Autoimmune hepatitis: A clinical study

By

E H Buchel

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Autoimmune hepatitis: A clinical study

Elwin Buchel

Thesis submitted to the University of Pretoria,
to obtain the degree of

Doctor in Medicine

March 2007

Promoters

PROFESSOR A. L. VAN GELDER
Professor J. Fevery

DEDICATION

This thesis is dedicated to the memory of my mother, who encouraged me to seek as much education as possible.

SUMMARY

This study involved the analysis of 112 patients with autoimmune hepatitis (AIH), during which process several specific issues or problems were identified. One subgroup included pregnant patients, and another patients diagnosed at an older age (>65 years). The diagnosis of AIH is not always easy, and the investigations revealed that some patients diagnosed with AIH had features of an overlap syndrome (with primary sclerosing cholangitis) or in fact never had AIH, but as retrospectively diagnosed, had toxic hepatitis masquerading as AIH. Some patients who were initially documented as clearly having chronic hepatitis C, also had autoantibodies in their serum and were (or could have been) incorrectly diagnosed as AIH. The results showed a large variability in symptoms and complaints at the time of diagnosis of AIH. The disorder can present as an acute or chronic disease. In some patients only vague symptoms are reported and in others, the diagnosis is an incidental discovery because of abnormal liver enzymes. In contrast, other patients present with acute liver failure. The diagnosis must be considered in patients with non-viral liver disease with significantly raised ANA and SMA and high serum gammaglobulin concentrations. No fixed age predisposition was found, but rather an even spread over various age groups. The male:female ratio is 1:2.5. This disorder can for the most part be well treated with a combination therapy of corticosteroids and azathioprine. Experience shows that high doses of corticosteroids can cause serious complications, particularly in older patients. A lower dose is mostly adequate, although biochemical normalisation may take longer. In approximately half of the patients, corticosteroid-therapy was discontinued, with azathioprine as the only treatment. The long-term survival of adequately treated patients is almost equal to that of the control group. In the cohort, ten patients died: three who had presented with acute liver failure, two who later developed a hepatocellular carcinoma and one who died because of cerebral lymphoma. Three older patients died of sepsis, possibly co-induced by high doses of steroids. Five patients underwent liver transplantation, of which one died of aspergillosis.

OPSOMMING

`n Ontleding van 112 patiënte met Autoimmune Hepatitis is onderneem. Gedurende hierdie studie is daar spesifieke sub-groepe geïdentifiseer. Een sub-groep het verskeie swangerskappe bevat terwyl `n ander groep slegs by `n ouer ouderdomsgroep (>65 jaar) gediagnoseer was. Die diagnose van AIH is nie altyd maklik nie, en die ondersoeke het getoon dat sommige pasiënte eienskappe van `n oorvleulingsindroom (AIH en Primêre Skleroserende Cholangitis) vertoon, of inderwaarheid nooit AIH gehad het nie, maar inderdaad retrospektief met toksiese hepatitis gediagnoseer was, wat as AIH vermom voorgekom het. Sommige patiënte was oorspronklik as duidelike chroniese hepatitis C lyers gedokumenteer, maar het ook antiliggame in hulle serum gehad en was (of kon) verkeerdelik as AIH gediagnoseer gewees het. Die resultate het `n groot verskeidenheid simptome en klagtes getoon ten tye van diagnose van AIH. Die kwaal kan voorkom as `n akute of chroniese siekte. In sommige pasiënte is slegs vae simptome gedokumenteer en in ander, was dit `n toevallige ontdekking na aanleiding van abnormale lewer ensieme. In teendeel, sommige gevalle het in akute lewer versaking aangemeld. Die diagnose moet oorweeg word in pasiënte met nie-virale lewersiekte, met `n betekenisvolle styging in ANA, en SMA antiliggame, wat met hoë serum gammaglobulien konsentrasies gepaard gaan. Geen vasgestelde ouderdoms-vatbaarheid is teëgekom nie, maar eerder `n gelyke verspreiding oor verskeie ouderdomssgroepe was gedokumenteer. Die manlike:vroulike verhouding is 1:2.5. Die kwaal kan grootendeels behandel word met `n kombinasie van kortikosteroide en azathioprine. Ondervinding het getoon dat hoë dosisse kortikosteroide aanleiding kan gee tot ernstige komplikasies, veral onder ouer pasiënte. `n Laer dosis is gewoonlik voldoende, alhoewel die biochemiese normalisering langer mag duur. In ongeveer die helfte van die pasiënte, kan die kortikosteroide gestaak word, met azathioprine as die enigste behandeling. Die langtermyn oorlewing van pasiënte wat voldoende behandel is, is byne dieselfde as dié van die kontrole groep. In die kohort, is tien pasiënte oorlede: drie wat akute lewerversaking gehad het; twee ander het later hepato-sellulêre karsinoom ontwikkel en een het weens serebrale limfoom gesterf. Drie ouer pasiënte is dood aan sepsis, moontlik presipiteer deur hoë dosisse steroide. Vyf pasiënte het leweroorplantings ondergaan, waarvan een oorlede is weens aspergillose.

AKNOWLEDGEMENTS

To everyone who supported and helped me during this research, I would like to say thank you. I would like to address a special word of gratitude to the following persons:

- My promoters, Prof. Johan Fevery and Prof. Antoine van Gelder for guidance, support and empathy;

- Prof. V. Desmet and Prof. T. Roskams, for the interpretation of the liver biopsies, and Prof. X. Bossuyt, for the immunological tests;

- Dr C. Verslype, Dr C. George, Prof. F. Nevens and Prof. W. van Steenbergen. for analytical help and sharing patient information;

- Prof. Michael Manns, Prof. Albert Cjaza and Dr Ian McFarlane, who for decades have researched and written about autoimmune hepatitis; I met all of them to discuss aspects of AIH;

- Appreciation is expressed to the staff of the medical library in Leuven Gasthuisberg Hospital complex for their unstinting help over an extended period both during the time of the initial research, and during three subsequent visits to Leuven thereafter.

- Appreciation is expressed for the unstinting help and valuable advice given by members of the library personnel at the Pretoria Academic Hospital, especially with the checking of references.

- Dr Abdullah el Tom, my colleague and friend, for his support;

- Ms Myleen Oosthuizen and Ms Magriet Lee from the University of Pretoria Medical Library for help with references;

- Ms Maria Prozesky for editing the text of this thesis;

- Ms U. Mather for assisting with the typing of this thesis;

- My wife Sjaan, for encouragement and help;

- My children, for their loyal support, encouragement and humour; and

- to God, in whom I trust.

AFFIDAVIT

I declare that:

Autoimmune hepatitis: A clinical study

is my own work and that all sources I have used or quoted have been indicated and acknowledged by means of recognition in the complete references in the bibliography.

Elwin Herbert Buchel March 2007

AUTHOR'S PREFACE

This text grew out of a long time passion for clinical hepatology. With the help and encouragement of my chief promoter Professor Johan Fevery and the patience and motivation of my other promoter Professor Antoine van Gelder it was finally completed. Professor Fevery was Chief of Medicine and Head of the Hepatology Unit at Gasthuisberg Academic Hospital in Leuven, Belgium, where the initial research was done. During this elective period the files of all patients diagnosed with AIH since 1975 were categorized according to general characteristics such as age, gender, initial symptoms, biochemistry, serology, therapy and acute flare ups etc. and then sub-categorized according to 'interesting or unique features' representing lesser-known trends in AIH which had been identified from analysis of these 112 patients. Each of these subgroups was then looked at more closely. Two important groups thus identified were, an elderly cohort (those patients 65 years old and older) and a group of female patients who, despite having chronic AIH, not only became pregnant but also successfully delivered. It transpired that differentiating patients with toxic hepatitis, which mimicked AIH, from those with true AIH was a very important issue, since the symptoms, biochemistry, serology and even histology of both conditions are very similar, yet toxic hepatitis was the consequence of medication. Also, the enigmatic association between AIH and hepatitis C was investigated mainly because of two unique patients who had both hepatitis C and AIH and were over time successfully treated for both conditions. Finally, and perhaps most interestingly, analysis of the total cohort revealed a few patients who had 'crossed over' from AIH to primary sclerosing cholangitis (PSC). Two case histories were selected, singular for their long period of follow up and thoroughness of investigation. This tendency of one immune-related disease to develop the clinical picture of another is known as the 'variant' or 'overlap' phenomenon.

Each chapter mainly focuses on specific aspects of AIH and its differential diagnosis, but some repetition was unavoidable, particularly in the introductory paragraphs. I however feel that this is not harmful to the study since each chapter is intended to stand as a separate contribution.

TABLE OF CONTENTS

ABBREVIATIONS

GENERAL INTRODUCTION **1**

PATHOGENESIS OF AIH **8**

RESEARCH METHODOLOGY **12**

TABLES:

Table 1: Revised criteria for autoimmune hepatitis **4**

Table 2: Occurrence of autoantibodies in chronic liver disorders **8**

Table 3: Evolution of liver enzymes in an older patient on low-dose steroids **11**

AIMS OF THIS STUDY **13**

CHAPTER 1

AUTOIMMUNE HEPATITIS: AN OVERVIEW OF 112 CONSECUTIVE PATIENTS

Introduction 15

Patients and diagnoses 15

Results of the overview of the 112 patients 15

Discussion 19

Summary 22

TABLES:

Table 4: Complaints and physical examination on diagnosis 16

Table 5: Laboratory tests at diagnosis 17

Table 6: Histological findings 18

FIGURES

Figure 1: Age distribution of the 112 patients with autoimmune hepatitis 16

CHAPTER 2

AUTOIMMUNE HEPATITIS AT AGE 65 OR OLDER

Introduction 23
Patients 23
Methods 23
Statistics 23
Results 24
Discussion 27

TABLES

Table 7: Age and gender in the cohort of 112 patients with AIH 24
Table 7a: Age and gender in the cohort of 112 patients with AIH 24
Table 8: Clinical characteristics of 28 elderly AIH patients compared to 84 younger AIH patients 26
Table 9: Laboratory tests at diagnosis 26
Table 10: Histology at diagnosis 27

FIGURES

Figure 2: Laboratory test results of the elderly AIH patients at diagnosis 25
Figure 3: Treatment with low-dose steroids and azathioprine in some illustrative cases 30

CHAPTER 3

AUTOIMMUNE HEPATITIS DURING PREGNANCY

Introduction 33

Description of patients 34

Discussion 38

TABLES

Table 11: Characteristics of pregnant patients 41

FIGURES

Figure 4: Pattern of ALT at diagnosis, during two pregnancies with flare ups following delivery, and effect of therapy 35

Figure 5: Autoimmune sclerosing cholangitis: effect of treatment and delivery on ALT levels in three pregnancies (one ending in miscarriage) 38

CHAPTER 4

AUTOIMMUNE HEPATITIS EVOLVING INTO PRIMARY SCLEROSING CHOLANGITIS

Introduction 42

Patient 1: A young female patient with evolution of AIH into PSC 44

Patient 2: A female patient with evolution of AIH into PSC 45

Discussion 47

TABLES

Table 12: Immune markers and therapy in autoimmune liver disease 44

Table 13: A young female with evolution of AIH into PSC 45

Table 14: Patient 2 liver function and serology tests 47

CHAPTER 5

TOXIC HEPATITIS WITH FEATURES OF AUTOIMMUNE HEPATITIS

Case 1: Nitrofurantoin 49

Case 2: Alpha-methyldopa 50

Case 3: NSAID hepato-toxicity 52

Case 4: Phenprocoumon (R marcoumar) 53

Case 5: Minocycline liver toxicity 55

Overall discussion on toxicity mimicking AIH 56

TABLES:

Table 15: Liver function tests of 61 year old woman who took alpha-methyldopa 51

Table 16: 50 years old male complaining of fatigue 53

Table 17: Evolution of laboratory tests 54

Table 18: HLA predispositions 58

CHAPTER 6

HEPATITIS C VIRUS AND AUTOIMMUNE HEPATITIS

Introduction **60**

Case history 1: MH **61**

Case history 2: DS **63**

Comment and discussion **64**

Conclusion **65**

Summary **66**

CHAPTER 7

GENERAL CONCLUSIONS AND PERSPECTIVES **68**

REFERENCES **73**

Abbreviations:

DISEASES or CONDITIONS:

AIH autoimmune hepatitis

aHCV anti hepatitis C virus

ASC autoimmune sclerosing cholangitis

HAV hepatitis A virus

HBsAg hepatitis B surface antigen

HBV hepatitis B virus

HCV hepatitis C virus

HCV-RNA hepatitis C viral –RNA

LE Lupus erythematosis

NANBH non-A non-B Hepatitis

NASH non-alcoholic steatohepatitis

PBC primary biliary cirrhosis

PSC primary sclerosing cholangitis

SBP spontaneous bacterial peritonitis

SLE systemic lupus erythematosus

ENZYMES:

ALP alkaline phosphatase (normal value <260 U/L)

ALT alanine aminotransferase (normal values <41 U/L)

AST aspartate aminotransferase (normal value < 39 U/L)

GGT gamma glutamyl transpeptidase (normal value 7-32 U/L)

ANTIBODIES and OTHER CELLULAR BIOCHEMICALS:

AMA antimitochondrial antibodies

ANA anti-nuclear antibodies

ANF Antinuclear factor

anti-ASGPR antibodies to asialoglycoprotein receptor

anti-SLA/LP anti-soluble liver antigen and anti liver/pancreas

c-ANCA cytosolic anti-neutrophylic cytoplasm antibodies

LKM liver-kidney-microsomal

MHC major histocompatibility complex or HLA

p-ANCA perinuclear anti-neutrophylic cytoplasm antibodies

SMA smooth muscle antibodies (=anti-actine antibody)

DRUGS:

AZA azathioprine

IFN interferon

INH isoniazide

MP methylprednisolone

URSO ursodeoxycholic acid

DIAGNOSTIC TECHNOLOGIES:

CT computerised tomography scan

ERC endoscopic retrograde cholangiography

ERCP endoscopic retrograde cholangiopancreatography

GENERAL:

Bid twice daily

cf compare with

Lab. laboratory

GP general practitioner

N normal

ND not done

OD daily

s.c. subcutaneous

TIW 3 times per week

> More than

< Less than

Cellcept is mycophenolate mofetil

NB: azathioprine and Imuran are commonly used terms for the same medication. Because they are used so frequently I have not bracketed the alternative nomenclature

GENERAL INTRODUCTION

Liver diseases are an important health problem all over the world. In Germany in 1883, Lurman (1) first recognised the association of infective particles being transmitted in the blood and hepatitis. He reported a large group of persons who received smallpox vaccine prepared from human serum; jaundice developed in 15% of that group over the next several weeks to months but in none of the clinical staff who had not undergone immunization (1).

A vaccine derived from human serum was similarly implicated in the transmission of hepatitis during World War II when a high incidence of jaundice developed in a specific group of soldiers who received yellow fever vaccine made from human serum (2).

40 years ago a unique antigen was identified in the serum of an Australian aborigine patient with leukaemia. Subsequently the antigen was found to occur commonly in patients who had received multiple blood transfusions (3). The antigen was called the Australian antigen, which was ultimately recognized as the hepatitis B surface antigen (HBsAg). Electron microscopy identified virions then termed ‘Dane’ particles, which were subsequently shown to be the etiologic agents responsible for hepatitis B viral infection (4).

Viral hepatitis was now classified as either ‘serum’ or ‘infectious’, yet it was soon evident that there were more than two agents capable of causing viral hepatitis. Repeated bouts of hepatitis were seen to occur in intravenous drug users and haemophiliacs and this suggested the existence of at least one additional blood-borne hepatotrophic virus.

The identification of serological markers for hepatitis A virus (HAV) and hepatitis B virus (HBV) indicated that the virus (or viruses) responsible for 50 to 75% of cases of viral hepatitis (especially parenterally transmitted) remained unidentified. These cases were designated by exclusion as non-A non-B hepatitis (NANBH) (5, 6,7).

Parenterally transmitted NANBH remained the primary infectious risk associated with the administration of blood products and presented a significant complication for individuals requiring transfusion.

The demise of the cumbersome term NANB hepatitis is primarily attributable to the discovery in 1989 that the major cause of post-transfusion hepatitis is the agent now called the hepatitis C virus (HCV). The importance of the discovery of HCV is best appreciated by considering the role of HCV in causing liver injury. In the USA HCV is responsible for about 20% of all cases of acute hepatitis and nearly 50% of the cases of chronic hepatitis. Chronic HCV can cause cirrhosis and end-stage liver disease; this condition can evolve into hepatocellular carcinoma at a yearly rate of 1%, usually after cirrhosis has developed. HCV is now also the number one reason for liver transplantation in the USA (8).

In addition, although HCV is a hepatotrophic virus it either causes or is associated with several other diseases both hepatic and non-hepatic (9). The introduction of routine screening of blood and organ donors for HCV infection produced a decline in the prevalence of hepatitis following transfusion and transplantation.

Despite vaccines against hepatitis A and B, the aetiology of both acute and chronic hepatitis is still very often traced to viral causes. However, chronic hepatitis is also caused by diverse non-viral aetiologies. Diseases such as Wilson's disease, alpha-1 anti-trypsin deficiency and autoimmune hepatitis (AIH) are rare, while alcohol-associated liver damage and hepatitis associated with medications and herbal remedies are frequently seen in liver clinics. Chronic cholestatic, or immune-related diseases such as primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC) remain relatively rare.

AIH is a chronic liver disease that in the spectrum of chronic liver diseases seldom takes centre stage yet is often in the spotlight. AIH is the most successfully treatable form of chronic hepatitis (10), but can arguably be described as the most enigmatic of chronic liver diseases. By the end of this text, I hope that the truth of this statement will be obvious.

The incidence of AIH is estimated at 1.9 cases per 100,000 per annum and the prevalence at 16.9 per 100,000 for adults (11). AIH is as frequent in Norway as PBC, SLE and myasthenia gravis (11). AIH can be defined as a chronic necro-inflammatory liver disorder of unknown aetiology associated with the presence of circulating autoantibodies and a high serum gammaglobulin level (12), and with lympho-plasmocytic infiltration of the liver. The plasmocytes produce the gammaglobulins. AIH was first described by Waldenström in 1950 (13) and redefined by MacKay in 1956 (14).

Initially the diagnosis appeared to be confined to young women who presented with acute hepatitis or with chronic illness characterized by lethargy, epigastric pain, arthralgias or myalgia or with oligomenorrhea, fluctuating jaundice and occasionally a cushingoid appearance with striae, hirsutism and acne. However, it is now apparent that AIH can also affect men and that it occurs at all ages (15).

Diagnostic criteria were now needed to support the diagnosis of AIH. At the meeting of the International Association for the Study of the Liver held at Brighton in the United Kingdom, in June 1992, an international panel was convened to review all the features of AIH and determine whether a consensus could be reached on criteria. These criteria were subsequently reviewed and revised (Table 1, p4). The currently used criteria (16) have a >90% specificity compared to the diagnosis of other chronic liver diseases such as PBC, PSC and hepatitis C (17).

Some patients however, are difficult to classify, because they show symptoms of two disease patterns. These patients are classified as suffering from a combination or ‘overlap’ condition between AIH and PBC, AIH and AMA-negative PBC (also called autoimmune cholangitis) or AIH and PSC. This has implications not only for diagnosis but also for the treatment (if not immune suppression). In rare cases an evolution is seen from PBC to autoimmune hepatitis, with an analogue evolution of the autoantibodies (18), or from AIH to PSC (19). The combination of AIH and PSC is seen more frequently in children; this is referred to as autoimmune sclerosing cholangitis (20).

Table 1. Criteria for Autoimmune hepatitis (adapted Brighton report)

Marker			Points
- Female gender			**2**
- Δ ALP divided by ΔALT	**<1.5**		**2**
	>3		**-2**
- Δ IgG	**>2x**		**3**
	1.5-2x		**2**
	1.0-1.5x		**1**
- ANA, SMA, LKM 1		**>1/80**	**3**
		=1/80	**2**
		=1/40	**1**
- AMA positive		**≥1/40**	**-4**
- viral markers neg			**3**
▪ (if pos)			**-3**
- medication intake		**neg**	**2**
▪ (if pos)			**-2**
▪			
- alcohol intake	**<25g/d**		**2**
	>60g/d		**-2**
-Biopsy: interface hepatitis			**3**
lympho-plasmocyte infiltration			**1**
liver cell rosettes			**1**
biliary changes			**-3**
characteristics of other diseases			**-3**
-other autoimmune disorders (only counts if ANA/SMA is neg)			**2**
-HLA DR3/DR4			**1**
- successful response to therapy; complete			**2**
or relapse after discontinuation			**3**

Score: definite AIH > 15 before therapy or >17 on treatment
[Adapted from Alvarez (16)]

There are two classical types of AIH and probably a type III AIH. **Type I autoimmune hepatitis** is characterized by the presence of SMA and/or ANA in the serum. Type I disease occurs at any age but very often has a bimodal distribution. 78% of patients are women (female-to-male ratio, 3.6:1), and 41% have concurrent extra-hepatic immunologic diseases, including autoimmune thyroiditis (12%), Graves disease (6%), ulcerative colitis (6%),

rheumatoid arthritis, pernicious anaemia, systemic sclerosis, Coombs' positive haemolytic anaemia, idiopathic thrombocytopaenic purpura, leukocytoclastic vasculitis, nephritis, erythema nodosum, and fibrosing alveolitis (1% each). The occasional presence of ulcerative colitis compels cholangiography to exclude PSC (21).

40% of patients with severe type I disease have an acute onset of symptoms, and the disease may present in a fulminant fashion (22, 23). Typically, patients with an acute presentation often have clinical signs of cirrhosis with ascites, oesophageal varices, spider naevi, with thrombocytopaenia, hypoalbuminemia, hypergammaglobulinaemia and histological changes of cirrhosis that indicate chronic liver disease (24). This acute presentation with changes reflecting chronic liver disease, reflects a pre-existent subclinical disease that is unmasked by disease progression or a spontaneous exacerbation (23, 24, 25). 8% of patients have no features of chronicity and are initially indistinguishable from an acute viral or toxic hepatitis (24).

Type II Autoimmune hepatitis is characterized by the presence of anti-LKM 1 in the serum (26) and is far less prevalent than type I. Type II AIH afflicts mainly children between 2 and 14 years old and only 20% of patients are adults. Type II has clinical, laboratory, genetic and prognostic differences from type I. An acute or fulminant presentation is possible, and it is essential to screen all patients with an acute decompensation for type-specific autoantibodies. Patients with type II disease seem to progress to cirrhosis more frequently than those with type I disease (82% vs 43% within 3 years) (26). This suggests that type II has a poorer prognosis than type I AIH. The target antigen of type II AIH is cytochrome mono-oxygenase P-450 IID6 (CYP2D6), a 50-dalton microsomal drug-metabolising enzyme (27). The antigen can be expressed on human hepatocyte membrane surfaces, and this expression can be modulated by interleukins and tumour necrosis factor. Antibodies to LKM1 inhibit the activity of P-450 IID6 *in vitro* but not *in vivo*, and lymphocytes extracted from the liver tissue of patients with the disease exhibit immunoreactivity specific to the antigen (28). Recombinant P-450 IID6 has been used to define the epitopes of anti-LKM1, and reactivity is restricted mainly to a short linear 33-amino acid sequence (27). Of the sera reactive to this sequence, 50% are reactive to an even shorter 8-amino acid sequence. Sera from type II patients bind mainly to the 54 – 271 peptide sequence of recombinant P-450 IID6, so this region has been designated as its 'core motive' (29).

