FACULTY OF MEDICINE AND HEALTH SCIENCES

CHARACTERIZATION OF PATIENTS WITH AUTOIMMUNE HEPATITIS IN THE GHENT AREA:

A RETROSPECTIVE COHORT STUDY IN TERTIARY AND SECONDARY HEPATOLOGY CENTERS

Cedric Simoen
Student number: 01110551

Supervisor(s): Prof. dr. Hans Van Vlierberghe, Prof. dr. Jeoffrey Schouten, dr. Xavier Verhelst

A dissertation submitted to Ghent University in partial fulfilment of the requirements for the degree of Master of Medicine in Medicine

Academic year: 2016 – 2018

GHENT UNIVERSITY

FACULTY OF MEDICINE AND HEALTH SCIENCES

CHARACTERIZATION OF PATIENTS WITH AUTOIMMUNE HEPATITIS IN THE GHENT AREA:

A RETROSPECTIVE COHORT STUDY IN TERTIARY AND SECONDARY HEPATOLOGY CENTERS

Cedric Simoen
Student number: 01110551

Supervisor(s): Prof. dr. Hans Van Vlierberghe, Prof. dr. Jeoffrey Schouten, dr. Xavier Verhelst

A dissertation submitted to Ghent University in partial fulfilment of the requirements for the degree of Master of Medicine in Medicine

Academic year: 2016 – 2018

GHENT UNIVERSITY

TABLE OF CONTENT

LIST OF USED ABBREVIATIONS

AIH	Autoimmune hepatitis
AILD	Autoimmune liver diseases
ALP	Alkaline phosphatase
ALT	Alanine transaminase
AMA	Antimitochondrial antibody
ANA	Anti-nuclear antibody
ANCA	Anti-neutrophil cytoplasmic antibody
Anti-ds DNA	Anti-double stranded DNA
Anti-LC1	Anti-liver cytosol antibody type 1
Anti-LKM	Anti-liver kidney microsomal antibody
AST	Aspartate transaminase
EBV	Epstein-Barr virus
ECM	Extracellular matrix
GGT	Gamma-glutamyl transpeptidase
HAV	Hepatitis A virus
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HLA	Human leukocyte antigen
HSV-1/2	Herpes simplex virus type 1 and 2
IAIHG	International Autoimmune Hepatitis Group
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
LTX	Liver transplantation
MHC	Major histocompatibility complex
MMF	Mycophenolate mofetil
PBC	Primary biliary cholangitis / Primary biliary cirrhosis
PSC	Primary sclerosing cholangitis
SMA	Anti-smooth muscle antibody
UDCA	Ursodeoxychol acid
ULN	Upper limit of normal

1. ABSTRACT (EN)

Background: Autoimmune hepatitis (AIH) is a rare autoimmune liver disease. Without proper diagnosis and treatment, AIH leads to the development of cirrhosis and end-stage liver disease. The diagnosis is based on an internationally recognized scoring system. Patients with early initiation of treatment have an excellent prognosis that is similar to a matched control population. However, data for Belgian patients with AIH are scarce.

Objective: To assess the prevalence and general characteristics of patients with AIH in the Ghent area and to look for predictors of long-term outcome in patients with AIH.

Materials and methods: In this retrospective study, data of 212 patients with AIH were reviewed in the hepatology department of Ghent University Hospital, AZ Nikolaas (Sint-Niklaas) and AZ Maria Middelares (Ghent). Patients with follow-up time shorter than 1 year were excluded. Clinical, biochemical and outcome data, including development of cirrhosis, hepatocellular carcinoma (HCC), need for liver transplantation and death, were collected. Statistical analysis included Kaplan-Meier survival analysis, Mann Whitney U test and Cox regression analysis.

Results: Development of cirrhosis with decompensation occurred in 11 patients (6.7%), whereas ascites was the most common form of decompensation (n=8, 72.7%). Nine patients (5.1%) developed HCC during follow-up, 8 patients died (4.9%). Five-year and ten-year survival was respectively 96.9% and 83.1%. A prognostic scoring system, including the variables 'bilirubin, AST, ALP and GGT' after 6 months, was developed based on response on first treatment. The hazard ratio of this score was 1.519 (p=0.003). The AUC for this score was 0.82 (p=0.001). Using a cut-off derived from the ROC-curve (Log Rank: p=0.002), sensitivity (72.7%) and specificity (82.6%) was determined for the prediction of an adverse outcome, including decompensation, HCC development of death. The Kaplan-Meier survival analysis could differentiate patients from responders with an excellent prognosis after 6 months of therapy.

Conclusion: Response on treatment of AIH after 6 months using a newly developed scoring system is an excellent predictor of long-term event-free survival in AIH. A validatation in two independent Belgian cohorts is ongoing.

2. ABSTRACT (NL)

Achtergrond: Auto-immune hepatitis (AIH) is een zeldzame auto-immuunziekte. Zonder de juiste diagnose en behandeling leidt AIH tot de ontwikkeling van cirrose en terminale leverziekte. De diagnose is gebaseerd op een internationaal erkend scoresysteem. Patiënten waarbij de behandeling vroeg gestart wordt, hebben een uitstekende prognose die vergelijkbaar is met een gematchte controlepopulatie. Gegevens voor Belgische patiënten met AIH zijn echter schaars.

Doel: Het bepalen van de prevalentie en algemene kenmerken van patiënten met AIH in regio Gent en het zoeken naar voorspellers van langetermijnuitkomsten bij patiënten met AIH.

Materialen en methoden: In dit retrospectief onderzoek werden gegevens van 212 patiënten met AIH beoordeeld op de afdeling hepatologie van het UZ Gent, AZ Nikolaas (Sint-Niklaas) en AZ Maria Middelares (Gent). Patiënten met een follow-up tijd korter dan 1 jaar werden uitgesloten. Klinische, biochemische en outcome gegevens, waaronder de ontwikkeling van cirrose, hepatocellulair carcinoom (HCC), noodzaak voor levertransplantatie en overlijden, werden verzameld. Statistische analyses omvatte Kaplan-Meier overlevingsanalyse, Mann Whitney U-test en Cox-regressieanalyse.

Resultaten: Ontwikkeling van cirrose met decompensatie trad op bij 11 patiënten (6.7%), waarvan ascites de meest voorkomende vorm van decompensatie was (n=8, 72.7%). Negen patiënten (5.1%) ontwikkelden HCC tijdens de follow-up, 8 patiënten stierven (4.9%). De overleving na vijf jaar en tien jaar was respectievelijk 96.9% en 83.1%. Een prognostisch scoresysteem die de variabelen 'bilirubine, AST, ALP en GGT' na 6 maanden bevat, werd ontwikkeld op basis van de respons op de eerste behandeling. De hazard ratio van deze score was 1.519 (p=0.003). De AUC voor deze score was 0.82 (p=0.001). Met behulp van een cut-off waarde afgeleid van de ROC-curve (Log Rank: p=0.002), werd de sensitiviteit (72.7%) en specificiteit (82.6%) bepaald voor de voorspelling van een nadelige outcome, waaronder decompensatie, de ontwikkeling van HCC en mortaliteit. De Kaplan-Meier overlevingsanalyse kon patiënten met een nadelige outcome onderscheiden van patiënten met een uitstekende prognose na 6 maanden therapie.

Conclusie: De respons op de behandeling van AIH na 6 maanden met een nieuw ontwikkeld scoresysteem is een uitstekende voorspeller van een langetermijnevrije overleving bij AIH. Een validatie in twee onafhankelijke Belgische cohorten is lopende.

3. INTRODUCTION

3.1 THE NORMAL LIVER

3.1.1 Anatomy, histology and physiology

The liver is both the largest gland and the largest organ in the adult body. [1-2] Weighing about 1.5-2.0kg, it constitutes 2.5% of the total body weight. [1-3] The liver, which is situated underneath the diaphragm [1], contains two main lobes (left and right), each divided into hexagonic hepatic lobules. [2] These lobules are the smallest functional unit of the liver. [3-4] They are irrigated by the portal triad (hepatic artery, portal vein and bile duct) [1,5] and consist of five types of specialized cells [2]; hepatocytes, Kupffer cells, stellate cells, sinusoidal endothelial cells and cholangiocytes. [1,5] The main functions of these liver cells are listed in Table 1.

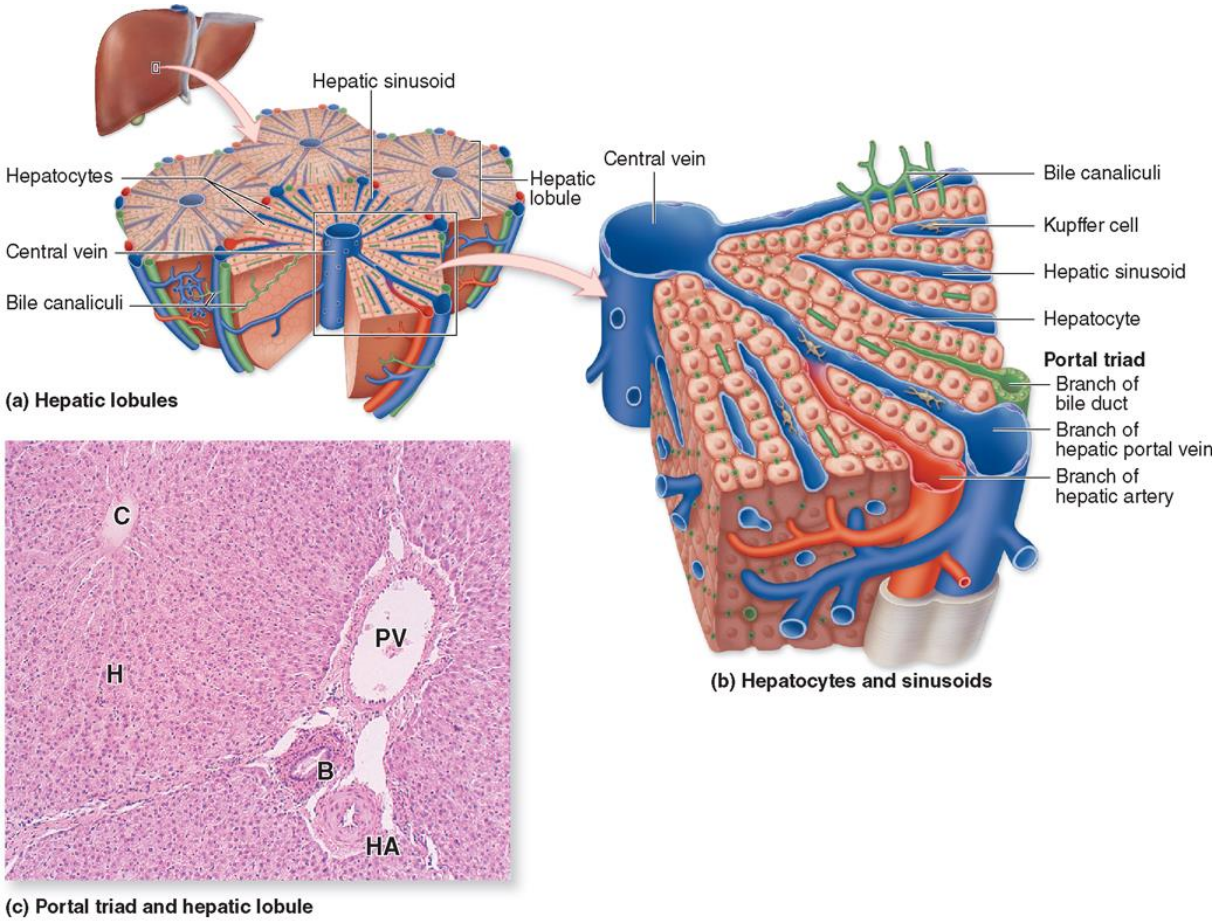


Figure 1. Anatomy and histology of the hepatic lobule. Abbreviations: B, Bile duct; C, Central vein; H, Hepatocyte; HA, Hepatic artery; PV, Portal vein. (Adapted from Mescher A.L. Junqueira's Basic Histology Text And Atlas. The McGraw-Hill Companies, New York, 2013. Available from: https://archive.org/details/JunqueirasBasicHistologyTextAndAtlas13thEd)

Table 1: Main functions of normal liver cells. [3-4]

Liver cell	Function in the normal liver
Hepatocytes	Fat metabolism Carbohydrate metabolism Protein metabolism Storage of glycogen, vitamins and iron First pass metabolism of drugs and toxins Synthesis and secretion of bile
Kupffer cells	Phagocytosis of antigens and erythrocytes
Stellate cells	Storage of vitamin A Production of extracellular matrix (ECM) and collagen
Sinusoidal endothelial cells	Lining the hepatic sinusoids
Cholangiocytes	Lining the bile duct

3.1.2 Pathology and physiopathology

Multiple causes of liver inflammation, including infections, malnutrition, intoxication, metabolic hereditary disorders and immune mediated diseases, can lead to liver damage. The consequences are derived from the functions of the normal liver cells. [3] For example, destruction of Kupffer cells causes less capturing of antigens, inducing an increased amount of immunoglobulin G (IgG). [6] Devastation of hepatocytes leads to an increased release of hepatic enzymes, such as alanine transaminase (ALT) and aspartate transaminase (AST), but also to a decreased production of albumin, resulting in a decreased elimination of soluble bilirubin and consequently an increased serum level of total bilirubin [7].

Damage of stellate cells are thought to produce an increased amount of collagen. [3-4] The hepatic fibrosis resulting from this process can be considered as a reversible wound healing response of the liver, although the amount of fibrosis becomes problematic in case of persistent injury. [8] Repetitive damage leads to destruction of the liver architecture, ending in an irreversible cirrhosis. [4] Hepatic cirrhosis is the most advanced stage of chronic liver disease and is linked to hepatocellular failure, leading to the development of portal hypertension, with hepatic encephalopathy and jaundice, and hepatocellular carcinoma (HCC). In some cases, there is a need for liver transplantation. [3]

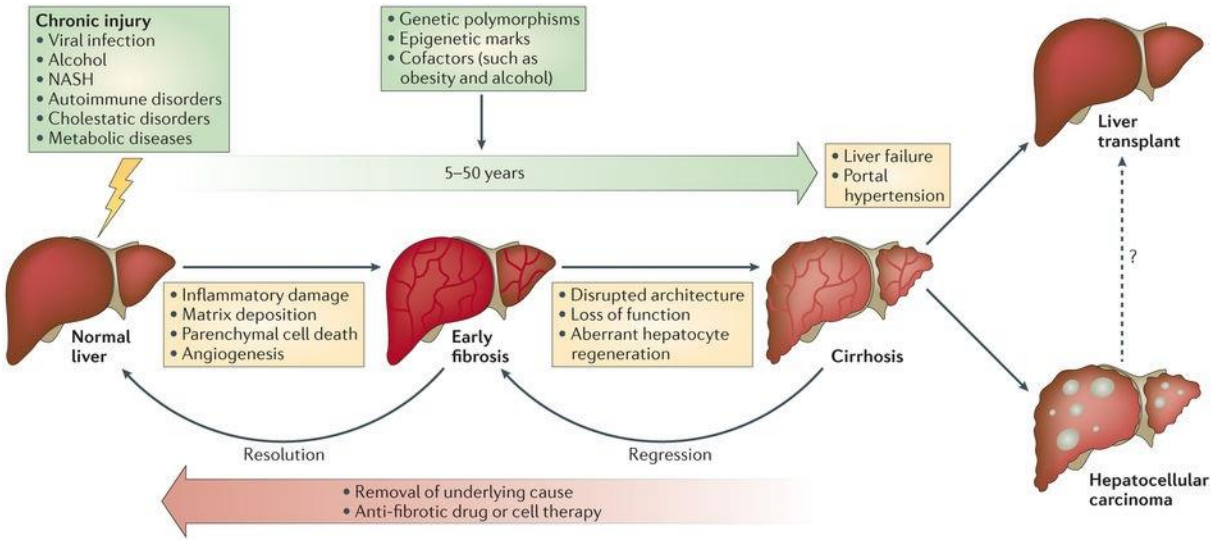


Figure 2. Evolution of chronic liver disease. (Adapted from Pellicoro A., Ramachandran P., Iredale J.P. and Fallowfield. J.A. Liver fibrosis and repair: immune regulation of wound healing in a solid organ. Nat Rev Immunol. 2014 Mar;14(3):181-94. doi: 10.1038/nri3623.)

3.2 AUTOIMMUNE HEPATITIS

3.2.1 Definition

Autoimmune hepatitis (AIH) is a relatively rare disease [9], belonging to the group of the autoimmune liver diseases (AILD). [10] AIH is a chronic hepatitis [9,11-12], characterized by self-perpetuating and progressive inflammation of the liver [9-10,13-14], due to aberrant autoreactivity. Ultimately, this disease can progress to cirrhosis and subsequently to hepatic failure [13] and HCC [15]. Consequently, AIH is an important cause of liver morbidity and mortality. [16]

Since it was first recognized in 1942 by Amberg [17] and described in the early 1950s by Jan Gösta Waldenström and Henry George Kunkel as a chronic form of hepatitis predominantly in young women [14,18-21], this disease has been known by a variety of terms, such as chronic active hepatitis, plasma cell hepatitis and lupoid hepatitis. [11,18-19,21] The term 'Autoimmune hepatitis' was used for the first time in 1965 by Ian Mackay et al. [19,22-23] and was widely accepted by the Autoimmune Hepatitis Group in 1992 as the most appropriate term for this disease. [11,18-19]

Like other rare diseases, studies about AIH are hampered by the limited number of patients that can be included. [9] Most of the data are collected from studies conducted in the United Kingdom, North America and Japan [13], but similar data are lacking from Belgium. One goal of this paper is to map Belgian AIH-patients by means of a retrospective cohorte.

