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Alcoholic liver disease – epidemiology, prognosis and risk factors

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ACADEMIC DISSERTATION

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II Sahlman P, Nissinen M, Pukkala E, Färkkilä M: Cancer incidence among alcoholic liver disease patients in Finland: A retrospective registry study during years 1996-2013. *Int J Cancer* 2016;138(11):261-21

III Sahlman P, Nissinen M, Simonen P, Färkkilä, M: Noncholesterol sterols as surrogate markers in patients with severe alcoholic hepatitis. *Lipids* 2018;53(3):323-34

IV Sahlman P, Nissinen M, Puukka, P, Jula A, Salomaa V, Männistö S, Lundqvist A, Valsta L, Perola M, Färkkilä M, Åberg F: Genetic and lifestyle risk factors for advanced liver disease among men and women. *J Gastroenterol Hepatol. 2019 Jul. doi: 10.1111/jgh.14770. [Epub ahead of print]*

ABBREVIATIONS (in alphabetical order)

ALD	Alcoholic liver disease
ABIC	Age, serum bilirubin, INR, and serum creatinine -score
AH	Alcoholic hepatitis
ASH	Alcoholic steatohepatitis
AUC	Area under the curve
AUDIT	Alcohol use disorders identification test
CI	Confidence interval
ECBL	Early change of bilirubin level
FXR	Farnesoid X receptor
GAHS	Glasgow alcoholic hepatitis score
GLC	Gas-liquid chromatography
HCC	Hepatocellular carcinoma
HDL	High density lipoprotein
ICD	International classification of diseases
INR	International normalised ratio
LDL	Low density lipoprotein
MELD	Model for end-stage liver disease
MBOAT7	Membrane bound O-acyltransferase domain containing protein 7
NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis
PBC	Primary biliary cholangitis
PSC	Primary sclerosing cholangitis
PNPLA3	Phospholipase-containing domain 3
ROC	Receiving operating characteristics
SIR	Standardised incidence ratio
SMR	Standardised mortality ratio
SEM	Standard error of mean
TM6SF2	Transmembrane 6 superfamily 2
WHR	Waist-hip ratio

ABSTRACT

Introduction

Alcoholic liver disease (ALD) is one of the main consequences of alcohol abuse. Data on epidemiology of alcoholic liver disease in Finland is scarce. Both liver cirrhosis and alcohol consumption are risk factors for malignancies, but cancer incidence among patients with all forms of advanced alcoholic liver disease is not thoroughly studied. All persons with excessive alcohol consumption do not develop advanced liver disease, and other risk factors besides alcohol are poorly understood. Severe alcoholic hepatitis (AH) is treated with corticosteroids. Patients lacking response to corticosteroids have a very poor prognosis. Prognostic markers predicting response to corticosteroids at baseline are needed. The purpose of the study was to examine incidence, risk factors, prognosis and malignant comorbidities of ALD and to search for prognostic factors in AH.

Patients and methods

A cohort of patients with alcoholic liver cirrhosis (n=7746) and AH (n=4127) as an inpatient diagnosis during the years 1996-2012 (41209 person-years) was identified from the Hospital Discharge Registry from the National Institute for Health and Welfare. The incidence of AH and alcoholic liver cirrhosis was calculated. Survival rates of the patients were determined. The cohort was combined with the Finnish Cancer Registry and National Causes of Death Register in Statistics Finland to calculate standardised mortality ratios (SMR) for various causes of death. Standardised incidence ratios (SIR) for various cancers were calculated by comparing cancer incidence of the cohort to that of the general population. From a study analysing patients hospitalised with severe AH and treated with prednisolone and randomised to get either prednisolone alone or in combination with ciprofloxacin, twenty-four patients were enrolled in a substudy analysing cholesterol metabolism in severe AH. The response to prednisolone was assessed with the Lille model at day 7. Serum levels of cholesterol and non-cholesterol sterols and various standard biochemical parameters, including ferritin, were determined. The results were compared with two control groups: patients with primary sclerosing cholangitis (PSC) (n=156) and healthy individuals (n=124). Persons participating in Health2000 or FINRISK studies during 1992-2012 without an underlying liver disease comprised a cohort of 41260 individuals in a study assessing risk factors for advanced liver disease. Alcohol consumption, metabolic, lifestyle-related and anthropometric parameters were analysed with Cox regression analysis using severe liver disease, cancer or death as the end-point. Viral liver diseases were excluded. The follow-up was 511789 person-years (mean 12.4 years).

Results

The incidence of alcoholic liver cirrhosis increased by 66% among men and 75% among women from year 2001 to year 2012. Respectively, the incidence of AH increased 76% among men and 108% among women. The relative 5-year survival rates for male and female alcoholic liver cirrhosis patients were 0.28 (95%CI 0.27-0.30) and 0.39 (95%CI 0.36-0.41). The 5-year survival rate of all patients with AH was 0.46 (95%CI: 0.44-0.48) without difference between the two sexes. Sixty-five percent of the deaths were alcohol-related. Mortality from several other causes was increased: cancers (SMR 6.82; 95%CI 6.35-7.29), digestive diseases (SMR 27.95; 95%CI 24.78–31.31), respiratory diseases (SMR 7.86; 95%CI 6.70–9.10) and circulatory diseases (SMR 6.13; 95%CI 5.74–6.52). The risk of accidental or violent death was also increased (11.12; 95%CI 10.13-12.15).

The risk of cancer among the cohort was increased, and the SIR for all cancers was 2.86 (95%CI 2.69-3.03). The incidence of several other cancers was increased: liver (SIR 59.20; 95%CI 53.11–65.61) pancreas (SIR 3.71; 95%CI 2.72-24.94), pharynx (SIR 9.25; 95%CI 6.05–13.56), mouth (SIR 8.31; 95%CI 4.84–13,29), tongue (SIR 7,21; 95%CI 3.60–12.89), oesophagus (SIR 7.92; 95%CI 5.49–11.07), larynx (SIR 5.20; 95%CI 2.77–8.89), lung (SIR 2.77; 95%CI 2.27– 3.32), stomach (SIR 2.76; 95%CI 1.79–4.07), kidney (SIR 2.69; 95 CI 1.84–3.79), colon (SIR 2.33; 95%CI 1.70–3.11), cervix uteri (SIR 4.93; 95%CI 1.34–12.63) and non-melanoma skin cancer (SIR 1.89; 95%CI 1.18–2.86).

AH patients had a distinct profile of cholesterol metabolism compared to patients with PSC and healthy subjects. Responders to prednisolone therapy had 56-60% higher (p-range 0.032-0.044) ratios of phytosterols to cholesterol, while the lathosterol to campesterol ratio was 76% (p=0.031) lower compared to non-responders. The serum ratio of stigmasterol to cholesterol predicted the response to corticosteroid therapy. The median ferritin concentration at baseline was ~37% lower (p=0.011) among responders.

Among FINRISKI and Health2000 participants, 355 severe liver events occurred during the_mean 12,4 years of follow-up. Age (HR 1.03, p=0.0083 for men, HR 10.4, p=0.0198 for women), waist-hip ratio (WHR) (HR for WHR/1 standard deviation 1.52, p=0.0006 for men and 1.58, p=0.0167 for women) PNPLA3 mutations (HR 1.9, p=0.024 for men, HR 2.7, p=0.0109 for women) and weekly binge drinking (HR 2.4, p=0.0024 for men, HR 7.4, p<0.0001 for women) predicted advanced liver disease. Additionally, among men, diabetes (HR 2.7, p=0.0002), average alcohol consumption (HR for 10g of ethanol 1.1, p=0.0022), non-married status (HR 1.9, p=0.0397 for single and HR 2.4, p=0.0002 for widow/separated) and serum HDL (HR 2.2, p=0.0022) and non-HDL cholesterol (HR 1.2, p=0.0237) were risk factors. Alcohol intake increased the risk, especially among persons with a high WHR

Conclusions

The incidence of alcoholic liver cirrhosis and AH increased during the study period. The survival of the patients was poor. A majority of the patients die from alcohol-related causes. Cancer incidence is increased among patients with advanced ALD. AH patients have a distinct profile of sterol metabolism compared to patients with PSC and healthy individuals. Certain non-cholesterol sterols (*i.e.* plant sterols) putatively reflecting the nutritional status of the patients are related to response to corticosteroids. A high serum level of ferritin at baseline might predict poor response to corticosteroid therapy. Age, PNPLA3 haplotype and WHR increase the risk for advanced liver disease. There is synergism between alcohol intake and central obesity to the risk. Binge drinking poses an additional risk factor.

1. INTRODUCTION

Alcohol has been recognised as a major public health problem (WHO, 2014). Alcohol consumption has increased in Finland during the past decades, although there has been a slight decrease in the total annual consumption in recent years (National Institute for Health and Welfare, 2018). The burden of the alcohol-related liver diseases and liver mortality are related to the amount of alcohol consumed at the population level (Ramstedt, 2001, Stein *at al.*, 2016). Hazardous drinking exceeding 24 drinks per week in men and 16 per week in women is common in Finland; the prevalence is 5.8% (8.5% in men and 3.1% in women) (Halme *et al.*, 2010). As deaths from ALD are decreasing in traditionally wine-drinking southern European countries, there is marked increase in liver-related deaths in many European countries, especially in Finland (Sheron, 2016).

Alcoholic liver disease (ALD) is one of the major consequences of excessive alcohol consumption (Rehm 2009). In Finland, a great majority (87%) of liver mortality is from ALD (Sheron, 2016).

The spectrum of ALD ranges from asymptomatic and potentially reversible alcoholic steatosis to acute alcoholic hepatitis (AH), liver cirrhosis and ALD-related hepatocellular carcinoma (HCC) (O'Shea *et al.*, 2010).

Due to the relatively high level of alcohol consumption, Finland offers an optimal ground for studies on ALD. However, epidemiological data on ALD during the 21st century in Finland is scarce. Since alcohol and advanced liver disease are both risk factors for cancer, epidemiology of malignancies among ALD patients is particularly interesting. Previous studies (Kalaitzakis *et al.*, 2011, Sørensen *et al.*, 1998) cover cancer risk of liver cirrhosis in general rather than alcoholic cirrhosis, and there is no data on cancer incidence among AH patients. Although alcohol is the only essential aetiological factor needed for development of ALD, there is wide variation in individual risk among persons consuming similar amounts of alcohol. Thus, recognition of other potential risk factors of ALD are important for health promotion, both on an individual level and in society. Treatment options in severe AH are unsatisfactory, and assessment of prognosis of individual patients is difficult.

The objective of this thesis was to examine incidence and cause-specific mortality of severe ALD and associated malignant comorbidities by utilising comprehensive nationwide patient registries and extensive cohorts in Finland. Furthermore, the aim was to identify new prognostic indicators in severe AH and to examine the interactions of alcohol and other risk factors of advanced liver disease.

In this thesis, the term advanced liver disease is used to describe severe liver events requiring hospitalisation. In context of ALD, they include alcoholic liver cirrhosis, AH and unspecified alcohol-induced liver failure cases.

2. REVIEW OF THE LITERATURE

Clinical picture and diagnosis of alcoholic liver disease

The clinical spectrum of ALD consists of three different stages (Figure 1). The mildest form is simple steatosis which may be completely asymptomatic or diagnosed coincidentally by elevated liver enzymes (Bruha *et al.*, 2012). Alcoholic steatosis is reversible after cessation of drinking. Longstanding drinking leads to chronic hepatitis, which can progress into hepatic fibrosis and clinical liver cirrhosis (Singal *et al.*, 2018). A distinctive form of ALD resulting from a longer period of hard drinking is AH manifesting as an acute syndrome of advanced liver disease. The most difficult form of acute AH clinically resembles decompensation of liver cirrhosis (O'Shea *et al.*, 2010).

The diagnosis of ALD is mostly clinical and is based on anamnesis or objective findings of excessive alcohol consumption combined with clinical picture of liver disease (EASL, 2018a). However, liver biopsy can be used to determine the severity and type of the liver damage, to clarify the diagnosis in atypical cases and to rule out concomitant liver diseases (Torruellas *et al.*, 2014).

Alcoholic steatohepatitis (ASH) is defined histopathologically as an inflammation in alcoholic fatty liver. It can be asymptomatic, but as a clinical term, AH refers to a symptomatic condition often associated with jaundice, pyrexia, leucocytosis and elevated liver

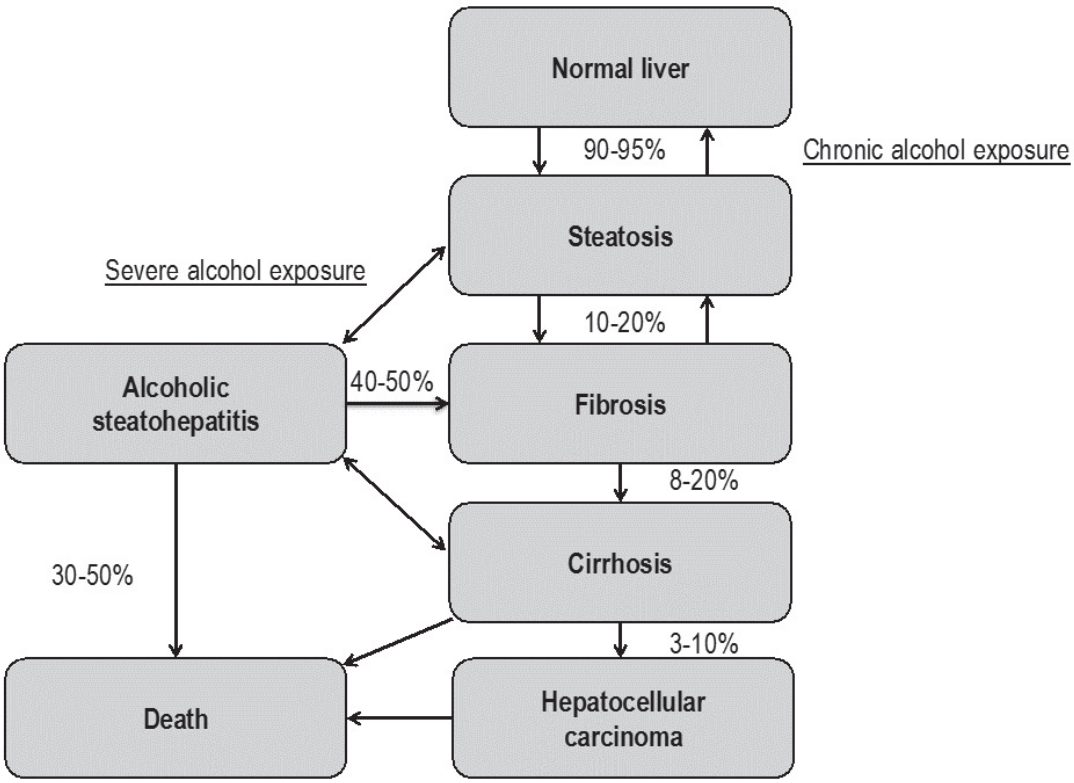

Figure 1. The course of alcoholic liver disease and the lifetime risk (%) of progression. The figure is modified from EASL Clinical Practice Guidelines. Management of alcoholic liver disease. J Hepatol 2012;57(2):399-420.

enzymes. Clinical picture of severe AH with ascites and liver failure can resemble decompensation of liver cirrhosis. Most patients with a severe form of AH have significant fibrosis or even underlying cirrhosis. In the latter case, it represents a form of acute-on-chronic liver failure (Menachery & Duseja, 2011). AH occurs in 10-35% of heavy drinkers and may lead to progressive fibrosis (Gao & Bataller, 2011). Diagnosis of definite ASH is based on liver biopsy, while probable or possible AH diagnosis can be made on a clinical basis (Singal *et al.*, 2018). Although liver biopsy is the gold standard in diagnosing AH and provides prognostic information, it is not widely used in clinical practice outside trials (Dhanda *et al.*, 2013). European guidelines recommend liver biopsy in uncertain cases of AH requiring specific medical therapy (EASL, 2018a). Examples of the histological picture of alcoholic liver cirrhosis and ASH are shown in figures 2 and 3.