Type II autoimmune hepatitis is associated with HLA-B14, HLA-DR3 and HLA-C4A-QO (30). The B14 allele is found in 26% of patients and only 4% of normal subjects. DR3 is found in 70% and C4A-QO in 90% of the patients. These findings suggest that the disease has a distinctive genetic predisposition (28). In most cases of type II AIH, other autoimmune diseases such as diabetes mellitus type I, vitiligo and autoimmune thyroiditis are also present (31). Sometimes type II AIH forms part of the rare autoimmune polyglandular syndrome type I, a disorder that is predominantly characterized by ectodermal dysplasia, mucocutaneous candidiases and multiple endocrine abnormalities (31). The three patients with type II AIH had no other associated disorders.

In the literature one other type of autoimmune hepatitis is mentioned. This so-called **Type III autoimmune hepatitis** is characterised by the presence of an anti-soluble liver antigen/ liver/pancreas (anti-SLA/LP). Patients with type III AIH usually lack ANA and anti-LKM1, but commonly have SMA (35%), mitochondrial antibodies (22%), rheumatoid factor (22%) and antibodies to liver-membrane antigen (26%) (32). The lack of mutual exclusivity between anti-SLA and other conventional autoantibodies weakens their case for being hallmarks of a distinct subgroup (33). These markers are absent in chronic viral hepatitis, but they are found in 11% of patients with type I disease (33). Seropositivity does not identify patients with distinctive clinical or prognostic features, and the antibodies may be variant manifestations of type I AIH (33). Antibodies to liver/pancreas have also been proposed as markers of type III AIH, but their candidacy suffers similarly from a lack of specificity (34). Since these tests are only available in a few laboratories, not much is known about this group. These patients are identical to patients with type I AIH in age, male-female ratios, clinical presentation, prevalence and other antibodies. They do however react less positively to corticosteroid therapy and also have a greater chance of relapse when the prednisone dose is reduced (9). In all probability they represent a subgroup of patients with type I AIH with a more serious form of the disorder. Until anti-SLA and anti-LP are associated with distinctive clinical, laboratory and prognostic features, neither can be accepted as markers of valid subtypes.

When all the diagnostic criteria are not present it is difficult to decide if the disease is indeed AIH or rather so-called cryptogenic cirrhosis. This term is used when the origin of the cirrhosis is unknown. A Turkish group argue that a proportion of patients with cryptogenic cirrhosis might well actually have a form of ‘burnt out’ or end-stage AIH (35). These

patients' symptoms also fluctuate, with normalization of liver function tests and loss of immune markers co existing with active disease processes visible in the histology. A study done in Bern showed that 18% of AIH patients who had an earlier spontaneous normalization of previously raised liver enzymes lost their immune markers (35). This was also the case in three of the patients, in whom serum autoantibodies disappeared during the follow-up period. This could make a definite diagnosis of AIH more difficult, if these markers were absent in the initial stages. In the patients the disappearance of the autoantibodies was only noted during therapy with immunosuppressants.

It must be pointed out that ANA and to a lesser degree SMA are not specific for AIH, and that these antibodies can also be present in other disorders, including toxic or medication-induced liver disease (36, 37, 38). It can therefore be extremely difficult to differentiate between *bona fide* AIH and 'toxic' hepatitis. In toxic hepatitis the same symptoms, immune serology and even histology may present as in AIH. The presence of eosinophilic granulocytes in the liver biopsy however may suggest medication-induced liver disorder (39). The International AIH Group (16) therefore suggests that a negative point be noted against a diagnosis for AIH if medicines that could cause liver injury were taken previously. Autoantibodies are also sometimes found in some chronic hepatitis C patients [See table 2, p8 (16)].

It is important to remember that it used to be difficult to differentiate HCV from AIH, since false-positive reactions frequently occurred with the use of the first generation of anti-HCV test kits (40), because the high gammaglobulin level in AIH could interfere with the ELISA test. It is therefore not surprising that in the past patients with autoimmune hepatitis were treated with interferon, which caused flare ups of jaundice due to the underlying autoimmune disorder (41). Since the next generations of aHCV tests have become far more specific and HCV-RNA is now routinely available, it has become easier to differentiate between chronic viral hepatitis C and an underlying autoimmune hepatitis.

Table 2: Occurrence of autoantibodies in chronic liver disorders

	AIH	Hep B	Hep C	PBC	PSC
ANA≥ 1/80	**90%**	**30%**	**20%**	**20%**	**<20%**
SMA≥1/40	**80%**		**20%**	**20%**	**10%**
LKM 1	**3%**		**6%**		
p-ANCA	**60-90%**				**>60%**
AMA	**6-12%**			**>90%**	
Type Ig	**IgG**			**IgM**	

Apart from these antibodies, several other antibodies can occur in AIH, such as the perinuclear anti-neutrofilic cytoplasm antibodies (p-ANCA). These p-ANCAs are mostly of the IgG_1 subtype, a feature that might differentiate them from the p-ANCAs that occur in PSC and ulcerative colitis (42), but distinguishing these antibodies is an expensive service often not provided. The presence of p-ANCA may be of help in the differentiation of AIH from so-called cryptogenic cirrhosis, since p-ANCA might be present in a case of autoimmune hepatitis in which ANA or SMA are no longer significantly present (42). Another antibody that may be important in the future in the diagnosis of AIH is anti-ASGPR (antibodies to asialoglycoprotein receptor). These antibodies can however also occur in chronic hepatitis B and C, alcoholic liver disease and in PBC. They are therefore of little use in making a correct diagnosis of AIH, though they do seem to be correlated to disease activity in AIH. Their quantity may play a role in determining the dosage of medication and the duration of treatment. Patients with anti-ASGPR often display rapid flare ups of the disease after decrease of corticosteroids (43). There is currently no commercial test available for determining anti-ASGPR levels.

PATHOGENESIS OF AIH

The basic pathogenesis of autoimmune diseases in general is a loss of tolerance against self-antigens. The aetiology is thought to be an as yet unknown trigger(s) initiating a self-perpetuating disease process. Potential candidates for provoking or triggering autoimmune diseases are infectious agents such as viruses and bacteria, or chemicals like drugs and other environmental agents. How and why the immune response develops and which auto-antigens are involved is currently still unexplained. In addition, a specific immunogenetic background seems to be a prerequisite. Three factors are presently considered most important, namely the female gender, specific HLA-DRB alleles and the CTLA4*G allele. The association of AIH

with genes coding for the major histocompatibility complex has been extensively investigated (31). In type I AIH patients, 85% have DRB1*0301 or DRB1*0401 on one or both alleles. These genotypes correspond to phenotypes HLA-DR3 and HLA-DR4. The relative risk of developing AIH is 2.7 for HLA type A1; 4.1 for HLA B8; 4.6 for DR3; and 12 for haplotypes A1, B8 and DR3. This does not mean that there are no AIH patients with other HLA-typing, rather that there is a clear genetic predisposition in people with these haplotypes. There is also a relationship between HLA-type and clinical picture as well as response to therapy. Progressive fibrosis is associated with heterozygosity for HLA DR3/DR4 (44). Patients with HLA-DR3 are usually younger and respond less positively to corticosteroids than patients with HLA-DR4. This phenomenon is particularly evident in Japan, where there is a very low prevalence of the DRB1*0301 allele and autoimmune hepatitis is typically associated with HLA-DR4. Patients with AIH who have the HLA-DR4 haplotype are usually older, have less active disease and respond better to corticosteroid therapy (45). The basis for this genetic predisposition is not yet known. Amino acid sequences coded by DRB1*0301 and DRB1*0401 in the antigen-bonding grooves possibly play a role in class II MHC, because they determine which antigen will be presented to the T-cell receptor. The HLA-system is however not the only system that plays a role in genetic predisposition. A number of other genes are currently under scrutiny. Most probably a combination or interaction of different genes makes an individual predisposed to develop AIH.

The pathogenesis of AIH and other autoimmune disorders remains unknown, except that it involves an increasing immunological reaction against elements of the self. Significantly liver function tests in AIH improve markedly and often normalize during pregnancy, during which immune tolerance increases (46). Presumably a decreased reaction with regard to own liver cells develops. There is however often a strong flare up in these AIH patients after confinement.

Autoimmune diseases are usually characterized by a predominance of female patients, chronic inflammatory disease process with lymphocytic infiltration into tissue, hypergammaglobulinaemia and characteristic autoantibodies. These autoantibodies are often valuable diagnostic markers and are sometimes the only hallmark of autoimmunity. Some autoimmune diseases show related pathogenic mechanisms of tissue destruction, e.g. receptor autoantibodies in endocrine diseases. However, in most autoimmune diseases tissue-infiltrating T-lymphocytes are regarded as mediators of the autoimmune process, i.e. they

represent the effector limb of the immune system for self-destruction. The antigen-specificity of these tissue-infiltrating lymphocytes is usually unknown. It is important to diagnose AIH, since appropriately adapted treatment almost completely suppresses the disease process in most patients, resulting in almost normal survival. The 5-year mortality in untreated patients can be as high as 50% especially if 'bridging' necrosis or cirrhosis is already present (31). A study in Mainz showed that the survival of patients in whom disease activity could be suppressed was equal to that of a control population (47).

The classic treatment of AIH consists of suppressing the overactive immune response. Therapy has traditionally begun with high doses of steroids (for example 60mg Prednisone) with azathioprine (Imuran) added later (48). This approach has lost credibility because the steroid-sparing effect of Imuran only reaches its optimum after 2-3 weeks. There is therefore little reason not to include Imuran from the start unless a thrombocytopaenia and/or leucopaenia or any other contraindication is present. It is also not necessary, to wait until all liver enzymes have normalized before decreasing the corticosteroid dose. The initial high dose of steroids may reduce transaminase levels more rapidly, but may also lead to more side effects such as intercurrent infections and septicaemia. This study finds that a lower dose (for example 8-12mg methylprednisolone together with 50mg azathioprine) can lead to equally positive results, as was the case in three of the older patients who developed osteoporosis (see table 3, p11). The treatment of AIH must therefore be individualized. Not all patients need to be treated. A limited number of patients, specifically those with only mild liver enzyme changes, can be kept under observation only. The absolute indications for treatment are symptomatic disease with fatigue, arthralgias or icterus and laboratory values that indicate high or serious activity of the disease, namely AST>10N or AST >5N in combination with gammaglobulines >2N, and the presence of 'bridging' necrosis or of multi-acinar collapse in the biopsy (49). The aim of treatment is to suppress liver inflammation. It must be remembered that prednisone is converted to prednisolone in the liver, and this prednisolone bonds to albumin. The hypo-albuminaemia that occurs in cases of cirrhosis thus leads to a larger fraction of free prednisolone. This may be responsible for the side effects of the corticosteroid therapy in this patient group. Cirrhosis with concomitant inflammation is one indication for treatment and the therapeutic results are the same as in patients without cirrhosis (50). In approximately half of the patients a permanent suppression of the disease process could be achieved with maintenance dose of azathioprine only. This confirms the findings of Johnson *et al* in 1995 (51).

Table 3: Evolution of liver enzymes in an older patient with type I AIH on low dose steroids

	Feb 1999	May 1999	July 1999
ALT (<31 U/ml)	**364**	**73**	**27**
Gammaglob. (g/l)	**31.4**	**26.7**	**18.3**
Methylpredn mg	**8**	**6**	**4**
Azathioprine mg	**50**	**50**	**50**

[Adapted from table in Verslype *et al* (52)].

The literature refers to a few patients who do not tolerate azathioprine (31, 49). Experience indicates that it is advisable to take the Imuran in the evening. Should intolerance occur alternatives are available, such as 6-mercaptopurine, a metabolite of azathioprine (53). A number of new immunosuppressants, known from the transplant world, could eventually play a role in the treatment of autoimmune hepatitis. Mycophenolate mofetil (Cellcept), which has a similar working mechanism to azathioprine but a greater specificity for lymphocytes, gave positive results in 5 of 7 patients who had not reacted well to the classic treatment with azathioprine (54). Other medications such as cyclosporin (as an induction treatment followed by maintenance treatment with corticosteroids and azathioprine) and tacrolimus (55, 56) look promising, but as yet no randomised comparative studies with the standard treatment have been conducted, and these drugs are mostly only used in an academic or research environment in patients who do not respond to classic treatment. When budesonide became available, this steroid alternative, which causes less side effects than classical corticosteroids, was hoped to significantly change standard therapy of AIH. However the first results with budesonide were very disappointing. In one study in which 10 patients with corticosteroid-dependent autoimmune hepatitis were switched over to maintenance treatment with budesonide, 7 of the patients suffered flare ups of the disease, and a greater frequency of side effects was also reported. This could be explained by the fact that a large section of the glucocorticoid receptors were already occupied by oral corticosteroids, which left only a few binding sites for budesonide (57). A study is currently being carried out in Europe to investigate the effects of administering budesonide immediately upon diagnosis.

Liver transplantation is necessary in rare cases. The 5 to 10-year survival rate of transplant patients with AIH is about 75%. Determining the optimal time for transplantation can be difficult, because the course of the disease is unpredictable in most cases. The disease can recur after transplantation. The literature describes a small series of patients who, after

transplantation for a non-autoimmune disorder, developed autoimmune hepatitis (58). There is however usually a good response to restarted treatment with corticosteroids. In a number of cases the recurrence of AIH leads to the loss of the transplanted liver. There is also a higher incidence of chronic rejection reported for liver transplantation in AIH (58).

RESEARCH METHODOLOGY

The initial research was done in Leuven, Medical data relating to 97 patients diagnosed with AIH were researched, 15 patients were subsequently added. The patient data was tabulated in summary. The essential facts were then divided into specific categories. These categories related to; age, gender, medication used, concomitant Hepititis C and features relating to a billary component, observations relevant to the study, i.e. relapse after confinement in the AIH plus pregnancy group, and the age above 65 at diagnosis group, were further developed via intensive searches by the researcher in the Leuven Library.

Regular meetings with Prof. Johann Fevery helped the researcher to develop clarity of purpose and direction.

The data collected by the researcher for chapter on AIH at age 65 or older was collated by Dr Verslype who developed the tables for the said data.

AIMS OF THIS STUDY

Careful analysis of a cohort of 112 patients with AIH (the first chapter of the results section), revealed several aspects of AIH which deserved further analysis. The focus of this thesis is to clarify or explore these areas, which have in the past either attracted little interest or warranted review in the light of the cases that were identified. These aspects are:

1**. Age distribution of AIH**

Classical textbooks describe AIH as a disease mainly seen in young women. Initial analysis of the cohort suggested that this is not the case. AIH in 28 elderly patients diagnosed at age 65 or older was therefore analysed and compared with 84 younger patients.

2. **Pregnancy in AIH**

Pregnancy in chronic liver disease and therefore also in autoimmune hepatitis was traditionally thought to be rare, but it appears to be becoming more frequent as therapy improves. The study aimed to analyse the consequences for mother and child in AIH patients.

3. **Overlap between AIH and PSC**

Diagnostic criteria for AIH have been put forward and validated. Despite this, some cases remain difficult to classify because they exhibit features of other immune-related liver diseases. Other cases are initially classified as definite AIH but go on to develop features of PSC or PBC. Overlap behaviour between AIH and PSC is reported in two cases together with a review of the 'overlap' literature.

4. **Toxic hepatitis presenting as AIH**

Acute or chronic drug-related hepatic reactions may be indistinguishable from idiopathic AIH. The patients' symptoms, serology and even histology can be very similar. The development of toxic hepatitis masquerading as AIH in five patients is documented and the relevant literature researched.

5. Autoimmunity and hepatitis C

Hepatitis C is a chronic liver disease whose biochemical, serological and clinical features may be indistinguishable from those of AIH. It is also well recognised that the autoantibodies present in AIH are also frequently present in hepatitis C patients. In addition, an array of immune-mediated symptoms and diseases also sometimes occur in patients with chronic hepatitis C. Two patients who had or were thought to have both AIH and hepatitis C and who were treated at different times with both corticosteroids and Interferon were recorded.

CHAPTER 1

AUTOIMMUNE HEPATITIS: AN OVERVIEW OF 112 CONSECUTIVE PATIENTS

Adapted from an article by C George, E Buchel, W van Steenbergen, F Nevens, C Verslype, P Yap, J de Groote, J Fevery. Tijdschr voor Geneeskunde 2004; 60: 880-9.

Introduction

In this chapter a series of 112 consecutive patients with autoimmune hepatitis who were diagnosed and treated in the Gasthuisberg University Hospital, Leuven, Belgium between 1975 and 2001 is analysed in detail.

Patients and diagnoses

112 patients were diagnosed with AIH according to the diagnostic criteria laid down by the International Autoimmune Hepatitis Group [Table 1, p 4 (16)] and subsequently adapted and published in J Hepatology (16). Laboratory tests were done according to the classical prescribed methods and pathologists with a particular interest in liver disease interpreted the biopsies. The tissue antibodies in the serum were traced with immuno-fluorescent techniques, mostly on human hepatoblastome (Hep G_2) cells.

Results of the overview of the 112 patients

The distribution according to age is set out in figure 1. The disorder presented in a male: female ratio of approximately 1:2,5. A particular age predominance could not be demonstrated in these patients.

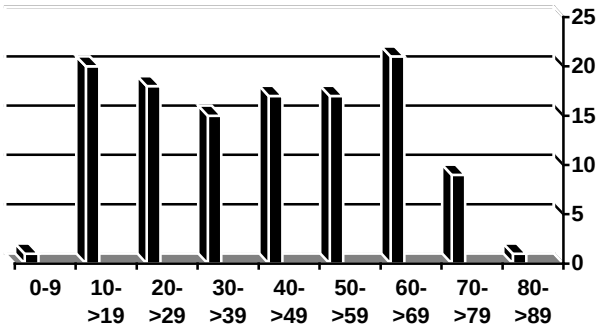

Figure 1: Age distribution of the 112 patients with autoimmune hepatitis

The symptoms and findings that motivated the diagnoses were very diverse, varying from incidental findings of raised serum transaminases to a picture of fulminant hepatitis with threatening liver coma. The most frequent symptoms were fatigue, malaise, anorexia, weight loss, abdominal pain and arthralgias (table 4). In the series, 15% of the patients were asymptomatic; in these cases the diagnoses were incidental findings or were postulated within the framework of an investigation for another autoimmune disorder such as thyroiditis, vasculitis or arthritis.

Table 4: Complaints and physical examination on diagnosis

Symptoms*	**%**	**Physical examination***	**%**
Asymptomatic	15	normal	35
Fatigue, malaise	40	icterus	50
Abdominal pain	12	ascites	15
Arthralgias	20	encephalopathy	3
Itching	10		

*combinations present

The physical examination was completely normal in 35% of the patients; in the others hepato-splenomegaly, ascites or peripheral oedema were seldom present. Approximately half of the patients were icteric at presentation or reported one previous episode of jaundice.

The most important biochemical deviations at the time of diagnosis are given in table 5; considerable variations are evident. The elevation of transaminases is very varied. Bilirubin

concentration is frequently raised with otherwise normal or almost normal alkaline phosphatase (ALP) levels. An important diagnostic element is the hypergammaglobulinaemia (13); which is of the IgG type in contrast to IgM elevation in PBC and IgA elevation in alcoholic liver disease.

Table 5: Laboratory tests at diagnosis

ALT U/L	%	Bil. mg/dl	%	Gamma glob. g/l	%
>200	33	< 1	28	<15	27
200-600	30	1-2,5	17	15-25	22
600-1000	15	2,5-5	14	25-50	41
> 1000	22	5-8	11	> 50	10
		>8	30		

As indicated in the diagnostic criteria, the presence of a significantly raised titre of tissue antibodies in the serum is a very important diagnostic tool (15, 16). On the basis of the presence of these antibodies, AIH is divided into two types. Type I is characterized by the presence of positive ANA or of antibodies directed at smooth muscles (SMA or anti-actine). Type II is characterized by the presence of liver-kidney microsomal (LKM) antibodies. The group of patients included three young females in which the diagnosis of type II AIH was made at the ages of 14, 22 and 25 years respectively, on the basis of the clinical findings, a liver biopsy and the isolated presence of LKM antibodies in the serum. Most of the patients belong to the type I group. In many instances, both ANA and SMA were present. In 26% only a high ANA titre was found, while an isolated rise in SMA was present in 16% of the patients. In two patients both antibodies were negative but all other criteria and the response to long-term treatment with corticosteroids were proof of the diagnosis. In one of these two patients chronic hepatitis C was suspected (the HCV antibodies later proved to be a false positive). During treatment with Interferon a flare up of icterus occurred, due to the underlying autoimmune hepatitis. p-ANCA was positive in 23 of the 44 patients in whom these antibodies were looked for. AMA which are classically found in PBC, were positive in 6% of the cases; the literature reports figures of up to 12% (see chapter 1; table 2).

The histological picture of the liver was also very varied (table 6, p18).

Table 6: Histological findings

-Minimal changes	0,9%
-Interface hepatitis	54%
-Fulminant necrosis	15%
- Cirrhosis	25%
- Biopsy refused or too risky	5%

A light non-specific hepatitis was found in one person, while severe necrotising hepatitis was present in 16 patients. In 54% a characteristic picture was demonstrated that consisted of interface hepatitis with portal or peri-portal lympho-plasmocytic infiltration. Cirrhosis was already present at the first biopsy in 25% of the patients in spite of acute presentation. This shows that the disease is present sub-clinically in many patients in whom the histological process is following an aggressive course. In 16 patients the interlobular bile ducts were also seen to be affected. This biliary component was so pronounced in eight patients that the diagnosis was made of an overlapping syndrome AIH/PBC or of autoimmune cholangitis (called AMA-negative PBC in the literature). In two other patients a cholestatic syndrome developed after many years, and the diagnosis of sclerosing cholangitis was made after ERCP. These patients demonstrate the so-called overlap phenomenon between AIH and PSC.

Associated immunological disorders were found in 35% of the AIH type I patients. These disorders were mainly autoimmune thyroiditis, vitiligo, lupus, arthritis, Crohn's disease and autoimmune pericarditis. This concurs with the 41% quoted in chapter 1 on page 4.

A positive response to the prescribed immunosuppressive treatment is one of the criteria for the diagnosis of AIH. In a series 15 patients initially received only corticosteroids, while the others were treated with a combination of steroids and azathioprine (Imuran). Of the total group all treatment could be discontinued in only 15% of patients. The corticosteroids were decreased and stopped in 50% of the patients; they were treated further with azathioprine only. At the last evaluation 25% of all patients still had discreetly raised transaminases.

Complications during therapy usually presented early. These consisted mostly of bleeding esophageal varices, which necessitated the insertion of a porta-caval shunt in two patients, and of spontaneous bacterial peritonitis (SBP) in patients with ascites.

Of the entire group, five patients received liver transplants. One patient, in whom the diagnosis of AIH in acute liver failure was made at admission, was transplanted immediately. The other four initially responded well to treatment, but eventually had to be transplanted due to a less than adequate response to treatment, respectively two, 12, 14 and 24 years after diagnosis. One of these patients died immediately after transplantation due to an invasive aspergillosis.