3.2.2 Epidemiology

AIH is a relatively rare disease, with a prevalence that ranges from 10 to 17 per 100.000 in the Caucasion population. [18-19,24-26] Some studies mention a prevalence of 24.5 and 42.9 cases per 100000 in respectively New Zealand and Alaska. [24,26] Thus, the prevalence in our area may be underestimated. [24] A Norwegian study by Boberg et al. reported an incidence of 1.9 per 100000 in Caucacian patients. [19,27] AIH has a worldwide distribution [28] and can occur at any age, in both genders and in every ethnic group. [19,24] AIH was originally described in young adult women and was rarely seen at older age. With the improvement of the technical investigations, AIH is nowadays also diagnosed in the elderly. [29] The disease can occur at any age, but a bimodal age pattern is mostly seen, with one peak during childhood and a second peak around the menopause. [24,26,30] However, recent studies proved that an increasing number of AIH patients are diagnosed at ages of 60 years and older [24,31], it might even be a disease predominantly in older women. [14] The mean age of initial diagnosis is nowadays in the forties. [25] Both sexes can be affected, although AIH is predominantly observed in women. [24,28,30,32] Three quarters of the patients are women [33], resulting in a female:male sex ratio of around 3-3.6:1. [19,24]

3.2.3 Etiology

The etiology of AIH remains still unknown, although both environmental and genetic factors are tought to be involved in the initiation of this AILD. [21-23] The most accepted theory of the mechanism inducing AIH, postulates that an environmental agent triggers the immune system of an genetic susceptible patient. [21,24,34]

The environmental agents are summarized in Table 2. Drugs can be involved in 9% of patients with the phenotype of AIH [35], of which nitrofurantoin and minocycline account for 90% of this drug-induced AIH. [24,35] Not only a few viruses are associated with AIH [36-38], also some case reports where AIH would be caused by hepatitis A/B vaccination have been documented. [39-40] Finally, several herbs have also reported to trigger AIH. [19,23]

Table 2: Environmental triggering agents. [19,23-24,41-42]

Triggering agents	Examples			
Drugs	Adalimumab	Ezetimibe	Methylphenidate	Propylthiouracil
	Atommoxetine	Imatinib	Minocycline	Pyrazinamide
	Atorvastatin	Indomethacin	Natalizumab	Ranitidine
	Beta Interferon	Infliximab	Nitrofurantoin	Rifampin
	Diclofenac	Methotrexate	Ornidazole	Risperidone
	Doxycycline	Methyldopa	Oxyphenisatin	Statins
Viruses	CMV	HAV/HBV/HCV	HIV	Measles virus
	EBV	Hepatitis A/B vaccine	HSV6	
Herbs	Black cohosh	Dai-saikoto	Khat	Sho-saikoto
	Chinese herbal tea	Ephedra	Melatonin	

Several observations confirm a genetic predisposition for AIH. [43] AIH does not follow a Mendelian pattern of inheritance, meaning that no single gene has been identified as responsible for this illness. [18,34] The entire genetic base is still not elucidated. [43] Because of this, the complete genetic mechanism is beyond the scope of this dissertation, only a general description will be given.

A study by Muratori et al. [44] and Hasan et al. [45] demonstrated a distinct genetic association of the human leukocyte antigen (HLA) region with AIH. [44-45] Located on chromosome 6p21.3 [46], the strongest association can be found in the major histocompatibility complex (MHC) class II region (HLA-DR). [22,43] Various HLA serotypes are associated with different ethnicities and races. [47-48] The two main alleles, HLA-DR3 and HLA-DR4, listed in Table 3, confer a 6-7 fold increased disease risk. Though it plays the dominant role [49], the HLA-region alone cannot explain the whole genetic base of AIH. In addition, a genome-wide association study (GWAS) has identified several genetic loci outside the HLA region, considering to take part in the disease mechanism. [45]

Table 3: Features of the HLA-serotypes. [18-19,44,50]

	HLA-DR3	**HLA-DR4**
Gender	Mostly females	Female/male
Age	Young adults	Older adults
Ethnicity	Caucasian	Japanese
Extrahepatic manifestations	Less common	Common
Response to corticosteroids	Decreased response	Responds well

3.2.4 Pathogenesis

Despite the discovery of the disease more than 60 years ago, the underlying pathogenesis of AIH is complex and still unclear. [51] Both etiologic agents are able to induce a loss of immune tolerance, leading to an immunological mediated destruction of hepatocytes. [19,23,52] Only a general description will be given in this dissertation.

In the presence of auto-antigens, A dysregulation or decrease of the Treg-cells ensures an activation of autoreactive T-cells. [50,53] The production of TGF-β by these autoreactive cells, leads to an increase of IL-6, which triggers the induction of Th17. [50] IL-17, produced by these T-cells, are approved to contribute in other autoimmune diseases, although a study by Czaja et al. approved that several cytokines (see Figure 3) have been linked to the pathogenesis of AIH. [54]

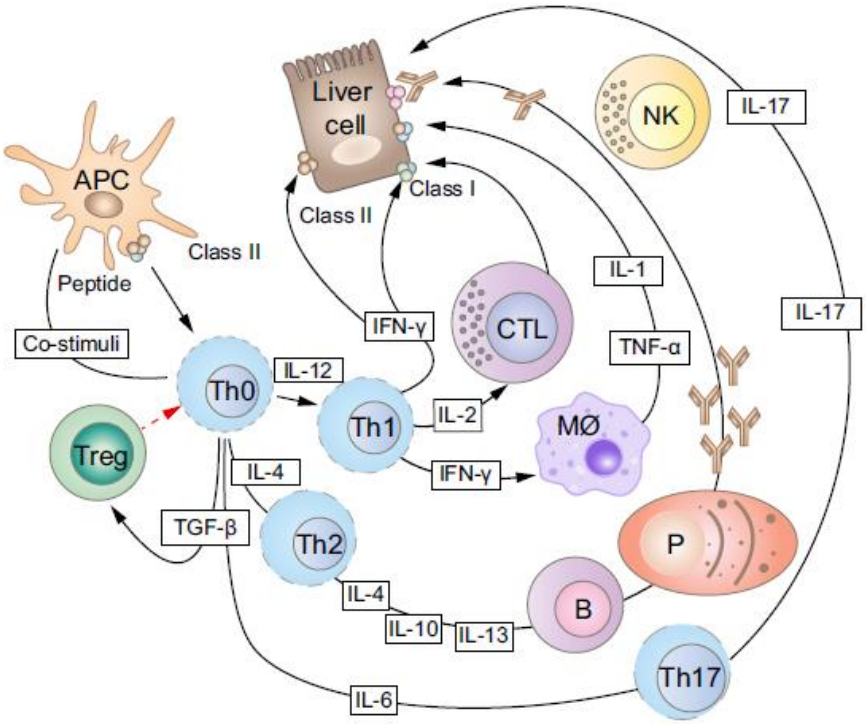


Figure 3. Pathogenesis of AIH. (Manns M.P., Lohse A.W., Vergani D. Autoimmune hepatitis - Update 2015. J Hepatol. 2015 Apr;62(1 Suppl):S100-11. doi: 10.1016/j.jhep.2015.03.005.)

3.2.5 Clinical features

The spectrum of clinical features is very heterogeneous and is characterized by a fluctuation of decreased and increased disease activity. [24,26,49] The presentation may vary from asymptomatic to acute fulminant liver failure [11,19,55] and depends on ethnicity. [31] Up to 12-35% of patients have no symptoms at diagnosis [9,24,26,56], up to 70% become symptomatic. [31] Approximately 25-49% of patients show clinical features similar to acute viral or toxic hepatitis [9,23-24,26], or more rarely as fulminant liver disease [20], characterized by jaundice and hepatic encephalopathy. [57] The most common symptoms of acute hepatitis are summarized in Table 4. Other symptoms are non-specific and include lethargy, malaise, anorexia, nausea, weight loss and itching. [11,20,24,49] According to a study of Peng et al., 76.9% of symptomatic patients present with three symptoms, four symptoms can be seen in 23.1% of AIH patients. [58] Patients may also present with signs of a chronic liver disease [59], such as spider naevi and palmar erythema. [21,26,59]

Table 4: Most common clinical features of AIH. [23,27,31,43,52,57]

Clinical features	Prevalence (according to a study by Choudhuri et al.)
Jaundice	50-55%
Fatigue	45%
Right upper-quadrant abdominal pain	24%
Arthralgia in the small joints	18%

Physical examination will be mostly normal in patients with AIH. [24,59] In an advanced stage of liver disease, the abdomen is characterized by hepatomegaly, splenomegaly and ascites. [49] Table 5 shows the prevalence of these features. Characteristics of cirrhosis, such as (bleeding) esophageal varices and hepatic encephalopathy can be present. [59] The liver may be enlarged, swollen and tender. Nodularity may be palpable in some patients. [25]

Table 5: Physical examination. [27]

Physical examination	Prevalence (according to a study by Abdollahi et al.)
Hepatomegaly	13%
Splenomegaly	50%
Ascites	17%

3.2.6 Histology

Unless there is a contraindication, a liver biopsy should be considered in every patient with suspected AIH. [24,26,52] The aim of the biopsy is to identify histological features and to rule out other liver diseases. [23,52] The histology of AIH is very heterogeneous [21] and differ between patients [55]. The findings on liver biopsy are not pathognomonic for AIH [9,52,56,60-61] and are those of a chronic hepatitis, although with some characteristics compatible with AIH [49,55]. A histological feature suggesting AIH includes interface hepatitis, also called piecemeal necrosis. It refers to the process whereby inflammatory cells migrate from the portal triad into the liver parenchym and erode and replace the hepatocytes at the edge of a lobule [24,43,55], with an absence of biliary duct damage [62]. It occurs in 84-98% of patients, but it can also be seen in other forms of hepatitis. [43] Similar to interface hepatitis, is the process called emperipolesis, defined as endocytosis of lymphocytes within hepatocytes, seen in the interface hepatitis area. [19,26,63] It can be seen in about 34% of AIH patients. [64] A third characteristic that may indicate AIH, is rosette formation. These clusters of reactive hepatocytes, surrounded by inflammatory cells, are a form of hepatic regeneration. [62] Forty percent of the patients have this histological feature. [64] However, the sensitivity and specificity of liver biopsy are respectively 40% and 81%, which is not enough to use histology as only marker for diagnosis. [65] Other histological features that may be seen in liver biopsy, include eosinophils, steatoses and plasmacells. [9,55] Almost every AIH-patient has a certain degree of fibrosis. [19,25-26,55] The several stages of fibrosis are summarized in Table 6. [66] If left untreated, fibrosis results into cirrhosis. Up to 30% of AIH-patients already have cirrhosis at diagnosis. [24-26,56]

Table 6: stages of fibrosis. [66]

Stage	IASL	Batts and Ludwig	Metavir[a]
No fibrosis	No fibrosis	Stage 0	F0
(Peri)portal fibrosis	Mild fibrosis	Stage 1	F1
Septal fibrosis	Moderate fibrosis	Stage 2	F2
Bridging fibrosis	Severe fibrosis	Stage 3	F3
Cirrhosis	Cirrhosis	Stage 4	F4

A non-invasive fibroscan or elastometry can be used to quantify the amount of fibrosis. Values between between 2.5 and 7.0 indicate an absent (Metavir F0) or mild (Metavir F1) fibrosis. Values up to 9.5 indicate Metavir F2 fibrosis. Values between 9.5 and 12.5 point to Metavir F3 fibrosis, while values higher than 12.5 prognosticate cirrhosis (Metavir F4). [67]

3.2.7 Biochemical markers

AIH is characterized by elevated aminotransferases, hypergammaglobulinemia and circulating autoantibodies. [68-71] Elevated aspartate aminotransferase (AST) and alanine aminotransferase (ALT) reach levels of <500U/L, in rare cases up to 1000U/L and higher. [70,72] Increased levels of serum immunoglobulin G (IgG) can reach levels of 1.2-3.0 times higher than the upper limit of normal (ULN). [49,70,73] IgA and IgM remain typically normal. [21] A small elevation of alkaline phosphatase (AP) is also frequent. [72-73] Serum bilirubin can be slightly elevated during a period of inflammatory activity. [24,72] However, it should be noticed that up to 25-40% of patients may present with an acute and atypical onset of the disease, where IgG serum levels are normal and circulating autoantibodies are not detectable. [23,43,31,74] Biochemical remission is obtained when there's a normalization of both IgG and transaminases. [21] It is important to mention that there is no correlation between biochemical and histological activity. [24, 26,63]

The presence of circulating autoantibodies is frequently seen in AIH. [75] Autoantibodies are considered to be positive when they are present at a dilution ≥1:40. [76] However, no autoantibody is pathognomonic. [75]

Autoantibodies that can be observed in AIH are antinuclear antibodies (ANA), smooth muscle antibodies (SMA), antibodies to liver/kidney microsome type 1 (anti-LKM1) and antibodies to soluble liver antigen/liver pancreas (anti-SLA/LP). [75] ANA is a marker for immune reactivity and has several nuclear targets. [77] It's the most seen autoantibody in AIH patients, although they can also be found in other autoimmune diseases, even in healthy persons. Up to 70-80% have positive titers of ANA or SMA, around 3-4% have positive titers for LKM-1, while up to 20% of patients is seronegative for these autoantibodies. [43]

3.2.8 Diagnostic criteria

Due to the absence of a pathognomonic marker, the heterogeneity of the disease and the large number of differential diagnoses, diagnosis of AIH is difficult. [21,24-25,78] In 1993, the IAIHG developed a scoring system to differentiate AIH from other forms of hepatitis. A revision with a high sensitivity and specificity of respectively 95% and 90% was made in 1999 to simplify the system. [70,79] Nevertheless, the amount of criteria, consisting of 13 categories and 29 grades, remained complex and difficult to use in clinical practice. Therefore, a simplified scoring system was developed in 2008. Only four independent categories were included; autoimmune antibodies, IgG levels, liver histology and absence of viral hepatitis. [70,80] The distribution of points for every variable is shown in Table 7.

Table 7: Simplified scoring system. [21,25]

Variable	Cut-off	Points[a]
Autoantibodies	ANA or SMA or LKM > 1:40	1
	ANA or SMA or LKM > 1:80	2
IgG	Upper normal limit	1
	>1.10 times normal limit	2
Liver histology[b]	Compatible with AIH	1
	Typical for AIH	2
Absence of viral hepatitis	Yes	2
	No	0

[a]: A score < 6 indicates not being AIH, a score ≥ 6 indicates problable AIH; a score ≥ 7 indicates definite AIH.
[b]: Typical for AIH: presence of (1) interface hepatitis, (2) emperipolesis and (3) rosette formation; Compatible with AIH: any chronic hepatitis with lymphatic infiltration that is not suggestive for non-alcoholic steatohepatitis (NASH) or drug-induced inflammation.

A score between 3 and 5 identify patients as being not AIH, a score of at least 6 points indicates probable AIH, A score of 7 or 8 indicate definite AIH. [76] The simplified scoring system retained their specificity, but is less sensitive than the original system. A score of at least 6 have a sensitivity and specificity of respectively 88% and 97%, while definite AIH have a sensitivity and specificity of 81% and 99%. [19,43,60,80] The working hypothesis for daily use is shown in Figure 4.

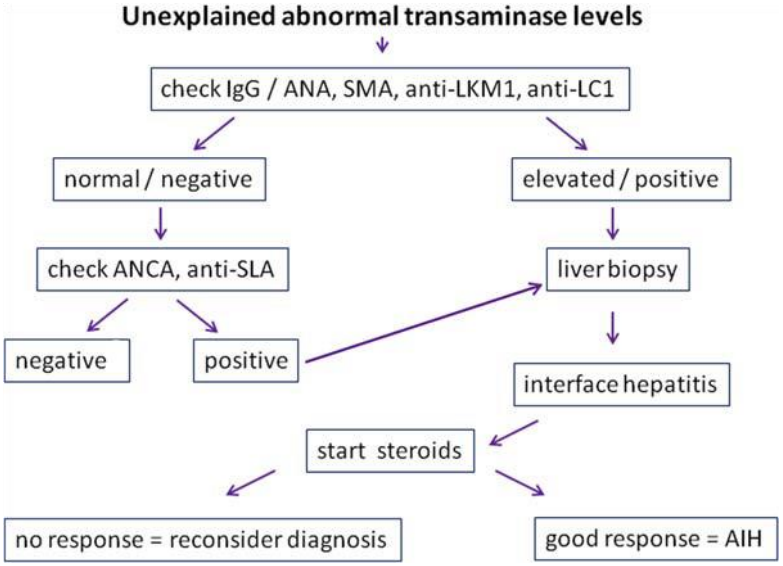


Figure 4: Diagnostic algorithm. (Adapted from: Vergani D., Mieli-Vergani G. Autoimmune Hepatitis: Diagnostic Criteria and Serological Testing. Clin. Liver Dis. 2014 Feb;3(2):38-41. doi: 10.1002/cld.321.)

However, it's important to mention that this simplified scoring system is not a golden standard, but only a guideline for the clinician to make the diagnosis of AIH. [24] Several atypical cases with a score less than 6 have been reported yet. [12] Therefore, It can be helpful to rescore these patients by the original revised score. [24] In addition, the simplified scoring system performs less efficiently in patients who present with a acute form of AIH and patients with an associated cholestatic disease [23,81].

3.2.9 Associated autoimmune diseases

Characteristically for AIH is the presence of extrahepatic autoimmune or immune-mediated diseases. [9,20-21,56] Forty percent of the AIH patients are diagnosed with an associated autoimmune disease [43,49,56], but it can also be seen in first-degree relatives [9,24]. The most common associated diseases are autoimmune thyroiditis (Hashimoto's disease, Graves' disease), ulcerative colitis, coeliac disease, diabetes mellitus type-1, vitiligo and rheumatoid arthritis. [24] Table 8 has listed the other autoimmune diseases that have already been described in an AIH patient.

