

From Department of Gastroenterology and Hepatology,
Institution of Medicine

Karolinska Institutet, Stockholm, Sweden

STUDIES ON ALCOHOLIC LIVER DISEASE

Knut Stokkeland



**Karolinska
Institutet**

Stockholm 2006

Reprinted from “Autoantibodies against cytochromes P-4502E1 and P-4503A in alcoholics“ by Lytton SD, Helander A, Zhang-Gouillon ZQ, Stokkeland K, Bordone R, Arico S, Albano E, French SW, Ingelman-Sundberg M from *Molecular Pharmacology* 1999, 55: 223-233, with permission of the American Society for Pharmacology and Experimental Therapeutics.

Reprinted from “Morbidity and mortality in liver diseases in Sweden 1969-2001 in relation to alcohol consumption“ by Stokkeland K, Brandt L, Ösby U, Ekbom A, Hultcrantz R from *Scandinavian Journal of Gastroenterology*, www.tandf.no/gastro, 2006, 41, 463-468, by permission of Taylor & Francis.

Reprinted from “Improved Prognosis for Patients Hospitalized With Esophageal Varices in Sweden 1969-2002 “ by Stokkeland K, Brandt L, Ekbom A, Hultcrantz R from *Hepatology* 2006, 43: 500-506, with permission from John Wiley & sons, Inc.

Published and printed by Karolinska University Press
Box 200, SE-171 77 Stockholm, Sweden
© Knut Stokkeland, 2006
ISBN 91-7140-853-3

ABSTRACT

The overall aim of this thesis was to explore the impact of alcoholic cirrhosis in Sweden, and the relation between drinking patterns and beverage types and alcoholic cirrhosis, and to evaluate the possible role of autoantibodies in the pathogenesis of alcohol related liver disease.

We found that rats had appearance of IgG against rat CYP3A1 and CYP2E1 during chronic ethanol feeding. We found that patients had reactivity against CYP3A4 and CYP2E1 in about 20 to 30% and 10 to 20% of the alcoholic sera. Western blotting confirmed anti-human CYP2E1 reactivity in 8 of 85 alcoholic sera, and 3 of 58 control sera. Anti-CYP3A4 reactivity was detected in 18 of 85 alcoholic sera and 4 of 58 control sera. The results suggest that autoantibodies toward both CYP2E1 and CYP3A could be generated after exposure to alcohol in both humans and experimental animals and it may contribute to the development of liver cirrhosis.

We found that the liver disease mortality increase from 1969 to 1976 coincided with the increase in spirit sales. Both mortality and spirit sales decreased thereafter, whereas there was no decrease in beer or wine sales. Hospitalization rates were reduced after 1987. Depending on age and sex there was a 30-80 percent five-year mortality following discharge. The same patients could be diagnosed as having both alcoholic and non-alcoholic liver diseases in the Hospital Discharge Register and the Cause of Death Register. This was most often found among men. The results suggest that liver diseases in Sweden during the last thirty years have a reduced mortality that may be associated with the reduction in spirit consumption on the aggregate level of the Swedish population. There are difficulties in differing between alcoholic and non-alcoholic liver disease using register data.

We investigated and followed up 12 281 patients who were hospitalized with esophageal varices. There was an increase in five-year survival between 1990 and 2002 compared to the years between 1969 and 1979 for all patients. There was a better survival for women than men, for younger patients compared to older and for patients hospitalized in the latest decade compared to the earlier decades. We found a significant decrease in mortality due to esophageal varices during the years studied but no decrease due to other causes. An improved prognosis for patients with esophageal varices may be related to improvement in therapeutic strategies towards acute variceal bleeding and secondary prophylactic treatment.

We interviewed 140 patients (50 with alcoholic cirrhosis, 50 with alcohol dependence without cirrhosis and 40 with non-alcoholic cirrhosis). We found that women drank less than men, and patients with alcoholic cirrhosis did not drink more than patients with alcohol dependence without cirrhosis. Women with alcoholic cirrhosis drank 14 009 drinks of alcohol during their lifetime compared to 45 658 drinks consumed by men with alcoholic cirrhosis. Women with alcoholic cirrhosis reported 9 198 drinks consumed as binge drinking compared to 25 890 drinks for women with alcohol dependence. Women with alcoholic cirrhosis drank less beer compared to women with alcohol dependence. The results suggest that patients with alcoholic cirrhosis seem to be predisposed to the hepatotoxic effects of alcohol - with a more pronounced sensitivity in women.

LIST OF PUBLICATIONS

- I. Lytton SD, Helander A, Zhang-Gouillon ZQ, Stokkeland K, Bordone R, Arico S, Albano E, French SW, Ingelman-Sundberg M. Autoantibodies against cytochromes P-4502E1 and P-4503A in alcoholics, *Molecular Pharmacology* 1999, 55: 223-233
- II. Stokkeland K, Brandt L, Ösby U, Ekbom A, Hultcrantz R: Morbidity and mortality in liver diseases in Sweden 1969-2001 in relation to alcohol consumption, *Scandinavian Journal of Gastroenterology* 2006, 41: 463-468
- III. Stokkeland K, Brandt L, Ekbom A, Hultcrantz R: Improved Prognosis for Patients Hospitalized With Esophageal Varices in Sweden 1969-2002, *Hepatology* 2006, 43: 500-506
- IV. Stokkeland K, Hilm G, Spak F, Franck J, Hultcrantz R: Different Drinking Patterns in Patients with Alcohol Cirrhosis Compared to Patients with Alcohol Dependence, submitted

CONTENTS

1. Thesis Summary
 - 1.1. Epidemiology of Alcoholic Cirrhosis
 - 1.2. Measures of Alcohol Consumption
 - 1.3. Definitions of Alcoholism
 - 1.4. Clinical Complications of Liver Cirrhosis
 - 1.5. Pathogenetic Mechanisms of Alcohol Cirrhosis

2. Aims of the study

3. Methods
 - 3.1. Methods used in Paper I
 - 3.2. Methods used in Paper II
 - 3.3. Methods used in Paper III
 - 3.4. Methods used in Paper IV
 - 3.5. The Statistical Methods used in the Papers

4. Results
 - 4.1. Paper I
 - 4.2. Paper II
 - 4.3. Paper III
 - 4.4. Paper IV

5. Discussion
 - 5.1. Methods used in the Studies
 - 5.2. Findings and Implications
 - 5.3. Conclusions

6. Acknowledgements

7. References

LIST OF ABBREVIATIONS

AUDIT	Alcohol Use and Disorder Test
LDH	Lifetime Drinking History
NO	Nitric Oxide
eNOS	Endothelial Nitric Oxide synthase
TNF α	Tumor Necrosis Factor alpha
CYP2E1	Cytochrome P450 2E1
CYP1A2	Cytochrome P450 1A2
CYP3A4	Cytochrome P450 3A4
CYP3A1	Cytochrome P450 3A1
CYP4A	Cytochrome P450 4A
CYP2D6	Cytochrome P450 2D6
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IgA	Immunoglobulin A
HER	1-hydroxyethyl radical
MAA	Malondialdehyde-acetaldehyde
ICD	International Classification of Diseases
DSM	Diagnostic and Statistical Manual of Mental Disorders
LAI	Lifetime Alcohol Intake
OA	Onset Age
DAC	Daily Alcohol Consumption
DD	Drinks per Day
DDD	Drinks per Drinking Day
CDT	Carbohydrate-Depleted Transferrin
ELISA	Enzyme-Linked Immunosorbent Assay
S.E.M.	Standard Error of the Mean
ALD	Alcohol related Liver Disease
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis

1 THESIS SUMMARY

This thesis includes results from a broad research program which aimed to assess the interactions between alcohol consumption and alcoholic cirrhosis in Sweden both on the epidemiological and individual level.

Specifically the following associations were studied:

- The possible development of autoantibodies to cytochromes after long-term exposure to alcohol as part of the development of immunological reaction, and its relation to the initiation and continuation of alcohol related liver disease.
- Alcohol sales and liver cirrhosis mortality and morbidity and the prognosis of liver disease in patients hospitalized with liver disease.
- The change in prognosis for patients hospitalized with esophageal varices regarding their age, sex and periods of discharge.
- The lifetime exposure to alcohol in patients with alcoholic cirrhosis and their drinking pattern in comparison with patients with alcohol dependence without cirrhosis and patients with non-alcoholic cirrhosis.

1.1 EPIDEMIOLOGY OF ALCOHOLIC CIRRHOSIS

Alcohol dependence is a huge problem world-wide, and the problems arising from extensive consumption has been known to man from the Curse of Ham (1). The epidemiology of liver cirrhosis has been extensively studied, because the disease itself is important but also because it has been seen as a marker of total alcohol consumption in a population. Whether total consumption trends or beverage-specific consumption trends are associated with cirrhosis mortality on an aggregate-level has been a longstanding focus of epidemiological interest (2-6).

The risk of acquiring alcoholic cirrhosis has been suggested to increase when the alcohol intake increases. In the National Survey on Drug Use and Health of 2004 the risk of cirrhosis due to alcohol was 1.30 for males with an average consumption of alcohol between 40 and 60 g daily and 13.00 for females (7). In a case-control study from Australia the risk of acquiring cirrhosis increased when the alcohol consumption exceeded 40 g per day (8). From a large cohort in northern California there was an increased risk of cirrhosis with increasing alcohol intake (9).

Whether alcohol intake increases the risk of acquiring liver disease in a linear fashion, or if there is a threshold of consumption, permitting liver disease to be developed above the threshold is a matter of discussion. From cross-sectional, case-control and prospective cohort studies a threshold has been observed. The threshold has been estimated to 20–40 g/day, 30 g/day, 12–24 g/day (women) and 24–36 g per day (men), 40 g/day, 40–80 g/day in fatty liver and alcoholic hepatitis and 80–160 g/day in fibrosis and cirrhosis, and <50 g/day (10-17). In a meta-analysis even low levels resulted in a significantly increased risk of liver disease (18).

Only a small percentage of individuals who have a high alcohol intake will develop cirrhosis. Population studies have measured the risk of acquiring alcoholic liver disease. They have found a frequency of 4.2 % of alcoholic liver disease among Northern Italians consuming 60 g or more of alcohol (19). In Danes who drank more than five drinks daily the risk of cirrhosis was estimated to be 5.9% of the study population (20). In addition, other external factors than alcohol consumption may adjust the risk of developing cirrhosis. Two US studies has found a possible lower risk for development of liver disease associated with coffee consumption with a hazard ratio of 0.46 (0.26-0.8) for more than 2 cups, and one-fifth the risk of non-coffee drinkers with a consumption of four or more cups per day (16, 21). Another Italian study showed an increased risk with the intake of non-fruit sugar and animal products, and a decreased risk of developing cirrhosis with the intake of vegetables and fruit (22).

The rate of total cirrhosis mortality is the most commonly used measure when studying alcohol's aggregate-level association with liver disease. In Sweden there has been a decrease in the mortality in liver cirrhosis since 1976, despite slightly increased total alcohol consumption, this change has been seen in France, Norway, Austria, Spain, Italy, and the USA (4, 23). In Britain an alarming increase is found when analyzing data from 1950 to 2002, which contrasts the decline seen in the rest of Europe, and the alarming development of mortality in liver cirrhosis for women in Scotland is highlighted – more so after recalculating their original data (24, 25). Sometimes the distinction between alcohol-related and non-alcohol related cirrhosis mortality rates are made in the analysis (26-32).

