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LIVER CIRRHOSIS -

EPIDEMIOLOGY, PROGNOSIS AND CANCER

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Liver cirrhosis - epidemiology, prognosis, and cancer

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

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All interest in disease and death is only another expression of interest in life.

Thomas Mann

To Pappi

POPULAR SCIENCE SUMMARY OF THE THESIS

Liver cirrhosis is the end-stage of all chronic liver diseases. Cirrhosis is caused by repeated or continuous injury to the liver that cause significant scarring in the liver. The scar tissue replaces liver tissue and destroys the architecture of the liver cells, blood supply and bile drainage seen in the healthy liver. A partially scarred liver is usually tolerated, and liver cirrhosis usually goes without symptoms in the beginning of the disease. In some individuals with cirrhosis, the scarring becomes so severe that the liver is no longer able to perform its normal functions and complications may develop. Common complications are abdominal swelling caused by accumulation of fluid in the abdomen (ascites), dilated and potentially bleeding veins in the esophagus, bacterial infections, and liver cancer to mention a few.

The most common liver disease is non-alcoholic fatty liver disease (NAFLD), a condition in which fat is accumulated in the liver. It is mostly seen in individuals with overweight and type 2 diabetes. Other common liver diseases causing cirrhosis, in our part of the world, are hepatitis C virus and alcohol-related liver disease due to chronic overconsumption of alcohol. Autoimmune liver diseases, when the body's immune defense attacks cells in the liver and bile ducts causing an inflammation, can also cause cirrhosis. Rare diseases such as hereditary hemochromatosis when iron is accumulated in the liver and Wilson's disease when copper is deposited in the liver can also cause cirrhosis.

The overall aim of this PhD-thesis was to improve understanding of prognosis and severe consequences of cirrhosis to be able to identify patients with a higher risk to develop complications to cirrhosis, such as liver cancer (study I and III) and bacterial infections (study IV). In study I, we focused on NAFLD and liver cancer, in study III, we assessed the risk of liver cancer in all cirrhosis patients in Sweden and in study IV, we examined if a specific blood test could predict the risk to be diagnosed with an infection in cirrhosis patients. We also performed a separate study on health-administrative coding for cirrhosis to examine the reliability of the codes.

In study I, we identified all liver cancer patients at Karolinska University Hospital from 2004-2018 and focused on those with NAFLD as an underlying liver disease to look for specific features that characterize patients with liver cancer and NAFLD. This is of particular interest as patients with NAFLD have been reported to differ from other liver cancer patients and because large studies with detailed information are lacking. We specifically studied patients with NAFLD with and without cirrhosis since absence of cirrhosis is more common in patients with NAFLD.

In study II, we wanted to examine health administrative codes, ICD-codes, related to liver diseases. ICD-codes are registered by a physician at contact with healthcare in Sweden and when a person dies. The codes describe what disease or condition that was the cause of the healthcare contact or the cause of death. These codes provide data used in settings such as healthcare economics, for reimbursements, in statistics and research. We studied the accuracy of codes related to cirrhosis. Patient charts with an ICD-code related to liver cirrhosis were

randomly collected from hospitals across Sweden. The charts were reviewed to assess whether the code was correctly registered or not.

In study III, we used ICD-codes validated in study II to identify patients with cirrhosis in Sweden and examined the risk of developing liver cancer after the diagnosis of cirrhosis. The patients with cirrhosis were divided by age, sex, and underlying liver disease to compare if the risk of liver cancer differed within such subgroups.

In study IV, we included patients with cirrhosis from the hepatology clinic at the Karolinska University Hospital. A blood sample was obtained from participants where a cell, important in the immune defense against bacteria, mucosal associated invariant T (MAIT) cell, were analyzed. We wanted to investigate if a low level of MAIT cells was associated with a higher risk of developing bacterial infections in patients with cirrhosis.

We found that patients with NAFLD and liver cancer are older, have larger tumors at the time of cancer diagnosis, but have the same risk of dying as other patients with liver cancer. Liver cancer patients with NAFLD have cirrhosis less often compared to liver cancer patients with other types of liver diseases. NAFLD as an underlying liver disease was more common in 2018 towards the end of the study compared to 2004 when the study began. In study II, we found that ICD-codes for cirrhosis and esophageal varices were accurate in over 90% of cases, but for ascites, we found that only 43% of patients had ascites caused by a liver disease. For liver cancer the accuracy was 84%. By using an algorithm where another ICD-code corresponding to liver-related disease was required in patients with a code for ascites or a code for liver cancer, the number of patients with a correctly registered code increased. In study III, we found that each year, on average 2.3% of patients with cirrhosis were diagnosed with liver cancer. Patients with liver cirrhosis frequently die from other causes than liver cancer, therefore, the percentage of patients that had been diagnosed with liver cancer ten years after cirrhosis diagnosis, was lower than expected - 12.2%. The risk of being diagnosed with liver cancer was higher in men, older individuals and in patients with hepatitis B and C compared to other underlying causes of liver cirrhosis. Finally, in study IV, we found that patients with cirrhosis and *higher* levels of MAIT cells more frequently had bacterial infections.

In conclusion, our findings are important as they point to the different risks for individuals with liver cirrhosis to be diagnosed with complications. We report several risk factors for developing liver cancer and specific characteristics for patients with NAFLD and liver cancer. Our findings can support decision-making about how to design medical follow-up for patients with cirrhosis. Other researchers conducting epidemiological, and other type of research where liver-related ICD-codes are used, will benefit from knowing to what extent they can “trust” the ICD-code for identifying individuals with cirrhosis and liver cancer. The association of higher MAIT cells and higher risk of bacterial infections is an interesting finding but it needs to be further investigated in larger studies to evaluate if it can be used in the future to foresee the risk of infection.

ABSTRACT

Liver cirrhosis is a major risk factor for hepatocellular carcinoma (HCC). Patients with liver cirrhosis also have a high risk to develop infections leading to deterioration of liver function and increased mortality. In this PhD-thesis, our aim was to improve the ability to predict risk of developing liver cancer and infections in patients with cirrhosis.

In study I, all patients diagnosed with HCC at the Karolinska University Hospital between 2004 and 2018 were included. Patients with HCC and underlying non-alcoholic fatty liver disease (NAFLD) were characterized in detail to investigate their prognosis compared to that of other patients with HCC. In study II, we included randomly selected patients with an international classification of disease (ICD)-code corresponding to cirrhosis and cirrhosis complications registered in the national patient registry (NPR) between 2000 and 2016 to investigate the positive predictive value (PPV) of liver-related ICD-codes. In study III, all patients with cirrhosis registered in the outpatient part of the NPR were included to investigate rate and risk of HCC in cirrhosis. In study IV, we included patients with cirrhosis seen at the Hepatology clinic at the Karolinska University Hospital and obtained a blood test from the participants analyzed for fractions of mucosal-associated invariant T (MAIT) cells. Patient were followed prospectively for risk of bacterial infection and hepatic decompensation.

In study I, we included 1,562 patients with HCC, and 225 (14%) of these had NAFLD. We report that NAFLD is a growing cause of HCC. One third of the patients with NAFLD-HCC had no clinical signs of cirrhosis. NAFLD patients were older than non-NAFLD patients, and non-cirrhotic NAFLD patients were even older than NAFLD patients with cirrhosis. Survival was similar between patients with NAFLD and non-NAFLD and between patients with cirrhotic and non-cirrhotic NAFLD. In study II, we found that ICD-10 codes for cirrhosis and esophageal varices had a PPV above 90%, whereas HCC had a PPV of 84%. Ascites had an unsatisfactorily low PPV of 43% for liver-related ascites, but when combined with a code indicating chronic liver disease, the PPV increased to 91%. In study III we included 15,215 individuals with cirrhosis and report that the rate of HCC in cirrhosis is 23/1,000 person-years with a lower-than-expected cumulative risk at five and ten years of 8.3% and 12.2% respectively. The cancer risk varied significantly depending on sex, age, and etiology of liver disease. In study IV, we included 106 patients with cirrhosis and found that relatively preserved MAIT cell fractions were associated with a higher risk of bacterial infections in patients with cirrhosis.

In conclusion, we describe NAFLD HCC-patients with and without cirrhosis and found that patients with non-cirrhotic NAFLD are older. We suggest that any surveillance attempts in this patient group should take age into account. ICD-codes for cirrhosis and esophageal varices have a high PPV, but when using ICD-10 code for ascites to identify patients with cirrhosis, we recommend adding another code for chronic liver disease to obtain a PPV above

90%. In study III, we report that the incidence for HCC in cirrhosis and the cumulative risk at five and ten years highly depends on sex, age, and type of liver disease, indicating that HCC-surveillance should be individually tailored. In study IV, the association of bacterial infections and a relatively preserved MAIT cell fraction is an interesting finding that needs to be investigated further.

LIST OF SCIENTIFIC PAPERS

I. **Characteristics and outcome of hepatocellular carcinoma in patients with NAFLD without cirrhosis**
Bengtsson B, Stål P, Wahlin S, Björkström N, Hagström H.
Liver International. 2019 March;39:1098-1108.

II. **Validity of administrative codes associated with cirrhosis in Sweden**
Bengtsson B, Askling J, Ludvigsson J, Hagström H.
Scandinavian Journal of Gastroenterology. 2020 October;55(10):1205-1210.

III. **The risk of hepatocellular carcinoma in cirrhosis differs by etiology, age and sex: A Swedish nationwide population-based cohort study**
Bengtsson B, Widman L, Wahlin S, Stål P, Björkström N, Hagström H.
United European Gastroenterology Journal. 2022 May;10(5):465-476.

IV. **Evaluation of mucosal-associated invariant T-cells as a potential biomarker to predict infection risk in liver cirrhosis**
Bengtsson B, Maucourant C, Sandberg J, Björkström N, Hagström H.
Re-submitted Liver International, major revision

CONTENTS

LIST OF ABBREVIATIONS

AFP	∝-fetoprotein
APC	Antigen-presenting cell
AIH	Autoimmune hepatitis
ARLD	Alcohol-related liver disease
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BCLC	Barcelona Clinic Liver Cancer system
BMI	Body mass index
CD	Cluster of differentiation
CI	Confidence interval
DAMPs	Damaged-associated molecular patterns
ECOG	Eastern Cooperative Oncology Group
HCC	Hepatocellular carcinoma
HCV	Hepatitis C
HR	Hazard ratio
HRS	Hepatorenal syndrome
HVPG	Hepatic venous pressure gradient
ICD	International classification of diseases
IQR	Interquartile range
MAIT cell	Mucosal-associated invariant T-cell
MDT conference	Multidisciplinary therapy conference
MELD	Model for end-stage liver disease
NAFLD	Non-alcoholic fatty liver disease
NK cell	Natural killer cell
NPR	The Swedish National Patient Register
PAMPs	Pathogen-associated molecular patterns
PBC	Primary biliary cholangitis

PSC	Primary sclerosing cholangitis
INR	International normalized ratio
RF	Radiofrequency therapy
SBP	Spontaneous bacterial peritonitis
SHR	Subdistribution hazard ratio
TACE	Trans-arterial chemoembolization
TIPS	Transjugular intrahepatic portosystemic shunt
T2DM	Type II diabetes
UCSF	University of California San Francisco criteria

1 INTRODUCTION

1.1 LIVER PHYSIOLOGY

The liver is a large, beautiful, and important organ that interacts with nearly every other organ in the body. The liver has many different tasks and is sometimes referred to as a factory with production, storage, and degradation as the main tasks. The most common cells in the liver, the hepatocytes, *produce* bile that is secreted into the bowel, and many other important proteins that are secreted into the bloodstream. Various plasma proteins including albumin and coagulation factors are synthesized in the liver as well as glycogen and other building blocks for the metabolism. Glycogen is not only produced but also *stored* in the liver and is a source of energy when blood levels of glucose is low. The liver also stores vitamin A, vitamin D, iron, and copper. Another important task of the liver is the *metabolism* and detoxification of different substances and waste products. Bilirubin, which comes from the degradation of hemoglobin, is conjugated in the liver, and secreted with the bile to the bowel. The liver is also responsible for metabolizing many pharmaceutical drugs, alcohol, and other toxins.[1]

