Autoimmune hepatitis -
Life, death and in-between

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To all patients with Autoimmune hepatitis
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**Abstract**

**Background** Autoimmune hepatitis (AIH) is a chronic autoimmune liver disease that is overrepresented in women (75% of cases). Studies have described a 10-year survival after diagnosis near to that of the general population, but less is known about the long-term survival. The inflammation in AIH causes fibrotic tissue to form in the liver and about 1/3 of AIH patients have cirrhosis at diagnosis. Studies have shown that treatment of the underlying liver disease can reverse fibrosis, and sometimes even cirrhosis, but only a few studies have examined the response to treatment in AIH. AIH affects all ages and some women will have cirrhosis during pregnancy, which is a risk factor for an adverse outcome. Cirrhosis is also a risk factor for hepatocellular carcinoma (HCC), but the true risk for HCC in cirrhotic AIH patients is not known.

**Aim** To study the epidemiology of AIH in Sweden, the causes of death and the risk of cancer for AIH patients, the efficacy of medical treatment on fibrosis and cirrhosis, and outcomes for the mother and child in pregnancy.

**Material and methods** A cohort of 634 AIH patients was established at the Swedish University hospitals. Prevalence and incidence were calculated, and a relative survival analysis was performed in which survival after AIH diagnosis was compared to that of the general population. Causes of deaths were retrieved from the Cause of Death Registry.

The Cancer Registry was used to calculate standard incidence ratios (SIR) and compare cancer risk to that of the general population.

Two hundred fifty-eight liver biopsies from 101 patients were analyzed by a single pathologist and classified according to the Ishak grading and staging system. Liver histology was stratified according to the temporal changes of fibrosis stage, and groups were compared.

A questionnaire was answered by 138 women with AIH about medication, pregnancies, disease behavior during and after pregnancy, and pregnancy outcomes.

**Results** The incidence and prevalence of AIH were 1.2/100 000 and 17.3/100 000, respectively. The relative survival started to decline after 4 years compared to the reference population, and was even more pronounced after 10 years. Men were diagnosed (33.5 years versus 48.0 years, p<0.001) and died (59.7 versus 75.4 years, p=0.002) at a younger age than women. Patients with cirrhosis at diagnosis had an inferior survival (p<0.001). Liver-related death was the most common cause of death (32.7%). Among AIH
patients a higher incidence of cancer was found compared with that of the general Swedish population, SIR of 2.08 (95% Confidence Interval (CI) 1.68-2.55). SIR for non-melanoma skin cancer was 9.87 (95% CI 6.26-14.81) and hepatobiliary cancer was 54.55 (95% CI 19.92-99.99). HCC was found in 4% of the cirrhotic patients and the incidence rate was 0.3% per year. A reduction of fibrosis stage from first to last biopsy was common (62.4% of patients) and patients on a continuous glucocorticoid medication more often had a decreased fibrosis stage than those with withdrawal attempts (p=0.002). One hundred children were born by 58 women with AIH, of which 23 women had 43 children after diagnosis of cirrhosis. Malformations were reported in 3%, and pre-term births (<week 38) in 22% of the pregnancies. Cirrhotic women gave birth without more complications than others, but with a higher frequency of caesarean sections than non-cirrhotic women (p=0.047).

Conclusion Contrary to previous reports, AIH patients' life expectancy was significantly inferior to that of the control population already 4 years after onset of disease, and liver disease was the most common cause of death. AIH patients had an overall enhanced risk for cancer, mainly from an increased risk of non-melanoma skin cancer and HCC. However, the annual risk of HCC was only 0.3% in cirrhotic patients. Histological improvement of liver fibrosis was common in AIH. The proportion of pre-term births was high, but overall pregnancy and childbirth appear to be safe in AIH, even in compensated cirrhosis.

Keywords Autoimmune hepatitis, autoimmune liver disease, hepatocellular carcinoma, surveillance, pregnancy, pregnancy outcome, cirrhosis, fibrosis, epidemiology, cause of death
Original papers

The thesis is based on the following articles:

**Paper I**
Hepatocellular and extrahepatic cancer in patients with autoimmune hepatitis – a long-term follow-up study in 634 Swedish patients.


**Paper II**
Pregnancy and childbirth in women with autoimmune hepatitis is safe, even in compensated cirrhosis.


**Paper III**
Histological improvement of liver fibrosis in well-treated patients with autoimmune hepatitis – a cohort study.


**Paper IV**
Epidemiology and the causes of death in a Swedish cohort of patients with autoimmune hepatitis.


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Abbreviations

6-MP – 6-Mercaptopurine
AIH – Autoimmune hepatitis
ALT – Alanine aminotransferase
ALP – Alkaline phosphatase
AMA – Anti-mitochondrial antibody
ANA – Antinuclear antibody
Anti-Lc1 – Anti-liver cytosol type 1
AST – Aspartate aminotransferase
AZA – Azathioprine
BCLC – Barcelona Clinic Liver Cancer Staging System
CI – Confidence Interval
ECC – Extrahepatic cholangiocarcinoma
GCS – Glucocorticosteroids
GGT – Gamma-glutamyl transferase
HBV – Hepatitis B virus
HCC – Hepatocellular carcinoma
HCV – Hepatitis C virus
HLA – Human leukocyte antigen
IBD – Inflammatory bowel disease
ICC – Intrahepatic cholangiocarcinoma
ICD – International Classification of Disease

IgG – Immunoglobulin G

IgM – Immunoglobulin M

LKM-1 – Liver/Kidney microsome type 1

MELD – Model End Stage Liver Disease

MMF – Mycophenolate mofetil

MULL – Referring to patients in the AIH cohort from the primary catchment areas of Malmö, Umeå, Lund and Linköping

MULLÖ – Same as MULL, but also patients from the primary catchment area of Örebro

NMSC – Non-melanoma skin cancer

PBC – Primary biliary cholangitis

PSC – Primary sclerosing cholangitis

SILK – Swedish Internal Medicine Club

SIR – Standard incidence ratio

SLA/LP – Soluble liver antigen/liver-pancreas antibody

SMA – Smooth muscle antibody

TPMT – Thiopurine methyltransferase
Populärvetenskaplig sammanfattning

Autoimmun hepatit (AIH) är en relativt ovanlig autoimmun leversjukdom som oftast drabbar kvinnor (cirka 75% av patienter med AIH är kvinnor). Orsaken till sjukdomen är inte klarlagd, men det är troligast en blandning av genetiska förutsättningar i kombination med en eller flera utlösende faktorer. Sjukdomen finns beskriven över hela världen och kan drabba alla åldersgrupper, från barn till äldre. Upp till en tredjedel av patienterna har tecken till skrumplever (cirrhos) vid diagnos. Behandlingen är medicinsk med kortison, oftast med tillägg av annan immunmodulerande behandling.

Avhandlingen baseras på en kohort med 634 AIH-patienter från de svenska universitetssjukhusen och består av fyra delarbeten med olika fokus:

Första delarbetet studerade risken för cancer hos AIH-patienter, med speciellt fokus på levercancer. Här fann vi att den generella risken för cancer efter AIH-diagnos var dubblerad jämfört med en ålders- och könsmatchad befolkning, men att risken för cancer i lever och gallvägar var 55-faldigt ökad. Levercancer drabbade bara de som hade cirrhos och 4% av alla patienter i kohorten med cirrhos hade fått levercancer i slutet av uppföljningstiden. Den årliga takten för insjuknande i levercancer bland patienter med cirrhos var 0,3% per år. Vi fann också att det fanns en ökad risk för hudtumörer (10-faldigt ökad), troligast kopplad till användandet av immunmodulerande behandling.


Aims

The general aim of the thesis was to give a broad clinical and epidemiological picture of the AIH population in Sweden.

More specifically the aims of the different studies were:

**Paper I:** To study the cancer risk among AIH patients, with a special focus on the risk of developing hepatocellular carcinoma (HCC).

**Paper II:** To assess the outcome of women with AIH and their children during pregnancy, childbirth and post-partum. Further, to study if the presence of maternal cirrhosis affects the outcome.

**Paper III:** To study the course of inflammation and fibrosis in AIH patients over time, and to evaluate the efficacy of medical treatment for protection of progression of fibrosis.

**Paper IV:** To study the prevalence and incidence of AIH in the Swedish population, as well as the causes of death and long-term prognosis in the AIH cohort.
Introduction

Physiology and anatomy of the liver
The liver (Greek; *hepar*) is the body's largest solid organ. It weighs about 1500 grams and has multiple and complex functions. For example, the liver produces and secretes bile and is involved in the metabolism and excretion of foreign and endogenous substances. It is a storage site for fat-soluble vitamins (especially vitamin A and D) and iron. It is involved in gluconeogenesis, and it stores and releases glycogen. The liver produces plasma proteins and coagulation factors (for example albumin, fibrinogen, Factor VII and prothrombin) and metabolizes hormones. It is also involved in the turnover of red blood cells, and is a large reservoir for blood.

The liver anatomy can be described by using either an anatomical or functional description. Anatomically it is divided into a right and left lobe, separated by ligamentum falciforme. The right lobe is larger than the left lobe. However, the functional division going through an imaginary line from the fundus of the gallbladder to vena cava inferior separates the liver into two equally large parts with separate arterial and portal blood flow as well as drainage of bile.

Autoimmune hepatitis
Autoimmune hepatitis (AIH) was first described by the Swedish physician Waldenström in the 1950s, as a chronic liver disease typically affecting women, presenting with jaundice, elevated gamma globulins and amenorrhea. The disease eventually led to liver cirrhosis (1). Almost 70 years later AIH still remains enigmatic due to its heterogeneity in presentation (from asymptomatic to fulminant), age at onset (from childhood to elderly), and the at large unknown causes of etiology. This introduction will give a brief overview of the field of AIH.

Pathogenesis and etiology of AIH
AIH is an autoimmune disease initiated by one or more triggering events that lead to damage of the hepatocytes (and possibly other liver cells); this damage in turn leads to the presentation of liver auto antigens on the hepatocyte (most often via Major Histocompatibility Complex class I molecules) or cross-presentation by antigen-presenting cells. Cytokines are released and attract and activate Th0 cells to become predominantly Th1 cells and Th17 cells. Furthermore, B cells and Natural Killer cells are attracted, and all participate in the inflammatory process. In addition, regulatory T cells, which are supposed to control the response to auto antigens recognition, are numerically and functionally impaired in AIH (2).
Other autoimmune diseases (thyroiditis, diabetes mellitus, Sjögrens Syndrome, celiac disease, rheumatoid arthritis, inflammatory bowel disease etc.) are common in both patient and first-degree relatives (3).