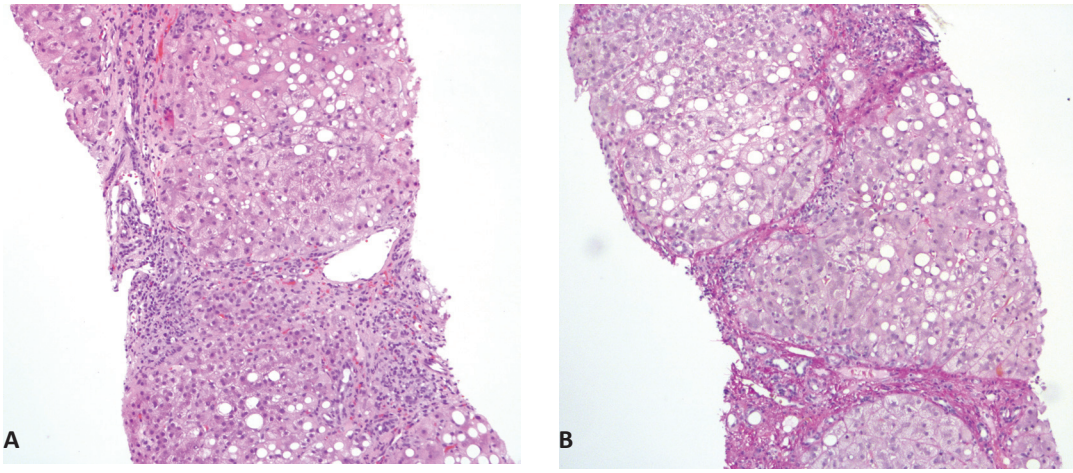

Figure 2. Microscopic picture of a liver biopsy in alcoholic liver cirrhosis showing parenchymal steatosis, fibrous septae and regenerative cirrhotic nodules, haematoxylin-eosin (A) and Herovici (B) stain. Photo by Dr Anu Mäkelä.

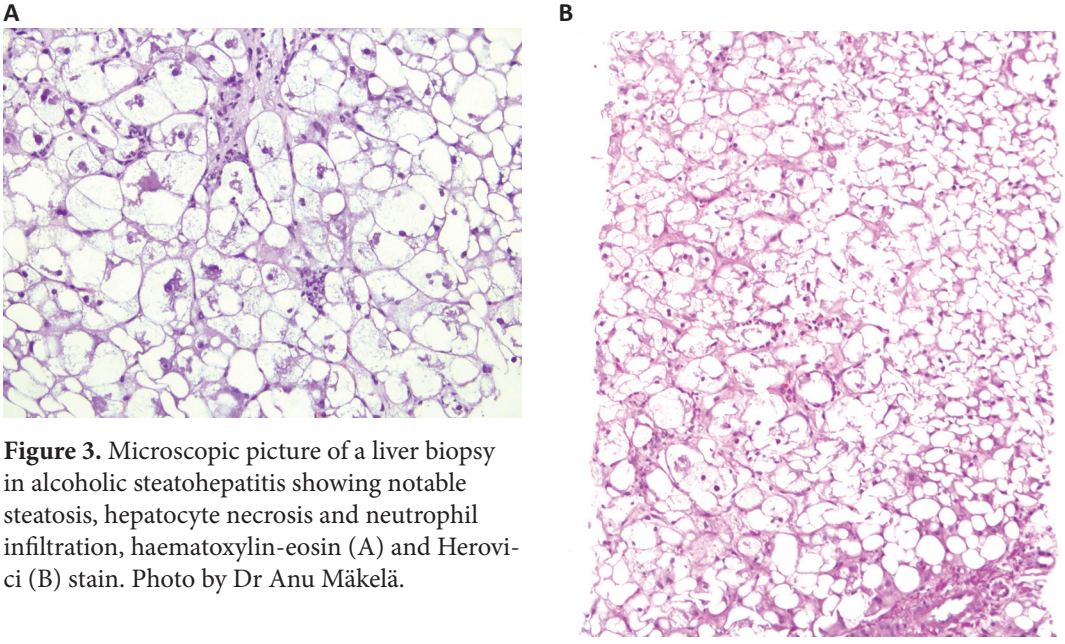

Figure 3. Microscopic picture of a liver biopsy in alcoholic steatohepatitis showing notable steatosis, hepatocyte necrosis and neutrophil infiltration, haematoxylin-eosin (A) and Herovici (B) stain. Photo by Dr Anu Mäkelä.

Epidemiology

Alcohol consumption of more than 60 g/day leads to a fatty liver in the great majority of patients, but in susceptible persons even less alcohol can cause fatty degeneration (O'Shea *et al.*, 2010). Thus, the majority of people drinking alcohol excessively are expected to have at least a mild form of ALD.

ALD can be defined as elevated liver enzymes in persons with a history of an excessive intake of alcohol. In a Korean study, the prevalence of ALD with such definition was 1.7% of the population, while 6,7% of people were defined as heavy alcohol consumers (Park *et al.*, 2011). Solid data about the prevalence of early stages of ALD in the general population is lacking globally, and true prevalence is hard to assess due to the asymptomatic nature of mild disease (Basra *et al.*, 2011). AH is present in 10%-35% of patients hospitalised due to alcoholic diseases (O'Shea *et al.*, 2010). Among hazardous drinkers, 15% have normal liver histology, steatosis is found in 27%, ASH in 24% and cirrhosis in 26% (Parker *et al.*, 2019).

Epidemiology of AH has been studied in a nationwide population-based study in Denmark (Sandahl *et al.*, 2011a). The annual incidence rate of AH requiring hospital admission was 46 per million in men and 34 per million in women. From the year 1999 to 2008 the increase of incidence of AH was 27% for men and 41% for women (Sandahl *et al.*, 2011a). However, there has been a decrease in ALD incidence in Denmark during recent years (Deleuran *et al.*, 2015). In another Danish study, the incidence of liver cirrhosis was 33/100,000 persons per year (Fialla *et al.*, 2012). Thus, cirrhosis seems to be a much more common form of ALD requiring hospitalisation than acute AH. During recent years, the incidence of AH-related hospitalisations has been increasing in the USA (Jinjuvadia *et al.*, 2015). In China, the incidence of severe AH increased by 2.43 times from 2002 to 2013 (Wang *et al.*, 2019).

The liver-related mortality correlates strongly with *per capita* alcohol consumption (Ramstedt, 2001; Polednak, 2012). In the USA, the ALD-related mortality decreased from 6.9/100,000 to 4.4/100,000 between the years 1980 and 2003 (Paula *et al.*, 2010). Alcohol consumption has reduced in many countries worldwide especially in countries with traditionally high liver-related mortality, and, as a result, worldwide mortality from liver cirrhosis has been declining during the past decades (Bosetti 2007). Unfortunately, in certain European countries the mortality is increasing. In Finland, Denmark and UK, the cirrhosis mortality increased among both men and women from the 1990s to 2002 (Bosetti *et al.*, 2007). In the beginning of this century, annual mortality from liver cirrhosis in Finland was 13.58/100,000 for men and 4.93/100,000 for women. There was a 9% increase in ten years in men and 5.6% in women. The mortality was especially high in the age group of 34-64 years; 34.33/100,000 for men and 11.78/100,000 for women. Unlike in many other countries, there was a rising trend in cirrhosis mortality among women; a 34% increase in a decade (Bosetti *et al.*, 2007). In 2004 major changes were made in Finnish alcohol policy. Alcohol taxes were cut, and limitations on the import of alcoholic beverages from the EU were reduced. As a result, alcohol consumption increased 10%, while alcohol-related liver deaths increased even by 46% (Mäkelä & Österberg, 2009).

Alcohol seems to be related to a significant proportion of deaths in Nordic countries. In the age group of 20-64 years, alcohol is related to 22% of all deaths in Finnish men and to 11% in Finnish women (Poikolainen *et al.*, 2008). The most important alcohol-related cause of death in Nordic countries is ALD. In Finland 5.9% of all causes of deaths among men and 4.6% among women are due to alcoholic liver cirrhosis. Alcoholic liver mortality in men is at the same level in Denmark but

lower in Sweden for both men and women (Poikolainen *et al.*, 2008). Interestingly, ALD is not the leading cause of death in the group of the most malignant alcoholics. In a Finnish study following heavy-drinking antisocial alcoholics, almost half of the patients died in 15 years but 57% of the deaths were violent. However, liver cirrhosis was the leading non-violent cause of death (Saarnio, 2005).

The burden of ALD for the health care system is significant and seems to be increasing. In Australia, a significant increase in hospitalisations due to AH was observed during 1993-2005. In the age group 20-29 years, there was a 10-fold increase in hospital admissions due to ALD (Liang *et al.*, 2011). In the USA, the rate of hospital admissions is 4.5/100,000 for AH and 13.7/100,000 for alcoholic liver cirrhosis (Yang *et al.*, 2008). Moreover, ALD causes higher in-patient costs compared to other alcohol-related hospital admissions in the USA (Heslin *et al.*, 2017).

Risk factors of alcoholic liver disease

ALD is caused by excessive consumption of ethyl alcohol. Thus, ethanol is the only essential aetiological factor needed for the development of this disease (Askgaard *et al.*, 2015). The risk is dose-dependent on the amount of alcohol consumed (Becker *et al.*, 1996). Most people consuming alcohol excessively develop a fatty liver, but only some of them will have cirrhosis (Bruha *et al.*, 2012). Women have a higher risk of ALD with similar alcohol intake compared to men at any level of alcohol consumption (Becker *et al.*, 1996). Daily alcohol intake exceeding 30 g ethanol per day was a risk factor for developing ALD among both sexes in an Italian study (Bellentani *et al.*, 1997). Traditionally, the threshold level for men is 30 g per day and 20 g per day for women (EASL, 2018a). However, the threshold of daily alcohol ingestion for developing ALD might be considerably lower. In a meta-analysis, alcohol consumption exceeding 12-24 g (1-2 doses) per day increased mortality from liver cirrhosis, and there were no distinct threshold amounts of alcohol for morbidity nor mortality from ALD (Rehm *et al.*, 2010). Hence, current guidelines conclude that if there is any threshold level of alcohol intake for developing ALD, it is very low (EASL, 2018a). In a recent follow-up study, a dose dependent increase in the risk of severe liver disease was demonstrated even among persons consuming less than 30 g of alcohol per day (Hagström *et al.*, 2018).

The effect of drinking patterns and type of alcohol consumed on the risk of ALD is controversial. It has been suggested that total alcohol intake consisting principally of wines would be less harmful for the liver (Becker *et al.*, 2002). However, there might be confounding factors related to other habits and lifestyle among wine drinkers, since persons consuming wine tend to consume more healthy food compared to beer and spirit drinkers (Johansen *et al.*, 2006).

Binge drinking (drinking too much too fast) seems to be a risk factor for ALD (Mathurin & Deltenre, 2009, Åberg *et al.*, 2017). However, daily drinking contributes to mortality from liver diseases more than heavy episodic drinking (Hatton *et al.*, 2009). Both overall consumption of alcohol and binge drinking have increased in Finland during recent years. Binge drinking is especially common in young age groups (Härkönen & Mäkelä, 2011). Besides binge drinking, alcohol consumption outside mealtimes is an independent risk factor for ALD (Bellentani *et al.*, 1997), especially among women (Simpson *et al.*, 2019).

The exact mechanisms as to why heavy alcohol consumers develop various stages of liver injury are poorly known. In addition to direct hepatotoxic effects of ethanol, its metabolite acetaldehyde affects structural elements of the hepatocyte, interferes with gene expression by interaction with DNA and

binds to various macromolecules inducing an immune response (Setshedi *et al.*, 2010). Alcohol metabolism in hepatocytes interferes with lipid metabolism by various mechanisms and results in intracellular lipid accumulation (You & Arteel, 2019). Progression from simple steatosis to ASH results from inflammatory response triggered by alcohol-induced cell death and gut microbes translocated via portal circulation into the liver (Gao *et al.*, 2019). Cell death of the hepatocytes and inflammation induce fibrogenesis leading to cirrhosis. Additionally, ethanol inhibits anti-fibrogenic mechanisms (Lackner & Tiniakos, 2019).

Various factors may have an impact on the risk of ALD. One putative explanation to this could be the difference between individuals in intracolonic microbial acetaldehyde production from ingested ethanol (Seitz *et al.*, 2005; Jokelainen *et al.*, 2000). Several genetic factors increase the risk of development of liver cirrhosis in persons with excessive alcohol consumption. Variations in genes regulating hepatic lipid metabolism, *i.e.* patatin-like phospholipase-containing domain 3 (PNPLA3), transmembrane 6 superfamily 2 (TM6SF2) and membrane bound O-acyltransferase domain containing 7 (MBOAT7) are related to increased risk of alcoholic liver cirrhosis (Tian *et al.*, 2010; Stickel *et al.*, 2011; Friedrich *et al.*, 2014; Buch *et al.*, 2015). Moreover, rs738409:G homozygosity in PNPLA3 increases the risk of mortality in severe AH (Atkinson *et al.*, 2017).

Traditionally, fatty liver is categorised as alcoholic and non-alcoholic. ALD is distinguished from non-alcoholic fatty liver disease (NAFLD) by history of alcohol intake exceeding certain threshold levels (O'Shea *et al.*, 2010). NAFLD and ALD share common histopathological features (Lefkowitch, 2005). Furthermore, many individuals consuming alcohol excessively might have risk factors of NAFLD. Excess body weight is an independent risk factor for all stages (steatosis, AH, cirrhosis) of ALD (Naveau *et al.*, 1997). In fact, coexistence of obesity and excessive alcohol consumption has a supra-additive effect on liver disease (Hart *et al.*, 2010).