1.2 LIVER HISTOLOGY

The pathophysiology of liver cirrhosis is complex. Cirrhosis appears when liver cells are replaced with fibrotic tissue. There are different liver diseases that cause liver cirrhosis, but a common feature for them all is that they cause an injury to the liver that directly or indirectly damage the hepatocytes and the microscopical organization of the liver lobule **(Figure 1),** to an extent that the injury is permanent. Although the hepatocytes can regenerate, the scar tissue destroys the hepatic architecture of the liver cells, the bile ducts and the blood vessels and the liver function will eventually be impaired. Fibrosis is usually graded into five severity levels from zero to four with increasing amount of fibrosis-replaced liver parenchyma for each stage. Fibrosis stage four is equivalent to cirrhosis and is histologically defined by regenerative noduli surrounded by thick fibrotic septa (**Figure 1).**

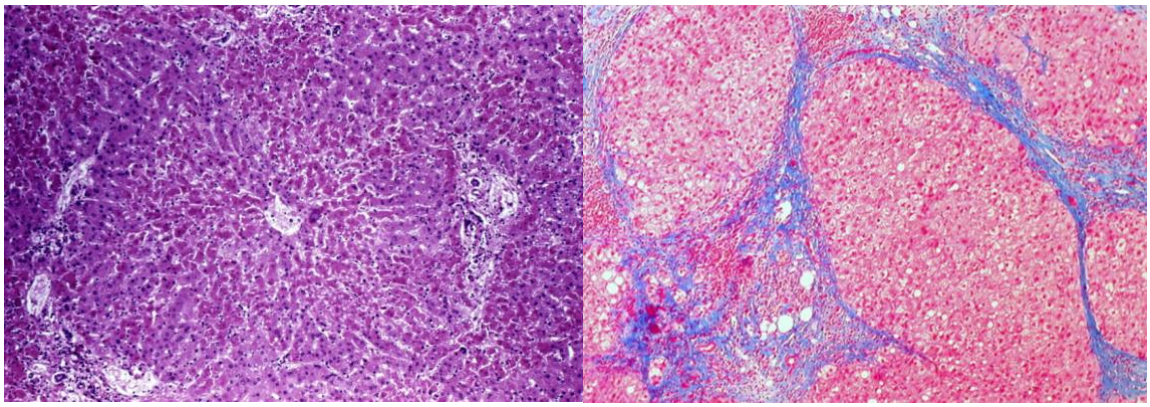


Figure 1. Liver lobule in a healthy liver (left) and in a cirrhotic liver (right). Printed with permission, in courtesy of Department of Histology, Jagiellonian University and Dr Ed Uthman.

1.3 CHRONIC LIVER DISEASES AND CIRRHOSIS

In Sweden and other Western countries, alcohol-related liver disease (ARLD), and hepatitis C (HCV) are the most common etiologies of cirrhosis[2] although the risk attributed to HCV has decreased in recent years owing to new effective and curative treatments.[3, 4] Hepatitis B is the most common etiology of liver cirrhosis in Africa and south-east Asia, but vaccination campaigns are changing this scenario.[4, 5] Another important cause of liver cirrhosis is non-alcoholic fatty liver disease (NAFLD) often seen in patients with the metabolic syndrome when fat is accumulated in the liver cells.[6-8] NAFLD affects around 25%-30% of the global population and is a fast-growing cause of cirrhosis although most of the individuals with NAFLD will not develop cirrhosis.[9-11]

There are also inflammatory diseases affecting the liver and the bile ducts such as primary biliary cholangitis (PBC), primary sclerosing cholangitis (PSC) and autoimmune hepatitis (AIH). Rare liver diseases caused by a pathological deposition of copper (Wilsons's disease) and iron (hemochromatosis), can also cause cirrhosis, as well as other rare diseases such as hepatic porphyria.[12, 13]

In the initial phase, liver cirrhosis goes without symptoms. This phase is referred to as *compensated cirrhosis*. As the pressure in the portal vein increases and the liver function decreases, symptoms such as ascites, variceal bleeding, and encephalopathy occur and the disease is then considered to have turned into *decompensated cirrhosis* with a poor prognosis and a median survival of less than two years.[14] Opinions differ about which clinical conditions that should be regarded decompensating events. Most hepatologists agree on bleeding esophageal or gastric varices, ascites, and encephalopathy[15] and some would add jaundice[16] and hepatorenal syndrome (HRS).[17] Others suggest that bacterial infections[18] should be added to the list of decompensating events.

1.4 PORTAL HYPERTENSION

The portal circulation drain blood from the gallbladder, spleen, pancreas, stomach, and small and large intestines to the liver **(Figure 2)**. When liver fibrosis progress, the intrahepatic portal vascular resistance increases. The resulting increase in portal vein blood pressure is referred to as portal hypertension defined by a hepatic venous pressure gradient (HVPG) above 10 mmHg.[19] HVPG measurement is invasive and therefore not often measured as clinical signs often are evident and the exact HVPG-value is of limited clinical value. In portal hypertension, the blood is congested in the gastrointestinal tract, with dilated veins as a result. The blood in the portal circulation then takes other paths to bypass the liver. Together this will cause dilated veins and a risk of bleeding from hemorrhoids and esophageal or gastric varices. As the portal vein also drains the spleen, splenomegaly with low platelets as a result due to larger destruction of blood cells in the enlarged spleen can be seen. An additional explanation to low platelets in cirrhosis is a diminished production in the liver of thrombopoietin, a protein important to produce platelets.

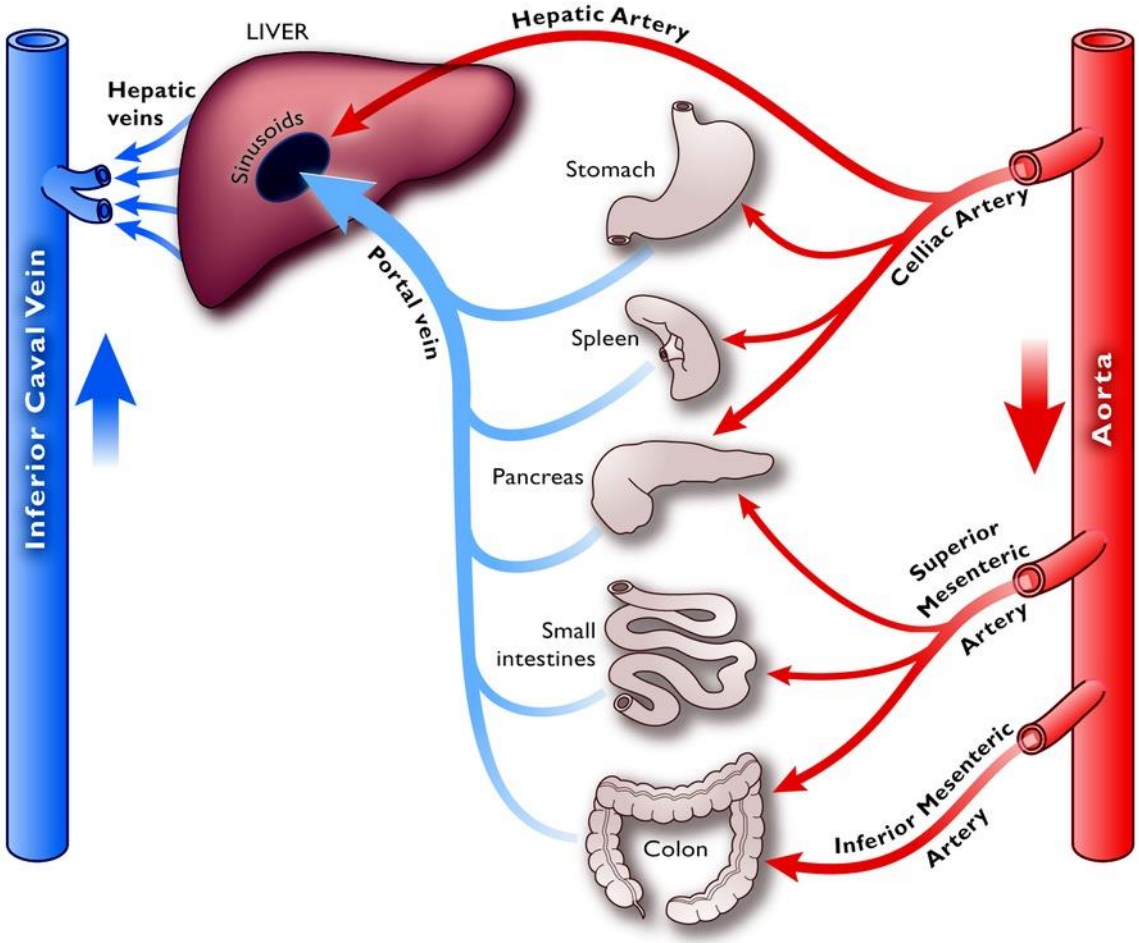


Figure 2. Portal circulation. Printed with permission from "Gelman S et al. Catecholamine-induced Changes in the Splanchnic Circulation Affecting Systemic Hemodynamics. Anesthesiology 2004; 100:434–439

1.5 IMPAIRED LIVER FUNCTION

The liver is involved in many different processes in the body, and therefore a myriad of clinical signs and symptoms can be seen in liver cirrhosis. The production of plasma proteins is decreased and biochemical changes such as a low p-albumin and an elevated INR[20] can be found in the laboratory list. In addition to the reduced synthesis of coagulation factors I, II, VII, and X, the decreased number of platelets, impaired absorption of vitamin K due to cholestasis together with a slower degradation of coagulations factors create an imbalance in coagulation hemostasis.[21] In the past, individuals with cirrhosis were considered to have an increased risk of bleeding based on the laboratory abnormalities but evidence for this not being true has emerged. In fact, cirrhosis poses an increased risk of both bleeding and thrombosis and the coagulation equilibrium is more accurately described as rebalanced.[22] The impaired liver function also results in a diminished storage of vitamins and trace elements. The metabolism of bilirubin is sometimes compromised and cause clinical symptoms such as jaundice, pruritus, and nausea.[23, 24]

Ascites and impaired kidney function as seen in hepatorenal syndrome (HRS) are other complications to cirrhosis. The pathophysiological explanation for development of ascites and HRS has recently been revised and the fact that liver cirrhosis is an inflammatory condition has been introduced in the hypothesis. Due to an increased intestinal permeability,

bacteria can bypass the intestinal wall. The bacteria are often killed by the immune defense, but the byproducts called pathogen-associated molecular patterns (PAMP's) are released in the systemic circulation.[25] PAMP's are detected by receptors on cells, which in turn excrete pro-inflammatory cytokines in the blood stream resulting in a systemic inflammatory condition with a decreased effective arterial blood volume and a hyperdynamic circulatory state and the activation of compensating mechanisms.[26] Small molecules derived from the inflammation in necrotic liver tissue, called damage associated molecular patters (DAMPs), are further contributing to an inflammatory state with high levels of circulating cytokines and chemokines found in the blood in patients with decompensated cirrhosis. Of note, the inflammatory hypothesis does not exclude previous explanations that were based on a decreased albumin and an increased portal pressure, to contribute to the development of ascites and HRS.