Several genes have been linked to an increased risk for developing AIH. Of special interest in North American and European patients are the human leukocyte antigen (HLA) alleles HLA-DR3 (DRB1*03:01) and HLA-DR4 (DRB1*04:01) (2, 3).

Differences in ethnical backgrounds also exert an impact on the presentation and course of disease. For example, African-American patients, primarily male patients, were found to have a more aggressive disease at presentation, and poorer outcome and response to conventional medical therapy (4). Cirrhosis at diagnosis appears to be more common in patients with Hispanic origin. (5).

**Laboratory and clinical features of AIH**

**Laboratory findings**
The presence of elevated immunoglobulin G (IgG) and autoantibodies, in combination with elevated transaminases (alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are the laboratory hallmarks of AIH (6). The first autoantibody identified that was associated with the disease was the antinuclear antibody (ANA). Later, the smooth muscle antibody (SMA) was described, and thereafter multiple antibodies such as the Soluble liver antigen/liver-pancreas antibody (SLA/LP), the anti-liver cytosol type 1 (anti-LC1) and the Liver/Kidney microsome type 1 (LKM-1) were found (7, 8). Several additional antibodies are also described in AIH but will not be further discussed in this thesis.

**Subgrouping**
AIH is conventionally grouped into AIH type I and AIH type II where type I is the most common (75%) (9-11). The grouping is based on the pattern of antibodies. AIH type I usually presents with one or more of ANA, SMA and/or SLA/LP, whereas LKM-1 and/or anti-LC1 characterize type II. AIH type II often debuts in childhood or adolescence, and is usually regarded as more aggressive and difficult to treat (7). A third variant of AIH, AIH type III, has been suggested, where the patient displays SLA/LP antibodies but lack other conventional antibodies (12). SLA/LP is highly specific for AIH and is proposed to be a marker for a more severe course of disease and a dismal outcome (13). AIH type III has been debated and is currently not a generally accepted subclass (14, 15).
It is important to remember that not all patients exhibit antibodies, and that autoantibodies can vary with time. In some patients with acute onset, the antibodies will not become positive until later (16). Furthermore, most antibodies are not exclusive for AIH and may be encountered in the general population as well.

Clinical presentation
The clinical presentation in AIH is variable and ranges from asymptomatic, insidious, to an acute and fulminant hepatitis. Patients may present with signs of decompensated liver disease such as ascites (7-15%) and/or variceal bleeding (4%) (9, 17). The most common presentation is with vague symptoms that include fluctuating jaundice, abdominal pain in the right upper quadrant, fatigue, arthralgia and pruritus (9, 17, 18). Physical signs of the insidious onset may have been present at some time at the discovery of AIH.

About 25% of AIH patients will present with symptoms of an acute hepatitis, resembling the viral hepatitis (19). In this group of patients, there might be two different patient entities – one with patients that present acute but previously have had a subclinical AIH, and the other the genuinely acute AIH. In contrast to the first, the liver biopsy in the latter will not display chronic histological findings (15, 20).

Precipitating events and drug-induced liver injury
Occasionally onset of disease can come after viral infections (for example, Epstein-Barr or hepatitis A) (21), or after the administration of certain drugs (especially nitrofurantoin, minocycline) (22). According to Björnsson et al (22) drug-induced AIH has a more favorable prognosis, and discontinuation of immunomodulating medication without relapse is possible.

Sometimes it is difficult to distinguish drug-induced liver injury from a debut of AIH, since liver histological samples can resemble one another (23). Drug-induced liver injury can also be treated with glucocorticosteroids (GCS), and sometimes it is the feature of relapse of disease after tapering of GCS that will separate one diagnosis from the other (15).

Epidemiology
About 75% of all patients with AIH are women (10, 24). Some studies suggest an even higher proportion of women (i.e. 87-95%) (25, 26). AIH onset can occur at any age, but often displays a bimodal age distribution with the first peak around puberty and the second around the fifth to the seventh decade of life (9, 24).
AIH has been described worldwide (17, 26-29). However, the prevalence and incidence in different populations vary. It appears that ethnicity has an impact on the risk of developing AIH, the clinical presentation and the outcome; this implies that genetic variations play a role in the pathogenesis of AIH and perhaps also in the response to treatment (5, 29, 30).

The highest reported prevalence of AIH so far is from the native Alaskan population at 42.9 per 100 000 inhabitants (30). The prevalence in the Western European populations ranges from 11 to 24 per 100 000 inhabitants, and the incidence from 0.8 to 3 per 100 000 inhabitants and year (6). For Sweden and Norway the reported prevalence has been 10.7 and 16.9 per 100 000 inhabitants and the incidence at 0.85 and 1.9 per 100 000 inhabitants and year, respectively (9, 31). In a population-based study in Denmark from 2014, (24), the reported prevalence was 23.9 per 100 000 inhabitants and an almost doubled incidence rate during the study period (1994 to 2012) from 1.37 to 2.33 per 100 000 inhabitants was noted.

**Treatment**

The aim of the treatment of AIH is to induce and sustain complete remission of inflammation, and hence to hinder the progression of the liver disease.

**Standard therapy**

Our modern treatment regimens are based on studies preferably from the 1970s (32-34). These studies revealed that untreated moderate to severe AIH had a poor prognosis. Therefore medical treatment is advocated in most patients today. A group of patients where one can consider abstaining from treatment is in asymptomatic elderly patients with only mild inflammatory activity in liver biopsies (15). The decision not to treat is still controversial, because of the difficulties to predict the course of disease (35). Patients with burned-out cirrhosis and absence of inflammatory activity is another group where the benefits of medical therapy are outweighed by the side effects of treatment (15).

The standard treatment regime is based upon an induction treatment with GCS (0.5-1 mg/kg), with an addition of Azathioprine (AZA) (1-2 mg/kg) after a couple of weeks. GCS is then tapered under several months as the more slowly in-setting effect of AZA occurs (15, 36). The combination therapy strategy reduces the side effects of GCS, and the delay in introducing AZA helps distinguish between hepatotoxic side effects of AZA and treatment failure.

AZA as monotherapy for induction is not recommended since it was shown that the mortality rate was increased (33). The delayed introduction also
gives the possibility to test the patient’s ability to metabolize AZA by genotyping the gene of the enzyme thiopurine methyltransferase (TPMT), which metabolizes the drug into its inactive metabolites. There are 0.3% of patients that are homozygous for low activity of TPMT (TPMT\text{low}/TPMT\text{low}), which may lead to serious complications with myelotoxicity.

*Alternative medical therapies*

Side effects of GCS therapy are feared and include weight gain, osteopenia, hypertension, acne, Cushingoid features (buffalo hump, striae, etc.), diabetes, and emotional instability including psychosis (36). Budesonide has therefore emerged as an interesting drug in AIH treatment. Budesonide is a synthetic GCS with a high affinity for the GCS receptor. The treatment is appealing since the first passage metabolism in the liver is high (90%) and the risk for systemic effects reduced. Studies have shown efficacy even as an induction therapy, followed by the introduction of AZA (37, 38). Budesonide should not be used in cirrhotic patients or those with peri-hepatic shunting due to the increased risk for systemic GCS effects in these conditions.

Second line treatment in the case of non-responsiveness to the standard regime of GCS and thiopurines (i.e. AZA or 6-Mercaptopurine (6-MP)) often includes Mycophenolate mofetil (MMF) or calcineurin inhibitors as Cyclosporine or Tacrolimus (36). Tacrolimus was also used as an induction treatment in a study of Marlaka et al (39) on pediatric patients that investigated if Tacrolimus could be a treatment option since the long-term effects of standard treatment regimens are dreaded. Seventy percent of the patients in the study had a complete remission, but additional low doses of GCS and AZA were often required. Only three patients came to a complete remission on Tacrolimus alone.

More infrequently other immunomodulators have been used (cyclophosphamide, methotrexate, rituximab, infliximab, sirolimus), but there are no general recommendations on treatment with these drugs (15).

*Treatment length and withdrawal*

Treatment of AIH is recommended to continue for at least 18 to 24 months after a complete normalization of transaminases and IgG levels. The treatment is always individualized, and the GCS are kept in a maintenance dose during several months. The goal of treatment is a GCS free regime but this is not always possible to achieve (14, 15). It is well known that the histological resolution of inflammation lags behind the biochemical, and findings of histological activity in liver biopsies as well as findings of biochemical activity, predict a relapse after discontinuation of medication (40). A follow-up biopsy before considering discontinuation of medication is
recommended, and if there is histological activity drug withdrawal is not recommended (15). Even if biochemical remission is achieved, an inflammatory activity can sometimes still be found (41).

The frequency of relapse, no matter if histological and biochemical resolution have been achieved, is common (47-89%) (40, 42). If a treatment withdrawal is attempted close follow-up is recommended, and since a relapse can occur after several years, patients should be followed life-long (15).

**Autoimmune liver diseases and AIH variant syndromes**

**Primary biliary cholangitis**
Primary biliary cholangitis (PBC) is a chronic autoimmune cholestatic liver disease that predominantly affect women (10:1) in their middle ages (>45 years) (43). Formerly the name was Primary biliary cirrhosis, a misleading name since not all patients have or will develop cirrhosis (44). The incidence has been described to be 0.33 to 5.8 per 100 000 inhabitants and year in large population-based studies, and the prevalence 1.91 to 40.2 per 100 000 inhabitants (45).

The pathogenesis is believed to be multifactorial with interactions between genetics and environmental triggers (43, 46). The disease affects the small intrahepatic bile ducts leading to progressive fibrosis and may lead to liver cirrhosis if left untreated. Anti-mitochondrial antibodies (AMA) are found in about 95% of all PBC patients (47). Other common serological findings are elevated Immunoglobulin M (IgM) and gamma-glutamyl transferase (GGT) (48).

**Primary sclerosing cholangitis**
Primary sclerosing cholangitis (PSC) is a chronic cholestatic liver disease, but unlike PBC and AIH, the disease predominantly affects men (2:1) (48). Prevalence and incidence in Sweden was described by Lindkvist et al (49), and a prevalence of 16.2 per 100 000 inhabitants and a mean annual incidence of 1.22 per 100 000 and year was found. The pathogenesis is unknown and multifactorial but genetic factors contribute to the risk of getting the disease (50, 51). There are no specific autoantibodies found in PSC (48, 51). About 80% of patients with PSC also have an inflammatory bowel disease (IBD), preferably ulcerative colitis (47, 49).

Diagnosis is made if the patient persistently has had elevated Alkaline phosphatase (ALP) and GGT, as well as a cholangiogram showing multifocal
strictures throughout the intra- and extrahepatic biliary tree. Other cholestatic conditions must be excluded. (48).

PSC is progressive and may lead to liver cirrhosis. The main cause of death is malignancy (40-50% of all deaths) and there is especially an enhanced risk for cholangiocarcinoma and colorectal cancer (52). Surveillance with annual radiological examinations of the biliary tree and colonoscopies in case of IBD is recommended.