Alcoholic liver disease and malignancies

Alcohol intake increases the risk of HCC (Trichopoulos *et al.*, 2011) and various other cancers (Bagnardi *et al.*, 2015). Liver cirrhosis, regardless of aetiology, is a risk factor for several malignant neoplasms (Sorensen *et al.*, 1998, Kalaitzakis *et al.*, 2011). The role of alcohol in the development of cancer is related to the carcinogenic metabolites of ethanol affecting DNA methylation (Varela-Rey *et al.*, 2013). Alcohol abuse may often coexist with unhealthy lifestyle and, e.g. smoking (Grucza *et al.*, 2005). Exact mechanisms behind the interaction between liver cirrhosis and carcinogenesis are not thoroughly understood.

Treatment

Medical treatment

Abstinence from alcohol is crucial in the treatment of ALD. In pure steatosis, the continuing alcohol intake is a risk factor for progression towards liver cirrhosis (Teli *et al.*, 1995). Complete abstinence is mandatory in every stage of ALD (Singal *et al.*, 2018).

In alcoholic liver cirrhosis, abstinence determines the long-term prognosis of the patient (Bell *et al.*, 2004; Masson *et al.*, 2014; Lackner *et al.*, 2017). Fortunately, even in decompensated disease, abstinence may render compensation (O'Shea *et al.*, 2010). Other treatment modalities focus merely on the complications of cirrhosis and portal hypertension. They include salt restriction, diuretics and tapping for ascites (EASL, 2018b), lactulose and antibiotics (*e.g.* rifaximin) in hepatic encephalopathy (Vilstrup *et al.*, 2014), albumin and terlipressin in hepatorenal syndrome (EASL, 2018b) and vasoactive medication (octreotide, terlipressin) along with endoscopic therapy (band ligation, cya-

noacrylate injection, oesophageal stenting) in varices (Garcia-Tsao *et al.*, 2017), and they do not differ from therapy of these complications in cirrhosis of other aetiologies.

Similarly, in AH abstinence is essential. It is the main determinant of the long-term prognosis of AH patients (Potts *et al.*, 2013a). In severe cases of AH, the short-term mortality is high, up to 30-50% (Cuthbert *et al.*, 2014; Liangpunsakul, 2011). Corticosteroid treatment has been used in the treatment of severe AH for several decades (Maddrey *et al.*, 1978). However, the efficacy of corticosteroids has become controversial. Several studies (Carithers *et al.*, 1989; Mathurin *et al.*, 2002; Mathurin *et al.*, 2011) have demonstrated the efficacy of corticosteroids in reduction of mortality, while other studies (Theodossi *et al.*, 1982; Rambaldi *et al.*, 2008) have failed to indicate any survival benefit. In a recent STOPAH-trial there was no difference in short- or long-term mortality between patients treated with prednisolone or placebo (Thursz *et al.*, 2015). Prednisolone seems to increase infections and infection-related mortality of AH patients (Vergis *et al.*, 2017). Nonetheless, meta-analyses including the results from STOPAH confirmed the beneficial effect of prednisolone in reducing short-term mortality (Njei *et al.*, 2016; Lee *et al.*, 2017). Current treatment guidelines recommend prednisolone in severe AH (Singal *et al.*, 2018) in order to reduce short-term mortality (EASL, 2018a). The outcome of the patients not responding to corticosteroid therapy is very poor (Mathurin *et al.*, 2011a). A recent Cochrane report concluded that the efficacy of corticosteroids in AH remains uncertain (Pavlov *et al.*, 2019).

Pentoxifylline has also been studied in the therapy for severe AH. In an initial placebo-controlled trial, it improved the outcome (Akriviadis *et al.*, 2000). In an open-label trial, pentoxifylline was inferior to prednisolone (Park *et al.*, 2014). A recent trial has proven it ineffective in reducing mortality (Thursz *et al.*, 2015). However, it might decrease the incidence of hepatorenal syndrome in AH patients when combined with prednisolone (Lee *et al.*, 2017).

Various other treatment modalities have been studied in severe AH, *e.g.* tumour-necrosis-factor-alpha antagonists. Both inflximab (Sharma *et al.*, 2009) and etanercept (Boetticher *et al.*, 2008) trials failed to show improvement in survival due to increased infection-related mortality related to these highly immunosuppressive therapies.

Acetylcysteine infusion improved short-term mortality in severe AH patients in a French trial but had no effect on long-term survival (Nguyen-Khac *et al.*, 2011).

There are conflicting results of the effect of intensive enteral nutrition on the survival of AH patients (Moreno *et al.*, 2016; Fialla *et al.*, 2015). It can be speculated that correction of poor nutritional status in general is beneficial for hospitalised alcoholic patients.

Potential treatment modalities in severe AH include granulocyte stimulating factor (Singh *et al.*, 2014) and granulocyte apheresis (Horie, 2012), but despite promising results in single trials their efficacy is still to be confirmed.

Infections play an important role in early mortality of AH patients (Louvet *et al.*, 2009). Consequently, vigorous screening and treatment of infections in essential (Karakike *et al.*, 2017). Corticosteroid treatment should be postponed until a severe infection is under control (EASL, 2018a). Systemic inflammatory response determines the short-term mortality in severe AH regardless of the presence of infection (Michelena *et al.*, 2015) suggesting that AH is, in fact, a systemic disease.

General supportive measures in treatment of acutely ill ALD patient include correction of malnutrition and fluid balance, substitution of thiamine and vigorous surveillance and treatment of infections (EASL, 2012). After an acute episode of AH, the patients should be referred to an addiction specialist (Thursz

& Morgan, 2016). Therapeutic measures are summarised in Table 1.

Liver transplantation in alcoholic liver disease

Liver transplantation is an option for a small minority of decompensated alcoholic liver cirrhosis patients. While alcohol is the leading cause of liver diseases, ALD is becoming more and more important indication for liver transplantation worldwide (Lucey, 2011). Although ALD is among the most common indications of liver transplantation in many countries, only a small minority of alcoholic cirrhosis patients are evaluated for transplantation. Pre-transplant abstinence is usually required for 6-12 months, depending on the transplantation centre. It is not clear if requirement for abstinence predicts post-transplant sobriety. One important indication for the period of abstinence is to give to the liver an opportunity for spontaneous recovery (Varma *et al.*, 2010). Long-term survival after liver transplantation for alcoholic liver cirrhosis seems to be good. Among almost ten thousand European transplant patients, survival rates were significantly higher than survival of patients transplanted for viral hepatitis or cryptogenic cirrhosis. (Burra *et al.*, 2010).

Cessation of alcohol consumption at the time of diagnosis of ALD predicts sustained alcohol abstinence (Altamirano *et al.*, 2012). Relapse rate of alcohol consumption is 39% among transplanted Finnish ALD patients (Koljonen *et al.*, 2015). In Sweden, 33% of patients continue to consume some amount of alcohol after transplantation due to ALD and 18% are abusive drinkers. Despite these numbers the survival rates at 1 and 5 years are comparable to patients transplanted for non-alcoholic liver cirrhosis (Björnsson *et al.*, 2005). In a German study, the relapse rate to drinking after transplantation for ALD was 19%. In this study pre-transplant sobriety of 6 months predicted long-term abstinence. Shorter pre-transplant abstinence period and certain social factors predicted relapse after transplantation. Abusive drinking was also associated with poor survival (Pfitzmann *et al.*, 2007). In another German study, the recurrence of abusive drinking was found in 27% of patients. 5-year survival was high regardless of alcohol abuse but after 10 years there was a significant difference favouring the abstinent patients (82% *versus* 68% 10-year survival) (Schmeding *et al.*, 2011). The deleterious effect of alcoholic relapse on the graft is confirmed in later studies (Erard-Poisont *et al.*, 2016, Dumotrier *et al.*, 2015). Hence, abusive consumption of alcohol impairs the long-term outcome after liver transplantation.

An acute AH is traditionally considered an absolute contraindication to transplantation (Varma *et al.*, 2010). This is partly due to suspected poor outcome and partly due to the requirement of a 6-12 -month abstinence which is not applicable in acute settings of severe AH with rapid deterioration of condition. However, these arguments have been recently questioned as data about the importance of the six-month abstinence rule is somewhat conflicting (Wu *et al.*, 2018). Additionally, outcomes of patients with a coincidental histological finding of acute AH in their explanted livers seem to be comparable to patients with cirrhosis only (Singal *et al.*, 2011). In the USA, the five-year survival in patients with AH was not different from patients with alcoholic cirrhosis (Singal *et al.*, 2012). Consequently, it has been suggested that liver transplantation could be an option for occasional carefully selected patients who fail intensive medical therapy and fulfil all other criteria, excluding a 6-month abstinence (Dureja & Lucey 2010; EASL, 2016). In a French study, the transplantation improved the survival of AH patients and the rate of recidivism was low (Mathurin *et al.*, 2011b). This is not necessarily generalisable to other countries and non-selected patient groups outside clinical trials. Liver transplantation in treatment of severe acute AH is still highly

Table 1. Key points in the management of acute presentations of alcoholic liver disease. (Based on various clinical practice guidelines from American College of Gastroenterology, American Association for the Study of Liver Diseases and European Association for the Study of the Liver). ALD – alcoholic liver disease, SBP – spontaneous bacterial peritonitis.

<table>
<tr><th colspan="2">Alcoholic hepatitis</th><th>Decompensated alcoholic liver cirrhosis</th></tr>
<tr><td colspan="3">▪ Screen for infections
▪ Evaluate and monitor renal function
▪ Assess and correct fluid balance, electrolyte disturbances and nutritional status
▪ Administer intravenous thiamine substitution</td></tr>
<tr><td colspan="2">▪ Confirm diagnosis
o clinical suspicion
o exclusion of obstructive jaundice (imaging)
o exclude other liver diseases as necessary
o consider liver biopsy
▪ Define severity
o clinical scoring systems (Maddrey, GAHS, ABIC)
▪ Consider corticosteroids in a severe case
o evaluate the response after 7 days (Lille, ECBL)
o discontinue if no response</td><td>▪ Recognise precipitating factors of decompensation
▪ Treat complications of portal hypertension as necessary:
o <u>Ascites</u>
• tapping
• diuretics
• antibiotics and albumin for SBP
o <u>Varices and variceal bleeding</u>
• vasoactives (octretiode, terlipressin) in acute bleeding
• upper endoscopy
• banding for oesophageal varices
• cyanoacrylate for gastric varices
o <u>Encephalopathy</u>
• lactulose
• antibiotics (quinolones, rifaximin)
o <u>Hepatorenal syndrome (type 1)</u>
• albumin
• terlipressin
▪ No specific pharmacotherapy available for alcoholic cirrhosis</td></tr>
<tr><td colspan="3">▪ Evaluate and treat alcohol withdrawal syndrome
▪ Demand <u>total abstinence</u>
▪ Assess alcohol dependency
o consult addiction specialist
o consider pharmacotherapy (only baclofen is safe in advanced ALD)</td></tr>
</table>

debatable (Fung 2017; Im *et al.*, 2019). New treatment modalities are desperately needed in severe AH (Louvet & Mathurin, 2015).

Assessment of prognosis in alcoholic liver disease

Alcoholic liver cirrhosis

The five-year mortality of all cirrhosis patients is approximately 60%, and the higher mortality is related to male gender and alcoholic aetiology of the cirrhosis (Fialla *et al.*, 2012). The clinical course and prognosis of alcoholic liver cirrhosis seems to be related to a prevalence of complications at the time of diagnosis and development of complications during the course of the disease (D'Amico *et al.*, 2006). In Danish population-based study, the one-year mortality in alcoholic liver cirrhosis patients varied from 17% to 64% depending on the number of complications, and the five-year mortality was between 58% and 85% (Jepsen *et al.*, 2010). The course of the alcoholic liver cirrhosis and pattern of decompensation also seem to differ from patients with cirrhosis of other aetiology (Wiegand *et al.*, 2012). The long-term prognosis of alcoholic liver cirrhosis seems very pessimistic. A poor prognosis of the disease is strongly related to continuing alcohol consumption, but considerable improvement is often seen after total abstinence, even in patients with hepatic decompensation (O'Shea *et al.*, 2010). Abstinence from alcohol improves the outcome at all stages of ALD. Early abstinence immediately after diagnosis of alcoholic liver cirrhosis is the key factor in the long-term prognosis (Verrill *et al.*, 2009). The survival of patients with end-stage liver cirrhosis can be estimated with the Model for End-Stage Liver Disease (MELD) consisting of serum bilirubin and creatinine levels, International Normalised Ratio (INR) for prothrombin time, and aetiology of liver disease (Kamath *et al.*, 2001).

Alcoholic hepatitis

The most severe form of ALD is AH. Both the short-term and long-term prognosis of this condition is poor. Mortality at 30 days is 15%-30% and 40% at 1 year. In the most severe forms of AH, the 1-month mortality can be up to 50%-60%. More than 1/3 of the survivors will develop cirrhosis later (Basra *et al.*, 2011).

In a Danish nationwide population-based study, the 28-day mortality after hospitalisation due to an episode of AH was 15%, while mortality rates at day 84 and 5 years were 24% and 56%, respectively. The short-term prognosis seems to be worsening mostly because patients nowadays are older at the time of diagnosis (Sandahl *et al.*, 2011). Mortality rates in randomised trials are high for placebo-treated patients. The overall mortality rate in median time of 160 days is 34% and one-month mortality approximately 20%. The main causes of death among these patients are hepatic failure, gastrointestinal bleeding and infection (Yu *et al.*, 2010). There was no improvement in survival over time in a study comparing outcomes of AH patients in randomised trials between the years 1971 and 2016 (Hughes *et al.*, 2018). The prognosis of recurrent AH due to alcohol recidivism is especially poor with the 1-month mortality being approximately 60% (Potts *et al.*, 2013 b).

Short-term mortality in AH seems to be related to the severity of the disease, whereas long-term prognosis is determined by abstinence from alcohol (Louvet *et al.*, 2017). Several prognostic models have been created to predict the outcome in AH. Only patients with severe disease benefit from medical therapy. Mild cases do not need corticosteroids (*i.e.* there is a threshold for treatment) and it is also suggested that extremely severe cases may get more harm than benefit from corticosteroids (*i.e.* a possible ceiling effect) (O'Shea *et al.*, 2010). Clinical scoring systems are needed to identify patients who benefit from medical treatment, to predict outcome and to define a stopping rule for non-responding patients.

In Maddrey's pioneering corticosteroid study, severe AH was defined by using Maddrey's discriminant factor (Maddrey *et al.*, 1978). The formula was later modified, and the current formula is: 4.6 x (patient's prothrombin time in seconds – control prothrombin time in seconds) + total bilirubin (mg/dL). A value over 32 implies severe disease. A score less than 32 is related to a 93% survival at day 28 without corticosteroid therapy. The formula was adapted into the Finnish practice by Julkunen: 300 x (INR – 1) + bilirubin (µmol/l). Severe disease is defined by a value over 300 and is used as an indication for corticosteroid therapy (Julkunen, 2003).