A study by Czaja et al. reported that female patients are distinguished from male patients by higher frequencies of associated autoimmune diseases. This may be due to a difference in the HLA-DR status between men and women. HLA-DR4 is a marker for higher frequency of associated autoimmune diseases, especially autoimmune thyroiditis. [32]

Table 8: Extrahepatic autoimmune diseases related with AIH. [25,49,56,82]

Extrahepatic autoimmune diseases related with AIH	
Addison's disease	Multiple sclerosis
Autoimmune gastritis	Panniculitis
Collagen colitis	Polyglandular autoimmune syndrome, type 1
Conjunctivitis	Polymyalgia rheumatica
Crohn's disease	Polymyositis
Glomerulonephritis	Sarcoidosis
Hemolytic anemia	Sjögren's syndrome
Hypophysitis	Sweet's syndrome
Idiopathic thrombocytopenic purpura	Systemic lupus erythematosus
IgA deficiency	Urticaria pigmentosa
Mixed connective tissue disease	Uveitis
Mononeuritis	

3.2.10 Overlap syndromes

Approximately 18% of patients with AIH present with cholestatic features that can resemble primary biliary cirrhosis (PBC) or primary sclerosing cholangitis (PSC). [19,43] No international consensus has been reached regarding their classification, so many terms have been used so far, like “the hepatic form of PBC”, “autoimmune sclerosing cholangitis” or “combined hepatitic/cholestatic syndrome”. However, “overlap syndrome” is the most appropriate terminology. [9,24] An overlap syndrome should be considered in every patients with atypical cholestatic characteristics, either clinical, biochemical, serological and/or histological, or in those who fail to respond to conventional therapy of AIH. [83-84] Due to the lack of international criteria defining the overlap, diagnosis is difficult. [24] Yet, no international consensus has been reached for the diagnosis of AIH-PBC or AIH-PSC [83], although arriving to the correct diagnosis as early as possible is important to be able to set the correct treatment in an early stage of the disease. [84] The Paris criteria can be useful in making the diagnosis of AIH-PBC overlap syndrome. These criteria require two features of AIH and two features of PBC. [35]

3.2.11 Types of AIH

Depending on the presence of the circulating autoantibodies, AIH can be subclassified into two main types. [21,43] AIH type 1 (AIH-1) is positive for anti-nuclear antibodies (ANA) and/or anti-smooth muscle antibodies (SMA), while AIH type 2 (AIH-2) has a positivity for anti-liver kidney microsomal antibody type 1 (anti-LKM1), anti-LKM3 and/or anti-liver cytosol type 1 antibody (anti-LC1). [21] AIH type 1 is the most common form. [20] The incidence ratio of AIH-1 to AIH-2 is 2:1 in Europe. [18] Originally, a third type was described in patients with AIH. Patients with the circulating soluble liver antigen antibodies (SLA) and liver pancreas antibodies (LP), who were classified as AIH-3, present with the same clinical and pathological features of AIH-1 and

are therefore classified to AIH-1. [19,60] Anti-SLA/LP can be seen in 10-30% of patients with AIH-1. [55]

Comparing to AIH-2, AIH-1 is more benign [85] and is associated with a lower frequency of associated autoimmune diseases [56], although inflammatory bowel diseases and PSC are frequently associated in AIH-1, diseases that normally are not observed in AIH-2. [18] The bimodal age pattern is typically seen in type 1 AIH. [23,85] AIH-1 is linked to HLA-DR3 in Caucasians and HLA-4 in Japanese patients. [24,55] Children have commonly an assocation between AIH-1 and HLA-DR13. [55]

AIH-2 is more seen in Northern Europe [52]. Especially children and young adults are affected, [23,26,43,56], with a mean age at onset of 10 years old. [52] Patients with AIH-2 present more with acute and severe clinical features at diagnosis. [26,56] Their histology is more advanced in liver biopsy [26] and progression to cirrhosis is more seen in AIH type 2. [85] Treatment failure, relapse after withdrawal drugs and need for a long-term therapy is common, when compared to type 1 AIH. [26] AIH-2 is linked to the genes HLA-DR3 and HLA-DR7. [24,52]

3.2.12 Differential diagnosis of AIH

Many of the characteristics seen in AIH, may also occur in other liver disorders. [11] When a patient presents with acute elevation of serum transaminases, it is therefore important to mind the full spectrum of chronic liver diseases. [52] Diagnosing retains difficult, as the autoantibodies can appear in several of these disorders. In addition, chronic liver diseases, like AIH, are often complicated with necroinflammation and fibrosis or cirrhosis. [11] The differential diagnosis of AIH is illustrated in Table 9.

Table 9: Differential diagnosis of AIH. [9,11,52,86]

AILD	Chronic viral hepatitis	Other forms of chronic hepatitis
PBC	Chronic hepatitis B	Chronic drug-induced hepatitis
PSC	Chronic hepatitis C	Alpha-1 antitrypsin deficiency
AIH/PBC overlap syndrome	Chronic hepatitis delta	Wilson disease
AIH/PSC overlap syndrome	Chronic hepatitis due to other viruses	Cholangiopathy related to AIDS
Autoimmune cholangiopathy		(non)Alcoholic steatohepatitis
		Granulomatous hepatitis
		Systemic lupus erythematosus
		Graft-versus-host disease
		Cryptogenic chronic hepatits
		Hemochromatosis

3.2.13 Treatment

According to the guidelines and clinical experience, treatment should be given to each patient in which the diagnosis of AIH was made. [43,60,87] Other indications to start the AIH-therapy

include patients with serum AST/ALT level greater than 10 times the upper limit of normal (ULN), serum AST/ALT level greater than 5 times the ULN combined with an elevated γ-globulin twice the ULN, histological features of interface hepatitis on liver biopsy [9,43 ,60,87-88] and symptoms of arthralgia or jaundice [60]. The potential side effects of the drugs are a relative contraindication for the use of the first-line therapy, while treatment does not seem beneficial in patients with decompensated liver cirrhosis in absence of an inflammatory activity and on the waiting list for liver transplantation (LTX). [87] The golden standard therapy of AIH, depicted in Figure 5, consists of corticosteroids and immunosuppression [49].

Treatment to obtain remission, consists of monotherapy with a high dose of predniso(lo)ne or a reduced dose of corticosteroids in combination with azathioprine. [9,86] Every two months or depending on the evolution of the laboratory exams, there is a reduction of the corticosteroids. [18,24] Response to corticosteroid treatment is similar between male and female patients, whereof treatment failure depends on their HLA-status. [32] Long-term treatment with corticosteroids leads in up to 80% of the patients after two years to predictable side-effects, such as cosmetic changes, osteoporosis, diabetes, psychosis, hypertension or malignancy and should therefore be avoided. [9,24,88-89] Changing predniso(lo)ne with budesonide, a next-generation corticosteroid, reduces the corticosteroid-induced side effects (28% versus 53%), because of the rapid degradation and the 90% first-pass metabolism in the liver. [24,35,89] Side-effects of budesonide, especially headache and respiratory infections [31], are mostly seen in cirrhotic patients [90]. However, budesonide is more expensive to be used as first-line therapy. [24]

Once remission with a corticosteroid is achieved, maintenance with an immunosuppressive therapy, in monotherapy or combined with low-dose corticosteroids, should be actively pursued. [9,24] Azathioprine is mostly a well tolerated drug and its side-effects, such as gastrointestinal problems, rash, pancreatitis and myelosuppression, are the most common reason to discontinue the immunosuppressive therapy. [91]

First-line therapy with corticosteroids and immunosuppression is in up to 10-20% of AIH-patients unsuccessful. [43,92-94] Besides of failure of standard therapy, in 2 other cases second-line therapy can be considered; avoiding side effects of the drugs or as experimental therapy. [60] These alternative treatment includes cyclosporine, tacrolimus, methotrexate, cyclophosphamide or mycophenolate mofetil (MMF), though their role haven't been established yet. [55] The role of Treg-cells in the pathogenesis of AIH provides researchers nowadays to develop biologicals as a new therapy against the disease. [21,60] Examples of biologicals include anti-TNF antibodies (e.g. infliximab) and antibodies against CD3 and CD20 (e.g. rituximab). [21,86,91] In the future, it is the intention to strive to individualized therapy. [70,91,94]

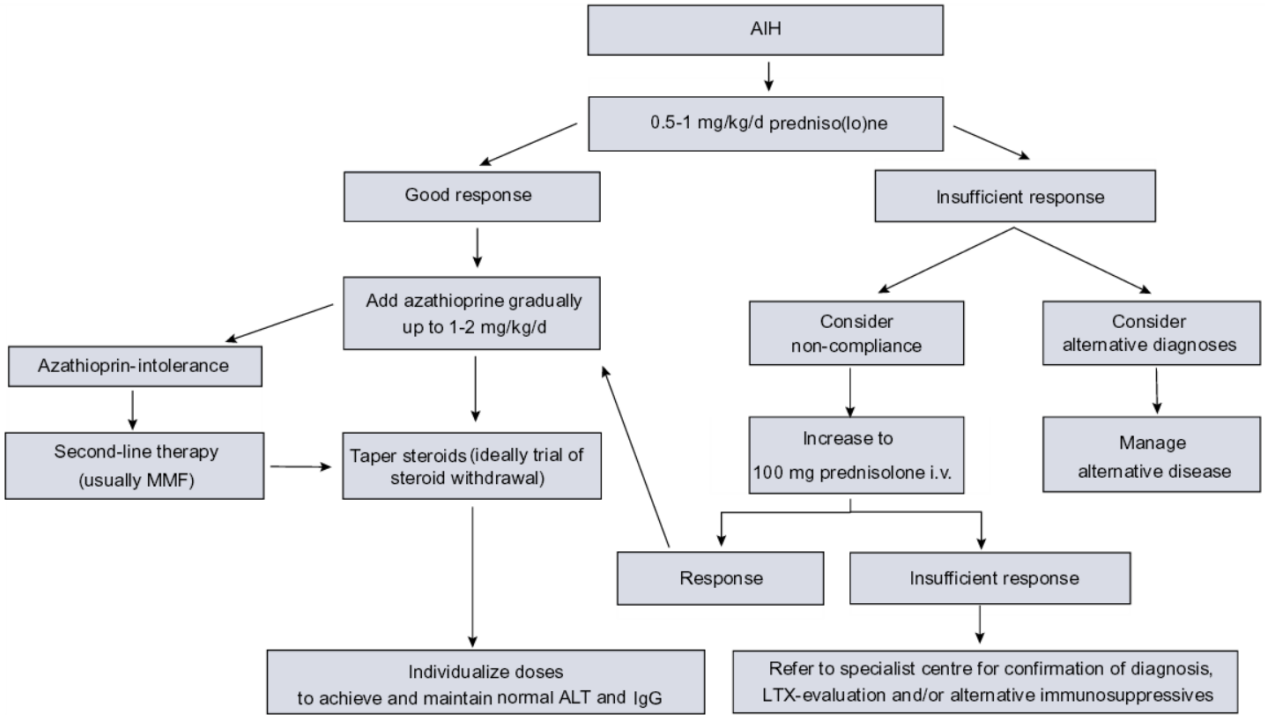


Figure 5. Therapeutic strategy of AIH. Abbreviations: MMF: mycophenolate mofetil; LTX: liver transplantation (Adapted from: Lohse A.W., Chazouillères O., Dalekos G., Drenth I., Heneghan M., Hofer H. et al. EASL Clinical Practice Guidelines: Autoimmune hepatitis. J Hepatol. 2015 Oct;63(4):971-1004. doi: 10.1016/j.jhep.2015.06.030.)

3.2.14 Outcome and prognosis

The aim of treatment is to achieve complete remission of the disease, meaning a recovery of symptoms [23], a normalization of both transaminases and IgG [9,21,60,89] and an abolishement of inflammation on the liver biopsy [23,35]. In this way, further disease progression to liver cirrhosis can be avoided. [9,21]

When biochemical remission is obtained, maintenance therapy with azathioprine should be continued to improve the rate of histological remission from 25% to 80%, respectively after 12 and 36 months. [23,89] When a liver biopsy shows absence of inflammation, the frequency of relapses reduces from 50-87% to 20-40%, compared with patients who still have an inflammatory activity. [35,86] Subsequently, there is scarce evidence for how long azathioprine should be given. [49] It has been proposed to withdraw the immunosuppressive therapy when a patient is in stable remission for at least 4 years. [49] Though, a large study from the Netherlands (N=131) showed an almost universal relapse when azathioprine was interrupted in AIH-patients who received a long-term remission therapy. [24] Other studies confirm the occurrence of relapses, independently from how long therapy was taken. [95] Due to the chronicity of the disease, it is recommended to continue the therapy for lifelong. [96] The overall prognosis of AIH is especially determined by the use and the response to therapy. [49] Long-

term survival, shown in Table 10, is excellent and compatible with those of the normal population. [19,43,49,53]

Table 10: Long-term survival. [19,43,53,86,97-98]

Survival	Rate
10-year survival	75-90%
20-year survival	80%

According to studies, 40% of the untreated patients will die within 6 months of diagnosis [19,43] and the overall AIH mortality rate of untreated patients is 80%. [53,99] Of the survivors, another 40% will develop cirrhosis. [19] The 10-year survival for AIH-patients with cirrhosis is similar to that seen in non-cirrhotic patients (62%) [100], however the prognosis after 10-20 years is lower [24,87-88], leading to ascites, hepatic encephalopathy or HCC [35]. HCC occurs in 1-9% of AIH patients [43,56,70,72,88] and is less common comparing with other liver diseases [23-24,43,47,70]. In all cases of decompensated liver cirrhosis with a MELD-score ≥15, liver transplantation (LTX) seems to be the only option [70]. Overall, approximately 10% of AIH-patients will require LTX. [52,87-88,101-102] Other indications for LTX in AIH patients includes clinical deterioration, bleeding esophageal varices and coagulation abnormalities despite adequate therapy with azathioprine. [87] The 5-year and 10-year survival after LTX is excellent (70-90%) [43,55-56,60,70,87], although around 10-50% will develop de novo autoimmune hepatitis, meaning recurrence of AIH in the graft liver [43,52,56,60,88,94,101], over a median time of 2 years [101]. Risk factors to develop de novo AIH have not been identified yet. [87]
One purpose of this thesis is to develop a system that allows to predict the long-term prognosis.

4. MATERIAL AND METHODS

4.1 LITERATURE RESEARCH

This thesis has been achieved by extensive literature research. After the identification of the central hypothesis, the databases Medline (PubMed) and Embase were consulted for the literature review.

The MeSH-term 'Hepatitis, Autoimmune' was used in PubMed. The following subheadings were added: 'analysis', 'anatomy and histology', 'blood', 'cytology', 'diagnosis', 'enzymology', 'etiology', 'immunology', 'pathology', 'physiology' and 'physiopathology'.
In Embase, the following search term was used: ''Emtree - major focus exp.: 'autoimmune hepatitis' NOT Emtree - major focus exp.: 'overlap syndrome'''. In order to get as much as relevant results, the following floating subheadings were used: 'endogenous compound', 'drug therapy', 'diagnosis', 'etiology', 'drug combination', 'adverse drug reaction', 'surgery', 'drug dose', 'pharmacology', 'epidemiology', 'therapy', 'prevention', 'drug comparison', 'drug toxicity', 'pharmacokinetics', 'drug concentration' and 'drug resistance'.
Further relevant literature about normal liver anatomy, histology and physiology has been obtained through the courses of the education.

The references has been listed via the Vancouver style using the 'EndNote' software program.

4.2 STUDY SETTING

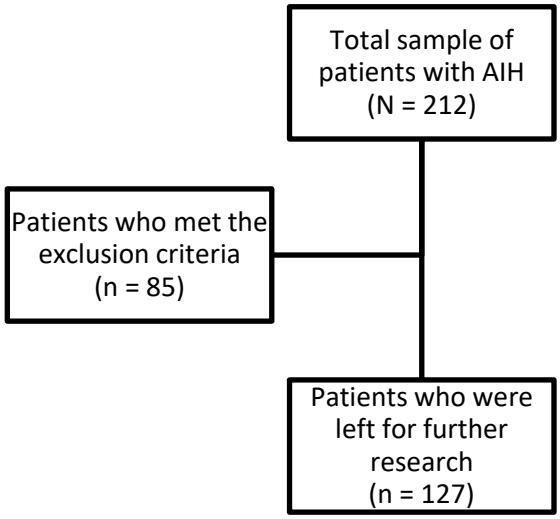
This thesis is a retrospective multi-centre study. It was conducted at the tertiary care Ghent University Hospital (UZ Gent) and the secondary care general Hospitals of Sint-Niklaas (AZ Nikolaas) en Ghent (AZ Maria Middelares Gent) in Belgium.
The study was approved by the ethics committee of the University of Ghent and the general Hospitals of Sint-Niklaas and Ghent. At the Ghent University Hospital and the general Hospital of Ghent, there was a need for a written informed consent. Patients who didn't want to participate in our study, had the choice to resend an opting-out document (see attachment). However, no patient returned this document.

4.3 STUDY POPULATION

All patients diagnosed with AIH, irrespectively their age, sex or ethnic background, were considered for inclusion. This resulted in a total sample size of 212 patients. Except for the descriptive statistics, patients are excluded for the following reasons:

- resending the opting-out document
- follow-up time less than 12 months or longer than 240 months

85 patients met these exclusion criteria. Therefore, 127 patients were left for further research.



4.4 PATIENT DATA

Data were derived from the Electronic Patient Record of the several hospitals. Collected clinical and laboratory data included general data at start of follow-up (age, sex, length, weight), other autoimmune disorders (Hashimoto's disease, Graves' disease, ulcerative colitis, Crohn's disease, coeliac disease, diabetes mellitus type 1, vitiligo, scleroderma, rheumatoid arthritis, pernicious anemia, systemic lupus erythematosus, Sjögren's syndrome), clinical signs (fatique, anorexia, losing weight, pruritus, abdominal pain, arthralgia, jaundice), medical history of viral infections (HSV-1/2, HAV, HBV, HCV, EBV), time and age at diagnosis, laboratory data at diagnosis (total bilirubin, AST, ALT, ALP, GGT, IgG, IgM, IgA, total cholesterol, eosinophils, platelets), autoantibodies (ANA, AMA, SMA, ANCA, anti-LKM, anti-LC1) liver biopsy (number, interface hepatitis, ballooning, cholate stasis, steatosis, bilirubinostasis, ceroid macrophages, rosette formation, plasma cells, fibrosis, degree of fibrosis, cirrhosis, necrosis), fibroscan, elastometry, overlap syndromes (PSC, PBC), treatment (type of medication, dosage), events (liver transplantation, HCC, relapse), outcome (remission, liver decompensation, death). Another database was created for follow-up laboratory data (total bilirubin, AST, ALT, ALP, GGT, IgG, platelets).

4.5 STATISTICAL ANALYSES

All data were processed with the 'SPSS Statistics 22' software programme. The following variables were newly created: BMI, age at diagnosis, type of AIH, duration of the individual

treatment (predniso(lo)ne, budesonide, UDCA, azathioprine, purinethol, MMF, ciclosporine), time of follow-up, time between diagnosis and the several events and outcome and time between diagnosis and first event. Events are in this paper defined as HCC, LXT and relapses. Outcome is defined as remission, liver decompensation and mortality. After extended data cleaning, descriptive statistics of the overall population (N=212) was performed. Continuous data were presented as mean ± standard deviation (SD), categorical data as number and frequency distribution.