An improving trend in the survival experience of liver cirrhosis cases could decrease the apparent mortality rate in spite of increasing prevalence of cirrhosis. Hence the number of patients hospitalized with liver cirrhosis would then be a better measure of severe liver disease in the population. Few studies have compared the morbidity measured as hospitalization rates, to the consumption of different types of beverage in liver disease (26, 27, 29, 30, 33). The difficulty in understanding the complex relations between aggregate-level studies on alcohol consumption and liver cirrhosis mortality, and case-control studies with individual information on drinking patterns are recently reviewed, and the value of the hypothesis-generation made possible by associations found on the aggregate-level are emphasized (34).

Few studies have reported on differences in drinking pattern in liver cirrhosis. A Danish group have estimated the risk of becoming an excessive drinker (as defined by drinking above 14 and 21 drinks per week for women and above 21 and 35 drinks per week for men), and the risk of developing alcoholic cirrhosis were decreased for wine drinkers (35). In three Danish cohorts individuals with a total intake of wine of more than 51 % of their total alcohol intake had a risk of 0.3 of developing alcoholic cirrhosis, as reported from the Danish Death Certificate Register or Hospital Discharge Register, compared to no wine-drinkers whose risk were defined as 1.0. It is to be noted that these cohorts only had been responding to their consumption of alcohol in a standard week, and that more detailed questions regarding prior consumption were not asked (20, 36). They did find an increase in risk with increasing total intake of alcohol. In a case-control study using the Lifetime Drinking History questionnaire (LDH), a questionnaire which in detail assesses the prior consumption of different beverages, a protective effect of wine could not be found (37). In USA and in five predominantly beer-drinking countries the spirit consumption was associated with the mortality rates in liver cirrhosis on the aggregate level (4, 5).

1.2 MEASURES OF ALCOHOL CONSUMPTION

Alcohol consumption may be assessed by using the recorded legal sales of alcohol. In Sweden the Swedish State Monopoly provided a limited source of alcohol, and alcohol sales could be measured with some accuracy. After 1955 the alcohol sales are registered by the different beverage types, as spirits, wine (including cider and mixed drinks) and beer, and they are easily available and can be used as an approximation of actual alcohol consumption in Sweden (38).

Consumption surveys exist that address the alcohol consumption in the total population and in subgroups. In Sweden several consumption surveys examine the total consumption of alcohol at different points in time: 1990, 1998, 2000 and 2002. The surveys show an increase in consumed alcohol bought from the State Monopoly or at restaurants, from 4.8 litres of pure alcohol per inhabitant above the age of 15 in 1998 and up to 7.0 litres in 2002. Including legally and illegally imported alcohol and home production of alcohol the consumption increased from 8.0 to 9.9 litres of alcohol (39, 40).

The mortality for aggregated diagnoses of alcohol-related diseases has been used as an indirect measure of alcohol consumption. Patients who die with a diagnosis of liver disease represent a majority of the number of patients deceased from alcohol-related diagnoses, thus rendering this indirect way of measuring alcohol consumption unsuitable for studying liver disease mortality in relation to alcohol, but useful when controlling time-trends in survey results for changes in unrecorded consumption (41).

The consumption of alcohol may also be assessed by the use of questionnaires. In Sweden the Alcohol Use and Disorder Test (AUDIT) are used as a screening tool. It has been used in a random sample of Swedes where 18% of men and 5% of women showed signs of a hazardous drinking pattern. Questions about both frequency and quantity of drinking are included in the questionnaire (42-44).

There are questionnaires regarding alcohol consumption that span the whole lifetime, floating time period interviews asks the patients to define some points in their lives where the drinking pattern changed in a significant way. Their consumption is assessed with detailed questions regarding their drinking pattern between points of change (45). There exists an interview with fixed time periods that divides the patients lives into specific age periods. Similar detailed questions are asked as in the floating time period interview (46). Interviews can be administered as questionnaires to be filled in by the patients, and they can be performed as person-to-person interviews. The reliability of the LDH-interview is reported to be good, and the questionnaire has been reliability-tested and reliability is better when it is administered as an interview (47, 48). The questionnaire has been used in patients with alcohol dependence and a variety of liver diseases (37, 49-55). The lifetime drinking history has also been assessed in some non-hepatic disorders and diseases (56-59).

1.3 DEFINITION OF ALCOHOLISM

According to the International Classification of Diseases 10 (ICD-10) the Alcohol Dependence Syndrome is defined as a cluster of physiological, behavioral, and

cognitive phenomena in which the use of alcohol takes on a much higher priority for a given individual than other behaviors that once had greater value.

A definite diagnosis of alcohol dependence should usually be made only if three or more of the following have been experienced or exhibited at some time during the previous year: a strong desire or sense of compulsion to take alcohol, difficulties in controlling alcohol-taking behavior, a physiological withdrawal state when alcohol use has ceased or been reduced or use of alcohol with the intention of relieving or avoiding withdrawal symptoms, evidence of tolerance, progressive neglect of alternative pleasures or interests because of alcohol use, persisting with alcohol use despite clear evidence of overtly harmful consequences, such as harm to the liver through excessive drinking.

Narrowing of the personal repertoire of patterns of alcohol use has also been described as a characteristic feature (e.g. a tendency to drink alcoholic drinks in the same way on weekdays and weekends, regardless of social constraints that determine appropriate drinking behavior). It is an essential characteristic of the dependence syndrome that either alcohol taking or a desire to take alcohol should be present; the subjective awareness of compulsion to use alcohol is most commonly seen during attempts to stop or control alcohol use.

In this thesis the definition of alcoholism is used in paper I and II and III, and even earlier definitions from ICD-8 and ICD-9 in paper III (60-62).

The Diagnostic and Statistic Manual of Mental Disorders (DSM) IV relies on symptoms for its definition. The DSM says that addiction, or dependence, is present in an individual who demonstrates any combination of three or more of the following symptoms occurring at any time in the same 12-month period: Preoccupation with use of the chemical between periods of use, using more of the chemical than had been anticipated, the development of tolerance to the chemical in question, a characteristic withdrawal syndrome from the chemical, use of the chemical to avoid or control withdrawal symptoms, repeated efforts to cut back or stop the drug use, intoxication at inappropriate times, or when withdrawal interferes with daily functioning, a reduction in social, occupational or recreational activities in favor of further substance use and a continued substance use in spite of the individual having suffered social, emotional, or physical problems related to drug use (63). This definition of alcoholism is used in paper IV.

1.4 CLINICAL COMPLICATION OF LIVER CIRRHOSIS

Liver cirrhosis is a slow and progressive disorder. Liver cirrhosis may present itself first with unspecific symptoms as fatigue and nausea. In many cases, even of fibrosis and cirrhosis, a thorough investigation and history will reveal no specific symptoms and signs. In hepatic decompensation or acute or chronic liver failure specific liver-related symptoms and signs may occur. Liver related symptoms are related to the diminishing liver function, or to the portosystemic shunting of blood. The shunting transports products not metabolized by the liver into the systemic circulation. Other signs of liver cirrhosis are related to the decompensation, and they occur with a significant loss of hepatic function, as jaundice caused by the decreased ability of the liver to metabolize bilirubin. Hypoalbuminemia results from the reduced synthesis of albumin, and it will result in ascitic fluid and peripheral edema. Coagulopathy caused by reduced synthesis of coagulation factors will lead to bleedings in the skin or in the

mucosa. The increasing fibrosis and cirrhosis increases the portal pressure and leads to ascitic fluid, hepatic encephalopathy and esophageal varices. In addition: hepatic fetor, palmary erythema, caput medusa and spider naevi occurs (64).

Portal hypertension is a main complication of cirrhosis. It occurs when the resistance in the portal vein increases after fibrosis and cirrhosis has occurred in the liver and destroyed liver architecture. Primary intrahepatic vasoconstriction adds to the effect, and esophageal varices may develop. Postprandial hyperemia and peristaltic contractions gives rise to increased pressure in the esophageal varices and may contribute to rupture of the varices. The impaired intrahepatic vasodilatory response may further increase the pressure (65).

Haemorrhage of the esophageal varices is a frequent cause of death among cirrhotic patients, and the average mortality after the first variceal bleeding are reported to be 30-50% (66, 67). The current state-of-the-art treatment for acute variceal haemorrhage includes endoscopic and pharmacological control with intravenous administration of vasoactive drugs, and antibiotics. This has been reported to reduce mortality (68, 69). Secondary prophylaxis includes pharmacological agents, such as propranolol, and follow-up programs with endoscopical band ligation and in addition surveillance for varices and in some cases interventional radiological procedures (70). Randomized controlled trials exist on well-defined patient groups treated for bleeding and prophylaxis against rebleeding, but there are few long-term studies in a population-based setting (71-74).

1.5 PATHOGENESIS OF ALCOHOLIC CIRRHOSIS

In addition to the effect of alcohol many host factors influence the development of alcoholic cirrhosis, as the hepatotropic viruses – especially hepatitis C, polymorphisms in alcohol metabolizing genes and polymorphisms in inflammatory genes. Other factors such as obesity may worsen alcoholic liver disease. Heterozygosis for alpha-1-antitrysin deficiency and for hemochromatosis does not seem to predispose to advanced alcoholic liver disease (75).

Ethanol is metabolized to acetaldehyde by the alcohol dehydrogenase and the microsomal ethanol oxidizing systems. The microsomal ethanol oxidizing systems is located in the endoplasmic reticulum of the hepatocytes. Ethanol intake results in proliferation of the smooth endoplasmic reticulum, and it increases the amount of enzyme activities of the microsomal membranes in the hepatocytes (76-78). The microsomal ethanol oxidizing systems are inducible by chronic ethanol feeding (79, 80). The key enzyme of the microsomal ethanol oxidizing systems is the ethanol-inducible cytochrome P4502E1 (CYP2E1), which is increased fourfold to 10-fold in liver biopsy with a corresponding rise in mRNA with chronic alcohol exposure (81, 82). The CYP2E1 gene has been isolated and characterized in humans, and it is localized to chromosome 10 (83). This induction of the CYP2E1 contributes to the metabolic tolerance to ethanol that develops with an elevated alcohol intake, apart from the CNS tolerance. Cytochrome P450s other than CYP2E1 such as CYP1A2 and CYP3A4 may also contribute to ethanol metabolism in the microsomes (84).

The damage caused by oxidative stress in alcoholic liver injury includes mitochondrial injury, which exacerbates the oxidative stress (85). CYP2E1 leaks oxygen radicals and when they exceed the cellular defense systems they result in oxidative stress and

induction of fibrosis (86-90). Alcohol adducts are reactive compounds, which can bind to proteins. They develop both from ethanol metabolism and ethanol-induced oxidative stress. Adducts change proteins and may induce autoimmunity (91). Acetaldehyde binds to many compounds, and may act as an adduct. Acetaldehyde may bind to reactive lysine residues, aromatic amino acids, cysteine or hemoglobin (92-94). Elevated IgG and IgA antibodies against acetaldehyde-adducts were found in patients with alcoholic liver disease (95). Hydroxyethyl radicals (HER) are the product of the interaction between ethanol and hydroxyl radicals. The targets of human anti-HER IgG have been identified by immunoblotting as four microsomal proteins, including CYP2E1 (96). In both rats and humans the presence of anti-HER antibodies shows a strict correlation with CYP2E1 activity (97, 98).