Another prognostic model is the Glasgow alcoholic hepatitis score (GAHS). It consists of the patient's age, white cell count, urea, INR and bilirubin. In the initial study, GAHS seemed to be more accurate in predicting mortality at 28 and 84 days and was more specific for death. Patients with a GAHS score greater than or equal to 9 have a very poor prognosis without corticosteroid treatment. Patients with GAHS less than 9 do not benefit from corticosteroid even if their modified Maddrey's score is over 32 (Forrest *et al.*, 2007).

A simple method for identification of non-responders to corticosteroid therapy and predicting poor outcome was created by Mathurin. Patients who had a decrease in their bilirubin level after seven days of treatment with corticosteroids had 83% survival at 6 months while patients with no change or increasing bilirubin level had only 23% survival. Early change in the bilirubin level (ECBL) is a very simple prognostic model for assessing the response to corticosteroid therapy (Mathurin *et al.*, 2003).

A more complex scoring system is the so-called Lille model which takes into account liver synthesis capacity (prothrombin time or INR and albumin), renal function (creatinine) and age of the patient combined with the change in bilirubin level in seven days. The cut-off level for defining non-response to corticosteroids is 0.45. Patients above this level have only 25% survival in 6 months while

	Bilirubin	P-TT/INR	Crea/Urea	Leukocytes	Age	Albumin	Decrease in bilirubin
Maddrey	+	+	–	–	–	–	–
MELD	+	+	+	–	–	–	–
GAHS	+	+	+	+	+	–	–
ABIC	+	+	+	–	+	+	–
Lille score	+	+	+	–	+	+	+
ECBL	+	–	–	–	–	–	+

P-TT	Prothrombin time
INR	International Normalised Ratio
MELD	Model-for-End-Stage-Liver-Disease score
GAHS	Glasgow Alcoholic Hepatitis Score
ABIC	Age, Bilirubin, INR and serum Creatinine score
ECBL	Early Change of Bilirubin Level

Figure 4. Comparison of various prognostic models in alcoholic hepatitis. The figure is adapted from Mathurin P, Lucey M: Management of alcoholic hepatitis. J Hepatol 2012;56:S39-45.

patients below this level have 85% survival. In a large cohort, 40% of the patients with severe AH were non-responders defined by the Lille model (Louvet *et al.*, 2007). A recent study showed that the Lille score at day 4 predicts the response as accurately as at day 7, suggesting that the decision of the continuation of corticosteroid therapy might be done at an earlier time point (Garcia-Saenz-de-Sicilia *et al.*, 2017).

The ABIC (Age, Bilirubin, INR and Creatinine) score consists of age, serum bilirubin, INR and serum creatinine: (age x 0.1) + (serum bilirubin in mg/dL x 0.08) + (serum creatinine in mg/dL x 0.3) + (INR x 0.8). By using cut-off values of 6.71 and 9.0 patients can be grouped into low, intermediate and high risk of death at 90 days with survival rates 100%, 70% and 25%, respectively (Dominguez *et al.*, 2008).

The MELD score determined at admission (with cut-off value 18) and after 1 week (with cut-off value 20) predicts in-hospital mortality of severe AH patients (Srikureja *et al.*, 2005).

A Danish study compared the predictive performances of GAHS, Lille model and ABIC score in clinical practice. All the prognostic models had similar performance in predicting the actual survival (Sandahl *et al.*, 2011b). However, recent data from the STOPAH-trial suggests that other scores than Maddrey's discriminant function are superior in assessment of the prognosis (Forrest *et al.*, 2017). Various prognostic models are compared in Figure 4.

Certain histological features are related to the prognosis of AH. Polymorphonuclear infiltration reflecting hepatic regeneration is related to better outcome, whereas advanced fibrosis is related to higher mortality (Altamirano *et al.*, 2014).

Non-cholesterol sterols in liver diseases

Cholesterol in the human body originates from dietary absorption and from endogenous synthesis in tissues, especially in the liver. Serum concentrations of cholesterol, non-cholesterol sterols, including cholesterol precursors, cholestanol, and phytosterols can be measured by gas-liquid chromatography (GLC). Non-cholesterol sterols are either precursors of cholesterol in endogenous synthesis (lanosterol, cholestenol, desmosterol and lathosterol) or absorbed phytosterols from digested plants (*e.g.* sitosterol, campesterol, avenasterol and stigmasterol). Consequently, the former serve as surrogate markers of cholesterol synthesis and reflect the synthesis function of the liver, while the latter are surrogates of absorption (Tilvis & Miettinen 1986, Miettinen *et al.*, 1990, Miettinen *et al.*, 1989, Nissinen *et al.*, 2008). Moreover, since certain non-cholesterol sterols (*e.g.* cholestanol) are secreted into the bile, they are surrogate markers of cholestasis, as demonstrated in primary biliary cholangitis (PBC) (Gylling *et al.*, 1996). Furthermore, serum sterol parameters indicate the severity and prognosis of PBC and acute liver failure (Nikkilä *et al.*, 2005, Nikkilä *et al.*, 2008). Serum desmosterol levels are also associated with inflammation in non-alcoholic steatohepatitis (NASH) (Simonen *et al.*, 2013). Thus, measurement of serum levels of non-cholesterol sterols provides potential means to examine the pathophysiology of liver diseases of various aetiologies. Since the levels of lipoproteins carrying non-cholesterol sterols vary between individuals, sterols are usually reported as a ratio to cholesterol (MacKay & Jones, 2012).

3. AIMS OF THE STUDY

This study examines epidemiological and prognostic aspects of ALD including incidence, survival, cause-specific mortality, associated malignancies and clinical prognostic factors as well as risk factors of ALD.

The specific aims of the study were:

1. To investigate the epidemiology of ALD in Finland:
    - incidence of ALD
    - changes in incidence of ALD in Finland over time
    - survival of AH and alcoholic liver cirrhosis patients
    - causes of death among ALD patients
2. To investigate incidence of malignant neoplasms among ALD patients
3. To examine alterations in cholesterol metabolism in severe AH, and to identify potential prognostic factors to predict response to corticosteroid therapy at baseline
4. To determine potential risk factors besides alcohol for advanced liver disease separately in men and women, and to assess interaction of alcohol intake and various other risk factors for advanced liver disease

4. PATIENTS AND METHODS

This study was carried out in the Clinic of Gastroenterology at Helsinki University Hospital. In Studies I and II, a cohort of patients with ALD requiring hospitalisation during the years 1996-2012 was identified from the national Inpatient Register Database (HILMO) of the National Institute for Health and Welfare.

In Study III, 24 hospitalised patients with severe AH during years 2015-16 were enrolled from Peijas Hospital, Jorvi Hospital, Porvoo Hospital and Kuopio University Hospital. Control groups consisted of 124 randomly selected healthy persons and 156 patients with primary sclerosing cholangitis (PSC).

In Study IV, the study cohort (41260 individuals) comprised of the participants of two population-based surveys (FINRISK and Health2000) during the years 1992-2012 representing Finnish general population.

The Helsinki University Central Hospital Scientific Board approved all the studies. For Studies I and II an approval from the National Institute of Health and Welfare was obtained. In addition, we obtained an approval from the Finnish Cancer Registry for Study II and from the Statistics Finland for Study I. The Ethics Committee of the hospital district of Helsinki and Uusimaa and Finnish Medicines Agency (Fimea) approved Study III. The study protocol conforms to the ethical guidelines of the 1975 declaration of Helsinki. All patients in Study III gave their informed consent. For Study IV an approval of the Coordinating Ethical Committee of the Helsinki and Uusimaa Hospital District was obtained.

Studies I and II

All patients during years 1996-2012 with ALD as a primary or secondary diagnosis were identified from the national Inpatient Registry of the National Institute for Health and Welfare. The 10th revision of the International Classification of Diseases (ICD-10) has been used since 1996. AH was defined by ICD-10 codes K70.1 and K70.4 and liver cirrhosis by codes K70.3 and K70.2. A validation procedure was performed by checking the correspondence of the diagnosis between the hospital record and the inpatient registry in a randomly selected group of 106 patients. The diagnosis was accurate in 99% of cases.

Table 2. Study characteristics

	n	SUBJECTS	STUDY DESIGN	INTERVENTION	PRIMARY ENDPOINT
I	11875	Persons in HILMO database with ALD as in-patient diagnosis	Retrospective registry study	None	Death
II	11875	Persons in HILMO database with ALD as in-patient diagnosis	Retrospective registry study	None	Cancer diagnosis
III	24	AH patients	Prospective intervention study	Prednisolone Ciprofloxacin	Death, steroid response assessed with the Lille model
IV	41260	Persons in Health2000 and FINRISK studies	Cohort study	None	Severe liver events

The cohort consisted of 8798 male and 3077 female patients (Table 3).

In Study I the incidence of AH and alcoholic liver cirrhosis was determined by dividing the observed number of cases with ALD as a primary or secondary first-time diagnosis by mean population of each year obtained from the Statistics Finland. To exclude prevalent cases diagnosed during years before the study period, only patients since the year 2001 were included in the incidence analysis. The incidence rates were standardised to the European standard population (www.who.int/health-info/paper31.pdf).

The time of death and causes of death were obtained from Statistics Finland. The standardised mortality ratio (SMR) was calculated by dividing the observed number of deaths in each category by the number of deaths in the general population.

The relative survival ratio was calculated by dividing the observed survival of the patients with the expected survival derived from mortality ratios of the Finnish population stratified with age and sex by using the Ederer II method.

In Study II, the cohort was combined with the database of the Finnish Cancer Registry. The incidence of cancers in the cohort was compared to the incidence in the general population.

The calculations were carried out with R software (R® Development Core Team 2015) using popEpi package.

Study III

During the years 2015-2016 twenty-four patients hospitalised with severe AH were enrolled in the study from 4 hospitals, *i.e.* Peijas Hospital (Vantaa, Finland), Jorvi Hospital (Espoo, Finland), Porvoo Hospital (Porvoo, Finland) and Kuopio University Hospital (Kuopio, Finland). The original study design was an intervention study to investigate the effect of ciprofloxacin in addition to prednisolone therapy on the survival of patients with severe AH. The goal was 150 patients. However, the recruitment of the patients turned out to be extremely slow, and the drop-out rate was high. Therefore, the study had to be terminated. The study samples were utilised to examine the metabolism of non-cholesterol sterols in severe AH.

AH was diagnosed on a clinical basis, and the diagnosis was confirmed histologically by liver biopsies in patients who gave their consent to the biopsy and did not have contraindications (9 patients out of 24). The severity of AH was defined by using the modified Maddrey score (Julkunen, 2003). Only patients with a modified Maddrey score over 300 were included. Exclusion criteria were age

Table 3. Number of male and female alcoholic liver disease patients under followup (N) and number of person-years at risk in 1996–2013, by age.

	Total	Males		Females	
	N	N	Person-years	N	Person-years
Age[a]	11877	8798	29127.8	3077	12105
< 30 years	93	67	148.4	26	93.2
30-44 years	1496	1100	3069	396	1093.6
45-59 years	5992	4450	14242.8	1542	5354.6
60-74 years	3862	2866	10411.0	996	4900.1
>74 years	434	315	1256.3	119	677.6

[a] Age of persons defined at the beginning of follow-up

under 18 or over 65, recent gastrointestinal haemorrhage (within 4 days before recruitment), uncontrolled bacterial infection, acute or chronic viral hepatitis A, B or C, autoimmune hepatitis, metabolic liver disease other than ALD (i.e. abnormal results in tests for hepatitis C virus antibodies, hepatitis B surface antigen, class IgM hepatitis A antibodies, antinuclear antibodies, anti-smooth-muscle antibodies, antimitochondrial antibodies, liver-kidney microsomal antibodies, serum immunoglobulin G, serum transferrin saturation, serum ceruloplasmin or serum antitrypsin), significant renal insufficiency (serum creatinine level over 150 μmol/l), malignancy and mental impairment. Patients were randomised to receive either prednisolone treatment alone or prednisolone combined with ciprofloxacin. The dose of prednisolone was 40 mg once a day. The effect of prednisolone therapy was assessed after 7 days by using the Lille model. Prednisolone was discontinued in patients whose Lille score was 0.45 or above at day 7. Otherwise, the treatment continued for 4 weeks and was tapered down subsequently. The dose of ciprofloxacin was 500 mg twice a day. All patients in the ciprofloxacin group received the full regimen for 3 months regardless of steroid response assesses by the Lille model.

Blood and serum samples were collected after initiation of the treatment. Additional serum samples were collected after 7, 30, 90 and 180 days of treatment.

he polymorphism of the PNPLA3 gene was determined from the blood samples in United Medix Laboratories Ltd by genotyping with the Taqman® polymerase chain reaction method.

The levels of serum lipids, non-cholesterol sterols and squalene were analysed from the serum samples using GLC (Agilent Technologies, Wilmington, DE, USA) as described by Miettinen (1988). The procedure uses 5-alpha-cholestane as the internal standard, and it measures the serum concentrations of squalene, cholesterol, cholestanol, cholestenol, desmosterol, lathosterol, campesterol, sitosterol, stigmasterol and avenasterol, in this order of retention time. Since serum levels of lipoproteins transporting non-cholesterol sterols vary, the values were calculated as ratios to 100 x mmol/mol of cholesterol measured in the same GLC run.

The serum lipid and non-cholesterol sterol results were compared with control groups of 124 healthy individuals with similar age randomly selected from the Finnish Population Register Centre and 156 patients with PSC, whose samples were collected when they visited Helsinki University Hospital for endoscopic retrograde cholangiography.

Statistical analyses were performed using SPSS software (IBM® *SPSS* Statistics®, Armonk, New York, USA) and the Number Crunching Statistical Software® (NCSS®, Statistical Solutions Ltd., 2007, Kaysville, Utah, USA). The descriptive statistics are shown as median, range, numbers and percentages. The differences between groups were analysed by the Chi-square test, Student's t-test, Mann-Whitney's U-test, Fisher's exact test and analysis of variance (ANOVA) as appropriate. Correlations were analysed by the Pearson's correlation test and Spearman rank test as appropriate. Multivariate linear regression analysis was used in identifying variables predicting the Lille score. p-values <0.05 were considered statistically significant.

Study IV

The study population consisted of individuals in two population-based cohorts, FINRISK and Health2000. The mean follow-up was 12.4 years (511789 person-years). The FINRISK survey was carried out among a randomly selected cohort representing the entire Finnish population from 1972 until 2012 every five years by interviews and health examinations. Health2000 was a comprehensive study with health interviews and examinations including

questionnaires, physiological measurements and blood samples carried out in 2000-2001. FINRISK cohorts from 1992, 1997, 2002, 2007 and 2012 were included into this study. Lifestyle habits of the individuals were collected from the questionnaires. Average alcohol consumption, frequency of physical exercise and consumption of coffee were determined. The presence of diabetes and hypertension were recorded. A diagnosis of liver disease (ICD8: 571.0, 571.8, 571.9, 573.0, 573.9; ICD-9: 571.1, 571.2, 571.3, 571.5, 571.8; ICD10: K70.1, K70.2, K70.3, K70.4, K70.9, K72.0, K72.1, K72.9, K74.0, K74.1, K74.2, K74.6), liver cancer (C22.0) and liver related death (ICD8/9:570-573, 155.0; ICD10: K70-K77, C22.0) were collected by linking the cohort with the national inpatient registry from the Institute of Health and Welfare and the Cause of Death Registry from Statistics Finland. Persons with any liver diagnosis at baseline were excluded.