Besides this one patient, AIH proved fatal in nine others. Three died of acute liver failure before they could be transplanted. Three others died later (that is, more than 15 years after diagnosis), two due to hepatocellular carcinoma (developed 10 and 26 years respectively after diagnosis), and one died of cerebral lymphoma. Three elderly patients died of sepsis, probably co-induced by the high doses of corticosteroids that were then still the standard treatment. In one of the patients a neuro-endocrine tumour was diagnosed and removed curatively at an early stage.

DISCUSSION

The classical description of AIH as a disease affecting young woman and women in their middle years (59) was not borne out by the results of this study. A particular age distribution could not be demonstrated in the patients of this study. Indeed a significant number (twenty-eight) were above the age of 65 years and form the basis of a separate analysis (see chapter 2). Similarly, the most often described and most common presenting symptoms of fatigue, anorexia, weight loss, abdominal pain and arthralgias were prevalent in the majority (85%) of the cohort of 112 patients. This is in keeping with most other series and underlines the imperative of considering AIH as a possible diagnosis when a patient's symptomatology appears vague; malaise, lack of appetite and non specific aches and pains can indeed fulfil the criteria of a multiplicity of diagnoses, from depression to anaemia!

The presence of jaundice in half the patients was undoubtedly fundamental in directing further clinical investigations towards a liver-related aetiology. It is highly unlikely that a diagnosis of AIH was initially considered in these patients until after viral studies were found to be negative. It was also only after thorough and repeated history-taking enquiring into alcohol, medications and herbal substances had excluded toxic hepatitis that AIH became a serious consideration. Conversely, the finding of several cases of toxic hepatitis which were

first diagnosed as AIH focussed attention on an important subgroup of patients whose clinical presentation, biochemistry, serology and even histology were so similar to those of AIH that the masquerade was not initially suspected. These misdiagnoses were recognised when further clinical developments, such as flare ups after restarting or improvements after stopping the offending medication or persisting improvement after cessation of steroids threw doubt on the original diagnosis of AIH in these patients (see chapter 5).

An important observation is the relatively high proportion of cases with severe liver damage i.e. cirrhosis and portal hypertension with complications such as ascites, at the time of diagnosis. These patients had until then been unaware of having chronic hepatitis. This underlines the known tendency of AIH to progress sub-clinically for several years before manifesting itself, and then only with vague symptoms such as tiredness and joint pains. Other researchers have also observed this tendency. This study's finding of biopsy-proven, established cirrhosis in approximately one third of patients is similar to that reported in other series (60, 61).

Biochemistry results show considerable variation. In acute viral hepatitis the level of transaminases are mostly hepatitic i.e. >10 times the normal upper level, however in only slightly more than one third of the patients (37%) was the ALT initially above 600. However a raised gammaglobulin i.e. above 15mg/l was found in 73% of the patients, and therefore, as MacKay, Taft and Cowling mentioned in 1956, hypergammaglobulinaemia remains a cornerstone feature of the diagnosis of AIH.

The immune serology of the group of patients studied, (i.e. ANA and/or SMA positive) categorized all except three patients as belonging to type I AIH. The three patients who were anti-LKM positive and therefore suffering from type II AIH were under the age of 25 years and female. The lack of anti-LKM in patients above 25 years of age in less than 3% of the total cohort makes It is considered to be cost-ineffective to initially test for LKM antibodies and suggest that this test only be requested after all other serologies are negative in a patient with liver disease of unknown cause which could feasibly be AIH.

Liver biopsy remains an important cornerstone of the diagnosis of AIH, and in the cohort was particularly useful in pointing us towards the 'overlap' or overlapping 'variant' syndrome in

eight patients. Two of these patients who later developed a cholestatic syndrome were shown to have progressed to PSC although they had initially met the criteria for 'definite' AIH. The clinching investigation in these two patients was of course endoscopic retrograde cholangiography (ERC) (see chapter 4). The reality is that the diagnosis of AIH in a patient presenting with disturbed liver functions is not always straightforward but requires time and reflection on the individual patient's symptomatology and test results. This is one factor that often contributes to the known extended time to diagnosis.

The finding of associated immunological disorders in 35% of the patients, particularly thyroiditis, arthritis, vitiligo, lupus and pericarditis, is in line with other series analyses. This suggests that if the diagnosis of AIH is made additional immune-related organ involvement should always be looked for.

Finally, the experience gained in treating 112 patients over a quarter of a century can be summarised in two sentences: Firstly – it is not necessary and may be harmful to initially give high doses of steroids to patients – lesser doses i.e. $^{1}/_{2}$mg per kg are just as effective in the long term, and although transaminases take longer to normalize, these patients manifest fewer side-effects.
Secondly – the early initiation i.e. at the time of initial diagnosis, of azathioprine (Imuran) and the subsequent tapering of steroids while maintaining or increasing azathioprine is, it is felt, could be beneficial. Indeed in patients above 65 years of age it was found that side-effects, notably sepsis, were more frequent in patients initially given high doses of steroids.

So in summary, the lessons learned from this cohort of 112 patients have contributed to an adjusted approach to the investigation and initial and long-term treatment of AIH patients. This fine-tuning of proven standards will shorten the time to diagnosis, lessen the morbidity and increase the longevity of AIH patients.

Furthermore, the division of the total cohort into specific themed groups such as AIH and pregnancy, AIH in the elderly, AIH and hepatitis C and AIH and hepatotoxic medications, the subsequent analysis of each of these groups and the recognition of hitherto undescribed features as well as the confirmation of already described aspects of AIH justify this study. Indeed, these unanticipated observations made the whole project a satisfying experience.

SUMMARY

An overview is given of 112 consecutive patients with autoimmune hepatitis. The results show a large variability in symptoms and complaints at diagnosis. The disorder can present as acute or chronic, and can be diagnosed because of incidentally found transaminitis during liver function tests or acute liver failure. The diagnosis must be considered in patients with non-viral liver disease with significantly raised gammaglobulin and positive ANA and/or SMA. There is no fixed age predisposition but rather an even spread over various age groups. The male:female ratio is 1:2,5. This disorder can for the most part be effectively treated with a combination therapy of corticosteroids and azathioprine. This study showed that high doses of corticosteroids could cause serious complications, particularly in older patients. A lower dose is mostly adequate, although biochemical normalisation may take longer. In approximately half of the patients, cortisone could be discontinued. The long-term survival of adequately treated patients is almost that of the control group. In total ten patients died: three presented with acute liver failure, two died later of hepatocellular carcinoma, one died of cerebral lymphoma, three older patients died of sepsis, possibly co-induced by high doses of steroids. Five patients underwent liver transplants, after which one died of aspergillosis.

CHAPTER 2
AUTOIMMUNE HEPATITIS AT AGE 65 OR OLDER

INTRODUCTION

Autoimmune hepatitis (AIH) is a chronic necro-inflammatory disease of the liver, which mostly, and often silently progresses to cirrhosis. Classical textbooks describe AIH as occurring mainly in young females and in postmenopausal women (12, 13, 59). It was thought to be uncommon in older patients (62). However, more recent studies show it can occur at any age (15) and, though predominantly observed in females, is also diagnosed in males. Criteria for the diagnosis have been outlined (15) and improved (16).

In reviewing 112 patients with AIH in follow up it was found that the diagnosis was made at age 65 or above in 28 patients. The aim of this chapter is to analyse whether this group of elderly AIH patients has special features or requires another therapeutic approach. Other groups have also reported on AIH in the elderly (63, 64, 65, 66).

PATIENTS

In the Gasthuisberg Academic Hospital, Leuven, 112 patients were diagnosed with AIH between 1975-2002. Of the total group, 77 (68.5 %) are female and 35 (31.5 %) are male. The diagnosis was made according to the criteria put forward by the International AIH Group (16), based on clinico-laboratory characteristics, presence of autoimmune antibodies in serum, liver biopsy specimens and response to steroids. Of these 112 patients, 28 (25 %) were diagnosed at age ≥ 65 yr. This group is analysed in detail. It was made up by 21 females and seven males.

METHODS

Liver function tests, virology and serology were carried out using standard methodology.

STATISTICS

The Mann-Whitney rank sum test was used to analyse significant differences between the elderly and younger patients.

RESULTS

The disease was almost equally spread over the different decades, but a bimodal distribution was apparent in the female patients (table 7). In 28 patients (25%) the diagnosis was made at age 65 or older. The gender spread was 21 females and seven males (Table 7a and table 8, p26). All except three had an AIH score greater than 17 under treatment; three patients scored 'probable AIH,' two because of a lack of a biopsy, and the third because of not having the gammaglobulin result at diagnosis. The presenting symptoms consisted mainly of typical complaints such as fatigue, general malaise, vague pain or dyspepsia in ten patients, and of arthralgias in one patient (Table 8, p26). The diagnosis was made incidentally during a preoperative examination in two patients. Acute jaundice was the most frequent presenting symptom, seen in 14 patients. Two of these 14 patients also had pruritus, two encephalopathy and two jaundice with ascites. In three patients, ascites was the predominant symptom. The clinical signs are summarized in Table 8; p26. They do not differ significantly from those of the younger patients except for the lower incidence of arthralgias. Jaundice was present in half of the patients.

Table 7: Age and gender in the cohort of AIH patients

Age/ years Gender	0-9	10-19	20-29	30-39	40-49	50-59	60-69	70-79	80-89	Total
Male	1	7	5	7	3	5	2	5		35
Female	-	13	15	2	14	10	18	4	1	77
Total	1	20	20	9	17	15	20	9	1	112

Table 7a: Age and gender in the cohort of AIH patients

Age/ years Gender	0-9	10-19	20-29	30-39	40-49	50-59	60-64	65-69	70-79	80-89	Total
Male	1	7	5	7	3	5	-	2	5		35
Female	-	13	15	2	14	10	2	16	4	1	77
Total	1	20	20	9	17	15	2	18	9	1	112

An overview of the laboratory tests at presentation is given in Figure 2, p25 and table 9, p26. The test results show a large spread but this is not different from the results of the younger patients. ANA was negative or present only in low titre in three patients, positive (titre ≥ 1/80) in 20 patients and strongly positive (>1/1280) in five patients; SMA was below 1/40 in 14 patients and ≥ 1/40 in 14 patients. The three patients with negative or low ANA were all positive for SMA.

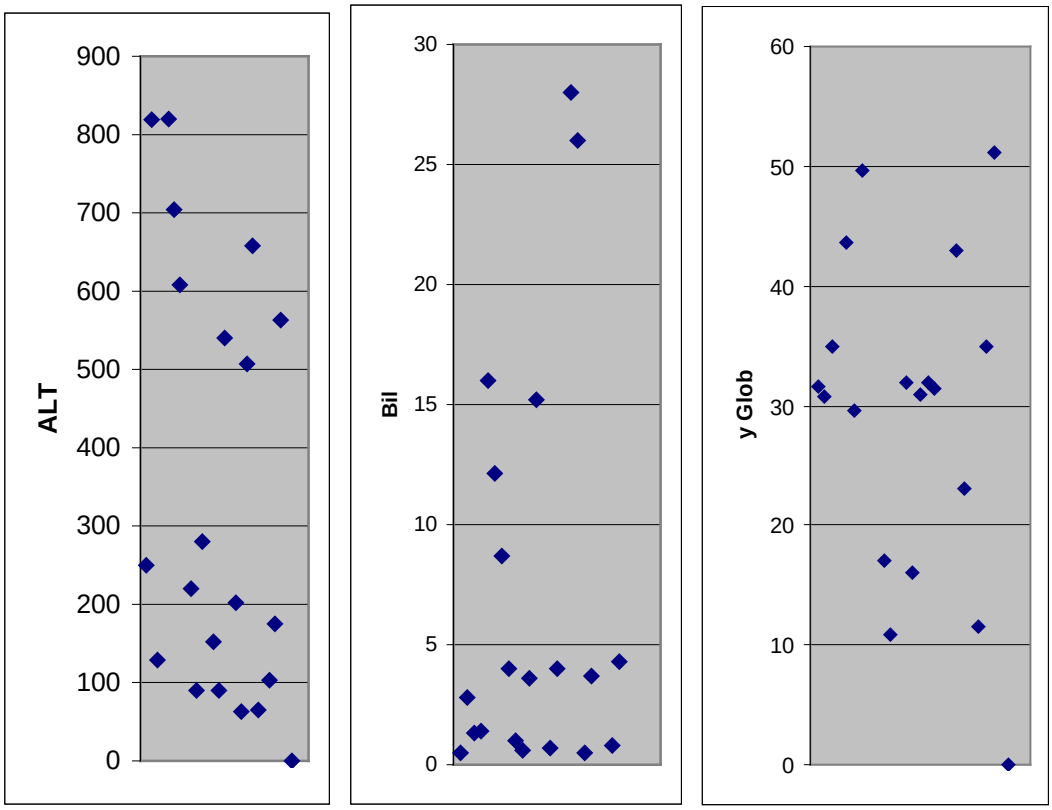

Figure 2: Laboratory test results of the elderly AIH patients at diagnosis

Three patients refused liver biopsy. Biopsy showed in five of the remaining patients severe necrotising hepatitis with areas of collapse, in eight mild chronic hepatitis with portal lympho-plasmocytic infiltration, in four severe chronic hepatitis with marked piecemeal necrosis (interphase hepatitis), in three active cirrhosis and in five cirrhosis with mild inflammatory activity. Thus overall eight (29%) had cirrhosis at the time of diagnosis. Two patients died shortly after the diagnosis was made: one of hepatic encephalopathy and one due to combined *Aspergillus* pneumonia and *Klebsiella* septicaemia, possibly partly due to the high dose of corticosteroids favoured as treatment at that time.

All patients, except one, received corticosteroids combined with azathioprine (1 mg/kg) as initial treatment; the one exception did not receive azathioprine because of marked thrombocytopaenia. A severe respiratory infection necessitated temporary interruption of

azathioprine in two patients. The amount of methylprednisolone given ranged from a low dose of 8 mg per day in four patients, to 16 mg in most patients, 24 mg in five patients and 32 mg in one patient. In recent years, it has been found prudent to decrease the dose of steroids given. At present, half of the patients are on azathioprine alone, with the others receiving azathioprine with an additional 2-4 mg of methylprednisolone. At present, liver enzyme tests are normal or nearly normal in all patients. All medication could be stopped in only one patient.

Table 8: Clinical characteristics of 28 elderly AIH patients compared to 84 younger AIH patients

	N = 28 elderly ≥ 65 years	**N = 84 younger < 65**
Gender M:F	7 : 21	28 : 56
Presenting symptoms:		
- incidental findings	2(7)	2(2)
- fatigue, malaise	10(36)	20(24)
- arthralgias	1(3.5)	11(13)*
- jaundice+ other symptoms	14(50)	44(52)
- ascites	3(11)	7(8)
Physical examination:		
- insignificant	10(36)	29(34)
- jaundice	14(50)	44(52)
- ascites	5(18)	12(14)
- encephalopathy	2(7)	1(1)

***P<0.05 Values expressed in parentheses are percentage.** Adapted from Verslype *et al.* (52)

Table 9: Laboratory tests at diagnosis

	Older patients (n=28)	Younger patients (n=84)	P-value
ALT*	535(114-904)	300(156-795)	0.936
T.bili	4.69(1.00-10.50)	3.00(0.93-9.68)	0.429
γ-globulin	31.1(23.9-43.2)	27.6(20.2-36.9)	0.295
ANA≥1/180(%)			
positive	25(89)	69(82)	0.116
negative	3(11)	15(18)	
SMA ≥ 1/40(%)			
positive	14(50)	70(83)	0.090
negative	14(50)	14(17)	
Anti-LKM (%)			
positive	0	3(4)	
AMA (%)			
Positive	0	5(5.9)	

***Median and lower (first) and upper (third) quartile**
ALT=alanine aminotransferase. Adapted from Verslype *et al* (52)

Table 10: Histology at diagnosis

Biopsy:	Patients*: **Older (n=28)**	**Younger (n=84)**
- minimal changes	-	1 (1)
- severe necrotizing hepatitis (+ collapse)	5 (19)	15 (18)
- mild hepatitis + lympho-plasmocytic infiltration	8 (30)	14 (17)
- active interphase hepatitis (piecemeal necrosis)	4 (15)	21 (25)
- cirrhosis+ interphase hepatitis	3 (11)	12 (14)
- cirrhosis	5 (19)	13 (15)
- biopsy refused or too risky	3 (11)	9 (10)

* percentage given between brackets

Adapted from Verslype *et al* (52)

DISCUSSION

The diagnosis of AIH was equally spread over all age decades but 25% of the patients were 65 years or older at the time of diagnosis. Similar cases have recently been documented (64, 65, 66). Diagnosis seems to be delayed in the elderly, presumably because AIH is erroneously considered to be a disease of youth (66). In this study , the median time to diagnosis was more than twice as long in the older patients than in the rest of the cohort: 8,5 months {range,0-348 months} p=0,048. AIH must therefore be included in the differential diagnosis of liver disease in the elderly population. This contrasts with the classical nomenclature for AIH, as 'juvenile cirrhosis' (13). In the young females in the cohort, jaundice, amenorrhoea and arthralgias were the major presenting symptoms. Except for amenorrhoea, these symptoms were the same in the elderly population, a fact also noted in other studies (63, 64, 65, 66). These same authors also note that not many patients with AIH presenting in advanced age have been described.

The clinico-biochemical or histological spectrum in the elderly group is also very similar to that of the younger patients. An acute presentation, with pronounced jaundice and a biopsy showing severe necrotizing hepatitis with collapse, was present in 19%. Five patients had ascites, and two had encephalopathy, underlining the severe presentation. Although an acute presentation was most frequently seen, 30 % already had cirrhosis in the biopsy at the time of diagnosis (Table 10). This supports the data of Nikias *et al*. (23), who find no significant histological differences in a group of 12 AIH patients with acute presentation versus 14 with clinically chronic disease. Nikias *et al* suggest the presence of a pre-existent sub-clinical

disease only unmasked later on as explanation of this finding. At the other end of the spectrum, eight patients in the cohort were diagnosed because of mild, asymptomatic elevation of transaminases, only incidentally detected in one patient. Most often these last mentioned patients complained of mild fatigue, and one them only complained of arthralgias.

The elderly patients included in this sub-group were selected on the basis of the internationally defined criteria. Overall, ANA ≥ 1/80 was absent in three patients, and SMA absent or below 1/40 in half; the latter incidence is less than in the younger population. A decade ago both Czaja *et al*. (67) and Kaymakoglu *et al* (68) pointed out that cryptogenic acute or chronic hepatitis has pronounced similarities to AIH and suggested that the presence of ANA or SMA might not be an absolute prerequisite for the diagnosis. The cases described in these two studies all had active disease either acute or chronic. However, cryptogenic cirrhosis diagnosed because of ascites or variceal bleeding is not a rare event. Some of these cases could feasibly represent burnt-out AIH or otherwise could be late presentations of post-alcoholic or non-alcoholic steatohepatitis (NASH)-induced cirrhosis.

In the case of an acute presentation, differentiating between AIH and toxic necrotizing hepatitis is not always easy. The elderly patient often takes a series of medications, and several drugs such as isoniazide (INH), α-methyldopa and nitrofurantoin were frequently taken in the past. At present it is more often NSAIDs and antidepressants, which can induce positive serum ANA or SMA (36, 37, 38). A biopsy revealing (sub) acute hepatitis with confluent necrosis can result from both AIH or toxic hepatitis, and differentiation can be difficult, especially when the predominance of plasmocytes (favouring AIH) or alternatively eosinophils (suggesting a toxic origin) infiltrating the liver is not very pronounced (39). Amelioration of symptoms or signs upon cessation of the suspected drugs can be helpful but such improvement may take a long time to happen (69). Also, steroid therapy is sometimes introduced with improvement in both AIH and toxic hepatitis! The response to steroids is thus of little diagnostic help, though relapse upon cessation of steroids points to AIH rather than to a toxic cause. One of the patients had experienced a previous episode of acute hepatitis thought to be of toxic origin due to intake of cimetidine (70). In retrospect, it is difficult to differentiate whether this episode was really due to cimetidine or rather a first upsurge of AIH. It turned out that three (11%) of the patients had experienced a documented episode of ‘unexplained’ hepatitis several years prior to the current diagnosis of AIH. This tendency is not uncommon, and occurred in up to 18% of 51 Swiss patients with AIH (35). The elderly

patients with AIH responded very well to corticosteroids and azathioprine. Two patients (one 63 years and one 67 years old) died, as a result of *Salmonella* and *Klebsiella* septicaemia respectively. This last patient also had an *Aspergillus* pneumonitis, which manifested during treatment with high doses of corticosteroids. Severe respiratory infection with ensuing cardiac decompensation was seen in another case. Furthermore, osteoporosis often pre-exists in female patients and steroid therapy may lead to further rapid deterioration and even vertebral collapse. The policy is to use lower dosages of steroids in the older population with AIH. The experience indicates, lower steroid doses still lead to sufficiently rapid improvement in general condition and laboratory test results (Figure 3, p30 - 32). This is clearly at odds with the advice given by a German group (66), however generally speaking, most recent overviews tend to advise lower dosages (71) than previously used (31). In the absence of severe thrombocytopaenia, it also does not seem logical to wait one or more months before starting azathioprine, since this drug takes 6-8 weeks to become fully active (71). Azathioprine has a 'steroid-sparing' action and has proven to be effective in the prevention of relapse even when given as a single drug (51). Since its pharmacological action takes a few weeks to take effect, azathioprine should be added at the start of therapy.

Case 1.

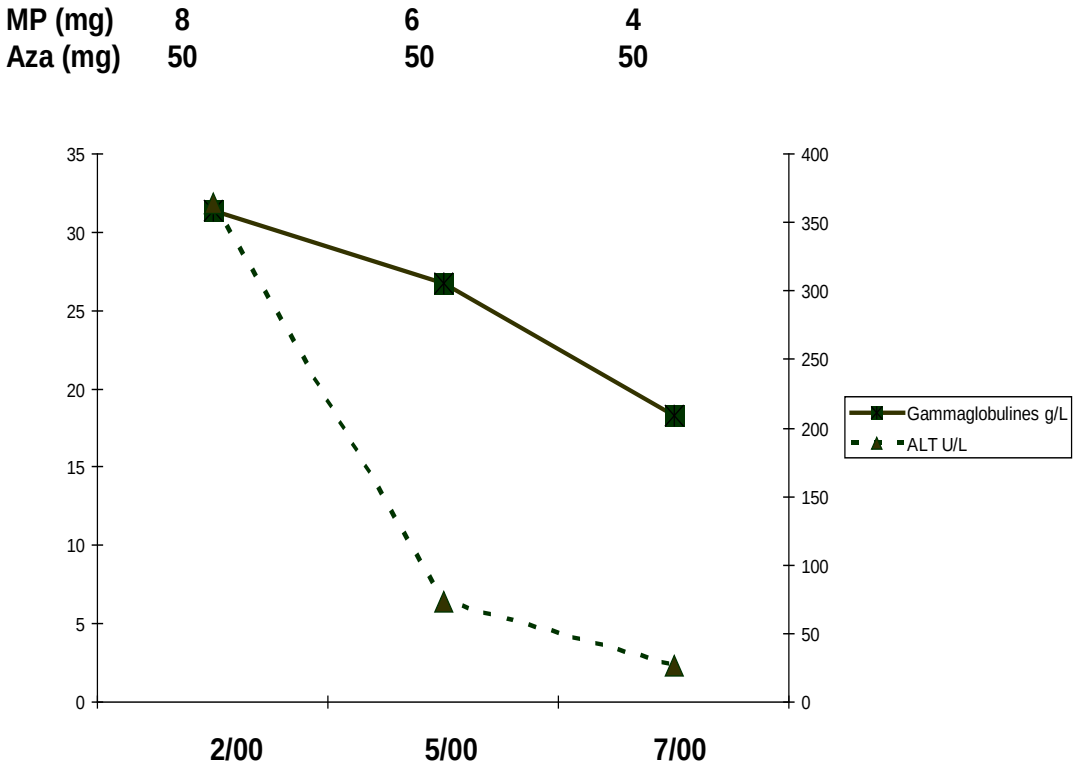

Figure 3: Treatment with low dose of steroids and azathioprine in some illustrative cases. Adapted from Verslype *et al* (52)

Case 2.