Malondialdehyde and 4-hydroxynonenal forms adducts with proteins. Hybrid adducts with acetaldehyde and malondialdehyde have been designated as malondialdehyde-acetaldehyde adducts (MAA adducts) (99). Antibodies towards MAA adducts have shown correlation with alcoholic liver disease in humans (100). In humans, protein adduct formation is in addition associated with induction of cytochromes 2E1, 2A, and 3A (101).

Immunohistochemical studies have found acetaldehyde-protein adducts in zone 3 hepatocytes in the early phase of alcoholic liver disease (102-106). In advanced alcoholic liver disease adduct distribution is more irregular and widespread (107-110).

The oxidative stress in the hepatocytes caused by CYP2E1 induction and mitochondrial injury results in lipid peroxidation and membrane damage and impaired oxygen capacity and may lead to a vicious circle of increasing acetaldehyde accumulation and more mitochondrial injury (111, 112). Lipid peroxidation products stimulate fibrosis, which is also increased through decreased feedback inhibition of collagen synthesis because acetaldehyde forms adducts with the carboxyl-terminal propeptide of procollagen (113). Oxidative stress promotes inflammation, which is aggravated by an increase of the proinflammatory cytokines in Kupffer cells. Kupffer cells are a major source of cytokines and they harbor CYP2E1 which is increased after chronic alcohol consumption (114).

The hepatic stellate cell plays a culprit role in the fibrogenetic pathway leading to liver fibrosis and cirrhosis. The transition of the hepatic stellate cells from indolent retinoid storing cells to an activated myofibroblast-like cell may be induced by acetaldehyde and acetaldehyde adducts (115). Other stimuli may activate the stellate cell, as paracrine stimuli from neighbor cells, endothelial cells and Kupffer cells as well as changes in the extra-cellular matrix. The "leaky-gut" hypothesis suggests that increased amount of gut-derived endotoxins activates Kupffer and other inflammatory cells, and by production of inflammatory cytokines the activation of the hepatic stellate cell occurs. The gut-derived endotoxins may directly activate the hepatic stellate cell as well (116). This contributes to the perpetuation of liver damage in alcoholic cirrhosis. The activated hepatic stellate cell increases the collagen production, and the collagen degradation is impaired, thus increasing the fibrosis further towards cirrhosis (116).

The stellate cell is the main responsible cell for the increased sinusoidal resistance leading to intrahepatic vasoconstriction in the liver (117). The vasoconstriction may be caused by impaired nitric oxide (NO) production and decreased endothelial nitric oxide synthase (eNOS) activity. The portal hypertension is maintained even after collaterals

have been formed by an increased splanchnic blood inflow secondary to vasodilatation. The splanchnic and systemic vasodilatation leads to the development of a hyperdynamic circulatory state that worsens all complications of cirrhosis. The vasodilatation seems to be caused by a splanchnic increase in eNOS-derived NO. Bacterial translocation increases production of tumor necrosis factor alpha (TNF α) and NO which further increases the vasodilatation (118). This hyperdynamic state is in addition responsible for increased blood flow in esophageal varices, their growth and eventual rupture.

2 AIM OF THE STUDY

To study the pathogenetic mechanisms in alcoholic liver disease, in order to assess if alcohol intake could induce autoantibodies towards CYP2E1 and CYP3A.

To study the associations between mortality and morbidity in alcoholic liver disease, and to determine the relation of consumption of different alcoholic beverages, in order to elucidate if different beverages influence the mortality and morbidity. Furthermore we wanted to study the prognosis for patients with alcoholic liver disease.

To study if there had been changes in prognosis for patients with esophageal varices, in order to find changes in survival for patients with severe liver disease.

To study the consumption pattern of alcohol in patients with alcoholic cirrhosis in order to determine the role of different drinking patterns and drinking volumes of different beverages in the development of alcoholic cirrhosis.

3 METHODS

3.1 METHODS USED IN PAPER I

Three groups of Swedish patient were enrolled. In the first group there were 85 patients who were admitted for alcohol detoxification. A second group of 18 patients with alcoholic cirrhosis were recruited from the Department of Gastroenterology and Hepatology at Karolinska University Hospital. A third group consisted of 58 social drinkers and 14 individuals abstaining from alcohol. Sera from these patients were retrieved and prepared through centrifugation.

From Italy first a group of 25 patients were included with alcohol abuse and steatohepatitis or alcoholic cirrhosis. A second group 12 heavy drinkers without clinical evidence of liver disease were included. These patients had an estimated mean alcohol intake of ethanol of more than 100 g per day. A group of healthy control subjects, consisted of 14 subjects abstaining from alcohol, was recruited within and through research staff. In all patients their alcohol abuse were assessed by standardized questionnaire and confirmed by positive CDT testing.

Male Wistar rats were pair-fed ethanol or isocaloric dextrose continuously infused via a permanent intragastric cannula for 2 months in the amount necessary to maintain a constant high blood ethanol level greater than 200 mg/100 ml (119). Two other groups were treated with dextrose plus chlormethiazole (80 mg/kg/day in the diet or in a bolus 80-120 mg/kg/day) or with ethanol plus chlormethiazole. Blood samples for preparation was taken before and after 1 and 2 months of treatment. At the end of the experiment, livers were removed and microsomes were prepared. The relative amounts of CYP2E1, CYP3A1, and CYP4A in the different microsomal preparations were assessed in ELISA using rabbit anti-CYP3A1 antibodies.

Polystyrene microwell plates for ELISA were coated with purified CYPs or serum albumin. After incubation, the antigen solutions were removed. Rabbit sera against CYP3A1 or CYP2E1 were added. The secondary were incubated. The results were expressed as difference between the absorbances in the well containing CYPs and those containing human serum albumin.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed using stacking gel and separating gels. After electrophoresis the proteins were electrotransferred. Blots were washed and then probed with diluted rabbit anti-CYP2E1 or rabbit anti-CYP3A1 sera or different human sera. Immunoblots were performed for and developed on photographic film.

3.2 METHODS USED IN PAPER II

All Swedish residents have a national registration number, this is a unique personal identifier that is assigned to them (120). The National Board of Health and Welfare has collected information of the primary and the secondary causes of death for all Swedish residents in The Cause of Death Register since 1952 in a computerized form (121).

The Swedish Hospital Discharge Register has data on individual hospital discharges in Sweden since 1964. It increased in coverage, by increasing numbers of completely covered counties until it was complete for the whole country in 1987. When using the

national identification number one can identify all hospital care for one individual patient since 1964. The register has a high validity (122).

The Cause of Death register and the Swedish Hospital Discharge Register were used to identify all patients with alcohol-related and non-alcohol related liver diseases diagnosed according to the ICD –8, -9 and –10 (60-62). We chose to use the last three ICDs, starting with ICD-8 in 1969, to explore the trends in liver diagnoses.

We defined non-alcohol related liver diseases as 070, 999 and E939, 570.9, 570.01, 570.02, 570.08, 570.09, 571.90, 571.98, 571.99, 572.99, 573.00, 573.01, 573.02, 573.08 and 573.09 according to ICD-8, and 570, 571 E-W, 572, 573 and 070 in ICD-9. B18 and K71-77 were used in ICD-10. Non-alcoholic cirrhosis was defined as 571.90 in ICD-8, and 571F in ICD-9. It is not possible to classify non-alcoholic cirrhosis in ICD-10, because the patients, who have developed cirrhosis caused by a specific non-alcohol related liver disease, will be diagnosed according to their original diagnoses causing cirrhosis (e.g. patients with primary biliary cirrhosis will have the diagnose K74.3 from the initial phase of the disease to end-stage liver disease).

Alcohol related liver diseases were defined as 571.00 and 571.01 in ICD-8, 571A-D in ICD-9, and K70 in ICD-10. Alcoholic cirrhosis was defined as 571.00 in ICD-8, 571C in ICD-9, and K70.3 in ICD-10.

The diagnosis of alcoholism was defined as 291 and 303 in ICD-8, 303 and 305A in ICD-9, and F10 in ICD-10.

We have used registered sales of alcohol from the State Alcohol Monopoly (38). The different types of alcohol were divided into spirits, wine and beer.

3.3 METHODS USED IN PAPER III

By using the national registration number for Swedish residents we used data from The Swedish Hospital Discharge Register at the National Board of Health and Welfare and linked the data with The Cause of Death Register as described in paper I (121-122).

These registers were used to identify all patients with esophageal varices diagnosed according to ICD–8, -9 and –10 (60-62). All patients who were hospitalized with esophageal varices as primary and secondary diagnosis were included. To calculate the cause-specific mortality all patients with a diagnosis of esophageal varices as primary and secondary diagnosis in the Death Certificate Register were included.

We defined esophageal varices as 456.00 according to ICD-8, and 456 A (with bleeding) and 456 B (without bleeding) in ICD-9. In ICD-10 we defined esophageal varices as I 85.0 (with bleeding) and I 85.9 (without bleeding).

3.4 METHODS USED IN PAPER IV

Fifty patients with alcoholic cirrhosis and 40 patients with non-alcoholic cirrhosis were recruited from the Department of Gastroenterology and Hepatology, Karolinska University Hospital in Stockholm and from the Department of Internal Medicine, Visby Hospital. Fifty patients with alcohol dependence were recruited from the Magnus Huss Clinic, Karolinska University Hospital and the Alcohol Clinic, Visby Hospital during

the same period. They all had alcohol dependence according to DSM-IV criteria (63). Ten of the patients with alcoholic cirrhosis had Child-Pugh's grade A, 23 had grade B and 17 had grade C (123). All patients with alcoholic cirrhosis had been hospitalized previously for alcohol dependence. Twenty-two patients with non-alcoholic cirrhosis had Child-Pugh grade A, 14 had grade B, and four had grade C. Eighteen of the patients with alcoholic cirrhosis in this paper participated in paper I.

The diagnosis of liver cirrhosis was based on clinical signs of complications due to liver disease or by persistent biochemical abnormalities indicating cirrhosis. Exclusion criteria were terminal disease, psychiatric disorders which made interviewing impossible or untreated encephalopathy.

The interview Lifetime Drinking History (LDH) was used to provide quantitative indices of past alcohol consumption. The questionnaire was designed to be scanned and interpreted by computer software (48). The interview included questions regarding beer, wine and spirits. The definition of a standard drink was 12 grams of pure alcohol. The onset of alcohol intake was registered, and identical questions were asked for the age intervals from 12 to 18, 19-27, 28-44, 45-60 and for the age above 60 years.

Based on the answers in the questionnaire we could determine the duration of alcohol consumption (DAC), the drinking days (DD), the lifetime alcohol intake (LAI), the average number of drinks per drinking day (DDD) and the volume of binge drinking.

3.5 THE STATISTICAL METHODS USED IN THE PAPERS

3.5.1 In paper I

The values presented in this work are the mean standard error of the mean (S.E.M.). Unless mentioned otherwise, all statistical analyses were performed with StatMost program using parametric unpaired Student's *t* test.