The simplified scoring system could only be approximately calculated. Titers of the auto-antibodies in the UZ Gent are standard determined with a dilution of 1:40. The intensity of the auto-antibodies on fluorescence microscopy is scored by a value between 0 en 4. These values cannot be converted to a dilution, although scores of at least 2 correspond approximately to a dilution of at least 1:80. In addition, the viral status was not always known. Emperipolesis was not standard determined in the biopsies, although the outcome of liver biopsy (no evidence for AIH, probable AIH, definite AIH) was mentioned in the reports of the hospitals.

There were a few outliers in our population with a follow-up duration of more than 20 years. These patients could influence the further analyses. Therefor, a filter was used for the statistical analyses of survival and prognosis. Only patients with a follow-up duration more than 12 months (1 year) and less than 240 months (20 year) were included.

The Kaplan-Meier survival analysis was used to calculate the event-free survival, once with hospital and gender as covariate, once without to compute the overall survival.

The amount of individual events (HCC and LXT) and outcomes (liver decompensation and mortality) were to small to make a ruling. A new computed variable combined these 4 events and outcomes. The Mann-Whitney U test was used to find a correlation between this newly created variable and the other data in the database. The Cox Regression was used to determine the hazard ratio of these correlated variable on an event or outcome. In a first time, variables were put in separately. Significant resuls in the univariate analysis were combined in a multivariate analysis, although no combinations were significant. A new variable was created with the results of the hazard ratios of the univariate analysis. The Cox Regression, ROC curve and Kapan-Meier survival analysis were determined for this prognostic system.

5. RESULTS

5.1 GENERAL CHARACTERISTICS

The distribution of the patients in the several hospitals is listed in Table 11.

Table 11: Distribution of patients.

Hospital	Total (N=212)
UZ Gent, n (%)	174 (82.1)
AZ Nikolaas, n (%)	13 (6.1)
AZ Maria Middelares, n (%)	25 (11.8)

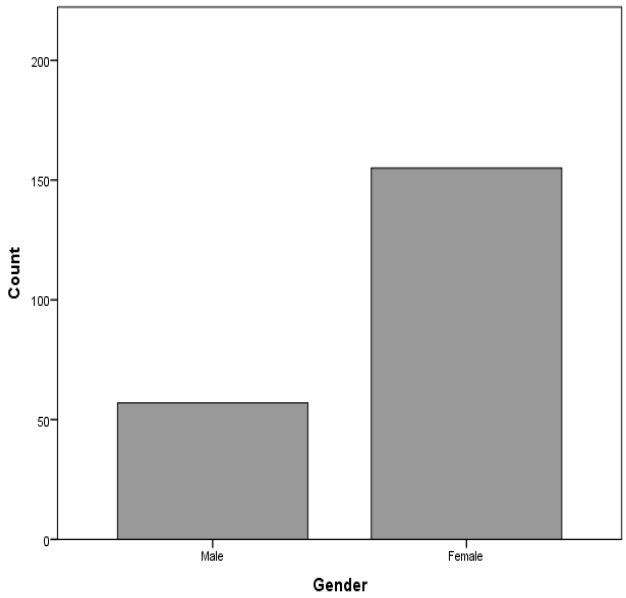
5.2 BASELINE CHARACTERISTICS

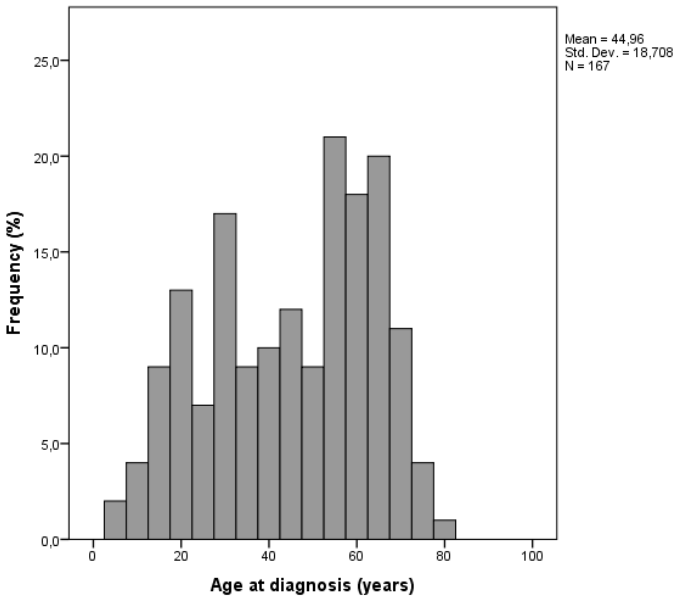
The baseline characteristics of the population are illustrated in Table 12. Associated autoimmune diseases were seen in 47 patients (22.2%), of which 6 patients (2.8%) had more than one associated autoimmune disease.

Table 12: Baseline characteristics.

Variables	UZ Gent (n=174)	AZ Nikolaas (n=13)	AZ Maria Middelares (n=25)	Total (N=212)	Total range
Age, y (mean ± SD)	52.2 (17.7)	59.2 (13.6)	60.8 (18.5)	53.7 (17.8)	10 – 87
Female gender, n (%)	125 (71.8)	9 (69.2)	21 (84.0)	155 (73.1)	
Length, cm (mean ± SD)	168.2 (11.8)[a]	168.3 (8.0)[a]	168.7 (4.7)[a]	168.2 (11.6)	111 – 197
Weight, kg (mean ± SD)	69.0 (15.6)[b]	77.0 (15.9)[b]	73.2 (8.8)[b]	69.5 (15.3)	18.4 – 110.0
BMI, kg/m² (mean ± SD)	24.4 (4.4)[c]	27.7 (8.4)[c]	28.5 (1.0)[c]	24.6 (4.5)	14.9 – 37.7
Time of diagnosis, y (mean ± SD)	2009 (6.2)[d]	2011 (7.9)[e]	2010 (7.7)[f]	2009 (6.5)	1975 – 2017
Age at diagnosis, y (mean ± SD)	43.5 (18.6)[d]	52.5 (13.9)[e]	50.2 (20.1)[f]	45.0 (18.7)	5 - 79
Associated autoimmune disease, n (%)	41 (23.6)	2 (15.4)	4 (16.0)	47 (22.2)	
Hashimoto's disease, n (%)	14 (9.6)	2 (15.4)	1 (4.0)	17 (9.2)	
Graves' disease, n (%)	5 (3.4)	0 (0.0)	0 (0.0)	5 (2.7)	
Diabetes mellitus type 1, n (%)	3 (2.1)	0 (0.0)	0 (0.0)	3 (1.6)	
SLE, n (%)	4 (2.8)	0 (0.0)	0 (0.0)	4 (2.2)	
Celiac disease, n (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
CU, n (%)	8 (5.5)	0 (0.0)	1 (4.0)	9 (4.9)	
Vitiligo, n (%)	1 (0.7)	0 (0.0)	0 (0.0)	1 (0.5)	
Scleroderma, n (%)	1 (0.7)	0 (0.0)	0 (0.0)	1 (0.5)	
RA, n (%)	6 (4.1)	0 (0.0)	2 (8.0)	8 (4.3)	
Crohn, n (%)	2 (1.4)	0 (0.0)	0 (0.0)	2 (1.1)	
Autoimmune gastritis, n (%)	0 (0.0)	0 (0.0)	1 (4.0)	1 (0.5)	
Pernicieuze anemie, n (%)	1 (0.7)	0 (0.0)	0 (0.0)	1 (0.5)	
Sjögren, n (%)	2 (1.4)	0 (0.0)	0 (0.0)	2 (1.1)	

[a]: Length was available for respectively 104 (UZ Gent), 3 (AZ Nikolaas) and 3 (AZ Maria Middelares) patients.
[b]: Weight was available for respectively 95 (UZ Gent), 3 (AZ Nikolaas) and 6 (AZ Maria Middelares) patients.
[c]: BMI could be calculated in respectively 87 (UZ Gent), 3 (AZ Nikolaas) and 3 (AZ Maria Middelares) patients.
[d]: Data could be collected and calculated in 135 patients.
[e]: Data could be collected and calculated in 11 patients.
[f]: Data could be collected and calculated in 21 patients.





5.3 SYMPTOMS

In some patients, the initial diagnosis was made in another hospital and patients were referred for second opinion. In these patients, initial symptoms were unknown. Data could be collected within 134 patients. 27 patients (20.1%) were asymptomatic at time of diagnosis. Features of the 107 symptomatic patients (79.9%) at diagnosis and their frequencies are listed in Table 13.

Table 13: Symptoms at diagnosis.

	UZ Gent (n=104)	AZ Nikolaas (n=9)	AZ Maria Middelares (n=21)	Total (N=134)
Asymptomatic, n (%)	23 (22.1)	2 (22.2)	2 (9.5)	27 (20.1)
Symtomatic, n (%)	81 (77.9)	7 (77.8)	19 (90.5)	107 (79.9)
Fatigue, n (%)	39 (37.5)	2 (22.2)	12 (85.7)	53 (41.7)
Anorexia, n (%)	20 (19.2)	1 (11.1)	7 (58.3)	28 (22.4)
Losing weight, n (%)	24 (23.1)	3 (33.3)	6 (54.5)	33 (26.6)
Itching, n (%)	11 (10.6)	0 (0.0)	2 (33.3)	13 (10.9)
Abdominal pain, n (%)	23 (22.1)	4 (44.4)	5 (45.5)	32 (25.8)
Arthralgia in the small joints, n (%)	12 (11.5)	0 (0.0)	4 (44.4)	16 (13.1)
Icterus, n (%)	34 (32.7)	3 (33.3)	5 (50.0)	42 (34.1)

5.4 LABORATORY FINDINGS

Laboratory findings and the presence of auto-antibodies at diagnosis are respectively given in Table 14 and Table 15. Multiple auto-antibodies can occur in the same patient.

Table 14: Laboratory findings at diagnosis.

	UZ Gent	AZ Nikolaas	AZ Maria Middelares	Total	Range
Viral history					
HSV Ig, n (%)	16 (84.2)	0 (0.0)	-	16 (80.0)	
HAV Ig, n (%)	22 (34.9)	5 (83.3)	2 (66.7)	29 (40.3)	
HBsAb, n (%)	18 (21.4)	3 (42.9)	1 (8.3)	22 (21.4)	
HBcAb, n (%)	8 (10.0)	0 (0.0)	1 (11.1)	9 (9.7)	
HCV Ig, n (%)	5 (4.0)	0 (0.0)	0 (0.0)	5 (3.4)	
EBV, n (%)	54 (94.7)	3 (75.0)	6 (85.7)	63 (92.6)	
Biochemical findings					
Total bilirubin, mg/dL (mean ± SD)	4.7 (8.1)[a]	2.6 (3.3) [a]	4.5 (7.8) [a]	4.5 (7.8)	0.2 – 46.0
AST, U/L (mean ± SD)	528.2 (684.3)[b]	599.9 (686.1)[b]	430.2 (738.6)[b]	519.8 (687.6)	19 – 4977
ALT, U/L (mean ± SD)	599.5 (740.2)[c]	774.1 (799.7)[c]	501.5 (668.5)[c]	597.4 (731.5)	11 – 4524
ALP, U/L (mean ± SD)	206.5 (172.1)[d]	233.6 (101.3)[d]	195.7 (141.5)[d]	207.1 (164.2)	54 – 1188
GGT, U/L (mean ± SD)	227.0 (234.2)[e]	333.6 (180.1)[e]	239.2 (161.1)[e]	235.7 (224.2)	9 – 1065
γglobuline, g/L (mean ± SD)	25.3 (8.5)[f]	18.1 (9.7)[f]	26.3 (13.4)[f]	24.2 (9.0)	5.8 – 45.9
IgG, g/L (mean ± SD)	21.7 (11.3)[g]	18.5 (5.7)[g]	25.3 (16.9)[g]	21.8 (11.5)	8.5 – 81.1
IgM, g/L (mean ± SD)	1.9 (1.5)[h]	4.1 (6.6)[h]	2.3 (1.3)[h]	2.1 (2.4)	0.3 – 20.5
IgA, g/L (mean ± SD)	3.3 (1.7)[i]	2.8 (1.6)[i]	3.6 (2.1)[i]	3.3 (1.7)	0.06 – 9.40
Total cholesterol, mg/dL (mean ± SD)	169.8 (56.8)[j]	137 (-)[j]	169.7 (41.9)[j]	169.4 (55.8)	63 – 371
Eosinophils, /µL (mean ± SD)	161.7 (162.3)[k]	212.0 (265.7)[k]	620.0 (-)[k]	168.7 (172.4)	0 – 754
Platelets, x10³/µL (mean ± SD)	240.6 (98.3)[l]	222.7 (56.2)[l]	212.6 (69.7)[l]	236.9 (93.7)	52 – 604

[a]: Total bilirubin was available for respectively 106 (UZ Gent), 9 (AZ Nikolaas) and 16 (AZ Maria Middelares) patients.
[b]: AST was available for respectively 107 (UZ Gent), 9 (AZ Nikolaas) and 18 (AZ Maria Middelares) patients.
[c]: ALT was available for respectively 107 (UZ Gent), 9 (AZ Nikolaas) and 19 (AZ Maria Middelares) patients.
[d]: ALP was available for respectively 104 (UZ Gent), 9 (AZ Nikolaas) and 15 (AZ Maria Middelares) patients.
[e]: GGT was available for respectively 107 (UZ Gent), 9 (AZ Nikolaas) and 15 (AZ Maria Middelares) patients.
[f]: γglobuline was available for respectively 18 (UZ Gent), 4 (AZ Nikolaas) and 2 (AZ Maria Middelares) patients.
[g]: IgG was available for respectively 99 (UZ Gent), 9 (AZ Nikolaas) and 9 (AZ Maria Middelares) patients.
[h]: IgM was available for respectively 86 (UZ Gent), 9 (AZ Nikolaas) and 9 (AZ Maria Middelares) patients.
[i]: IgA was available for respectively 82 (UZ Gent), 9 (AZ Nikolaas) and 11 (AZ Maria Middelares) patients.
[j]: Total cholesterol was available for respectively 75 (UZ Gent), 1 (AZ Nikolaas) and 3 (AZ Maria Middelares) patients.
[k]: Eosinophils were available for respectively 96 (UZ Gent), 5 (AZ Nikolaas) and 1 (AZ Maria Middelares) patients.
[l]: Platelets were available for respectively 106 (UZ Gent), 9 (AZ Nikolaas) and 11 (AZ Maria Middelares) patients.

Table 15: Presence of auto-antibodies at diagnosis.

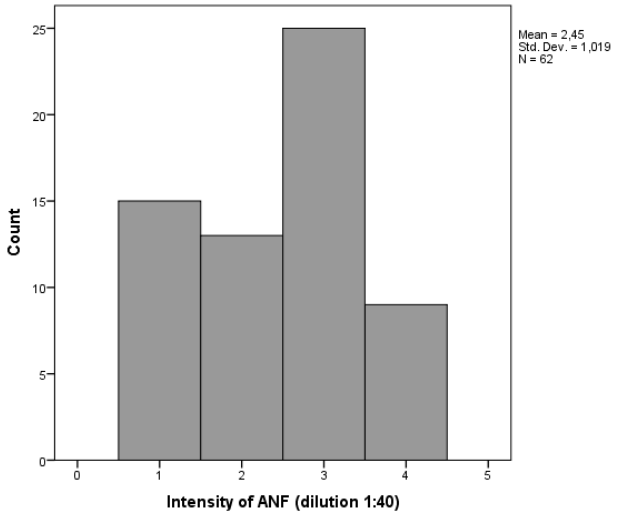
Auto-antibodies	UZ Gent	AZ Nikolaas	AZ Maria Middelares	Total
ANA, n (%)	70 (76.1)	10 (100.0)	15 (75.0)	95 (77.9)
AMA, n (%)	12 (13.3)	2 (50.0)	5 (25.0)	19 (16.7)
SMA, n (%)	43 (46.7)	0 (0.0)	9 (52.9)	52 (46.4)
ANCA, n (%)	18 (58.1)	-	1 (12.5)	19 (48.7)
Anti-LKM, n (%)	2 (2.4)	0 (0.0)	0 (0.0)	2 (2.1)
Anti-LC1, n (%)	3 (8.8)	-	-	3 (8.8)

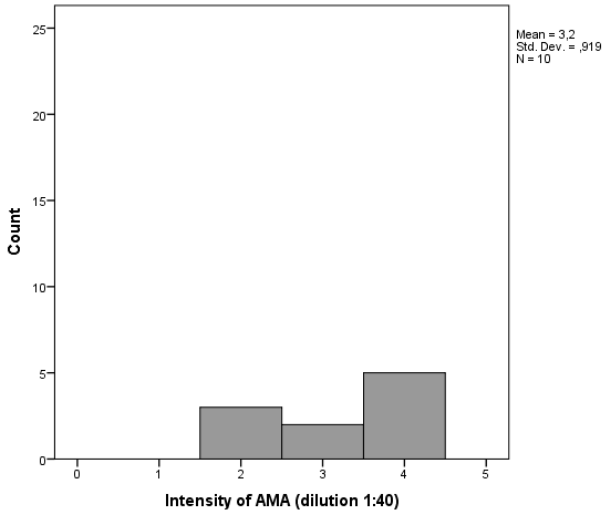
In the UZ Gent hospital, dilutions other than 1:40 are obtained by other hospitals. In the general hospitals of Gent and Sint-Niklaas are titers obtained by ever-increasing dilutions until no auto-antibody can be observed.

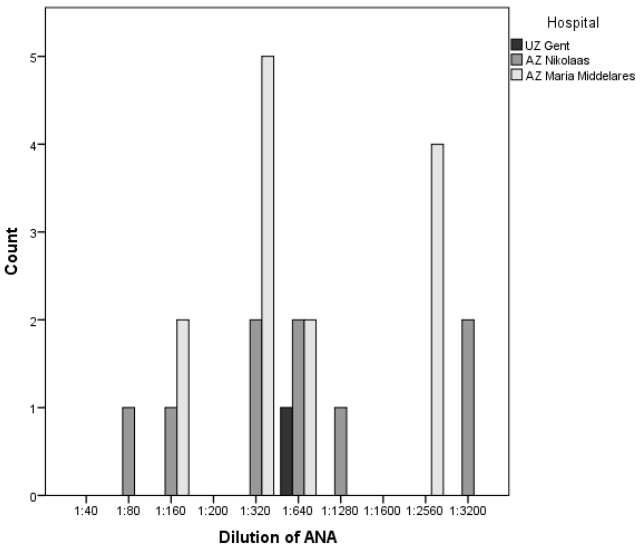
The numbers of patients doesn't fit with the numbers of Table 15, because referrals from other hospitals doesn't always mention the dilution or intensity. The dilutions and intensity of autoantibodies are given in Table 16.