All individuals in the study had given their informed consent for future registry linkage.

A cox regression analysis was performed separately for both sexes with a severe liver event (advanced liver disease, liver cancer or liver-related death) as an endpoint and age, marital status, education (low, average, high), employment (part- or full-time employment, retired, other), diabetes, waist-hip ratio (WHR), physical exercise, smoking status (former, current, never), alcohol consumption status (lifetime abstainer, current abstainer, user), average daily alcohol consumption (10 g of ethanol dose), frequency of binge drinking (weekly, monthly, less often), serum levels of non-HDL cholesterol, HDL cholesterol and triglycerides, elevated blood pressure, mutations in patatin-like phospholipase-containing domain 3 (PNPLA3), transmembrane 6 superfamily 2 (TM6SF2) HFE C282Y and H63D as independent variables using a backward stepwise elimination process. All independent risk factors were further tested in subgroup analyses (diabetes, alcohol intake, WHR median, BMI, marital status and PNPLA3 carrier status). Alcohol-related (ICD8: 571.0; ICD-9: 571.1, 571.2, 571.3; ICD10:K70.1, K70.2, K70.3, K70.4, K70.9) and non-alcoholic diseases (ICD8: 571.8, k71.9, 573.0, 573.9; ICD-9: 571.5, 571.8; ICD10: K72.0, K72.1, K72.9, K74.0, K74.1, K74.2, K74.6) as an outcome were further analysed with fixed-model Cox regression analysis using the risk factors from the primary analysis as independent variables. Analyses were performed with SAS, SPSS and R software.

5. RESULTS

Incidence of alcoholic liver disease

There was 66% increase in the incidence of alcoholic liver cirrhosis among men from year 2001 to year 2012. The incidence among men increased from 8.8/100 000 to 14.6/100 000. Among women, the increase of incidence was 75% from 2.4/100 000 to 4.2/100 000. The incidence of AH increased 76% among men from 3.7/100 000 to 6.5/100 000. The increase was highest in AH among women (108%) from 1.3/100 000 to 2.7/100 000.

Survival of alcoholic liver disease patients

The 5-year absolute survival rates were 0.37 (95%CI: 0.36-0.38) for all ALD patients, 0.29 (95% CI: 0.28-0.30) for alcoholic liver cirrhosis patients and 0.46 (95% CI: 0.44-0.48) for AH patients. The relative 5-year survival rates were 0.35 (95% CI: 0.34-0.37), 0.31 (95% CI: 0.30-0.32) and 0.48 (95% CI: 0.46-0.50), respectively. AH patients had better survival compared to the alcoholic liver cirrhosis patients. The relative survival of male alcoholic liver cirrhosis patients was worse compared to female patients: 5-year survival rates were 0.28 (95%CI 0.27-0.30) and 0.39 (95%CI 0.36-0.41), respectively. There was no difference in survival between men and women in AH. The age-standardised cumulative survival of alcoholic liver cirrhosis and AH patients is presented in Figure 5. The age patterns between cirrhosis and hepatitis patients and between men and women were so similar that age adjustment of the survival ratios did not lead to visible changes of the cumulative relative survival curves compared to unadjusted figures. The total number of person-years during the follow- up was 8468.3.

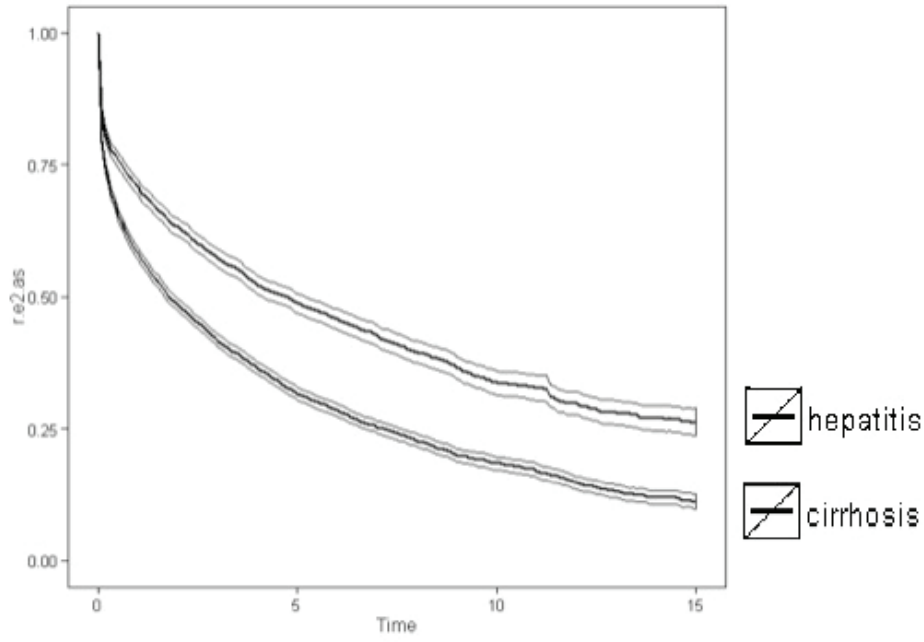

Figure 5. Age-standardised relative survival figures of patients with alcoholic hepatitis and alcoholic liver cirrhosis (time in years).

Causes of death among alcoholic liver disease patients

8440 patients died during the follow-up. The number of expected deaths was 423.6. Consequently, the SMR for death was 19.93 (95%CI 19.50-20.35). The majority (5485 out of 8440 deaths, *i.e.* 65%) were alcohol-related, presumably from ALD itself. Other diseases causing deaths among the cohort were cancers (SMR 6.82, 95%CI 6.35-7.29), digestive diseases (SMR 27.95; 95%CI 24.78–31.31), respiratory diseases (SMR 7.86; 95%CI 6.70–9.10) and circulatory diseases (SMR 6.13; 95%CI 5.74–6.52). The risk of death from accident or violence was increased (11.12; 95%CI 10.13-12.15). The causes of death with corresponding SMRs for male patients are presented in Table 4, and for female patients in Table 5, respectively. The SMR for death from any cause was higher among women (SMR 25.87; 95%CI 24.75-27.00 versus 18.58; 95%CI 18.13-19.03). The SMR was higher among men for malignant neoplasms (SMR 7.29; 95%CI 6.74-7.85 versus 5.31; 95%CI 4.50-6.19) and diabetes (SMR 11.92; 95%CI 8.76-15.85 versus 9.96; 95%CI 4.43-18.39). The SMR was higher among women for respiratory disease (SMR 13.34; 95%CI 9.70-17.91 versus 6.83; 95%CI 5.66-8.10), circulatory disease (SMR 7.91; 95%CI 6.82-9.07 versus 5.80; 95%CI 5.39-6.22) non-alcoholic digestive disease (SMR 38.92;95%CI 31.26-47.90 versus 24.72; 95%CI 21.35-28.34) and accidents and violence (SMR 18.81; 95%CI 15.18-22.03 versus 10.09; 95%CI 9.09-11.14).

Table 4. Observed (Obs) and expected (Exp) numbers of deaths of various causes and standardised mortality ratios (SMR) with 95% confidence intervals (CI) among Finnish male patients with alcoholic liver disease patients in 1996-2013.

Cause of death	Obs	Exp	SMR	95% CI
TOTAL DEATHS	6415	345.30	18.58	18.13 – 19.03***
ALL DISEASES	6027	307.18	19.62	19.13 – 20.11***
Specific infections				
Tuberculosis	2	0.42	4.79	0.58 – 17.30NS
Malignant neoplasms	652	89.44	7.29	6.74 – 7.85***
Oral and pharyngeal	26	2.07	12.55	8.30 – 18.38***
Oesophagus	31	2.77	11.17	7.59-15.86***
Colon cancer	20	4.96	4.03	3.46 – 6.22***
Rectal cancer	15	3.30	4.54	2.54 – 7.49***
Liver cancer	319	3.84	83.11	74.24 – 92.48***
Pancreatic cancer	43	7.49	5.74	4.15 – 7.73***
Cancer of lung and larynx	83	24.92	3.33	2.65 – 4.12***
Prostate cancer	18	8.89	2.02	1.20 – 3.20**
Kidney cancer	10	3.21	3.11	1.49 – 5.72**
Cancer of urinary bladder	9	2.16	4.16	1.90 – 7.90***
Diabetes	47	3.94	11.92	8.76 – 15.85***
Dementia[a]	29	8.95	3.24	2.12 – 4.65***
Circulatory system	741	127.84	5.80	5.39 – 6.22***
Ischaemic heart disease	382	81.64	4.68	4.22 – 5.16***
Other heart diseases	139	12.37	11.24	9.45 – 13.18***
Cerebrovascular disease	168	21.55	7.80	6.66 – 9.01***
Respiratory system	120	17.57	6.83	5.66 – 8.10***
Influenza	1	0.16	6.22	0.16 – 34.63NS
Pneumonia	49	5.30	9.24	6.84 – 12.21***
COPD[b]	49	9.51	5.15	3.81 – 6.81***
Asthma	4	0.31	12.92	3.52 – 33.07***
Digestive diseases (non-alcohol)	192	7.77	24.72	21.35 – 28.34***
Genitourinary diseases	18	1.46	12.29	7.28 – 19.42***
Alcohol related disease[c]	4110	33.70	121.95	118.25 - 125***
ACCIDENTS AND VIOLENCE	372	36.87	10.09	9.09 – 11.14***
All accidents	289	23.23	12.44	11.05 – 13.91***
Traffic	24	2.56	9.38	6.01 – 13.95***
Accidental falls	151	8.67	17.41	14.74 – 20.29***
Drowning	8	1.88	4.25	1.84 – 8.37***
Poisoning (non-alcohol)	31	2.54	12.21	8.30 – 17.33***
Suicide	55	11.45	4.80	3.62 – 6.25***
Assault	15	1.14	13.15	7.36 – 21.68***

$p < 0.05$ ** $p < 0.01$ *** $p < 0.001$ NS – Non-significant

[a] including Alzheimer's disease [b] Chronic obstructive pulmonary disease [c] Including alcohol poisoning

Table 5. Observed (Obs) and expected (Exp) numbers of deaths of various causes and standardised mortality ratios (SMR) with 95% confidence intervals (CI) among Finnish female patients with alcoholic liver disease patients in 1996-2013.

Cause of death	Obs	Exp	SMR	95% CI
TOTAL DEATHS	2025	78.29	25.87	24.75 – 27.00***
ALL DISEASES	1931	73.09	26.42	25.25 – 27.60***
Specific infections				
Tuberculosis	2	0.11	18.73	2.27 – 67.66*
Malignant neoplasms	150	28.23	5.31	4.50 – 6.19***
Oral and pharyngeal	9	0.32	28.03	12.82 – 53.21***
Oesophagus	9	0.34	26.14	11.96 – 49.62***
Colon cancer	8	1.79	4.47	1.93 – 8.80**
Rectal cancer	3	0.78	3.86	0.80 – 11.27NS
Liver cancer	46	0.79	58.33	42.71 – 77.80***
Pancreatic cancer	11	2.52	4.37	2.18 – 7.82***
Cancer of lung and larynx	20	3.68	5.43	3.32 – 8.38***
Breast cancer	19	5.54	3.43	2.07 – 5.35***
Cancer of cervix uteri	2	0.31	6.47	0.78 – 23.35NS
Cancer of uterus	2	0.89	2.25	0.27 – 8.11NS
Kidney cancer	1	0.75	1.32	0.03 – 7.39NS
Cancer of urinary bladder	2	0.24	8.37	1.01 – 30.25*
Diabetes	9	0.93	9.69	4.43 – 18.39***
Dementia[a]	15	4.33	3.47	1.94 – 5.71***
Circulatory system	189	23.90	7.91	6.82 – 9.07***
Ischaemic heart disease	80	12.09	6.62	5.25 – 8.23***
Other heart diseases	20	2.50	8.02	4.90 – 12.37***
Cerebrovascular disease	71	6.56	10.83	8.46 – 13.65***
Respiratory system	44	3.30	13.34	9.70 – 17.91***
Influenza	1	0.05	21.39	0.54 – 119NS
Pneumonia	18	1.18	15.31	9.07 – 24.19***
COPD[b]	23	1.39	16.53	10.48 – 24.80***
Asthma	1	0.21	4.67	0.12 – 26.02NS
Digestive diseases (non-alcohol)	89	2.29	38.92	31.26 – 47.90***
Genitourinary diseases	11	0.55	19.83	9.90 – 35.48***
Alcohol related disease[c]	1375	3.71	370.49	351.16 - 390***
ACCIDENTS AND VIOLENCE	93	4.95	18.81	15.18 – 22.03***
All accidents	75	3.11	24.13	18.98 – 30.25***
Traffic	1	0.42	2.37	0.06 – 13.22NS
Accidental falls	49	1.22	40.01	29.60 – 52.89***
Drowning	3	0.16	18.30	3.77 – 53.48**
Poisoning (non-alcohol)	12	0.56	21.52	11.12 – 37.59***
Suicide	10	1.55	6.45	3.09 – 11.86***
Assault	2	0.16	12.17	1.47 – 43.97*

$p < 0.05$ ** $p < 0.01$ *** $p < 0.001$ NS – Non-significant

[a] including Alzheimer's disease [b] Chronic obstructive pulmonary disease [c] Including alcohol poisoning

Cancer incidence among alcoholic liver disease patients

During the follow-up period of 41209 patient-years (mean 3,47 years), 1052 cancer cases occurred among the cohort. The expected number of cases was 367.8. Thus, the SIR for all cancers was 2.86 (95%CI 2.69-3.03). The SIRs of various cancers were: liver cancer (SIR 59.20; 95%CI 53.11–65.61) pancreas (SIR 3.71; 95%CI 2.72.24.94), pharynx (SIR 9.25; 95%CI 6.05–13.56), mouth (SIR 8.31; 95%CI 4.84–13,29), tongue (SIR 7,21; 95%CI 3.60–12.89), oesophagus (SIR 7.92; 95%CI 5.49–11.07), larynx (SIR 5.20; 95%CI 2.77–8.89), lung (SIR 2.77; 95%CI 2.27–3.32), stomach (SIR 2.76; 95%CI 1.79–4.07), kidney (SIR 2.69; 95 CI 1.84–3.79), colon (SIR 2.33; 95%CI 1.70–3.11), cervix uteri (SIR 4.93; 95%CI 1.34–12.63) and non-melanoma skin cancer (SIR 1.89; 95%CI 1.18–2.86). ALD did not decrease the risk of any cancer**.** The SIRs of cancers in various sites according to follow-up periods among AH and liver cirrhosis patients are presented in Tables 6 and 7. The SIR of all cancers was highest during the first year of follow-up both among cirrhosis (7.27; 95%CI 6.43-8.16) and AH patients (SIR 3.95; 95%CI 3.01-5.08), but remained increased throughout the whole period of follow-up. The risk of cancer of larynx increased over time. The risk of liver cancer was highest during the first year both in liver cirrhosis (SIR 207; 95%CI 172.37-245.00) and in AH (SIR 66.14; 95%CI 37.02-109.00), and it remained increased until the end of follow-up.