3.5.2 In paper II

Mortality and incidence rates of liver disease were directly age standardized with the age structure of the population in Sweden 1986 as reference. Temporal trends were analysed graphically and tested as linear trends in a Poisson regression model, taking age and sex into account (124). Prognosis of patients hospitalized with liver disease was analyzed in survival curves by the actuarial method and tested with log rank test.

3.5.3 In paper III

The mortality rates were calculated for all patients by the Kaplan-Meyer method and comparisons were made with the use of log rank tests.

The cause-specific mortality was defined as the patients hospitalized with esophageal varices that were diagnosed according to ICD-8, ICD-9 and ICD-10 as deceased from esophageal varices. The data from patients who died of other causes than esophageal varices are not taken into account when calculating the cause-specific mortality. Non-variceal mortality was defined as the mortality for the patients deceased from all other causes.

Proportional hazard Cox regression models were performed on discharge groups, sex and age (125). Hazard ratios were expressed as the relative hazard of death for men compared to women, and for patients older than 70 years, and those between 50 and 69 years compared to those younger than 50 years. The hazard ratio was also calculated for the later discharge groups compared to the patients hospitalized between 1969 and 1979.

3.5.4 In paper IV

Overall differences in drinking parameters between the three patient groups were analyzed using the Kruskal-Wallis test combined with exact Wilcoxon post-hoc tests to detect differences between groups (126, 127). The p-values from the paired comparisons were adjusted by the Holm method (128).

4 RESULTS

4.1 PAPER I

Rats receiving an ethanol-containing diet by intragastric feeding to maintain high blood alcohol levels developed changes that resembled those seen in ALD, including fatty change, focal inflammation, necrosis and fibrosis (134). Analysis by ELISA for the expression of autoimmune reactivity against CYPs demonstrated that by 1 month after ethanol administration, rats fed ethanol had an appreciable increase of IgG reactivity against either CYP3A1 or CYP2E1 compared with baseline values. When anti-CYP reactivity was expressed as percent of the baseline values, ethanol-fed rats exhibited a 2- to 3-fold increase over control animals not receiving ethanol.

The sera from alcoholics and patients with alcoholic liver disease and from social drinkers were evaluated for the presence of IgG reacting with CYP2E1 and CYP3A4, using purified recombinant human CYP2E1 and CYP3A4. Positive ELISA reactions against human CYP3A4 and CYP2E1 were found at a higher frequency among the alcoholics and the patients with alcoholic liver disease than among control subjects. The mean anti-CYP3A4 reactivity among Swedish alcoholics was $A_{490} = 0.38 (\pm 0.28)$, and among patients with alcohol liver disease the value was similar $0.4 (\pm 0.24)$, whereas among control subjects, $A_{490} = 0.27 (\pm 0.13)$. The reaction with CYP2E1 was less intense in the sera, and the corresponding mean values were 0.12 ± 0.07 among alcoholics, 0.19 ± 0.02 in patients with alcoholic liver disease and 0.09 ± 0.05 in control subjects.

The presence of anti-CYP2E1 and anti-CYP3A4 reactivity was further examined in the Italian population. The mean IgG autoreactivity against recombinant human CYP3A4 was $0.43 (\pm 0.22)$ among Italian alcoholics and $0.36 (\pm 0.21)$ among ALD patients versus $0.21 (\pm 0.07)$ for the control subjects. IgG autoreactivity against CYP2E1 was elevated among the Italian alcoholic groups to similar levels: $0.43 (\pm 0.3)$ in alcoholics and $0.28 (\pm 0.13)$ in alcoholic liver disease (ALD) patients versus $0.24 (\pm 0.07)$ for the control subjects.

The reactivity of sera from alcoholics against recombinant human CYP2E1 and CYP3A4 was also investigated by immunoblotting. We observed that 18 of 85 sera (21%) from alcoholics and 3 of 18 sera (20%) from ALD patients recognized CYP3A4, whereas 8 of 85 (10%) alcoholic sera and 2 of 18 ALD sera (11%) tested positive against human CYP2E1.

4.2 PAPER II

There were 26 005 persons, 17 095 men and 8 910 women, who died of liver disease as a primary or secondary cause of death between 1969 and 2001. Of the deceased diagnosed with a non-alcohol related liver disease as cause of death 22% of men and 9% of women had been hospitalized for alcoholism, and 35% of men and 18% of women had previously been hospitalized with a diagnosis of alcohol related liver disease.

Total mortality for men from all liver diseases increased to 19 per 100 000 in 1976. The mortality gradually decreased after 1976. For men with alcohol-related liver disease

there was an increase up to 1980. The increase was followed by a decrease to about 4 per 100 000 deaths per year in the last 15 years.

For women there was no significant linear trend in total mortality in liver disease before 1976. The mortality gradually decreased from 1976. There seems to be an increase in mortality since 1997. For women with alcohol-related liver disease the mortality increased to 1980, with no significant linear trend after 1981.

The spirits sales increased from 1969 up to 3.8 litres in 1976, and then decreased to 1.2 litres in 1998, which seems to follow the same pattern as total liver disease mortality while sales of wine and beer did not decrease during the period.

There was a decrease in the number of hospitalized patients with liver disease between 1987 and 2001 although there was an increase in hospitalized patients with liver disease between 1992 and 1996. There were 32 225 patients hospitalized with liver diseases between 1987 and 2001 and 13 073 of these died after discharge. The prognosis was poor for all patients; younger patients had a much better prognosis than older. Men had poorer prognosis than women in all age groups.

4.3 PAPER III

Between 1969 and 2002 there were 12 281 patients hospitalized with the diagnosis of esophageal varices. The percentage of women increased from 29.3 to 33.6%. The incidence of patients hospitalized with esophageal varices was stable at a level between 4 and 6 per 100 000 inhabitants hospitalized per year after 1972.

There was high overall mortality during the first year after discharge. There was a statistically significant improvement in survival over time (log rank-test $p < 0.0001$). Women had a better survival rate than men in all age and discharge groups. The multivariate hazard ratio of death showed an improved survival for women compared to men and the hazard ratio was higher in the earlier discharge groups.

The differences in cause-specific mortality between the three discharge groups were significant, improving over time (log rank test of $p < 0.0001$). We did not find any differences in non-variceal mortality between men and women, or between the discharge groups. The main cause of death for patients hospitalized with esophageal varices was liver disease.

4.4 PAPER IV

The mean onset age for alcohol consumption was between 16.5 and 20.9 years for patients with alcoholic cirrhosis and patients with alcohol dependence. Patients with alcoholic cirrhosis did not have a significantly different duration of alcohol consumption compared to the patients with alcohol dependence. Patients with alcoholic cirrhosis did not have a statistically different number of drinking days when compared to patients with alcohol dependence. The number of days and number of years the patients with alcohol cirrhosis and the patients without cirrhosis were exposed to alcohol were the same.

Women with alcoholic cirrhosis drank 4.4 drinks per drinking day compared to 5.8 drinks for women with alcohol dependence ($p<0.01$). Men with alcoholic cirrhosis had consumed 6.2 drinks per drinking day as compared to men with alcohol dependence who had been drinking 8.5 drinks per drinking day ($p<0.05$). Patients with alcoholic cirrhosis drank significantly less drinks per drinking day compared to patients with alcohol dependence.

Women with alcoholic cirrhosis had drunk 14 010 drinks during their lifetime whereas women with alcohol dependence had drunk 27 440 drinks, although the difference between the mean value was large it did not reach statistical significance ($p=0.09$). Men with alcoholic cirrhosis had drunk 45 660 drinks during their lifetime, and men with alcohol dependence had drunk 49 760 drinks, which did not differ significantly ($p=0.78$). The lifetime alcohol intake did not differ significantly between patients with alcoholic cirrhosis and patients with alcohol dependence.

A large difference was seen when comparing women with alcoholic cirrhosis to women with alcohol dependence when we analyzed the total amount of alcohol they had consumed as beer during their lifetime. Women with alcohol dependence had drunk 11 350 glasses of beer compared to women with alcoholic cirrhosis who had drunk 1 611 glasses ($p<0.01$). Women with alcoholic cirrhosis had drunk significantly fewer drinks of beer than women with alcohol dependence.

When comparing women with alcoholic cirrhosis to men with alcoholic cirrhosis women had a later onset of alcohol consumption ($p=0.04$). Women consumed less alcohol during their lifetime than men ($p<0.0001$). The total consumption of beer was significantly less for women ($p<0.0001$), as well as spirits consumption ($p=0.0009$), but there was no significant difference regarding their consumption of wine ($p=0.776$). Women with alcoholic cirrhosis drank less alcohol as binge drinking than men ($p<0.0004$). In addition women had fewer drinking days ($p=0.007$), and drank fewer drinks per drinking day ($p=0.014$), and their onset age were significantly later than men ($p=0.04$), although both women and men had a similar duration of alcohol consumption ($p=0.99$).

5 DISCUSSION

5.1 METHODS USED IN THE STUDIES

This thesis consists of papers where different study designs are used. In paper II and III we have used cohort studies. They are well suited for the studies of rare outcome, and are less prone to selection bias and selective recall of exposure especially when the information is collected through well-functioning registers. Cohort studies do not represent impossible expenses and can be performed using register data. This design could be the most efficient, especially since the cost will be independent of the sample included in the study. In study II and III we used a registry based cohort to maximize the efficacy and to minimize the role of chance and biases. In paper I and IV we have used case-control designed studies. These studies have advantages when studying multiple exposures and even multiple causes. The main disadvantage is that the selection of cases and controls may give rise to biased assessment of the exposure. We have studied a rather small patient population with a detailed assessment of the exposure to alcohol retrospectively in paper IV. A prospective study design could have been used in paper IV, although this would have increased the duration of the study to several decades and increased the risk of introducing interventional bias in the study.

Errors in study results may occur with any study design used. There are at least four major problems with study design which may alter the results of the studies. These problems are selection bias, recall bias, confounding and chance.

Selection bias in cohort studies may even occur when careful measures have been taken to select the right patients to the cohort, this may be beyond the control of the investigators. In paper II and III the patients were selected from the total Swedish population, but even so there are patients that never are hospitalized and even patients that die of these diseases unrecognized by the physicians reporting to the Hospital Discharge Register or to the Death Certificate Register. In addition, physicians may have recognized the disease but not registered it. This is probably more frequent with the diagnoses of liver diseases than when diagnosing esophageal varices. Esophageal varices may be missed if the patients die of an acute upper gastrointestinal bleeding without known liver disease or alcohol dependence, especially if an upper endoscopy was not performed. The widespread use of upper endoscopies in Sweden since the 1970s does not support the notion that this should be a large group of patients. In addition, patients hospitalized with esophageal varices were included from the start of the study without a washout period, and during the period when the Swedish Hospital Discharge Register did not cover the whole of Sweden. This could have biased our results regarding the mortality of the patients in the first discharge group, because some patients would have been hospitalized earlier with esophageal varices. This bias would lead to a larger improvement of mortality if a washout period was applied. We did not see regional differences for the mortality rates in the different counties in Sweden during our study period. We think that neither the inclusion of all patients with esophageal varices at the start of the study period, nor the incomplete coverage of the Swedish Hospital Discharge Register rates have impaired our results. Therefore, we think that selection bias is not a major problem related to study II and III. In paper I and IV we have selected patients to the studies and the possibility of selection bias is clearly present, but we have tried to avoid that by applying a consecutive enrollment procedure. In addition to the careful selection of cases we have used controls with alcohol dependence where the exposure-time to alcohol is similar. Thus we think that

the differences found in drinking patterns represent true differences between the two groups. We do think that even in paper I and IV the selection bias is not a factor that would change our findings.