Variant syndromes
Sometimes patients with AIH show features of PBC or PSC, also known as overlap syndromes or variant syndromes. These features can co-exist from time of diagnosis or develop during the course of the disease. Sometimes the disease will shift from mostly having features of one autoimmune liver disease to another. All autoimmune liver diseases have criteria to settle the diagnosis, but the diseases also share histological, immunological and serological markers. As of today, there are no validated diagnostic criteria for these variant syndromes, and the treatment of overlap disease is mostly empiric and extrapolated from data of the primary autoimmune liver diseases (47, 53, 54). Chazouillères et al (55) proposed criteria for diagnosis of AIH-PBC overlap syndrome (the Paris criteria) that is currently the most widely used, but no consensus in international guidelines exist (14, 15). No similar criteria exist for AIH-PSC. Hence the percentage of patients with suspected overlap syndrome of both AIH-PBC and AIH-PSC varies among clinical material (5-20%) (47, 53).

A special form of overlap called autoimmune sclerosing cholangitis has been described, particularly in children. These patients have autoantibodies resembling AIH, but cholangiographic findings suggestive of sclerosing cholangitis (47).

Prognosis and long-term survival
The natural clinical course of AIH was studied in the 1970s and showed the greatly improved survival by immunomodulator treatment (32-34). Even though the response to treatment is generally good, with the majority of patients responding to treatment, some patients will progress and need a liver transplantation, either acute or later during the disease. Since the nature of the disease is chronic and reoccurring, patients need to be followed regularly.
The AIH patient’s clinical presentation and variable outcomes were described in an exemplary manner by Kriese and Heneghan (56) (Figure 1).

**Figure 1** Modes of presentation, course of disease and clinical outcomes in AIH. Adopted and modified from Kriese and Heneghan (56).

The overall and transplant free survival have been studied, but the studies are often characterized by limited time of follow-up and/or small numbers of included patients. The short-term survival (≤10 years) has often been good, and close to that of the general population (57, 58). Sometimes studies have focused on overall survival (10 year overall survival, 90%) (25, 59). Others have included transplant free survival (10 year transplant free survival, 73.5-83%) (60, 61). Further studies have focused on the mortality compared to an age- and gender matched reference population, and found an increased risk for death and especially due to a liver-related cause (24, 62). The long-term prognosis after diagnosis is not as widely studied, but seems to be worse than expected (transplant free survival 48% +/-5% at 20 years) (63).

**Liver pathology in AIH**

A liver biopsy is usually considered to be a prerequisite for diagnosis, and is often performed at the time of diagnosis (64, 65). The histological picture together with clinical and laboratory findings will lead to a diagnosis. There are no specific histopathological findings for AIH but there are several features, of which some are more typical of AIH than others (Figure 2). The following are common findings in AIH: *Interface hepatitis* is a mononuclear cell infiltrate in the portal tracts (of predominantly lymphocytes and often plasma cells), but also extends outside the portal tracts and into the liver lobule (6, 66-68). This is often described as a histological hallmark of AIH,
but can also be found in other liver diseases (viral hepatitis) or liver injury (drugs, toxins) (6). *Rosetting or rosette formation* is another typical finding, where hepatocytes are arranged around a small lumen (68). Sometimes *emperipolesis* is found, which is the migration and penetration of a smaller cell (plasma cell or lymphocyte) into and through a larger cell (hepatocyte) (66). About 30% of all AIH patients have cirrhosis already at diagnosis (69).

**Figure 2** Liver biopsy, with interface hepatitis (blue arrows) and rosette formation (red arrows). Originally published in Nationella riktlinjer för handläggande av Autoimmun hepatit 2014 (70).

There are several classification systems that are used to describe the changes that come with liver disease such as Metavir, Batts and Ludwig and Ishak score. The scores are all constructed to give an indication of the severity of the disease and how far the disease has progressed (71).

Even though a liver biopsy is one of the best ways of evaluating inflammation and degree of fibrosis in patients with liver disease, the method has its limitations. *Sampling error* is a term to describe that the biopsy will only represent a small part of the liver and that the sample may not be representative for the whole liver (72). A liver biopsy is an invasive procedure with risk for morbidity and mortality, even though the risk of
death is low with modern ultrasound guided procedures (0.01%) (73). Therefore, there has been a great interest in non-invasive procedures and finding biochemical markers of fibrosis. Transient elastography uses ultrasound to assess liver fibrosis and has especially been studied in viral hepatitis, but also in other liver diseases (74). It has not yet been fully validated in AIH, and is limited by ongoing inflammatory activity (75). Biochemical markers (for example AST to platelet ratio index, AST to ALT ratio, FibroQ, etc.) can give an indication of liver fibrosis but fail to differentiate between different stages of fibrosis (76).

**Liver dysfunction and cirrhosis**

Liver damage of any kind will lead to the destruction of liver cells. A chronic inflammation that is not properly suppressed will lead to the building of fibrotic tissue that replaces the normal liver tissue. The liver will regenerate with initially micro-nodular and subsequently macro-nodular appearance, as the liver tries to regenerate but is restricted by the fibrotic tissue. Eventually the liver becomes cirrhotic.

The fibrotic tissue will start to contract the intrahepatic blood vessel, restricting the blood flow through the liver and raising the pressure in the vena porta, and when the pressure exceeds 6 mmHg it is defined as portal hypertension. The higher the pressure gets, the greater the risk becomes for complications. The hypertension will force the blood to take alternative routes than through the portal venous system, for example, via the spleen and esophagus leading to enlargement of the spleen with secondary thrombocytopenia and the risk for gastroesophageal varices. Shunting of the blood past the liver will also increase the amount of ammonium in the blood that can pass the blood-brain barrier and contribute to hepatic encephalopathy. An intricate chain of events will ultimately lead to increased levels of anti-diuretic hormone, which results in retention of potassium and water and at last to the development of ascites.

Liver cirrhosis is first compensated; i.e. that the liver can compensate for the progressively stiffer tissue and functional impairment. Eventually, it can become decompensated, which means the development of at least one of following findings: ascites, hepatic encephalopathy, jaundice or variceal bleeding. The patient with chronic liver cirrhosis can also present clinical stigmata like, e.g. palmar erythema, flapping tremor (asterixis), gynecomastia, hypogonadism and spider naevi.

Decompensated liver cirrhosis is a catabolic state, and the cirrhotic patient will lose weight and muscle mass. The synthesizing of coagulation factors
and albumin will decrease, the liver's metabolic functions will deteriorate (carbohydrate, fat and protein metabolism), and vitamin D deficiency will occur, etc.

The severity of the cirrhosis can be estimated by different risk scores where the most common are the Child-Pugh score (77) and Model for End-Stage Liver Disease (MELD) (78). The former is often used to predict the long-term survival (years) in cirrhotic patients, and the latter for short-term prognosis (months).

**Hepatocellular carcinoma**

Hepatocellular carcinoma (HCC) is globally a major contributor to both morbidity and mortality, and is closely linked to the occurrence of viral hepatitis. Most cases of HCC (>80%) occur in Eastern Asia and Sub-Saharan Africa, reflecting the prevalence of the hepatitis viruses. Hepatitis B (HBV) and C (HCV) both promote cirrhosis, which is the greatest risk factor for the development of HCC (79). About 80-90% of all patients with HCC has cirrhosis at the time of diagnosis (79). The risk for developing HCC is not the same for all cirrhotic patients, as it will vary according with the etiology of the cirrhosis. The highest risk has been reported in cirrhotic hepatitis C patients with a yearly incidence between 3.7-8% per year (80), and the risk remains increased even after a sustained virological response is achieved (81, 82).

The risk in AIH is not as well studied as in viral hepatitis, but there is an enhanced risk for HCC (83), and it is closely related to the presence of cirrhosis (83-85). A systematic review and meta-analysis by Tansel et al (86) suggested an incidence of 1.0% per year among patients with AIH. Several risk factors have been suggested in AIH, i.e. cirrhosis at diagnosis and long-standing cirrhosis, signs of portal hypertension, persistent inflammation, male sex, older age, and use of alcohol (86, 87).

Surveillance is the use of repeated screening, at fixed intervals, to discover neoplasms at an early stage. Early detection is essential for finding HCC at a curable stage. Surveillance is generally considered cost-effective when the yearly risk for developing HCC exceeds 1.5 % (88, 89). As described, the risk in AIH has not been found to be as high, but still surveillance has been recommended, based on a lack of reliable studies (14).

Only patients eligible for a curative intended treatment of HCC are recommended to be included in surveillance programs i.e. patients with Child-Pugh A-B (or Child-Pugh C if the patient can be a subject for liver
transplantation). In some non-cirrhotic patients surveillance is also recommended (e.g. active HBV or HBV carrier with a family history of HCC, Asian background, etc.). The recommended modality and interval for surveillance is ultrasound examination every 6 months (90).

HCC diagnosis can be based upon histopathological examination of biopsy specimen, or preferably radiological measures. Both American and European guidelines on management of HCC (88, 90, 91) support the Barcelona Clinic Liver Cancer Staging System (BCLC), that systematizes the patient into categories according to status of the liver, performance status and tumor characteristics. The staging system separates patients that may benefit from a curative therapy from those who should be subjected to a palliative treatment (92, 93). Today curative therapies are based on liver resection in very early stages (stage 0) of HCC, and in the early stage (stage A) on liver transplantation or radiofrequency ablation. Palliative treatment is practiced with transarterial chemoembolization in intermediate stages (stage B) and Sorafenib (a tyrosine kinase inhibitor) in the advanced stages (stage C). In the terminal stage (stage D) the treatment is symptomatic (90, 91).

Liver transplantations in AIH

Some patients will present with an acute and severe AIH that does not respond to medical therapy. Others will not have a sufficient response or are intolerant to medical treatment, and they will therefore slowly progress to end-stage liver disease. In these patients liver transplantation can be an effective and lifesaving procedure. Liver transplantation in AIH is therefore recommended in patients with acute, medically unresponsive disease, in patients with decompensated liver cirrhosis (MELD >15), and as already described in selected patients with HCC (14, 94, 95).

AIH accounts for about 4-5% of all patients undergoing liver transplantations in Europe and USA (14, 94, 96). In a study of the European Liver Transplant Registry the 5-year survival for AIH patients was found to be 73 % (95% Confidence Interval (CI), 67-77%) after liver transplantation, with an inferior survival in older patients than in younger patients (97).