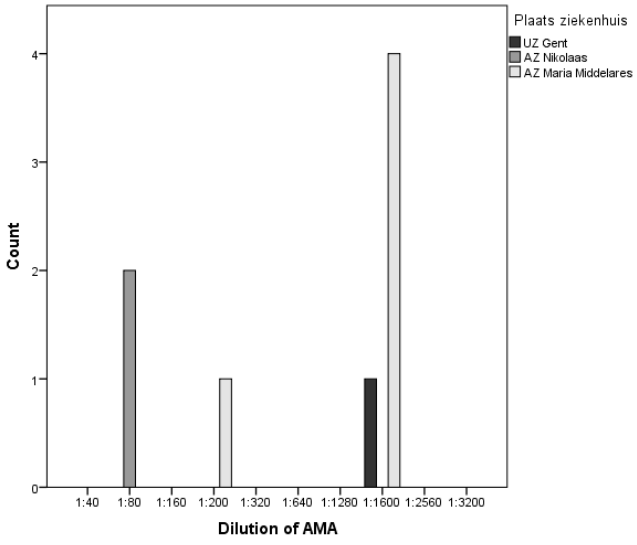
Table 16: Dilution and intensity of auto-antibodies.

	UZ Gent		AZ Nikolaas		AZ Maria Middelares		Total	
	ANA (n=63)	AMA (n=11)	ANA (n=9)	AMA (n=2)	ANA (n=13)	AMA (n=5)	ANA (n=23)	AMA (n=8)
1:40, n (%)	62 (98.4)	10 (90.9)	-	-	-	-	-	-
1, n (%)	15 (24.2)	0 (0.0)	-	-	-	-	-	-
2, n (%)	13 (21.0)	3 (30.0)	-	-	-	-	-	-
3, n (%)	25 (40.3)	2 (20.0)	-	-	-	-	-	-
4, n (%)	9 (14.5)	5 (50.0)	-	-	-	-	-	-
1:80, n (%)	-	-	1 (11.1)	2 (100.0)	-	-	1 (4.3)	2 (25.0)
1:160, n (%)	-	-	1 (11.1)	-	2 (15.4)	-	3 (13.0)	-
1:200, n (%)	-	-	-	-	-	1 (20.0)	-	1 (12.5)
1:320, n (%)	-	-	2 (22.2)	-	5 (38.5)	-	7 (30.4)	-
1:640, n (%)	1 (1.6)	-	2 (22.2)	-	2 (15.4)	-	5 (21.7)	-
1:1280, n (%)	-	-	1 (11.1)	-	-	-	1 (4.3)	-
1:1600, n (%)	-	1 (9.1)	-	-	-	4 (80.0)	-	5 (62.5)
1:2560, n (%)	-	-	-	-	4 (30.8)	-	4 (17.4)	-
1:3200, n (%)	-	-	2 (22.2)	-	-	-	2 (8.7)	-









5.5 LIVER BIOPSY

For the same reason as for the laboratory findings, several patients already had their diagnosis when consulting one of the investigated hospitals for their first time. Consequently, liver biopsies were retrieved in 157 cases. Table 18 shows the histological features and their frequencies. Liver biopsy was not carried out in 7 patients. Reasons included refusal of the patient and rapid deterioration with death as consequence.

Table 17: Liver biopsy.

	UZ Gent (n=132)	AZ Nikolaas (n=11)	AZ Maria Middelares (n=20)	Total (N=163)
Liver biopsy, n (%)	129 (97.7)	11 (100.0)	17 (85.0)	157 (96.3)
Interface hepatitis, n (%)	59 (72.0)	9 (100.0)	7 (63.6)	75 (73.5)
Ballooning, n (%)	19 (29.7)	2 (50.0)	2 (40.0)	23 (31.5)
Cholate stasis, n (%)	13 (18.6)	2 (33.3)	4 (57.1)	19 (22.9)
Steatosis, n (%)	4 (20.0)	0 (0.0)	3 (75.0)	7 (21.9)
Bilirubinostasis, n (%)	15 (21.1)	2 (50.0)	0 (0.0)	17 (21.5)
Ceroid macrophages, n (%)	47 (54.7)	6 (100.0)	1 (100.0)	54 (58.1)
Rosette formation, n (%)	3 (5.4)	0 (0.0)	1 (16.7)	4 (6.3)
Plasmacells, n (%)	80 (84.2)	9 (100.0)	15 (100.0)	104 (87.4)
Fibrosis, n (%)	78 (80.4)	11 (100.0)	10 (76.9)	99 (81.8)
Cirrhosis, n (%)	24 (34.8)	2 (25.0)	5 (41.7)	31 (34.8)
Necrosis, n (%)	37 (52.9)	4 (100.0)	8 (50.0)	49 (54.4)

The level of fibrosis on biopsy, listed in Table 19, was given in 24 patients. A non-invasive fibroscan or elastometry can also be used to quantify the amount of fibrosis. This equipment is not yet available in AZ Nikolaas and it has only been operational for about 2-3 years in the hospitals of Gent, which explains the small number of investigated patients (N=51). The values of the fibroscan and elastometry of this population are illustrated in Table 20.

Table 18: Level of fibrosis on biopsy.

	UZ Gent (n=17)	AZ Nikolaas (n=5)	AZ Maria Middelares (n=2)	Total (N=24)
Metavir F0, n (%)	2 (11.8)	0 (0.0)	0 (0.0)	2 (8.3)
Metavir F1, n (%)	7 (41.2)	2 (40.0)	0 (0.0)	9 (37.5)
Metavir F2, n (%)	5 (29.4)	1 (20.0)	1 (50.0)	7 (29.2)
Metavir F3, n (%)	2 (11.8)	0 (0.0)	1 (50.0)	3 (12.5)
Metavir F4, n (%)	1 (5.9)	2 (40.0)	0 (0.0)	3 (12.5)

Table 19: Fibroscan and elastometry.

	UZ Gent (n=49)	AZ Nikolaas (n=0)	AZ Maria Middelares (n=2)	Total (N=51)	Range
Fibroscan, kPa (mean ± SD)	10.0 (7.0)	-	8.8 (3.2)	9.9 (6.9)	3.2 – 33.8
2.5 – 7.5 kPa, n (%)	20 (52.6)	-	1 (50.0)	21 (52.5)	
7.5 – 9.5 kPa, n (%)	4 (10.5)	-	0 (0.0)	4 (10.0)	
9.5 – 12.5 kPa, n (%)	5 (13.2)	-	1 (50.0)	6 (15.0)	
>12.5 kPa, n (%)	9 (23.7)	-	0 (0.0)	9 (22.5)	
Elastometry, kPa (mean ± SD)	6.2 (1.8)	-	-	6.3 (1.8)	3.8 – 10.0
2.5 – 7.5 kPa, n (%)	8 (72.7)	-	-	8 (72.7)	
7.5 – 9.5 kPa, n (%)	2 (18.2)	-	-	2 (18.2)	
9.5 – 12.5 kPa, n (%)	1 (9.1)	-	-	1 (9.1)	
>12.5 kPa, n (%)	0 (0.0)	-	-	0 (0.0)	

5.6 OVERLAP SYNDROME

33 patients (15.6%) are diagnosed with an overlap between AIH and PSC/PBC. Table 21 shows the frequency of these overlap syndromes.

Table 20: Frequency of overlap syndromes.

	UZ Gent (n=174)	AZ Nikolaas (n=13)	AZ Maria Middelares (n=25)	Total (N=212)
AIH–PBC overlap syndrome, n (%)	16 (9.2)	1 (7.7)	3 (12.0)	20 (9.4)
AIH–PSC overlap syndrome, n (%)	11 (6.3)	0 (0.0)	2 (8.0)	13 (6.1)

5.7 TYPES OF AIH

Data of the two types was available for 113 patients (53.3%). Table 22 shows the frequency of these types.

Table 21: Frequency of types of AIH.

	UZ Gent (n=85)	AZ Nikolaas (n=10)	AZ Maria Middelares (n=18)	Total (N=113)
AIH–1, n (%)	80 (94.1)	10 (100.0)	18 (100.0)	108 (95.6)
AIH–2, n (%)	5 (5.9)	0 (0.0)	0 (0.0)	5 (4.4)

5.8 SIMPLIFIED SCORING SYSTEM

The simplified scoring system could only be calculated in 34 patients (16.0%). Lack of data was frequent, especially because of the large absence of the viral hepatitis status in the several hospitals. Data are listed in Table 23.

Table 22: Simplified scoring-system.

	UZ Gent (n=30)	AZ Nikolaas (n=3)	AZ Maria Middelares (n=1)	Total (N=34)
No evidence for AIH, n (%)	12 (40.0)	1 (33.3)	0 (0.0)	13 (38.2)
Probable AIH, n (%)	9 (30.0)	2 (66.7)	1 (100.0)	12 (35.3)
Definite AIH, n (%)	9 (30.0)	0 (0.0)	0 (0.0)	9 (26.4)

5.9 TREATMENT

Of the 212 patients with AIH, 166 received treatment (78.3%). The treatment regimen was unknown for 40 patients (18.9%). 6 patients (2.8%) did not receive one of the treatments mentioned in Table 24. Reasons include refusal, uncertain diagnosis and mortality after fulminant hepatitis.

Table 23: Treatment of AIH.

	UZ Gent	AZ Nikolaas	AZ Maria Middelares	Total	Total range
Steroids, n (%)	133 (97.1)	11 (91.7)	18 (81.8)	162 (94.7)	
Predniso(lo)ne, n (%)	101 (75.9)	5 (41.7)	9 (40.9)	115 (68.9)	
Start dose, mg/day (mean ± SD)	31.3 (18.0)	42.7 (18.5)	29.7 (6.0)	31.6 (17.4)	3 – 125
Duration, months (mean ± SD)	47.2 (64.0)	50.6 (65.2)	21.6 (25.7)	45.3 (61.9)	0 – 510[a]
Budesonide, n (%)	73 (55.3)	10 (83.3)	13 (65.0)	96 (58.5)	
Start dose, mg/day (mean ± SD)	8.0 (1.9)	8.7 (0.9)	8.8 (0.8)	8.2 (1.8)	3 – 9
Duration, months (mean ± SD)	42.7 (38.1)	32.4 (26.1)	27.6 (37.5)	39.6 (37.2)	0 – 153[b]
Immunosuppressiva, n (%)	98 (74.8)	10 (83.3)	17 (77.3)	125 (75.8)	
Azathioprine, n (%)	87 (64.4)	10 (90.9)	16 (76.2)	113 (67.7)	
Start dose, mg/day (mean ± SD)	80.9 (30.8)	72.2 (26.4)	80.4 (46.2)	79.9 (33.0)	25 – 175
Duration, months (mean ± SD)	63.6 (52.7)	48.2 (71.9)	39.2 (43.7)	58.1 (53.9)	0 – 249[c]
Mycophenolate mofetil, n (%)	20 (15.2)	1 (8.3)	0 (0.0)	21 (12.7)	
Start dose, mg/day (mean ± SD)	1296.9 (725.8)	1000.0 (-)	-	1279.4 (706.5)	250 – 3000
Duration, months (mean ± SD)	69.9 (37.0)	9.0 (-)	-	66.5 (38,6)	9 – 142[d]
Ciclosporine, n (%)	14 (10.7)	0 (0.0)	1 (4.8)	15 (9.1)	
Start dose, mg/day (mean ± SD)	158.5 (58.2)	-	200.0 (-)	162.3 (56.6)	60 – 200
Duration, months (mean ± SD)	63.3 (58.1)	-	28.0 (0)	60.1 (56.1)	0 – 170[e]
Other drugs					
UDCA, n (%)	53 (41.1)	1 (8.3)	4 (19.0)	58 (35.8)	
Start dose, mg/day (mean ± SD)	901.7 (290.4)	900.0 (-)	800.0 (264.6)	895.3 (284.1)	75 – 1800
Duration, months (mean ± SD)	76.2 (64.9)	15.0 (-)	20.3 (22.7)	70.7 (64.2)	0 – 237[f]
Purinethol, n (%)	5 (3.8)	0 (0.0)	1 (4.8)	6 (3.7)	
Start dose, mg/day (mean ± SD)	65.0 (28.5)	-	-	65.0 (28.5)	25 – 100
Duration, months (mean ± SD)	41.6 (32.0)	-	1.0 (-)	34.8 (33.1)	1 – 86[g]

[a]: 42 of 113 patients (40.7%) still use predniso(lo)ne. 2 missing data.
[b]: 40 of 85 patients (47.1%) still use budesonide. 11 missing data.
[c]: 69 of 104 patients (66.3%) still use azathioprine. 9 missing data.
[d]: 14 of 18 patients (77.8%) still use mycophenolate mofetil. 3 missing data.
[e]: 6 of 13 patients (46.2%) still use ciclosporine. 2 missing data.
[f]: 38 of 56 patients (67.9%) still use UDCA. 2 missing data.
[g]: 3 of 5 patients (60.0%) still use purinethol. 1 missing data.

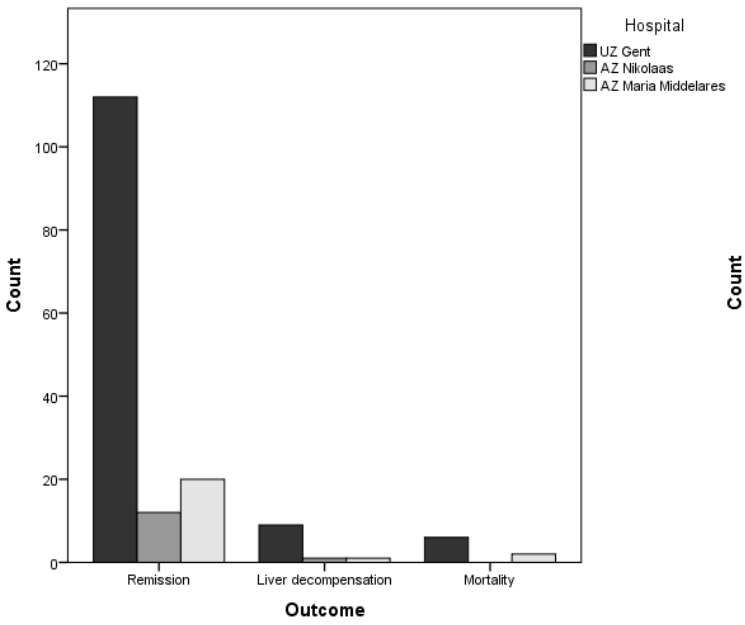
5.10 OUTCOME AND EVENTS DURING TREATMENT

Long-term outcome and events during treatment are summed up in Table 25. Forty-nine missing values were due to lost-to-follow-up and too recent diagnoses.

Table 24: Outcome and events during treatment.

	UZ Gent	AZ Nikolaas	AZ Maria Middelares	Total
Outcome[a]				
Remission, n (%)	112 (88.2)	12 (92.3)	20 (87.0)	144 (88.3)
Decompensation, n (%)	9 (7.1)	1 (7.7)	1 (4.3)	11 (6.7)
Ascites, n (%)	6 (66.7)	1 (100.0)	1 (100.0)	8 (72.7)
Varices, n (%)	1 (11.1)	0 (0.0)	0 (0.0)	1 (9.1)
Hepatic encephalopathy, n (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Mortality, n (%)	6 (4.7)	0 (0.0)	2 (8.7)	8 (4.9)
Events				
Relapse, n (%)	53 (44.5)[b]	5 (41.7)[b]	7 (29.2)[b]	65 (41.9)
HCC, n (%)	8 (5.7)[c]	0 (0.0)[c]	1 (4.2)[c]	9 (5.1)
LxT, n (%)	21 (14.9)[d]	0 (0.0)[d]	0 (0.0)[d]	21 (11.9)

[a]: Outcome was available for respectively 127 (UZ Gent), 13 (AZ Nikolaas) and 23 (AZ Maria Middelares) patients.
[b]: Relapse was available for respectively 119 (UZ Gent), 12 (AZ Nikolaas) and 24 (AZ Maria Middelares) patients.
[c]: HCC was available for respectively 140 (UZ Gent), 12 (AZ Nikolaas) and 24 (AZ Maria Middelares) patients.
[d]: LxT was available for respectively 141 (UZ Gent), 12 (AZ Nikolaas) and 24 (AZ Maria Middelares) patients.



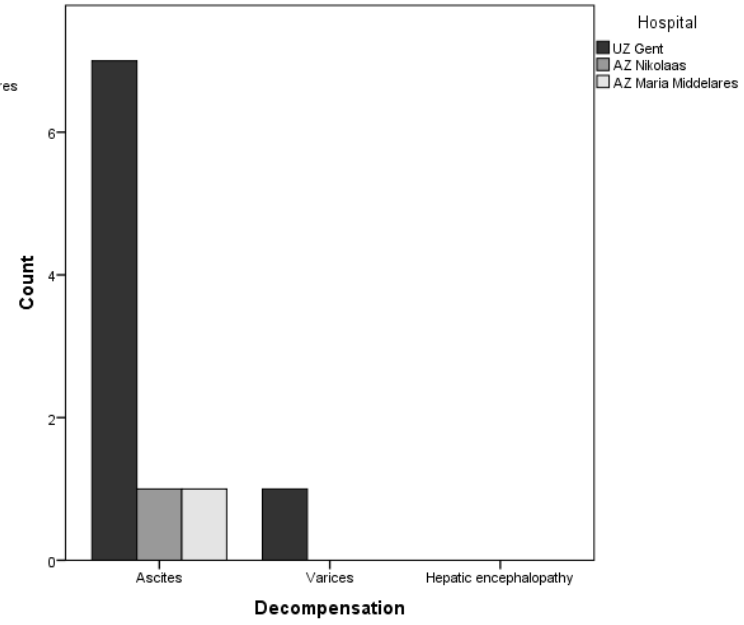


Table 25: Mean time to the occurrence of an event.