The problems regarding recall bias are the second problem in regard to study design, and it is best exemplified by paper IV. Biased answers regarding the alcohol patients have consumed in the past may occur even if the questionnaire used is reliable. All patients with alcoholic cirrhosis had been hospitalized at least once for alcohol dependence, thus we think that the recall bias may have played little role, and we think that the interview and technique used rendered the most valid report possible of their consumption of alcohol.

Confounding factors are a third problem with epidemiological studies. Confounding factors are themselves associated with the exposure; they are risk factors for outcome, but not intermediate steps from exposure to disease. It can be exemplified as finding an association for the wrong reason. Factors which may interfere with outcome in paper I would be smoking status, a change in weight distribution and the number of patients drinking coffee in the study population. This is also the case for the cohort of patients with esophageal varices, and in that population the cause of liver disease could be another confounder. Information regarding the etiology of their liver disease does exist but has not been analyzed. It should be noted that the confounders affecting the mortality in paper II and III would require a large effect on mortality to change our analysis as the five years mortality rates are high. In paper I and IV, other factors which are not registered such as current medication, coexisting diseases and disabilities, smoking status and coffee drinking could affect the outcome.

The fourth methodological problem which may affect the outcome of our studies is chance. Both null results and positive findings may be due to chance. Statistical tests of significance and the application of confidence intervals may judge the probability that a given result is due to chance rather than causal. In paper II and III the patient material consist of a large cohort of patients thereby ruling out insufficient number of patients as a cause of chance. In paper II the study design can never establish a causal relationship between the spirit sales and liver disease mortality and morbidity although the changes in mortality for men were highly significant. The differences in prognosis between men and women, and the worsening of the prognosis with increasing age, are probably not caused by chance. In paper III the cohort size exceeds the sum of all earlier cohorts of patients with esophageal varices were the prognosis has been assessed. The relative risk clearly indicates that there has been a reduction in mortality the first year after hospitalization with esophageal varices. In paper I, and also in paper IV, the patients we have examined represent a small sample size. Therefore chance may have played a part in the results we found both in drinking pattern and in levels of antibodies against cytochromes. Because of the small sample size and the small power of these studies the results should be interpreted with caution. In paper IV we did use non-parametrical methods to analyze the results to avoid misinterpretations. However the differences we found with regard to the drinking pattern in women with alcoholic cirrhosis compared to men with alcoholic cirrhosis were all highly significant, making chance a less probable cause. In addition there exist earlier studies that have suggested that the increased susceptibility to alcohol could be explained by interactions by alcohol with the female sex hormones. It is known that estrogen enhances the immune response in women, and this enhancement could increase the female risk for developing cirrhosis (129). The reduction of fibrosis when exposed to estrogen has even been found in experimental animals and cell lines (130, 131). These experiments provide a biological

explanation to our findings, and reduce the possibility of chance as an explanation for our findings. The differences found in drinks per drinking day for all patients with alcoholic cirrhosis compared to the patients with alcohol dependence without cirrhosis are more likely to be a result of chance, but do support the conclusion that patients with alcoholic cirrhosis do not drink more than the patients with alcohol dependence without cirrhosis.

After checking for biases, confounding and chance as mentioned above, we want to see if our findings are applicable to other populations. The ability to generalize our findings to other populations than the one studied is called external validity. This relies on the characteristics of the study population, and on the effect of exposure. In this thesis both population-based studies and cohort studies are included. Whether the results regarding the mortality rates found in paper II are valid for all patients with liver diseases in Sweden are more unlikely. There are certainly many patients who are never hospitalized with the diagnosis of a liver disease, and some are probably never in contact with health care regarding their liver disease. They may have low virulent liver diseases without flares, as fatty liver disease, hepatitis C, alcoholic liver disease and hemochromatosis. These patients will lack information regarding their liver disease in their health records. In addition complications of cirrhosis could be misinterpreted as unrelated to liver disease. Hepatorenal failure may be misinterpreted as primarily caused by renal or cardiovascular disease, or liver failure misinterpreted as heart failure when concomitant cardiovascular disease exists. In paper III, patients who have esophageal varices that never have been hospitalized may exist, but patients with bleeding esophageal varices almost certainly will be admitted to emergency departments as upper gastrointestinal bleedings, and correctly diagnosed varices are probable. Thus our results should be applicable to all patients with esophageal varices, with the exception of the few with small varices found en passant on upper endoscopy and those where upper gastrointestinal bleeding is wrongly diagnosed as caused by other diseases than varices, thus making paper III externally valid. In studies I and IV consecutive patients were recruited from two Departments of Medicine in Sweden. Severe cases of alcoholic cirrhosis with severe encephalopathy have been excluded in paper IV; these patients may have had a drinking pattern and levels of autoantibodies against cytochromes that differed from the patients we recruited. This is of concern regarding the interpretation of the results in paper IV, but we consider the number of patients that we excluded to be very small. These patients' age and sex distribution matched the patients hospitalized for liver diseases in Sweden and we therefore have proposed that the results found are externally valid for the population of patients with alcoholic cirrhosis seen both as in-patients and out-patients at hospitals in Sweden.

5.2 FINDINGS AND IMPLICATIONS

As paper I was one of the first papers reporting on autoantibodies toward CYP2E1 and CYP3A4 in patients with elevated alcohol consumption the paper has been the basis of much recent research. The interaction of CYP2E1 in the induction of hepatocyte injury by the formation of acetaldehyde, the formation of adducts and the interaction with the hepatic stellate cell in inducing liver fibrosis was reviewed recently (132). There even exist studies that indicate that CYP2E1 may not play a role in alcohol-induced liver disease (133, 134). The findings imply that we should continue to elucidate the mechanisms involved in the development of alcoholic cirrhosis, and that autoantibody formation may play a role both in the initiation and the continuation of the pathogenetic process. Whether the development of antibodies in paper IV is related to the drinking pattern, the prognosis (studied in paper II and III), the gender of the patients or the

morbidity in liver cirrhosis (as in paper IV) has not been studied in this thesis, but will be studied in detail in the future.

The relation between spirits sales and mortality in liver disease that we found in paper II is supported by some existing papers (4, 5, 34). The poor differentiation between alcohol and non-alcohol related liver disease both regarding the mortality and morbidity is in line with earlier studies (27-31). The prognosis we found is in line with the results found in our paper III, where the patients had a poorer prognosis and also had esophageal varices as a marker of the end-stage of liver cirrhosis. Even poorer survival has been reported in previous studies (135-139). In paper IV we could not find that the spirit consumption was increased compared to alcohol dependent patients without cirrhosis. This was maybe due to a small sample size, or maybe due to that the patients we have interviewed were the survivors among the patients with alcoholic cirrhosis. Their drinking pattern may differ from the average patient with cirrhosis. The findings in paper II imply that we should identify the subgroup of the population who predominantly drink spirits, and examine the possible link between spirit consumption and the risk of developing liver disease so an increase in liver-related mortality can be avoided. In addition we should examine if there are other factors in the predominately spirit drinking population to elucidate if there exist confounders who increases the mortality in liver disease.

The improvement in total mortality for patients hospitalized with esophageal varices in paper III - which is the world's largest cohort study with patients with esophageal varices - confirms earlier findings in smaller selected cohorts (71-74). The earlier studies compare well with our 1-year total mortality, but are far worse than our 1-year cause-specific mortality. Comparison of two cohorts of patients with esophageal variceal hemorrhage showed an improvement in 30 days mortality of 29.6% in the patient cohort from 1981-82 compared to 20.8% in the cohort from 1988-91 which probably is in line with our patients even if we have included all patients hospitalized with esophageal varices without making the differentiation between bleeding and non-bleeding varices (73). Our finding of a decrease in cause-specific mortality implies that we should continue to focus on the education of gastroenterologists in the treatment of esophageal varices to ensure a continuous improvement. In addition, as the non-variceal mortality does not improve during the study period this emphasizes the imminent need for improved treatment strategies for liver cirrhosis.

The drinking volumes found in paper IV are similar to the volumes of alcohol drunk by Swedish patients with hepatitis C and lower than in other cohorts of patients with liver disease (18, 19, 37, 52, 54). This is the first paper to report on lifetime drinking habits in Swedish patients with alcoholic cirrhosis, and the first paper to include both alcohol dependant without cirrhosis and non-alcoholic cirrhotics as control groups. The papers that have measured higher total lifetime intakes have measured the lifetime intake in all patients with decompensated liver cirrhosis, regardless of etiology (18, 19, 52). In addition the papers originate from Italy and France who still have a different drinking culture and dietary pattern compared to Sweden.

Women with alcoholic cirrhosis are fewer than men, as we showed in paper II, both when we compared their mortality in liver disease and the number of women hospitalized with liver diseases. There seems to be a better consistency in the reported diagnosis of alcoholic and non-alcoholic liver disease in women compared to men. They have a better prognosis when they have acquired liver disease. Even when their liver disease has progressed onto decompensated liver cirrhosis with esophageal varices

the prognosis is significantly better compared to men – both in the short-term and in the long-term after hospitalization, as we showed in paper III. To acquire alcoholic cirrhosis women seem to require less alcohol than men implying that there probably is an increased susceptibility to the hepatotoxic effects of alcohol in a certain group of women. Not all women do develop alcoholic cirrhosis when exposed to the same level of alcohol consumption or same drinking pattern; if this would be the case then the women in paper IV with alcohol dependence *without* liver disease should have developed cirrhosis. Sex specific hormones alone could probably not fully explain our findings (129). Whether the lower lifetime intake of alcohol in women with alcoholic cirrhosis could explain the difference in prognosis is unknown for the time being, but highly interesting. One might also consider the possibility that there exist subgroups of women who differs in their genes or proteins that regulates the fibrosis process in the liver.

5.3 CONCLUSIONS

Autoantibodies toward both CYP2E1 and CYP3A4 could be generated after chronic exposure to alcohol in both humans and experimental animals. They may be a part of the pathogenetic mechanism behind the development of alcoholic cirrhosis.

A reduction in mortality in liver diseases in Sweden coincided with a reduction in spirit consumption, and may be caused by the decrease in spirit consumption. In addition the separation between alcoholic and non-alcoholic liver disease was an impossible distinction to make in this register study. Furthermore, the patients with liver disease had a grave prognosis.

The reduction in mortality over time in patients with cirrhosis with esophageal varices may be related to improved therapeutical strategies towards acute variceal bleeding and secondary prophylactic treatment. We may have insufficient therapeutical strategies towards other complications in end-stage liver cirrhosis to show effects on the epidemiological level.

Patients with alcoholic cirrhosis did not drink more alcohol than patients with alcohol dependence without cirrhosis. Women with alcoholic cirrhosis had a specific drinking pattern that differed from men, they had been drinking less both when compared to women with alcohol dependence without cirrhosis and compared to men with alcoholic cirrhosis. This may indicate that some women may be at a high risk of acquiring liver cirrhosis despite a low level of alcohol consumption.