Table 6. Observed (Obs) and expected (Exp) numbers of various cancer cases and standardised incidence ratios (SIR) with 95% confidence intervals (CI) among patients with alcoholic liver cirrhosis according to follow-up periods from years 2001-2013.

Site	Follow-up 0-0.99 years				Follow-up 1-4.99 years				Follow-up 5-9.99 years				Follow-up > 10 years			
	Obs	**Exp**	**SIR**	**95%CI**	**Obs**	**Exp**	**SIR**	**95%CI**	**Obs**	**Exp**	**SIR**	**95%CI**	**Obs**	**Exp**	**SIR**	**95%CI**
All sites	268	36.86	7.27	6.43-8.16***	189	79.64	2.37	2.05-2.72***	71	28.10	2.53	1.97-3.18***	9	2.77	3.25	1.49-6.17**
Tongue	4	0.15	26.22	7.14-67.12***	2	0.33	6.15	0.74-22.22	2	0.11	17.67	2.14-63.81*	0	0.01	0.00	0.00-351.00
Mouth	2	0.21	9.65	1.17-34.87*	4	0.44	9.06	2.47-23.19**	0	0.15	0.00	0.00-23.87	0	0.01	0.00	0.00-256.00
Pharynx	8	0.29	27.26	11.77-53.71	1	0.62	1.62	0.04-9.03	4	0.21	19.04	5.19-48.76***	1	0.02	54.48	1.38-303.00*
Oesophagus	8	0.44	18.08	7.80-35.61***	6	0.94	6.40	2.35-13.93***	1	0.33	3.06	0.08-17.07	0	0.03	0.00	0.00-118.00
Stomach	7	0.88	7.94	3.19-16.36***	2	1.82	1.10	0.13-3.97	2	0.61	3.29	0.40-11.90	1	0.06	16.87	0.43-94.01
Colon	8	1.91	4.20	1.81-8.26**	8	4.22	1.90	0.82-3.73	2	1.55	1.29	0.16-4.64	1	0.16	6.19	0.16-34.50
Liver	124	0.60	207.24	172.37-245.00***	73	1.32	55.12	43.20-69.30***	21	0.49	42.99	26.61-65.71***	1	0.05	19.95	0.50-111.00
Pancreas	17	1.24	13.76	8.02-22.03***	3	2.70	1.11	0.23-3.24	1	0.98	1.02	0.03-5.67	1	0.10	9.91	0.25-55.23
Larynx	1	0.26	3.91	0.10-21.76	4	0.51	7.82	2.13-20.01**	2	0.17	12.10	1.46-43.72*	1	0.01	67.53	1.71-376.00*
Lung	19	3.81	4.98	3.00-7.77***	20	8.10	2.47	1.51-3.81***	12	2.83	4.23	2.19-7.39***	0	0.28	0.00	0.00-13.17
Skin[a]	0	1.12	0.00	0.00-3.28	9	2.67	3.37	1.54-6.38**	1	1.07	0.93	0.02-5.20	1	0.12	8.44	0.21-47.03
Kidney	7	1.18	5.95	2.93-12.25***	2	2.51	0.80	0.10-2.87	1	0.89	1.12	0.03-6.25	0	0.09	0.00	0.00-42.07

*$p<0.05$ **$p< 0.01$ ***$p <0.001$

[a]non-melanoma

Table 7 . Observed (Obs) and expected (Exp) numbers of various cancer cases and standardised incidence ratios (SIR) with 95% confidence intervals (CI) among patients with alcoholic hepatitis according to follow-up periods from years 2001-2013.

Site	Follow-up 0-0.99 years				Follow-up 1-4.99 years				Follow-up 5-9.99 years				Follow-up > 10 years			
	Obs	**Exp**	**SIR**	**95%CI**	**Obs**	**Exp**	**SIR**	**95%CI**	**Obs**	**Exp**	**SIR**	**95%CI**	**Obs**	**Exp**	**SIR**	**95%CI**
All sites	60	15.19	3.95	3.01-5.08***	82	41.62	1.97	1.57-2.44***	35	21.15	1.65	1.15-2.30***	3	2.06	2.25	0.30-4.25
Tongue	0	0.07	0.00	0.00-52.13	0	0.19	0.00	0.00-37.53	0	0.09	0.00	0.00-39.77	0	0.01	0.00	0.00-462.00
Mouth	1	0.09	10.64	0.27-59.26	1	0.26	3.90	0.10-21.75	0	0.13	0.00	0.00-29.04	0	0.01	0.00	0.00-306.00
Pharynx	0	0.14	0.00	0.00-26.71	3	0.38	7.90	1.63-23.08	2	0.19	10.61	1.28-38.32*	0	0.02	0.00	0.00-219.00*
Oesophagus	3	0.18	16.31	3.36-47.66**	4	0.51	7.86	2.14-20.12**	2	0.26	7.57	0.92-27.32	0	0.03	0.00	0.00-146.00
Stomach	4	0.36	11.04	3.01-28.26**	0	0.95	0.00	0.00-3.86	0	0.46	0.00	0.00-8.02	0	0.04	0.00	0.00-8.02
Colon	8	1.91	4.20	1.81-8.26**	8	4.22	1.90	0.82-3.73	2	1.55	1.29	0.16-4.64	1	0.16	6.19	0.16-34.50
Liver	15	0.23	66.14	37.02-109.00***	13	0.65	20.04	10.67-34.26***	9	0.36	24.97	11.42-47.40***	1	0.04	27.46	0.69-152.00
Pancreas	3	0.49	6.15	1.27-17.97*	3	1.36	2.21	0.46-7.88	1	0.71	1.41	0.04-7.88	0	0.07	0.00	0.00-51.63
Larynx	0	0.11	0.00	0.00-33.86	1	0.29	3.45	0.09-19.28	1	0.14	7.24	0.18-40.34	0	0.01	0.00	0.00-299.00
Lung	9	1.47	6.14	2.81-11.65***	9	4.06	2.21	1.01-4.20*	4	2.13	1.87	0.51-4.79	0	0.21	0.00	0.00-17.70
Skin[a]	1	0.40	2.51	0.06-13.98	0	1.21	0.00	0.00-3.05	0	0.73	0.00	0.00-5.021	0	0.09	0.00	0.00-43.16
Kidney	7	0.50	2.00	0.05-11.14	7	1.37	5.12	2.06-10.54**	0	0.70	0.00	0.00-5.30	0	0.07	0.00	0.00-54.94

*$p<0.05$ **$p< 0.01$ ***p <0.001

[a]non-melanoma

Results of the prospective intervention study

24 patients (18 males and 6 females) were enrolled in the study. Their median age was 57.5 (26-65) years. The median Alcohol Use Identification Test (AUDIT) score was 22 (7-32). The median Maddrey score was 421 (319-987). Ten patients achieved a response to prednisolone therapy based on the assessment with the Lille model with the cut-off point 0.45. Patients with a Lille score above the cut-off value of 0.45 were older (61.5 vs 47.5 years, p=0.021). Otherwise there was no difference between corticosteroid responders and non-responders in baseline characteristics. Seven patients (29%) died. Deaths occurred after 7-44 days from randomisation, and the causes of death were sepsis (1 patient), pneumonia (1 patient), progression of AH combined with hepatorenal syndrome (2 patients) and progression of AH alone (3 patients). Ten patients were lost to follow-up. The results of the routine laboratory tests of the patients during the follow-up are presented in Table 8.

Efficacy of ciprofloxacin in severe alcoholic hepatitis

10 out of 11 patients (90.9%) in group 1 (receiving ciprofloxacin in addition to prednisolone) survived 6 months, whereas 7 out of 13 patients (53.8%) survived in group 2 (treated with prednisolone only). In terms of this endpoint the study was underpowered, and, due to the small number of patients, the difference was not statistically significant (p=0.078). There was no difference in response to prednisolone therapy assessed with the Lille model on day 7 between the two groups.

Cholesterol metabolism in severe alcoholic hepatitis

At baseline, AH patients had higher ratios of cholestanol (539±26 100 x mmol/mol of cholesterol), desmosterol (82.1±4.3) and sitosterol (268±26) to cholesterol compared to PSC (226±10, 70.7±1.1 and 237±10) and healthy subjects (139±12, 75.9±1.4 and 122±12) (p-range 0.0164 - < 0.0001). The serum cholesterol level (164±10 mg/dl) was lower among AH patients compared to PSC patients (193±4) and healthy controls (237±4) (p<0.0001). Serum ratios of lathosterol (45.9±18.3), cholestenol (9.3±4.4) and squalene (19.6±2.3) to cholesterol were lower among AH patients compared to PSC patients (161.4±7.2, 18.7±1.7 and 33.1±1.0) and healthy controls (194.8±8.1, 13.3±1.9 and 33.5±1.1) (p<0.0001). The serum campesterol to cholesterol ratio (395±42) among AH patients was lower than that of PSC patients (413±17) but higher than that of healthy subjects (191±19) (p<0.0001). Lathosterol to cholestanol (0.05±0.2) and lathosterol to campesterol 0.17±0.13) ratios were lower among AH patients compared to PSC patients (1.00±0.06 and 0.58±0.05) and healthy subjects (1.50±0.07 and 1.26±0.06) (p<0.0001). On average, AH patients were older (54.4±2.3 years) than PSC patients (41.1±1.0) and healthy subjects (52.3±0.2) (p<0.0001). There was male predominance among AH patients compared to healthy subjects (18 males and 6 females *versus* 63 males and 61 females; p=0.042), but no significant difference to PSC patients (90 males and 66 females, p=0.122).

During the follow-up, the mean bilirubin level decreased gradually, while the cholestanol to cholesterol ratio decreased more slowly by 40% reduction until day 90 (p=0.014). Serum campesterol/cholesterol, sitosterol/cholesterol and avenasterol/cholesterol increased from baseline to day 7, and to day 30 by 18-51% for campesterol, by 25-65% for sitosterol, and by 8-21% for avenasterol (p-range 0.049 - <0.001). These ratios returned to the baseline level partially on day 90 and completely on day 180. The evolution of the bilirubin level and serum non-cholesterol sterol surrogate markers of cholestasis are presented in Table 9.

Table 8. Basic laboratory values in patients with Lille score below 0.45 and above 0.45 at day 7.

	Baseline			Day 7			Day30			Day 90			Day 180		
	<0.45 (n=10)	>0.45 (n=12)	p*	<0.45 (n=10)	>0.45 (n=12)	p*	<0.45 (n=9)	>0.45 (n=9)	p*	<0.45 (n=6)	>0.45 (n=6)	p*	<0.45 (n=5)	>0.45 (n=3)	p*
Bilirubin umol/l	199 (49-337)	231.5 (119-564)	0.582	92 (21-204)	81.5 (70-509)	0.069	54 (11-110)	42 (31-406)	0.863	19 (9-54)	24.5 (8-60)	1.000	17 (5-52)	10 (10-12)	0.250
AST U/l	129 (51-271)	149.5 (111-668)	0.315	100 (59-215)	131 (70-309)	0.211	71 (30-155)	77 (41-144)	0.815	67 (31-109)	47 (20-65)	0.26	59 (34-62)	28 (21-39)	0.114
ALT U/l	43 (13-95)	73.5 (23-131)	0.254	48 (21-252)	81.5 (41-156)	0.254	43 (30-173)	44 (26-113)	0.546	41.5 (16-65)	18.5 (14-102)	0.310	24 (14-52)	31 (21-53)	0.786
ALP U/l	181 (81-373)	177 (96-472)	0.710	158 (91-516)	176.5 (74-290)	1.000	189 (82-435)	107 (77-268)	0.534	144 (60-179)	92.5 (80-183)	1.000	127 (98-280)	75.5 (73-78)	0.133
Albumin g/l	18 (18-66)	19.5 (15-29)	0.069	24 (18-31)	21.5 (14-26)	0.382	26 (25-33)	23 (17-30)	0.063	29.6 (24-44)	18.5 (14-102)	0.699	37.2 (25-44)	38 (25-44)	1.000
P-TT[1] %	38.5 (18-66)	31.5 (16-54)	0.582	41 (24-76)	48 (32-88)	0.882	48 (30-90)	44 (33-80)	0.730	55.5 (50-79)	53.5 (39-87)	0.485	57 (41-85)	76 (63-96)	0.250
Creatinine umol/l	64.5 (44-145)	58.5 (32-109)	0.346	70.5 (51-260)	68 (35-117)	0.381	64 (52-117)	69 (43-108)	0.666	65.5 (54-88)	59 (48-81)	0.394	59 (54-74)	60 (46-71)	1.000
Haemoglobin g/l	104 (86-131)	116 (81-153)	0.552	112 (84-141)	127 (97-140)	0.603	126.5 (95-149)	121 (111-150)	1.000	144 (89-164)	136.5 (117-151)	1.000	134 (99-169)	144 (141-150)	1.000
Leukocytes x 10^9/l	9,3 (4.4-43.5)	8.9 (4.1-20.5)	1.000	12.9 (6.3-82.0)	12.8 (7.1-29.8)	0.824	10.0 (6.6-106)	7.0 (4.6-27.2)	0.093	7.1 (4.5-11.3)	8.8 (6.0-10.1)	0.329	5.9 (5.2-6.3)	9.8 (8.1-12.8)	0.057
Platelets x 10^9/l	131 (41-416)	158 (43-263)	0.824	164 (76-341)	179 (122-353)	0.710	143 (69-296)	191 (95-440)	0.200	133 (63-251)	242 (142-319)	0.052	169.5 (93-261)	264 (152-271)	0.229
CRP mg/l	14 (0-72)	37 (26-74)	0.152	9 (0-53)	16 (5-63)	0.201	6 (0-16)	17 (4-46)	0.046*			0.017*	2 (0-5)	4 (0-14)	0.629
NH4 umol/l	63 (24-94)	72 (31-122)	0.447	52 (28-90)	55 (19-99)	0.717	66 (41-107)	41 (22-66)	0.021*	68 (21-83)	41 (29-96)	0.792	41.5 (26-88)	32 (30-78)	1.000
Ferritin ug/l	471.5 (58-1833)	1266 (245-4318)	0.011	368 (79-1287)	1016 (265-2879)	0.004	141 (31-1957)	478 (135-1996)	0.059	60 (10-547)	134.5 (42-684)	0.662	96.5 (9-528)	31 (21-35)	0.400

*p compared between groups, *p <0.05 ALT – alanine aminotranspherase, AST- aspartate aminotranspherase, ALP – alkaline phosphatase , CRP – C-reactive protein
[1]Thromboplastin

There was no change in the serum cholesterol level during the follow-up. The surrogate markers of cholesterol synthesis remained low during the follow-up. The serum campesterol to sitosterol ratio increased by 16-25% on days 90 and 180 from the baseline (*p*-range 0.036-0.018). The evolution of the serum cholesterol level and serum non-cholesterol surrogate markers of cholesterol synthesis are presented in Table 10.

