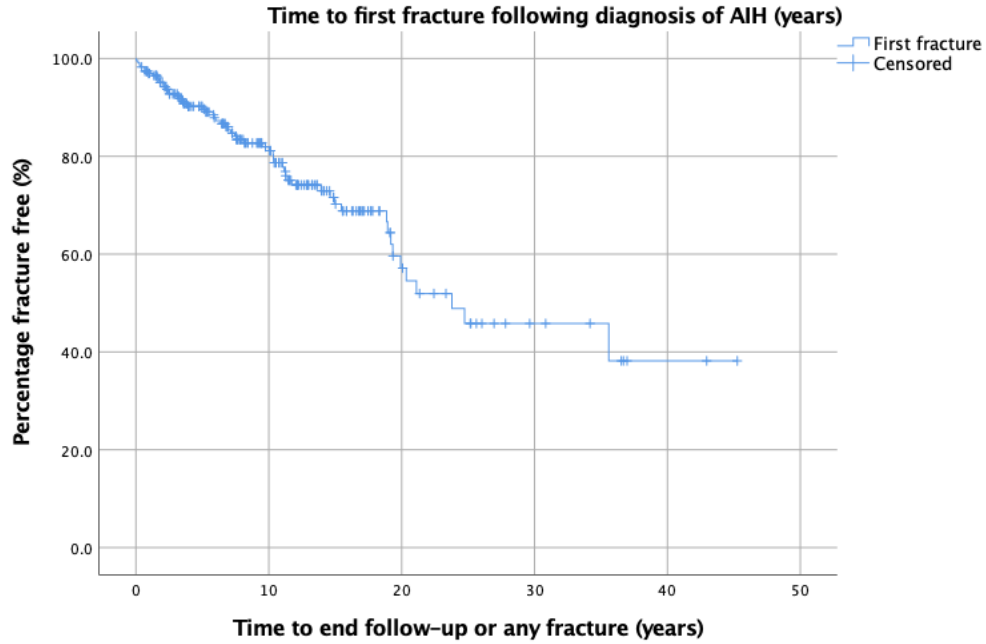
6.3.3 New fractures in AIH patients

Characteristic	First fracture	All fractures
Vertebral	**26**	**52**
Wrist	**7**	**10**
Femur	**6**	**9**
Humerus	**2**	**5**
Tibia/fibula	**5**	**5**
Finger/toe	**5**	**5**
Sternum	**3**	**3**
Foot	**2**	**2**
Pelvis	**1**	**2**
Patella	**1**	**1**

Table 6.7: Fracture numbers after diagnosis of AIH

Following diagnosis of AIH, 94 fractures occurred in 58 patients. First fracture was diagnosed 7((0-36) years (median(range)) after AIH diagnosis at an age of 72(28-104) years (median(range)). In 54 of the 59 patients, post-diagnosis fractures occurred over the age of 50. Details of fractures are given in table 6.7. 56 fragility fractures occurred in 48 patients.

With analysis confined to the 48 first fragility fractures, there were significant independent associations with hip BMD and with cumulative Prednisolone dose (to first DEXA scan) but no longer, with major osteoporosis score or with alcohol excess. Hip Z score, sex, spinal BMD, fracture pre-AIH diagnosis, and fibrosis score in initial biopsy showed no associations with fracture rate.



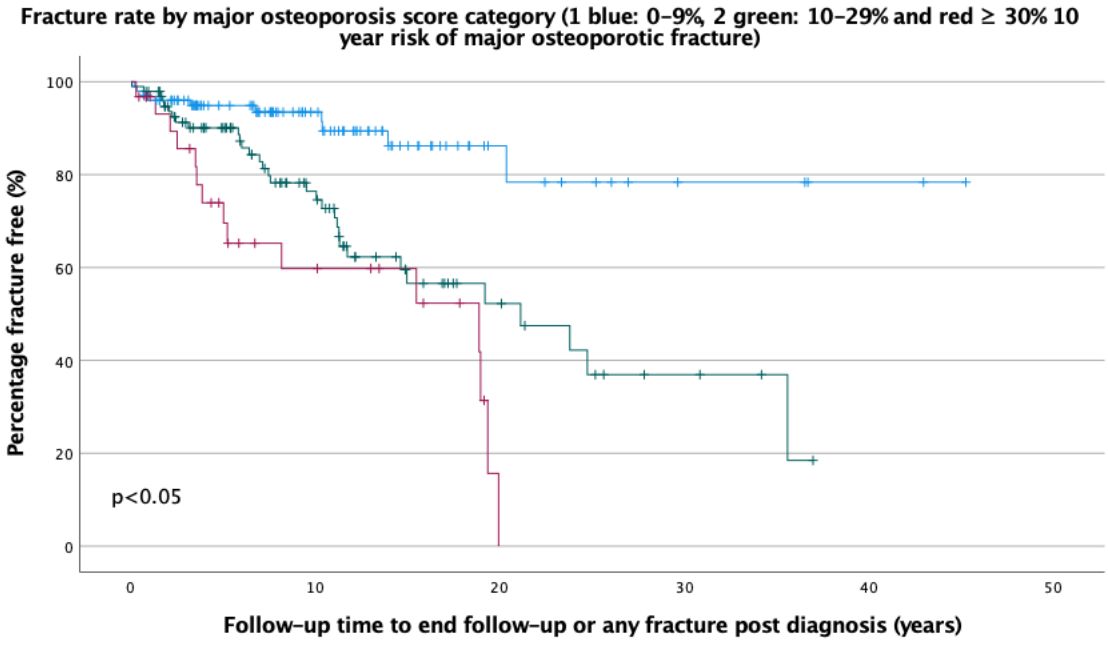
Patients at risk (n):

First fracture	232	190	132	106	88

Figure 6.2: Overall first fracture rate following diagnosis of AIH (any fracture)

By life-table analysis, the first fragility fracture rate was 18% and 36% 10 and 20 years respectively after AIH diagnosis (figure 6.1).

6.3.4 Factors associated with fracture rate

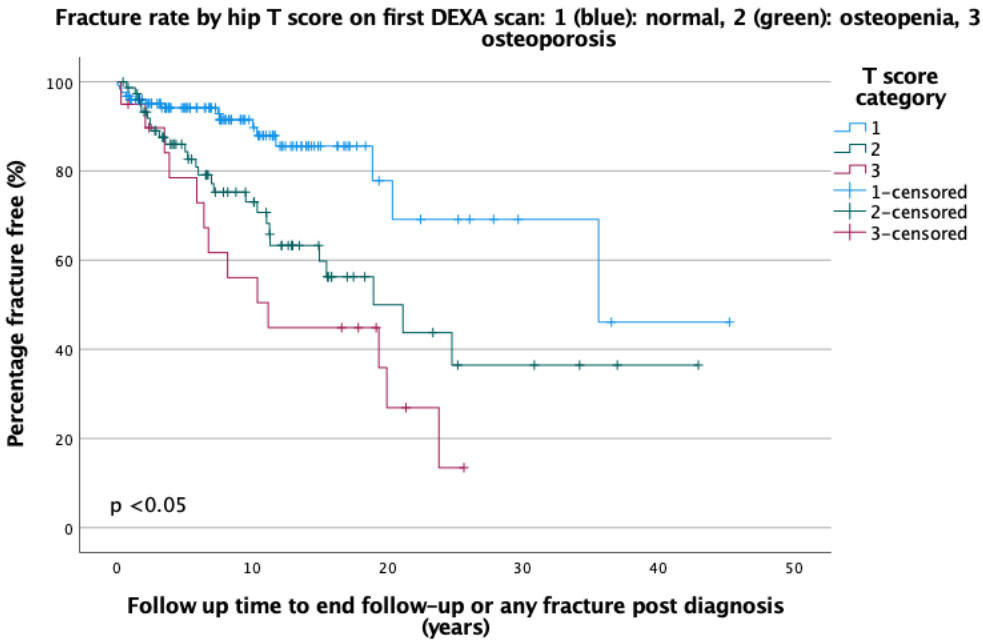


Patients at risk (n):

MOP 1	100	93	86	78	78
MOP 2	96	70	50	35	
MOP 3	30	17	0		

Figure 6.3: Fracture rate by FRAX Major Osteoporosis Score category

There was a significant association between the FRAX output, major osteoporosis score and fracture free survival. Patients with a major osteoporosis score of 0-9% had a 92% fracture free survival at 10 years compared to 60% in patients with a MOP score of over 30% **(figure 6.3)**.



Patients at risk (n):

Cat. 1	125	112	97	86	57
Cat. 2	76	70	38	27	27
Cat. 3	19	10	5		

Figure 6.4: Fracture rate by Hip T score on first DEXA scan

Fracture-free survival was significantly lower (56%) in patients with osteoporosis (green line figure 6.4) compared to patients with a normal hip T score (92%) (blue line).