5.4 IMPLICATION FOR FUTURE RESEARCH

On the basis of the results in the studies I-IV, the following questions are of importance for future research:

Can a probable cause-effect relationship between the spirit consumption during the 1970s and the increase in hospitalization rates for liver diseases in the later 1990s be found?

What factors regulate the mortality in patients with alcoholic cirrhosis? Are viral infections involved, or could patients infected with other agents (as for example the

Helicobacter Pylori) have a more severe course of liver disease leading to increased hospitalization and mortality?

Will the increase in hospitalization rates for liver diseases in the later 1990s lead to an increase in mortality in liver diseases in the future decade, unrelated to the increase in mean alcohol consumption in Sweden?

Does alcohol consumption represent an individual risk factor for decreased survival after a period of hospitalization with the diagnosis of esophageal varices?

What role do the different drinking patterns and types of beverage play in relation to hormones, genetics and other external factors as for example diet in the development of liver cirrhosis? Are there other agents than alcohol involved in the initiation and continuation of liver disease in patients with increased alcohol consumption?

Are there specific genetic polymorphisms in women who have acquired alcoholic cirrhosis that renders them susceptible to a rather small amount of alcohol they have drunk? Does the lifetime alcohol intake act as a prognostic marker for survival in women with alcoholic cirrhosis, and is it applicable to men as well?

What role do autoantibodies, both against cytochromes and against other substances, play in the regulation and continuation of the inflammatory and the fibrogenetic pathways in alcoholic cirrhosis? Does drinking pattern or gender influence the process?

6 ACKNOWLEDGEMENTS

First and foremost I would like to thank Professor Rolf Hulcrantz, my supervisor, for invaluable support, inspiration and patience. The endless hours he has spent on the phone and his encouragement have been essential. His ever-lasting enthusiasm has made this journey altogether very enjoyable.

The present studies were made possible by the generous support and contributions from many colleagues and friends. In particular I would like to thank:

Assistant Professor Johan Franck, and Professor Anders Ekblom who have both in their unique and personal ways been of great inspiration and help. They have both shared their expert knowledge in their fields with me,

Nils Stobaeus, M.D., Lars Gustafsson, M.D., Thomas Kunze, M.D., as former and present Directors of the Department of Medicine at Visby Hospital, who has inspired me to continue my research and provided financial support and time off from clinical work,

Lena Brandt, B.Sc., for invaluable support and all the time she has spent on the figures and tables and for explaining the wonderful world of epidemiology to me,

Gunnar Hilm, B.Sc., for so many hours of hard work and fun,

Professor Hans Bergman, Urban Ösby, M.D, for invaluable support and fruitful discussions,

Professor Magnus Ingelmann-Sundberg, Steve Lytton and Inger Johansson for invaluable support,

all my colleagues at the Department of Medicine in Visby who have been doing my share of the work at our Department when I've been away and for all their patience with me and their support, especially former colleague Assistant Professor Eva Forbes who has been inspiring me in my efforts to continue research while living in Visby,

all my good friends and colleagues at the Department of Gastroenterology and Hepatology at Karolinska University Hospital, who have encouraged me and inspired me,

Margareta Gard-Hedander, RN, Else-Britt Hillner, RN, for their invaluable support and help, specifically with the interviews, but in addition and not least as truly supportive when needed,

Kicki Prior, RN, Kerstin Bohlin, RN, Lully Mjörnell, RN and Linda Malmsröm, RN at the Department of Gastroenterology and Hepatology at Karolinska Hospital for invaluable help and a lot of fun,

Anna Nilsson at Statisticon AB for her calculations and fruitful discussions,

Barbro Norrström Mittag-Leffler and Ulf Mittag-Leffler and Julia Gitchamo
Norrström, for always making me feel like coming home when I go away –you’re the
best!

The Norwegian Church in Stockholm for their hospitality,

Julia Coles and Philippa Gillström for excellent help with the English language,

my two children, Astrid and Andreas, for being the two most wonderful human beings
on earth, and having great patience and understanding,

My darling Cecilia for being of immense support and loving inspiration,

Kristina Okemia Jonsson, for ideas and inspiration

and finally, a moment of gratitude should be offered to all my patients - past, present
and future - for inspiring me, for their contribution to the thesis and for their patience
with me during the studies and the preparation of this thesis.

7 REFERENCES

1. Genesis, 9: 19-25.
2. Norström T. The Impact of Per Capita Consumption on Swedish Cirrhosis Mortality. *British Journal of Addiction* 1987; 82: 67-75
3. Pequignot G, Tuyns AJ, Bertha JL. Ascitic cirrhosis in relation to alcohol consumption. *Int J Epidemiology* 1978; 7: 113-120
4. Roizen R, Kerr WC, Filmore KM. Cirrhosis mortality and per capita consumption of distilled spirits. United States 1947-94: trend analysis. *British Medical Journal* 1999; 319: 666-70
5. Kerr WC, Fillmore KM, Marvy P. Beverage-specific alcohol consumption and cirrhosis mortality in a group of English-speaking beer-drinking countries. *Addiction* 2000; 9: 339-346
6. Gruenewald PJ, Ponicki WR. The Relationship of Alcohol Sales To Cirrhosis Mortality. *J Stud Alcohol* 1995; 56: 635-641
7. Substance Abuse and Mental Health Administration. Results from the National Survey on Drug Use and Health: National findings. Office of Applied Studies, NSDUH Series H-28, DHSS publication no. SMA 05-4062. Rockville, Maryland: Substance Abuse and Mental Health Administration; 2005.
8. Batey RG, Burns T, Benson RJ, Byth K. Alcohol consumption and the risk of cirrhosis. *Med J Aust* 1992; 156: 413-416.
9. Klatsky AL, Armstrong MA. Alcohol, smoking, coffee, and cirrhosis. *Am J Epidemiol* 1992; 136: 1248-1257
10. Kamper-Jorgensen M, Gronbaek M, Tolstrup J, Becker U. Alcohol and cirrhosis: dose-response or threshold effect. *J Hepatol* 2004; 41: 25-30
11. Corrao G, Baghardi V, Zambon A, La Vecchia C. A meta-analysis of alcohol consumption and the risk of 15 diseases. *Prevent Med* 2004; 38: 613-619
12. Willner IR, Reuben A. Alcohol and the liver. *Curr Opin Gastroenterol* 2005; 21: 323-330
13. Meyerhoff DJ, Bode C, Nixon SJ, et al. Health risks of chronic moderate and heavy alcohol consumption: how much is too much? *Alcoholism Clin Exp Res* 2005; 29: 1334-1340
14. US Department of Health and Human Services. Dietary Guidelines for Americans 2005. US Department of Agriculture, HHS publication no. HHS-ODPHP-2005-01-DGA-A. Home and Garden bulletin no. 232. Washington DC: US Government Printing Office; 2005
15. Ruhl CE, Everhart JE. Coffee and caffeine consumption reduce the risk of elevated serum alanine aminotransferase activity in the United States. *Gastroenterology* 2005; 128: 24-32
16. Ruhl CE, Everhart JE. Coffee and tea consumption are associated with a lower incidence of chronic liver disease in the United States. *Gastroenterology* 2005; 129: 1928-1936
17. Inoue M, Yoshimi I, Sobue T, Tsugahi S, JPHC Study Group. Influence of coffee drinking on subsequent risk of hepatocellular carcinoma: a prospective study in Japan. *J Natl Cancer Inst* 2005; 977: 293-300
18. Corrao G, Bagnardi V, Zambon A, Torchio P. Meta-analysis of alcohol intake in relation to risk of liver cirrhosis. *Alcohol Alcohol* 1998; 33: 381-392

19. Belletani S, Saccoccio G, Costa G Tiribelli C, Manenti F, Sodde M, Saveria Croce L, Sasso F, Pozzato G, Cristianini G, Brandi G. Drinking habits as cofactors of risk for alcohol induced liver damage. *Gut* 1997; 41: 845-50
20. Becker U, Gronbaek M, Johansen D, Sorensen TI. Lower risk for alcohol-induced cirrhosis in wine drinkers. *Hepatology* 2002; 35: 868-875
21. Klatsky and M.A. Armstrong, Alcohol, smoking, coffee, and cirrhosis. *Am J Epidemiol* 1992;136: 1248–1257
22. Corrao G, Zambon A, Bagnardi V, Arico S, Loguercio C, D'Amicis A; Collaborative SIDECIR Group. Nutrient intakes, nutritional patterns and the risk of liver cirrhosis: an explorative case-control study. *Eur J Epidemiol.* 2004; 19: 861-869
23. Ramstedt M. Per capita alcohol consumption and liver cirrhosis mortality in 14 European countries. *Addiction* 2001; 96 (Supplement): s19-s34
24. Leon DA, McCambridge J. Liver cirrhosis mortality rates in Britain from 1950 to 2002: an analysis of routine data. *Lancet* 2006; 367: 52-56
25. Leon DA, McCambridge J. Liver cirrhosis mortality rates in Britain, 1950 to 2002. *Lancet* 2006; 367: 645
26. Kreitman N, Duffy J. Alcoholic and Non-alcoholic Liver Disease in Relation to Alcohol consumption in Scotland, 1978-84. Part I: Epidemiology of Liver Diseases. *British Journal of Addiction* 1989; 84: 607-618
27. Lúdvíksdóttir D, Skúlason H, Jakobsson F, Thórisdóttir A, Carigila N, Magnússon B, Thjodleifsson B. Epidemiology of liver cirrhosis morbidity and mortality in Iceland. *European Journal of Gastroenterology & Hepatology* 1997; 9: 61-66
28. Noble JA, Caces MFE, Steffens RA, Stinson FS. Cirrhosis Hospitalization and Mortality Trends, 1970-87. *Public Health Reports* 1993; 108, 2: 192-197
29. Smith I, Burvill PW. Epidemiology of liver cirrhosis morbidity and mortality in Western Australia, 1971-82: Some preliminary findings. *Drug and dependence* 1985; 15: 35-45
30. Poikolainen K. Increase in Alcohol-Related Hospitalizations in Finland 1969-1975. *British Journal of Addiction.* 1980; 75: 281-291
31. Mendez-Sanchez N, Villa AR, Chavez.-Tapica NC, Ponciano-Rodriguez G, Almeda-Valdes P, Gonzalez D, Uribe M. Trends in liver disease prevalence in Mexico form 2005 to 2050 through mortality data. *Ann Hepatol* 2005; 4: 52-55
32. Ramstedt M. Alcohol consumption and liver cirrhosis mortality with and without mention of alcohol – the case of Canada. *Addiction* 2003, 98: 1267-1276
33. Adang RP, Wensing WM, Stockbrugger RW. Alcohol consumption and alcohol-related liver disease in the Netherlands. *Scand J Gastroenterology* 1998; 33 Supplement 225: 70-74
34. Kerr WC, Fillmore KM, Marvy P. Additional comments on the aggregate-level associations between spirits consumption and cirrhosis mortality. *Addiction.* 2000; 95: 1271-1272
35. Gronbaek M, Jensen MK, Johansen D, Sorensen TI, Becker U. Intake of beer, wine and spirits and risk of heavy drinking and alcoholic cirrhosis. *Biol Res.* 2004; 37: 195-200
36. Everhart JE In vino Veritas. *J Hepatol* 2003; 28: 245-46