Serum lathosterol, cholestenol and desmosterol ratios to cholesterol were mostly inversely related to those of cholestanol and phytosterols in healthy persons (*r*-range -0.155 - -0.530, *p*-range 0.055 - <0.001), and consistently in subjects with PSC (*r*-range -0.155 - -0.530, *p* <0.001 for all). Patients with AH had no such interaction at baseline and on day 7, nor on day 30. On day 90, cholestenol and lathosterol – but not desmosterol – negatively reflected cholestanol and phytosterols (*r*-range -0.721 - -0.952, *p*-range 0.045 - <0.001). Serum bilirubin was associated on days 7 and 30 with cholestanol (*r*-range +0.475 - +0.694, *p*-range 0.045-0.001) and on day 90 with sitosterol only (*r*=+0.809, *p*=0.005). Serum ferritin was inversely related to ratios to cholesterol of campesterol, sitosterol and avenasterol at baseline (*r*-range -0.494 - -0.697, *p*-range 0.017 – 0.001), and on day 7 (*r*-range -0.486 - -0.573, *p*-range 0.040 - 0.013). Serum ferritin level was positively related to the bilirubin level at baseline (*r*= +0.539, *p*= 0.017 and on day 90 (*r*= +0.752, *p*= 0.008).

Predictors of response to corticosteroids in severe alcoholic hepatitis

The baseline values of serum ratios to cholesterol (100x mmol/mol of cholesterol) of phytosterols campesterol, sitosterol and avenasterol were higher among the patients with

Table 9. Evolution of mean bilirubin level (μmol/l) and ratios to cholesterol of cholestanol, campesterol, sitosterol, avenasterol and stigmasterol (100 x mmol/mol) with SEM during the follow-up of alcoholic hepatitis patients (p-values compared to the baseline). **p*<0.05

	Day 0	Day 7		Day 30		Day 90		Day 180	
Bilirubin	224±26	174±26	*p*=0.002*	78±26	*p*=0.002*	28±5	*p*=0.003*	18±5	*p*=0.009*
Cholestanol	527±37	539±40	*p*=0.121	468±47	*p*=0.055	316±37	*p*=0.014*	231±27	*p*=0.030*
Campesterol	395±42	467±70	*p*=0.003*	598±82	*p*<0.001**	536±121	*p*=0.014*	464±112	*p*=0.364
Sitosterol	268±26	334±48	*p*=0.003*	442±48	*p*<0.001	333±82	*p*=0.154	275±76	*p*=0.944
Avenasterol	61±7	66±9	*p*=0.044*	74±11	*p*=0.049*	73±16	*p*=0.541	73±20	*p*=0.624
Stigmasterol	29.9 ±3.2	31.2 ±2.8	*P*=0.799	33.1 ±2.6	*P*=0.765	27.5 ±4.6	*P*=0.966	27.1 ±3.4	*P*=0.808

Table 10. Evolution of mean serum cholesterol level (mg/dl, conversion to mmol/l dividing by 38.67), lathosterol/cholesterol ratios, desmosterol/cholesterol (100 x mmol/mol) ratios, lathosterol/campesterol ratios and campesterol/sitosterol ratios with SEM during the follow-up of alcoholic hepatitis patients (p-values compared to baseline). **p*<0.05

	Day 0	Day 7		Day 30		Day 90		Day 180	
Cholesterol	172±18	185±19	*p*=0.290	190±25	*p*=0.369	172±25	*p*=0.363	156±25	*p*=0.159
Lathosterol	45±7	44±8	*p*=0.832	30±4	*p*=0.029*	50±14	*p*=0.543	51±13	*p*=0.417
Desmosterol	80±4	72±4	*p*=0.041*	69±5	*p*=0.023*	69±7	*p*=0.837	75±8	*p*=0.499
Lathosterol/ campesterol	26±26	25±9	*p*=0.263	8±2	*p*=0.001*	18±2	*p*=0.318	18±5	*p*=0.593
Campesterol/ sitosterol	154±7	142±7	*p*=0.040*	154±10	*p*=0.660	179±16	*p*=0.036*	193±19	*p*=0.018*

response to corticosteroid therapy (316±76 *versus* 507±89, *p*=0.044; 219±54 *versus* 342±52, *p*=0.032; 49.9±8.5 *versus* 76.9±12.2, *p*=0.032). There was no difference in these values between survivors and non-survivors. Responders to prednisolone had lower values of ferritin at baseline (471.5 *versus* 1266 μg/l; *p*= 0.011) and on day 7 (368 *versus* 1016 μg/l; *p*=0.004). The receiving operating characteristics (ROC) curve with its corresponding area under the curve (AUC) for ferritin is presented in Figure 6. In conclusion, a high ferritin level at baseline and lower levels of campesterol, sitosterol and avenasterol were related to lack of response to prednisolone therapy.

In the multivariate linear regression model with the value of the Lille Score as the dependent variable, the stigmasterol to cholesterol ratio (β= -0.348, *p*=0.031) was the only statistically significant independent predictor of the model, suggesting that a higher stigmasterol to cholesterol ratio is related to a lower Lille score and poorer response to corticosteroid therapy.

Polymorphism in PNPLA3 gene among patients with severe alcoholic hepatitis

A DNA sample was available from 15 patients. From those samples, PNPLA3 gene variations rs738409:C>G were determined. Seven patients were CC homozygotes, and 8 were CG heterozygotes. There were no GG homozygotes among studied patients. There was no difference in the survival at 6 months between CC and CG patients (85,7% *versus* 75%, *p*=0.400). The PNPLA3 gene status was not related to response to corticosteroids, either.

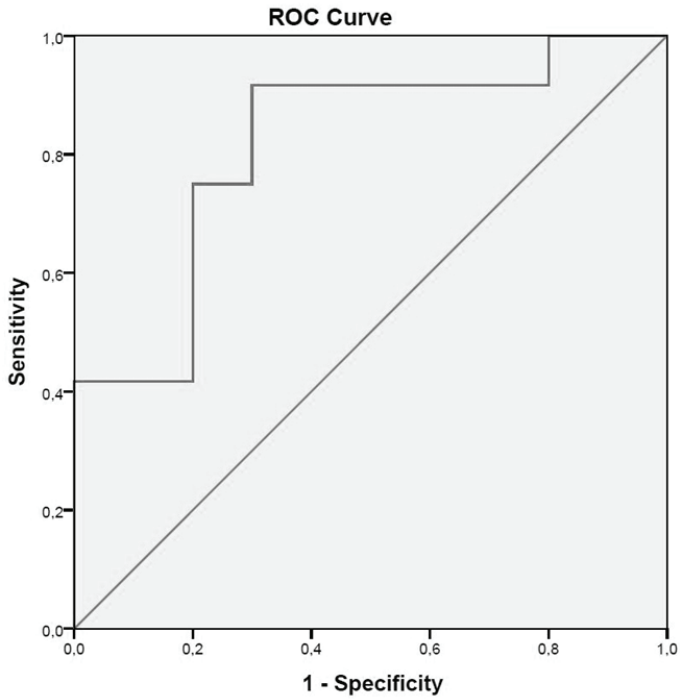

Figure 6. Receiver operating characteristics (ROC) curve for ferritin for separation of corticosteroid responders from non-responders in severe alcoholic hepatitis treated with prednisolone. Area under the curve (AUC) is 0.817 (*p*=0.012). The optimal cut-off value is 634 μg/l with 91.7% sensitivity and 70.0% specificity.

Risk factors of alcoholic liver disease

After exclusion of individuals with liver disease at baseline (*n*=288) and with viral hepatitis (*n*=100), among the participants of FINRISK and Health2000 population surveys, the study cohort consisted of 41260 persons. During the follow-up of 511789 person-years, there were 355 liver events (245 among men and 110 among women), 196 of which were liver-related deaths and 51 liver cancers.

In the final multivariate model age (HR 1.03; 95%CI 1.01-1.05, *p*=0.0083), diabetes (HR 2.72; 95%CI 1.61-4.61 *p*=0.0002), WHR (HR for WHR/1 standard deviation 1.52; 95%CI 1.20-1.92, *p*=0.0006), average alcohol consumption (HR for 10 g of ethanol 1.13; 95%CI 1.08-1.19, *p*<0.0001), weekly binge drinking (HR 2.36; 95%CI 1.35-4.11, *p*=0.0024) serum HDL (HR 2.21; 95%CI 1.33-3.67, *p*=0.0022) and non-HDL cholesterol (HR 1.19; 95%CI 1.02-1.39, *p*=0.0237), PNPLA3 carrier status CG/GG *versus* CC (HR 1.88; 95%CI 1.25-2.81, *p*=0.0024) and marital status (HR 1.85; 95%CI 1.03-3.33, *p*=0.0397 for single *versus* married and HR 2.37; 95%CI 1.43-3.91, *p*=0.0008 for divorced or widow *versus* married) predicted severe liver disease among men, and age (HR 1.04; 95%CI 1.01-1.07, *p*=0.0198), WHR (HR for WHR/1 standard deviation 1.58; 95%CI 1.09-2.30, *p*=0.0167), weekly binge drinking (HR 7.38; 95%CI 2.85-19.12, *p*<0.0001) and PNPLA3 carrier status CG/GG *versus* CC (HR 2.73; 95%CI 1.26-5.92, *p*=0.0109) among women.

In an age-adjusted subgroup analysis, where independent risk factors (diabetes status, alcohol intake, sex-specific WHR, median BMI, marital status, and PNPLA3 carrier status) were tested, alcohol intake was a risk factor in all subgroups without evidence of effect modification of BMI, diabetes, PNPLA3 or marital status on alcohol intake as a risk factor of severe liver disease. In fixed-model Cox regression analysis with the significant independent risk factors from the primary analysis and alcoholic and non-alcoholic liver diseases as end-points, weekly binge drinking was a risk factor (HR 3.9, 95%CI 1.3-11.6 for men and HR 8.1, 95%CI 1.6-40.4 for women) but average alcohol intake was not (Table 11 and 12).

Table 11. Fixed-model Cox regression with significant independent risk factors in men from primary analysis using alcoholic versus non-alcoholic liver cirrhosis and liver dysfunction as an outcome.

	Alcoholic		Non-alcoholic	
	HR (95%CI)	p	HR (95%CI)	p
Weekly binge drinking	**3.27 (1.54-1.96)**	**0.002**	**3.86 (1.28-11.60)**	**0.016**
PNPLA3 carrier CG/GG *vs* CC	**5.96 (2.76-12.89)**	**<0.001**	**6.63 (1.69-26.07)**	**0.007**
WHR x 100	**1.05 (1.00-1.09)**	**0.048**	**1.08 (1.01-1.15)**	**0.025**
Marital status: single *vs* married	**2.57 (1.34-4.93)**	**0.004**	2.24 (0.77-6.50)	0.137
Diabetes	**2.89 (1.41-5.89)**	**0.004**	**3.30 (1.22-8.97)**	**0.019**
Alcohol intake 10 g/dose	**1.18 (1.12-1.24)**	**>0.001**	1.02 (0.89-1.17)	0.0778
HDL cholesterol (mmol/l)	**2.09 (1.07-4.10)**	**0.032**	**3.39 (1.29-8.91)**	**0.013**
Age (years)	1.01 (0.98-1.04)	0.497	1.03 (1.00-1.07)	0.087

Table 12. Fixed-model Cox regression with significant independent risk factors in women from primary analysis using alcoholic versus non-alcoholic liver cirrhosis and liver dysfunction as an outcome.

	Alcoholic		Non-alcoholic	
	HR (95%CI)	p	HR (95%CI)	p
Weekly binge drinking	**9.54 (2.75-33.08)**	**<0.001**	**8.14 (1.64-40.43)**	**0.010**
PNPLA3 carrier CG/GG *vs* CC	2..7 (0.70-6.19)	0.518	2.91 (0.32-26.14)	0.340
WHRx100	**1.13 (1.07-120)**	**<0.001**	0.99 (0.90-1.08)	0.711
Age (years)	0.99 (0.95-1.04)	0.711	**1.09 (1.04-1.15)**	**<0.001**

6. DISCUSSION

Incidence of alcoholic liver disease

The incidence of severe forms of ALD requiring hospitalisation increased during the study period. Consumption of alcohol increased in Finland during the study period (Varis & Virtanen, 2015). Presumably, changes in alcohol consumption are reflected in AH incidence more rapidly, since AH is an acute manifestation of ALD, while increase of alcohol consumption is reflected in incidence of alcoholic cirrhosis later, since the process of disease progression is slower. The incidence of AH was higher in Finland compared to Denmark (Sandahl *et al.*, 2011a). Moreover, the incidence of AH in Denmark has been decreasing recently (Deleuran *et al.*, 2015). Total annual alcohol consumption *per capita* in Finland is similar to several European countries and among the highest in Nordic countries (Hallberg & Österberg, 2014). Furthermore, there are certain differences in drinking patterns between the countries. Strong spirits are more common in Finland compared to other Nordic countries (Nordic Alcohol Statistics, 2011). This might partly explain the difference, since alcohol drinking patterns are related to the risk of ALD (Askgaard *et al.*, 2015). Other putative reasons might be genetic and other contributing factors. Unfortunately, these factors are not completely understood nor widely studied in different populations. The absolute increase in incidence of alcoholic liver cirrhosis among men was very notable, but the relative increase was higher among women especially in AH, which is somewhat surprising, since despite the increase in total annual consumption, excess alcohol consumption and binge drinking did not increase during the study period among women (National Institute for Health and Welfare, 2018). However, this alarming result warrants public health measures to prevent severe adverse events of alcohol abuse among both sexes.

Prognosis of alcoholic liver disease

The overall prognosis of ALD turned out to be poor. Survival of AH patients was better compared to cirrhosis patients. The survival of AH patients has been poor in various studies (Cuthebert *et al.*, 2014; Liangpunsakul, 2011). Our registry study included all hospitalised AH patients without data of the severity of the disease. Thus, our results represent the prognosis of all AH patients in general regardless of the severity of the disease.