Another type of decompensation is *hepatic encephalopathy* characterized by an altered mental state. The encephalopathy can range from mild, with troubles to concentrate and remember things, to severe when the patient is in coma. Hepatic encephalopathy is commonly graded into four grades according to West Haven criteria, four being the most severe type.[27] The hypothesis is that encephalopathy is caused by an increased amount of circulating, neurotoxic substances such as ammonia. Ammonia normally enters the portal circulation and is cleared in the liver in the urea cycle. Due to portal hypertension, and the development of portosystemic collaterals and shunting of portal blood, the urea is bypassing the liver and the urea is degraded in the brain or skeletal muscles instead of the liver until this mechanism is oversaturated and symptoms of hepatic encephalopathy appears.[28]

1.6 DIAGNOSING LIVER CIRRHOSIS

Liver cirrhosis is typically diagnosed by weighting of several findings - clinical signs, radiology, lab tests, endoscopy, elastography and liver biopsy. Elastography is a non-invasive technique that has been available the past fifteen years where stiffness of the liver can be measured and presented as a surrogate of the amount of fibrosis in the liver. The different findings indicating cirrhosis are summarized in **Table 1.**

FINDINGS INDICATING CIRRHOSIS

CLINICAL SIGNS	Ascites and jaundice both have a sensitivity of < 0.4[29], spider naevi a sensitivity of 0.5 and a specificity of 0.88. For palmar erythema, accuracy differs between studies,[29, 30] in one study it was found in 70% of patients with cirrhosis. Caput Medusa in uncommon, and found in 8% of patients with cirrhosis.[31]
RADIOLOGY	Surface nodularity seen on ultrasound has a sensitivity of 52-63% and a specificity of 93-95%.[32, 33] Atrophy of the posterior segment in the right and left lobe and hypertrophy of the caudatus and lateral segment in the left lobe are less accurate. Signs of portal hypertension is an important, but late, finding in CT and MRI.[33]
LAB WORK	Decreased platelets are the earliest indicator of cirrhosis.[34] Elevated INR and bilirubin, decreased albumin are all late signs of cirrhosis. AST:ALT ratio>1 is a poorly sensitive test for cirrhosis but the specificity is better, especially in combination with decreased platelets.[35]
GASTROSCOPY	Signs of portal hypertension (esophageal and gastric varices and portal hypertensive gastropathy) have a high specificity but low sensitivity.[36]
ELASTOGRAPHY	Reliable to discriminate between cirrhosis and no cirrhosis. In HCV a value of > 12.5 kPa has a high sensitivity (87%) and specificity (91%) for cirrhosis. The accuracy is even better for NAFLD cirrhosis.[37, 38]
LIVER BIOPSY	Gold standard but invasive, 3% risk of complication requiring hospitalization[39] and not without risk of sampling error.[40]

Table 1. Diagnostics in cirrhosis. Abbreviations: AST aspartate transaminase ALT alanine transaminase, HCV hepatitis C virus, NAFLD non-alcoholic fatty liver disease

1.7 TREATMENT OF CIRRHOSIS COMPLICATIONS

1.7.1 Liver transplantation

There is only one curative treatment for cirrhosis, namely liver transplantation. Liver transplantation is an extensive procedure where the cirrhotic liver is replaced by a healthy liver from another human (**Figure 3)**. Due to several reasons, liver transplantation is not available to all patients with cirrhosis. First, there is a lack of organs, and the healthcare system therefore needs to prioritize between patients in need for a new liver. Secondly, the surgery is associated with risks if the patient is not in a good physical shape. Further, the patient must be able to adhere to the lifelong vital immuno-suppressant treatment after liver transplantation to avoid rejection of the new organ.[41]

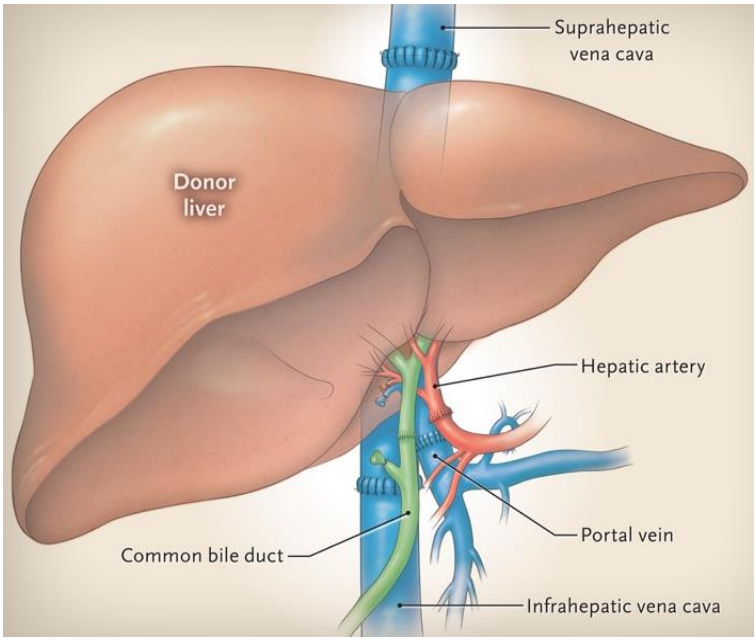


Figure 3 Liver transplantation. Reproduced with permission from (Diensag JL et al. Liver Transplantation - A Vision Realized, Diensag JL et al. N Engl J Med 2012. 367:1483-1485), Copyright Massachusetts Medical Society.

1.7.2 TIPS

Transjugular intrahepatic portosystemic shunt (TIPS) is a symptom-relieving procedure that may be relevant for patients with cirrhosis. A stent is inserted into the cirrhotic liver to connect a branch from the portal vein to a branch from the hepatic vein to decrease the pressure in the portal circulation **(Figure 4)**. This is typically done in variceal bleeding in individuals with high risk of recurrent bleeding and in refractory ascites. TIPS is also recommended as a rescue therapy in variceal bleeding that persists or rebleed and there is also a discussion about "early" preemptive TIPS within 72 hours that should be considered in patients with a high-risk of rebleeding.[42] Available research suggest that TIPS for refractory ascites and variceal bleeding has a survival benefit.[42-45].

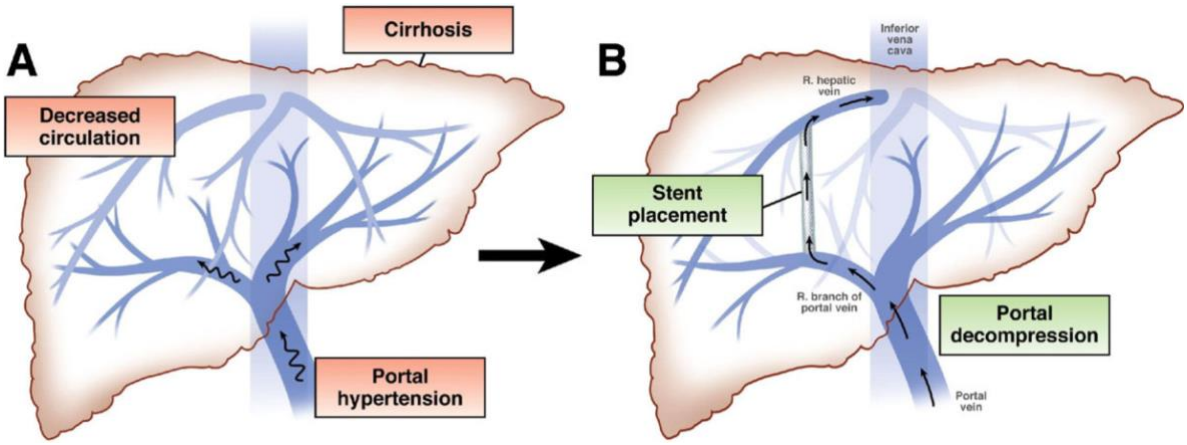


Figure 4. A. Cirrhotic liver with portal hypertension. **B** Cirrhotic liver after TIPS procedure. Printed with permission from "Bhogal HK. Transjugular intrahepatic portosystemic shunt: An overview. Clin Liver Dis (Hoboken). 2012;1(5):173-176." (Wiley).

A common side effect from TIPS, is encephalopathy. In early studies with bare stents, this was reported to affect up to 50% of patients [46], but with covered stents the risk is lower.[47] Encephalopathy is an expected side effect as the whole point with the TIPS-procedure is to let some portal blood bypass the liver directly into a hepatic vein and further to the brain, instead of passing through the sinusoids in the liver where the detoxification process takes place. The TIPS-procedure is reversible and if encephalopathy occurs, the diameter of the TIPS-stent can be decreased, or the stent completely closed.[28, 48]

1.7.3 Esophageal and gastric varices

Varices in the esophagus and fundus are caused by portal hypertension and present in 30-40% of patients with compensated cirrhosis and 70-85% of patients with decompensated cirrhosis.[36] As long as no bleeding occur, the varices are asymptomatic. Bleeding, however, is associated with a high risk of death, six-week mortality ranges between 15 and 25%[49], and is even higher in patients with other decompensating events.[50] Therefore, esophageal varices are treated prophylactically to prevent bleeding, with unselective betablockers or band ligation, which involves endoscopically placing a rubber band around the esophageal varices in repeated sessions until they are obliterated. Fundus varices are more difficult to treat, and the recommendation is histoacryl injection. TIPS can also be recommended as a treatment for *bleeding varices* as mentioned in the previous section.[51]

1.7.4 Ascites and spontaneous bacterial peritonitis

Large amount of fluid in the abdomen is associated with obvious complaints such as troubles breathing and eating and difficulties moving around. Paracentesis is recommended for larger volumes whereas diuretic medication with aldosterone receptor inhibitor and furosemide is recommended for smaller amounts of ascitic fluid and to maintain effect of paracentesis.[52, 53] Neither paracentesis or diuretics are associated with a survival benefit.[16] Ascites that cannot be managed by medical therapy either because of a lack of response to maximum doses of diuretics or because patients develop complications related to diuretic therapy that preclude

the use of an effective dose of diuretics, is defined as refractory ascites.[54] Patients with ascites are predisposed for developing bacterial infection in the peritoneum referred to as spontaneous bacterial peritonitis (SBP). SBP is diagnosed by the finding of an increased concentration of polymorphonuclear leucocytes in the ascitic fluid. It is reported to be one of the most common infections in cirrhosis[55] and symptoms are often mild but mortality is as high as 19%.[56, 57] Antibiotic treatment is recommended for treatment during infection and prophylactic antibiotics should be prescribed after the first episode of SBP.[58]

1.7.5 Hepatic encephalopathy

Hepatic encephalopathy refers to an often temporary altered mental status seen in some patients with cirrhosis. Lactulose, a non-absorbable disaccharide, has a demonstrated effect to evacuate proteins and making the stool acidic which in turn decrease ammonia absorption from the intestine and is the first-line acute and prophylactic treatment for hepatic encephalopathy.[59] Combining lactulose with rifaximin, an antibiotic with less than 5% systemic absorption, has been associated with an increased survival.[60] Oral branched-chain amino acids are also an available treatment option for encephalopathy.[61]

1.8 DISEASE SEVERITY SCORES

The most common cirrhosis severity score is the Child-Pugh score (**Table 2**), and it consists of laboratory markers (INR, bilirubin, albumin) and clinical findings (encephalopathy and ascites). The score was originally created to predict survival in surgery for patients with cirrhosis.[62] There are three classes, A, B and C where the best survival is seen in A and the worst in Child Pugh C. Patients in Child C have an overall mortality of 55% in one year.[63] A disadvantage with the Child-Pugh score is that the grading of encephalopathy and ascites requires a subjective assessment.

POINTS	*1*	*2*	*3*
ENCEPHALOPATHY	None	Grad 1-2	Grade 3-4
ASCITES	Absent	Easily controlled	Poorly controlled
BILIRUBIN (μmol/L)	<34	34-51	>51
ALBUMIN (G/L)	>35	28-35	<28
INR	<1.7	1.7-2.3	>2.3

CHILD PUGH A=5-6 POINTS, CHILD-PUGH B=7-9 POINTS AND CHILD-PUGH C = 10-15 POINTS

Table 2 Child-Pugh classification.