MP (mg)	8	8	6	4	3	2	2
Aza (mg)	25	25	25	25	25	25	25

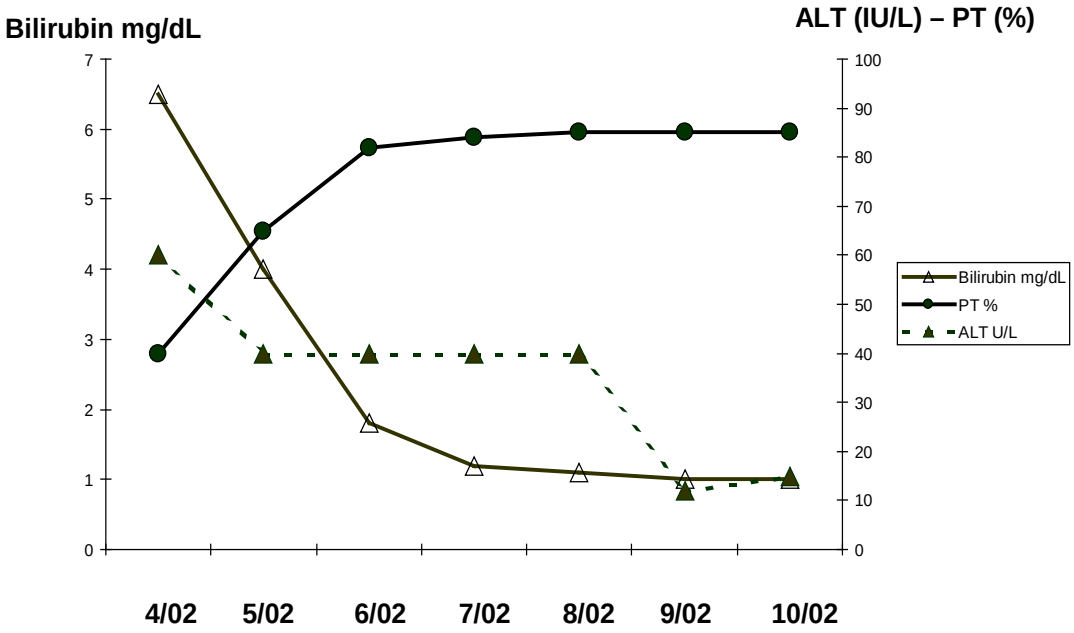

Figure 3: Continued

Case 3.

MP (mg)	**6**	**4**	**4**	**3**
Aza (mg)	**50**	**75**	**75**	**75**

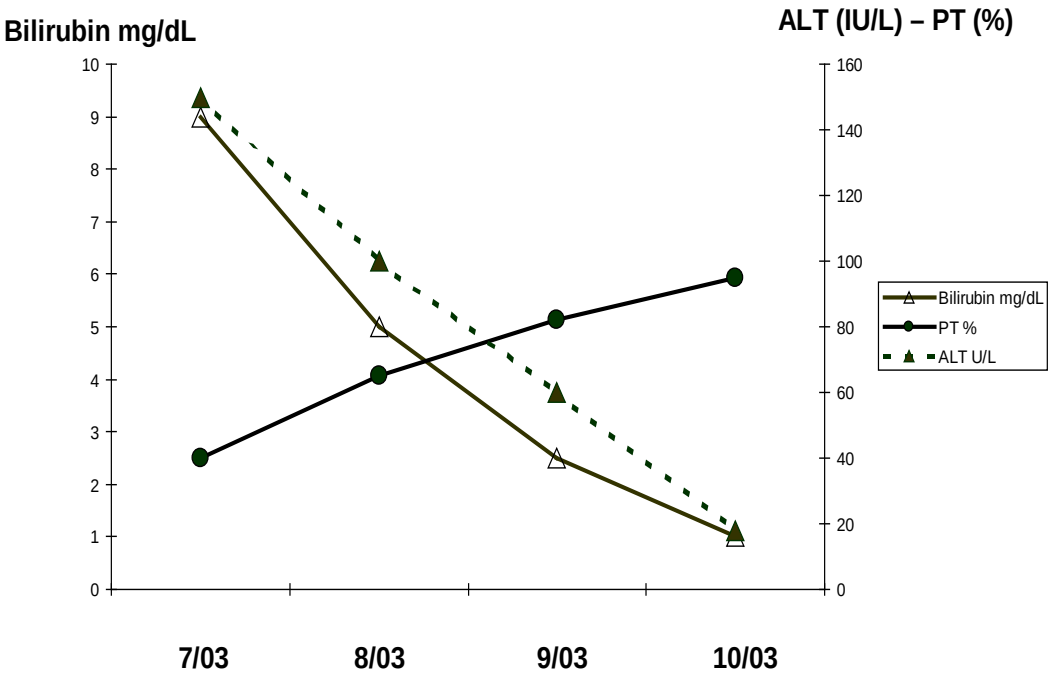

Figure 3: Continued

In conclusion, AIH has to be considered as a possible diagnosis in patients aged 65 or older who present with acute jaundice, chronic liver disease or nearly quiescent 'cryptogenic cirrhosis'. With regard to treatment, steroid therapy should be individualized but preferably started immediately, and kept, at low dose (if possible) to avoid complications. azathioprine should be added from the start of therapy.

CHAPTER 3
AUTOIMMUNE HEPATITIS DURING PREGNANCY

INTRODUCTION

AIH is a chronic necro-inflammatory disease with a diverse spectrum (16, 31, 72,). The pathogenesis and possible triggering factors remain unknown. In Caucasians, more than half the patients have HLA type A1, B8, DR3 or 4 (16, 30, 73), which underlines a genetic predisposition. The disease is subdivided into type I, characterized by the presence in serum of ANA and/or SMA in high titre, and type II consisting mostly of female children with aLKM as discussed in the general introduction.

AIH is prevalent in young women in their childbearing years (72).These woman often desire to fall pregnant, but since most cases already have cirrhosis at the time of diagnosis, this is not to be expected. Indeed, oligo-amenorrhoea or anovulation, presumably due to hypothalamic-pituitary dysfunction, is often one of the presenting symptoms (74). Under adequate immunosuppressive therapy, disease activity mostly regresses and normal menstruation returns. The question remains whether pregnancy is a threat for a patient with AIH, the foetus or the newborn child. In other liver diseases, stillbirths occur quite often and premature birth can frequently lead to foetal or neonatal wastage (75). However, the incidence of congenital malformations does not seem to be enhanced in AIH (76, 77, 78). Bleeding from oesophageal varices can occur during Valsalva manoeuvres at delivery, but less frequently than was previously thought. This can be avoided by improved obstetrical handling and the preventive administration of vasoactive drugs such as propranolol combined with spironolactone or isosorbide-5-mononitrate, as demonstrated by experience in two patients with portal vein thrombosis and oesophageal varices, who spontaneously delivered three neonates under this combined therapy (79).

A total of 98 patients with AIH and eight with autoimmune sclerosing cholangitis (ASC or overlap AIH-PSC) attend the Liver Clinic in Leuven. Among the 72 females with AIH and the four with ASC, six have been followed up during several pregnancies. This chapter describes the clinical history of five patients with AIH and one with ASC, who between them had 14 pregnancies. In all, liver function tests improved markedly after the third month of

pregnancy, but flares-ups were noted in ten of 14 following delivery. Therapy should therefore be adapted.

DESCRIPTION OF PATIENTS

Patient 1

This woman developed severe arthralgia on her twenty-fifth birthday. At that time, she had an ALT of 643 IU/L, and her ANA was weakly and SMA strongly positive. A liver biopsy confirmed AIH. Methylprednisolone (MP) was initiated at 24 mg and slowly decreased as AZA was added. A control biopsy after one and a half years showed significant improvement. AZA was stopped and MP gradually reduced and stopped after three and a half years of treatment. At age 29, she was found to be pregnant. Her ALT was 81 IU/L, and normalized from the fourth month onwards. Her term delivery was followed by an increase of ALT to 519 IU/L. The MP was reintroduced at 24 and later at 32 mg.

The patient became pregnant again one and a half years later. ALT decreased from 372 to near-normal values at month 6 and MP was reduced to 10 mg. The patient had a premature delivery at week 32; this was followed by a rapid doubling of ALT (with further increase to 210 IU/L three months after delivery) and an increase of gammaglobulins. Since than, she remains under therapy with MP 8-12 mg and AZA 75mg.

Patient 2

AIH type I with cirrhosis was diagnosed at age 17 years, and treated with 16 mg MP and AZA 50 mg per day. Five years later, she still had active disease and irregular menstruation; the steroids were increased to 24 mg, and AZA to 100 mg. Transaminases settled down and MP was gradually reduced to 12 mg. At age 23, her pregnancy test proved positive; at that time ALT was 110 IU/L and serum bilirubin 2.23 mg/dl. The AZA was stopped and MP reduced over the next month to 8 mg. She delivered a healthy female infant in August 1993. One month later, her ALT had risen to 252. The MP was increased to 16 mg and AZA restarted at 75 mg.

One year after delivery, she mentioned that she was again 19 weeks pregnant. She was on 8 mg MP and had stopped the AZA herself just recently. The MP was gradually reduced to 2

mg per day. She delivered a healthy full-term baby. Her hepatitis flared up immediately after this second delivery despite a proactive increase of the steroid therapy to 24 mg and reintroduction of AZA 50 mg. At the time of writing she was still maintained on MP 6 mg and AZA 50 mg.

Patient 3

This patient was diagnosed with AIH at age 29 years after presenting with diffuse arthralgias, hypergammaglobulinaemia and positive SMA, ANA and AMA. Her liver biopsy was compatible with AIH. AMA can be positive in up to 8 % of cases with AIH (33). She expressed the wish to become pregnant and refused all therapy. She became pregnant after two years and her ALT decreased from 200 IU/L to normal from the sixth month onwards. Her hepatitis flared two weeks after delivery with an ALT of 206 IU/L, but she continued to refuse therapy. A second pregnancy progressed without problems, again with normalization of transaminases. It was ended full-term with a caesarean section because of foetal distress during delivery in April 1996. Within two weeks, she developed severe arthralgias and pericarditis (possibly of auto-immune origin), together with fever and mastitis. Her transaminases increased four-fold and her gammaglobulins rose to 28 g/L. MP 16 mg and AZA 75 mg were started, together with antibiotic treatment. Liver tests normalized rapidly. The steroids could be gradually reduced. She started oral contraception. Liver tests remained normal on therapy with AZA 75 mg alone.

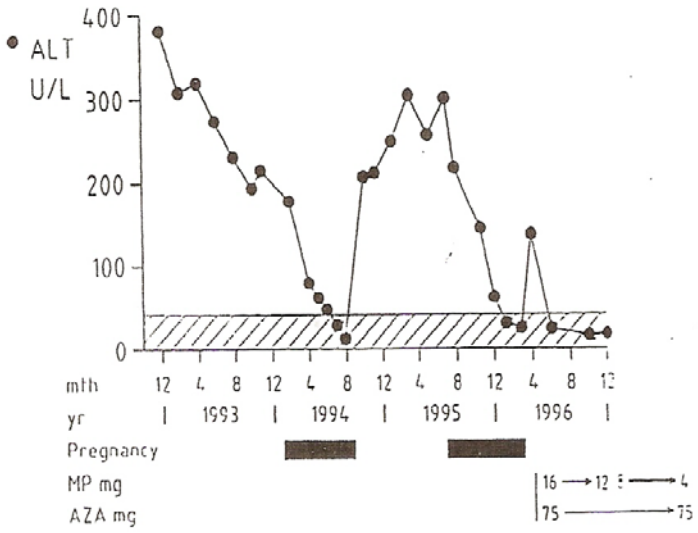

Figure 4: Pattern of ALT at diagnosis, during two pregnancies with flare ups following delivery, and effect of therapy. Adapted from Buchel *et al* (46).

Patient 4

At age 12 years, this patient presented with AIH and cirrhosis. She responded well to steroid therapy for two years, but later on her hepatitis flared up markedly. When questioned, it became clear that she had discontinued her steroids because of her pending marriage. The steroids were reintroduced and AZA added. This was followed by five years of normal liver tests, allowing the decreasing of her medication. She became pregnant whilst taking 4 mg MP. She delivered a healthy baby. Her ALT remained normal after delivery.

Eight months later, the MP was reduced to 2 mg and finally stopped. Her liver tests remained normal and she became pregnant again. Throughout this second pregnancy, following delivery of a normal baby, her liver tests have remained normal without medication.

Patient 5

This patient was diagnosed with AIH at age 19 years, and treated with 32 mg MP and 25 mg AZA (higher doses of AZA were not tolerated because of nausea) and responded well.

At age 31, she was found to be 10 weeks pregnant, whilst taking MP 6 mg. She had discontinued AZA four weeks earlier. After three months MP was reduced to 4 mg per day because ALT had decreased from 78 to 34. A healthy 3.45 kg baby was born by caesarean section, because of failure of induction at 41 weeks. To avoid a flare up, MP was immediately increased to 16 mg per day and AZA restarted.

In January 1997 she reported that she was again 14 weeks pregnant; she was on 4 mg MP, with normal liver enzymes. MP was gradually reduced to 2 mg on alternate days only. Her second child was born normally and because of a desire to breast-feed, AZA was not restarted, but MP was temporarily enhanced to 12 mg. One month later she still had normal liver tests and MP was reduced to 4 mg. She became pregnant again in July 2000 and the MP could again be reduced to 2 mg on alternate days. She underwent a caesarean section in March 2001; MP was temporarily enhanced to 12 mg daily for one week, to be reduced with 2 mg every two weeks; currently (at the time of writing), taking 4 mg with normal tests.

Patient 6 (ASC)

This young woman, who had delivered a normal child one year before, presented with a rash at age 23; ALT was initially four to five times normal levels but increased markedly within three months, together with raised gammaglobulins (from 22 to 49 g/L) and two to fourfold increase in ALP (552 to 1053 IU/L); ANA was strongly positive. A biopsy was compatible with AIH, but a single bile duct showed mild periductular fibrosis suggesting that PSC also be considered. A week after the liver biopsy, she developed a severe sub-capsular haemorrhage, which required a laparotomy. A second liver biopsy, taken at surgery, was interpreted as compatible with sclerosing cholangitis. Because of the high transaminases and the ANA, the patient was considered to have ASC (20, 80) and was treated with MP and AZA. Each attempt to reduce the immunosuppression was followed by flare ups.

In 1991 the patient fell pregnant and AZA was discontinued. She became asymptomatic and her liver tests normalized, so MP was reduced to 10 mg. She had a normal term delivery. A month later she flared up clinically and biochemically. The MP was increased to 20 and two weeks later to 40 mg and AZA added initially at 100 mg and later at 150 mg. MP was gradually reduced, but frequent subsequent flare ups required that MP be kept at 15-20 mg and AZA at 75-100 mg.

The patient continued this medication until April 1995 when she became pregnant again. AZA was stopped and MP increased to 30 mg per day. However, this time, she became symptomatic at the end of April and a biochemical flare up was documented. The pregnancy ended in a miscarriage on 11 May 1995. The patient’s persistent wish for another child was rewarded with a third pregnancy, with improvement of transaminases and gammaglobulins; her third baby was born full term. A flare up was seen again after her delivery. Her condition has continued to be problematic since then. She received a successful liver transplant in September 2000 because of increasing cholestasis with itching and malabsorption.

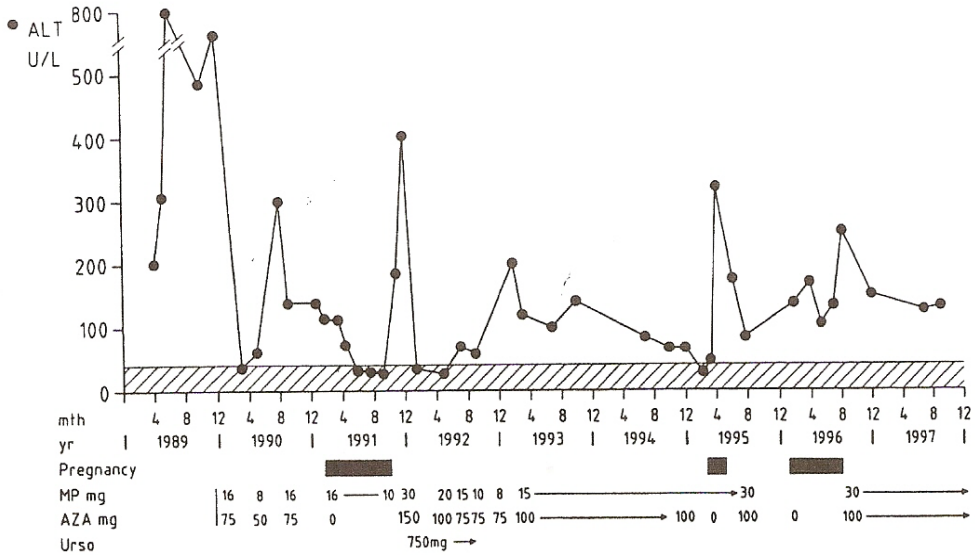

Figure 5: A case of ASC with effect of treatment, three pregnancies (one ending with a miscarriage) and delivery on ALT levels. Adapted from Buchel *et al* (46**)**

DISCUSSION

The present series describes five patients with AIH and one with ASC who between them had 14 pregnancies after diagnosis. In all pregnancies except two, liver tests normalized after the third to fourth month of pregnancy (in the additional patient, near-normal values were achieved, despite the fact that AZA had been discontinued. In all patients on immunosuppression, the dose of corticosteroids could be reduced. A similar course in two cases was recently reported (81). In patient No 6, a flare up of hepatitis occurred during the first trimester, but this pregnancy aborted spontaneously at 12 weeks. It seems likely that the flare up was subsequent to the intrauterine death of a blighted foetus. Not all cases might show improvement, since overall five cases with deterioration during pregnancy have been reported (82, 83). Following delivery, a flare up of hepatitis was noted in 12 of the 14 pregnancies, despite the proactive increase of steroids and AZA dosages immediately after delivery instituted in the six most recent pregnancies. In their series of 18 women with autoimmune hepatitis and pregnancy, Heneghan *et al* (82) described two cases of *de* novo diagnosis of autoimmune hepatitis during pregnancy. They also observed relatively infrequent exacerbations of AIH during pregnancy (84). It seems therefore that pregnancy mostly leads to attenuation of the immune process, allowing a considerable decrease in immunosuppressive therapy, but with a risk of flare up after delivery. These observations point to a marked immunosuppressive effect of pregnancy on autoimmune liver disease.

Several (but not all) autoimmune diseases tend to run a more benign course during pregnancy and to possibly relapse following delivery, presumably due to the disappearance of the peculiar steroid environment of the pregnant state. Indeed, the outcome of pregnancy in patients with inflammatory bowel diseases (IBD) approached that of a control population, although the IBD patients had more (11.5 %) premature births. The outcome was similar in those taking mesalazine or AZA (85). Similarly, pregnancy improved the symptoms of rheumatoid arthritis in approximately 75 % of patients, but relapses occurred in 90 % during the postpartum period (86). Rheumatoid arthritis is considered a T-cell mediated and T-helper 1 (T_H1) immune response driven disorder. Since pregnancy leads to a shift from a T_H1 to T_H2 response (87), it may ameliorate the disease. In contrast, pregnancy worsens systemic lupus erythematosus (SLE), a disease more dependent on T_H2-stimulated humoral response (86). In general, females react preferentially with a T_H1 response to antigens except during pregnancy, when a T_H2 environment prevails (87, 88). As long as the pathogenesis of autoimmune hepatitis is not clarified in detail, assessment of the relevance of the shift from T_H1 to T_H2 predominance on the disease remains speculative.

In pregnancy, it is not yet clear whether one or more hormones induce the altered immune response. Estrogens at low doses seem to enhance immune activity but at high doses (such as during pregnancy) they may inhibit such activity. Progesterone and testosterone promote T_H2 cells and have anti-inflammatory properties (88, 89). Two recent studies (90,91) document a decrease in serum transaminases, and one of these studies (91) shows an increase in serum HCV RNA during pregnancy in patients with chronic hepatitis C. These observations represent an additional example of pregnancy-induced tolerance (87), allowing the HCV to replicate. They also support the view that the liver damage in chronic hepatitis C is mainly due to the host response to the virus.

Corticosteroids and AZA is the mainstay of treatment for AIH. Such treatment induces a marked improvement in morbidity and mortality (12, 92, 93). In cases of IBD patients who become pregnant, side effects of AZA for neither mother nor foetus have not been encountered (94). Experience based on several thousands of pregnancies in IBD or rheumatoid sufferers and transplant patients, has led to the overall consensus that AZA can safely be continued during pregnancy (95, 96). In a cohort of IBD patients who unknowingly became pregnant, side effects of AZA for both mother and foetus were not encountered (71, 82).

Since flare ups occur quite often following delivery, it seems wise to augment immune suppressive therapy (including AZA) soon after parturition. Breast feeding during treatment with AZA is not recommended (96), although only 1.2% of the absorbed amount of AZA seems to be excreted in breast milk (97). A recent report on six women with kidney transplants who were taking AZA during breast feeding reports no side effects in the newborns (98). The drug was therefore reclassified as ‘probably safe’ for the neonate receiving breast-feeding (99).

In conclusion, pregnancy induces a situation of immune tolerance. This is advantageous in patients with AIH, IBD or rheumatoid arthritis, but not in cases of SLE, and might be related to the transition from a T_H1 into a T_H2 environment. In the former group of disorders, immunosuppressive therapy can usually be decreased during pregnancy but it is wise to enhance therapy following delivery in order to prevent a serious flare up. Because not all cases improve during pregnancy, careful attention and individual follow-up is needed. AZA has not been shown to induce malformations, but the drug can usually be stopped during pregnancy because the disease activity is reduced.