	UZ Gent (n=59)	AZ Nikolaas (n=5)	AZ Maria Middelares (n=8)	Total (N=72)	Total range
Time to decompensation[a], months (mean ± SD)	2.3 (5.2)	-	-	2.3 (5.2)	-2 – 12
Time to relapse[b], months (mean ± SD)	64.3 (83.7)	63.2 (58.5)	42.6 (32.9)	61.6 (76.9)	2 – 459
Time to HCC[c], months (mean ± SD)	17.3 (36.2)	-	0 (-)	15.3 (34.3)	-20 – 68

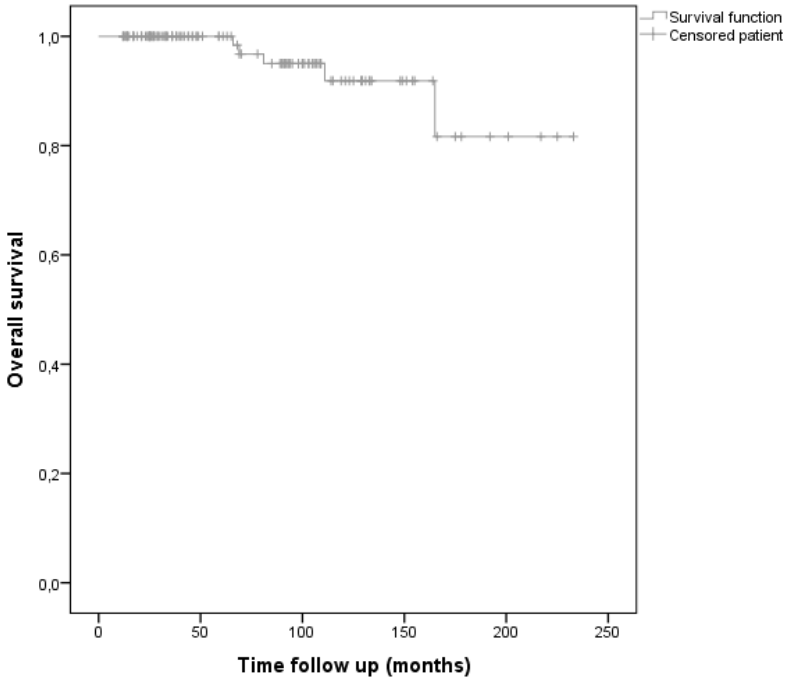
[a]: Time to decompensation was available for respectively 6 (UZ Gent), 0 (AZ Nikolaas) and 0 (AZ Maria Middelares) patients.
[b]: Time to relapse was available for respectively 45 (UZ Gent), 5 (AZ Nikolaas) and 7 (AZ Maria Middelares) patients.
[c]: Time to HCC was available for respectively 8 (UZ Gent), 0 (AZ Nikolaas) and 1 (AZ Maria Middelares) patients.

5.11 OVERALL SURVIVAL

The long-term survival is listed in Table 26.

Table 26: Overall survival.

	UZ Gent (n=107)	AZ Nikolaas (n=9)	AZ Maria Middelares (n=11)	Total (N=127)
5-year survival rate, % (mean ± SD)	96.6 (2.4)	-	-	96.9 (2.1)
6-year survival rate, % (mean ± SD)	-	-	75.0 (21.7)	95.2 (2.7)
9-year survival rate, % (mean ± SD)	93.4 (3.8)	-	-	92.3 (3.9)
13-year survival rate, % (mean ± SD)	84.1 (9.5)	-	-	83.1 (9.4)



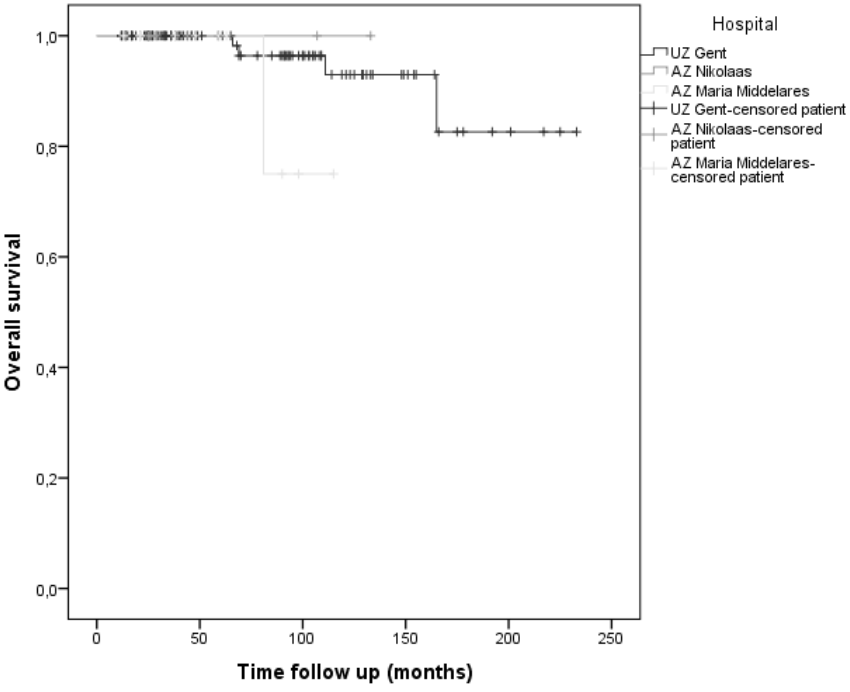
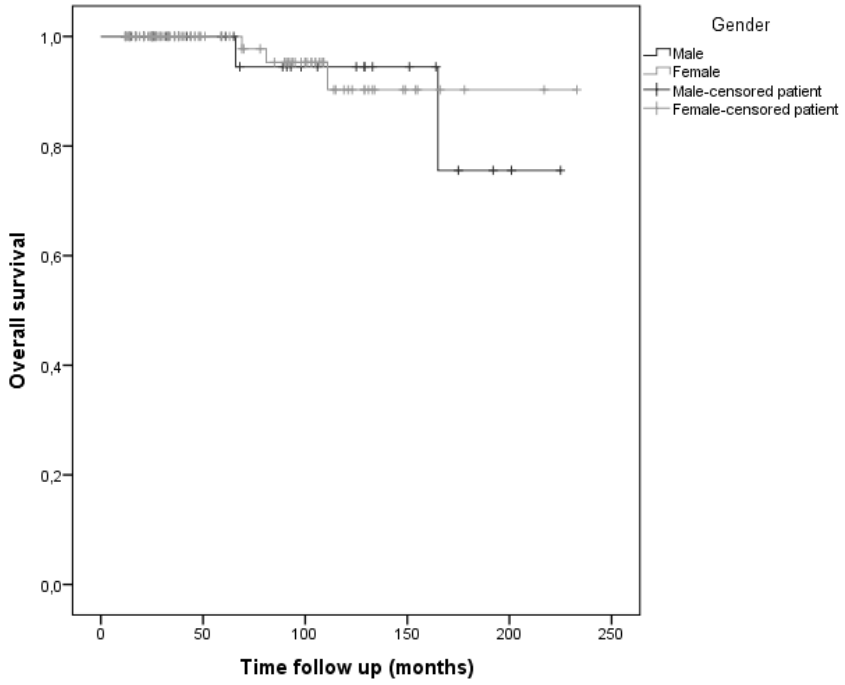


Table 27 shows the survival rate in function of the gender. Two men out of 35 male patients (5.7%) died of AIH against 3 women out of 92 female patients (3.3%). The small number of male patients provides a higher mortality ratio and consequently a lower overall survival.

Table 27: Overall survival in function of the gender.

	Male (n=35)	**Female (n=92)**	**Total (N=127)**
5-year survival rate, % (mean ± SD)	95.0 (4.9)	97.8 (2.2)	96.9 (2.1)
6-year survival rate, % (mean ± SD)	-	95.4 (3.2)	95.2 (2.7)
9-year survival rate, % (mean ± SD)	-	91.1 (5.2)	92.3 (3.9)
13-year survival rate, % (mean ± SD)	79.2 (15.0)	-	83.1 (9.4)



5.12 PROGNOSTIC SCORES

A compound variable was created with the variables HCC, LTX, liver decompensation and mortality. These variables are correlated with an adverse outcome. The Mann-Whitney U Test found a correlation between the compound variable and 4 variables in the blood (see Table 28). Foundings of the univariate and multivariate analyses of the Cox Regression are given in respectively Table 28 and Table 29.

Table 28: Mann-Whitney U test.

Variables	**P-value**
Bilirubin after 6 months	0.034
AST after 6 monts	0.036
ALP after 6 months	0.013
GGT after 6 months	0.000

Table 29: Cox Regression: univariate analysis.

Variables	P-value	Hazard ratio[a]	95.5% confidence interval for Hazard ratio	
			Lower	Upper
Bilirubin after 6 months	0.001	2.222	1.360	3.630
AST after 6 monts	0.008	1.026	1.007	1.045
ALP after 6 months	0.013	1.011	1.002	1.019
GGT after 6 months	0.016	1.004	1.001	1.008

[a]: Estimated relative risk on the occurence of an event.

Table 30: Cox Regression: multivariate analysis.

Variables	P-value	Hazard ratio[a]	95.5% confidence interval for Hazard ratio	
			Lower	Upper
Bilirubin after 6 months	0.293	1.822	0.596	5.572
AST after 6 monts	0.854	0.996	0.956	1.038
ALP after 6 months	0.676	1.003	0.988	1.020
GGT after 6 months	0.173	1.004	0.998	1.009

[a]: Estimated relative risk on the occurence of an event.

No combinations were significant in the multivariate Cox Regression analysis. A new variable was created, based on the hazard ratio of the univariate Cox Regression analyses:

$$Prognostic\ score = [Bilirubin_{6m} * 2.222] + [AST_{6m} * 1.026] + [ALP_{6m} * 1.011] + [GGT_{6m} * 1.004]$$

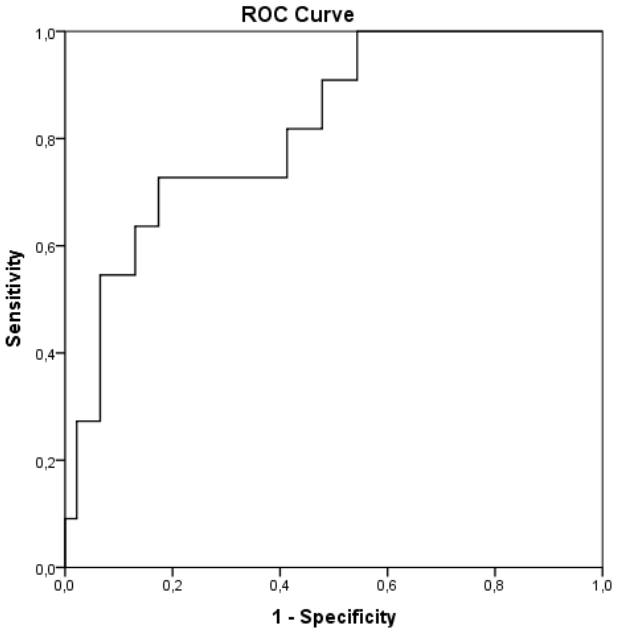
Every variable is the value measured after 6 months of therapy. The significance of this prognostic scoring system was calculated with the Cox Regression analysis and is illustrated in Table 31. If the prognostic score elevates with 1 point, does not change much. Therefor, the prognostic scoring system will be divided by 100.

Table 31: Cox Regression analysis of the prognostic scoring system.

Variables	P-value	Hazard ratio[a]	95.5% confidence interval for Hazard ratio	
			Lower	Upper
Prognostic scoring system	0.003	1.519	1,151	2,004

[a]: Estimated relative risk on the occurence of an event.

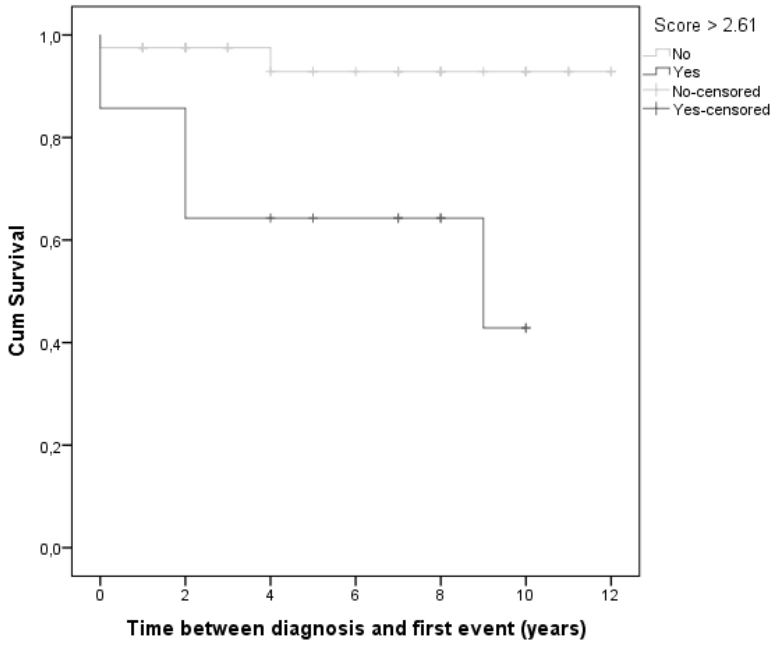
The AUC for this prognostic score was 0.82 (p=0.001, 95%CI=0.69-0.95). The Log Rank of the ROC-curve was p=0.002. Using a cut-off (score=2.61) derived from the ROC-curve, the sensitivity (72.7%) and specificity (82.6%) could be determined for the prediction of an adverse outcome.



The Kaplan-Meier survival analysis was calculated, with the variable 'score > cut-off (2.61)' as covariate. The results are shown in Table 32.

Table 32: Kaplan-Meier survival analysis of the prognostic scoring system.

Survival	Prognostic score ≤ 261	Prognostic score > 261
2-year survival rate, % (mean ± SD)	-	64.3 (12.8)
4-year survival rate, % (mean ± SD)	92.9 (5.1)	-
9-year survival rate, % (mean ± SD)	-	42.9 (19.5)



6. DISCUSSION

This study maps 212 AIH patients in the University Hospital of Ghent and the general Hospitals of Sint-Niklaas (AZ Sint-Nikolaas) and Ghent (AZ Maria Middelares), respectively with 174, 13 and 25 patients. Overall, there were 155 women (73.1%) and 57 men (26.9%) included in this cohort. The female-to-male sex-ratio is 2.7:1, which is similar to the ratio in the literature (3-3.6:1). The mean length and weight are respectively 168.2cm and 69.5kg, resulting in an overall mean BMI (n=87, 41.0%) of 24.6kg/m². This is equivalent to the mean BMI of 23 in the Belgian population without AIH [3].

Forty-seven patients (22.2%) were diagnosed with an associated extrahepatic autoimmune disease, wherof 6 patients (2.8%) had more than one associated autoimmune disease. This frequency is much lower than represented in earlier studies (40%). Autoimmune thyroiditis, such as Hashimoto's disease and Graves' disease, are the most common associated diseases (n=22, 11.9%) and were seen in respectively 17 patients (9.2%) and 5 patients (2.7%). The literature also mentions autoimmune thyroiditis as the most common associated autoimmune disease in AIH. Other associated autoimmune diseases in our population were ulcerative colitis (n=9, 4.9%), rheumatoid arthritis (n=8, 4.3%), systemic lupus erythematosus (n=4, 2.2%), diabetes mellitus type 1 (n=3, 1.6%), Crohn's disease (n=2, 1.1%), Sjögren's syndrome (n=2, 1.1%), vitiligo (n=1, 0.5%), scleroderma (n=1, 0.5%), autoimmune gastritis (n=1, 0.5%) and pernicious anemia (n=1, 0.5%). No patient was diagnosed with celiac disease. The frequent appearance of ulcerative colitis, rheumatoid arthritis and diabetes mellitus type 1 is in line with what is mentioned in the literature. On the other hand, the frequency of vitiligo and celiac disease is slightly lower than in earlier studies. Although it should be remembered that the number of patients in our cohort with one of these autoimmune diseases is very low. Consequently, a low frequency can explain these deviations.
An overlap syndrome between AIH and PSC or PBC was seen in 33 patients (15.6%). A similar frequency of 18% is recorded in the literature. Overlap between AIH and PBC was most common (n=20, 9.4%), AIH-PSC overlap syndrome was seen in 13 patients (6.1%).

Dates of included follow-up range from 1975 to 2017. Their mean age at start of diagnosis and follow-up was 45.0 years old (range 5 – 79 years old), but a bimodal distribution can be seen, with a peak at the age of 20 – 30 years old and a peak at the age of 55 – 65. The same peaks were founded in the literature.

Of the 134 available patients, 27 patients (20.1%) were asymptomatic at time of diagnosis. These findings are completely similar to the results in the literature, where up to 12-35% of the AIH patients do not have symptoms at diagnosis. Of the symptomatic patients (n=107, 79.9%),

fatigue was the most common complaint at diagnosis (n=53, 41.7%). Other symptoms were icterus (n=42, 34.1%), losing weight (n=33, 26.6%), abdominal pain (n=32, 25.8%), anorexia (n=28, 22.4%), arthralgia in the small joints (n=16, 13.1%) and itching (n=13, 10.9%). These symptoms are also mentioned as the main symptoms of AIH in the literature, although, whereas jaundice is the most prevalent symptom (50 – 55%) in a study by Choudhuri et al., it's less common in our population. However, it has to be noticed that the study of Choudhuri et al. was done in India. The question that can be argued is whether this difference in frequency is a consequence of diversity between an Indian and mostly Occidental race or whether the smaller population in India (n=38). However, the unavailability of the ethnicity is a disadvantage of our research. The frequencies of fatigue, abdominal pain and arthralgia in the small joints are similar to the literature, respectively with a difference of 3, 2 and 5 percent. In the physical examination, ascites was observed in 8 patients (6.0%). This number is lower than the findings in the study of Abdollahi et al. (17%). Here too, a diversity in race (Iranian versus Occidental) and a rather small population (n=60) may explain the difference. Another explanation is an early diagnosis in our population.
In our study, the distribution from one till five symptoms at diagnosis was respectively seen in 43 (40.2%), 32 (29.9%), 20 (18.7%), 9 (8.4%) and 3 (2.8%) patients. According to the study of Peng et al., patients only had three (76.9%) or four (23.1%) symptoms at diagnosis. The diversity in race (Chinese versus Occidental) is a possible explanation of the difference in number of symptoms.