The model for end-stage liver disease (MELD) is another disease severity score used in chronic liver disease with the advantage of having only laboratory values as parameters. The MELD was originally developed to estimate risk of mortality in patients planned for a TIPS-procedure.[64] The score is also used to assess survival in different cirrhosis populations and in several countries MELD-score is used in allocation of organs for liver transplantation.[65] Several updates of the MELD-score have been presented and validated such as the MELD-Na and just recently the MELD 3.0.[66] MELD-Na has been shown to be superior to MELD in predicting 90-day mortality in patients on the transplantation list.[67]

1.9 LIVER CIRRHOSIS AND HEPATOCELLULAR CARCINOMA

Patients with a chronic liver disease have a sustained inflammation, fibrosis and hepatocyte regeneration in the liver that can favor the formation of dysplastic noduli that may develop into liver cancer.[68] In fact, 80-90% of patients with liver cancer have an underlying cirrhosis[69] which makes it a major risk factor of liver cancer.[70]The most common type of liver cancer is hepatocellular carcinoma (HCC) that comprises 90 % of all liver cancers.[71]

1.9.1 Surveillance, prognosis, and diagnosis

The overall prognosis in patients with HCC is poor, the five-year survival is only 17%,[72] mainly due to late diagnosis for most patients. Macro- and microvascular invasion, increased ∝-fetoprotein (AFP) and higher numbers of tumors are associated with shortened survival.[73, 74]

When HCC is diagnosed at an early stage and curative treatment is available, prognosis is better as seen in a five-year survival exceeding 70%.[68, 71, 75] Patients with liver cirrhosis are therefore recommended ultrasound of the liver bi-annually aiming to find HCC early on. Cohort studies and a meta-analysis have shown an association between HCC-surveillance and early tumor detection and improved survival after adjusting for lead-time and length-time bias,[76, 77] but no randomized controlled trial with results that can be generalized to a European setting has been performed. The sensitivity for HCC surveillance with ultrasound is reported to be 58-89% and the specificity > 90%.[78] To add the biomarker AFP to the screening provides no benefit as AFP is a marker for advanced tumor disease.[79] To make screening cost-effective, risk should be high enough and an annual rate of HCC of at least 1.5% has been proposed.[79-81] For screening to be recommended, patients should also be available for treatment if diagnosed with HCC and not to sick.[82]

To confirm the HCC-diagnosis, biopsy is not required in patients with cirrhosis. Radiological examination by MRI or CT with contrast showing a noduli larger than 1 cm with an increased uptake in the arterial phase and wash-out in the venous phase is consistent with HCC and has a diagnostic sensitivity of 89% and a specificity of 96%.[83] In patients without liver cirrhosis, biopsy is however required for the diagnosis of HCC.

1.9.2 Barcelona Clinic Liver Cancer system

The Barcelona Clinic Liver Cancer system (BCLC) shown in **Figure 5** is one of several protocols that have been developed to optimize matching between patient and treatment modality and is the staging system for HCC used in Sweden, also endorsed by the European Association for Study of the Liver.[82] In the BCLC, the severity of cirrhosis is estimated by the Child-Pugh score (**Table 2**) and the patient's performance status is assessed using the Eastern Cooperative Oncology Group (ECOG) classification (**Table 3**). Tumor characteristics such as size, number of tumors and extrahepatic manifestations are also added in the BCLC staging system. In the Stockholm region, patients with a liver mass should be referred to Karolinska University Hospital in Huddinge for a multidisciplinary therapy conference (MDT) where surgeons, hepatologists, oncologists, radiologists and pathologists attend and discuss diagnosis and suitable treatment based on this algorithm.

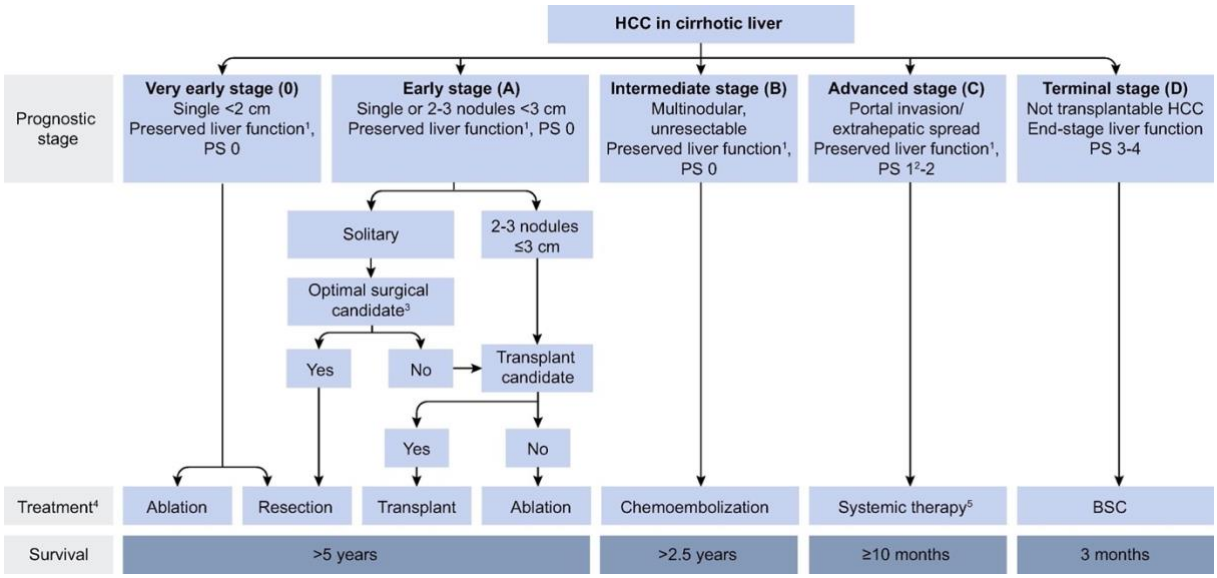


Figure 5. Barcelona Clinic Liver Cancer System (BCLC) Abbreviations: PS performance status, HCC hepatocellular carcinoma, BSC best supportive care. Printed with permission from "Galle et al. EASL Clinical Practice Guidelines: Management of hepatocellular carcinoma, J Hepatol. 2018;69(1):182-236."

GRADE	*ECOG- PERFORMANCE STATUS*
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light housework, office work
2	Ambulatory and capable of all selfcare but unable to carry on any work activities. Up and about more than 50% of waking hours
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair
5	Dead

Table 3. Performance status. Abbreviations: ECOG Eastern Cooperative Oncology Group

1.9.3 Transplantation criteria

There are several criteria used in different settings to determine whether a patient with HCC is eligible for liver transplantation. To fit the Milan Criteria, first presented 1996, the following criteria must be met: a single lesion ≤5 cm or 3 or fewer lesions all <3 cm and no evidence of macrovascular invasion, lymph node involvement, or extrahepatic metastasis. In 2001, the University of California San Francisco (UCSF) criteria[84] were published, expanding the tumor size condition. Patients with a single tumor ≤6.5 cm in diameter or up to 3 lesions each ≤4.5 cm in diameter with a total diameter of ≤8 cm fell within the UCSF criteria.[85] In Sweden, the UCSF criteria is used and embedded in the BCLC protocol.[86]

1.9.4 Treatment options for liver cancer

Liver function is an important factor to consider when deciding on appropriate treatment for HCC. For decompensated cirrhosis, there is a considerable risk that a liver resection will result in a remaining liver insufficient to meet the body's demand. Decompensated cirrhosis is also a risk factor for other treatments modalities due to a higher risk of adverse events. The overall poor prognosis in decompensated cirrhosis must also be considered when deciding on HCC treatment.

1.9.4.1 Liver transplantation and neoadjuvant treatment

Liver transplantation is primarily considered for a patient with *decompensated cirrhosis* and a tumor within the Milan or UCSF criteria and no other contraindications to transplantation. Liver transplantation has the best five-year survival among the HCC therapies (70%-79%)[84, 87, 88] and offers a treatment both for HCC *and* the underlying cirrhosis. To prevent tumor progression when a patient is on the transplantation waiting list, locoregional treatments such as trans-arterial chemoablation (TACE) and radiofrequency ablation (RF) have been used and found to reduce drop-out on waiting list and cancer recurrence after transplantation.[89, 90]

These procedures have also been used to reduce the tumor burden to fit within the transplantation criteria (downstaging) with encouraging post-transplantation results.[91, 92]

1.9.4.2 Liver resection

A tumor larger than 3 cm in a *liver with preserved function* is ideally resected if the tumor location allows it.[93] The risk of recurrence after hepatic resection is >50 %.[94] This high recurrence risk is also seen in patients diagnosed with HCC at the Karolinska University Hospital where recurrence was seen in 58% of resected patients (unpublished data). A recently published article from Karolinska University Hospital report the five-year survival in Sweden to be 60%.[88]

1.9.4.3 Radiofrequency treatment

Image-guided ablation with RF is a potentially curative treatment recommended for non-surgical early-stage liver tumor less than 3 cm and for patients with early stage HCC not suitable for resection due to liver dysfunction or several tumors where liver transplantation is contraindicated.[82] Median survival depends on tumor stage, for BCLC 0 overall survival after 4 years is similar to resected patients with the same disease burden, and reported to be 63-68%.[95, 96] The five-year survival for all patients receiving RF, is 35% in Sweden.[88]

1.9.4.4 Trans-arterial chemoablation

TACE is considered a non-curative treatment where a chemotherapeutic drug is directly administered in the arterial blood vessel that feeds the tumor and thereafter the same blood vessel is embolized cutting off the tumor blood supply. TACE is indicated for patients with intermediate HCC, as presented in the BCLC-algorithm in **Figure 5.** The median overall survival for patients treated with TACE is 26-30 months.[97, 98]

1.9.4.5 Systemic treatment

Sorafenib is a multikinase-inhibitor that reduce the angiogenesis and cell proliferation in the tumor. It was approved in 2007 as a monotherapy in patients with unresectable HCC although overall survival was only three months longer compared to placebo and side effects are common.[99] Levantinib is a newer drug with a similar mode of action, and was approved and introduced as another first line treatment after being shown to be non-inferior to Sorafenib, but with a different safety profile.[100]

Tyrosine-inhibitors (regorafenib and cabozatinib) and vascular endothelial growth factor receptor inhibitors (ramucirumab) were introduced as second-line treatment and has proven survival benefit as well as immune checkpoint inhibitors atezolizumab combined with bevacizumab that were introduced in Sweden in 2021.[101] Several new immunotherapies and combination of treatments are now emerging as novel systemic treatment options for HCC[102] and has challenged sorafenib as the first line treatment.[101]

1.9.4.6 Best supportive care

Patients with decompensated cirrhosis Child-Pugh class C and an impaired performance status is not eligible for any, but symptom relieving treatment, and have a median survival of 3-5 months.[99]

1.10 LIVER CIRRHOSIS AND INFECTION

One of the immunological functions of the liver is to screen the huge number of bacterial products, environment toxins and food antigens provided by the double blood supply from the portal vein and the hepatic artery. The liver will either remain tolerant or activate an immune response to protect against these potentially toxic agents. In the liver, there are several immune-cells able to detect, present and clear infection, such as antigen-presenting cells (APC), T-cells, B-cells, Kupffer cells, and natural killer (NK) cells.[103] APC present bacterial peptides for T-cells to activate the T-cells. T-cells are lymphocytes that are either T-helper or T-cytotoxic. B-cells are antibody-producing cells and Kupffer cells are liver resident macrophages. NK cells secrete cytokines that will activate or increase the immune response or kill an infected cell.