Table 11: Characteristics of patients

Patient			Pregnancy		ALT values				
No	Born	Age(yr) at diagnosis	No.	Year	Just before pregnancy	During pregnancy*	After delivery (wks)	Decrease in immunosuppression	Birth
1	1957	25	1	1986	81	41	519(6wk)	No	Term
			2	1988	327	48	103(4wk)	†	Premature
2	1969	17	1	1993	110	13	405 (5wk)	†	Term
			2	1994	129	14	96(4wk) ‡	§	Term
3	1963	29	1	1994	200	12	206(2wk)	No therapy	Term
			2	1996	302	28	156(2wk)	No therapy	Section†
4	1973	12	1	1995	Remained Normal		Kept Identical	No	Term
			2	1998	Remained Normal		Kept No therapy	No	Term
5	1963	19	1	1995	78	34	69 (3wk) ‡	†	Section§
			2	1997	55	17	34 (4wk) ‡	†	Section¶
			3	2000	45	14	32 (4wk) ‡	†	Section¶
6	1966	23	1	1988	nd	nd	nd	No therapy	Term
			2	1992	132	31	412 (4wk) ‡	§	Term
			3	1995	63	36	198	§	Stillbirth
			4	1996	Fluctuating		267(2wk)	§	Term

Patient no.6 has autoimmune sclerosing cholangitis nd= not determined
*Lowest level during the third trimester of pregnancy is given
†Caesarean section because of foetal distress during delivery
‡Despite proactive increase in immunosuppressive therapy
§Caesarean section because of failure to deliver inspite of induction at 41 weeks
¶Caesarean section because of previous caesarean sections
Adapted from Buchel *et al* 2002 (46).

CHAPTER 4
AUTOIMMUNE HEPATITIS EVOLVING INTO PRIMARY SCLEROSING CHOLANGITIS

INTRODUCTION

AIH is a chronic hepatitis of unknown aetiology characterized by hypergammaglobulinaemia, the presence of circulating auto antibodies, and inflammatory changes on liver histology. Variant syndromes have been described whereby patients have features of AIH as well as features of a coexistent different liver disease such as PSC or PBC. The diagnostic criteria for these variant forms have not been standardized nor has an appropriate treatment strategy been established (28).

An international panel proposed a numerical scoring system for ‘definite’ and ‘probable’ diagnoses of AIH (15). This allows for standardisation and comparison between patients. The sensitivity and specificity of this scoring system has been studied and commented on by a number of investigators (100, 101, 102, 103, 104, 105). The logical consequence of all this comment and ‘criticism’ was that certain criteria were revisited and an updated consensus with improved specificity published (16).

In a study done at the Mayo clinic of 162 patients with the clinical diagnosis of type I AIH, 11 (7 %) satisfied the criteria for autoimmune cholangitis and eight (5 %) were redesignated as having AIH and PBC. Interestingly, seven of the 37 patients with PBC (19 %) satisfied the criteria for AIH and PBC and 14 of the 26 patients with PSC (54 %) were redesignated as having AIH and PSC. Variant syndromes are found more commonly in patients with the original diagnosis of PSC than in patients with the original clinical diagnosis of type I AIH or PBC (106). PSC does not display the typical characteristics of classical autoimmune-mediated disease; for example it does not show the usual preponderance of female patients seen in AIH and PBC. In addition, classical PSC does not respond well to immune suppression.

In children, features of AIH and of PSC can quite often present together. In a retrospective study Mieli-Vergani (20) diagnosed ASC in 31 children on bile duct abnormalities at

cholangiography in association with positive antibodies (20). 30 children had positive ANA/SMA and one was LKM positive. The majority had greatly increased gammaglobulin levels. Eight of the 31 (23 %) had histological features compatible with AIH and would have been diagnosed with AIH if ERCP had not been performed at presentation.

AIH-PSC overlap appears to be rare in adults. Minak and associates reported two cases (107) and ten years later a report in the American Journal of Gastroenterology (108) reported five cases, all of whom fulfilled the criteria for 'definite' AIH but also had evidence of PSC.
Two female patients who fulfilled all the criteria for AIH but who gradually developed cholestatic features were followed up. In one of these patients, who were regularly followed up, over a period of 20 years and who in this time had three liver biopsies, the liver enzyme profile began to show cholestatic features with a raised ALP and GGT. The patient was subsequently shown by ERCP to have PSC. The second young female patient was originally also diagnosed as 'definite AIH'. She too developed the enzyme profile of PSC and was subsequently diagnosed as having crossed over to PSC. These data suggest either that young adults with PSC have a broadly directed and severe immune reaction, or that in some the autoimmune reaction is initially directed against hepatocytes and later attacks bile duct epithelium. In a comprehensive study of 119 PSC patients Broome *et al* (109), a significant association of PSC with other autoimmune diseases was noted, most commonly diabetis mellitus and Graves disease (110).

TABLE 12: Profile of known features, HLA haplotype, immune markers and therapy in autoimmune liver disease

	PBC	"Overlap syndrome"	Autoimmune hepatitis	Autoimmune sclerosing cholangitis	Primary sclerosing cholangitis
Gender predominantly	F	F	F	F>M	M>F
Age	>40		12 -15 and >55	12-->25	20 - 50
HLA A1			+	+	
B8			+	+	
DR2/3				+	-or+
DR4					+
DR52w				+	+
ERCP	-	-	-	++	++
ANA	+/-	++	+++	+++	+/-
AMA	+++	++	+/-	+/-	-
pANCA	+-	-	++	++	+++
ALP	+++	++	+	++	+++
Biopsy	bile duct lesions	hepatitis and bile duct	hepatitis	hepatitis and bile duct	bile duct lesions
Treatment	Urso	steroids AZA and Urso	Steroids and AZA	steroids AZA and Urso	Urso

Urso = Ursodeoxycholic acid

Patient 1: A young female patient whose AIH evolved into PSC

This young girl was first seen in 1982 at age 8years, complaining of fatigue, and mild fever. Laboratory test results are given in table 13, p45. ANA was positive at 1/80, SMA at 1/40. A liver biopsy prompted diagnosis of AIH because of the dense portal and periportal lymphocytic infiltrate with large numbers of plasmocytes. Treatment with prednisone 20 mg daily led to rapid normalization of transaminases and to a decrease of IgG from 37.6 g/l to 23.7g/l. HLA typing showed positive for HLA A1/A2, B8 and Cw1/5; DR was not tested at that time. A control biopsy in 1983 showed chronic active hepatitis with a dense lympho-plasmocytic infiltrate, and some bile ducts seemed slightly injured; PBC was considered but thought unlikely since AMA was negative and ALP levels were normal. A third biopsy done in 1985, after the patient developed arthralgia and a surge in transaminases, revealed early macronodular cirrhosis. It was also noted that the epithelium of one bile duct was eroded and that this duct was surrounded by a dense lymphoid infiltrate. Other bile ducts had a dense collagenous wall. This biopsy seemed compatible with early PBC, but again serum alkaline phosphatase levels (192 U/L) remained normal and AMA negative.

In 1992, ten years after the initial diagnosis, liver enzyme tests suddenly showed a marked increase of ALP and GGT. ERCP revealed abnormal intrahepatic ducts, compatible with sclerosing cholangitis, and a normal common bile duct. A repeat biopsy demonstrated macronodular cirrhosis with normal size bile ducts surrounded by a ring of dense collagen, compatible with PSC. Ursodeoxycholic acid was added to the patient's therapy with marked improvement of liver enzymes. In 1997, she experienced her first variceal bleeding, which was successfully treated by repeated endoscopic sclerotherapy. The patient was also treated with propranolol 80 mg, isosorbide-5-mononitrate 2 x 20 mg and spironolactone 25 mg daily to prevent recurrent variceal haemorrhage. The follow up has remained uneventful.

Table 13: A young female with evolution of AIH into PSC

Normal values	1982	1983	1984	1985	1988	1990	1992	1994	1995	1997	1998	2000
ALP(<260U/L)	204	126	197	192	271	188	1233	139	144	195	225	306
ALT(<40U/L	970	51	98	168	140	30	197	79	68	44	47	116
γ glob (g/L)	37.6	23.7		30.2	21.2	22.2		31	34	29	17.5	
ANA	1/80.	pos									1/40.	
SMA	1/40.	pos		pos	Pos				>1/80		1/40.	
AMA					Neg						Neg	
PANCA									>1/80		1/160.	
Predn. (mg)	>20	20	10	0/ 15	15	10				10	10	1`0
AZA							75	75	75	75	75	75
Urso (750mg)								+	+	+	+	+

AIH score before treatment =21 or 22 depending on HLA DR (not tested)

Patient 2: A female patient whose AIH evolved into PSC

The patient is a 34-year-old unmarried woman who was diagnosed with AIH in 1994. At that time she had a significantly elevated ANA (titre1/320), no viral markers and a liver biopsy compatible with AIH. The AMA and LKM antibodies were negative. Her past history included thyroid disease and amenorrhoea. Treatment with prednisone (60 mg OD) was initiated and, possibly due to a communication problem or extended time intervals between follow up visits, this dose was continued for longer than intended. The patient was referred to a liver unit in April 2001. Her medication at that time was prednisolone 10mg and AZA 150 mg daily. Her ANA titre was 1/160, the AST 134 and ALT 120. As the patient had indicated that she did not want to take any more steroids, she was started on Cellcept (mycophenolate mofetil) 500mg Bid.

Follow up after 2001 was at times erratic and between March 2003 and September 2003 no follow up visits are documented. In September 2003 the patient was admitted to hospital complaining of fatigue and pruritus. She was jaundiced and had obstructive liver enzymes with a total bilirubin of 4.4 (direct 3.1), ALP 974, GGT1206, and ALT and AST slightly above 200 (table 14, p47). It was established that she had not taken any medication for at least three months prior to admission. Therapy with AZA 150 mg, Cellcept 1 gm OD and prednisolone 60 mg daily was started. Ultrasound and CT scan showed a normal biliary tract. A month later, on 8 October, when the patient's bilirubin was 27 mg/dl (17 mg/dl direct) and ALT and AST still above 100, with an albumin of 2.2 as well as ascites, she was transferred to a transplant unit in Leuven, Belgium. The suspicion was that her AIH had now crossed over to PSC. In Leuven a MRCP and ERCP were done. Brush cytology did not reveal malignancy and a biliary stent was placed because of a dominant stricture. The stent only led to a very modest decrease in the patient's total bilirubin.

The patient's situation became more complicated with the development in November 2003 of CMV pneumonia. After the pneumonia improved she underwent a liver transplant at the end of December 2003, receiving a right liver lobe donated by her brother. The graft functioned well after the transplant, and her bilirubin and transaminases normalised. Her ALP and GGT remained mildly elevated due to a slightly narrowed bile duct anastomosis. Through 2004 her ALP and GGT continued to increase and when her bilirubin also began to climb it was evident that the anastomosis was now significantly stenotic. The patient returned to Leuven in October 2005. After several attempts at endoscopic dilatation failed, the hepaticojejunostomy was reviewed operatively. She is maintained on Cellcept 1 gm Bid and prednisone 5 mg daily.

Table 14: Patient 2: Liver Fuction and Serology Tests

	June 2002	March 2003	Sept 7 2003	Sept 30 2003	Oct 21 2003	Dec 2003	Dec 2004	N Values
T.Bili	0.23	5.64	4.43	15.3	27.49	LIVER TRANSPLANT LEUVEN BELGIUM	0.4	Up to 1.1
D.Bili		4.74	3.65	11.3	17.67			
ALP	243	597	974	326	281		270	N to 135
GGT		881	1206	584	301		306	N to 85
ALT	62	103	227	109	134		132	N to 67
AST	37	65	269	86	87		61	N to 37
Glob	4.4	4.4	5	4.3	2.9		3.6	N to3.5
ANA			pos. 1:100		neg.		pos. 1:160	
SMA			pos. 1:100		Neg.		pos. 1:160	
AMA			Neg.		ND		Neg.	
ANCA			ND		pos. 1:320		pos. 1:320	

ND: Not Done **E.H. Buchel 2007**

DISCUSSION

PSC is a chronic, often progressive disease characterized by raised levels of cholestatic liver enzymes, although ALP levels may be normal in up to 8.5 % of patients at the time of diagnosis (109), which makes thinking of PSC more difficult. In addition, the early features of the biopsy are not very specific, and inflammatory lesions may prevail above fibro-sclerotic lesions, further hampering the differentiation of PSC from chronic hepatitis. The final correct diagnosis may thus be retarded or kept in doubt, especially when ERC is not (yet) carried out or is inconclusive. PSC is considered an immune disease, but the exact pathogenesis remains unknown. Recent studies suggest a genetic predisposition related to the predominance of HLA type B8 DR3/DR4 (73). However, individuals with HLA type B8 DR3 are also prone to develop AIH (111). In general, the autoantibody profile differs among the various liver diseases (table 12, p44) but this is not always the case in an individual patient. As documented in the present cases, liver enzyme elevations may especially in children and in the early phase point more to hepatitis than to cholestasis. Furthermore, features of both AIH and PSC may be present together in what has been called ‘autoimmune sclerosing cholangitis’ (20). In young adults, several sporadic cases combining features of AIH and PSC have been reported (100, 101, 102, 103, 104, 105, 108, 112), and immune phenomena are not rare (113). In children, AIH and PSC can be very difficult to diagnose on clinical and biochemical grounds. Indeed, both in a series from King’s College Hospital (80, 114, 115) and in one from Toronto (116), approximately one in three patients with the final diagnosis of

PSC presented clinically as AIH; also their liver biopsies were initially read as hepatitis. In addition, serum markers of autoimmunity were present in high titres in more than two thirds of the patients. Active IBD was present in more than half of the children while in adults this incidence was far lower and the colon disease mostly inactive. The similarity of clinico-biochemical features of AIH and PSC in children was further substantiated by the London group (115) in a study of 47 children presenting with hepatitis and pronounced serum markers of autoimmunity. In all these children, an ERC were carried out which was indicative of PSC in 25 children. These two disease groups, AIH and what is called autoimmune sclerosing cholangitis (ASC), differed only by a slight difference in gender distribution, a higher incidence of positive atypical pANCA and by the presence of lympho-plasmocytic infiltrate in the biopsy. The initial favourable response to immunosuppressive therapy was comparable in both groups.

Clear-cut AIH disease, which later on progresses to PSC, is rare. It was seen in the paediatric groups mentioned above and in some random case reports, and is explored by Abdo *et al* (19). One such patient was first seen in Leuven, but she moved to Pretoria and was than temporarily followed up in South Africa before returning to Leuven. She has now been followed up for approximately 20 years.

CHAPTER 5
TOXIC HEPATITIS WITH FEATURES OF AUTOIMMUNE HEPATITIS

The clinical and biochemical spectrum of AIH is very diverse and so the condition is sometimes difficult to differentiate from other causes of (sub)acute or chronic hepatitis (117). The following case reports testify to problems in differentiating toxic or drug-induced hepatitis from autoimmune liver disease.

CASE 1: NITROFURANTOIN

A 65-year old woman was referred because of tiredness and dyspnoea. She had previously complained of recurrent bladder infections. A month prior to admission, she developed myalgia, fevers and a dry cough. Her general physician documented disturbed liver and renal function tests and referred her.

On examination, the patient had mild cyanosis, dyspnoea and fine crepitations at both lung bases. Heart and abdominal examination revealed nothing abnormal. A recent intra-venous pyelogram and cystoscopy reported no abnormalities. Thrombocytopaenia was present and she had multiple ecchymoses. Biochemistry and serology tests revealed abnormal liver function tests, including raised gammaglobulins and a positive ANA (titre > 1/180). Chest x-ray showed fine diffuse infiltrates of both lungs.

The diagnosis was not immediately clear. The patient was treated with oxygen and she improved gradually. A few days after admission, she requested ‘her nitrofurantoin’ tablets, which she had been taking during the preceding two years but had not mentioned in her history, despite being questioned about her medication used!

She continued to improve and after her platelets had increased a liver biopsy was done. The biopsy showed moderate hepatocellular acidophilic necrosis with ballooning, mild cholestasis and portal and periportal mononuclear cell infiltration with several eosinophils but no plasmocytes. This biopsy was suggestive of toxic hepatitis.

Comment on nitrofurantoin toxicity

Females make up 90% of cases of nitrofurantoin-induced hepatitis (118). Common presenting symptoms include arthralgias, myalgia, low-grade fever and a pruritic rash. The onset is

variable, and since nitrofurantoin is mostly taken intermittently, hepatotoxicity is often only noted after the third or fourth exposure. The most frequent finding is hepatitis with mild cholestasis (119, 120). Depending on the duration of intake, chronic hepatitis and even cirrhosis can develop (38, 121). Improvement usually follows after cessation of the offending substance.

The pathogenesis of nitrofurantoin toxicity remains unclear but since eosinophilia is often present both in the blood and in the liver, together with positive ANA and sometimes even LE cells, an immuno-allergic mechanism has been proposed. The presence of eosinophils in the liver is considered a marker of toxic hepatitis independent of the mechanisms involved (122). Nitrofurantoin might function as a hapten and elicit antibodies when bound to a protein. The elicited immune complexes could be hepatotoxic as well as toxic to alveolo-capillary membranes and as such induce hepatitis, alveolitis and interstitial oedema with eosinophilic lung infiltrates. SMA are rarely present. It is unclear whether genetic (e.g. HLA pattern) or environmental factors such as other medication predispose an individual to toxicity. Nitrofurantoin toxicity mostly affects middle-aged or older female patients. It has been suggested that HLA type DR2 and DR6 are more frequent than other HLA types in patients with nitrofurantoin hepatitis. Haematological toxicity such as haemolytic or megaloblastic anaemias and even aplasia has been reported. Megaloblastic anaemia might be due to folic acid antagonism, since the chemical structure of nitrofurantoin resembles that of phenytoin, a known antagonist of folic acid. Neurological toxicity in the form of polyneuropathy with vertigo and nystagmus has been described (122, 123). Pulmonary infiltrates and chronic interstitial pneumonitis are often present (124).

CASE 2: ALPHA-METHYLDOPA

Five weeks after onset of therapy with alpha-methyldopa (Aldomet) a 61-year old woman complained of fever, itching and jaundice. Serum transaminases and ALP levels were elevated (table 15, p51) while virological markers were negative. ANA was positive > 1/360. Both AMA and SMA were negative. A liver biopsy showed a late phase of acute hepatitis with some fibrosis in the portal tracts and piecemeal necrosis suggesting the possibility of transition into chronicity. Since the patient did not have hypertension while in the hospital, her alpha-methyldopa was stopped. At this time the association of her symptoms with alpha-methyldopa had not yet been made. The patient returned home with the diagnosis of acute

hepatitis probably autoimmune in origin. However, when her transaminases normalised without further medication, the diagnosis was changed to acute hepatitis of unknown origin.

One year later, the patient's general physician again noted mild arterial hypertension and again prescribed alpha-methyldopa. Four weeks later, she developed general malaise, jaundice, dark urine and pruritus, but no arthralgias. She was referred to the academic hospital where a repeat liver biopsy showed post-necrotic collapse, fibrous septa and centrilobular confluent necrosis. After the alpha-methyldopa was withdrawn her liver enzymes normalized and a diagnosis of alpha-methyldopa-induced hepatitis was considered. Eight months later elevated blood pressure was again noted and it was decided to test the diagnosis with alpha-methyldopa. Within days of the administration of a single dose the patient's liver enzymes increased and, within a week normalized again. This re-exposure thus confirmed the diagnosis.

Table 15: Liver function tests of 61 year old woman who took Alpha-methyldopa

	Nov 1971 1st week after exposure	Oct 1972 4 weeks after 2nd exposure	May 1973 After single dose challenge	Normal Values
Bili mg/100ml	6	2	N	<1mg%
ALT (1u/l)	350	550	220	<30 1u/l
ALP (1u/l)	275	290	N	<120 1u/l
GGT (1u/l)	N	175	150	<30 1u/l

Comment on alpha-methyldopa

Liver enzyme disturbances due to alpha-methyldopa have been noted, but are very rare according to De Groote et al (125), but are estimated to occur in between 1 % and 6 % of individuals taking alpha-methyldopa (124, 126). Clinically overt hepatitis is much less frequent (127, 128). The association of hepatitis and repeated administration of alpha-methyldopa was confirmed by Elkington *et al* (126). These authors prove this association by repeating administration and observing consequent recurrence of liver dysfunction. The relapses described after re-exposure are clinically and biochemically rather mild and less severe than the first attack. However a case of rechallenge with the drug which was followed by a fatal hepatitis, has been described (129). A liver biopsy performed nine days after the appearance of jaundice and six days before death revealed severe hepatitis with confluent centrilobular necrosis, enlarged portal tracts with inflammatory infiltrate and occasional

eosinophils. Five cases of chronic active hepatitis associated with alpha-methyldopa were reported from a consecutive series of 21 cases. The relationship was substantiated in one patient by an unintentional rechallenge. Liver function tests improved in all these patients after withdrawal of the drug (130). The case of a 40year-old pregnant Afro-Caribbean woman who developed hypertention and was given alpha-methyldopa has recently been reported (131). Two weeks after starting the drug she developed fatigue, myalgias and fevers. However, she continued to take the drug for another two weeks until her urine became dark and scleral icterus was noticed. Her ALT was 251 iu/l (N to 52 iu/l), and her ANA was positive (1:180). All viral markers were negative. The alpha-methyldopa was discontinued and her symptoms and liver enzymes gradually normalised.

The hepatitis in the patient presented in this study was doubtlessly caused by alpha-methyldopa. Indeed, the symptoms were observed twice during the drug's administration. Liver dysfunction with transaminitis also occurred promptly after re-challenge. The biopsies showed changes suggestive of chronicity with cellular necrosis and collapse of septa as well as disturbance of lobular architecture.

The histology of alpha-methyldopa toxicity is hepatitis-like and difficult to distinguish from viral or autoimmune disease. It is therefore necessary to stop treatment with this drug whenever significant liver dysfunction is observed. This means liver enzymes should initially be followed up in all patients receiving alpha-methyldopa.

CASE 3: NSAID HEPATO-TOXICITY

A 50year-old male patient was referred because of fatigue and elevation of transaminases. All viral markers were negative, but ANA and SMA were both positive. A liver biopsy showed discrete hepatitis, local congestion and steatosis. When the patient was seen at outpatients two weeks later ostensibly, to start steroids and AZA on the basis of the presumed diagnosis of AIH, the transaminases had improved and ANA and SMA were now negative (table 16, p53). Steroid treatment was withheld and upon thorough retaking of the history, it became evident that the patient had taken Ibuprofen for ten days before the onset of symptoms because of arthralgias. His HLA markers were A1 positive, B8 negative, DR3 negative, DRw52 positive, which differs from the usual HLA type A1, B8, DR3/DR4 seen in AIH (16, 132). Upon long-term follow-up all tests remained normal. The final diagnosis of toxic hepatitis due to NSAIDS (Ibuprofen) was made.

Table 16: Male 50 years old complaining of fatigue

	1997 12 June	1997 26 June	1997 20 Sept
AST(<37)	54	49	24
ALT (<40)	126	103	67
ALP (260)	161	262	
GGT (<40)	208	201	113
HBV		neg	neg
AHCV		neg	neg
ANA	pos	neg	neg
SMA	pos 1:180	neg	neg
ANCA	ND	neg	neg

Haplotype: HLA A1 pos, B8 neg, DR3 neg, DR52 pos.

Discussion of NSAID hepatotoxicity

The apparent mechanism by which NSAIDs produce hepatic injury is immunologic or metabolic idiosyncrasy (128). Despite the overall low incidence of NSAID-induced hepatotoxicity, the enormous consumption of NSAIDs has caused them to become an important cause of toxic hepatitis (133, 134). Because of the divergent chemical structures of NSAIDs, the mechanisms and clinicopathological manifestations of their hepatotoxicities vary widely. Reactive metabolite syndrome, in which rash, eosinophilia and other forms of tissue injury are common, may be provoked by several NSAIDs. Female gender, age above 50 years, and (for some drugs), certain types of arthritis may be risk factors for drug-induced liver injury. The spectrum of NSAID-related hepatotoxicity continues to expand, with reports of interactive toxicity in adults with hepatitis C and recognition of rare cases of liver disease associated with non-selective, selective and preferential COX-2 inhibitors (135).