AIH can recur after transplantation in the donor allograft. The reported frequency of recurrence varies between 15-40% in different studies depending on diagnostic criteria, protocol for immunosuppression and biopsies and length of follow-up (98, 99). Patients generally respond to intensified immunosuppression, and the prognosis of patients with recurring AIH is comparable to those without (14).
De novo AIH can sometimes develop in patients transplanted for other reasons than AIH, and has particularly been observed during treatment for HCV with interferon. It can occur at any time after transplantation, and a prompt diagnosis and treatment are necessary to avoid graft dysfunction and graft loss (100).

**Pregnancy in liver disease, including AIH**

**Pregnancy in AIH**

Pregnancy in AIH was described as early as in the 1960s and 1970s with high risks of adverse outcomes that included high rates of fetal losses, caesarean sections, low birth weights, and high risk for maternal complications that included maternal death (101, 102). However, these studies were published before the discovery of the hepatitis C virus and the establishment of the International AIH Group’s AIH score for establishing the diagnosis (64).

Studies published in the twenty-first century have shown more favorable results (103-106). However, an increased risk for pre-term birth (105-107) and fetal loss has been found (103, 108). Schramm et al suggested that the risk for an adverse outcome could be connected to the presence of antibodies to SLA/LP and Ro/SSA, since they were found in 7 out of 11 pregnancies with unfavorable outcome (108). There have also been reports of adverse events for the mothers (103, 106, 108), that include decompensation of liver disease and need for transplantation, and sporadic descriptions of maternal death (106, 108). The usual scenario is a disease that goes into a more immune tolerant state during the pregnancy and to flare up post-partum (30% of the pregnancies), but with an outcome that is generally good for both mother and child (103, 104). International guidelines emphasize that the risk for adverse events increases with the severity of the liver disease (14, 15).

**Medical therapy during pregnancy in AIH**

The main therapeutic regimen of AIH is founded upon GCS with or without the addition of thiopurines. The use of oral GCS is generally believed safe in pregnancy, even though some studies have suggested an increased risk for orofacial cleft lip and palate (109, 110). Recent studies have not been able to verify this connection (111, 112). The use of AZA and its pro-drug 6-MP in pregnancy has been debated since animal studies have shown an increased risk for birth defects (113). The American Food and Drug Administration categorize AZA as a class D medication. This classification states that there is a fetal risk, but that the risk may be outweighed by the benefits. The first animal studies on AZA used higher doses than used in the human setting, and since then there has been considerable clinical experience from solid organ transplanted patients (114) and other patient categories (e.g. IBD)
Today the European guidelines support the continuation of thiopurines during pregnancy, whereas the American guidelines do not (14, 15). Mycophenolate mofetil (MMF) should be avoided due to the high risk for teratogenicity (116).

**Pregnancy and cirrhosis**

Historically, pregnancy in women with liver cirrhosis was uncommon due to various changes that lead to endocrine dysfunction, anovulation, amenorrhea and hence to infertility (117-119). Additionally, cirrhosis in general more often develop after a woman’s reproductive years. AIH is different in this aspect, as about 30% of patients have cirrhosis already at diagnosis, and women of all ages are affected (11, 24). With the medical progressions over the last decades more women are entering adulthood with compensated cirrhosis and a reproductive potential.

The pregnant cirrhotic woman puts herself at risk for hepatic decompensation (120). As the blood volume expands in the pregnant woman so does the risk for variceal bleeding due to increased portal hypertension. Variceal bleeding is the leading cause of maternal death in liver cirrhosis (118, 121), and therefore endoscopic screening for varix is recommended during the second trimester (118). Westbrook et al suggested pre-conceptional use of the MELD-score with a cut-off of <6 to ensure an uneventful pregnancy in women with cirrhosis (121).

**Liver transplantation and pregnancy**

Liver transplantation often restores the fertility in cirrhotic women, and pregnancy is therefore not uncommon among liver transplanted patients (119). However, it is recommended to avoid pregnancy the first year after transplantation to minimize the risk of graft rejection (118).

**Materials and methods**

**Collection of data**

The Swedish Internal Medicine Club (SILK) is a Swedish collaboration of hepatologists and pathologists at the Swedish University hospitals, with a special interest in the study of liver diseases. In 1999 SILK decided to collect all patients with AIH from the Swedish University hospitals to create a well-characterized cohort of AIH patients for epidemiological studies. Sweden’s unique possibilities for register studies are based on the Swedish citizens’ personal identification numbers, which enabled the establishment of a robust epidemiological database on this relatively rare disease.
A detailed protocol was created and after several revisions was finalized in 2001. After ethical approval a retrospective collection of patients took place between 2001 and 2003. Patients were retrieved from the medical records as far back in time as possible. It was estimated that about 1/2 to 2/3 of the Swedish AIH population had been included.

The University hospitals of Malmö, Umeå, Lund and Linköping (MULL) had made a full retrospective search from 1990 and therefore it was decided to base epidemiological data (survival analyses, prevalence and incidence) on the catchment areas of these centers in the first epidemiological analyses carried out on the cohort.

The first inclusion formed a cohort of 473 patients (the original AIH cohort), with a sub cohort of 163 patients (MULL). In 2010 the cohort was updated to include new patients and data on cirrhosis development, liver transplantations and deaths. The protocol used in this inclusion was simplified, compared to the original protocol. One hundred and sixty-one patients were included and the cohort now consisted of 634 patients (the updated AIH cohort).

**Database**

All data from the first inclusion of patients were entered into a database, and transferred to Statistical Package for the Social Sciences (version 21, SPSS Inc, USA), which was used for the statistical analyses throughout the thesis. In the first inclusion of patients an internal validation was carried out through re-entering 10% of the case report forms twice, and the errors were found to be less than 2%. In the second inclusion data were transferred directly into SPSS and a similar procedure of cross-checking the data for errors was performed. All errors were corrected before analyses.

**AIH score**

To ensure that the included patients were exclusively patients with AIH, the diagnosis was validated with an AIH score. The International Autoimmune Hepatitis Group (IAHG) created an AIH score in the early 1990 to form homogenous groups of patients for research purposes (122). The original score was revised in 1999 (64) for simplification and improvement of specificity.

Since our cohort was retrospectively collected, and sometimes included patients with diagnosis from several decades ago, the data at times could be incomplete and therefore a modified revised AIH score was created (Table 1). In 2008 Hennes et al (65) published a simplified version of the original
revised AIH score, but since the original cohort was validated with the former score we used the same score for the new inclusion.

The cut off levels for definite AIH was >17 points and probable AIH was 12-17 points (post-treatment calculations). The AIH score was calculated from the time of diagnosis, or when data were missing from 6 months before or after diagnosis.

Based on the calculations we found that 356 patients in the updated AIH cohort had definite AIH, 251 probable AIH and 27 patients scored less than 12 points. Patients with scores <12 were re-evaluated by searching their medical records for establishing the diagnosis. Low scores were often a result of missing data, due to the retrospective nature of the study.

**Patient demographics of the updated AIH cohort**

The updated cohort of 634 AIH patients consists of 464 women (73.2%) and 170 men (26.8%). Five hundred and twelve patients (80.2%) were from the primary catchment areas. The median follow-up from diagnosis until death, liver transplantation or end of follow-up was 11.3 years (range 0–51.5 years) and total follow-up time was 8067 years. In Paper I a total and median follow-up time of 8084 years and 11.4 years were presented. These numbers were corrected in Paper IV since we discovered that the Swedish National Board of Health and Welfare had missed to report the deaths of two patients, as well as that we discovered that one patient classified as liver transplanted was not transplanted. Hence, the frequency of liver transplanted patients in the cohort is 33 and not 34 (6 men and 27 women). Further, we have corrected the status of cirrhosis at diagnosis for one patient.
### Table 1: Original revised AIH score adapted from Alvarez et al (64) and modified revised score.

<table>
<thead>
<tr>
<th>Features/Parameters</th>
<th>Original revised score</th>
<th>Modified revised score</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>Female</td>
<td>+2</td>
<td>Men = 0</td>
</tr>
<tr>
<td>ALP/ALT (or AST) ratio</td>
<td>&lt;1.5</td>
<td>+2</td>
<td>+2</td>
</tr>
<tr>
<td></td>
<td>1.5-3.0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Serum globulines or IgG levels above normal</td>
<td>&gt;2</td>
<td>+3</td>
<td>+3</td>
</tr>
<tr>
<td></td>
<td>1.5-2</td>
<td>+2</td>
<td>+2</td>
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<tr>
<td></td>
<td>1-1.5</td>
<td>1</td>
<td>+1</td>
</tr>
<tr>
<td></td>
<td>&lt;1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ANA, SMA or LKM-1</td>
<td>&gt;1:80</td>
<td>+3</td>
<td>+3</td>
</tr>
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<td></td>
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<td>+2</td>
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<tr>
<td></td>
<td>1:40</td>
<td>+1</td>
<td>+1</td>
</tr>
<tr>
<td></td>
<td>&lt;1:40</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>AMA</td>
<td>Positive</td>
<td>-4</td>
<td>-4</td>
</tr>
<tr>
<td>Hepatitis Ag or Anti-HCV</td>
<td>Positive</td>
<td>-3</td>
<td>-3</td>
</tr>
<tr>
<td>Drugs, history of recent use of known or suspected hepatotoxic drug</td>
<td>Positive</td>
<td>+1</td>
<td>+1</td>
</tr>
<tr>
<td>Drugs, history of recent use of known or suspected hepatotoxic drug</td>
<td>Negative</td>
<td>-4</td>
<td>-4</td>
</tr>
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<td>+2</td>
<td>No abuse</td>
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<td>&gt;60 g/d</td>
<td>-2</td>
<td>Unknown 0</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Suspected -2</td>
</tr>
<tr>
<td>Liver histology</td>
<td>Interface hepatitis</td>
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<td>+3</td>
</tr>
<tr>
<td></td>
<td>Predominant lympho-plasmacytic infiltrates</td>
<td>+1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rosetting None</td>
<td>+1</td>
<td>+1</td>
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<tr>
<td></td>
<td>Biliary changes</td>
<td>-5</td>
<td>-3</td>
</tr>
<tr>
<td></td>
<td>Other changes</td>
<td>-3</td>
<td>-3</td>
</tr>
<tr>
<td>Other autoimmune diseases (in patient or first-degree relative)</td>
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<td>+2</td>
<td></td>
</tr>
<tr>
<td>Other autoantibodies</td>
<td>pANCA, anti-Lc1, anti-SLA/LP, anti-ASPG</td>
<td>+2</td>
<td>+2</td>
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<tr>
<td>HLA DR3 or 4</td>
<td>+1</td>
<td>+1</td>
<td>Not tested, all patients +1</td>
</tr>
<tr>
<td>Response to therapy</td>
<td>Complete Relapse</td>
<td>+2</td>
<td>+2</td>
</tr>
<tr>
<td></td>
<td>+3</td>
<td>+3</td>
<td>Scores if response or relapse</td>
</tr>
</tbody>
</table>

At diagnosis or +/− 6 months
IgG levels converted to g/l. From time of diagnosis or +/− 6 months
Revision due to different titers at different hospitals. From time of diagnosis or +/− 6 months

We only asked for septa building, fibrosis, cirrhosis and interface hepatitis
Thyroid, celiac or psoriatic disease, diabetes, Sjögrens syndrome, Systemic Lupoid Erythematosus, IBD, sarcoidosis, glomerulonefritis, arthritis or other autoimmune disease

+2 if more than 1/40-1/100 for the aforementioned antibodies, as well as ENA.
Liver cirrhosis was diagnosed in 248 patients (39.1%) by liver biopsy (n=215), radiology (n=17), the finding of ascites (n=5), esophageal varices (n=2) or through other clinical findings (n=9). Follow-up time from date of cirrhosis was in total 3018 years and in median 11.4 years (range: 0 - 40.5 years). In the cohort we found six patients that tested positive for HCV but none for HBV. The prevalence of HCV in the Swedish population is around 0.5% (123), and in our cohort it was 1%. According to the medical records of these patients they still fulfilled the criteria for AIH.