37. Pelletier S, Vaucher E, Aider R, Martin S, Perney P, Balmès JL, Nalpas B. Wine consumption is not associated with a decreased risk of alcoholic cirrhosis in heavy drinkers. *Alcohol Alcohol* 2002; 37: 618-621
38. http://www.systembolaget.se/NR/rdonlyres/25DDE957-9727-4A99-B932-B30077298C23/0/arsredovisning_04_eng.pdf, accessed May 10th 2006.
39. http://www.can.se/docs/press_rapporter/CAN_RS_79.pdf, accessed May 10th 2006
40. Kuhlhorn E, Ramstedt M, Hibell B, Larson S, Zetterberg, Swedish consumption of alcohol from legal and illegal sources in the middle of the 1990s, Department of Health and Social Affairs, Stockholm, Sweden
41. Norstrom T, Estimating changes in unrecorded alcohol consumption in Norway using indicators of harm. *Addiction*. 1998; 93: 1531-1538
42. <http://www.beroendecentrum.com/pages/topmenu/formular/audit.pdf>, Accessed May 10th 2006
43. Bergman H, Kallmen H. Alcohol use among Swedes and a psychometric evaluation of the alcohol use disorders identification test. *Alcohol Alcohol*. 2002; 37: 245-251
44. Reed DN, Saxe A, Montanez M, Kotlowski R, Brancheau DA, Coffey DC, Pollard M, Fitzgerald H, Barber K, Wolf B. Use of a single question to screen trauma patients for alcohol dependence. *J Trauma*. 2005; 59: 619-23
45. Skinner HA. Instructions for the lifetime drinking history. Alcohol Research Foundation, Toronto Canada 1982
46. Lemmens P: Gebruik van bier, wijn, sterke drank. personal communication, 1997
47. Friesma HM, Veenstra, MY, Zwietering PJ, Knottnerus JA, Garretsen HFL; Lemmens PHHM. Measurement of Lifetime Alcohol Intake: Utility of a self-administrated Questionnaire. *American Journal of Epidemiology* 2004; 159: 809-817
48. Lemmens PH: Measuring Lifetime Drinking Histories. *Alc Clin Exp Res* 1998; 22: 29S-36S
49. Summers T. Validity of alcoholics' Self-Reported drinking History. *Qart J Stud Alc* 1970; 31: 972-974
50. Yates W, Labreque DR, Pfab D. The Reliability of alcoholism history in patients with alcohol-related cirrhosis. *Alcohol Alcohol* 1998; 33: 488-494
51. Corrao G, Arico S, Lepore AR, Valenti M, Torchi, P, Galatola G, Tabone M, DiOrio F. Amount And Duration Of Alcohol Intake As Risk Factor Of Symptomatic Liver Cirrhosis: A Case-Control Study. *J Clin Epidemiol* 1993, 46: 601-607
52. Corrao G, Torchio P, Zambon A, Ferrari P, Arico S, di Orio F. Exploring the combined action of lifetime alcohol intake and chronic hepatotropic virus infections on the risk of symptomatic liver cirrhosis. *European Journal of Epidemiology* 1998;14: 447-456
53. Donato F, Tagger A, Gelatti U, Pariello G, Boffetta P, Alberitni A, Decarli A, Trevisi P, Ribero L, Martelli D, Porru S, Nardi G. Alcohol and Hepatocellular Carcinoma: The effects of Lifetime Intake and Hepatitis Virus Infection in Men and Woman. *Am J Epidem* 2002; 155: 323-331
54. Westin J, Lagging M, Spak F, Aires N, Svensson E, Lindh M, Dhillon A, Norkrans G, Wejstahl R. Moderate alcohol intake increases fibrosis progression in untreated patients with hepatitis C virus infection. *Journal of Viral Hepatitis* 2002; 9: 235-241

55. Hayashi PH, Harrison SA, Torgerson S, Perez TA, Nochajski T, Russell M. Cognitive lifetime drinking history in nonalcoholic fatty liver disease: some cases may be alcohol related. *Am J Gastroenterol.* 2004 Jan; 99: 76-81
56. Ahmad S, Rohrbaugh JW, Anokin AP, Sirevaag EJ, Goebel JA. Effects of ethanol consumption on postural control: A computerized dynamic posturography study. *Journal of Vestibular Research* 2002; 12: 53-64
57. Ji BT, Gao Y-T, Hsing AW, McLaughlin JK, Fraumeni Jr. JF, Chow W-H. Cigarette and alcohol consumption and the risk of colorectal cancer in Shanghai, China. *European Journal of Cancer Prevention* 2002; 11: 237-244
58. Tjønneland A, Christensen J, Thomsen B, Olsen A, Stripp C, Overvad K, Olsen JH. Lifetime Alcohol Consumption and Postmenopausal Breast Cancer Rate in Denmark: a prospective Cohort Study. *Journal of Nutrition* 2004; 134: 173-178
59. York JL, Hirsch JA. Association between blood pressure and lifetime drinking patterns in moderate drinkers. *Journal of Studies on Alcohol.* 1997; 58: 480-485
60. International Classification of diseases Eight Version, WHO, 1966.
61. International Classification of diseases Ninth Version, WHO, 1987.
62. International Classification of diseases, Tenth Version, WHO, 1997.
63. Diagnostic and Statistical Manual of Mental Disorders, Washington DC, American Psychiatric Association, 1994
64. Dennis L. Kasper, Eugene Braunwald, Anthony S. Fauci, Stephen L. Hauser, Dan L. Longo, J. Larry Jameson, and Kurt J. Isselbacher, Eds. In *Harrison's Principals of Medicine*, Ch 289, Cirrhosis and its complications 16th Edition, McGrawHill 2006, through Harrison Online: <http://www.accessmedicine.com.proxy.kib.ki.se/content.aspx?aID=92107>, accessed May 10th 2006
65. Miller LS, Kim JK, Dai Q, Mekapati J, Izanec J, Chung C, Liu JB, Sanderson A, Bohning M, Desipio J, Gandegok J, Harberson JJ, Schneck C, Nicosia MA, Thangada V, Thomas B, Copeland B, Miller E, Miller A, Ahmed N, Brasseur JG. Mechanics and hemodynamics of esophageal varices during peristaltic contraction. *Am J Physiol Gastrointest Liver Physiol.* 2004; 4: G830-835
66. D'Amico G, Lucas A. Natural history. Clinical-hemodynamic correlations. Prediction of the risk of bleeding. *Baillieres Clin Gastroenterol* 1997; 11: 243-256
67. Graham DY, Lacey Smith J. The course of patients after variceal hemorrhage, *Gastroenterology* 1981; 80: 800-809
68. Iouannou G, Doust J, Rockey DC. Terlipressin for acute esophageal variceal hemorrhage, the Cochrane database of Systemic Reviews, Volume 4, 2004
69. Patch D, Sabin CA, Goulis J, et al. A randomized, controlled trial of medical therapy versus endoscopic ligation for the prevention of variceal rebleeding in patients with cirrhosis, (comment) *Gastroenterology* 2002; 123: 1013-1019.
70. Wung S, Sung JJ. Update on treatment of variceal hemorrhage. *Digestive Diseases* 2002; 20: 134-144
71. Carbonell N, Pauwels A, Serfaty L, Fourdan O, Levy VG, Poupon R. Improved survival after variceal bleeding in patients with cirrhosis over the past two decades. *Hepatology* 2004; 40: 652-9
72. McCormick PA, O'Keefe C. Improving prognosis following a first variceal hemorrhage over four decades. *Gut* 2001; 49: 682-685

73. El-Serag HB, Everheart JE. Improving prognosis after variceal hemorrhage over an 11-year period in the Department of Veterans Affairs. *American Journal of Gastroenterology* 2000; 95: 3566-3573
74. Chalasani N, Kahi C, Francois F, Pinto A, Marathe A, Bini EJ, Pandya P, Sitaraman S, Shen J. Improved Patient Survival After Acute Variceal Bleeding: A Multicenter Cohort Study. *American Journal of Gastroenterology* 2003; 98: 653-659
75. Reuben, A. Alcohol and the Liver. *Curr Opin Gastroenterol* 2006; 22: 263-271
76. Iseri, OA, Gottlieb, L S, Lieber, C. S. The ultrastructure of ethanol-induced fatty liver. *Fed. Proc.* 1964; 23: 579A
77. Iseri OA, Lieber, CS, Gottlieb, LS. The ultrastructure of fatty liver induced by prolonged ethanol ingestion. *Am. J. Pathol.* 1966; 48: 535-555
78. Lane, BP, Lieber, CS. Ultrastructural alterations in human hepatocytes following ingestion of ethanol with adequate diets. *Am. J. Pathol.* 1966; 49: 593-603
79. Lieber, CS, DeCarli, LM. Ethanol oxidation by hepatic microsomes: adaptive increase after ethanol feeding. *Science* 1968; 162: 417-418
80. Lieber, CS, DeCarli, LM. Hepatic microsomal ethanol oxidizing system: in vitro characteristics and adaptive properties in vivo. *J. Biol. Chem.* 1970, 245: 2505-2512
81. Tsutsumi, M, Lasker, J M, Shimizu, M, Rosman, AS, Lieber CS. The intralobular distribution of ethanol-inducible P450IIE1 in rat and human liver. *Hepatology* 1989; 10: 437-446
82. Takahashi, T, Lasker, JM, Rosman, AS, Lieber CS. Induction of cytochrome P4502E1 in the human liver by ethanol is caused by a corresponding increase in encoding messenger RNA. *Hepatology* 1993; 17: 236-245
83. Umeno Y, Matsushima E, Maniwa M, Marunaka T. Human ethanol-inducible P450IIE1: complete gene sequence, promoter characterization, chromosome mapping, and cDNA-directed expression. *Biochemistry.* 1988 Dec 13; 27: 9006-9013
84. Salmela KS, Kessova IG, Tsyrllov, IB, Lieber CS. Respective roles of human cytochrome P4502E1, 1A2, and 3A4 in the hepatic microsomal ethanol oxidizing system. *Alcohol. Clin. Exp. Res.* 1998; 22: 2125-2132
85. Arai M, Leo MA, Nakano M, Gordon ER, Lieber CS. Biochemical and morphological alterations of baboon hepatic mitochondria after chronic ethanol consumption. *Hepatology.* 1984; 4: 165-74
86. Caro AA, Cederbaum AI. Oxidative stress, toxicology, and pharmacology of CYP2E1. *Annu Rev Pharmacol Toxicol* 2004; 44: 27-42
87. Cederbaum AI, Wu D, Mari M, Bai J. CYP2E1-dependent toxicity and oxidative stress in HepG2 cells. *Free Radic Biol Med* 2001; 31: 1539-1543
88. Kessova I, Cederbaum AI. CYP2E1: biochemistry, toxicology, regulation and function in ethanol-induced liver injury. *Curr Mol Med* 2003; 3: 509-518
89. Albano E, Tomasi A, Gorla-Gatti L, Dianzani MU, Spin trapping of free radical species produced during the microsomal metabolism of ethanol. *Chem. Biol. Interact.* 1988; 65: 223-234
90. Albano E, Tomasi A, Gorla-Gatti L, Persson JO, Terelius Y, Ingelman-Sundberg M Dianzani MU. Role of ethanol-inducible cytochrome P-450 (P450IIE1) in catalyzing the free radical activation of aliphatic alcohols. *Biochem. Pharmacol.* 1991; 41: 1895-1902