CASE 4: PHENPROCOUMON (MARCOUMAR)

A 35year-old lady complained of recent onset of progressive dyspnoea after exercise. She was overweight (BMI 26) and had hypercholesterolaemia. She had previously undergone an endoscopic cholecystectomy and recent exertion had caused tendonitis.

Initial clinical examination was unimpressive, but pulmonary ventilation and perfusion scintigraphy revealed multiple perfusion defects in the upper lobe of the right lung. A CT chest demonstrated a pronounced right pulmonary artery suggestive of pulmonary hypertension. A venogram demonstrated post-thrombotic changes in the left popliteal vein.

Ultrasonography showed a hyperechoic (presumably fatty) liver. Blood test results are given in Table 17. Protein C and S as well as antithrombin III levels were normal. Treatment was started with oral phenprocoumon (Marcoumar) and oral contraceptives were stopped.

Five months later, the patient was readmitted because of fatigue, dyspepsia, enlarged liver and dark urine. Blood tests (table 17) showed elevated transaminases (AST 910); negative HAV, HBV and HCV; and negative ANA, AMA and LKM1. A liver biopsy was suggestive of 'florid' AIH.

Table17: Evolution of laboratory tests

	Aug 1996	Feb 1997	May 1997	Sept 1997	April 1998
T.Bili (≤ 1 mg/dl)	-	4			
AST (<39 U/l)	29	910	23	22	15
(ALT ≤ 40 U/l)					
ALP (<270 U/l)					
Chol (< 220 mg/dl)	309			285	224
Prothrombin (T% of N1)		20%		97%	99%
ANA		neg	neg	neg	neg
SMA		neg	neg	neg	neg
AMA		neg	neg	neg	neg
Heparin	pos	neg			
Phenprocoumon	pos	pos			
LMW heparin				pos	
Prednisone mg/day			50 to 30	0	0

The patient was treated with 50 mg prednisolone, and the phenprocoumon was stopped and replaced with low molecular weight heparin s.c. The liver enzymes normalized and the immune serology remained negative. Prednisolone was rapidly tapered to zero because autoantibodies remained negative, and because the HLA type of the patient was negative for the A1, B8 and DR3 alleles, implying that AIH was very unlikely.

The initial slight elevation of ALT could be due to mild steatosis secondary to the patient's overweight condition and hypercholesterolaemia. Her pulmonary emboli were not so easily explained. One likely explanation is the fact that in addition to taking oral contraceptives and to having high blood cholesterol, the patient was shown to have high homocysteine (14.8 micro mol/l) and lipoprotein (877 mg/l; N<300) blood levels. The homocysteine levels decreased following the intake of folic acid 1 mg a day combined with pyridoxine 250 mg a week. Hyperhomocysteinaemia predisposes to vascular problems (136). Increased homocysteine can be reduced by folic acid (137). Proteins C and S, antithrombin III and APC

resistance as well as cardiolipin antibodies and lupus anticoagulant were all within normal values.

Comments on phenprocoumon toxicity

The initial interpretation of the liver biopsy indicated a diagnosis of AIH. However, when scored against the revised criteria of the International Expert Committee (16), the patient's disorder did not even qualify as 'possible' AIH, since autoantibodies were negative and the HLA type was non-specific. In addition, steroid therapy could be rapidly tapered with liver enzyme tests remaining normal (a feature which in itself is inconsistent with AIH). The patient was therefore considered to be a case of toxic hepatitis presumably induced by phenprocoumon.

Hepatotoxicity due to phenprocoumon is rare and although the drug is widely taken, only a small number of cases of toxic liver disease have been reported (138, 139, 140, 141). The interval between initial intake of the drug and onset of liver disease varies between two and six months. This long delay renders the link between intake of the drug and the toxic reaction easy to overlook. It might also suggest gradual accumulation of a metabolite, which eventually causes hepatotoxicity in a predisposed individual. 70 % of phenprocoumon is recovered in urine while part of the drug and hydoxylated metabolites appear as conjugates in bile (142). In all cases reported, recovery occurred within two to five months with or without steroid therapy.

Two reports mention cross-toxicity with other coumarin derivatives such as warfarin (141, 143).

CASE 5: MINOCYCLINE LIVER TOXICITY

A 27 year-old lady presented with a three month history of arthralgias, myalgias, low-grade fever and weight loss of 10 kg. Her previous medical history was unremarkable. Clinical examination was normal except for acne vulgaris for which she had taken minocycline intermittently over the previous two years. She had been referred with the presumptive diagnosis of AIH based on grossly elevated transaminases, hypergammaglobulinaemia and a significantly positive ANA (titre 1/160). SMA was negative. Her HLA pattern was not consistent with the pattern frequently found in AIH (i.e.$A_1B_8Dr_3/Dr_4$). A possible immunoallergic reaction to minocycline was considered and a liver biopsy performed. The

biopsy showed conservation of the lobular architecture and a mild to moderate infiltration of lymphocytes in the portal tracts. These features were judged to be more compatible with a toxic drug effect than AIH. The minocycline was stopped and after prednisone therapy the patient's transaminases normalised. The steroids were then tapered and stopped with no subsequent rise in her transaminases.

Comments on minocycline toxicity

Minocycline hydrochloride is a semi-synthetic tetracycline commonly used for acne vulgaris and frequently prescribed for long periods. This prolonged use is where the problem of minocycline and the development of autoimmune-like phenomena sometimes begins.

Elkayem *et al* (36) reviewed adverse reactions to minocycline in 82 patients and found that three specific drug toxicity syndromes were identifiable, namely serum sickness, drug induced lupus vasculitis and an AIH-like disease. The majority of the cases 70 % (66 of 82), had either the lupus like syndrome or autoimmune-like hepatitis. In addition, the autoimmune-like hepatitis was observed only to manifest after protracted use of minocycline (mean 25.3 months). The patients were all young. The presenting symptoms were arthralgias, arthritis, fever and rash. ANA was positive in 63 of the 68 tested and antineutrophyllic cytoplasm antibodies (cANCA) found in 20 of 24 tested

Several facets of minocycline hepatic injury deserve to be mentioned. It has long been known that the toxic lesion of tetracycline derivatives is micro-vesicular steatosis occurring as a closely related intrinsic toxic property of the molecule (128). However, minocycline leads to idiosyncratic injury with strong autoimmune characteristics. Particularly distressing is the SLE-type illness that can present with or without hepatic injury. A recent publication reported a case of minocycline-induced AIH with subsequent cirrhosis (144). The paucity of published reports probably reflects an underestimate of the frequency of autoimmune-associated manifestations in young women taking minocycline.

OVERALL DISCUSSION ON TOXICITY MIMICKING AIH

Drug-induced liver disease can mimic AIH. This was first realised when oxyphenisatin, a substance present in some laxative preparations, was identified as causing autoimmune-like hepatitis (145). Since then, alpha-methyldopa, nitrofurantoin, clometacin and minocycline

have been recognized as occasionally causing hepatitis with the presence in serum of ANA and/or SMA. These toxicities therefore mimic type I AIH (37). Other agents have been implicated in only a small number of AIH type I-like disease; they include benzarone, phenofibrate, germander, papaverine, pemolines, propylthiouracil, diclofenac and, as shown above, ibuprofen (rarely) (146). Hepatitis with positive LKM (mimicking type II AIH) has been reported with dihydralasine, tienilic acid, phenytoin, carbamazepine and halothane (147).

Tienilic acid	development of	LKM 2 antibodies	directed against	CYP 2C9
Dihydralasine	development of	LKM antibodies	directed against	CYP 1A2
Halothane	development of	LKM antibodies	directed against	CYP 2E1
Phenytoin & carbamasepine	development of	LKM antibodies	directed against	CYP 3A

The diagnosis of a drug-induced disease is most often based on exclusion of all other causes, and usually needs thorough and often repeated history taking. In up to 20 % of cases, viral hepatitis, PBC and PSC may present with autoantibodies (148) most often in low titres in the serum. Viral causes therefore need to be excluded before thinking of AIH (16). This has to be followed by careful checking of potentially toxic causes. As shown in the cases presented, a toxic cause is often not immediately evident and most times is only thought of later on. Knowledge of the substances that mimic AIH is helpful. When toxicity is suspected, the drug should be discontinued. This approach usually leads to amelioration or even total resolution of the necro-inflammatory lesion. The clinical characteristics of drug-induced AIH-like disease are seldom helpful in discriminating toxicity from real AIH since both disorders are mostly (> 80 %) seen in female patients. The symptoms and clinical signs are identical and often only present after taking the offending drug from two to 24 months (or even longer). This long period diverts attention from the drug.

Drug-induced injury as a possible trigger of AIH goes back to 1956 when Mackay *et al* described what they called Lupoid hepatitis in the *Lancet* (14) and although this hypothesis still remains to be confirmed a syndrome with clinical, biochemical and serologic features closely resembling AIH has been clearly shown to result from the effects of some drugs (149). The *New England Journal of Medicine* reported in 1971 that oxyphenisatin, an ingredient of laxative preparations, could cause hepatic injury, which mimics AIH (145). Subsequent to this report, descriptions of AIH-like cases in recipients of alpha-methyldopa (150), diclofenac (151) and minocycline (152) appeared.

Immune-related drug hepatotoxicity tends to be acute in onset. In rare cases continued use of the drug may lead to chronic hepatitis and cirrhosis, which is indistinguishable from idiopathic AIH and includes the presence of ANA and/or SMA (146). A recent report describes three patients (one male and two female) who developed hepatitis after the initiation of statin therapy; the hepatitis was severe and persisted even after the medication was discontinued (153). The patients developed positive titres of ANA (1/40 to 1/160), and hypergammaglobulinaemia. Features of all three cases met the criteria of the International Autoimmune Hepatitis Expert Committee (16). Liver biopsies in all three showed varying stages of fibrosis and plasma cell infiltration, compatible with AIH (153). These cases indicate that severe ongoing AIH can on rare occasions be caused by drugs (153).

Evidence suggests that an underlying genetic predisposition may impair immunologic control of auto-reactivity and thereby cause loss of self-tolerance to liver auto-antigens (154). In some instances, a predominance of specific HLA haplotypes has been noted with drug-induced autoimmune liver injury similar to that of idiopathic autoimmune disease. In a survey of 71 patients with drug-induced hepatitis, the allele A11 was found in 75 % of patients with diclofenac injury and in 29 % of those with halothane hepatitis. DR6 was present in 80 % of patients with chlorpromazine injury and DR6 and DR2 in over 50 % of those with nitrofurantoin injury (122). A8 was seen in 70 % of patients with clomethacin injury (table 18, p58). However, no consistent relationship between HLA haplotype and susceptibility to the drug-induced chronic hepatitis syndrome has been demonstrated (155). Indeed, lack of association with a particular haplotype distinguishes drug-induced AIH from cryptogenic hepatitis (156).

Table 18: HLA predispositions

DRUG	HLA
Halothane	A11
Diclofanac	A11
Tricyclic antidepressants	A11
Clomatacin	A8
Nitrofurantoin	DR6 and DR2
Chlorpromazine	DR6
Amoxycillin-clavulanic acid	DR15/DQ6

The therapy of any case of hepatitis which may be drug-related starts with the immediate cessation of the (possible) offending medication. A short course of high dose corticosteroids

in severe reactions is of unproven value, yet given the possible (or probable) immune-mediated pathogenesis, steroids may be ‘worth considering’ (146), while prolonged cholestatic reactions may benefit from empiric treatment with URSO.

In conclusion, drug-induced AIH-like disease is not frequent but can be difficult to differentiate from *bona fide* AIH.

CHAPTER 6

HEPATITIS C VIRUS AND AUTOIMMUNE HEPATITIS

INTRODUCTION

This text presents a case history, and examines the background and reviews past and present perceptions of the association between AIH, auto-immune features and chronic hepatitis C. When the hepatitis C virus (HCV) was recognized in 1989, many cases that until then had been labelled as idiopathic chronic hepatitis could because of the OrthoHCV enzyme immunoassay test for antibodies to HCV be diagnosed as chronic HCV infection. Since 1990, the role, if any, of the HCV in the pathogenesis of AIH has been debated (157).

Refinement of diagnostic tests for HCV as well as other antibody diagnostic serology led to a spate of publications documenting the presence of HCV in autoimmune diseases (158). The HCV is especially associated with LKM-1 antibodies. These antibodies are mainly directed against cytochrome P450 2D6 (159). When LKM-1 antibodies are present in patients with chronic hepatitis who have no markers for hepatitis C then their presence reflects a probable diagnosis of type II AIH.

In the1990s multiple reports of the presence of HCV infection in patients with anti-LKM-1 were published. Studies by Lenzi *et al* (160) and Todros *et al* (161) in 1991 indicate that the prevalence of HCV infection in patients with anti-LKM was 66 % in Italian patients, nearly 49 % in French and 48 % in German patients yet 0% British and North American patients! A possible conclusion is Vogel's, caveat (162) that epidemiological evaluations have limited usefulness in assessing the role of HCV in AIH. Subsequent studies have found the prevalence of anti-LKM-1 in patients with chronic HCV to be between 3 % - 5 % (162).

A Japanese group (163) investigated 25 adult Japanese patients with AIH who were positive for anti-LKM-1 antibody and who were also infected with HCV. The majority of the patients (22 of 25) did not present with any unusual symptoms or any associated diseases during the course of their chronic infection. Histology of their

liver biopsies showed only the usual characteristics of chronic hepatitis C and lacked characteristics of AIH type I. There were also no differences in the genotype spectra of these patients when compared to anti-LKM-1 negative Japanese patients with chronic HCV; no disease-specific HLA haplotypes were noted. HLA-DR4, detected in 88,7 % of Japanese patients with AIH was detected in only 50 % of the 25 patients. In addition prednisone was ineffective in the six patients treated with it, while interferon was effective in six of ten patients treated (60 %). The conclusion drawn from this study is that these patients should not be categorized as AIH, since their disorder is essentially chronic HCV in which an auto-antibody was present before or has been produced during the course of the chronic HCV infection.

Clinical data studies have further widened the gap between classical AIH and HCV associated with anti-LKM-1. Type II AIH patients (i.e. anti-LKM-1 positive with no HCV) are usually young, mostly (90 %) female, with high serum ALT, high titres of anti-LKM-1, frequent extra-hepatic autoimmune-manifestations, who show a prompt response to immune-suppressant therapy and a dramatic aggravation or exacerbation of disease when given interferon. Conversely, patients with positive results for HCV RNA and anti-LKM-1 are older, and equally likely to be male or female, typically have only mild elevation of ALT and low titres of anti-LKM and show poor association with HLA-DR4 or extra-hepatic autoimmune syndromes (164).

A clear distinction must be made between HCV infection and AIH because the appropriate therapy needs to be prescribed with confidence and not in fear of a possible unintended catastrophic response. The following case reports illustrate the need for cautious use of IFN in two cases that were thought to be hepatitis C but in fact were AIH.

CASE HISTORY 1: MH

In 1986 MH was thought to have chronic NANB hepatitis. This diagnosis was made by exclusion, after all viral markers and tests for ANA and SMA were repeatedly negative. The patient's transaminases continued to show cyclical rises and in 1991, after a further set of negative serology (ANA and SMA), anti-HCV tested positive. He was started on IFN α2b, 3mu TIW s.c. Shortly after IFN had been started the patient became deeply jaundiced with raised total and direct bilirubin total 6.34 (N<1), direct

4.70, ALP 385, (N to 135) and GGT 72, (N to 28). The transaminases increased dramatically: ALT 489 (N to 40) and AST 584 (N to 37). Subjectively the patient complained of tiredness, loss of appetite and pruritis. The IFN was immediately discontinued. One month after discontinuing IFN, the gammaglobulin level was twice the upper limit of normal and SMA was positive (1/20). ALT was now 113 and AST 110. On 18 June 1991 MH started corticosteroid therapy (24 mg MP OD). One week later his transaminases and bilirubin had normalised and his gammaglobulin was only marginally raised. At this point MH was less tired, had a better appetite and no longer complained of pruritis. His liver was still slightly enlarged 4 cm under the rib edge. His clinical course was carefully observed over the following five years. After his transaminases had normalised, steroid treatment were tapered down gradually. He was now regarded as AIH and maintained on steroids. His SMA remained positive but all other autoantibodies were persistently negative. However, his HCV RNA now tested positive. (It could later be shown that this test was positive due to contamination by other samples tested at the same time.) Despite this, therapy with low dose corticosteroids was continued and in September 1994, subsequent to another flare up AZA 100mg/day was added. MH's HCV RNA tested negative in September 1996 and again in 1998.

This case illustrates the danger of giving IFN to a patient who in fact has AIH. The immediate deterioration in MH's liver functions after IFN was started (3mu TIW) was dramatic, as was the normalisation of his liver functions when corticosteroids were begun. Very possibly, the anti-hepatitis C test was falsely positive due to the high gammaglobulins, since it was later shown that the HCV RNA sample had been contaminated. One could speculate as to whether or not he did indeed have hepatitis C, which triggered the AIH. Whatever the exact sequence of events, the patient probably had an insidious mildly active AIH at least since 1986. The mistaken diagnosis is not surprising, since transaminases had risen slightly but all attempts to make a serological diagnosis of AIH were initially negative, while anti-hepatitis C did test positive.

The second case history is of a patient who appears to have had both AIH and hepatitis C concomitantly and was treated at different times with IFN and ribavirin as

well as steroids and AZA. This thesis also documents how over a decade the two diseases fluctuated and flared until finally settling.

CASE HISTORY 2: DS

A 50 year-old Italian engineer was referred for evaluation in December 1992. He gave a past history of a recurrent bleeding duodenal ulcer, and multiple blood transfusions in 1965 and 1967. His symptoms on presentation were fatigue, dark urine and light stool. Liver enzymes were grossly elevated: AST 450 and ALT 570 and the patient had a monoclonal gammopathy with raised IgG. His HCV RNA was positive. The ANA was strongly positive (1/64O), SMA slightly positive (1/320) and AMA negative. He was regarded as having chronic hepatitis C due to the blood transfusions received earlier, and treatment with IFN as monotherapy was initiated, since in 1992 ribavirin was neither available nor recognised as treatment. The patient tolerated the IFN well and his AST and ALT levels reduced over time. Repeat serology three months after starting IFN showed that ANA and SMA were still positive in significant titres.

By January 1994, exactly twelve months after beginning IFN therapy, the patient's transaminases had normalized (AST 35 and ALT 25). IFN therapy was reduced to 3mu TIW and six months later, in June 1994, when the transaminases were again found to be normal, the IFN was further decreased to 2mu twice a week. In July 1994, repeat HCV RNA results were still positive and the IFN treatment was continued until December 1994 when, despite positive HCV RNA, it was finally stopped.

In December 1995, IFN treatment was restarted this time in combination with ribavirin 800 mg per day. At this stage both ALT and AST were three times normal levels. Bilirubin was not elevated (0.85 mg/dl, N to 1). IgG was 3.5 (N to 2.2.) In April 1997, a sudden massive rise of ALT 435, (N to 40) and AST 411,(N to 37) and an increase in IgG were noted. HCV RNA now tested negative. Immune serology reflected a high titre ANA while SMA and AMA tested negative. These results strongly favoured a diagnosis of AIH and a follow-up biopsy was advocated. Treatment with MP and AZA was started and the enzymes gradually returned to

normal. The patient continued to take a maintenance dose of MP and AZA until a sudden flare up in December 2001 necessitated an increase in this therapy to MP 24mg and AZA 100mg. Liver enzyme tests became normal and have stayed normal under low doses of steroids and AZA.

COMMENT AND DISCUSSION

This patient was treated for hepatitis C for 24 months with IFN and then diagnosed with AIH and successfully treated with steroids and AZA. There is no doubt that he had hepatitis C. The repeatedly positive HCV RNA and initial positive ELIZA tests is adequate proof. At the time of his initial diagnosis he probably already had AIH, since the significantly positive ANA and SMA and the raised gammaglobulin are strongly indicative of AIH. The liver biopsy of December 1992 is however more suggestive of hepatitis C and this together with the viral serology is irrefutable evidence of hepatitis C and therefore the IFN was justified. IFN therapy is mostly poorly tolerated in patients with AIH and should in fact be avoided (as seen in case 1, discussed above) yet despite this, the patient received IFN for a total of 24 months from January 1993 to December 1994 without any apparent serious side effects.

The patient's AST and ALT normalized during this phase of treatment (January 1994 AST 35, ALT 25). In December 1995, one year later, IFN was stopped briefly but was restarted together with ribavirin 800mg per day. This combination was continued until April 1997 when AST and ALT suddenly increased. HCV RNA now tested negative but ANA was strongly positive, though SMA and AMA tested negative. A follow-up biopsy was done and as the serology and histology now very much favoured AIH, treatment with medrol and AZA was started. The transaminases again normalized and the patient continued with maintenance doses of medrol and AZA until December 2001 when a flare up of enzymes necessitated an increase in both drugs.

It is possible, indeed probable, that the patient had both HCV and AIH at the same time. Hepatitis C seems to have been more dominant initially, which is why the patient tolerated the IFN. Neither genotype nor viral quantification was available in 1992 when he was initially diagnosed, but he probably had a high viral load with genotype 1 or 1a, as genotype 1 is common in Italy. Also, HCV RNA remained

positive during the initial two years of IFN treatment, and only became negative after a further 16 months of IFN and ribavirin, which supports a diagnosis of genotype 1, which is less sensitive to IFN than genotypes 2 or 3. It is possible that the hepatitis C caused the immune serology results of December 1992, and that the IFN treatment then provoked a loss of immune tolerance, which led to full-blown AIH by April 1997.

CONCLUSION

Clinically, serologically, biochemically and even histologically, chronic liver disease cases caused only by hepatitis C infection and those with both AIH and Hepatitis C can be very difficult to differentiate. This distinction must be made, however, because the treatment of each condition is not only totally different but also potentially harmful for a sufferer of the other disease entity. IFN is a stimulant of cellular response, which increases autoantibody production, and cytotoxic T cell and B cell activity, and decreases suppressor T-cell activity. If inadvertently given to a patient with AIH, IFN may exacerbate autoimmune disease and provoke a severe hepatitis. Similarly, immunosuppressive drugs such as AZA and steroids, which are essential for AIH treatment, decrease T-helper cell and monocyte activity, decrease interleukin 2 production and increase T-suppresser cell activity and by so doing increase viraemia when mistakenly given to patients with hepatitis C. So if both AIH and hepatitis C are present in the same patient, then theoretically at least the risk of treating either condition exceeds the possible benefit! This is fortunately not the case in practice, and the following therapeutic recommendations can be made for treating patients with chronic hepatitis C with autoimmune features:

- Borderline positive titres of ANA or SMA reactivities occur often in all forms of liver disease; their frequency increases with age and with the inflammatory activity of the underlying liver disease. In the absence of other signs of autoimmunity they should be disregarded.
- In patients with LKM antibodies or significant titres of ANA and SMA (greater than 1:40 in indirect immuno-fluorescence) the diagnosis of HCV infection should be confirmed or excluded by testing for HCV RNA in serum.

If HCV RNA is consistently negative, the disease should first be considered autoimmune and a trial course of steroids should then be given.