**Patients and methods in different papers**

**Paper I**
In Paper I we used the updated AIH cohort (n=634) for all analyses. We used the Swedish Cancer Registry (data updated to December 31, 2009) and the Swedish Cause of Death Registry (data updated to December 31, 2010). Person-years at risk calculations started at the time of AIH diagnosis and at the date of cirrhosis, and ended at liver transplantation or death.

The Swedish Cause of Death Registry was searched for date, underlying cause and concomitant causes of death. We made an extra effort to find any HCCs that might have been missed being reported to the Swedish Cancer registry, and two additional cases were found.

**Paper II**
In Paper II we updated a previous questionnaire study on women with AIH that was published in 2007 (104). In the first questionnaire study 106 women with AIH diagnosis before the age of 42 years of age participated. A questionnaire form was developed in collaboration with colleagues from the pediatric and obstetric departments at Umeå University hospital, with questions concerning the patients’ AIH, their miscarriages, abortions, infertility problems, pregnancies, medications, well-being of the children, etc.

In 2012 we retrieved all women born 1970 or later from the updated cohort. The upper age limit of 42 years was chosen to make sure that the women would have their pregnancies in good memory. A slightly revised version of the questionnaire was sent to 84 women. Some women (n=32) therefore participated two times in the studies (Figure 3). For the first questionnaire study a search was made in the national registers of Congenital Malformations and Medical Birth Register, data that were reused in this paper. However, no new search was made in these registers for the present paper.
**Figure 3** Flow chart of the inclusion of patients to questionnaire studies from the AIH cohort in 2004 and 2012.

Paper III
The base for Paper III was the original AIH cohort (n=473). The study was initiated in 2006 before the inclusion of new patients. The collection of the biopsies from the different centers and the analyses took time, and therefore the statistical analyses of the data were not initiated until 2015.
The paper investigated the temporal changes of fibrosis and inflammation through re-analyzing biopsies from the cohort. A total of 828 biopsies in 433 patients were assessable for inclusion (1963 to 2003). However, for practical reasons only biopsies that were taken at the University hospitals were included. Only patients who had a paired biopsy (for comparison) were included. In result 353 biopsies were analyzed, but only 258 biopsies from 101 patients were included. A flow chart for inclusion of biopsies is shown in Figure 4.

**Figure 4** Flow chart of the inclusion of biopsies from the University hospitals in Paper III.

The histological assessments of the biopsies were made by a single pathologist (Professor Richard Palmqvist) in a standard manner but who was blinded for patient information, such as treatment, outcome and order of biopsies.

Routinely stained sections according to each laboratory’s staining protocol were retrieved. Biopsy material including at least five portal tracts or a length of at least 10 mm was considered suitable for interpretation. Light
microscopy was used to evaluate the biopsies, and fibrosis stage and inflammatory activity were classified according to the Ishak score. The Ishak score has one score for grading (0-18 points), which assesses periportal or periseptal interface hepatitis (0-4 points), degrees of confluent necrosis (0-6 points), as well as focal (spotty) lytic necrosis/apoptosis and focal inflammation (0-4 points). Finally, portal inflammation is assessed (0-4 points). The staging of the disease validates architectural disturbances, fibrosis and cirrhosis, and is staged between 0-6 where 6 equals liver cirrhosis (124).

A change of fibrosis stage from the first to the last biopsy was considered significant if the change was one or more steps on the Ishak scale of fibrosis. We also made a sub analysis with stricter criteria, with two steps in the scale of fibrosis as a significant change from first to last biopsy. For inflammatory activity the change was considered significant if the change was two steps or more.

**Paper IV**

For Paper IV we used the updated AIH cohort of 634 patients. In the first epidemiological analyses the sub cohort MULL was used. For this follow-up study of prevalence and incidence the patients from the primary catchment area of Örebro University hospital were also included (the MULLÖ sub cohort), since Örebro now had a complete retrospective enrolment of their patients (from 1990). The sub cohort of MULLÖ included 299 patients, of which 212 were diagnosed between 1990 and 2009, and the total follow-up time was 3433 years. The patients from the primary catchment areas of MULLÖ were used for incidence and prevalence calculations, and were compared to a gender and age matched population from the same areas. The population in the primary catchment areas covered nearly 1 000 000 individuals in 2010.

To validate if our cohort’s prevalence data reflected the prevalence of AIH in Sweden, we compared the point prevalence on the 31th of December of 2009 with that in Västerbotten County at the same time (125). We retrieved medical records (from January 1, 2007 to December 31, 2011) from patients with International Classification of Disease (ICD)-10 codes for AIH (K75.4), PBC (K74.3), PSC (K83.0A) and cholangitis (K83.0 and K83.0X). The diagnoses were validated through the medical records, searching for laboratory parameters, biopsies and x-ray findings. All three hospitals in Västerbotten County (Umeå, Lyckeåle and Skellefteå) share the same system for medical records, and therefore we believe we retrieved all adult patients with a diagnosed autoimmune liver disease (AIH and AIH overlap syndrome).
For calculations of relative survival it was decided to include all patients in the updated AIH cohort since we now had observed the old cohort for at least 5 years, and these patients were compared to the Swedish reference population of 2000. Patients included in the study are listed in Table 2.

Table 2 Patients included in the study, primary catchment areas and hospitals.

<table>
<thead>
<tr>
<th>University Hospital</th>
<th>Patients (n)</th>
<th>Primary catchment areas (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linköping</td>
<td>87</td>
<td>66</td>
</tr>
<tr>
<td>Lund</td>
<td>99</td>
<td>87</td>
</tr>
<tr>
<td>Malmö</td>
<td>44</td>
<td>41</td>
</tr>
<tr>
<td>Karolinska, Huddinge, Department of Medicine</td>
<td>90</td>
<td>64</td>
</tr>
<tr>
<td>Karolinska, Huddinge, Department of Infectious Diseases</td>
<td>48</td>
<td>48</td>
</tr>
<tr>
<td>Karolinska, Solna</td>
<td>29</td>
<td>17</td>
</tr>
<tr>
<td>Sahlgrenska, Gothenburg</td>
<td>36</td>
<td>32</td>
</tr>
<tr>
<td>Sahlgrenska, Östra</td>
<td>26</td>
<td>25</td>
</tr>
<tr>
<td>Umeå</td>
<td>88</td>
<td>54</td>
</tr>
<tr>
<td>Uppsala</td>
<td>30</td>
<td>27</td>
</tr>
<tr>
<td>Örebro</td>
<td>57</td>
<td>51</td>
</tr>
<tr>
<td>Total</td>
<td>634</td>
<td>512</td>
</tr>
</tbody>
</table>

We also used the Cause of Death Registry (data searched through December 31, 2010), for all deaths, causes of death and concomitant causes of death. The deaths were then compared to the actual statistics of Sweden in the year 2010 (126).

**Statistical methods**

A two sided p-value <0.05 was considered statistically significant. Values were presented as median, range, percent, and occasionally mean when appropriate. The Chi Square-test and Fisher’s exact test (for small samples) were used to evaluate categorical variables.

For non-parametrical analyses the Wilcoxon signed-rank test (Paper III) was used for comparing matched samples, Mann Whitney U for comparisons between two groups (Paper III and IV) and Kruskal-Wallis for multiple groups (Paper III).

Standard incidence ratios (SIRs) were calculated to estimate the cohort’s cancer risk (Paper I). SIRs were only calculated for patients from the primary
catchment areas of the hospitals. The expected number of cases to calculate SIRs was obtained by multiplying age- and calendar-specific person-years in the cohort with the corresponding matched incidence in the Swedish population. Cancer diagnosed before the AIH diagnosis rendered in the cancer being excluded from SIR analyses. A cancer was considered to have developed after AIH diagnosis (incident cancer) if the date of cancer diagnosis was at least one year after AIH diagnosis.

Logistic regression analysis was performed to investigate if age, sex and inflammatory activity at diagnosis would affect the outcome of fibrosis stage at last biopsy (Paper III).

The observed overall survival in the cohort was compared to that of the reference population (from the year 2000), and a relative survival was calculated. Kaplan Meier curves were used for comparisons within the cohort to predict time to event, defined as liver transplantation or death (Paper IV).

Data were analyzed with SPSS version 21 (SPSS Inc. USA).

**Ethics**

The committee for human ethics at Umeå University had approved all studies. The ethical approval for the AIH project was granted in 2005 (Dnr 04-174M) (Paper I-IV). Several amendments for the different projects have since then been approved (i.e. 2007, 2009, 2011 and 2016). The studies were conducted according to the 1975 Helsinki declaration of ethical guidelines.
**Results**

**Risk for cancer in AIH (Paper I)**

In Paper I we set out to investigate the risk for cancer in AIH patients. We found a total of 206 malignancies in 148 patients. Excluded from statistical analyses were 68 cases since they were not considered as true malignancies (for example, cervical dysplasia) and were not included within the reference population. Thirty-two cancers were diagnosed before and within one year of the diagnosis of AIH (*prevalent cancers*), and were therefore also excluded from SIR analyses. We found 106 incident cancers, of which all but 14 were from primary catchment areas, which left 92 cancers for SIR analyses. Thirty-three patients had more than one cancer reported; this was mostly non-melanoma skin cancer (NMSC). The overall risk of any form of cancer from date of birth in the AIH cohort was increased with a SIR of 1.47 (95% confidence interval (CI) 1.22-1.75) compared to the reference population. The cancer incidence after AIH diagnosis was higher, SIR of 2.08 (95%, CI 1.68-2.55). The rationale of investigating both incidences from birth and from AIH diagnosis was to see if the AIH diagnosis had an impact on the cancer incidence.