Patients with cirrhosis are more often diagnosed with bacterial infections compared to other patients admitted to hospital.[55, 104] It is also a common finding that patients with cirrhosis admitted to hospital have an infection at admission but the primary cause of hospital care is another.[105] This might be explained by bacterial infections being one of the most important reasons for developing liver-related decompensating events. One example of this is the increased risk of esophageal varices to rebleed in the presence of bacterial infection and the survival benefit when antibiotics are given prophylactically to patients with variceal bleeding.[106, 107] The most common infection in cirrhosis has historically been reported to be SBP, but in later studies respiratory- and urinary tract infections seem to be more common.[108-110] It is unknown if prophylactic antibiotics given after a first episode of SBP or other factors have changed this. Approximately 30% of the mortality seen in patients with cirrhosis is attributable to infections.[111]

The cirrhosis-associated immune dysfunction in decompensated cirrhosis, is due to a wide range of immune alterations seen in cirrhosis.[103] The synthesis of proteins important for the immune system is impaired and the antimicrobial surveillance function in the liver by the Kupffer cells, is also downregulated with an impaired liver clearance of DAMPs and PAMPs as a result.[112, 113]. A dysregulated bacterial translocation occur due to an increased intestinal permeability seen in decompensated liver cirrhosis.[103] In conclusion, the immunodeficiency and the systemic inflammation occurring in liver cirrhosis are the key components for the increased burden of infections in cirrhosis.

Early recognition of infections is of great importance but unfortunately the clinical picture is often atypical and sometimes only seen as a decompensating event or an organ dysfunction.[56, 114] Furthermore, conventional laboratory markers for infection (CRP and

procalcitonin) perform differently in patients with cirrhosis compared to patients without cirrhosis.[115]

2 LITERATURE REVIEW

2.1 LIVER CIRRHOSIS AND LIVER CANCER - EPIDEMIOLOGY

The risk of HCC in cirrhosis has increased in the latest years in many geographic areas. In an Australian study investigating HCC incidence from 1986 to 2014, HCC incidence increased a little over 4% per year.[116] This finding is also confirmed by Ioannou et al. reporting an

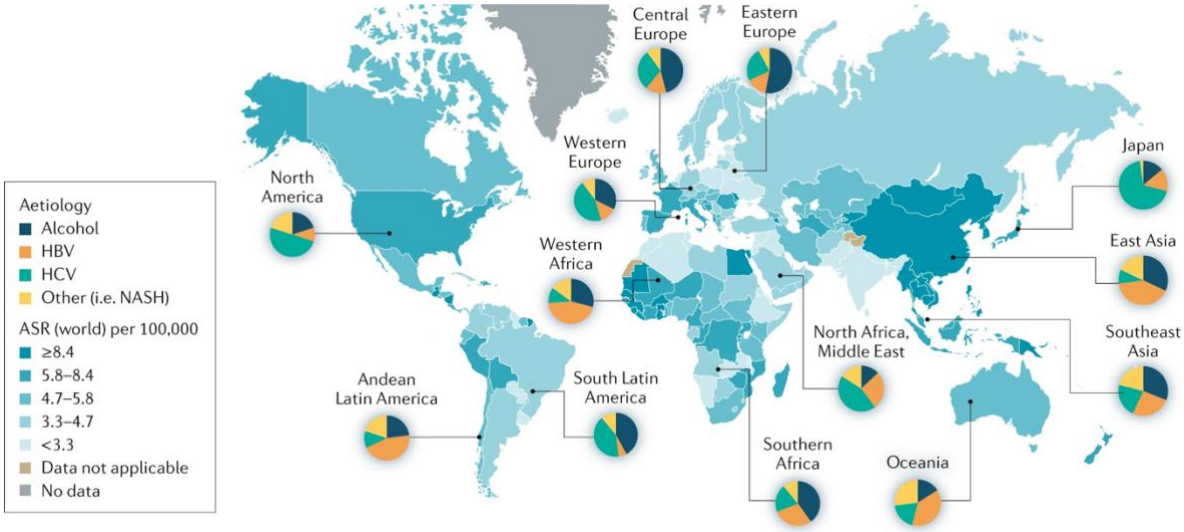


Figure 6. The incidence and major etiological factors. The main etiology included in "other" is NAFLD.. Abbreviations: ASR age standardized incidence rate, HBV hepatitis B virus, HCV hepatitis C virus. Printed with permission from "Llovet et al. Hepatocellular carcinoma. Nat Rev Dis Primers 7, 2021;6".

increased incidence of HCC for several etiologies of cirrhosis over time in a cohort of military veterans in the USA and by Jepsen et al. in a Danish cohort.[117, 118] HCC is more common in men than in women with a factor of 2-2.5:1[119] and the risk of HCC also depends on the etiology of liver disease, age,[120, 121] diabetes,[122] portal pressure and disease severity.[123, 124]

Incidence rates for HCC differs between studies and geographical areas as shown in **Figure 6**. West et al. examined 3,107 patients with cirrhosis in primary care in England and linked each case to cancer registry data. The authors found an overall incidence rate for HCC in cirrhosis of 3.9/1,000 person-years.[125] In a study of HCC-risk in cirrhosis by Sörensen et al. from 1998 the risk is reported to be 60-fold elevated compared to the general population.[126]

2.1.1 HCC risk in different liver diseases

The different risk of HCC in different liver diseases is pointed out in a Canadian study from a liver center in Toronto where a scoring system to predict risk of HCC in cirrhosis was designed and externally validated. Sharma and colleges found that HCC occurred most frequently in patients with hepatitis B and C (26 and 22/1,000 person-years respectively), followed by patients with ARLD (18/1,000 person-years) and NAFLD (14/1,000 person-years). Patients with autoimmune liver diseases had a lower incidence of HCC ranging from 1/1,000 person-years in AIH to 7/1,000 person-years in PSC.[127] The incidence rates in the

Canadian study are higher than in the study from England that reported an incidence of 3.9/1,000 person-years[125], but the settings are also different and this might be a part of the explanation.

2.1.2 HCC in NAFLD

NAFLD has become a leading cause of chronic liver disease in many western countries and the number of HCC patients with NAFLD is growing. In an American study of military veterans, the risk of HCC in NAFLD cirrhosis ranged from 2-24/1,000 person-years depending on age, race and other demographic characteristics.[128] Another study from the United States report the annual incidence of HCC in NAFLD cirrhosis to be 2.6%[129] and in a third study 1.6%.[130]

2.1.2.1 Non-cirrhotic liver cancer in NAFLD

Most patients with liver cancer have underlying cirrhosis, but in approximately 10-20% of patients, HCC develop in a non-cirrhotic liver.[131] An association between NAFLD and HCC in non-cirrhotic liver has been presented by several authors.[132-135] Mittal et al. found that 34% of 106 patients with NAFLD-HCC did not have cirrhosis in a study of American male veterans.[136] An Italian study of 146 patients with NAFLD-HCC by Piscaglia et al. reported that 50% of patients with NAFLD were non-cirrhotic[133] and in a meta-analysis of previously conducted NAFLD-HCC studies 39% of patients were reported to be non-cirrhotic.[137]

There are a few publications focusing mainly on *non-cirrhotic* NAFLD-HCC.[131, 138] To summarize, these studies report that patients with non-cirrhotic NAFLD-HCC have larger tumors [138, 139] and are older.[138] Patients with non-cirrhotic NAFLD-HCC underwent liver resection and received palliative treatment more often than patients with cirrhosis and liver cancer but liver transplantation and locoregional treatments such as TACE or RF were less often applied.[138]

Guidelines recommend HCC-surveillance only in patients with NAFLD and cirrhosis and therefore the high frequency of NAFLD-HCC with *no* cirrhosis is problematic. To perform HCC-surveillance in all individuals with NAFLD, with and without cirrhosis, is not an option due to the very high number of individuals affected by NAFLD and the rather low risk of HCC. In general, previous studies in this field have been small with limited power to identify differences between groups and few have examined any impact on survival. Data on non-cirrhotic NAFLD-HCC are lacking and research is needed to characterize high-risk individuals to develop HCC so that these individuals can be identified among all individuals with NAFLD.

2.1.3 HCC in viral hepatitis

Viral hepatitis is responsible for most cases of HCC worldwide. A Taiwanese study of 2,443 men positive for *hepatitis-B*, report the HCC incidence rate to be 4/1,000 person-years.[140] An Italian study with 214 *hepatitis-C* positive patients with cirrhosis were followed over an average period of nine and a half years, and 32% were diagnosed with HCC. The authors

reported an annual incidence rate of HCC of 4%[141], which is in line with 1-4% as reported in a Japanese study.[142]

2.1.4 HCC in alcohol-related liver disease

The incidence rates of HCC in ARLD is lower than that of viral hepatitis. Ioannou et al. present an incidence rate of 8.6/1,000 person-years in a study of American war veterans[117] and a Swedish study of patients with biopsy-proven ARLD cirrhosis report exactly the same incidence rate.[143] In a Danish registry-based study of HCC-risk in ARLD in 4,553 outpatients, the authors report an annual risk of only 0.7%.[144]

2.1.5 HCC in autoimmune liver diseases

A Danish study by Grønbæck et al. report that *AIH-cirrhosis* entailed a risk of 1.9/1,000 person-years.[145] In a meta-analysis of HCC-risk in autoimmune hepatitis, Tansel and colleges report a pooled HCC incidence rate in AIH cirrhosis of 10/1,000 person-years with a very wide range 0.8-27/1,000 person-years.[146] Liang et al. performed a meta-analysis of 17 publications of *PBC* and cancer risk and found an increased risk although the heterogenicity between included studies were noticeable.[147]

2.1.6 HCC in rare liver diseases

In a Swedish study of 1,847 patients with both cirrhotic and non-cirrhotic *hereditary hemochromatosis*, the risk of HCC was 20-fold compared to the general population.[148] In a study where medical charts of 363 *Wilson* patients with and without cirrhosis from Sweden and England were reviewed, the authors conclude that patients with Wilsons disease seems to be vulnerable to abdominal malignancies[149], but reliable data in this rare disease is lacking.

2.1.7 What more is there to know about risk of HCC in cirrhosis?

Why conduct research in this already explored area with numerous studies published? First, most cohorts are from specialized liver centers implicating a highly selected group of patients with cirrhosis and most studies have not included patients nationwide. Secondly, the risk of HCC is commonly estimated for one specific liver disease and due to diverse populations, comparisons of HCC risk between different liver diseases from different studies are often difficult to make and comparison with the background population is rarely done. Thirdly, previous studies are often underpowered to be able to investigate differences between subgroups such as age and sex and the competing risk of dying from something else than HCC are rarely considered. Thus, studies with a higher quality are important to further describe the risk for HCC in patients with cirrhosis.