	Any Fracture		Fragility Fracture	
Parameter (no. of patients)	Univariate P value	Multivariate P value (HR(CI))	Univariate P Value	Multivariate P Value (HR(CI))
Excluding MOP score				
Age at presentation (n= 233)	<0.001	<0.001 (1.06(1.04-1.08))	<0.001	<0.001(0.13(1.03-1.08))
Hip BMD on first DEXA	<0.001	0.02 (0.13(0.02-0.87))	<0.001	0.02(0.09(0.01-0.70))
Cumulative Prednisolone dose before DEXA	0.02	Ns.	0.08	Ns.
Spine BMD DEXA 1	Ns.	Ns.	0.02	Ns.
Including MOP score				
Age	<0.001	<0.001 (1.06(1.04-1.08))	<0.001	<0.001 (1.06(1.03-1.82))
MOP score (n=229)	<0.001	Ns.	<0.001	Ns.
Hip BMD on first DEXA	<0.001	0.04 (0.13 (0.02-0.87))	<0.001	0.02 (0.09(0.01-0.70))
Spine BMD DEXA 1	Ns.	Ns.	0.02	Ns.
Cumulative Prednisolone dose before DEXA	0.02	Ns.	0.07	Ns.

Table 6.8: Parameters associated with fracture risk following a diagnosis of AIH

In univariate Cox regression analysis, the risk of any fracture following diagnosis of AIH showed significant associations with older age, major osteoporosis score, first hip BMD and cumulative Prednisolone dose before DEXA 1 (table 6.8).

In multivariate analysis, only age and hip BMD were independently associated with fracture (Table 6.8).

6.4 Conclusions

In this steroid-treated AIH cohort, whose bone health was proactively managed with calcium and vitamin D supplements (89%) or bisphosphonates (56%) during follow-up, BMD remained similar to an age/gender-matched population (Z score 0.1) (table 6.3). However, BMD fell on consecutive DEXA scans as would be expected with older age. However, in the Schmidt et *al.* AIH study, the Z score was reduced.[277] This could be explained by the time lag in first DEXA from diagnosis in the German study. Like the Schmidt paper, we found that older age, lower BMI, Prednisolone (cumulative Prednisolone reported by here rather than duration) and fibrosis stage (reported here on histology rather than transient elastography) were significantly associated with lower BMD. Age, BMI and Prednisolone use are all factors which are considered when calculating the FRAX score.

Total fracture rate is higher than comparable data in the general population. Age and gender specific fracture incidence were estimated in a large epidemiological study in England and Wales using records from the General Practice Research Database. The 10-year risk of any fracture ranged from 9.8-21.7% in women aged 50-80 respectively and 7.1-8.0% in men aged 50-80 respectively. In a large Canadian Multicentre osteoporosis study, including 4322 women and 1732 men, incident fractures were seen in 14% (930) women and 9% (247) men at 10 years.[294]

All cause and fragility fracture risk was predicted by age and hip bone mineral density. Cumulative Prednisolone dose was significantly associated with all cause fractures and approached significance with fragility fractures (table 6.6).

This is the first time FRAX has been evaluated in a large cohort of AIH patients, who are steroid treated. A higher major osteoporosis score was significantly associated with lower hip and spine BMD and fracture risk. However, on multivariate analysis, only hip BMD was a significant predictor of fracture risk not the FRAX derived major osteoporosis score. One of the benefits of the FRAX calculator is that patients can be risk assessed by the clinician without the need for a DEXA scan. This enables bisphosphonate treatment to be started earlier, reducing the risk of incident fractures.

The strengths of this study include the long-term follow-up of patients (up to 36 years) with some patients having five DEXA scans available for analysis. Data on Prednisolone dose at time of DEXA and cumulative dose pre-DEXA was also available for many patients. In Sheffield, we also have links and management to a dedicated metabolic bone clinic.

7 Long-term Outcome of Autoimmune Hepatitis: Consecutive Patient Cohort and Data on the Second Twenty Years

The contents of this chapter have been published and adapted for this thesis.[295]

7.1 Introduction

Long-term outcome of AIH is incompletely characterised. De-novo cirrhosis develops, despite treatment in 14(6-40)% of patients.[296] Reported 10- and 20 year all-cause death/transplant rates are respectively 14(9-36)% and (32(18-53)% (all-cause) and 9(0-25)% and16(6-26)% (liver-related). [3,296] The reasons for the wide variation in these rates are unclear. There are virtually no published data beyond 20 years. Thus, it remains unclear whether AIH is a life-long disease or whether it "burns out".

Parameters associated with reduced survival in AIH include cirrhosis and decompensation at presentation[15,36,38,121,128,129,297] and older age (all-cause, not liver-related).[9,11] Reduced survival is also associated with failure to normalise serum transaminases [36,121,129,297] and with higher relapse rate.[36,123,129]

In our previous report of outcome in patients with AIH presenting to our (non-transplant) centre between 1971-2007[36], recruitment was retrospective and case-capture was complete only after 1/1/1987. In nearly all other studies recruitment has also been retrospective and so case capture may also have been incomplete.[125,298] Some studies explicitly exclude patients, with

decompensated cirrhosis,[124] ALF[123] and with follow-up <12 months.[69] In others, follow-up was not from the time of initial presentation[129] and/or was only 60-70% complete.[38,122]

Here, we report (a) long-term outcomes in all 330 patients presenting consecutively to our unit from 1987 to 2016 (b) novel data in an overlapping cohort of 65 patients already followed up for 20 years (c) the consequences of treatment withdrawal in 25 patients.

7.2 Patients and Methods

Our initial report of patients with AIH presenting to our Unit between 1971-2007 included all patients presenting between 1/1/87 and 31/12/2007.[36] We have continued to collect all cases prospectively since. In the present report, for the first analysis, we included all 330 patients presenting consecutively from 1/1/1987 to 31/12/2016. 24 patients were untreated, mostly due to spontaneous resolution of transaminases or normal transaminases at diagnosis (19 patients). Other reasons include mild fibrosis, liver transplant, comorbidity/unwell at presentation, drug-induced autoimmune hepatitis and patient choice. For analyses of the second 20 years of follow up and of consequences of immunosuppression withdrawal we also included patients presenting between 1971 and 1987 (prior to the period of complete case capture). All patients had probable or definite AIH, by the 1999 International AIH Group (IAIHG) revised criteria.[4]

Standard initial treatment regime was Prednisolone, usually with Azathioprine as recommended by UK [37], EASL[5] and AASLD guidelines.[42] Only one patient received Budesonide as initial treatment. After 2-3 years Prednisolone, patients with normal serum transaminases were offered a repeat biopsy (if deemed safe). If patients were found to be in

histological remission, Prednisolone was phased out. However, most patients remained on Azathioprine monotherapy long-term.

Biochemical remission was defined as serum ALT normalisation. Serum IgG was not routinely monitored until 2013 and insufficient data were available for analysis. Relapse (defined as ALT rising to > 3 times upper limit of normal) was treated by reintroduction (or increasing dose) of Prednisolone.

In patients not achieving biochemical or histological remission after 2 years of treatment the strategy until 2014 was to continue the Prednisolone and to double the Azathioprine dose to 1 to 2 mg/kg/day. Since 2014, Tacrolimus[299-302] has been used in such patients. Since 2000 patients unable to tolerate Azathioprine were switched to Mycophenolate Mofetil (23 after < 6 months Azathioprine). 26 patients discontinued immunosuppression treatment (IST). Outcome was compared to patients who remained on IST at the end of follow-up (267).

Data collection was up until the end of December 2016 for patients alive without a liver transplant (n=330). Where cause of death was uncertain from the clinical details, death certificates were obtained. Liver transplantation was also considered as a liver death. Of 56 patients who were discharged to their GP or moved out of area, living/deceased status was determined on 31/12/16. Of these, 14 patients had died and death certificates were obtained to clarify cause of death. 100 patients were censored at death and 8 at transplantation.

7.3 Statistical Analyses

Statistical analysis was performed using SPSS for windows version 25 (SPSS Inc, Chicago, IL). Categorical data was summarised as frequencies and percentages and continuous data as medians and ranges. Survival was calculated by life table analysis (Kaplan-Meier) and compared between subgroups using the log-rank test. First, we identified the baseline variables which showed associations with all-cause and liver-related death or transplantation using Cox regression analysis.

Three response parameters were assessed separately, along with the above baseline parameters: failure to normalise serum ALT after 6- and 12-months (despite being followed for at least those times) and relapse rate per decade of follow up.