**Hepatobiliary cancer**

We found 12 hepatobiliary cancers - 10 Hepatocellular Carcinomas (HCC; five men and five women), one intrahepatic cholangiocarcinoma (ICC), and one extrahepatic cholangiocarcinoma (ECC).

Included in SIR analyses of risk for hepatobiliary cancer from date of diagnosis of AIH were seven hepatobiliary cancers with a SIR of 8.24 (95% CI 3.30-16.97). Counting from date of cirrhosis, six hepatobiliary cancers gave a SIR of 54.55 (95% CI 19.92-99.99).

The 10 patients with HCC were diagnosed with cancer at a median of 59 years of age (range 43-78) and four underwent liver transplantations 4-11 months after HCC diagnosis. At the end of follow-up two patients were alive, of which one was liver transplanted. The transplanted patients that died had lived 1.5, 8 and 9 years after transplantation.

In the cohort we found 248 patients with cirrhosis that had been followed 3018 years after the diagnosis of liver cirrhosis. All patients with HCC had liver cirrhosis. Nine patients had a cirrhosis diagnosis in their medical journal (diagnosed in median 14 years after AIH diagnosis, range 0-29), and the last patient had indirect findings of cirrhosis (low albumin levels, elevated INR, etc.). This gives an incidence rate of 0.3% per year from
diagnosis of cirrhosis (10/3018=0.0033). Among the 248 cirrhotic patients the risk for HCC was 4.0% during the observational period (10/248=0.040).

**Extrahepatic cancer**
The only other cancer with an increased risk for AIH patients was NMSC, SIR of 9.87 (95% CI 6.26-14.81). We found 42 NMSC in 23 patients, of which 23 cases were included in SIR analyses. The finding of an enhanced risk for Non-Hodgkin Lymphoma in our previous study could not be confirmed in the present study (SIR 3.15, 95% CI 0.85-8.06) (83).

**Pregnancy and childbirth in AIH (Paper II)**
Paper II investigated the outcomes of women with AIH during pregnancy and the post-partum period, as well as their children, via a questionnaire study. The response rate to the 2012 questionnaire was 76.0% (64 out of 84 women). Together with the 74 women that participated in the 2004 study (104), 138 women answered the questionnaire, of which 32 women participated in both studies (Figure 3). Fifty-eight women gave birth to 100 children between 1959 and 2011 as a result of 99 pregnancies. The median age for pregnancy was 29 years (range 18-40 years). Fifty-seven women had liver cirrhosis, and of these women 23 gave birth to 43 children after diagnosis of cirrhosis.

**Fertility and miscarriages**
Ten women reported fertility problems, of which four had cirrhosis. Two women had successful pregnancies through in-vitro fertilization. Thirty-one miscarriages were reported in 24 women with no significant differences between cirrhotic and non-cirrhotic women (p=0.152). Two women had late miscarriages (week 19 and 20). The miscarriage rate was estimated at 23.6% (31 miscarriages/131 pregnancies).

Twenty-five women abstained from pregnancy, and 18 of these women reported that the decision at least partly was due to their AIH (14 had cirrhosis). Seven women had been advised by their physician not to become pregnant. We found four pregnancies in two liver transplanted women, of which one needed in-vitro fertilization to become pregnant. Thirteen women had terminated a pregnancy; eight of them after advice from their physician.

**Pregnancy**
Between 1959 and 2006 the most frequently used medical treatment during pregnancy was GCS (50.0%, n=34), whereas it was GCS in combination with AZA/6-MP between 2007 and 2012 (48.4%, n=15). Twelve women (17.6%)
did not have any medication during pregnancy between 1959 and 2006, and four women (12.9%) had none during pregnancy between 2007 and 2012.

Two patients were diagnosed with AIH during the pregnancy. The AIH remained stable in 65 pregnancies (65.7%). An increased disease activity was found in 13.1% (n=13) of the pregnancies. Almost one-third of the patients (32.7%) had one or more flares after the pregnancy. The flares were self-reported and no aminotransferase levels were known. Interestingly, we found that the frequency of flares during pregnancy and post-partum flares were more uncommon among the cirrhotic mothers (p=0.013 and p=0.02, respectively).

**Delivery and thereafter**

Delivery <38 weeks was noted in 22 pregnancies (22.0%). Caesarean sections were performed in 17 pregnancies (12 acute sections and 5 elective). No differences were noted between cirrhotic and non-cirrhotic mothers in pregnancy length (p=0.780), but the frequency of caesarean section was increased among the cirrhotic women compared to the non-cirrhotic women (p=0.047).

Eighty-one percent of the newborn children were reported to be doing well and had a normal status at birth. No differences were found between children to cirrhotic and non-cirrhotic mothers (81% in both groups). Among the 19% that were affected in some way, there were a variety of problems described from colic, poor growth to infections and problems associated with pre-term birth. Five children needed intensive care at birth, but all had been dismissed at one month. Two children died in the study - one was a stillborn infant (born at week 34) and the other a child with elevated liver enzymes already at birth that died after 3 months.

**Malformations**

One malformation was found in the 2012 follow-up - a child was born without a urethra. In total, three out of 100 children were found with physical malformations (hand and forearm defect, pre-auricular fibroma and urethra atresia). The medications of the mothers of these children were not fully documented.

**Histological changes in repeated liver biopsies (Paper III)**

Two hundred and fifty-eight biopsies from 101 patients (72 women and 29 men) from 1979 to 2003 were analyzed to investigate the histological changes over time in AIH patients. Sixty-four patients had undergone two biopsies, 23 patients had three biopsies, nine patients had four biopsies and
five patients had five biopsies. Median follow-up time between first and last biopsy was 3 years (range 0.5-23 years). Seventy-nine patients had biopsies from the time of diagnosis. Follow-up data until 2015 were available for 90 of the 101 patients.

**Changes in fibrosis and inflammation**

A decrease of fibrosis (≥1 stage) from first to last biopsy was the most common outcome, and was found in 62.4% of the patients (n=63). If the criteria for change of the fibrosis stage was increased to ≥2 stages, a stable fibrosis was the most common outcome and was found in 53.5% (n=54). The group with an increased fibrosis still remained the smallest group. If biopsies taken at the time of diagnosis were excluded, a decreased fibrosis stage was still the most common outcome (57.7%, n=30) (**Table 3**). The reason for excluding biopsies from the first year after diagnosis was to minimize the risk of overrating the fibrosis stage due to interference of inflammation, which is common in the untreated AIH patient. We could observe a higher inflammation score in biopsies taken at the time of diagnosis than in the first follow-up biopsy (7 versus 4, p<0.001).

**Table 3** Change in fibrosis stage from first to last biopsy. Patients are divided into groups of increased, stable or decreased fibrosis stage, and whether the change was one or two stages for all patients, and patients with paired biopsies from at least one year after AIH diagnosis. Data presented as percent (and number) of patients. Bold text refers to the largest group.

<table>
<thead>
<tr>
<th>Change in stage of fibrosis</th>
<th>All patients (n = 101)</th>
<th>Excluding biopsies from time of diagnosis* (n = 52)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased</td>
<td>27.8% (28)</td>
<td>19.2% (10)</td>
</tr>
<tr>
<td>Stable</td>
<td>19.8% (20)</td>
<td>23.1% (12)</td>
</tr>
<tr>
<td>Decreased</td>
<td>62.4% (63)</td>
<td>57.7% (30)</td>
</tr>
</tbody>
</table>

* Forty-nine patients did not have two biopsies one year after AIH diagnosis and were therefore excluded.

The absence of active inflammation (defined as an activity index ≤3) at least one year after diagnosis was more common among patients with a decreased fibrosis stage compared to the groups with increased or stable fibrosis (p<0.001). Patients with an absence of active inflammation at last biopsy mostly belonged to the group that had a decreased fibrosis stage (74.7%, 20 out of 27). Fibrosis stage at last biopsy was significantly higher in patients with an activity index >3 than in patients ≤3 (median 3 versus 2, p=0.01).
**Cirrhosis**

Twenty-one patients (20.7%) had at least one biopsy graded as cirrhotic, i.e. Ishak stage 6. Fifteen patients (71.4%) had a reduction of fibrosis stage from first to last biopsy, whereas three patients had an increased fibrosis stage. Only five of 21 patients (23.8%) were cirrhotic at their last biopsy.

**Response to treatment and medication**

Almost all patients had been on GCS (97.0%, n=98) or thiopurines (81.2%, n= 82) at some time, and the response to medical treatment was high. In patients with biopsies from the time of diagnosis (n=79), a complete (n=49) or a partial response (n= 26) to medication was achieved in 94.9%.

In comparison between the groups of patients with a decreased and increased fibrosis stage, we found that there had been fewer withdrawal attempts of GCS among the patients with a decreased stage of fibrosis at last biopsy compared to patients with an increased fibrosis stage (p=0.002). This was, however, not observed with thiopurines. Re-introduction of medication after withdrawal of GCS and/or thiopurines in both groups was common (41.7-50.0%).

There were no differences in stage of fibrosis at last biopsy or time between diagnosis and death between patients with and without withdrawal attempts. Neither could we observe any differences in the risk for long-term effects of GCS medication (hypertension, development of Cushing, weight gain, cataract, diabetes, osteoporosis and osteoporosis related fractures) between these two groups.

**Clinical outcome**

Thirty-two of the patients in the study were dead at the end of 2015. Four patients had had liver transplantations, of which two were dead. There were 39 patients in remission without cirrhosis and 17 with cirrhosis, of which two had decompensated cirrhosis. One of the patients with decompensated liver cirrhosis was waiting for a liver transplantation.

**Epidemiology of AIH in Sweden (Paper IV)**

In Paper IV epidemiology and causes of death were analyzed, as well as survival. In our cohort the age at diagnosis was 46 years (range 4-85 years), and men were diagnosed with AIH at a younger age than women (33.5 years, range 8-85 years versus 48.0 years, range 4-83 years, p<0.001). Women had the highest incidence in their fifties and sixties and men in their adolescence. Cirrhosis at diagnosis was common; almost one-third (n=178, 28.1%) had cirrhosis at diagnosis and 70 patients (11%) developed cirrhosis during
follow-up. Thirty-three patients underwent liver transplantations 20 days to 39.8 years after diagnosis.

**Prevalence and incidence**
The prevalence in 2009 was 17.3 per 100 000 inhabitants in the AIH cohort (Figure 5), and 18.6 per 100 000 inhabitants in Västerbotten county (December 31, 2009). The average yearly incidence for 1990 to 2009 was 1.2/100 000 inhabitants (95% CI 1.0–1.4). Men had a lower yearly incidence than women (0.7/100 000 inhabitants, 95% CI 0.6–0.9 versus 1.6/100 000 inhabitants, 95% CI 1.3–1.9).