2.2 MAIT CELLS IN LIVER CIRRHOSIS

The immune system is divided into the innate and the adaptive immune defense that coordinate their actions. The innate immune system consists of physical and anatomical barriers such as skin and endothelial tissues, but also antimicrobial peptides, microbiota, and immune cells such as leukocytes, APC and NK cells. The innate system provides a first line

of defense against many common microorganisms and is essential for the control of common bacterial infections the first days before the adaptive response takes effect. When the innate system do not manage to clear the infection, the inflammation caused by the cells engaged in the innate system will activate the adaptive responses and attract B- and T-lymphocytes that provide a more specific and complex type of defense. The adaptive response also offers an increased protection against subsequent reinfection with the same pathogen.[150]

The adaptive immune response is subdivided into the humoral immunity involving the B-lymphocytes that create antibodies and the cellular immunity involving the T-lymphocytes that secrete immune regulatory factors

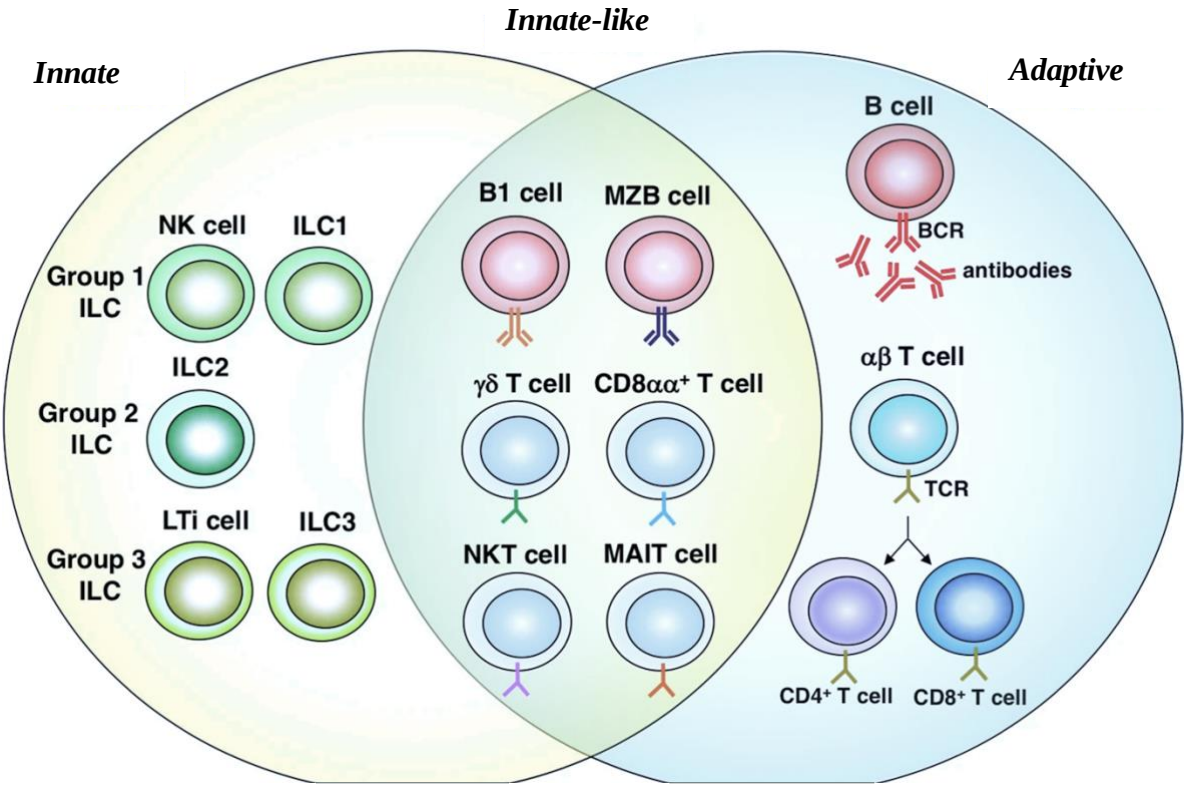
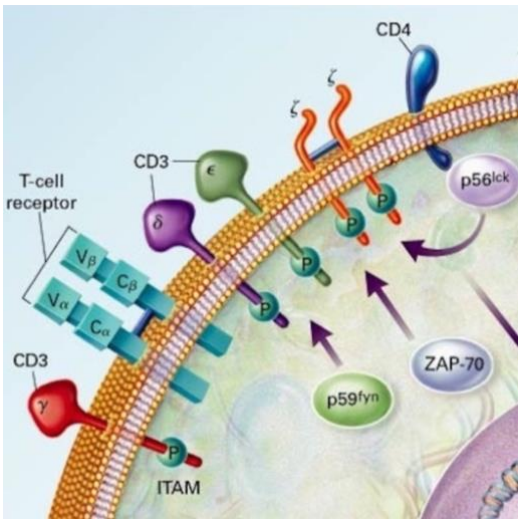


Figure 7. Classification of lymphocytes. Published with permission from "Van Kaer et al. Innate, innate-like, and adaptive lymphocytes in the pathogenesis of MS and EAE. Cell Moll Immunol. 2019;16(6):531-539.

There are also *innate-like* lymphocytes that do not fit into either side of the historical innate-adaptive classification. In this category you find the MAIT cell together with NK cells and innate-like B-cells as shown in **Figure 7**.[151] The MAIT cells are unconventional T-lymphocytes with both adaptive and innate immune defense characteristics, able to both detect bacteria and exert a cytotoxic effect on microorganisms.

MAIT cells are abundant in blood, the intestinal mucosa, lung and in liver tissue where they represent 10-40% of liver T-cells.[152, 153] MAIT cells are defined by a semi-invariant T-cell receptor that recognizes bacterial metabolites.[154] The T-cell receptor is always expressed with the associated cluster of differentiation(CD)3 complex, as shown in **Figure 8**. The CD-classification is often used to identify cells in flow cytometry and other immunophenotyping procedures and is based on expressed molecules found on the cell surface. CD4 and CD8 are



two CD molecules used to differentiate T-cells. CD4 is found on T-helper cells referred to as $CD4^+$ and CD8 is expressed on T-cytotoxic cells such as MAIT cells referred to as $CD4^-$. Some other leukocytes express CD4 and CD8 and therefore CD4 and CD8 is often used in combination with $CD3^+$ to differentiate T-lymphocytes from other leukocytes.[155]

Figure 8. T-cell receptor expressing $CD3^+$ and a coreceptor expressing CD4 (T-helper). Printed with permission from "Delves et al. The immune system. N Engl J Med 2000;343:108-117." Abbreviations: CD cluster of differentiation.

MAIT cells respond to a range of microbes and play an important role in the immune defense against infections.[156-158] The activation of MAIT cells results in the production of pro-inflammatory cytokines such as IFNγ, TNFα, IL-17 and IL-22 and release of perforin and granzyme B that can kill infected cells as shown in **Figure 9.**[159]

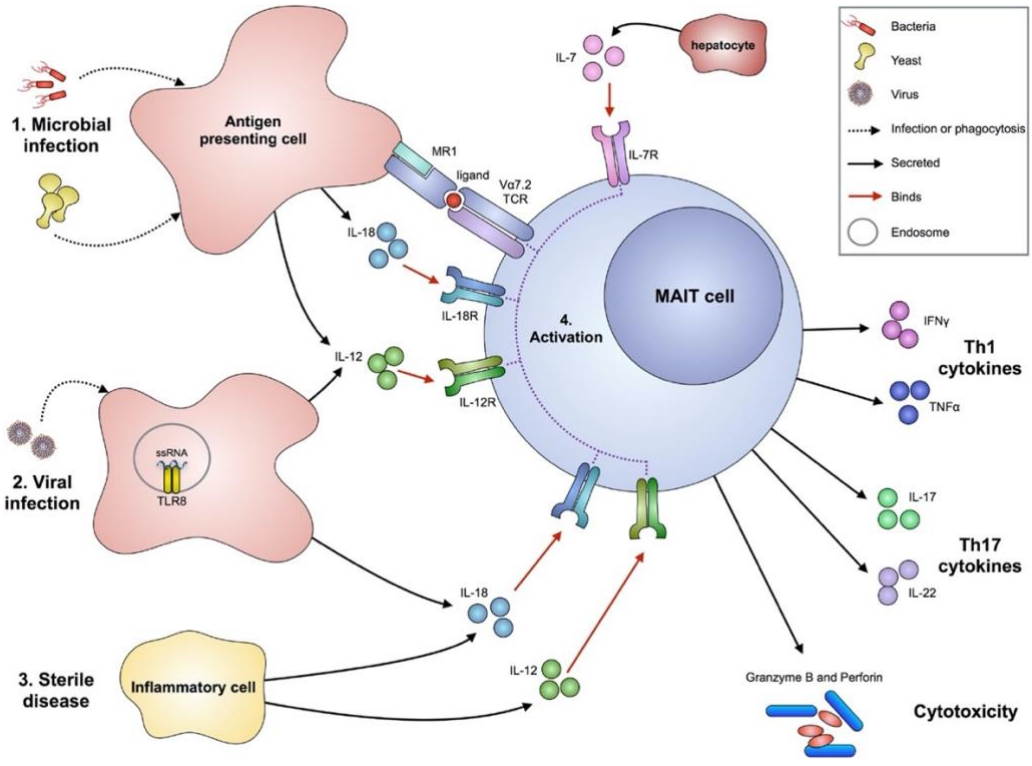


Figure 9. MAIT cell activation by 1) a microorganism phagocytosed by APC, 2) viral infection and 3) in sterile disease such as autoimmunity (3) Activated MAIT cell (4) produce cytokines but also granzyme B and perforin that directly can kill an infected cell. Printed with permission from Howson et al. MR1-restricted mucosal-associated invariant T-cells and their activation during infectious disease. Front Immunol. 2015;6:303.

Decreased levels of MAIT cells in blood have been described in several chronic liver diseases- viral hepatitis, AIH, alcoholic hepatitis, PSC and PBC.[160-166] Why levels of MAIT cells decrease in blood in patients with chronic liver diseases are not fully known. There is some evidence supporting migration of MAIT cells from blood to the tissue involved in the disease. MAIT cells are elevated in ascites in patients with decompensated cirrhosis[160], bile-MAIT cells are increased in PSC[165] and liver MAIT cells in NAFLD.[162] This is not reported for viral hepatitis,[163, 167] nor alcoholic hepatitis.[161] Decreased blood MAIT cells and elevated MAIT cells in affected tissue have also been reported for chronic *non-liver* diseases such as inflammatory bowel disease[168] multiple sclerosis[169], rheumatoid arthritis and systemic lupus erythematosus[170] also supporting the hypothesis that MAIT cells provide a response by T-cells to chronic inflammation common for various conditions.[171]

There are several studies on MAIT cells from a preclinical setting, but little is known about MAIT cell levels and impact on clinically relevant outcomes in patients with cirrhosis.

2.3 DATA SOURCES

The Swedish National Patient Register (NPR) contains International classification of diseases (ICD)-codes for all patient visits in specialized care. The NPR was established in 1964 with nationwide coverage occurring in 1987 and including specialized outpatient care since 2001.[172]

The Swedish personal identity number is a unique 12-digit code given to all Swedish residents. In research, the personal identity number can be used for linkages between registers, and data collected by the researcher.[173]

The Swedish Cancer Register is based on physicians' mandatory reporting of newly detected cancer and an independent mandatory reporting by pathologists on every cancer diagnosis made from pathological specimens. The register was established in 1958. The completeness differs depending on the type of cancer but is overall high, about 96%.[174] For HCC the completeness is reported to be lower than for other types of cancer, probably explained by the non-invasive methods to diagnose HCC, at least in cirrhosis.[175]

The National Causes of Death Register, established in 1961, comprises data on all deaths in Sweden and is highly reliable with over 99% of all deaths of Swedish citizens and residents with a personal identity number reported. However, due to misclassification, the *cause* of death is not as reliable. Misclassified deaths is estimated to be around 20% and even higher for liver related deaths.[176, 177] The physician who confirms the death write a death certificate that is sent to the Swedish tax office for registration. The death certificate must be completed before a burial can be authorized. A more detailed report of the cause of death is also filled in by a physician and sent to the National Board of Health and Welfare within three weeks.[178]

The Total Population Register is often used to link research participants to matched reference individuals for comparison and contains data on date of birth, migration, and death as well as other variables.[179]

2.4 ICD-CODES IN REGISTER-BASED STUDIES

ICD-codes, first presented a century ago, are used worldwide to convert clinical terms into a code that is used for health recording and statistics of health. ICD-codes are registered in primary care and hospitals but also for cause of death certificates in around 120 countries including Sweden.[180] ICD-codes for main and contributing diagnoses are made by the responsible physician at the time of the healthcare contact, these are kept locally and transferred to national registers on a regular basis. The codes classify disease and health problems causing death or contact with the healthcare. This data can thus be used to statistically describe diseases and is used in many aspects, such as reimbursement, health economic planning and to allocate resources. ICD-codes are also widely used in research as the codes enable summarization of large-scale data and comparisons between places and over time.