7.4 Ethics approval statement.

The study was part of a retrospective audit (registered in 2006) of long-term management and outcome of patients with AIH attending the Sheffield Teaching Hospitals Liver Unit. The project is also registered with the Sheffield Research Ethics Committee, reference number 014036.

7.5 Results:

(a) Overall outcome

Between 1/1/1987 and 31/12/16, 330 patients were diagnosed with AIH and followed for 8.5(0-29) years (median(range)). Table 7.1 shows their characteristics and initial treatment. Figure 7.1 shows overall outcome.

Characteristic	Number
Baseline	
Patients presenting 1987-2016	330
Sex (female/male)	265/65
Caucasian (n (%))	306 (93)
Age (median(range))	58 (3-87)
AIH diagnostic score (median(range))	16 (8-26)
Definite/probable AIH (n (%))	205/125 (62/38)
ALT U/L (median(range))	401.5 (19-2000)
AST U/L (median(range))	374 (8-2554)
Albumin grm/L (median(range))	35 (17-49)
Globulin grm/L (median(range))	42 (21-110)
IgG grm/L (median(range)(n=225))	24 (2.3-65.9)
PBC or PSC variant (n (%))	24 (7)
Cirrhosis (n (%))	85 (26)
Decompensation (n (%))	48 (15)
End of follow-up	
Treatment*:	
Prednisolone n (%)	289(88)
Dose(med(range))	30(2.5-60)
Prednisolone and Azathioprine	287 (87) †
Second and third-line agents (ever):	
Mycophenolate mofetil (MMF)	54
6-Mercaptopurine	3
Tacrolimus	21
Ciclosporin	5
Infliximab	1
Rituximab	1
ALT normalisation at 6/12 (n (%))	262 (86%)‡
ALT normalisation at 12/12 (n (%))	279 (91%)§
At least one relapse per decade (n (%))	65 (20%)

Table 7.1: Characteristics of 330 patients presenting between 1987-2016

De novo cirrhosis (n (%))		33 (11)
	Histology	17
	Imaging	2
	Fibroscan	2
	Endoscopic finding of varices	9
	Diagnosis of hepatocellular carcinoma	2
	Cytopenia	1
Follow-up time (years(median(range)))		8.5 (0-28.8)

* One patient treated with Budesonide and Azathioprine' † where treatment details could be clarified, ‡ proportion out of 306 patients, § proportion out of 305 patients

Table 7.1: Characteristics of 330 patients presenting between 1987-2016 continued

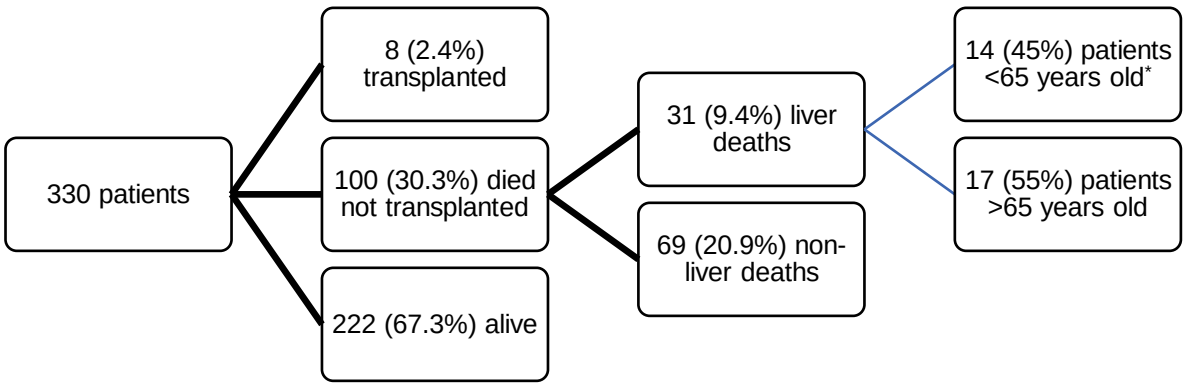
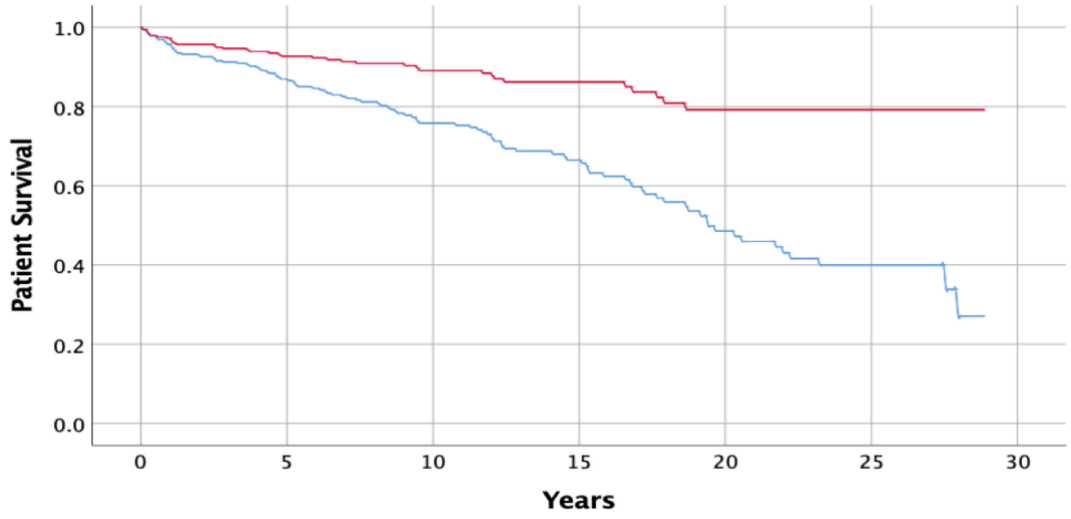


Figure 7.1: Flow-chart of overall outcome of patients presenting since 1987

Figure 7.2 shows overall all-cause and liver-related death/transplant rates were as follows: 24%(all-cause) and 11%(liver) after 10 years and 51% and 21% respectively after 20 years. In contrast to our previous report, both curves are approximately linear. The liver death curve is quite static from 15 years onwards. I suspect this is because is patients are more likely to have a non-liver related event as they get older and liver-related complications related to an AIH diagnosis are seen earlier. However, there is a steeper down-slope over the first year of follow-

up, during which there were sixteen death/transplant events (11 liver-related), and the death or transplant rate was 1.75- fold higher (5.0% versus 2.8%) than the overall annual rate over the first 10 years.



Time (years)	0	5	10	15	20	25	30
Patients at risk:							
Liver death	330	306	293	283	260	260	0
All cause	330	287	250	217	161	132	0

Figure 7.2: All-cause (bottom line) and liver-related (top line) death or transplantation in patients presenting since 1987

As shown in Table 7.2 death/transplantation showed independent positive associations with the following baseline/ treatment variables: presence of decompensation, lower serum ALT and not receiving Azathioprine (both all-cause and liver-related) and with older age and cirrhosis (all-cause only).

	All-cause death/transplantation		Liver death/transplantation	
Baseline variables				
Parameter (n=311)	**Univariate p value**	**Multivariate p value (HR(CI))**	**Univariate p value**	**Multivariate p value (HR(CI))**
Decompensation	0.00	0.00 (2.56(1.52-4.30))	0.00	0.00 (9.16(4.51-18.63))
Cirrhosis	0.00	0.03 (1.69(1.05-2.73))	0.00	Ns.
Age	0.00	0.00 (1.05(1.03-1.08))	Ns.	Ns
Non-treatment with Azathioprine	0.00	0.001 (0.42(0.25-0.70))	0.01	0.00 (0.29(0.13-0.65))
ALT	0.00	0.00 (0.999(0.999-0.999)	0.015	0.02 (0.999(0.999-0.999))
Ethnicity	Ns.	Ns.	Ns.	Ns.
Gender	0.023	Ns.	Ns.	Ns.
Globulin	Ns.	Ns.	Ns.	Ns.
Bilirubin	Ns.	Ns.	Ns.	Ns.
ALT normalisation at 6 months*	0.03	Ns.	0.04	0.01 (0.29(0.12-0.69))
Relapse rate†	0.00	0.00 (1.27(1.67-1.37))	0.00	0.01 (1.16(0.38-3.54))
ALT normalisation at 12 months‡	0.01	0.00 (0.11(0.05-0.27))	0.00	0.00 (0.13(0.04-0.41))
Relapse rate§	0.00	0.00 (1.32(1.19-1.45))	0.00	0.01 (0.18(1.05-1.32))

Table 7.2: Parameters associated with death/transplantation: baseline plus ALT normalisation within 12 months

* Patients followed up for at least 6 months, other baseline variables considered for multivariate analysis
† Patients followed up for at least 6 months, other baseline variables considered for multivariate analysis
‡ Patients followed up for at least 12 months, other baseline variables considered for multivariate analysis
§ Patients followed up for at least 12 months, other baseline variables considered for multivariate analysis

Death/transplantation was also associated with the response variables: failure to achieve normal serum ALT within 12 months and higher relapse rate per decade (all-cause and liver-related) and with failure to achieve normal ALT within 6 months (all-cause only).