The long-term prognosis of alcoholic liver cirrhosis is very poor. Less than one third of the patients survive over 5 years. The majority of ALD patients die from ALD. Due to poor prognosis of advanced stages of ALD, the prevention and early intervention is crucial to improve the survival of ALD patients. Even simple alcoholic steatosis is a precursor of fibrosis and further cirrhosis (Teli *et al.*, 1995). Thus, the main focus in treatment of ALD should be in the prevention of development and progression of alcohol-induced damage. Since there is obvious correlation between alcohol consumption and liver cirrhosis (Askgaard *et al.*, 2015) as well as liver-related mortality (Ramstedt, 2001), the main goal in the reduction of ALD mortality and morbidity is reduction of alcohol consumption both at the individual and society level. The former is carried out by early psycho-social interventions in persons with high alcohol consumption and by treatment of alcohol dependency, whereas the latter is a mission for alcohol politics (Hydes *et al.*, 2019). Detection of alcohol misuse in patients attending hospital with alcohol-related problems identifies persons in high risk of ALD and offers chances for targeted interventions (Westwood *et al.*, 2017). Detection of asymptomatic stages of ALD at the community level and early intervention seem feasible (Sheron *et al.*, 2013). The intervention should be implemented in conjunc-

tion with counselling of other health hazards including obesity (Cook *et al.*, 2015). Alcohol policy measures can reduce mortality from ALD (Sheron, 2016).

The most common cause of death among ALD patients was alcohol-related disease. The risk of death from other diseases was also increased 7-fold, reflecting the role of advanced liver disease as a risk factor of morbidity and mortality of various extrahepatic diseases. This might be due to increased incidence of various diseases in ALD patients and more severe course of concomitant diseases in patients with advanced ALD. SMR for any disease, and specifically for respiratory, circulatory and non-alcoholic digestive disease was higher among female patients suggesting that this interaction between ALD and extrahepatic disease might be more pronounced among women. SMR for accidental and violent death was higher in women, especially for accidental falls. It is not possible to conclude whether this is due to higher occurrence of falls or more severe consequence of these accidents among female ALD patients.

Malignancies in alcoholic liver disease

The risk of various cancers was increased among the ALD cohort. The risk of liver cancer was particularly high. Due to the high risk of liver cancer, ultrasound surveillance is recommended in liver cirrhosis patients (Corte Della *et al.*, 2012; EASL-EORTC, 2012). The incidence of liver cancer was highest during the first year after ALD diagnosis. This may result from profound imaging at the time of ALD diagnosis. Moreover, HCC may have been the first clinical presentation of ALD, and both diagnoses might have been made concomitantly. However, the incidence of liver cancer remained high during the follow-up, supporting the evidence of advanced liver disease as a risk factor of HCC. Currently, there are no recommendations for ultrasound surveillance for patients with a previous episode of AH. In our study, the risk of HCC was increased among patients who had survived an AH episode. However, the impact of HCC on the mortality of ALD patients seems minute (Jepsen *et al.*, 2012). Unfortunately, all ALD patients are not eligible for curative therapies for HCC. Liver transplantation is not available for active drinkers. Thus, ultrasound surveillance might be recommended only for selected patients after a severe AH episode, provided that they manage to remain abstinent. Moreover, the diagnosis of HCC is often delayed in ALD patients compared to other liver diseases (Schütte *et al.*, 2012).

The overall risk of cancer was highest during the first year of follow-up, but in addition to the risk of liver cancer, it remained increased during the whole follow-up. The risk for lung cancer was increased during the first 10 years of follow-up, while the risk for cancer of the larynx and pharynx increased significantly only after 5 years of follow-up among cirrhosis patients. Data is not sufficient to recommend surveillance of the ALD patients for extrahepatic malignancies, but it is important to recognise the risk of cancer in these sites in the management of individual patients.

The risks of various other cancers were increased among our cohort reflecting the possible synergistic effect of both alcohol and liver cirrhosis as risk factors of carcinogenesis. Increased risk of malignancies in the upper aero-digestive tract has been reported among cirrhosis patients before (Sorensen *et al.*, 1998). The role of alcohol in carcinogenesis in these sites might be related to acetaldehyde, a metabolite of ethanol, excreted into saliva (Seitz & Stickel, 2009). Since the cohort consisted only of ALD patients, the effect of advanced liver disease on carcinogenesis cannot be differentiated from the effect of alcohol. Furthermore, many of the cancers with increased SIRs in our cohort are related to smoking. Tobacco smoking and other confounding factors among the cohort were not

available. Smoking may be more common among persons with alcohol abuse (Grucza *et al.*, 2005).

There was no decreased risk for any cancer, suggesting that ALD does not protect from malignancies. From the clinical point of view, our result is relevant in the management of ALD patients. Routine screening can be recommended only for HCC among abstinent liver cirrhosis patients, but the ALD should be recognised as a risk factor for various other cancers, as well.

Cholesterol metabolism in alcoholic hepatitis

Phytosterols campesterol, sitosterol and avenasterol are derived from plants in diet, whereas cholestanol is synthesised in the liver (Miettinen *et al.*, 2011). Phytosterol levels reflect the intestinal absorption of cholesterol, and endogenous cholesterol precursors reflect the synthesis, while cholestanol, an enzymatically formed derivative of endogenous cholesterol having a low fractional absorption, is a relative indicator of absorption efficiency. There was an inverse correlation of absorption and synthesis markers among PSC patients and healthy controls, suggesting a mechanism of upregulation of cholesterol synthesis when intestinal absorption is low, and *vice versa*. Such correlation and homeostasis of cholesterol metabolism lacked among AH patients before day 90. Earlier studies showed that in acute non-alcoholic liver failure, the cholesterol homeostasis is maintained, suggesting further that despite fulminant liver failure, cholesterol synthesis *de novo* and LDL uptake by the hepatocytes maintain their sensitivity to detect serum cholesterol levels (Gylling *et al.,* 1996). Accordingly, the results of Study III indicate that severe AH may have a disease-specific pattern of cholesterol precursors and phytosterols in serum. Non-cholesterol sterols of patients from different study arms were not analysed separately, which is a limitation of the study, since the effects of the antibiotic therapy on cholesterol metabolism was not assessed.

Predictors of corticosteroid response in severe alcoholic hepatitis

Due to premature termination of the ciprofloxacin trial, the original goals were not achieved, and the study failed to show the efficacy of ciprofloxacin in severe AH. However, potential predictors of corticosteroid response were found. Lower levels of phytosterols campesterol, sitosterol and avenasterol were related to poor response to prednisolone therapy. However, further studies are needed before these markers can be suggested for clinical practice. Several phytosterols are surrogate markers of cholestasis and liver function in cholestatic diseases (Gylling *et al.*, 1996; Nikkilä *et al.*, 2005). In AH patients, their levels did not decrease along with bilirubin levels, but even increased during the follow-up. Moreover, phytosterol ratios did not correlate with bilirubin concentrations, unlike in PBC patients (Gylling *et al.,* 1996).Thus, plant-derived sterols presumably reflect the nutritional status of the patients suggesting that poor nutritional status at baseline might predict poorer response to corticosteroid therapy. An increase in phytosterols in survivors may reflect nutritional recovery. Efficacy of nutritional therapy in severe AH is controversial (Fialla *et al.*, 2015, Moreno *et al.*, 2016). However, it may be argued that poor nutritional status worsens the outcome in severe AH.

A high ferritin level predicted poor response to corticosteroids. All patients were screened for haemochromatosis with other iron parameters and only patients with normal iron and transferrin iron saturation were accepted into the trial. Thus, high ferritin was not related to undiagnosed concomitant haemochromatosis. Systemic inflammatory response is commonly present in severe AH

(Michelena *et al.*, 2015). High ferritin levels might be related to more severe inflammation. Ferritin is also related to the level of fibrosis in ALD (Raynard *et al.*, 2002, Machado *et al.*, 2009). Consequently, ferritin may reflect more advanced underlying liver disease. High ferritin levels are related to poor prognosis in acute liver failure (Anastasiou *et al.*, 2017), and predict early (1-month) mortality in patients with decompensated liver cirrhosis (Maiwall *et al.*, 2014).

In multivariate analysis, high levels of stigmasterol predicted better response to corticosteroids assessed with the Lille model. The result is surprising, since *in vitro*, stigmasterol is an antagonist of the farnesoid X receptor (FXR), which has hepatoprotective role in cholestasis (Carter *et al.*, 2007). Furthermore, in the animal model, FXR deficient mice are more susceptible to AH (Wu *et al.*, 2015). Our result suggests that nutritional status reflected by the stigmasterol level is an important determinant of prognosis and response to therapy in severe AH.

PNPLA polymoprhism in alcoholic liver disease

In a previous study, rs738409:G homozygosity in the PNPLA3 gene was associated with a worse outcome in severe AH (Atkinson *et al.*, 2017). There were no GG homozygotes among our patients, and CG heterozygosity was not related to survival nor response to corticosteroids, which is in line with the previous study. PNPLA3 CG and GG status was a risk factor of advanced liver disease in Study IV, which is in line with previous data recognising PNPLA3 as a risk gene for ALD (Friedrich *et al.*, 2014 Buch *et al.*, 2015¸Tian *et al.*, 2010).

Risk factors of advanced liver disease

Average alcohol consumption was an independent risk factor for severe liver disease among men in a dose-dependent pattern, as reported previously (Hagström *et al.*, 2018). Any safe threshold limit of alcohol intake can be questioned. Furthermore, amount of alcohol needed for liver damage may vary depending on other risk factors, *e.g.* obesity. Alcohol consumption was a risk factor only among men, but alcohol intake increased the risk in several female subgroups. Weekly binge drinking was a risk factor among both sexes. This has been reported in a previous study, too (Åberg *et al.*, 2017). Interestingly, in the present study, binge drinking predicted the risk of both alcohol-related and non-alcoholic liver disease.

A lower liver cirrhosis risk among wine drinkers has been suggested (Becker *et al.*, 2002). Furthermore, daily drinking and drinking outside meals seems to increase the risk of liver cirrhosis in women (Simpson *et al.*, 2019). Unfortunately, enough data for analysis of variation in risk with various alcoholic beverages was not available for analysis and detailed data about drinking patterns besides the frequency of binge drinking was not recorded.

Overweight is a known risk factor of ALD (Naveau *et al.*, 1997; Hart *et al.*, 2010), NAFLD (Li *et al.*, 2016) and HCC (Larsson *et al.*, 2007). In our study, WHR predicted severe liver disease. Metabolic syndrome is a risk factor of advanced liver disease (Åberg *et al.*, 2018). In the context of liver disease, WHR seems to be the best indicator of overweight and potential metabolic syndrome, as reported previously (Andreasson *et al.*, 2017). Furthermore, WHR is better predictor of severe liver disease than BMI (Andreasson *et al.*, 2017).

HDL and non-HDL cholesterol were related to the risk of advanced liver disease among men. Ethanol interferes with hepatic lipid metabolism in a complex way (You & Arteel, 2019). Exact mechanisms of this phenomenon are not easy to explain, and further studies are needed to examine the interaction of lipid metabolism and alcohol consumption in the context of liver damage.

The same risk factors were largely related to both alcoholic and non-alcoholic liver events. The diagnosis of ALD is traditionally made by the history of alcohol consumption (O'Shea *et al.*, 2010; EASL, 2018a). The majority of diagnoses in fatal liver cases in Finland are alcohol-related contrary to many other countries with a similar profile of overall alcohol consumption (Sheron, 2016). The coexistence and interaction of various risk factors of advanced liver disease make the traditional dichotomy of alcohol-related and non-alcoholic disease questionable. All potential risk factors of liver disease should be considered as a whole.

7. CONCLUSIONS

The incidence of severe forms of ALD, *i.e.* AH and alcoholic liver cirrhosis, has been increasing during the 21st century. The prognosis of ALD is poor. Over 70% of the liver cirrhosis patients die in 5 years. Less than half of the AH patients survive over 5 years. Most patients die from alcohol-related causes, mostly ALD itself. The risk of death from various other diseases is increased among ALD patients. Major causes of death are malignancies, digestive, circulatory and respiratory diseases as well as accidents and violence.

Since the majority of deaths are due to ALD, the efforts to reduce mortality of these patients should be focused on treatment and prevention of ALD itself. Unfortunately, the treatment options in severe AH are scarce. Abstinence from alcohol is crucial in every stage of ALD.

ALD patients have increased risk of various cancers. The risk of liver cancer is especially high. Furthermore, the cancers of the upper aero-digestive tract, pancreas and kidneys are increased among ALD patients. Since ALD and various cancers share alcohol as a common aetiologic factor, the reduction of alcohol consumption at the community level is essential in prevention of both ALD and several malignancies.

There is disease-specific profile in metabolism of cholesterol precursors and phytosterols in severe AH. Low serum levels of phytosterols may reflect nutritional status rather than cholestasis in AH, and they are related to poor response to corticosteroids, suggesting that nutritional status is a risk factor in severe AH. High serum ferritin is related to poor response to corticosteroid therapy. High stigmasterol to cholesterol ratio predicts better response to corticosteroids.

Alcohol consumption, especially binge drinking, and factors related to metabolic syndrome increase the risk of advanced liver disease among men and women. Genetic predisposition (PNPLA3) increases the risk. Marital status is related to the risk in men. Due to co-existence and interaction of risk factors, dichotomous distinction between alcoholic and non-alcoholic liver disease is often inappropriate.

The strength of Studies I and II is the large number of patient-years in follow-up of a validated nation-wide cohort representing all hospitalised ALD patients during the study period. In Study IV, the number of participants in nationally representative cohorts was considerable. Nation-wide registries used in Studies I, II and IV are considered precise. In Study III, cholesterol metabolism was thoroughly analysed and compared with a large number of controls. Limitations are the low number of patients and high number of drop-outs in Study III, lack of data about potential confounding factors and changes in risk factors and lifestyles of persons during the follow-up in Studies I, II and IV. Furthermore, the study populations consisted only of hospitalised patients, and the results are applicable only to advanced stages of ALD.

8. CLINICAL IMPLICATIONS AND SUGGESTIONS FOR FURTHER STUDIES

Our results demonstrated the poor prognosis of patients with advanced ALD. Since most of the deaths were alcohol-related, practically ALD itself, and effective therapy options in the most severe cases are scarce, the focus should be on the prevention of ALD and intervention in early stages of the disease to suspend alcohol consumption. The risk of various cancers and cancer-related mortality is increased among ALD patients. The risk of liver cancer is especially high, not only among cirrhosis patients but also after an AH episode. Ultrasound screening for HCC should be considered for cirrhosis patients and persons who have survived an AH episode if they are abstinent and eligible for curative treatment of HCC.

Serum phytosterol levels may reflect the nutritional status and nutritional recovery in severe AH, suggesting that nutritional status is related to outcome of the patients. High ferritin is related to poorer response to corticosteroid therapy. Further larger clinical studies are needed to confirm the role of sterol markers and ferritin as prognostic factors in clinical practice.

A distinct profile of disturbances in cholesterol metabolism among AH patients warrants further studies on the role of non-cholesterol sterols in the pathogenesis of AH.

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