The transaminases are both elevated. The mean value of AST and ALT is respectively 519.8 and 597.4. Serum IgG reach a mean level of 21.8, which is 1.6 times higher than the ULN. This value is similar to the values (1.2-3.0) indicated in the literature. In contrast to what is found in earlier studies, IgM and IgA are also (slightly) elevated. ALP and serum bilirubin are elevated as well.
95.6% of the patients had a positivity for ANA or SMA. This value differs from the 70-80% founded in the literature. The frequency of LKM-1 (2.1) is similar to the frequency that is mentioned in earlier studies (3-4%). Five patients (4.7%) were seronegative for circulating autoantibodies. The literature mentioned a frequency of 20%, which is a significant difference.

As reported in the literature, almost every patient (n=157, 97.7%) of our cohort population received a liver biopsy. The presence of plasmacells in the liver was the most common histological feature (n=104, 87.4%). Interface hepatitis and rosette formation, parts of the classic triad on liver biopsy, were respectively seen in 75 (73.5%) and 4 (6.3%) patients. This is significantly less than the frequencies in earlier studies (respectively 84-98% and 40%). Emperipolesis was not determined in the hospitals of Ghent and Sint-Niklaas. Other features

on biopsy included ceroid macrophages (n=54, 58.1%), necrosis (n=49, 54.4%), ballooning (n=23, 31.5%), cholate stasis (n=19, 22.9%), steatosis (n=7, 21.9%) and bilirubinostasis (n=17, 21.5%).
Similar to the literature, fibrosis was a frequent finding (n=99, 81.8%) on liver biopsy. The staging of fibrosis was determined in 24 patients. Stage one, two and three was respectively seen in 9 (37.5%), 7 (29.2%) and 3 (12.5%) patients. Cirrhosis (fibrosis stage four) was described in 31 patients (34.8%). This is similar to the 30% described in earlier studies.
Fifty one patients received a non-invasive fibroscan or elastometry to determine their stage of fibrosis. The mean level was 9.9kPa (SD=6.9) on fibroscan with a range between 3.2 – 33.8 and 6.3kPa (SD=1.8) on elastometry with a range between 3.8 – 10. Stage one, two, three and four was respectively seen in 29 (56.9%), 6 (11.8%), 7 (13.7%) and 9 (17.6%) patients.

The presence of AIH-1 and AIH-2 was respectively 95.6% and 4.4% in our population. This is significantly more than the 66.7% and 33.3%, described in the literature. The circulating autoantibodies anti-SLA/LP were not determined in our hospitals. Thus, the frequency of AIH-1 could be even higher. Associated autoimmune diseases were more seen in patients with AIH-1 compared to patients with AIH type 2, respectively 27 and 2 patients. However, the frequency is lower in AIH type 1 (25.0% vs. 40.0%). The bimodal age pattern can be seen in AIH-1, especially with a peak at the age of 55-65. No correlation was seen in patients with AIH-2, but this population is too small to make a statement.

All patients included in our cohort, already had the diagnosis of AIH. Therefore, it is expected that the patients have mainly probably or definite AIH. Though, 38.2% of the patients have no evidence for AIH, 35.3% and 26.4% have respectively probably AIH or definite AIH.

According to the literature, a large proportion of patients should receive treatment. Standard therapy wit steroids to obtain remission was induced in 162 patients (94.7%) of our cohort population, whereas 115 patients (68.9%) received predniso(lo)ne and 96 patients (58.5%) the second line therapy with budesonide. A larger percentage of patients in AZ Nikolaas (n=10, 83.3%) received therapy with budesonide, compared with the University Hospital (n=73, 55.3%) and the general hospital (n=13, 65.0%) of Ghent. A possible explanation comprises the fact that the mean date of diagnosis and follow-up was made more recently in Sint-Niklaas, compared with Ghent, whereby the standard guidelines are slightly changing and budesonide is given more the last few years. Budesonide is more and more welcomed to be used as standard therapy because of the few side effects. In the two other hospitals, there's nowadays also a trend, where one is tend to be more likely to start budesonide instead of predniso(lo)ne.

Another disadvantage of this study include the large range of follow-up of our cohort population and consequently a change in the medication schedule through the years.
In a second time, in 113 patients (67.7%), a therapy with azathioprine was started. Alternative second line therapy with MMF, ciclosporine, UDCA and purinethol was used in a smaller part of respectively 21 (12.7%), 15 (9.1%), 58 (35.8%) and 6 (3.7%) patients. 58 patients (35.8%) received therapy with UDCA. However, it should be noticed that UDCA therapy is the basic treatment in PBC, an associated disease that was seen in 9.4% of the AIH patients. A lower percentage of patients in the AZ Nikolaas and AZ Maria Middelares received a second line therapy, compared with the UZ Ghent. The explanation for this is that a general hospital is a secondary center, passing non-controllable patients with the standard therapy to a tertiary center, which in turn can start a secondary treatment. Survival based on therapy was not calculatable with our database, because of several cases with difficult treatment schedules.

In our hospitals, remission is determined by normal laboratory findings. A second liver biopsy to confirm histological remission was almost never performed. Out of 163 patients, 144 patients (88.3%) received biochemical remission. 8 patients (4.9%) died due their chronic autoimmune disease. Eleven patients (6.7%) suffered from liver decompensation, whereas ascites was the most common form (n=8, 72.7%). One patient (9.1%) developed varices, no patient developed hepatic encephalopathy. Data of the two other patients was not available. The mean time to develop decompensation at diagnosis was 2.3 months.
Relapses were seen in 41.9% of the patients (n=65). Although liver biopsies are not done to confirm histological remission, this percentage is between the 50-87% and 20-40%, respectively with abnormal and normal liver biopsy, mentioned in the literature. The mean time to develop relapse at time of diagnosis was 61.6 months.
The developing of HCC was seen in 9 patients (5.1%), LXT was necessary in 21 patients (11.9%). The frequency of both events correspond to what is investigated in earlier studies, respectively 1-9% and 10%. The mean time to develop HCC at diagnosis was 15.3 months.
Out of a total of 127, 5 patients (3.9%) eventually died as a result of their auto-immune hepatitis. Four patients died in the university hospital of Gent, 1 died in the general hospital of Gent. As a result of one death in a small number of patients followed up, the mortality rate in AZ Maria Middelares is higher than in the UZ Gent, consequently with a lower overall survival. No patient died in AZ Nikolaas, which means that the survival cannot be calculated in this hospital. The survival rate for men is lower for men, compared with women. Here also, a small male population with some deaths, leads to a greater mortality rate. The overall 5-year, 6-year, 9-year and 13-year survival rate could be calculated, which was respectively 96.6%, 95.2%, 92.3% and 83.1%. These frequencies correspond with the findings in earlier studies. The 10-year survival in the literature is around 75-90%, the 20-year survival swings around 80%.

As far as we found in the literature, no prognosis system has been developed yet. Interestingly, the evolution of liver enzymes during the first 6 months of treatment seemed to be correlated very well with their long-term outcome. The univariate Cox Regression analysis was significant for the variables 'bilirubin', 'AST', 'ALP', 'GGT'. Bilirubin had the highest hazard ratio (2,222) for the occurrence of an event after 6 months of therapy. This means that the risk for an AIH patient to undergo a poor outcome (HCC, LXT, liverdecompensation or mortality) on long-term, is 22.2% higher when the bilirubin value is one unit higher after 6 months of therapy, compared with another patient. The three other variables (AST, ALP and GGT) showed increased risk for adverse outcome. A new variable was composed with the hazard ratios of these 4 variables to create a new prognostic scoring system. This variable was strongly significant (p=0.003). The AUC for this score was 0.82 (p=0.001, 95%CI=0.69-0.95), meaning that this variable is useful. The newly developed prognostic scoring system has a sensitivity of 72.7% and a specificity of 82.6% for the prediction of an adverse outcome. The censored 5-years and 10-years survival for a score of 2.61 or less, were both 92.9%. In contrast, patients with a prognostic score of at least 2.61, showed a censored 5-years and 10-years survival of respectively 64.3% and 42.9%. A new study is ongoing in two independent Belgian cohorts to validate this new prognostic scoring system.

Data of Belgian AIH patients were scarce. This dissertation was able to compare and confirm data from AIH patients in the Ghent area with the existing literature. In addition, we developed a new prognostic scoring system that is an excellent predictor of long-term event-free survival in AIH after a 6 month during therapy. Further research to confirm this prognostic scoring system is needed, but is already ongoing in two independent centers.

Except the previously mentioned disadvantages, some other disadvantages are being part of this dissertation. Once diagnosis is made, lot of patients are being forwarded to a general practictioner or other hospitals. Because of this, their time to follow-up was lower than 6 months, being eligible for the exclusion criteria. In this way, 85 patients (40. 1%) were excluded for the survival rate and prognosis system. In addition, a big lack of data was a result of a not insignificant loss to follow-up.

There was no balance between the number of patients in the tertiary and secondary centers. Patients in a tertiary center often reports with a difficult-to-treat disease or have already liver decompensation at diagnosis. However, this tendency can not be confirmed, nor rejected due to the small number of patients in the secondary centers.

Finally, the HLA status is not determined in our hospitals. Several things, mentioned in the literature, could not be verified. Because of the important difference between male and female patients concerning epidemiology, type of AIH or survival rate, sex hormones could be a possible important link in the explanation of AIH, although they are not determined by default.

7. REFERENCES

[1] Juza R.M., Pauli E.M. Clinical and Surgical Anatomy of the Liver: A Review for Clinicians. Clin Anat. 2014 Jul;27(5):764-9. doi: 10.1002/ca.22350.

[2] Sear J. Anatomy and physiology of the liver. Bailliere's Clinical Anaesthesiology. 1992 Dec;6(4):697-727. doi: 1016/S0950-3501(05)80304-6.

[3] De Vos M., De Looze D., Van Vlierberghe H., Pattyn P., Van Nieuwenhove Y., Troisi R. et al. Leverlijden: from BLOK PROBLEMEN VAN DIGESTIEF STELSEL, ENDOCRIEN STELSEL EN VOEDING. Universiteit Gent, 2015; 3-114

[4] Young B., Lowe J.S., Stevens A., Health J.W. Wheater's Functional Histology, A text and Colour Atlas. Elsevier, Londen, 2006.

[5] Krishna M. Microscopic Anatomy of the Liver. Clinical Liver Disease. 2013 Mar;2(S5):109-112. doi: 10.1002/cld.147

[6] Parham P. The immune system. Garland science, New York, 2015

[7] De Vos M., De Looze D., Van Vlierberghe H., Pattyn P., Van Nieuwenhove Y., Troisi R. et al. Hepatocellulair falen: uit PROBLEMEN VAN DIGESTIEF STELSEL, ENDOCRIEN STELSEL EN VOEDING. GASTRO-ENTEROLOGIE EN HEELKUNDE IN ONDERLING VERBAND DEEL II. Universiteit Gent, 2015; 7.

[8] Pellicoro A., Ramachandran P., Iredale J.P. and Fallowfield. J.A. Liver fibrosis and repair: immune regulation of wound healing in a solid organ. Nat Rev Immunol. 2014 Mar;14(3):181-94. doi: 10.1038/nri3623.

[9] Lohse A.W., Chazouillères O., Dalekos G., Drenth I., Heneghan M., Hofer H. et al. EASL Clinical Practice Guidelines: Autoimmune hepatitis. J Hepatol. 2015 Oct;63(4):971-1004. doi: 10.1016/j.jhep.2015.06.030.

[10] Lian J.S., Liu W., Hao S.R., Chen D.Y., Wang Y.Y., Yang J.L. et al. A serum metabolomic analysis for diagnosis and biomarker discovery of primary biliary cirrhosis and autoimmune hepatitis. Hepatobiliary Pancreat Dis Int. 2015 Aug;14(4):413-21. Available from: http://www.hbpdint.com/fileup/HTML/2015-14-413.shtml

[11] Heneghan M.A.: Autoimmune hepatitis: Clinical manifestations and diagnosis. Online 2017. Picked up 28 August 2017, from https://www.uptodate.com/contents/autoimmune-hepatitis-clinical-manifestations-and-diagnosis#H16.

[12] Miyake Y., Iwasaki Y., Terada R., Onishi T., Okamoto R., Sakai N. et al. Clinical characteristics of fulminant-type autoimmune hepatitis: an analysis of eleven cases. Aliment Pharmacol Ther. 2006 May 1;23(9):1347-53. doi: 10.1111/j.1365-2036.2006.02894.x

[13] Ferrari R., Pappas G., Agostinelli D., Muratori P., Muratori L., Lenzi M. et al. Type 1 autoimmune hepatitis: patterns of clinical presentation and differential diagnosis of the 'acute' type. QJM. 2004 Jul;97(7):407-12. Available from: https://academic.oup.com/qjmed/article/97/7/407/1605727

[14] Chen J., Eslick G.D., Weltman M. Systematic review with meta-analysis: clinical manifestations and management of autoimmune hepatitis in the elderly. Aliment Pharmacol Ther. 2014 Jan;39(2):117-24. doi: 10.1111/apt.12563.

[15] Tomizawa M., Shinozaki F., Fugo K., Motoyoshi Y., Sugiyama T., Yamamoto S. et al. Anti-mitochondrial M2 antibody-positive autoimmune hepatitis. Exp Ther Med. 2015 Oct;10(4):1419-1422. doi: 10.3892/etm.2015.2694.

[16] Hoeroldt B., McFarlane E., Dube A., Basumani P., Karajeh M, Campbell M.J. et al. Long-term outcomes of patients with autoimmune hepatitis managed at a nontransplant center. Gastroenterology. 2011 Jun;140(7):1980-9. doi: 10.1053/j.gastro.2011.02.065.

[17] Kuntz E., Kuntz H.D. Autoimmune hepatitis. In: HEPATOLOGY TEXTBOOK AND ATLAS. Springer, Berlin, 2008; 655-667.

[18] Béland K., Lapierre P., Alvarez F. Influence of genes, sex, age and environment on the onset of autoimmune hepatitis. World J Gastroenterol. 2009 Mar 7;15(9):1025-34. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2655185/

[19] Malik T.A., Saeed S. Autoimmune Hepatitis: A Review. J Pak Med Assoc. 2010 May;60(5):381-7. Available from: http://jpma.org.pk/full_article_text.php?article_id=2051

[20] Goldberg A.C., Bittencourt P.L., Oliveira L.C., Ramasawmy R., Marin M.L.C., Palacio S.A. et al. Autoimmune Hepatitis in Brazil: An Overview. Scand J Immunol. 2007 Aug-Sep;66(2-3):208-16. doi: 10.1111/j.1365-3083.2007.01967.x

[21] Manns M.P., Lohse A.W., Vergani D. Autoimmune hepatitis--Update 2015. J Hepatol. 2015 Apr;62(1 Suppl):S100-11. doi: 10.1016/j.jhep.2015.03.005.

[22] Liberal R., Vergani D. Effect of ethnicity on the clinical presentation and outcome of autoimmune hepatitis. Expert Rev Gastroenterol Hepatol. 2012 Jun;6(3):267-9. doi: 10.1586/egh.12.17.

[23] Fallatah H.I., Akbar H.O. Autoimmune hepatitis as a unique form of an autoimmune liver disease: immunological aspects and clinical overview. Autoimmune Dis. 2012;2012:312817. doi: 10.1155/2012/312817.

[24] Zachou K., Muratori P., Koukoulis G.K., Granito A., Gatselis N., Fabbri A. et al. Review article: autoimmune hepatitis - current management and challenges. Aliment Pharmacol Ther. 2013 Oct;38(8):887-913. doi: 10.1111/apt.12470.

[25] Lohse A.W. Mieli-Vergani G. Autoimmune hepatitis. J Hepatol. 2011 Jul;55(1):171-82. doi: 10.1016/j.jhep.2010.12.012.

[26] Gatselis N.K., Zachou K., Koukoulis G.K., Dalekos G.N. Autoimmune hepatitis, one disease with many faces: Etiopathogenetic, clinico-laboratory and histological characteristics. World J Gastroenterol. 2015 Jan 7;21(1):60-83. doi: 10.3748/wjg.v21.i1.60.

[27] Abdollahi M.R., Somi M.H., Faraji E. Role of international criteria in the diagnosis of autoimmune hepatitis. World J Gastroenterol. 2013 Jun 21;19(23):3629-33. doi: 10.3748/wjg.v19.i23.3629.

[28] Granito A., Muratori L., Pappas G., Muratori P., Ferri S., Cassani F. et al. Clinical features of type 1 autoimmune hepatitis in elderly Italian patients. Aliment Pharmacol Ther. 2005 May 15;21(10):1273-7. doi: 10.1111/j.1365-2036.2005.02488.x

[29] Zhang Y., Sun W.L., Jin D.L., Jing-Hua D. Clinical features of elderly Chinese patients with autoimmune hepatitis. Turk J Gastroenterol. 2013;24(6):489-94. doi: 10.4318/tjg.2013.0592

[30] 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 Mar 15;21(6):695-9. doi: 10.1111/j.1365-2036.2005.02403.x

[31] Shariat A., Ariyo O., Daftary M.N. Therapeutic management of autoimmune hepatitis. U.S. Pharm. 2016 Jun;41(6):35-9. Available from: https://www.uspharmacist.com/article/therapeutic-management-of-autoimmune-hepatitis.

[32] Miyake Y., Iwasaki Y., Sakaguchi K., Shiratori Y. Clinical features of Japanese male patients with type 1 autoimmune hepatitis. Aliment Pharmacol Ther. 2006 Aug 1;24(3):519-23. doi: 10.1111/j.1365-2036.2006.03013.x

[33] Pereira T.C., Saron M.L.G., de Carvalho W.A., Vilela M.M., Hoehr N.F., Hessel G. Research on zinc blood levels and nutritional status in adolescents with autoimmune hepatitis. Arquivos de Gastroenterologia. 2011 Jan;48(1):62-65. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0004-28032011000100013&lng=en&tlng=en

[34] Francque S., Vongia L., Ramon A., Michielsen P. Epidemiology and treatment of autoimmune hepatitis. Hepat Med. 2012 Mar 16;4:1-10. doi: 10.2147/HMER.S16321.