Twenty-three Prednisolone-treated patients with early (within 6 months) intolerance of Azathioprine were switched to Mycophenolate. These patients had (compared with those tolerant of and continuing Azathioprine) similar histological responses on follow up biopsy and 5- and 10-year survival rates which were not significantly different from patients (93 ± 7% (5- and 10-year survival)) who were tolerant of and continued Azathioprine (92 ± 2% and 81 ± 3% respectively) (table 7.3).

	Switched to Mycophenolate due to Azathioprine intolerance (23)	Azathioprine continued/ changed due to unresponsiveness (212)	p
Men (number (%))	3 (13%)	44 (21%)	0.38
Age at diagnosis*	63 (19-80)	56 (2-80)	0.06
Follow-up (years)*	4.0 (1.0-17)	11.3 (0.5-29)	<0.05
Presentation: cirrhosis	4/22 (18%)	53/203 (26%)	0.19
decompensation	2 (9%)	23/209 (11%)	0.74
Serum ALT normal by 6/12	21/22 (95%)	189/201 (94%)	0.18
Serum ALT normal by 1 year	21/22 (95%)	197/201 (98%)	0.44
Biopsy 1: Necro-inflammatory (NI) score*	10 (2-16) n=23	12 (1-18) n=172	0.38
Fibrosis stage*	2 (1-6) n=23	3 (0-6) n=191	0.26
Biopsy 2: NI score*	4 (0-12) n=18	3 (0-12) n=153	0.37
Fibrosis stage*	2.5 (0-5) n=18	3 (0-6) n=152	0.15
% Histological remission on FU biopsy	9/18 (50%)	77/153 (50%)	0.96
All-cause death/transplant: 5year	7±7%	8±2%	ns.
10year	7±7%	19±3%	ns.
Liver death/transplant: 5 year	0%	4±1%%	ns.
10 year	0%	7±2%	

*Median (95% CI) ns. = not significant

Table 7.3: Patient characteristics and outcomes for patients switched to Mycophenolate Mofetil for Azathioprine intolerance

(b) Second 20 years of follow-up

In 65 patients, diagnosed 1971-1996, who had already been followed for at least 20 years, we compared outcomes over the subsequent 6.1(0.3-26) years with those in the 330 patients presenting 1987-2016 and followed from initial diagnosis. During the third/fourth decade of follow-up, five of these 65 patients relapsed and five developed de novo cirrhosis (table 7.4). Relapse rate per decade was not significantly different in patients followed up in the second twenty years, compared to patients followed from diagnosis (0.71 relapses/decade compared with 0.93 relapses/decade $p = 0.23$).

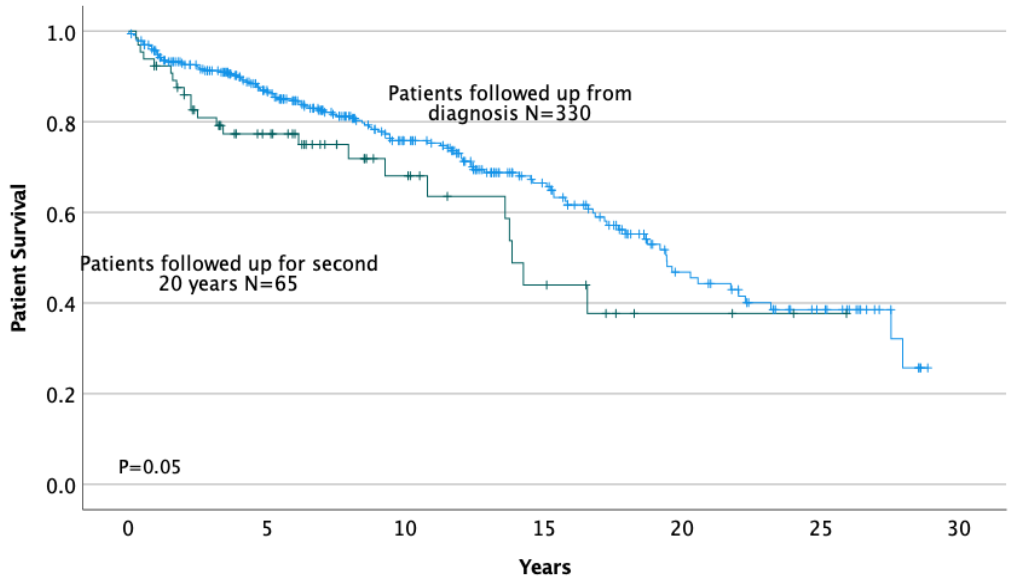
	Already followed up for 20 years: subsequent follow-up	Follow-up from initial diagnosis
Number (% female)	65 (80)	330 (81)
Years when followed-up	1991-2016	1987-2016
Age at start of follow-up ((years)(median(range)))	67 (22-91)	58 (2-87)
Follow-up time ((years)(median(range)))	6.1(0.3-26)	8.5(0-29)
Cirrhosis (n (%))		
At diagnosis	22 (34)	88 (27)
At start of second 20 years	34 (52)	
End of follow-up	39 (60) *	121 (37) †
Relapse rate/decade	0.71	0.93

* (5 (7.7%) patients de novo cirrhosis) †(33 (15%) patients de novo cirrhosis) P=<0.05

Table 7.4: Patients followed up for second 20 years compared with those followed up from initial diagnosis

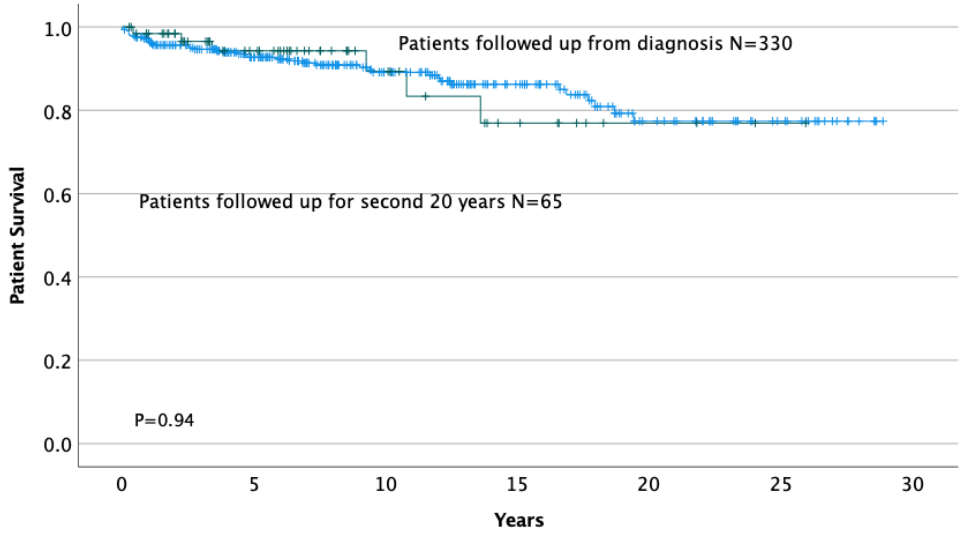
Liver-related death/transplant rates were identical (figure 7.3B), but all-cause death/transplant rate was higher in the cohort followed over the second 20 years (figure 7.3A).

A: All-cause death or transplant



Patients at risk:							
Followed from diagnosis	330	269	210	165	137	124	124
Second twenty years	65	52	35	31	26	24	24

B: Liver death or transplant



Patients at risk:					
Followed from diagnosis	330	250	174	118	76	57
Second twenty years	65	41	22	15	9	

Figure 7.3: Survival curves for patients followed up for the first twenty years compared with second twenty years

However, in multivariate analysis, incorporating predictive factors at the start of the respective follow-up periods, the death/transplant difference disappeared (HR 1.07 (0.65-1.76) p=ns.) and is thus likely to result from the older age in those followed over the third and fourth decades. Therefore, over the third and fourth decades after diagnosis, the course of AIH is similar to that soon after diagnosis.