- In patients with well-documented HCV infection, attention should be given to other autoimmune stigmata such as arthritis and haemolytic anaemia. A high level of IgG hypergammaglobulinaemia is unusual in chronic hepatitis C, except when the disease has reached the cirrhotic stage. To solve dubious cases, the revised International Autoimmune Hepatitis Group Scoring System (16) devised to identify AIH can be applied. If the aggregate score is non-diagnostic, therapy with IFN (started at low dosage) appears to be safe. If the score points to 'probable' or 'definite' AIH, steroids are advisable as the first therapeutic option.

SUMMARY

The following points about the relationship between HCV and AIH can now be accepted:

i. AIH and HCV may develop in the same patient, particularly in countries where there is a high prevalence of hepatitis C (165);
ii. Symptomatic AIH with all its usual biochemical and serological markers can develop in patients with hepatitis C who are treated with IFN (158); and
iii. Most patients with AIH have no association with chronic HCV infection and the HCV cannot by any criteria biochemical, serological, histological or immunogical, be recognized as a causal factor for AIH (166).

If these statements are taken as accurately reflecting the present state of knowledge about the relationship between AIH and chronic hepatitis C, then they suggest certain practical steps to be introduced into clinical practice before treating *bona fide* AIH with steroids and immunosuppressents and *bona fide* HCV with IFN, and after having initiated treatment in each case, certain surveillance to be ongoing during the treatment.

At present, the efficacy of IFN treatment in cases of hepatitis C complicated by AIH cannot be predicted. IFN treatment has been reported to be more effective in patients

whose serum levels of HCV RNA are low (167) or whose genotype is not 1b (168), indicating that IFN treatment is indicated for these HCV-positive AIH patients. But in the majority of HCV-complicated AIH, an effective dose of corticosteroid should be tried not only for therapeutic but also for diagnostic purposes. Finally the ability of the hepatitis C virus to induce autoimmune phenomena must be investigated. Although this question still needs precise elucidation, one attractive hypothesis is molecular mimicry, whereby a susceptible individual acquires an infection with an agent that exhibits antigens that are immunologically similar to the host antigens. These antigens have the ability to induce an immune response when presented to T cells. Linear amino acid sequences of the molecules or their conformational epitopes may be shared by host proteins, however, and as a result the immune response generated from this sharing crossreacts with host tissue antigens, and can potentially lead to tissue destruction. This immune attack may progressively spread to other self-antigens. The non-self antigens from the initiating infectious agent may or may not be present when the ensuing autoimmune disease becomes clinically apparent (162). Such mechanisms might explain the association of HCV with extra-hepatic disorders with an immunological background, such as lichen planus, mixed cryoglobulinaemia, nephropathies, thyroidopathies, sicca syndrome, chronic polyarthritis, idiopathic pulmonary fibrosis, diabetes, cardiomyopathy and atherosclerosis. A pathogenetic link between HCV and some extrahepatic manifestations was confirmed by these manifestations responsiveness to antiviral therapy, which is now deemed the first therapeutic option to consider. By contrast, the treatment of some other diseases with IFN is ineffective or dangerous (169). AIH is one such disease. At present, as these two cases illustrate there is undoubtedly risk in treating a patient with definite AIH and HCV with IFN and, in the author’s opinion initiation of therapy with steroids while monitoring their potential adverse effects should still be the first therapeutic choice.

CHAPTER 7
GENERAL CONCLUSIONS AND PERSPECTIVES

In Chapter 1 an overview is given of 112 consecutive patients with AIH, diagnosed in the University Hospital of Leuven, Belgium, during the period of 1975 to 2003. The results show a large variability in symptoms and complaints at the time of diagnosis. AIH can present as acute or chronic i.e. either as an incidentally found condition after routine liver tests, as acute hepatitis or even as fulminant liver failure. The evidence suggests that the diagnosis must be considered in non-viral liver disease with significantly raised ANA and/or SMA in a patient with gammaglobulinaemia. Contrary to classical thinking, no fixed age predisposition is found but rather an even spread over various age groups. The male:female ratio is 1:2.5. AIH can for the most part be successfully treated with a combination therapy of corticosteroids and AZA. However, high-dose corticosteroids can cause serious complications, particularly in older patients. Therefore, a lower dose of steroids is usually adequate, although biochemical normalisation may take longer. In approximately half of the patients corticosteroid therapy could be discontinued and treatment with AZA alone continued. The long-term survival of adequately treated patients is almost similar to that of the control group. In the cohort ten patients died, three who presented with acute liver failure, two died eventually of hepatocellular carcinoma and one died of cerebral lymphoma. Rather distressingly, three older patients died of sepsis, which possibly may have been co-induced by the high dose of steroids. Five patients underwent liver transplants, of which one subsequently died of Aspergillosis. Various sub-groups of these 112 patients were then researched further.

In Chapter 2, pregnancy and AIH are investigated. A report of 12 pregnancies in six patients with AIH supports the following: pregnancy in chronic liver disease is not a common event and the consequences for mother and child have not been thoroughly studied. AIH is often found in young women in their childbearing years, who thus often struggle to fall pregnant. This is because most of the patients are cirrhotic and have oligomenorrhea or anovulatory cycles. 12 pregnancies are documented in six patients and it was observed that all except one patient suffered a flare up of hepatitis shortly after delivery. The analysis of this data stresses the importance of adjusting

medication immediately after delivery so as to either prevent or reduce the severity of the anticipated AIH flare up.

Chapter 3 analyses 28 patients who were diagnosed at age ≥65 years. The group consisted of seven males and 21 females. Their characteristics were compared with those of the younger patient population. The gender ratio male:female is 1:3 (≥ 65), as opposed to 1:2 (<65yr). The presenting symptoms are not significantly different in the older versus the younger population and consist of identical findings in 9 % (vs 20 %), fatigue in 23 % (vs 30 %), jaundice in 40 % (vs 48 %), jaundice plus complications in 18 % (vs 5%), ascites in 9 % (vs 4 %). ANA ≥ 1/80 positive in 93 % (vs 86 %), SMA >1/40 positive in 50 % (vs 81 %), and aLKM always neg (vs 3 %). Histological assessment showed acute necrotizing hepatitis (collapse) in 19 % (vs 16 %), severe interphase hepatitis in 15 % (vs 25 %), and chronic hepatitis with plasmo-lymphocytic infiltrate in 30 % (vs 32 %), cirrhosis in 30 % (vs 27 %), while biopsy was refused in 11 %. Similarly in patients with cirrhosis inflammation was still very active in one third. Importantly, elderly patients responded very well to low doses of MP (8 mg) plus AZA (1 mg/kg). This schedule helps prevent the side effects seen with higher dosages including infectious complications that can lead to sepsis and death in some patients.

The recommendation is that AIH be considered when elderly persons present with liver disease with or without jaundice. Acute AIH in the above 65 year old patient may present with pronounced jaundice, ascites, and encephalopathy, so it is imperative that steroid therapy in adequate dose be instituted without delay if death is to be avoided. The steroid therapy should be individualised but preferably kept at the lowest effective dose. AZA should be introduced early and the dose increased as steroids are decreased as the patient's condition improves. Though this analysis only included patients 65 years or older, the same principles of diagnosis and treatment should be followed for all patients especially in the older age group (>40), in my opinion.

In Chapter 4 AIH evolving into PSC is examined. AIH and PSC are generally considered to be two distinct liver disorders. AIH affects women more often and is characterized histologically by infiltration of monocytes in portal spaces, migrating

into the liver lobule, while PSC mostly affects men and is characterised histologically by damage to small and large bile ducts (either or both are affected). However, some of the immunological and histological features of AIH are occasionally seen in PSC. Thus an overlap syndrome does exist. This chapter presents two cases of AIH in women who crossed over to PSC. When an AIH patient presents with raised ALP and GGT or cholestatic jaundice (direct bilirubin $\geq {}^{1}/_{3}$ of total), especially if these signs persist, then crossover to PSC or ASC needs to be considered. Investigation can be either by ERC or liver biopsy or both. Furthermore, if PSC is proven the therapy and surveillance of the patient needs to be reviewed. Patients with PSC, and chronic hepatitis benefit from URSO. The increased risk for cholangiocarcinoma in patients with PSC is well established, the prevalence varying in different studies between 5 % and 20 % (170). An interesting finding is that PSC not only predisposes to cholangiocarcinoma but is also possibly a risk factor for the development of colon carcinoma (110). Thus the clinician must maintain heightened vigilance in PSC patients, using regular liver ultrasound, CT scan, measurement of the billary tract tumour markers serum, Ca 19-9, and surveillance colonoscopy at intervals.

In Chapter 5 toxic hepatitis with features of AIH is analysed using five cases. The clinical and serological spectrum of AIH is very diverse, and AIH is often difficult to distinguish from other causes of acute or chronic hepatitis. The case reports document five patients who developed drug-related toxic hepatitis, which were initially indistinguishable from AIH. The histological features of drug-related hepatitis are often similar to those of viral or autoimmune hepatitis. Therefore all medications that could possibly have caused the hepatitis must be stopped and the patient treated for AIH. Follow up at intervals will reveal the diagnosis because the majority of cases of toxic hepatitis masquerading as AIH resolve slowly after cessation of the offending substance. Certain drugs (alpha methyldopa and nitrofurantoin) can occasionally provoke a chronic hepatitis and may require long-term follow up.

Chapter 6 reviews the problem of hepatitis C and AIH. From the early 1990s the role of the HCV in AIH has been explored, because of multiple reports of the high prevalence of hepatitis C infection in patients with anti-LKM, the diagnostic marker of type II AIH. In addition, the varying prevalence of anti-LKM occurrence in various geographical settings has not yet been explained. Subsequent studies with later

generation serology kits assessed the prevalence of anti-LKM in patients with chronic hepatitis as between 3 and 5 %. Distinguishing between hepatitis C and AIH is crucial, because IFN given to an AIH patient, can provoke a life-threatening flare up of the hepatitis, while steroids given to hepatitis C patients, can promote viral replication, though this increased viral count is never fatal. A case history of a patient who had both AIH and hepatitis C is presented, documenting how over a decade the two diseases fluctuated and flared. The literature on AIH and its association with hepatitis C is reviewed and guidelines suggested by the case and the literature are given.

Summary of this study's achievements:

This study has revealed and explored several important aspects of AIH. Some of these aspects are original observations.

1. Pregnancy in woman with AIH (especially if cirrhotic) is rare. All cases went into clinical remission during pregnancy and all (except one) relapsed shortly after delivery. **This flare up needs to be anticipated and appropriate dose increases in the standard therapy made directly after parturition in anticipation of the expected relapse.**
2. Elderly patients appear to do just as well with smaller doses of steroids as with the traditional high dose and because of steroid-related side affects (immune suppression, sepsis, osteopaenia, Cushingoid features etc.), early substitution with AZA is advocated.
3. AIH should be considered in elderly patients who present with hepatitis and have no markers for hepatitis A, B, C or E and no relevant drug history, because without treatment they may decompensate rapidly into liver failure, ascites, encephalopathy and death. In elderly patients, high-dose steroid treatment needs to be individualised and kept at the lowest effective dose because of the risk of sepsis and other steroid-related side effects.
4. Hepatitis C and AIH can occur in the same patient and great care needs to be taken if IFN is given to a patient whose predominant problem is AIH. Patients with AIH who are given IFN may flare up and decompensate acutely. Guidelines for an approach to and treatment of AIH-hepatitis C patients are reviewed.

5. Finally, the chapter describing patients with AIH and PSC crossover highlights the point that clinicians need to remain vigilant so that AIH patients who develop cholestatic features are investigated for the possibility of PSC. If crossover is confirmed by ERC and liver biopsy, appropriate therapeutic measures and follow up must be instituted.

I hope that those who read this thesis will have gained some new insights into AIH a fascinating and multifaceted disease which, though nearly always chronic, if recognized early, treated adequately and followed up endlessly need not be debilitating.

REFERENCES

1. Lurman A. Eine icterus epidemi. *Berl Klin Wochenschr. 1885; 22:20.*

2. Seeff LB, Beebe GW, Hoofnagle JH, Norman JE. Buskell-Bales Z. *et al*. A serologic follow up of the 1942 epidemic of post vaccination hepatitis in the United States Army. *N Engl J Med.* 1987; 316: 965-70

3. Blumberg BS, Alter HJ, Visnich S. A "new" antigen in leukemia serum. *JAMA.*1965; 191:541-6.

4. Dane DS, Cameron CH, Briggs M. Virus-like particles in serum of patients with Australia - antigen- associated hepatitis. *Lancet.* 1970;1: 695-8

5. Alter HJ, Holland PV, Purcell RH. The emerging pattern of post-transfusion hepatitis. *Am J Med Sci.* 1975; 270: 329-34.

6. Feinstone SM, Kapikian AZ, Purcell RH, Alter HJ, Holland PV. Transfusion-associated hepatitis not due to viral hepatitis type A or B. *N Engl J Med. 1975; 292:767-70.*

7. Gocke DJ. A prospective study of posttransfusion hepatitis. The role of the Australian antigen. *JAMA. 1972; 219: 1165-70.*

8. Sedlack RE, Viggiano TR, Poterucha JJ. Gastroenterology and Hepatology. in: Habermann TM. editor. Mayo Clinic Internal Medicine Review. 7th edition. Rochester: Mayo Clinic Scientific Press; 2006.

9. Kanzler S, Weidemann C, Gerken G, Löhr HF, Galle PR, Meyer zum Buschenfelde KH. *et al*. Clinical significance of autoantibodies to soluble liver antigen in autoimmune hepatitis. *J Hepatol. 1999; 31:635-40.*

10. Czaja AJ, Menon KV, Carpenter HA. Sustained remission after corticosteroid therapy for Type 1 autoimmune hepatitis: a retrospective analysis. *Hepatology. 2002;35(4): 890-7.*

11. Boberg KM, Aadland E, Jahnsen J, Raknerud N, Stiris M, Bell H. Incidence and prevalence of primary biliary cirrhosis, primary sclerosing cholangitis, and autoimmune hepatitis in a Norwegian population. *Scand J Gastroenterol.* 1998; 33: 99-103.

12. Krawitt EL. Autoimmune hepatitis. *N Engl J Med.* 1996; 334: 897-903.

13. Waldenström J. Leber, Blutproteine und Narungseiweiss. *Deutsch Z Verdau Stoffwechselkr. 1950;15:113-9.*

14. Cowling DC, Mackay IR, Taft LI. Lupoid hepatitis. *Lancet.* 1956; 271: 1323-6.

15. Johnson PJ, McFarlane IG. Meeting report: International Autoimmune Hepatitis Group. *Hepatology.* 1993; 18: 998-1005.

16. Alvarez F, Berg PA, Bianchi L, Burroughs AK, Cancado EL, Chapman RW. *et al*. International Autoimmune hepatitis Group report: review of criteria for diagnosis of autoimmune hepatitis. *J Hepatol.* 1999; *31: 929-938*.

17. Boberg KM, Fausa O, Haaland T, Holter E, Mellbye OJ, Spurkland A. *et al*. Features of autoimmune hepatitis in primary sclerosing cholangitis patients according to a scoring system for the diagnosis of autoimmune hepatitis. *Hepatology.* 1996; 23:1369-76.

18. Colombato LA, Alvarez F, Cote J, Huet PM. Autoimmune cholangiopathy: the result of consecutive primary biliary cirrhosis and Autoimmune hepatitis? *Gastroenterology*. 1994; 107: 1839-43.

19. Abdo A, Bain VG, Kichian K, Lee SS. Evolution of autoimmune hepatitis to primary sclerosing cholangitis: a sequential syndrome. *Hepatology*. 2003; 36: 1393-9.

20. Mieli-Vergani G. Autoimmune sclerosing cholangitis: a distinct clinical entity ? *J Hepatol. 2000; 32 (Suppl 2) : 7-8.*

21. Perdigoto R, Carpenter HA, Czaja AJ. Frequency and significance of chronic ulcerative colitis in severe corticosteroid-treated autoimmune hepatitis. *J Hepatol.* 1992; 14:325-31.

22. Crapper RM, Bhathal PS, Mackay IR, Frazer IH. Acute autoimmune hepatitis. *Digestion*. 1986; 34:216-25.

23. Nikias GA, Batts KP, Czaja AJ. The nature and prognostic implications of autoimmune hepatitis with an acute presentation. *J Hepatol.* 1994; 21: 866-71.

24. Davis GL, Czaja AJ, Baggenstoss AH, Taswell HF. Prognostic and therapeutic implications of extreme serum aminotransferase elevation in chronic active hepatitis. *Mayo Clin Proc.* 1982; 57: 303-9.

25. Dighiero G, Lymberi P, Monot C, Abuaf N. Sera with high levels of anti-smooth muscle and ant-mitochondrial antibodies frequently bind to cytoskeletal proteins. *Clin Exp Immunol.* 1990; 82: 52-6.

26. Homberg JC, Abuaf N, Bernard O, Islam S, Alvarez F, Khalil SH. *et al*. Chronic active hepatitis associated with antiliver / kidney microsome antibody type 1: a second type of “autoimmune” hepatitis. *Hepatology.* 1987; 7: 1333-9

27. Manns MP, Griffin KJ, Sullivan KF, Johnson EF. LKM-1 autoantibodies recognize a short linear sequence in P-45011D6, a cytochrome P-450 monooxygenase. *J Clin Invest.* 1991; 88: 1370-78.

28. Czaja AJ, Manns MP. The validity and importance of subtypes of autoimmune hepatitis: a point of view. *Am J Gastroenterol.* 1995; 90: 1206-11.

29. Yamamoto AM, Cresteil D, Homberg JC, Alvarez F. Characterization of the anti-liver-kidney microsome antibody (anti-LKM1) from hepatitis C virus-positive and -negative sera. *Gastroenterology.* 1993; 104: 1762-7.

30. Manns MP, Kruger M. Immunogenetics of chronic liver disease. *Gastroenterology*. 1994; 106: 1676-97.

31. Manns MP. Autoimmune hepatitis. In Bircher J, Benhamou JP, McIntyre N, Rizzetto M, Rodes JE. Editors. Oxford textbook of clinical hepatology. 2nd ed. Oxford: Oxford University Press; 1999 : 1109-20.

32. Manns M, Gerken G, Kyriatsoulis A, Staritz M, Meyer zum Buschenfelde KH**.** Characterisation of a new subgroup of autoimmune chronic active hepatitis by autoantibodies against a soluble liver antigen. *Lancet*. 1987; 1: 292-4.

33. Czaja AJ, Carpenter HA, Manns MP. Antibodies to soluble liver antigen, P450IID6, and mitochondrial complexes in chronic hepatitis. *Gastroenterology.* 1993; 105: 1522-28.

34. Stechemesser E, Klein R, Berg PA. Characterization and clinical relevance of liver-pancreas anti-bodies in autoimmune hepatitis. *Hepatology.* 1993; 18: 1-9.

35. Dufour JF, Zimmerman M, Reichen J. Severe autoimmune hepatitis in patients with previous spontaneous recovery of a flare. *J Hepatol.* 2002; 37: 748-53.

36. Elkayam O, Yaron M, Caspi D. Minocycline-induced autoimmune syndromes: an overview. *Semin Arthitis Rheum.* 1999; 28: 392-7.

37. Lewis JH, Zimmerman HJ. Drug-induced autoimmune liver disease. In Krawitt EL, Wiesner RH, Nishioka M. *Autoimmune Liver Diseases.* Amsterdam: Elsevier; 1998. : 627-48.

38. Sharp R, Ishak KG, Zimmerman HJ. Chronic active hepatitic and severe hepatic and severe hepatic necrosis associated with nitrofurantoin. *Ann Int Med.* 1980; 92: 14-19.

39. Pham BN, Bernuau J, Durand F, Sauvanet A, Degott C, Prin L. *et al*. Eotaxin expression and eosinophil infiltrate in the liver of patients with drug-induced liver disease. *J Hepatol* 2001; 34: 537-47.

40. Schvarcz R, Weiland O, Von Sydow M. False positive reactivity for antibodies against hepatitis C virus in patients with autoimmune hepatitis. *Scan J Infect Dis.* 1990; 22; 377-8.

41. Papo T, Marcellin P, Bernuau J, Durand F, Poynard T, Benhamou JP. Autoimmune chronic hepatitis exacerbated by alpha-Interferon. *Ann Intern Med* 1992; 116: 51-3.

42. Targan SR, Landers C, Vidrich A, Czaja AJ. High titer antineutrophil cytoplasmic antibodies in type I autoimmune hepatitis. *Gastroenterology.* 1995; 108: 1159-66.

43. Czaja AJ, Pfeifer KD, Decker RH, Vallari AS. Frequency and significance of antibodies to asialoglycoprotein receptor in type I autoimmune hepatitis. *Dig Dis Sci* 1996; 41: 1733-40.

44. Czaja AJ, Carpenter HA. Progressive fibrosis during corticosteroid therapy of autoimmune hepatitis. *Hepatology.* 2004; 39:1631-8.

45. Seki T, Kiyosawa K, Inoko H, Ota M. Association of autoimmune hepatitis with HLA-Bw54 and DR4 in Japanese patients. *Hepatology.* 1990; 12: 1300-4.

46. Buchel E, Van Steenbergen W, Nevens F, Fevery J. Improvement of autoimmune hepatitis during pregnancy but flare up after delivery. *Am J Gastroenterol.* 2002; 97: 3160-5.

47. Kanzler S, Lohr H, Gerken G, Galle PR, Lohse AW. Long-term management and prognosis of autoimmune hepatitis (AIH): a single center piece. *Z Gastroenterol.* 2001; 39: 339-48.

48. Summerskill WH, Korman MG, Ammon HV, Baggenstoss AH. Prednisone for chronic active liver disease: dose titration, standard dose, and combination with azathioprine compared. *Gut* 1975; 16: 876-83.

49. Al-Khalidi JA, Czaja AJ. Current concepts in the diagnosis, pathogenesis, and treatment of autoimmune hepatitis. *Mayo Clin Proc.* 2001; 76: 1237-52.

50. Roberts SK, Therneau T, Czaja AJ. Prognosis of histological cirrhosis in type I autoimmune hepatitis. *Gastroenterology*. 1996; 110: 848-57.

51. Johnson PJ, McFarlane IG, Williams R. Azathioprine for long-term maintenance of remission in autoimmune hepatitis. *N Engl J Med.* 1995; 333: 958-63.

52. Verslype C, George C, Buchel E, Nevens F, Van Steenbergen W, Fevery J. Diagnosis and treatment of autoimmune hepatitis at age 65 and older. *Aliment Pharmacol Ther.* 2005; 21:695-9.

53. Pratt DS, Flavin DP, Kaplan MM. The successful treatment of autoimmune hepatitis with 6-mercaptopurine after failure with azathioprine. *Gastroenterology*.1996; 110: 271-4.

54. Richardson PD, James PD, Ryder SD. Mycophenolate mofetil for maintenance of remission in autoimmune hepatitis patients resistant to or intolerant of azathioprine. *J Hepatol*. 2000; 33: 371-5.

55. Alvarez F, Giocca M, Canero-Velasco C, Ramonet M, Davila MTGd, Cuarterolo M. *et al*. Short-term cyclosporine induces a remission of autoimmune hepatitis in children. *J Hepatol.* 1999; 30: 222-7.

56. Van Thiel DH, Wright H, Carrol P, Abu Elmagd K, Rodrigues-Rilo H. McMichael J. *et al*. Tacrolimus: a potential new treatment for autoimmune chronic active hepatitis: results of an open-label preliminary trial. *Am J Gastroenterol*. 1995; 90:771-6.