[35] Czaja A.J. Review article: the management of autoimmune hepatitis beyond consensus guidelines. Aliment Pharmacol Ther. 2013 Aug;38(4):343-64. doi: 10.1111/apt.12381.

[36] Tanaka H., Tujioka H., Ueda H., Hamagami H., Kida Y., Ichinose M. Autoimmune hepatitis triggered by acute hepatitis A. World J Gastroenterol. 2005 Oct 14;11(38):6069-71. doi: 10.3748/wjg.v11.i38.6069.

[37] Singh G., Palaniappan S., Rotimi O., Hamlin P.J. Autoimmune hepatitis triggered by hepatitis A. Gut. 2007 Feb;56(2):304. doi: 10.1136/gut.2006.111864.

[38] Zellos A., Spoulou V., Roma-Giannikou E., Karentzou O., Dalekos G.N., Theodoridou M. Autoimmune hepatitis type-2 and Epstein-Barr virus infection in a toddler: art of facts or an artifact?. Ann Hepatol. 2013 Jan-Feb;12(1):147-51. Available from: http://www.annalsofhepatology.com/revista/numeros/2013/21_131_v12n1_2013_AutoimmuneHepatitis.pdf

[39] Berry P.A., Smith-Laing G. Hepatitis A vaccine associated with autoimmune hepatitis. World J Gastroenterol. 2007 Apr 21;13(15):2238-9. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4146852/

[40] Csepregi A., Treiber G., Röcken C., Malfertheiner P. Acute exacerbation of autoimmune hepatitis induced by Twinrix. World J Gastroenterol. 2005 Jul 14;11(26):4114-6. doi: 10.3748/wjg.v11.i26.4114.

[41] Ksouda K., Affes H., Atheimen R., Ezzeddine M., Zeghal K., Hammami S. Autoimmune hepatitis as an adverse effect of long-term methotrexate therapy. Indian J Pharmacol. 2014 Nov-Dec;46(6):649-50. doi: 10.4103/0253-7613.144940.

[42] van Heyningen C. Drug-induced acute autoimmune hepatitis during combination therapy with atorvastatin and ezetimibe. Ann Clin Biochem. 2005 Sep;42(Pt 5):402-4. Available from: http://journals.sagepub.com/doi/abs/10.1258/0004563054890105?url_ver=Z39.88-2003&rfr_id=ori:rid:crossref.org&rfr_dat=cr_pub%3dpubmed

[43] van Gerven N.M.F., de Boer Y.S., Mulder C.J.J., van Nieuwkerk C.M.J., Bouma G. Auto immune hepatitis. World J Gastroenterol 2016 May 21; 22(19): 4651-4661. doi: 10.3748/wjg.v22.i19.4651.

[44] Teufel A., Wörns M., Weinmann A., Cenntner C., Piendl A., Lohse A.W. et al. Genetic association of autoimmune hepatitis and human leucocyte antigen in German patients. World J Gastroenterol. 2006 Sep 14;12(34):5513-6. doi: 10.3748/wjg.v12.i34.5513.

[45] Baharlou R., Faghihi-Kashani A., Faraji F., Najafi-Samei M., Setareh M., Zamani F. HLA-DRB1 alleles of susceptibility and protection in Iranians with autoimmune hepatitis. Hum Immunol. 2016 Apr;77(4):330-5. doi: 10.1016/j.humimm.2016.01.007.

[46] Kaur N., Minz R.W., Anand S., Saikia B., Aggarwal R., Das A. et al. HLA DRB1 Alleles Discriminate the Manifestation of Autoimmune Hepatitis as Type 1 or Type 2 in North Indian Population. J Clin Exp Hepatol. 2014 Mar;4(1):14-8. doi: 10.1016/j.jceh.2013.12.002.

[47] Zahiruddin A., Farahmand A., Gaglio P., Massoumi H. Clinical characteristics and response to therapy of autoimmune hepatitis in an urban Latino population. Gastroenterol Hepatol Bed Bench. 2016 Summer;9(3):225-30. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4947138/

[48] Miyake Y., Takaki A., Onishi T., Okamato R., Takaguchi K., Ikeda H. et al. Human leukocyte antigen DR status and clinical features in Japanese patients with type 1 autoimmune hepatitis. Hepatol. Res. 2008 Oct;38(1):96-102. doi: 10.1111/j.1872-034X.2007.00204.x.

[49] Teufel A., Galle P.R., Kanzler S. Update on autoimmune hepatitis. World J Gastroenterol. 2009 Mar 7;15(9):1035-41. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2655176/

[50] Ohira H., Abe K., Takahashi A., Watanabe H. Autoimmune hepatitis: recent advances in the pathogenesis and new diagnostic guidelines in Japan. Intern Med. 2015;54(11):1323-8. doi: 10.2169/internalmedicine.54.4125.

[51] Kim T.I., Kagihara J.E., Tsai N.C., Roytman M.M. Autoimmune Hepatitis in Hawai'i. Hawaii J Med Public Health. 2015 Aug;74(8):270-4. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4536739/

[52] Corrigan M., Hirschfield G.M., Oo Y.H., Adams D.H. Autoimmune hepatitis: an approach to disease understanding and management. Br Med Bull. 2015 Jun;114(1):181-91. doi: 10.1093/bmb/ldv021.

[53] Malekzadeh Z., Haghazali S., Sepanlou S.G., Vahedi H., Merat S., Sotoudeh M. et al. Clinical features and long term outcome of 102 treated autoimmune hepatitis patients. Hepat Mon. 2012 Feb;12(2):92-9. doi: 10.5812/hepatmon.808.

[54] Kamijo A., Yoshizawa K., Joshita S., Yoneda S., Umemura T., Ichijo T. et al. Cytokine profiles affecting the pathogenesis of autoimmune hepatitis in Japanese patients. Hepatol Res. 2011 Apr;41(4):350-7. doi: 10.1111/j.1872-034X.2011.00773.x.

[55] Krawitt E.L. Clinical features and management of autoimmune hepatitis. World J Gastroenterol. 2008 Jun 7;14(21):3301-5. doi: 10.3748/wjg.14.3301

[56] Bittencourt P.L., Cançado E.L.R., Couto C.A., Levy C., Porta G., Silva A.E.B. et al. Brazilian society of hepatology recommendations for the diagnosis and management of autoimmune diseases of the liver. Arq Gastroenterol. 2015 Dec;52(Suppl 1):15-46. doi: 10.1590/S0004-28032015000500002.

[57] Lucey M.R., Vierling J.M. Clinical presentation and natural history of autoimmune hepatitis. Clinical Liver Disease. 2014 Jan;3(1):9-11. doi: 10.1002/cld.304.

[58] Peng M., Li Y., Zhang M., Jiang Y., Xu Y., Tian Y. et al. Clinical features in different age groups of patients with autoimmune hepatitis. Experimental and therapeutic medicine. 2014;7(1):145-148. doi: 10.3892/etm.2013.1363.

[59] Hennes E.M., Schramm C., Wiegard C., Lohse A.W. Autoimmune hepatitis. Medicine 2007 Feb;35(2):75-8. doi: 10.1016/j.mpmed.2006.11.018.

[60] Yeoman A.D., Longhi M.S., Heneghan M.A. Review article: the modern management of autoimmune hepatitis. Aliment Pharmacol Ther. 2010 Apr;31(8):771-87. doi: 10.1111/j.1365-2036.2010.04241.x.

[61] Alvarez F., Berg P.A., Bianchi F.B., Bianchi L., Burroughs A.K., Cancado E.L. et al. International Autoimmune Hepatitis Group Report: review of criteria for diagnosis of autoimmune hepatitis. J Hepatol. 1999 Nov;31(5):929-38. Available from: http://www.sciencedirect.com/science/article/pii/S0168827899802979?via%3Dihub

[62] Iwai M., Jo M., Ishii M., Mori T., Harada Y. Comparison of clinical features and liver histology in acute and chronic autoimmune hepatitis. Hepatol Res. 2008 Aug;38(8):784-9. doi: 10.1111/j.1872-034X.2008.00347.x.

[63] Geller S.A. Autoimmune hepatitis: Histopathology. Clin. Liver Dis. 2014 Feb;3(2):19-23. doi: 10.1002/cld.301.

[64] Suzuki A., Brunt E.M., Kleiner D.E., Miquel R., Smyrk T.C., Andrade R.J. et al. The use of liver biopsy evaluation in discrimination of idiopathic AIH vs drug-induced liver injury. Hepatology. 2011 Sep 2;54(3):931-9. doi: 10.1002/hep.24481.

[65] Hofer H., Oesterreicher C., Wrba F., Ferenci P., Penner E. Centrilobular necrosis in autoimmune hepatitis: a histological feature associated with acute clinical presentation. J Clin Pathol. 2006 Mar;59(3):246-9. doi: 10.1136/jcp.2005.029348.

[66] Goodman Z.D. Grading and staging systems for inflammation and fibrosis in chronic liver diseases. J Hepatol. 2007 Oct;47(4):598-607. doi: 10.1016/j.jhep.2007.07.006.

[67] Paula P., Margaret C. Evaluation and staging of liver fibrosis. Online 2015. Picked up 30 October 2017, from https://www.hepatitisc.uw.edu/go/evaluation-staging-monitoring/evaluation-staging/core-concept/all.

[68] Brissos J., Carrusca C., Correia M., Cabral J. Autoimmune hepatitis: trust in transaminases. BMJ Case Rep. 2014:1-3. doi: 10.1136/bcr-2014-203869.

[69] Maggiore G., Socie G., Sciveres M., Roque-Afonso A.M., Nastasio S., Johanet C. et al. Seronegative autoimmune hepatitis in children: spectrum of disorders. Dig Liver Dis. 2016 Jul;48(7):785-91. doi: 10.1016/j.dld.2016.03.015.

[70] Makol A., Watt K.D., Chowdhary V.R. Autoimmune hepatitis: a review of current diagnosis and treatment. Hepat Res Treat. 2011;1-11. doi: 10.1155/2011/390916.

[71] Healey R., Corless L., Gordins P., Holding S. Do anti-smooth muscle antibodies predict development of autoimmune hepatitis in patients with normal liver function? - A retrospective cohort review. Autoimmun Rev. 2016 Jul;15(7):668-72. doi: 10.1016/j.autrev.2016.03.001.

[72] Kriese S., Heneghan M.A., Autoimmune hepatitis. Medicine 2011 Oct;39(10):580-4. doi: 10.1016/j.mpmed.2011.07.005.

[73] Mackay I.R. Autoimmune hepatitis – from the clinic to the diagnostics laboratory. Lab. Med. 2011;42(4):224-33. doi: 10.1309/LM7EUBRRUPLF9V6R.

[74] Fujiwara K., Fukuda Y., Seza K., Saito M., Yasui S., Nakano M. et al. High level of persistent liver injury is one of clinical characteristics in treatment-naïve acute onset autoimmune hepatitis: experience in a community hospital. J Hepatobiliary Pancreat Sci. 2016 Mar;23(3):174-80. doi: 10.1002/jhbp.319.

[75] Yokokawa J., Kanno Y., Abe K., Saito H., Monoe K., Katsushima F. et al. Anti-nucleosome autoantibodies as markers for autoimmune hepatitis and their correlation with disease activity. Hepatol Res. 2014 Apr;44(4):420-8. doi: 10.1111/hepr.12130.

[76] Vergani D., Mieli-Vergani G. Autoimmune hepatitis: Diagnostic criteria and serological testing. Clin. Liver Dis. 2014 Feb;3(2):38-41. doi: 10.1002/cld.321.

[77] Czaja A.J. The role of autoantibodies as diagnostic markers of autoimmune hepatitis. Expert Rev Clin Immunol. 2006 Jan;2(1):33-48. doi: 10.1586/1744666X.2.1.33.

[78] Padelli M., Comacle P., Nousbaum I.B. Youinou P., Renaudineau Y. The diagnosis of autoimmune hepatitis (AIH) is difficult due to its clinical and biological heterogeneity. Immuno-Anal. Biol. Spec. 2011 Oct;26(5-6):255-5. doi: 10.1016/j.immbio.2011.10.002.

[79] Li Y., Peng M., Gong G. Evaluation of the revised versus the simplified scoring system in patients with autoimmune hepatitis. Exp Ther Med. 2014 Jan;7(1):131-136. doi: 10.3892/etm.2013.1366.

[80] Mileti E., Rosenthal P., Peters M.G. Validation and modification of simplified diagnostic criteria for autoimmune hepatitis in children. Clin Gastroenterol Hepatol. 2012 Apr;10(4):417-21 doi: 10.1016/j.cgh.2011.11.030.

[81] Takahashi H., Zeniya M. Acute presentation of autoimmune hepatitis: Does it exist? A published work review. Hepatol Res. 2011 Jun;41(6):498-504. doi: 10.1111/j.1872-034X.2011.00808.x.

[82] Volta U., Rostami K., Tovoli F., Caio G., Masi C., Ruggeri E. et al. Fulminant type 1 autoimmune hepatitis in a recently diagnosed celiac disease patient. Arch Iran Med. 2013 Nov;16(11):683-5. doi: 0131611/AIM.0015.

[83] Kriese S., Heneghan M.A. Autoimmune hepatitis and overlap syndromes. 2015 Nov 11;43(11):639-44. doi: 10.1016/j.mpmed.2015.08.005.

[84] Rojas C.P., Bodicharla R., Campuzano-Zuluaga G., Hernandez L., Rodriguez M.M. Autoimmune hepatitis and primary sclerosing cholangitis in children and adolescents. Fetal Pediatr Pathol. 2014 Aug;33(4):202-9. doi: 10.3109/15513815.2014.898721.

[85] Bellomo-Brandão M.A., Costa-Pinto E.A., De Tommaso A.M., Hessel G. Clinical and biochemical features of autoimmune hepatitis in 36 pediatric patients. Arq Gastroenterol. 2006 Jan-Mar;43(1):45-9. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0004-28032006000100012&lng=en&nrm=iso&tlng=en

[86] Czaja A.J. Challenges in the diagnosis and management of autoimmune hepatitis. Can J Gastroenterol. 2013 Sep;27(9):531-9. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3956012/

[87] Strassburg C.P. Autoimmune hepatitis. Dig Dis. 2013;31(1):155-63. doi: 10.1159/000347211.

[88] Heneghan M.A. Autoimmune hepatitis: Treatment. Online 2017. Picked up 28 September 2017, from https://www.uptodate.com/contents/autoimmune-hepatitis-treatment#H79576825.

[89] Manns M.P., Taubert R. Treatment of autoimmune hepatitis. Clin. Liver Dis. 2014 Jan;3(1):15-7. doi: 10.1002/cld.306.

[90] Csepregi A., Röcken C., Treiber G., Malfertheiner P. Budesonide induces complete remission in autoimmune hepatitis. World J Gastroenterol. 2006 Mar 7;12(9):1362-6. doi: 10.3748/wjg.v12.i9.1362.

[91] Than N.N., Jeffery H.C., Oo Y.H. Autoimmune Hepatitis: Progress from Global Immunosuppression to Personalised Regulatory T Cell Therapy. Can J Gastroenterol Hepatol. 2016;2016:7181685. doi: 10.1155/2016/7181685.

[92] Larsen F.S., Vainer B., Eefsen M., Bjerring P.N., Hansen B.A. Low-dose tacrolimus ameliorates liver inflammation and fibrosis in steroid refractory autoimmune hepatitis. World J Gastroenterol. 2007 Jun 21;13(23): 3232–3236. doi: 10.3748/wjg.v13.i23.3232.

[93] Zachou K., Gatselis N.K., Arvaniti P., Gabeta S., Rigopoulou E.I., Koukoulis G.K. et al. A real-world study focused on the long-term efficacy of mycophenolate mofetil as first-line treatment of autoimmune hepatitis. Aliment Pharmacol Ther. 2016 May;43(10):1035-47. doi: 10.1111/apt.13584.

[94] Lammert C., Loy V.M., Oshima K., Gawrieh S. Management of difficult cases of autoimmune hepatitis. Curr. Gastroenterol. Rep. 2016 Jan;18(2):1-9. doi: 10.1007/s11894-015-0484-7.

[95] Srivastava S., Boyer J.L. Psychological stress is associated with relapse in type 1 autoimmune hepatitis. Liver Int. 2010 Nov;30(10):1439-47. doi: 10.1111/j.1478-3231.2010.02333.x.

[96] Czaja A.J. Review article: permanent drug withdrawal is desirable and achievable for autoimmune hepatitis. Aliment Pharmacol Ther. 2014 May;39(10):1043-58. doi: 10.1111/apt.12701.

[97] Czaja A.J. Difficult treatment decisions in autoimmune hepatitis. World J Gastroenterol. 2010 Feb 28;16(8):934-47. doi: 10.3748/wjg.v16.i8.934.

[98] Access to primary care is associated with better autoimmune hepatitis outcomes in an urban county hospital. BMC Gastroenterol. 2015;15(91):1-7. doi: 10.1186/s12876-015-0318-y.

[99] Kil J.S., Lee J.H., Han A.R., Kang J.Y., Won H.J., Jung H.Y. et al. Long-term treatment outcomes for autoimmune hepatitis in Korea. J. Korean Med. Sci. 2010 Jan;25(1):54-60. doi: 10.3346/jkms.2010.25.1.54.

[100] Miyake Y., Iwasaki Y., Terada R., Nagano T., Kobashi H., Sakaguchi K. et al. A model for estimating cirrhosis in patients with type 1 autoimmune hepatitis. Hepatol Res. 2008 Jul;38(7):658-63. doi: 10.1111/j.1872-034X.2008.00329.x.

[101] Dbouk N., Parekh S. Impact of pre-transplant Anti-nuclear antibody (ANA) and Anti-smooth muscle antibody (SMA) titers on disease recurrence and graft survival following liver transplantation in autoimmune hepatitis (AIH) patients. J Gastroenterol Hepatol. 2013 March ; 28(3): 537–542. doi:10.1111/j.1440-1746.2012.07125.x.

[102] Yamamoto K., Miyake Y., Ohira H., Suzuki Y., Zeniya M., Onji M. et al. Prognosis of autoimmune hepatitis showing acute presentation. Hepatol Res. 2013 Jun;43(6):630-8. doi: 10.1111/j.1872-034X.2012.01109.x.