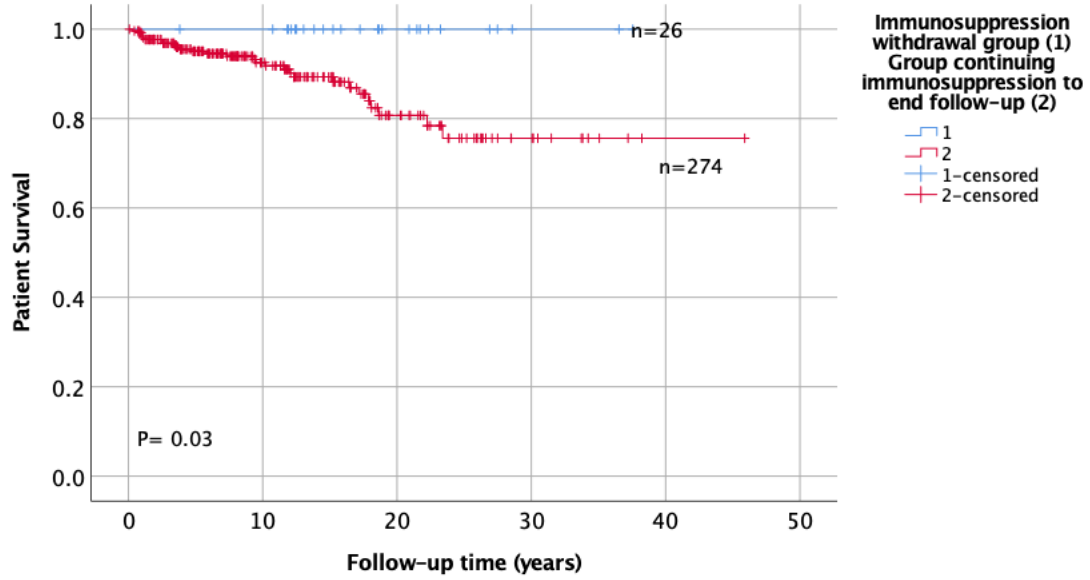
(c) Long-term consequences of immunosuppression withdrawal

Twenty-six patients presenting 1979-2016, discontinued IST. For details see Table 7.5. Six patients relapsed. There were no liver-related deaths in the group of patients who stopped IST over 2.3(0-23.1) years (figure 7.4).

Characteristic (n=26)	**Number**
Female(male)	21(5)
Cirrhosis at presentation	6
Age at diagnosis of AIH ((years)(median(range))	50(6-67)
Ever relapsed before treatment withdrawal (n(%))	9 (35)
One or more relapse per decade before immunosuppression withdrawal (n(%))*	3 (33)
Age at treatment withdrawal ((years)(median(range))	63(17-89)
Duration of treatment before IST withdrawal ((years)(median(range))	10.3(2.1-36.9)
Reasons for immunosuppression withdrawal:	
Side effects	5
Infection	3
Cancer	8
Patient choice	8
Frailty	1
Initial diagnostic doubt	2
Other	2
Relapses off immunosuppression (n(%))†	6(23)

*54 (20%) had over 1 relapse per decade) in 274 patients who continued IST (p=0.3),
†1.29(0.5-9.6) (median (range)) years after stopping IST

Table 7.5: Immunosuppression withdrawal patient characteristics



Patients at risk:	26 274	26 252	26 227	26 202	26 166

Figure 7.4: Survival curves comparing all-cause to liver deaths in patients withdrawing from immunosuppression (n=26) with patients continuing immunosuppression (n=274)

7.6 Discussion

Here we present data from a large unselected AIH patient cohort, with complete patient capture over 30 years.[296] We observed some differences from our previously reported patient cohort, followed from 1971-2007.[36] The increased mortality rate over the second decade is no longer apparent; survival curves are (after the first year) approximately linear. In the main analyses, we excluded patients presenting 1971-1987, who were inevitably selected by survival until 1987, as case capture was incomplete prior to then. Consequently, our current 10-year death/transplant rates are higher than we previously reported.[36]

Our patients received standard treatment for AIH: 86% achieved serum ALT normalisation within 12 months and only 15% developed de-novo cirrhosis. Despite this, our 10-year death/transplant rates are higher than in most single- or multi-centre cohort studies, with median values of (11(2-24)% all-cause and 6(0-17)% liver-related).[7,15,35,36,38,69,118-129] However, our results are similar to those reported in four recent national registry studies from the UK and Scandinavia:[2,3,130,131] 10 year death/transplant rates here were 30(20-32)% (all cause) and 10(9-12)% liver related.[303] Possible causes for these differences include firstly, older age at presentation: 58 years, higher than most other previous cohort studies (48(25-62) years; and secondly, possible case selection in some other cohort studies (as in our own previous study). A consistent feature in the national registry studies (unlikely to be selected) and also seen in our study was an excess mortality rate over the first year of follow-up (1.6-2.4-times the overall 10-year mortality). However, such an excess is apparent in only about half of reported cohort studies. Indeed, in some [69,118,119,123,127,128] no deaths or transplants occurred over the first year. Thus, case selection may have resulted in lower mortality rates in some of these cohort studies. This might be inadvertent (for example, by classification of fulminant AIH as another disease). However, in some studies, patients dying in the first few months were explicitly excluded.[69,123,125,129,134]

We provide further evidence for associations with failure of serum ALT to normalise within 6 months (liver deaths only) and within 12 months and also, with recurrent relapse. Other studies have supported the prognostic value of serum ALT response after 6[121,304] and after 12 months.[125,297] It is currently unclear which time point has better prognostic value. As we did not have sufficient data on serum IgG, we cannot address the prognostic value of complete biochemical response (CBR).

Virtually no data are available in patients with AIH followed into the third and fourth decade. Our data on 65 such patients suggests that de novo cirrhosis still develops and relapses still occur. Thus, outcome was not significantly different in the second twenty years of follow-up, suggesting that AIH is a long-term and probably life-long disease.

Data are also lacking regarding the longer-term outcomes of patients who stop IST. Our results and those of Hartl[133] demonstrate that patients who have been treated for several years, have a relatively low relapse rate. We also report, for the first time, absence of adverse liver-related outcomes after IST withdrawal. These data provide further support for recent recommendations[5,305] that immunosuppressive treatment withdrawal should be considered in many patients with AIH who are in sustained remission.

Our study has the weaknesses inherent in all retrospective single-centre cohorts such as potential difficulty recruituing enough patients to demonstrate statistical significance. However, its strengths are firstly, the reasonably large number of patients, relatively long duration of follow-up (8.5 years), complete data capture, and completeness of follow-up. Secondly, the novel observations on patients followed up into the third and fourth decade of AIH.

In summary, AIH is a life-long disease, with patients continuing to relapse and develop de novo cirrhosis for at least three decades into their disease course. In some patients, IST withdrawal appears to be safe, however, further studies are required to fully characterise such patients. Large multicentre studies combining databases should help to further characterise the long-term outcome of AIH.

8 Discussion

AIH is a lifelong disease. There are many challenges with regard to management. Key aims of treatment are to prevent liver-related death by induction of remission. Serum transaminases and g-globulin/IgG are used as surrogate markers for histological inflammation. However, they do not correlate closely with disease severity.[146,306] No or minimal inflammation on liver biopsy is associated with prevention of fibrosis progression.[44] Secondary aims are to prevent side effects and optimise quality of life. Despite treatment, 10-40% patients still progress to cirrhosis[36,138,297] which is an independent predictor of poor outcome.[138] Relapses still occur.

Evidence that treatment improves survival comes initially from randomised controlled trials in the 1970s[33,34,44]. Longer-term survival is reduced compared with matched controls. An SMR of 1.86 considering any death or transplantation was reported in a long-term outcome study and 20-year survival from liver death/transplantation of 70% at 20 years.[36] Treatment regimes remain largely unchanged since the 1970s. The mainstay of treatment is Azathioprine and Prednisolone. Despite treatment, around half of patients achieving biochemical remission, had persisting histological activity and were at higher risk of death/transplantation compared with patients in histological remission (SMR 1.4 vs. 0.7 $p<0.05$).[39]

Previously, our unit used a regime of Prednisolone 20-40mg/day (reducing dose) with AZA 1mg/kg and continued for at least two years, until demonstration of histological remission, when Prednisolone was withdrawn. I therefore carried out work to see if measuring AZA metabolites and increasing the AZA dose to 2mg/kg, aiming to obtain 6-TGN (the active metabolite) levels within the therapeutic range improved outcome. 88% and 96% patients obtained biochemical remission at 6 and 12 months respectively. 2mg/kg dosing was achieved

in 86% patients. However, the final dose was 1.3mg/kg with 6-TGN levels remaining within the therapeutic range (250-450pmol/8X10^8 RBC). However, rates of histological remission did not improve with this regime (50% patients in remission on biopsy 2). These results are important because they demonstrate that lower Azathioprine doses result in biochemical remission than previously reported. 6-TGN levels lower than observed in IBD have been associated with biochemical response[65,66,201] Dosing of 1.5-2.5mg/kg is required to maintain remission in IBD[202]. This work is limited by the small sample size (26) and the fact that not all patients had a second liver biopsy. An extension to the study would strengthen the findings. Further work is needed to evaluate the role of other agents such as tacrolimus in patients who fail to obtain histological remission on AZA.