The tenth revision, ICD-10, was released in 1992, and has been used in Sweden since 1997.[181] An eleventh version has been released by the World Health Organization. A translation into Swedish is planned to take place within a near future, the date of introduction is not decided but it will probably take another couple of years.[182]

For epidemiological studies in hepatology, administrative data from registers is useful for identifying liver-related events across different healthcare systems. A high accuracy of the coding system is vital to reduce the risk of false positives. An American validation study from 2013 by Nehra et al. validated ICD-9 codes from both inpatient and outpatient visits for the presence of cirrhosis. The cohort comprised patients from a teaching hospital but also from primary care centers. The authors found most individual ICD-9 codes, except that of ascites, to have high PPV ranging from 78-94% for identifying cirrhosis.[183] In another validation study from 2007 of liver-related diagnoses in 331 patients with ARLD and HCV in the Veteran Affairs system in the USA, the PPV for cirrhosis using ICD-9 codes was 90%.[184] In a cross-sectional study from 2012 with 266 randomly selected patients with ICD-9 codes corresponding to end-stage liver disease included from two university hospitals in Pennsylvania, Unites States, Goldberg et al. found that the PPV for cirrhosis was 94% when combining one code for cirrhosis with one code for a decompensation event such as variceal bleeding or ascites.[185]

One of few validation studies from the Nordic countries is a Danish study from 1997 by Vestberg et al. The cohort consisted of 198 patients who had received an ICD-8 code for cirrhosis from 1985-1989 and PPV for cirrhosis was estimated to be 85%.[186]

In summary, most validation studies for liver-related ICD-codes originate from the USA,[183-185, 187] where ICD-9 codes were used until 2015, unlike the Nordic countries where ICD-10 was introduced already in the 1990s.[181, 188] The healthcare system and the financing of care in the USA are also different from that in most European countries where publicly funded healthcare is more common. Because of reimbursement issues, ICD-coding might differ depending on the healthcare system.[189] Establishing the validity of ICD-codes from our

geographical area is therefore needed in order to make sure data from epidemiological studies using ICD-codes is as correct as possible.

2.5 COMPETING RISKS ANALYSIS

2.5.1 Competing risks and cumulative incidence

In cirrhosis and other chronic medical conditions with a high mortality, the possibility of competing events must be considered whenever you study something else than all-cause mortality. A competing event is defined as an event that prohibit observing the event of interest from occurring. In the Kaplan-Meier method, such events usually lead to censoring with the consequence that the cumulative incidence of the event of interest is overestimated.[190] The explanation for this is that the Kaplan-Meier method only takes one event into account at a time. The estimate derived from the Kaplan-Meier method is therefore the net survival, which provides a measure of how the survival would be if the outcome of interest was the only thing that could affect the patients. Kaplan-Meier is therefore an adequate statistical method to use if the outcome of interest is all-cause mortality but if the outcome of interest is cause-specific mortality, for example death from cirrhosis, one must consider competing events, such as death from other causes. This cannot be done in a good way in the Kaplan-Meier method where all other events are treated as censored observations.[191]

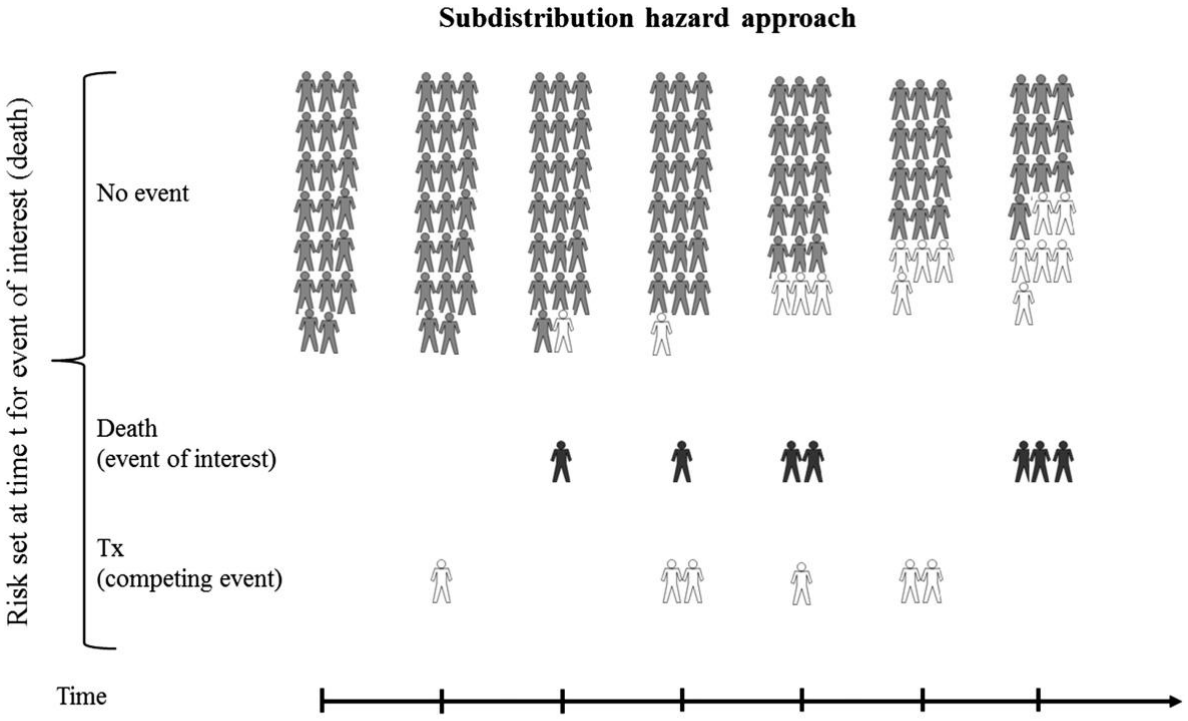
Why is censoring not an adequate way of handling competing risks in cause-specific mortality in chronic diseases such as cirrhosis? Common causes of censoring are when further follow-up is impossible or useless, when a patient is lost to follow-up during the study period or at the end of the study period if the event of interest has not occurred. Censoring should be independent, which means that those censored during the study period should be representative for those still at risk. In other words, censoring should not be a marker for good or bad prognosis. This assumption of independence is often true when censoring is performed due to things like emigration, that in most cases occur at random, but it might not be true for individuals censored because of an event that make further follow-up impossible, such as death from causes separate from the event of interest. It is not possible to statistically test if the assumption of independence is true or not, this must be decided based on knowledge in the research field. In a competing risk analysis the marginal probability, defined as the probability of individuals who developed the event of interest, regardless of whether they were censored or failed from other competing events, is estimated. In this way, the competing events are considered, and the competing risk analysis should be the method of choice when investigating cumulative incidence in cirrhosis.[191, 192]

2.5.2 Competing risks and prognostic factors

Research could also focus on prognostic factors, causal or predictive risk factors for a disease. For causal inference, there is no need to account for competing risks, and a Cox regression model can be used to investigate the association between the potential cause and the rate of an event. However, if the research question is descriptive or focus on finding predictive

factors, competing events are important to consider. Here, the risk in presence of competing risks, what happens in real life, is investigated, not the rate. For predictive studies, something that increase the rate might not increase the risk. This can happen when the predictor also increase the rate of the competing event. For example, male gender increase the rate of HCC but also the rate of death. Males will consequently die before being diagnosed with HCC to a larger extent which has to be considered in the analysis.

The Fine and Gray model is the most common statistical method to use for competing risk regression instead of a Cox regression model. In the Fine and Grey model, the subdistribution hazard ratio (SHR) is estimated, analogous to the hazard ratio obtained from the Cox proportional hazard model, except that it models a hazard function derived from the cumulative incidence function.[193] The interpretation is related to that of relative risk from which you can determine whether a predictive factor influences the risk of an event. **Figure 10** illustrates the different ways to handle the competing event of transplantation in the competing event analysis and the cause-specific hazard such as Cox regression model.



Hazard (t)	t=0	t=1	t=2	t=3	t=4	t=5	t=6
SDH	0/20=0	0/20=0	1/20=0.05	1/19=0.05	2/18=0.11	0/16=0	3/16=0.19
CSH	0/20=0	0/20=0	1/19=0.05	1/18=0.06	2/15=0.13	0/12=0	3/10=0.33

Figure 10. Calculation of the subdistribution hazards: The risk set starts with 20 individuals (grey). Over time, individuals may experience the event of interest (death, black) or the competing event (transplantation, white) and those having a competing event are maintained in the risk set as opposed to the cause specific hazard. Abbreviations: Tx transplantation, SDH subdistribution hazard, CSD cause specific hazard. Printed with permission from "Noordzij M et al. When do we need competing risks methods for survival analysis in nephrology. Nephrol Dial Transplant. 2013;2670–2677."

3 RESEARCH AIMS

The overall aim of this thesis was to improve the understanding of prognosis and the ability to predict severe outcomes in cirrhosis.

Our specific aims were:

1. To describe and compare prognosis for patients with NAFLD-HCC, with and without cirrhosis, and to identify mortality risk factors and temporal trends (Paper I)

2. To validate ICD-10 codes for liver-related events and calculate PPVs of these (Paper II)

3. To investigate the rate and cumulative incidence of HCC in patients with cirrhosis for various etiologies and subgroups (Paper III)

4. To evaluate if MAIT cell fractions in peripheral blood are associated with risk of bacterial infections in patients with cirrhosis (Paper IV)

4 MATERIALS AND METHODS

4.1 ETHICAL CONSIDERATIONS

All studies have ethical approval from the regional ethics committee of Stockholm County; dnr 2016/1772-31/2 (paper I); dnr 2017/1019-31/1 and dnr 2018/355-32/1 (paper II and III); dnr 2013/2285-31/3 (paper IV). In paper I-III, an informed consent was waived by the regional ethics committee with the motivation that the data collection was retrospective, and no direct contact was taken with the patients in these studies. In study IV, a written consent was obtained from all participants.

In study I-III, the patients could not choose whether they wanted to participate or not in the study. The principle of autonomy, referring to the right of patients to make decisions about their medical care and to be informed, is not considered when the requirement of an informed consent is removed as for the retrospective register-based studies. Many patients with liver disease suffer from addictive diseases and these patient's rights needs to be particularly addressed as this vulnerable group of patients tend not to raise their voice themselves. Furthermore, addictive diseases are still stigmatizing, and the integrity of these patients also needs to be taken into account.

On the other hand, the treatment and care of vulnerable patient groups are not promoted when no research is conducted about their diseases. Another thing to bear in mind is that most individuals in study I-III are diseased making it not only more time consuming and expensive, but also impossible to conduct the studies if an informed consent was required as these would never be obtained.

The principle of beneficence, aiming at producing net benefit over harm, is another principle to consider in medical ethics. I find it reasonable that this principle should be prioritized over autonomy and integrity to enable the studies being conducted, beneficial in the long run for this patient group.

4.2 OVERVIEW STUDY I-IV

	I- Characteristics and outcome of hepatocellular carcinoma in patients with NAFLD without cirrhosis	*II- Validity of administrative codes associated with cirrhosis in Sweden*	*III- The risk of hepatocellular carcinoma in cirrhosis differs by etiology, age, and sex*	*IV- Evaluation of MAIT cells as a potential biomarker to predict infection risk in liver cirrhosis*
Study design	Cohort study with historical data	Cross-sectional study	Cohort study with historical data	Cohort study with prospectively collected data
Study population	Liver cancer patients at Karolinska	Random patients with liver-related ICD-10 codes in Sweden	Patients with cirrhosis in Sweden	Patients with cirrhosis at Karolinska
Study period	2004–2018	2000–2016	2001–2016	2016–2019
Data sources	Medical records	NPR, medical records	NPR, the cancer registry, the total population registry	Medical records
Inclusion criteria	ICD-10 code C22.0 in the hospital registry	ICD-10 codes for esophageal varices, HCC, ascites, and cirrhosis in NPR	ICD-codes associated with cirrhosis in NPR	Patients with cirrhosis and no ongoing treatment for infection
Exclusion criteria	Previous liver cancer or liver transplantation	Charts with insufficient data	Previous liver cancer and liver transplantation	Previous cancer, HCV + HIV co-infection
Sample size	1,562	630	15,215	106
Statistical analysis	Mann-Whitney, Chi^2, Cox regression	Positive predictive value	Competing risk regression, Cox hazard	Chi^2, Kruskal-Wallis, competing-risk regression, Cox regression
Main factor analyzed	Differences between groups, incidence, time-trends, predictive factors for mortality	Accuracy of ICD-codes	Incidence rate, cumulative incidence function, time to event	Association between fractions of MAIT cells and risk of infection, decompensation, and death

Table 4. Overview study I-IV. Abbreviation: ICD international classification of diseases, HCV hepatitis C, HIV human immunodeficiency virus, MAIT mucosal associated invariant T-cells, NPR national patient register

4.3 STUDY DESIGN AND METHODS

4.3.1 Paper I

4.3.1.1 Study population

In study I, we included all patients ≥ 18 years receiving an ICD-10 code corresponding to HCC (C22.0) from 2004 to 2017 at the Karolinska University Hospital. By chart review, we excluded misclassified patients with other malignancies than HCC and benign lesions, patients with a previous HCC diagnosis or liver transplantation and cases with insufficient information in the medical chart to decide on whether the patient had cirrhosis or not. The final cohort included 1,562 patients with HCC (**Figure 11**) of whom 225 had NAFLD.