57. Czaja AJ, Lindor KD. Failure of budesonide in a pilot study of treatment-dependent autoimmune hepatitis. *Gastroenterology.* 2000; 119: 1312-6.

58. Neuberger J. Transplantation for autoimmune hepatitis. *Sem Liv Dis*. 2002;22:379-86.

59. Sherlock S, Dooley J. Diseases of the liver and biliary system, 9th ed. Oxford: Blackwell; 1993.

60. Feld J, Dinh H, Arenovich T, Marcus VA, Wanless IR, Heathcote EJ. Autoimmune hepatitis: effect of symptoms and cirrhosis on natural history and outcome. *Hepatology*. 2005; 42:53-62.

61. Kogan J, Safadi R, Ashur Y, Shouval D, Ilan Y. Prognosis of symptomatic versus asymptomatic autoimmune hepatitis: a study of 68 patients. *J Clin Gastroenterol.* 2002; 35: 75-81.

62. Lebovics E, Schaffner F, Klion FM, Simon C. Autoimmune chronic active hepatitis in postmenopausal women. *Dig Dis* Sci. 1985; 30: 824-8.

63. Al-Chalabi T, Boccato S, Portmann BC, McFarlane IG, Heneghan MA. Autoimmune hepatitis (AIH) in the elderly: a systematic retrospective analysis of a large group of consecutive patients with definite AIH followed at a tertiary referral center. *J Hepatol* 2006; 45: 575-83.

64. Newton JL, Burt AD, Park JB, Mathew J, Bassendine MF, James OF. Autoimmune hepatitis in older patients. *Age Ageing.* 1997; 29: 441-4.

65. Parker DR, Kingham JG. Type I autoimmune hepatitis is primarily a disease of later life. *Q J Med.* 1997; 90: 289-96.

66. Schramm C, Kanzler S, zum Büschenfelde KH, Galle PR, Lohse AW. Autoimmune hepatitis in the elderly. *Am J Gastroenterol.* 2001; 96: 1587- 91.

67. Czaja AJ, Carpenter HA, Santrach PJ, Moore SB, Homburger HA. The nature and prognosis of severe cryptogenic chronic active hepatitis. *Gastroenterology.* 1993; 104: 1755-61.

68. Kaymakoglu S, Cakaloglu Y, Demir K, Turkoglu S, Badur S, Gurel S. *et al*. Is severe cryptogenic chronic hepatitis similar to autoimmune hepatitis? *J Hepatol.* 1998;28: 78-83.

69. Geubel AP, Sempoux CL. Drug and toxin-induced bile duct disorders. *J Gastroenterol-Hepatol.* 2000; 15: 1232-8.

70. Van Steenbergen W, Vanstapel MJ, Desmet V, Van Kerckvoorde L, De Keyzer R, Brijs R. *et al*. Cimetidine-induced liver injury. Report of three cases. *J Hepatol.* 1985; 1: 359-68.

71. Heneghan MA, McFarlane IG. Current and novel immunosuppressive therapy for autoimmune hepatitis. *Hepatology.* 2002; 35: 7-13.

72. Sherlock S, Dooley J. Diseases of the Liver and Biliary System. 10th edition. Oxford: Blackwell; 1997.

73. Strettel MD, Donaldson PT, Thomson LJ, Santrach PJ, Moore SB. Czaja AJ. *et al*. Allelic basis for HLA-encoded susceptibility to type 1 autoimmune hepatitis. *Gastroenterology.* 1997; 112: 2028-35.

74. Cundy TF, Butler J, Pope RM, Saggar-Malik AK, Wheeler MJ, Williams R. Amenorrhoea in women with non-alcoholic chronic liver disease. *Gut.* 1991; 32: 202-6.

75. Steven MM, Buckley JD, Mackay IR. Pregnancy in chronic active hepatitis. *Q J Med.* 1979; 48: 519- 31.

76. Burroughs AK. Liver disease and pregnancy. In: Bircher J, Benhamou JP, McIntyre N, Rizzetto M, Rodés J. Editors. Oxford Textbook of Clinical Hepatology. 2nd edition. New York: Oxford University Press; 1999:1901 - 13.

77. Cheng YS. Pregnancy in liver cirrhosis and / or portal hypertension. *Am J Obstet Gynecol*. 1977; 128: 812- 22.

78. Schreyer P, Caspi E, El-Hindi JM, Eshchar J. Cirrhosis - pregnancy and delivery: a review. *Obstet Gynec Surv.* 1982; 37: 304-12.

79. Dave B, Danahy J, Fevery J, Nevens F. Treatment of acute esophageal variceal bleeding: a review. *Eur J Int Med.* 1999; 10: 140-5.

80. Gregorio GV, Portmann B, Karani J, Harrison P, Donaldson PT, Vergani D. *et al*. Autoimmune hepatitis / sclerosing cholangitis overlap syndrome in childhood: a 16 year prospective study. *Hepatology.* 2001; 33: 544-53.

81. Colle I, Hautekeete M. Remission of autoimmune hepatitis during pregnancy: a report of two cases. *Liver.* 1999; 19: 55-7.

82. Heneghan MA, Norris SM, O'Grady JG. Autoimmune hepatitis: optimal management in pregnancy and review of maternal and foetal outcomes. Hepatology. 1998; 28: 393A (abstract 921).

83. Laifer SA, Abu-Elmagd K, Fung JJ. Hepatic transplantation during pregnancy and the puerperium. *J Matern Fetal Med.* 1997; 6: 40-44.

84. Heneghan MA, Norris SM, O'Grady JG, Harrison PM, McFarlane IG. Management and outcome of pregnancy in autoimmune hepatitis. *Gut* 2001; 48:97-102.

85. Tennenbaum R, Marteau P, Elefant E, Rambaud JC, Modigliani R, Gendre JP. *et al*. Pregnancy outcome in inflammatory bowel diseases. *Gastroenterol Clin Biol.* 1999; 23: 464-9.

86. Ostensen M. Sex hormones and pregnancy in rheumatoid arthritis and systemic lupus erythematosus. *Ann NY Acad Sci.* 1999; 876: 131-43.

87. Wegmann TG, Lin H, Guilbert L, Mosmann TR. Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a T_H2 phenomenon? *Immunol Today*. 1993; 14: 353- 6.

88. Whitacre CC, Reingold SC, O'Looney PA. A gender gap in autoimmunity. *Science.* 1999; 283:1277-8.

89. Wilder RL. Hormones, pregnancy, and autoimmune diseases. *Ann NY Acad Sci.* 1998; 840: 45-50.

90. Conte D, Fraquelli M, Prati D, Colucci A, Minola E. Prevalence and clinical course of chronic hepatitis C virus (HCV) infection and rate of HCV vertical transmission in a cohort of 15,250 pregnant women. *Hepatology*. 2000; 31: 751- 5.

91. Gervais A, Bacq Y, Bernuau J, Martinot M, Auperin A, Boyer N. *et al*. Decrease in serum ALT and increase in serum HCV RNA during pregnancy in women with chronic hepatitis C. *J Hepatol.* 2000; 32: 293-9.

92. Czaja AJ. Autoimmune hepatitis. Evolving concepts and treatment strategies. *Dig Dis Sci.* 1995; 40.435-56.

93. Kirk AP, Jain S, Pocock S, Thomas HC, Sherlock S. Late results of the Royal Free Hospital prospective controlled trial of prednisolone therapy in hepatitis B surface antigen negative chronic active hepatitis. *Gut.* 1980; 21:78-83.

94. Korelitz BI. Inflammatory bowel disease and pregnancy. *Gastroenterol Clin N Am.* 1998; 27: 213- 24.

95. Friedman JM, Polifka JE. The effects of drugs on the fetus and nursing infant: a handbook for health care professionals. Baltimore : Johns Hopkins Univ Press; 1996.

96. Rayburn WF. Connective tissue disorders and pregnancy. Recommendations for prescribing. *J Reprod Med.* 1998; 43:341-9.

97. Coulam CB, Moyer TP, Jiang NS, Zincke H. Breast-feeding after renal transplantation. *Transplant Proc.* 1982; 14: 605-9.

98. Nyberg G, Haljamae U, Frisenette-Finch C, Wennergren M. Kjellmer I. Breast-feeding during treatment with cyclosporine. *Transplantation.* 1998; 65: 253 –5.

99. Begg EJ, Atkinson HC, Darlow BA. Guide to safety of drugs in breast feeding. In: Speight TM, Holford NHG. *Avery's Drug Treatment.* 4th edition. Auckland: Adis Press; 1997: 1701-23.

100. Ben-Ari Z, Dhillon AP, Sherlock S. Autoimmune cholangiopathy: part of the spectrum of autoimmune chronic active hepatitis. *Hepatology.* 1993; 18: 10-5.

101. Gohlke F, Lohse AW, Dienes HP, Lohr H, Marker-Hermann E, Gerken G, Meyer zum Buschenfelde KH. Evidence for an overlap syndrome of autoimmune hepatitis and primary sclerosing cholangitis. *J Hepatol*. 1996; 24: 699-705.

102. Griga T, Tromm A, Muller KM, May B. Overlap syndrome between autoimmune hepatitis and primary sclerosing cholangitis in two cases. *Eur J Gastroenterol Hepatol.* 2000; 12: 589-64.

103. Kim WR, Ludwig J, Lindor KD. Variant forms of cholestatic diseases involving small bile ducts in adults. *Am J Gastroenterol.* 2000; 95: 1130-38.

104. Lawrence SP, Sherman KE, Lawson JM, Goodman ZD. A 39 year old man with chronic hepatitis. *Sem Liver Dis*. 1994; 14: 97-105.

105. Rabinovitz M, Demetris AJ, Bou-Abboud CF, Van Thiel DH. Simultaneous occurrence of primary sclerosing cholangitis and autoimmune chronic active hepatitis in a patient with ulcerative colitis. *Dig Dis Sci*.1992; 37: 1606-11.

106. Czaja AJ. Frequency and nature of the variant syndromes of autoimmune liver disease. *Hepatology* 1998; 28: 360-5.

107. Minuk GY, Sutherland LR, Pappas SC, Kelly JK, Martin SE. Autoimmune chronic active hepatitis (lupoid hepatitis) and primary sclerosing cholangitis in two young adult females. *Can J Gastroenterol*. 1988;2: 22-7.

108. McNair AN, Moloney M, Portmann BC, Williams R, McFarlane IG. Autoimmune hepatitis overlapping with primary sclerosing cholangitis in five cases. *Am J Gastroenterol.* 1998; 93: 777-84.

109. Broomé U, Olsson R, Lööf L, Bodemar G, Hultcrantz R, Danielsson A. *et al*. Natural history and prognostic factors in 305 Swedish patients with primary sclerosing cholangitis. *Gut.* 1996; 38:610-5.

110. Lai G. Primary sclerosing cholangitis: a study of 123 patients and a review of the literature. [dissertation] Leuven: Katholieke Universiteit; 2004.

111. Maddrey W. How many types of autoimmune hepatitis are there? *Gastroenterology*. 1993; 105:1571-5.

112. Van Buuren HR, van Hoogstraten HJE, Terkivatan T, Schalm SW, Vleggaar FP. High prevalence of autoimmune hepatitis among patients with primary sclerosing cholangitis. *J Hepatol*. 2000; 33: 543-8.

113. Saarinen S, Olerup O, Broomé U. Increased frequency of autoimmune diseases in patients with primary sclerosing cholangitis. *Am J Gastroenterol.* 2000; 95: 3195-9.

114. el-Shabrawi M, Wilkinson ML, Portmann B, Mieli-Vergani G, Chong SKF, Williams R. *et al*. Primary sclerosing cholangitis in childhood. *Gastroenterology.* 1987; 92: 1226-35.

115. Gregorio GV, Portmann B, Reid F, Donaldson PT, Doherty DG, McCartney M. *et al*. Autoimmune hepatitis in childhood: a 20-year experience. *Hepatology*. 1997; 25: 541-7.

116. Wilschanski M, Chait P, Wade JA, Davis L, Corey M, St. Louis P. *et al*. Primary sclerosing cholangitis in 32 children: clinical, laboratory, and radiographic features, with survival analysis. *Hepatology.* 1995; 22: 1415-22.

117. Perez-Aquilar F, Berenguer M, Ramirez-Palanca JJ, Paya A, Vera-Sempere FJ. Sanchez-Cuenca JM. *et al*. Chronic autoimmune hepatitis following cholestatic hepatitis caused by droxicam. *Med Clin. (Barc)* 1996; 106; 460-2.

118. Stricker BH, Blok AP, Claas FH, Van Parys GE, Desmet VJ. Hepatic injury associated with use of nitrofurans. a clinicopathological study of 52 reported cases. *Hepatology.* 1988; 8: 599-606.

119. Jokela S. Liver disease due to nitrofurantoin. *Gastroenterology.* 1967; 53: 306-11.

120. Iwarson S, Lindberg J, Lundin P. Nitrofurantoin-induced chronic liver disease. Clinical course and outcome of five cases. *Scand J Gastroent.* 1979 14: 497-502.

121. Black M, Rabin L, Schatz N. Nitrofurantoin-induced chronic active hepatitis. *Ann. Int Med.* 1980; 92: 62-4.

122. Berson A, Freneaux E, Larrey D, Lepage V, Douay C, Mallet C. *et al*. Possible role of HLA in hepatotoxicity. An exploratory study in 71 patients with drug-induced idiosyncratic hepatitis. *J Hepatol.* 1994; 20: 336-42.

123. Loughridge LW. Peripheral neuropathy due to nitrofurantoin. *Lancet*. 1962; 2: 1133-5.

124. Prakash UBS. Pulmonary reaction to nitrofurantoin. *Sem Respir Med.* 1980; 2:70-5.

125. De Groote J, Fevery J, Desmet V, Verhaeghe M. Acute and chronic liver disease due to alpha-methyldopa. In: Gerok W, Sickling K. Editors. Drugs and the Liver. Stuttgart: FK Schattauer- Verlag; 1975: 327-9.

126. Elkington SG, Schreiber WM, Conn HO. Hepatic injury caused by L-alpha-methyldopa. *Circulation.* 1969; 40: 589-95.

127. Hoyumpa AM, Connell AM. Methyldopa Hepatitis. Report of three cases. *Amer J Dig Dis.* 1973; 18: 213-22.

128. Zimmerman HJ, Maddray WC. Toxic and drug-induced hepatitis. In: Schiff ER. Editor. Diseases of the Liver. 7th edition.. Philadelphia: Lippincott; 1993:707-69.

129. Rehman OU, Keith TA, Gall EA: Methyldopa-induced submassive hepatic necrosis. *JAMA.* 1973; 224: 1390-2.

130. Goldstein GB, Lam KC, Mistilis SP. Drug-induced chronic hepatitis. *Am J Dig Dis. 1973; 18:177-84.*

131. Phadnis SV, Sangay MR, Sanusi FA. Alpha-methyldopa-induced acute hepatitis in pregnancy. *Aust NZ J Obstet Gynaecol.* 2006; 46: 254-60.

132. Donaldson PT, Doherty DG, Hayllar KM, Mc Farlane IG, Johnson PJ, Williams R. Susceptibility to autoimmune chronic active hepatitis: human leucocyte antigens DR4 and A1-B8-DR3 are independent risk factors. *Hepatology.* 1991; 13: 701-6.

133. Brooks PM, Day RO. Nonsteroidal antiinflammatory drugs - differences and similarities. *N Engl J Med.* 1991; 324:1716-25.

134. Lewis JH. Hepatic toxicity of nonsteroidal anti-inflammatory drugs. *Clin Pharm.* 1984; 3:128-38.

135. Teoh NC, Farrell GC. Hepatotoxicity associated with non-steroidal anti-inflammatory drugs. *Clin Liver Dis.* 2003; 7: 4001-13.

136. Buchel O, Roskams T, Van Damme B, Nevens F, Pirenne J, Fevery J. Nodular regenerative hyperplasia, portal vein thrombosis, and avascular hip necrosis due to hyperhomocysteinaemia. *Gut.* 2005; 54:1021-23.

137. Schnyder G, Roffi M, Pin R, Flammer Y, Lange H, Eberli FR. *et al***.** Decreased rate of coronary restenosis after lowering of plasma homocysteine levels**.** *New Engl J Med. 2001; 345:1593-6.*

138. Den Boer W, Loeliger EA. Phenprocoumon-induced jaundice. *Lancet.* 1980;1:912.

139. De Man RA, Wilson JH, Schalm SW, ten Kate FJ, Van Leer E. Phenprocoumon-induced hepatitis mimicking non-A, non-B hepatitis. *J Hepatol.* 1990; 11: 318-321.

140. Hinrichsen H, Luttges J, Kloppel G, Folsch UR, Schmidt WE. Idiosyncratic drug allergic phenprocoumon-induced hepatitis with subacute liver failure initially misdiagnosed as autoimmune hepatitis. *Scand J Gastroenterol.* 2002;37(11):1345-6

141. Höhler T, Schnütgen M, Helmreich-Becker I, Mayet WJ, Meyer zum Büschenfelde KH. Drug-induced hepatitis: a rare complication of oral anticoagulants. *J Hepatology*. 1994; 21:447-9.

142. Toon S, Heimark LD, Trager WF, O'Reilly RA. Metabolic fate of phenprocoumon in humans. *J Pharm Sci.* 1985; 74: 1037- 40.

143. Ciorciaro C, Hartmann K, Stoller R, Kuhn M. Liver injury caused by coumarin anticoagulants: experience of the IKS (Intercanton Monitoring Station) and the SANZ (Swiss Center for Drug Monitoring). *Schweiz Med Wochenschr.*1996; 126: 2109-13.

144. Basude D, Dhawan A. Re: Minocycline-induced autoimmune hepatitis with subsequent cirrhosis. [comment]. J Ped Gastroent Nutr. 2007;44:389. Author reply : 389-90.

145. Reynolds TB, Peters RL, Yamada S. Chronic active and Lupoid hepatitis caused by a laxative, oxyphenisatin. *New Engl J Med.* 1971; 285: 813-20.

146. Liu ZX, Kaplowitz N. Immune-mediated drug induced liver disease. *Clin Liver Dis.* 2002; 6: 755-74.

147. Pessayre D, Mansouri A, Haouzi D, Fromenty B. Hepatotoxicity due to mitochondrial dysfunction**.** *Cell Biol Toxicol*. 15(6):367-73, 1999.

148. Larrey D. Hepatotoxicity of herbal remedies. *J Hepatol.* 1997; 26 Suppl 1: 47-51.

149. Maddray WL, Boitnott JK. Drug-induced chronic active hepatitis. In: Krawitt EL, Wiesner RH. Editors. Autoimmune Liver Diseases. New York: Raven Press; 1991: 219-232.

150. Rodman JS, Deutsch DJ, Gutman SI. Methyldopa hepatitis. A report of six cases and review of the literature. *Am J Med.* 1976; 60: 941-8.

151. Scully LJ, Clarke MD, Barr RJ. Diclofenac induced hepatitis. 3 Cases with features of autoimmune chronic active hepatitis. Dig Dis Sci. 1993; 38 : 744- 51.

152. Goldstein PE, Devier J, Cremer M. Acute hepatitis and drug-related lupus induced by minocycline treatment. *Am J Gastroentorol*. 1997; 92: 143-6.

153. Alla V, Abraham J, Siddiqui J, Raina D, Wu GY, Chalasani NP. Autoimmune hepatitis triggered by statins. *J Clin Gastroenterol.* 2006; 40: 757-61.

154. Zimmerman HJ. Drug induced liver disease. In: Schiff ER, Sorrel MF, Maddrey WC. Editors. Schiff's Diseases of the liver. 8th Edition. Philadelphia: Lippincott-Raven; 1999:973-1064.

155. Stricker BHC, Spoelstra P, Dukes MNG. Drug-induced hepatic injury. Amsterdam: Elsevier; 1995:1

156. Farrell GC. Drug-induced liver disease. Edinburgh: Churchill-Livingstone; 1994.

157. McFarlane IG, Smith HM, Johnson PJ, Bray GP, Vergani D, William R. Hepatitis C virus antibodies in chronic active hepatitis: pathogenic factor or false-positive result ? *Lancet.* 1990; 335:754-7.

158. Garcia-Buey L, Garcia-Monzon C, Rodriguez S, Borque MJ. Garcia-Sanchez A. Iglesias R. *et al*. Latent autoimmune hepatitis triggered during interferon therapy in patients with chronic hepatitis C. *Gastroenterology.* 1995; 108: 1770-7.

159. Gueguen M, Boniface O, Bernard O, Clerc F, Cartwright T, Alvarez F. Identification of the main epitope on human cytochrome P450 IID6 recognized by anti-liver kidney microsome antibody. *J Autoimmunity.* 1991; 4: 607-15

160. Lenzi M, Johnson PJ, McFarlane IG, Ballardini G, Smith HM, McFarlane BM. *et al*. Antibodies to hepatitis C virus in autoimmune liver disease: evidence for geographical heterogeneity. *Lancet.* 1991; 338: 277-80.

161. Todros L, Touscoz G, D'Urso N, Durazzo M. Albano E. Poli G. *et al*. Hepatitis C virus-related chronic liver disease with autoantibodies to liver-kidney microsomes (LKM). Clinical characterization from idiopathic LKM-positive disorders. *J Hepatol*. 1991; 13: 128-31.

162. Vogel A, Manns MP, Strassburg CP. Autoimmunity and viruses. *Clin Liver Dis.* 2002; 6:739-53.

163. Miyakawa H, Kitazawa E, Abe K, Kawaguchi N, Fuzikawa H, Kikuchi K. *et al*. Chronic hepatitis C associated with Anti-liver/kidney microsome-1 antibody is not a subgroup of autoimmune hepatitis. *J Gastroenterol.* 1997; 32: 769-76.

164. Michel G, Ritter A, Gerken G, Meyer zum Buschenfelde KH, Decker R, Manns MP. *et al*. Anti-GOR and hepatitis C virus in autoimmune liver diseases. *Lancet* 1992; 339:267-9.

165. Heathcote EJ. Autoimmune hepatitis and chronic hepatitis C: latent or initiated by interferon therapy? Gastroenterology. *1995;108:1942-4.*

166. Czaja AJ, Carpenter HA, Santrach PJ, Moore SB, Taswell HF, Homburger HA. *et al*. Evidence against hepatitis viruses as important causes of severe autoimmune hepatitis in the United States. *J Hepatol.* 1993; 18: 342-52.

167. Hagiwara H, Hayashi N, Mita E, Takehara T, Kasahara A, Fusamoto H. *et al*. Quantitative analysis of hepatitis C virus RNA in serum during interferon alfa therapy. *Gastroenterology.* 1993; 104: 877-83.

168. Kanai K, Kako M, Okamoto H. HCV genotypes in chronic hepatitis C and response to interferon. *Lancet* 1992; 339:1543.

169. Zignego AL, Ferri SA, Pileri SA, Caini P, Bianchi FB. Extrahepatic manifestations of hepatitis C virus infection: a general overview and guidelines for a clinical approach. *Dig Liver Dis* 2007 ; 39:2-17.

170. Bergquist A, Broomé U. Hepatobiliary and extra-hepatic malignancies in primary sclerosing cholangitis. Best Pract Res Clin Gastroenterol. 2001;15:634-56.