MMF is a suitable alternative to AZA in AZA intolerant patients, however it does not work where AZA has been ineffective.[5,86] My data demonstrates that patients switched to MMF because of intolerance did not have an inferior outcome compared to patients continuing AZA. This is in keeping with other published data.[95]

Fibrosis progression is associated with adverse outcome.[138] Over recent years, we have had access to newer methods of non-invasive fibrosis assessment including Fibroscan (vibration-controlled transient elastography), ARFI (acoustic radiation force impulse scanning) and MRE (magnetic resonance elastography). These methods provide the possibility to obtain serial measures of liver fibrosis in a safe manner which is better tolerated than liver biopsy. My results demonstrate Fibroscan showed good accuracy in excluding, but lower accuracy in predicting, Ishak fibrosis stage of 4 or more. Fibroscan was less accurate in predicting lower fibrosis stages. In a larger study, it was possible to differentiate between F0-2 and F3-4 fibrosis.[110] My

work was limited by sample size. In addition, not all scans were valid (accuracy markers acceptable: 10 valid measurements, IQR/Median <30%, success rate ≥ 60%). This technology is preferred by patients to liver biopsy.[307] This could result in improved adherence to treatment and outcome for patients. In future, Fibroscan may be useful to help demonstrate fibrosis regression associated with biochemical remission. Hartl *et al.* showed that improvements in liver stiffness were associated with biochemical remission and regression of fibrosis (-7.5%/year; 95% CI -11%to -2.0%; p=0.003), after 6 months treatment.[113]

AIH is a chronic liver disease associated with reduced BMD.[63,267] AIH is often steroid-treated which also results in reduced BMD, even at low doses.[78] The significance of lower BMD is that of increased fracture risk. I analysed a large database of proactively managed AIH patients. Importantly, patients had an excess risk of fragility fractures compared with the general population. First fragility fracture rate was 18% and 36% at 10 and 20 years. FRAX is a useful online tool for predicting 10-year fracture risk. These data demonstrate that the Major Osteoporosis Score (output of the FRAX calculator) predicts fracture risk. Recent osteoporosis guidelines advocate the calculation of FRAX and starting a bisphosphonate, pending formal bone mineral density assessment in indeterminate and high risk casesCh.[239] This data would support the use of this approach in AIH patients.

Data on long-term outcome in AIH (over 20 years) is lacking. Is AIH a life-long disease or does it burn out? In a cohort of patients with complete data capture from 1987, all-cause and liver-related death/transplant rates were: 24%(all-cause) and 11%(liver) after 10 years and 51% and 21% respectively after 20 years. Like others, I demonstrated baseline variables associated with death/transplantation including older age (all-cause, not liver-related),[9,11]

cirrhosis and decompensation [14,32,34,210-21]. They were also independently association with liver death. Our patient cohort is unique with complete data capture from 1987 and 65 patients who were followed for over 20 years. Relapse and cirrhosis progression still occur despite treatment in the second 20 years. There was no significant difference in death/transplantation rates between the group followed for the first 20 years compared with the second 20 years. Long-term follow-up should therefore be recommended in these patients.

Considering the implications of treatment: including drug monitoring, side effects and effects on quality of life, it is potentially desirable to limit treatment duration. Published relapse rates vary from 25%[132] to 100%[13]. Reviewing our own data, six out of 26 (23%) patients had relapses whilst off treatment over 1.29(0.5-9.6) (median (range)) years after stopping IST. Of note, treatment duration before IST withdrawal was long ((10.1(1-37)) years (median(range))). This is in keeping with published data.[96,134,153] Results in a small prospective observational study were similar where 12 patients, treated for at least 2 years, in histological remission had IST withdrawn. 8/12 (66%) remained in drug-free remission after 62(13-75) months median(range)) follow-up.[308] A possible strategy for selecting suitable patients for IST withdrawal was presented including a) factor predictive of low risk of relapse (e.g. drug precipitant), b) no cirrhosis or decompensation (further relapses unacceptable), c) good tolerance to steroids (no contraindication to further treatment) and d) new diagnosis of malignancy (known cause and effect relationship). A large prospective study would be needed to fully evaluate this strategy.

Long-term management of AIH is likely to evolve. Historically, the diagnosis of AIH was based on histology. A recent paper demonstrated that many of the classic histological features

of AIH were non-specific and not necessary to make a diagnosis (emperipolesis and hepatocyte rosettes).[32] Instead, a combination of normalisation of transaminases and IgG with transient elastography may be able to differentiate severe from non-severe fibrosis after 6 months.[110] Treatment regimes vary. In a large multicentre audit, non-cirrhotic patients treated with Budesonide did not have inferior outcomes compared with patients receiving Prednisolone.[35] This would have the potential benefit of fewer steroid-related side effects. Measurement of AZA metabolite 6-TGN, may improve rates of histological remission[66] and allow for reductions in doses of thiopurines and corticosteroids (also shown in my work).[198]

In conclusion, autoimmune hepatitis is a rare, heterogenous disease. There are many factors to consider during a patient's treatment journey and areas which require further research. Further work evaluating the use of Fibroscan in AIH would be desirable to assess inflammation resolution in addition to fibrosis stage to guide management decisions. Despite treatment, cirrhosis progression and relapses occur. Novel treatments are needed to improve outcomes for our patients. Ideally, a large multicentre, randomised controlled trial would provide rigorous data on which patients could be safely considered for immunosuppressive treatment withdrawal. Management should be patient focused, balancing good treatment outcome against risks of investigations (liver biopsy) and minimising side effects.

9 Appendices

9.1 NOGG guidelines

Article:
Gregson, C.L., Armstrong, D.J., Bowden, J. et al. (17 more authors) (2022) UK clinical guideline for the prevention and treatment of osteoporosis. Archives of Osteoporosis, 17 (1). 58. ISSN 1862-3522

https://doi.org/10.1007/s11657-022-01061-5

9.2 Supplementary tables (see Chapter 4)

	Six months follow-up				One year follow-up				Biopsy 2			End follow-up		
Patient	ALT (0-33 iU/L)	ALT Normal (N)	IgG (6-16 g/L)	6-TGN (68-150 (pM/8 X10^8 cells))	ALT (0-33 iU/L)	ALT N	IgG (6-16 g/L)	6-TGN (68-150 (pM/8 X10^8 cells))	ALT (0-33 iU/L)	IgG (6-16 g/L)	6-TGN (68-150 (pM/8X 10^8 cells))	ALT (0-33 iU/L)	IgG (6-16 g/L)	6-TGN (68-150 (pM/8X10^8 cells))
Median (range)	19 (9-58)	21	11.53 (9.87-35.3)	434 (128-1093)	18 (9-48)	22	14.2	322 (100-445)	15 (14-22)	9.2 (7.8 - 11.5)	NA	15.5 (8-47)	10.3 (5.3-12.8)	322 (294-350)

Supplementary Table 9.2.1: laboratory results

Supplementary Table 9.2.2: patient characteristics

Patient	Age at diagnosis	Biopsy 1		TPMT level (68-150 (pM/8X10^8 cells))	Initial AZA dose (mg)	Initial AZA dose mg/kg	6-TGN on initial dose (235-450 (pM/8X10^8 cells))	6-MMP on initial dose (0-5700 (pM/8X10^8 cells)))	Reason 2mg/kg dose not achieved or maintained	Biopsy 2						
		NIS	Fibrosis stage							NIS	Fibrosis	AZA dose (mg)	AZA dose (mg/kg)	6-TGN (235-450 (pM/8X10^8 cells))	In remission	Reason biopsy not done
Median (range)	52 (18-74)	13 (4-17)	3 (1-6)	97 (52-194)	100 (50-175)	1.0 (0.6-2.3)	223 (119-785)	641 (100-5588)		3.5 (2-12)	2.5 (1-5)	100 (25-150)	1.2(0.5-2.3)	332 (123-434)	7 (n=13)	
1	47	7	1	114	50	0.63	119	281	NA							Awaited
2	57	14	3	143	75	0.91	345	2380	NA	2	3	150	1.82	345	1	
3	63	4	3	97	75	0.93	183	433	NA							Done but inadequate for analysis
4	36	7	3	109	75	1.05	143	236	NA							Not done, patient choice
5	70	15	3	139	50	1.17	190	207	NA							Awaited