**Figure 5** Prevalence and incidence of AIH for men, women and for the AIH cohort.

**Survival**
Four years after diagnosis a significantly reduced relative survival was observed for AIH patients (98%, 95% CI 96.3–99.7), compared to an age and gender matched Swedish reference population (Figure 6).
Figure 6 Relative survival of the updated AIH cohort.

Women’s relative survival started to decline significantly from that of the reference population 12 years after AIH diagnosis (93.5%, 95% CI 89.1-97.9) and for men after 18 years (86.2%, 95% CI 75.3-97.1) (Table 4).

Table 4 Relative survival compared to the reference population of Sweden. Numbers are presented with 95% confidence intervals (CI) for the entire AIH cohort, and for men and women separately.

<table>
<thead>
<tr>
<th>Years after AIH</th>
<th>All %</th>
<th>CI</th>
<th>Men %</th>
<th>CI</th>
<th>Women %</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>96.7</td>
<td>94.6-98.8</td>
<td>95.5</td>
<td>90.9-1.01</td>
<td>97.1</td>
<td>94.7-99.5</td>
</tr>
<tr>
<td>10</td>
<td>96.3</td>
<td>93.3-99.3</td>
<td>94.6</td>
<td>88.4-1.01</td>
<td>96.8</td>
<td>93.4-1.00</td>
</tr>
<tr>
<td>15</td>
<td>91.7</td>
<td>87.2-96.2</td>
<td>91.2</td>
<td>82.5-99.9</td>
<td>91.7</td>
<td>86.5-96.9</td>
</tr>
<tr>
<td>20</td>
<td>86.0</td>
<td>79.9-92.1</td>
<td>84.7</td>
<td>73.1-96.3</td>
<td>86.2</td>
<td>79.0-93.4</td>
</tr>
<tr>
<td>25</td>
<td>77.9</td>
<td>69.8-86.0</td>
<td>70.4</td>
<td>53.8-87.0</td>
<td>80.0</td>
<td>70.7-89.3</td>
</tr>
</tbody>
</table>
No differences between men and women in overall or transplant free survival were observed. Patients diagnosed before 40 years of age had a better long-term prognosis than patients older than 40 years at diagnosis (p<0.001). Cirrhosis at diagnosis was a negative risk factor for both overall and transplant free survival (p=0.03 and p=0.002), but overlap syndrome was not associated with an inferior survival.

**Causes of death**
One hundred and fifty patients had died during follow-up (116 women, 34 men), with a median survival of 11.8 years after diagnosis (range 0.2-44.2 years). The median age of death was lower in men 59.7 (range 21.6-89.8 years) than in women 75.4 years (20.2-94.7 years) (p=0.002). Liver-related death was the most common cause of death in the AIH cohort (32.7%, n=49), followed by cancer (28.0%, n=42) and cardiovascular disease (22.0%, n=22).

Altogether 52% (n=78 patients) had liver disease as an underlying or concomitant cause of death. Patients with cirrhosis at the time of diagnosis and patients that were diagnosed with AIH before the age of 40 years were more likely to die from a liver related death (p=0.041 and p<0.001). Patients with cirrhosis at diagnosis also died at a younger age than patients without cirrhosis at diagnosis (65.5 versus 78 years, p<0.001).
Discussion

This clinical thesis focused on different perspectives of AIH – ranging from epidemiological to various outcomes of the disease, to the life in-between diagnosis and death. The studies are based on one of the world’s larger cohorts of patients with a validated AIH diagnosis, making the material fairly unique.

Studies on rare diseases like AIH are always subjected to the risk that observed numbers are too small to draw general conclusions. Collaborative multicenter studies are therefore a valuable way to collect patients, making the number of observed patients more substantial, and hopefully leading to that knowledge about the disease will become evidence-based.

Epidemiology and causes of death in AIH

In Paper IV we set out to investigate epidemiology and causes of death of AIH in Sweden and found a prevalence of 17.3/100 000 and incidence of 1.2/100 000 inhabitants and year (95% CI 1.0-1.4) between 1990 and 2009. As a follow-up to our previous study (9) we could now observe a tendency that the prevalence curve was evening out, as a sign that we might have reached the proper prevalence in the population. Of course this would preferably be confirmed by further follow-up studies. Our prevalence numbers are in line with most other observations in the European setting (15). However, the prevalence in Sweden is somewhat lower than in Denmark (23.9/100 000, 95% CI 22.6-25.2), where a rising incidence rate was noted (24). A rising incidence was also reported in a large epidemiological study from the Netherlands (10), but could not be confirmed in Paper IV. However, we did not include pediatric patients in the calculations of prevalence and incidence numbers, which was also the case in the study from the Netherlands.

Survival

The relative survival started to differ significantly four years after AIH diagnosis, but became even more obvious in the second decade. After 25 years the relative survival compared to the general Swedish population was only 77.9% (95% CI 69.8-86.0). Previous studies have often presented a favorable prognosis during the first decade (25, 57, 61, 127). Our study (Paper IV), as well as a study by Hoeroldt et al (63) investigated the prognosis beyond 10 years after AIH diagnosis, and indicated that the survival after this becomes poorer. We could also see that the most common cause of death was related to liver disease, in contrast to the Swedish
population where diseases of the gastrointestinal tract only represent a quite small part of the total deaths (3.2%) (126). A substantial increased risk for death due to liver disease was previously presented in AIH patients (24, 62, 63).

Patients with cirrhosis at the time of diagnosis had a worse prognosis of overall and transplant free survival than patients without cirrhosis. Feld et al (127) also found an inferior survival in patients with cirrhosis at diagnosis. Men were diagnosed with AIH at a younger age than women and died at a younger age, but there were no differences in overall or transplant free survival between the sexes. This is in contrast to the study of Al-Chalabi et al (128) where they actually found a better long-term survival in the men. The men in their study were also diagnosed at a younger age (39 years) than women (49 years), but the difference in the age of onset of AIH was not statistically significant. Based on the findings in Paper IV, we believe that the finding that men in our cohort died younger than women, but without differences in survival after AIH diagnosis, is not a result of a more aggressive disease in men but of an earlier onset of disease.

Our interpretation is that the long-term prognosis, after the first decade, is impaired compared to the general population and that this is mostly a result from death caused by liver-related disease. Suppressing the inflammation is of outmost importance since it is the only known way to avoid liver damage.

**Cancer risk in patients with AIH**

*Cirrhosis, HCC and surveillance*

In Paper I we found an increased risk of hepatobiliary cancer compared to the Swedish population (55-fold), but a yearly risk for HCC among the cirrhotic patients that by far fell short of the stipulated 1.5% per year suggested for cost-effectiveness in surveillance (89). The incidence rate among the cirrhotic patients was only 0.3% per year. In Sweden HCC is fairly unusual and only constituted 1.2% of all newly diagnosed cancers in men and 0.6% in women 2011 (129). Cirrhosis is considered a requirement of HCC in AIH, and longstanding cirrhosis is described as a major risk factor (87). About 1/3 of our AIH patients had cirrhosis at the time of diagnosis and all patients with HCC had cirrhosis. Unexpectedly, we found that 4 out 10 patients with HCC had developed cancer within 5 years from diagnosis of cirrhosis.

Several studies have investigated the risk for HCC in AIH, but as with all studies on AIH the size of the studied cohorts and observational periods can vary widely, as well as the knowledge about cirrhosis status (24, 62, 83, 85,
Therefore, it has been hard to establish the actual risk, and the question of whether it is cost-effective to include AIH patients in surveillance programs has been ongoing. In our study (Paper I) most patients had not been included in surveillance programs, since surveillance at the time had not been fully implemented in Sweden. As of today European and American guidelines on AIH recommend that cirrhotic AIH patients are included in surveillance (14, 15). Most studies on AIH and HCC have found the risk for HCC to be lower than 1.5% per year (24, 60, 85, 130, 131). The rationale of including AIH patients has been motivated with that the actual risk has not been established, and that some studies have found a risk close to 1.5% per year. Yeoman et al (131) found an annual risk of 1.1% and Wong et al (133) 1.9% in cirrhotic patients. Both studies were single-center studies at tertiary referral hospitals, which might have biased the results, i.e. potentially sicker patients, more aggressive disease, etc. A recently published meta-analysis of risk for HCC in AIH (86) confirmed that incidence rates from specialized tertiary centers were higher than in multicenter studies. The same meta-analysis found a pooled incidence rate of HCC of 3.06 per 1000 person-years (95% CI 2.22-4.23) in the AIH cohorts and 10.07 per 1000 patient-years (95% CI 6.90-14.70) in AIH patients with cirrhosis (86). The conclusion was that the incidence was lower than 1.5% per year, but since the upper 95% CI nearly encompassed 1.5%, AIH patients should be included in surveillance.

HCC has a poor prognosis when not found in an early stage. The median survival in an early stage is over 5 years, but declines rapidly with stage advancement (134). In our study (Paper I) four out of ten patients with HCC underwent liver transplantations, of which three died with a survival of 2 to 9 years after transplantation and the fourth was alive at the end of follow-up. Only two patients were alive at the end of follow-up, of which one had not been transplanted and had been diagnosed with HCC three years earlier. The other three patients with a date of HCC diagnosis (i.e. reported to the cancer registry) were dead <1 year after diagnosis.

The rationale for including some patient groups with cirrhosis and excluding others with enhanced cancer risk can be discussed. A meta-analysis on the efficacy on surveillance in patients with cirrhosis of any kind concluded that surveillance was associated with an improvement in early tumor detection, but also in patients offered a curative treatment and overall survival (135). American Association for the Study of Liver Diseases’ (AASLD) guidelines on the management of HCC from 2017 also endorse surveillance of adults with cirrhosis since it improves overall survival (136). Even if our data cannot support cost-effectiveness, I suggest including AIH patients in surveillance programs since they are an identified group with a higher risk for HCC and
since it is our best way to find the HCC in a curable, or at least treatable, stage.

**Potential limitations of Paper I**
There is of course the possibility that we might have missed cases of HCC, as implied by the finding of two patients through searching the Cause of Death Registry that had not been reported to the Cancer Registry. We also know that the autopsy rate in Sweden has gone down immensely (16% in 2010) (137), but to exceed 1.5% per year we would have to find 46 cases of HCC during 3018 cirrhotic observed years (46/3018= 1.5%).