91. Albano E, Parola M, Comoglio A, Dianzani MU, Evidence for the covalent binding of hydroxyethyl radicals to rat liver microsomal proteins. *Alcohol Alcohol.* 1993; 28: 453–459
92. Tuma DJ, Donohue TM, Medina A, Sorell MF. Acetaldehyde adducts with proteins binding of ¹⁴C-acetaldehyde to serum albumin. *Arc Biochem. Biophys* 1983; 220: 239-246
93. Stevens VJ, Fantl WF, Newman CB, Sima RV, Cerami A, Peterson CM. Acetaldehyd adducts with haemoglobin. *J. Clin. Invest* 1981; 67: 364-369
94. Jennet RB, Sorell MF, Saffari-Ward A, Ockner JL, Tuma DJ. Preferential covalent binding of acetaldehyde to the α -chain of purified rat liver tubulin. *Hepatology* 1989; 9: 57-62
95. Viitala K, Israel Y, Blake JE, Niemela O. Serum IgA, IgG and IgM antibodies directed against acetaldehyde-derived epitopes: relationship to liver disease severity and alcohol consumption. *Hepatology* 1997; 25:1418–1424.
96. Clot P, Albano E, Elliasson, E, Tabone, M, Aricò S, Israel Y, Moncada Y, Ingelman-Sundberg M. Cytochrome P4502E1 hydroxyethyl radical adducts as the major antigenic determinant for autoantibody formation among alcoholics. *Gastroenterology* 1996; 111: 206–216
97. Albano E, Clot P, Morimoto M, Tomasi A, Ingelman-Sundberg, M, French S. Role of cytochrome P4502E1-dependent formation of hydroxyethyl free radicals in the development of liver damage in rats intragastrically fed with ethanol. *Hepatology*1996; 23: 155–163
98. Klassen LW, Tuma D, Sorrell MF. Immune mechanisms of alcohol-induced liver disease. *Hepatology* 1995; 22: 355–357
99. Xu D, Thiele GM, Beckenhauer JL, Klassen LW, Sorrell MF, Tuma DJ Detection of circulating antibodies to malondialdehyde-acetaldehyde adducts in ethanol-fed rats. *Gastroenterology* 1998; 115: 686–692
100. Niemelä O, Parkkila S, Pasanen M, Iimuro Y, Bradford B, Thurman RG Early alcoholic liver injury: formation of protein adducts with acetaldehyde and lipid peroxidation products, and expression of CYP2E1 and CYP3A. *Alcohol. Clin. Exp. Res.* 1998; 22: 2118–2124
101. Niemelä O, Parkkila S, Juvonen RO, Viitala K, Gelboin HV, Pasanen M. Cytochromes P450 2A6, 2E1, and 3A and production of protein-aldehyde adducts in the liver of patients with alcoholic and non-alcoholic liver diseases. *J. Hepatol.* 2000; 33: 893–901
102. Niemelä O. Aldehyde-protein adducts in the liver as a result of ethanol-induced oxidative stress. *Front. Biosci.* 1999; 4: d506–d513
103. Niemelä O, Juvonen T, Parkkila S. Immunohistochemical demonstration of acetaldehyde containing epitopes in human liver after ethanol ingestion. *J. Clin. Invest.* 1991; 87: 1367–1374
104. Niemelä O, Parkkila S, Ylä-Herttua S, Halsted CH, Witztum JL, Lanca A, Israel Y. Covalent protein adducts in the liver as a result of ethanol metabolism and lipid peroxidation. *Lab. Invest.* 1994; 70: 537–546
105. Halsted H, Villanueva J, Chandler CJ, Ruebner B, Munn RJ, Parkkila S, Niemelä O. Centri-lobular distribution of acetaldehyde and collagen in the ethanol-fed micropig. *Hepatology* 1993; 18: 954–960
106. Niemelä O, Parkkila S, Ylä-Herttua S, Villanueva J, Ruebner B, Halsted CH. Sequential acetaldehyde production, lipid peroxidation and fibrogenesis in micropig model of alcohol-induced liver disease. *Hepatology* 1995; 22: 1208–1214

107. Niemelä O, Parkkila S, Pasanen M, Iimuro Y, Bradford B, Thurman RG. Early alcoholic liver injury: formation of protein adducts with acetaldehyde and lipid peroxidation products, and expression of CYP2E1 and CYP3A. *Alcohol. Clin. Exp. Res.* 1998; 22: 2118–2124
108. Paradis V, Scoazec JY, Kollinger M, Holstege A, Moreau A, Feldmann G, Bedossa P. Cellular and subcellular localization of acetaldehyde-protein adducts in liver biopsies from alcoholic patients. *J. Histochem. Cytochem.* 1996;44: 1051–1057
109. Trudell R, Ardies CM, Green CE, Allen K. Binding of anti-acetaldehyde IgG antibodies to hepatocytes with an acetaldehyde-phosphatidylethanolamine adduct on their surface. *Alcohol. Clin. Exp. Res.* 1991; 15: 295–299
110. Holstege A, Bedossa P, Poynard T, Kollinger M, Chaput JC, Houglum K, Chojkier M. Acetaldehyde-modified epitopes in liver biopsy specimens of alcoholic and nonalcoholic patients: localization and association with progression of liver fibrosis. *Hepatology* 1994; 19: 367–372
111. Baraona E, Matsumura T, Hernandez R, Kubota S, Soong J, Kawano S, Kato S, Inatomi N, Sato S, Lieber CS. Chronic alcohol intake impairs liver oxygenation by decreasing O₂ utilization, In K. Kuriyama, A. Takada, & H. Ishii (Eds.), *Biomedical and Social Aspects of Alcohol and Alcoholism: Proceedings of the Fourth Congress of the International Society for Biomedical Research on Alcoholism*: 387–390, Amsterdam, The Netherlands: Elsevier Science Publishers BV 1988
112. Hasumura Y, Teschke R, Lieber CS. Characteristics of acetaldehyde oxidation in rat liver mitochondria. *J Biol Chem* 1976; 251: 4908–4913
113. Ma X, Baraona E, Lieber CS. Alcohol consumption enhances fatty acid ω -oxidation, with a greater increase in male than in female rats. *Hepatology* 1993; 18: 1247–1253
114. Koivisto T, Mishin VM, Mak KM, Cohen PA, Lieber, CS. Induction of cytochrome P-4502E1 by ethanol in rat Kupffer cells. *Alcohol Clin Exp Res* 1996; 20: 207–212.
115. Friedman SL. Stellate cell activation in alcoholic fibrosis-an overview. *Alc Clin. Exp. Res.* 1999; 23: 904-910
116. Siegmund SV, Dooley S, Brenner DA. Molecular Mechanisms of Alcohol-Induced Hepatic Fibrosis. *Dig Dis* 2005; 23: 264-274.
117. Langer DA, Shah VH. Nitric oxide and portal hypertension: interface of vasoreactivity and angiogenesis. *J Hepatol* 2006; 44:209–216
118. Tazi KA, Moreau R, Herve P, Dauvergne A, cazals-Hatem D, Bert F, Poirel O, Rabiller A, Lebrec D. Norfloxacin reduces aortic NO synthases and proinflammatory cytokine up-regulation in cirrhotic rats: role of Akt signaling. *Gastroenterology* 2005; 129:303–314
119. Tsukamoto H, French SW, Benson N, Delgado G, Rao GA, Larkin EC, Largman C. Severe and progressive steatosis and fibrosis in rat livers induced by intragastric infusion of ethanol and low fat diet. *Hepatology* 1985; 5: 224-232.
120. <http://skatteverket.se/download/18.b7f2d0103e5e9ecb0800074/70407.pdf>, accessed May 11th 2006.
121. http://www.socialstyrelsen.se/Statistik/statistik_amne/dodsorsaker/Dödsorsaksregistret+engelsk.htm, accessed May 11th 2006
122. http://www.socialstyrelsen.se/Statistik/statistik_amne/dodsorsaker/Dödsorsaksregistret+engelsk.htm, accessed May 11th 2006

123. Pugh RNH, Murray IM, Dawson JL, Pietroni MR, Williams R. Transection of the esophagus for bleeding oesophageal varices, *British Journal of Surgery* 1973; 60: 646-9
124. McCullagh P, Nelder, JA. *Generalized Linear Models*. London, England: Chapman and Hall 1983
125. Cox, DR. Regression models and life tables. *J. R. Stat. Soc. B* 1972; 34: 187-220
126. Kruskal WH, Wallis WA. Use of ranks in one-criterion variance analysis. *Journal of the American Statistical Association* 1952; 47: 583-621
127. Siegel S, Castellan NJ *Non-parametric statistics for the behavioural sciences*. New York: McGraw-Hill 1988
128. Holm S A simple sequential multiple testing procedure. *Scandinavian Journal of Statistics* 1979; 6: 65-70
129. Kovacs EJ, Messingham KA. Influence of alcohol and gender on immune response, *Alcohol Research and Health* 2002; 26: 257-263
130. Brenner DM. Sex and Hepatic Fibrosis, *Hepatology* 1999; 29: 988-989
131. Yasuda H, Shimizu I, Shiba M, Ito S. Suppressive effects of estradiol on dimethylnitrosamine-induced fibrosis of the liver in rats. *Hepatology*; 29; 719-727
132. Albano E. Alcohol, oxidative stress and free radical damage. *Proc Nutr Soc.* 2006; 65: 278-90.
133. Koop DR, Klopfenstein B, Imuro Y, Thurman RG. Gadolinium chloride blocks alcohol-dependent liver toxicity in rats treated chronically with intragastric alcohol despite the induction of CYP2E1. *Mol. Pharmacol* 1997; 51: 944-950
134. Kono H, Bradford BU, Yin M, Sulik KK, Koop DR, Peters JM, Gonzalez DJ, McDonald T, Dikalova A, Kadiiska MB, Mason RP, Thurman RG. CYP2E1 is not involved in early alcohol-induced liver injury. *Am J Physiol.* 1999; 277: 1259-67
135. Roberts SE, Goldacre MJ, Yeats D. Trends in mortality after hospital admission for liver cirrhosis in an English population from 1968 to 1999. *Gut* 2005; 54: 1615-1621.
136. Powell WJ, Klatskin G Duration of Survival in Patients with Laennec's Cirrhosis. *An J Med* 1968; 46: 406-420
137. Brunt PW, Kew MC, Scheuer PJ, Sherlock S. Studies in alcoholic liver disease in Britain. *Gut* 1974; 15: 52-58
138. Borowsky SA, Strome S, Lott E. Continued Heavy Drinking and Survival in Alcoholic Cirrhotics. *Gastroenterology* 1981; 80: 1405-1409
139. Bouchier IA, Hislop WS, Prescott RJ. A prospective study of alcoholic liver disease and mortality. *Journal of Hepatology* 1992; 16: 290-297