Table 9.2.2: patient characteristics continued

Patient	Age at diagnosis	Biopsy 1		TPMT level (68-150 (pM/8X10^8 cells))	Initial AZA dose (mg)	Initial AZA dose mg/kg	6-TGN on initial dose (235-450 (pM/8X10^8 cells)))	6-MMP on initial dose (0-5700 (pM/8X10^8 cells)))	Reason 2mg/kg dose not achieved or maintained	Biopsy 2						
		NIS	Fibrosis stage							NIS	Fibrosis	AZA dose (mg)	AZA dose (mg/kg)	6-TGN (235-450 (pM/8X10^8 cells))	In remission	Reason biopsy not done
6	57		4		100	0.94	256	<100	NA							LFTs did not settle. Liver transplant 21/8/19
7	60	17	1	48	100		322	654	↑ 6-MMP							Awaited
8	19	13	2	92	50	1.12	187	427	↑6-TGN, ↑MMP and nausea	3	3	75	1.36	433	1	
9	65	15	1	98	75	0.98	127	880	↑ 6-MMP							Awaited
10	71	13	1	94	75	1.24	280	1960	↑ 6-TGN							Age
11	74	11	2	106	75		159	305	SCC/cancer							Age
12	50	11	3	52	50	1.10	406	689	↑ 6-TGN	2	1	50	0.89	418	1	

Table 9.2.2: patient characteristics continued

Patient	Age at diagnosis	Biopsy 1		TPMT level (68-150 (pM/8X10^8 cells))	Initial AZA dose (mg)	Initial AZA dose (mg/kg)	6-TGN on initial dose (235-450 (pM/8X10^8 cells))	6-MMP on initial dose (0-5700 (pM/8X10^8 cells))	Reason 2mg/kg dose not achieved or maintained	Biopsy 2						
		NIS	Fibrosis stage							NIS	Fibrosis	AZA dose (mg)	AZA dose (mg/kg)	6-TGN (235-450 (pM/8X10^8 cells))	In remission	Reason biopsy not done
13	52	12	1	55	75	1.12	785	<100	↑ 6-TGN							Awaited
14	51	11	3	71	100	1.00	315	378	In range at lower dose	2	2	150	1.18	252	1	
15	34	13	0	99	100	1.07	128	670	↑ 6-MMP	3	1	150	1.41	123	1	
16	62	15	3	121	100	0.99	409	1918	↑ 6-TGN	6	4	100	1.01	364	0	
17	41	7	0	52	50	0.96	1093	478	↑ 6-TGN	4	1	25	0.53	256	0	
18	36	14	3	95	50	1.11	571	240	↑ 6-TGN	3	1	50	0.91	332	1	

Table 9.2.2: patient characteristics continued

Patient	Age at diagnosis	Biopsy 1		TPMT level (68-150 (pM/8X10^8 cells))	Initial AZA dose (mg)	Initial AZA dose mg/kg	6-TGN on initial dose (235-450 (pM/8X10^8 cells))	6-MMP on initial dose (0-5700 (pM/8X10^8 cells))	Reason 2mg/kg dose not achieved or maintained	Biopsy 2						
		NIS	Fibrosis stage							NIS	Fibrosis	AZA dose (mg)	AZA dose (mg/kg)	6-TGN (235-450 (pM/8X10^8 cells))	In remission	Reason biopsy not done
19	22	8	3	123	100	0.98	432	1864	nausea	4	3	150	1.28	228	0	
20	57	15	1	132	100	1.06	264		↑ 6-MMP	5	1	150	1.43	235	0	
21	28	12	3	194	75		565	628	↑ 6-TGN							Lost to follow-up
22	55	14	4	81	75	0.88	534	5588	↑ 6-TGN	4	1	75	1.13	295	0	
23	55	15	5	125	75	1.10	296	754	↑ 6-TGN							On transplant list

Table 9.2.2: patient characteristics continued

Patient	Age at diagnosis	Biopsy 1		TPMT level (68-150 (pM/8X10^8 cells))	Initial AZA dose (mg)	Initial AZA dose mg/kg	6-TGN on initial dose (235-450 (pM/8X10^8 cells)))	6-MMP on initial dose (0-5700 (pM/8X10^8 cells))	Reason 2mg/kg dose not achieved or maintained	Biopsy 2						
		NIS	Fibrosis stage							NIS	Fibrosis	AZA dose (mg)	AZA dose (mg/kg)	6-TGN (235-450 (pM/8X10^8 cells))	In remission	Reason biopsy not done
24	48	6	6	97	75	0.89	528	1863	nausea	12	4	100	1.23	345	0	
25	51	13	1	89	75	0.89	434	2654	nausea	3	3	150	2.26	434	1	
26	69	16	1	138	75	1.06	315		Leucopenia and ↑ 6-TGN	6	5					On tacrolimus

9.3 Metabolic bone questionnaire

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Metabolic Bone Centre Questionnaire

Northern General Hospital, Tel: 0114 2715340

NHS number: _____

STH number: _____

Please complete this form and bring it with you to your appointment. The information will be used to advise you and your doctor about your bone health. **If you need any help filling in this form, please ask when you come for your appointment.**

When completing the form, please place a cross in the box to indicate your chosen answer like this: ☒

If you complete a box incorrectly, please black out the wrong one like this: ■

and put a cross in the correct box.

Forename	
Surname	
Address	Post code
Telephone No.	*Home* ☐☐☐☐☐☐☐☐☐☐☐ *Mobile* ☐☐☐☐☐☐☐☐☐☐☐
Gender	Male ☐ Female ☐ Prefer not to say ☐
Date of birth	D D M M Y Y Y Y — Age ☐☐
How tall were you as a young adult?	☐ Feet ☐☐ Inches or ☐☐☐.☐ cm
	Do you think you have lost any height? ☐ Yes ☐ No
Ethnic background (this is relevant for analysing your scans)	☐ White ☐ Black African ☐ Asian ☐ Arabic ☐ Mixed (describe) _____ ☐ Chinese/Oriental ☐ Black Caribbean ☐ Other (describe) _____

Name: _____ DOB: D D M M Y Y Y Y

Q1 Bone Health

If you have ever broken (fractured) a bone, please tell us about it in the boxes below				
Which bones you have broken (fractured)?	**How old were you** (approximate age if unsure)	**How severe was the incident that led to the broken bone?** *(put a cross in one box)*		
		Minor e.g. tripped over, knocked into something or no injury at all	**Moderate** e.g. fall whilst running or from a low height such as a step	**Severe** e.g. road accident, fall from great height
	☐☐ years old	☐	☐	☐
	☐☐ years old	☐	☐	☐
	☐☐ years old	☐	☐	☐
	☐☐ years old	☐	☐	☐
	☐☐ years old	☐	☐	☐
	☐☐ years old	☐	☐	☐
	☐☐ years old	☐	☐	☐
Please continue on page 11 if you require more space				

Q2 Back Health

Have you ever had severe back pain lasting for more than a few days?	☐ Yes ☐ No
If **Yes**, please describe **how** and **when** it started	_____ _____ _____

Name: _____ DOB: D D M M Y Y Y Y

Q3a About Your Health

Many medical conditions can affect bone health. Do you have or have you ever had any of the following conditions diagnosed by your GP or at the hospital? ***(Please put a cross in all that apply)***

- Addison's disease
- Anorexia or bulimia
  - ☐ Current
  - ☐ In past
- Arthritis:
  - ☐ Osteoarthritis
  - ☐ Rheumatoid
  - ☐ Don't know
- Asthma
- Cancer:
  - ☐ Breast
  - ☐ Prostate
  - ☐ Other (please specify)

  _____
- Coeliac disease
- Crohn's disease
- Cystic fibrosis
- Diabetes
  - ☐ Type 1
  - ☐ Type 2
  - ☐ Don't know
- Epilepsy
- Indigestion, acid reflux, stomach ulcer
- Kidney disease
- Liver disease
- Chronic lung disease:
  - ☐ COPD
  - ☐ Chronic bronchitis
  - ☐ Emphysema
- Lupus (SLE)
- Myeloma
- Multiple sclerosis
- Osteogenesis imperfecta
- Paget's disease
- Parathyroid disease
- Polymyalgia rheumatica
- Stone disease
  - ☐ Kidney stones
  - ☐ Gall stones
- Stroke
- Temporal arteritis
- Thyroid disease:
  - ☐ Overactive
  - ☐ Underactive
- Ulcerative colitis
- Vasculitis
- Vitamin D deficiency

Please list any other medical conditions you have:

_____

_____

Please continue on page 11 if you require more space

Name: _____ DOB: D D M M Y Y Y Y

Q3b About Your Health

Have you had any of the following kinds of operations which may affect your scan results?		Year of operation	Details
☐ Hip replacement/ other hip operation	☐ Right ☐ Left	Y Y Y Y Y Y Y Y	
☐ Bowel or stomach		Y Y Y Y	
☐ Endocrine	(e.g. thyroid, parathyroid adrenal, pituitary)	Y Y Y Y	
☐ Transplant	(e.g. heart, lung, kidney, liver, bone marrow)	Y Y Y Y	
☐ Spine		Y Y Y Y	
☐ Other: **Please tell us about any other operations you have had on page 11**			