**Overall cancer risk and NMSC**
We found a two-fold overall enhanced cancer risk, which consisted of an increased risk of hepatobiliary cancers and NMSC. The NMSC risk in solid organ transplant patients is well-known and multifactorial, but the strongest risk factor is the use of immunosuppression (138). The risk of NMSC has been described before in AIH patients (62, 139). AZA is known to photosensitize the skin for Ultraviolet A radiation as well as to enhance Ultraviolet-light tumorigenesis in mice (140). We do not have full knowledge on the use of thiopurines (doses, length of medication etc.), but we know that AZA has been frequently used in the cohort. Patients on thiopurines should be informed to regularly screen for skin lesions as well as to avoid direct sunlight.

**Reversal of fibrosis in AIH**
The study of repeated biopsies (Paper III) showed that patients with AIH responding to medical therapy have a good chance to improve or at least stabilize the fibrosis during a follow-up period of 3 years in median (0-23.5 years); it adds to the previous knowledge about fibrosis resolution in AIH patients (41, 59, 141-143). The study suggests that cirrhosis can be reversed in AIH. At the time of the last biopsy only five out of 21 patients that ever had a cirrhotic biopsy still had Ishak stage 6.

**Potential limitations of Paper III**
A selected cohort?
An observation was that the response to treatment was very high in the studied population; 94.9% of the patients had a complete or partial response to therapy. We also found that the frequency of liver transplantsations was lower compared to the cohort as a whole. In the remaining AIH cohort updated to the end of 2009, 5.8% had undergone liver transplantation at the end of follow-up compared to 2.0% in the biopsy study, i.e. 31 compared to
two patients, respectively. The most probable explanation for this is that patients with a very aggressive disease and/or acute onset will often only have one biopsy taken before transplantation or death (41). Furthermore, patients with a very advanced fibrosis or cirrhosis, where the risk of taking a biopsy is high and the clinical value is questionable, would also only have one biopsy. A follow-up biopsy after a couple of years to ensure histological resolution is not always performed. Therefore these patients would also be excluded from the study, and might have led to an unintentionally selected cohort of patients.

**Was the follow-up time sufficient?**

The majority of the biopsies were taken within 10 years after AIH diagnosis, and a legitimate question is what happened after this and if the follow-up time to substantiate a long-term prognosis on AIH liver histology was too short? Hard endpoints are the already discussed liver transplantations, but also liver-related death. From the findings in Paper IV we know that a considerable proportion of deaths (32.7%, n=49) in the AIH cohort was liver-related. Out of the 19 patients that had died by December 31th 2009 in the biopsy study (where we had information from the Cause of Death Registry), 26% (n=5) had died a liver-related death. The remaining 44 patients that died a liver-related death in the cohort constituted 33.6%, making the proportion of liver-related deaths somewhat higher in the remaining cohort (n=131). Three out of the five patients were in the increased stage of fibrosis group, but the other two patients had actually been in the group with a decreased fibrosis stage. The time between these patients’ last biopsies and deaths were 17 and 20 years, and our knowledge about flares, adherence to medication, etc., is unknown. As indicated by our study on the long-term survival (Paper IV), there is a possibility that the follow-up time was too short to pronounce how the long-term prognosis on liver histology will develop. This would require that we followed the patients over a longer period of time than in the present study (Paper III). Prospective studies with standardized biopsies taken at fixed intervals would shed further light on this.

**Sampling error**

The risk for sampling error must also be addressed when allowing a very small proportion of the liver to be representative for the whole organ (144). An irregular distribution of fibrosis can make biopsy samples unrepresentative, especially in macro-nodular cirrhosis, and therefore it is important that the biopsy is of an adequate size (145, 146). Preferably a biopsy should be 2-3 cm long with 11 complete portal tracts (146), but in the present study we included prepared biopsies on objective glasses that were ≥10 mm long or with at least five portal tracts. Sometimes the quality of the
biopsies was low due to the retrospective nature of the study, but if the interpretability was questionable they were sorted out. It has been suggested that the reversal of cirrhosis described in several liver diseases should be interpreted carefully, and that the improvements are a reflection of the resolution of collagen and not a full normalization of the liver parenchyma (145). At the end of the day, this does not diminish the finding of a considerable proportion of patients that showed an improvement of fibrosis stage.

**Absence of inflammation and tapering of glucocorticosteroids**

From the findings in our study (Paper III), it was more likely that patients achieved an improvement in fibrosis stage if they had an absence of inflammation (activity index <3) at last biopsy, as was also the finding in the study of Dahliwal et al (41). Furthermore, patients that tapered GCS were more likely to have an increased fibrosis stage at last biopsy, and it was common to reintroduce medication if it had been stopped. Our interpretation is that we should allow for as few exacerbations of disease as possible, and if cessation of medication is considered a biopsy should be performed beforehand. There is a risk that there is histological activity, even though a biochemical remission is achieved (41). It is also known that multiple relapses are associated with adverse outcomes (58, 63, 147).

**Future perspectives in supervision of fibrosis development**

Transient elastography has not yet been fully validated in AIH, albeit a recent paper found a strong positive correlation between liver stiffness and fibrosis stage (75). Hopefully this can be useful in the future for following the course of disease; but as a tool to assess low-grade inflammation, for example, before treatment withdrawal, currently it cannot replace the more invasive biopsy.

**Pregnancy and childbirth in AIH**

Since many women will be young when they fall ill the question if the disease or medication will affect their possibility to form a family will naturally be raised at some point. In our study the main finding was that women with AIH had a safe and for the most part successful pregnancy, and that this was also the case for women with compensated cirrhosis.

**Premature deliveries and flares**

However, there was a larger proportion of premature deliveries (22%) than expected compared to the general population. In Sweden in 2014 only 4.4% of all singletons (and 42.5% of all multiple births) were premature (148). In the study by Werner et al (104) it was decided to set the definition of
gestational week for premature birth at <week 38, and hence we kept this definition for the follow-up study (Paper II). Other studies on AIH and pregnancy have had other criteria, for example Stokkeland et al and Westbrook et al had <37 weeks and Terrabuio et al had <36 weeks. Westbrook et al had comparable results with ours (20%), but Stokkeland et al and Terrabuio et al found lower frequencies of prematurity (16.4 and 11.8%, respectively) (103, 105, 106). In the light of different criteria for prematurity the frequency of premature births in our study (Paper II) is probably in line with the other AIH populations, as one can assume that even premature births are normally distributed. Nevertheless, there is an increased risk for premature births in Swedish AIH patients, which the study of Stokkeland et al also confirmed (105). One-third of the patients had increased activity in the AIH after delivery, and therefore it is important to closely monitor laboratory parameters after delivery.

Risk for malformations
We could not find an enhanced risk for any specific deformities, or an apparent increased risk for malformations in general, even though the use of AZA during pregnancy had increased in the follow-up study. Stokkeland et al also found no enhanced risk for malformations in AIH patients compared to the general population in their study (105). The present study (Paper II) adds reassuring data to the previously published, that no significant risk for malformations is to be expected. However, we did not search the Congenital Malformation and Medical Birth Register for the follow-up study, and hence we have no objective validity to the reported births and outcomes. In the Werner et al study (104) the women actually reported more malformations than was registered; abnormalities that sometimes could not be classified as true malformations (e.g. liver disease, minor deafness). In the follow-up study there was only one report of a malformation, in a mother that was on GCS during the pregnancy. We do not know whether abortions were performed as a result of findings of malformations. No woman had commented on this.

Miscarriages
The frequency of miscarriages was relatively high (23.6% of the reported pregnancies, abortions excluded). A Swedish study on clinical miscarriages in a prospectively collected cohort concluded that 12% of all pregnancies ended as miscarriages (149); our results are undeniably higher. The underlying liver disease might have affected the outcome and it would have been interesting to learn more about medication(s) and laboratory parameters (transaminases and IgG) at the time of the miscarriages.
Limitations of the questionnaire study (Paper II)
This study has limitations that must be addressed. The first is that even if
our cohort of women and children born to women with AIH is large
compared to most other studies on the subject, it is still a small cohort and
therefore all results must be interpreted carefully. Secondly, the data are
self-reported, and therefore there is a risk for recall-bias. To minimize this
we chose to only include patients 42 years or younger to make sure that the
event was fairly close in time. We also used the information obtained from
women participating in the previous study (104), unchanged in this study
(Paper II), and only included information about new pregnancies,
miscarriages and abortions. Thirdly, we have no knowledge on
socioeconomic factors, and on how the children were doing and developing
beyond one month. This was beyond the scope of the study, but is also of
course an important aspect. However, Westbrook et al followed the 59
children in their study for a median of 7 years, and except for a boy that
developed Perthes disease, found no late events (106).

Information on risks with AIH in pregnancy
Eighteen out of 25 women that abstained from pregnancy reported that it
was in part due to their AIH. Some women may have had good reasons for
refraining from pregnancy such as an unstable disease, severe cirrhosis,
medication with MMF, etc. However, a lack of adequate information from
doctor to patient cannot be dismissed since at least a few women believed
that the risk of teratogenicity was high. Information about AIH, medication
and pregnancy is important, so that women that want to become pregnant
can enter a pregnancy well prepared (e.g. with adequate medication) and in a
stable phase of the disease. Women that have a more severe liver disease can
be informed about the risks for both mother and child, and patients where
the risk is low should also get that information. It is also important to ensure
that patients do not stop their medication during pregnancy, which was one
of the findings in the first study on the cohort (104).
Conclusions

Based on the findings in the studies of this thesis we conclude the following:

- There is an increased risk for hepatobiliary cancers in cirrhotic AIH patients. From the date of AIH diagnosis, the overall risk to develop cancer is about two times higher than in the general population, and this is due to the increased risk of hepatobiliary cancer and NMSC.
- Women with AIH have a higher risk for pre-term births and miscarriages than the general population. Maternal cirrhosis leads to a higher rate of caesarean sections but to a lower risk for flares during and after pregnancy. No increased risk of malformations due to the use of thiopurines was found in the cohort.
- The grade of fibrosis decreases or stabilizes in most AIH patients that respond to medical therapy. The absence of inflammation is linked to a decreased fibrosis stage at last biopsy. Our data suggest that a continuous low dose of GCS may be beneficial since an increase in fibrosis stage at last biopsy was more common among patients with discontinuation in GCS treatment. The study also suggests that cirrhosis in some patients is reversible.
- We found a prevalence of 17.3/100 000 inhabitants and an incidence of 1.2/100 000 inhabitants and year. The most common cause of death in Swedish AIH patients is related to liver disease. The relative survival was declining continuously over the observational period from four years after AIH-diagnosis as compared to the Swedish reference population.
- As a general conclusion it is important to continue collaborative research projects on unusual diseases like AIH to collect data of sufficient size and quality.

The overall conclusion of the thesis is that:

Even though AIH often responds very well to treatment, one cannot assume that long-term prognosis will always be favorable. Therefore, it is important to continuously aim for a complete remission, especially in younger patients, to avoid long-term complications.
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