Q4 Medication for Your Bone Health

Treatment for your bones - Please indicate if you have **ever** taken any of these treatments and approximate start and stop dates

Indicate	Medication	Start date (month / year)	Stop date (month / year)	Still taking (tick if taking)
◯	Alendronate (alendronic acid, Fosamax)	M M / Y Y	M M / Y Y	☐ Still taking
◯	Denosumab (Prolia)	M M / Y Y	M M / Y Y	☐ Still taking
◯	Etidronate (Didronel PMO)	M M / Y Y	M M / Y Y	
◯	Ibandronate (Bonviva, Bondronat, ibandronic acid)	M M / Y Y	M M / Y Y	☐ Still taking
◯	Raloxifene (Evista)	M M / Y Y	M M / Y Y	☐ Still taking
◯	Risedronate (Actonel, risedronate sodium)	M M / Y Y	M M / Y Y	☐ Still taking
◯	Strontium ranelate (Protelos)	M M / Y Y	M M / Y Y	☐ Still taking
◯	Teriparatide (Forsteo)	M M / Y Y	M M / Y Y	☐ Still taking
◯	Zoledronate (Aclasta, Zometa, zoledronic acid)	M M / Y Y	M M / Y Y	☐ Still taking
◯	Romosozumab (Evenity)	M M / Y Y	M M / Y Y	☐ Still taking
◯	Other bone treatments (specify):	M M / Y Y	M M / Y Y	☐ Still taking

Q5 Supplements for Your Bones

Do you take supplements of calcium with/without vitamin D ☐ Yes ☐ No If **Yes**, what is the name of the supplement? _____	
How often do you take your calcium supplement?	☐ Every day ☐ Most days ☐ Once or twice a week ☐ Occasionally
Do you take any of these supplements?	☐ Cod liver oil / fish oil ☐ Vitamin D ☐ Other(s): _____ _____

Q6 Steroid Use

If you have ever had treatment with steroids what type(s) of treatment have you had?
(Put a cross in all that apply)

☐ Tablets ☐ Inhalers ☐ Creams ☐ Joint/muscle injections ☐ Injection into a vein

↓

If you take **tablets,** please indicate which type(s) and how often:

☐ Prednisolone ☐ Budesonide ☐ Hydrocortisone ☐ Dexamethasone

Do you take a daily dose? ☐ Yes ☐ No

→ If **Yes**, please state the usual dose _____ mg

Do you have "Booster" doses? ☐ Yes ☐ No

→ If **Yes**, how often do you have a booster?

☐ Less than once a year ☐ 2-3 times a year ☐ 4+ times a year

What is/was the reason for your steroid treatment?	
If you are taking steroids now, how long have you taken them for?	☐☐ Years ☐☐ Months
If you are **not** taking treatment now, how long ago were you last treated with steroids?	☐☐ Years ☐☐ Months

Q7 Treatment for Other Conditions

Are you being treated with any of the following medications which can affect bone health:

- ○ Anastrazole (Arimidex)
- ○ Exemestane (Aromasin)
- ○ Letrozole (Femara)
- ○ Treatment for stomach ulcer, indigestion or acid reflux - specify: _____
- ○ Phenytoin (Epanutin)
- ○ Phenobarbital
- ○ Carbamazepine (Tegretol)
- ○ Sodium valproate (Epilim)
- ○ Other treatment for epilepsy Specify: _____

Please write down all the medications you use. This includes tablets, medicines and injections. Include everything from your GP, from the hospital and those you buy yourself.
Instead of completing the table you can bring a copy of your prescription if you'd prefer but please also tell us about any medication that you buy yourself

Medication	Dose	What do you take this for?

Please continue on page 11 if you require more space

Do you have any treatments that are given as an **injection** by your GP or at the hospital? ☐ Yes ☐ No

If **Yes**, please tell us the name of the treatment _____

and what the treatment is for _____

Do you have any allergies or sensitivity to medication? ☐ Yes ☐ No

Is **Yes**, please give details _____

Name: _____ DOB: D D M M Y Y Y Y

Q8 About Your Lifestyle

How much milk do you have each day? (e.g. in drinks, cereal etc.)	☐ None ☐ ½ to 1 pint ☐ Use non-dairy milk	☐ Less than ½ pint ☐ More than 1 pint
How often do you eat dairy foods? (e.g. cheese, yoghurts)	☐ Never ☐ Once/twice a week	☐ Less than once a week ☐ Most days/every day
In the summer how often do you spend 30 minutes out in the sunshine with your face and arms uncovered?	☐ Never ☐ Once/twice a week	☐ Less than once a week ☐ Most days

Q9 Alcohol and Smoking

How often do you drink alcohol? If so, please indicate how often

☐ Never ☐ Less than once/week ☐ 1 – 2 times/week

☐ Most days ☐ Every day

*one unit is ½ pint beer/lager, one small glass of wine, small pub measure of spirits

On **each** day that you drink, how many units * do you usually have? ☐☐ **units/day**

Do you smoke? (including e-cigarettes)

☐ Yes, I smoke now ☐☐ **per day**

☐ No, I stopped within the past 5 years

☐ No, I stopped more than 5 years ago

☐ No, I have never smoked

Tick here if you smoke e-cigarettes only ☐

Q10 Falls

Have you had any falls in the last 6 months? ☐ Yes ☐ No

If **Yes**, how many? ☐ 1 ☐ 2-3 ☐ 4 or more

What caused your falls? ☐ Felt dizzy/ lightheaded ☐ Blacked out ☐ Other: _____

Name: _____ DOB: D D M M Y Y Y Y

Q11 Mobility

Do you use any assistive/ mobility aids? ☐ Yes ☐ No If **Yes**, please indicate which:	**Please indicate how often**		**Please indicate where**	
	Sometimes	Most or all of the time	Outdoors only	Indoors and outdoors
☐ Wheelchair / mobility scooter	☐	☐	☐	☐
☐ Frame	☐	☐	☐	☐
☐ Stick(s) or crutches	☐	☐	☐	☐

Q12 Exercise

Put a mark on the line below to indicate how active you are:

Very inactive _____ Very active

Immobile | Active every day, no formal exercise or sport | Daily strenuous exercise or sport

Q13 About Your Family

Do/did any of your close relatives *(parent, brother, sister, child)* have osteoporosis? ☐ Yes ☐ No ☐ Unsure

If any of your close relatives have had a **broken hip,** please indicate who and when

Relationship to you (e.g. mother)	**Age at (first) hip fracture**
	☐☐ years old
	☐☐ years old
	☐☐ years old
	☐☐ years old

Q14 Menstruation and Menopause

How old were you when your periods started? ☐☐ years old

Do you still have regular periods? (8 or more each year) ☐ Yes ☐ No

If **No**, how old were you when you had your last period? ☐☐ years old

Did your periods ever stop for more than 3 months? (except during pregnancy and at the menopause) ☐ Yes ☐ No

If **Yes**, please describe when and why: _____

_____

Have you ever used contraceptive injections (Depo Provera)? ☐ Yes ☐ No

If **Yes**, please indicate your approximate start and stop age: ☐☐ Start ☐☐ Stop ☐ Still using

Do you have menopausal symptoms now? ☐ Yes ☐ No

Have you ever taken Hormone Replacement Therapy (HRT)? ☐ Yes ☐ No

If **Yes,** please indicate your approximate start and stop age: ☐☐ Start ☐☐ Stop ☐ Still using

Have you had a hysterectomy? ☐ Yes ☐ No

If **Yes**, how old were you? ☐☐ years old

Had your periods stopped before you had the hysterectomy? ☐ Yes ☐ No

Have you had either or both of your ovaries removed? ☐ Yes one ☐ Yes both ☐ No

If **Yes**, please indicate how old you were when they were removed: ☐☐ Age 1st ovary removed ☐☐ Age 2nd ovary removed

☐☐ Age if both were removed at the same time

Thank you for completing this questionnaire

Name: _____ DOB: D D M M Y Y Y Y

Continuation Sheet

- Please use this page to give us any further information about any of the questions on the form.
- Please include the question number with your answer.

Name: _____ **DOB:** D D M M Y Y Y Y

OFFICE USE ONLY
Please leave this box blank

Appointment Date
D D M M Y Y Y Y

Date of previous scan
D D M M Y Y Y Y

Current Height
. cm ft in

Previous Height
. cm

Current Weight
. kg lb

Previous Weight
. kg

LMP
D D M M Y Y

LMP age

Risk of pregnancy? Yes No

Comments:

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