4.3.1.2 Definitions

Cirrhosis diagnosis was based on an assessment made at an MDT-conference. In uncertain cases, cirrhosis was defined as either a liver biopsy with features of fibrosis stage IV, radiological evidence of cirrhosis or portal hypertension, gastroscopy with esophageal or gastric varices, or liver stiffness assessed by elastography (Fibroscan®) >14 kPa.

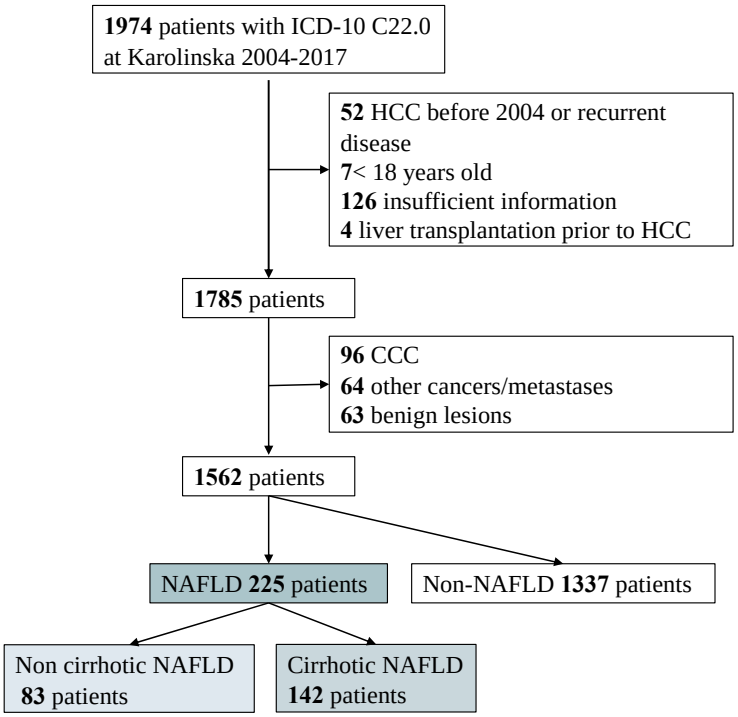


Figure 11. Flowchart study I. Printed with permission

NAFLD was considered present if other etiologies of liver disease could be ruled out *and* the patient had either a biopsy supporting NAFLD, a radiological finding supporting steatosis, body mass index (BMI) ≥25 kg/m^2 *and* type II diabetes (T2DM), *or* BMI ≥30 kg/m^2 in isolation.

The diagnosis of HCC was based on international guidelines[82, 194] and typically determined on an MDT-conference. The patient was defined as being in a surveillance program if ultrasound of the liver, performed without suspicion of HCC, was performed within a 12-month interval.

4.3.1.3 Statistics

We compared differences between HCC-patients with and without NAFLD with the Chi^2-test for categorical variables and Mann-Whitney U test for continuous variables. We divided the NAFLD group for comparison between cirrhotic NAFLD and non-cirrhotic NAFLD patients and further tested for significant differences with the previously mentioned tests. The overall mortality rate during follow-up was estimated using a Cox regression model. Uni- and multivariable hazard ratios (HRs) for mortality were calculated for patients with NAFLD-HCC compared with other causes of HCC and additional HRs were obtained for patients with

non-cirrhotic NAFLD-HCC compared with patients with cirrhotic NAFLD-HCC. Parameters with a p-value < 0.1 were considered in the regression model together with known clinically important variables that were forced into the model constructed using a forward-stepwise approach. The models for mortality rate were adjusted for age, BCLC stage, performance status, total number of tumors, size of the largest tumor, bilirubin, albumin, AFP, hyperlipidemia, T2DM and BMI. To investigate risk factors for mortality the HRs from previous calculations for overall mortality rate was used. The same forward-stepwise procedure was used after considering parameters with a p-value < 0.1 and again forcing clinically important factors into the model. The model for investigating risk factors for mortality in non-cirrhotic NAFLD included age, BCLC stage, number of tumors, albumin and T2DM.

4.3.2 Paper II

4.3.2.1 Study population

In study II, patients ≥18 years old with an ICD-10 code in the NPR corresponding to cirrhosis or cirrhosis-related complications from the time period 2000-2016 were randomly selected from the NPR by a request to the National Board of Health and Welfare. The personal identity numbers for these patients were obtained and medical charts were then requested from each healthcare provider. We requested 150 patient charts for each of the following ICD-10 codes: cirrhosis without a specified etiology (K74.6), alcohol-related cirrhosis (K70.3), esophageal varices with or without bleeding (I85.0 and I85.9), ascites (R18.9) and HCC (C22.0) including notes during a period of two years before and two years after the date of the registered ICD-code of interest. Cases with insufficient data in the medical charts to ascertain the investigated diagnosis were excluded. The flowchart for the study is presented in **Figure 12.**

4.3.2.2 Definitions

Cirrhosis (K70.3 and K74.6) was defined as present if one or several of the following criteria were present: a liver biopsy with features of fibrosis stage IV; radiological evidence of cirrhosis, ascites, or esophageal varices together with a doctors' note documenting cirrhosis. In alcohol-related cirrhosis (K70.3), we did not confirm if cirrhosis was due to alcohol but only if cirrhosis was present or not.

Esophageal varices (bleeding or not bleeding, I85.0/ I85.9) were defined as present if there was a gastroscopy note documenting esophageal varices. In several cases, notes from the endoscopy were missing but if a physician's note affirming esophageal varices treated with band ligation was found, varices were considered present in those cases as well.

Ascites (R18.9) were defined as present based on clinical examination by a physician or by radiology and the cause of ascites was defined as liver-related or not. Ascites was validated to what extent the ICD-code was used for ascites *caused by chronic liver disease* and thus here PPVs represent cirrhotic ascites.

The definition of hepatocellular carcinoma (C22.0) was based on biopsy or radiological reports of typical findings for HCC as described in international guidelines.[82]

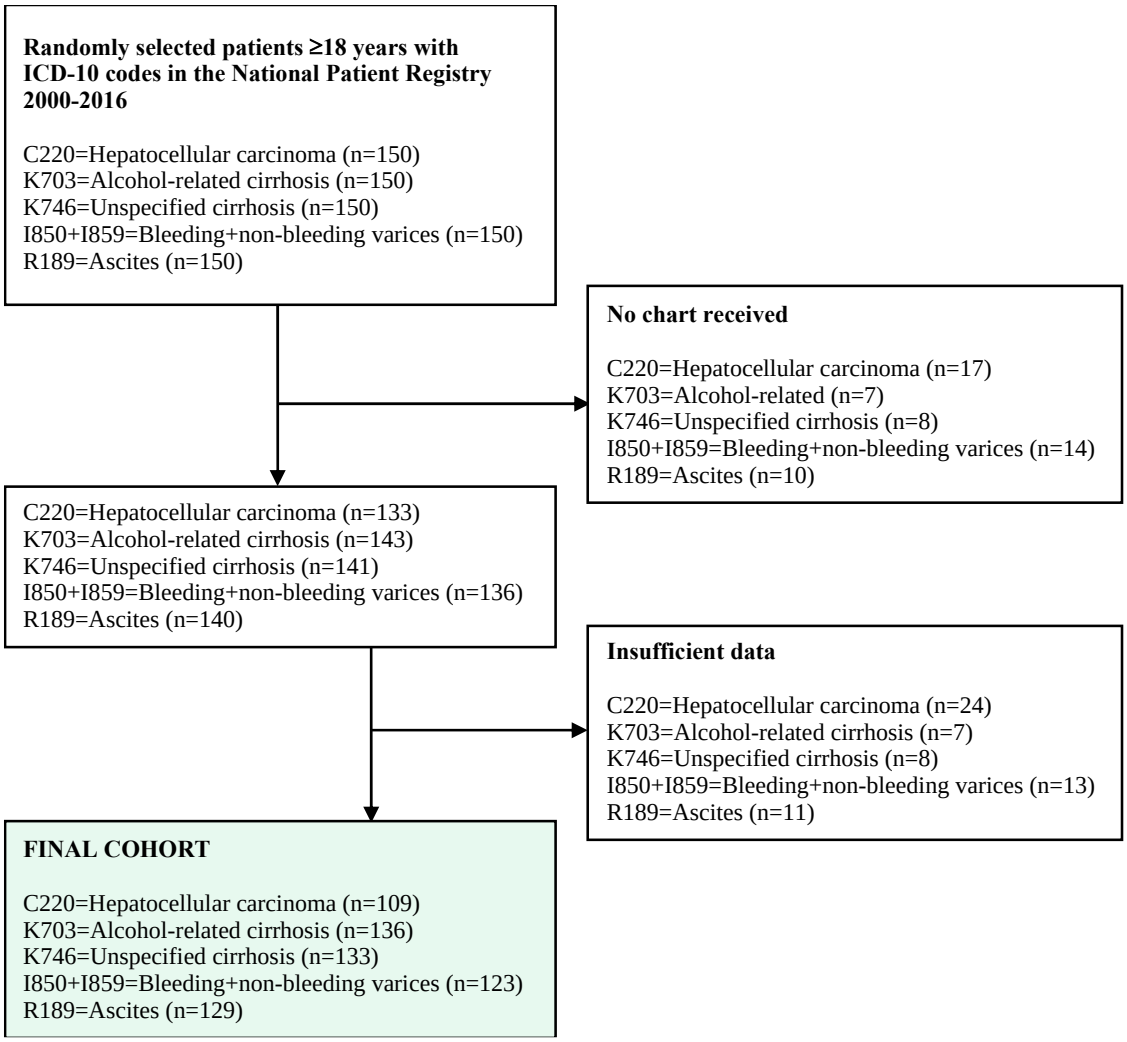


Figure 12. Flowchart study II. Printed with permission

4.3.2.3 Statistics

We used the interpretation of the patient chart as the gold standard and calculated PPVs for the ICD-10 codes. For ICD-codes with a PPV of <90% when used in isolation, we calculated if an additional code for chronic liver disease such as chronic viral hepatitis, increased the PPV. We also considered if codes were registered at university hospitals or other hospitals and whether ICD-codes were registered at a gastroenterology, internal medicine, or transplantation clinic vs. all other clinics.

4.3.3 Paper III

4.3.3.1 Study population

In paper III, all patients with an ICD-code potentially associated with liver cirrhosis registered in the outpatient part of the NPR from 2001-2016 were included. We excluded patients with HCC and liver transplantation before start of follow-up and patients with no reliable coding for cirrhosis such as an ICD-code for ascites but no coding for a specific liver

disease or cirrhosis. The inclusion and exclusion process is described more in detail in the flowchart in **Figure 13.** For each patient with cirrhosis, up to ten reference individuals matched for sex, age, county of residence, and year of cirrhosis diagnosis were randomly selected from the Total Population Register.

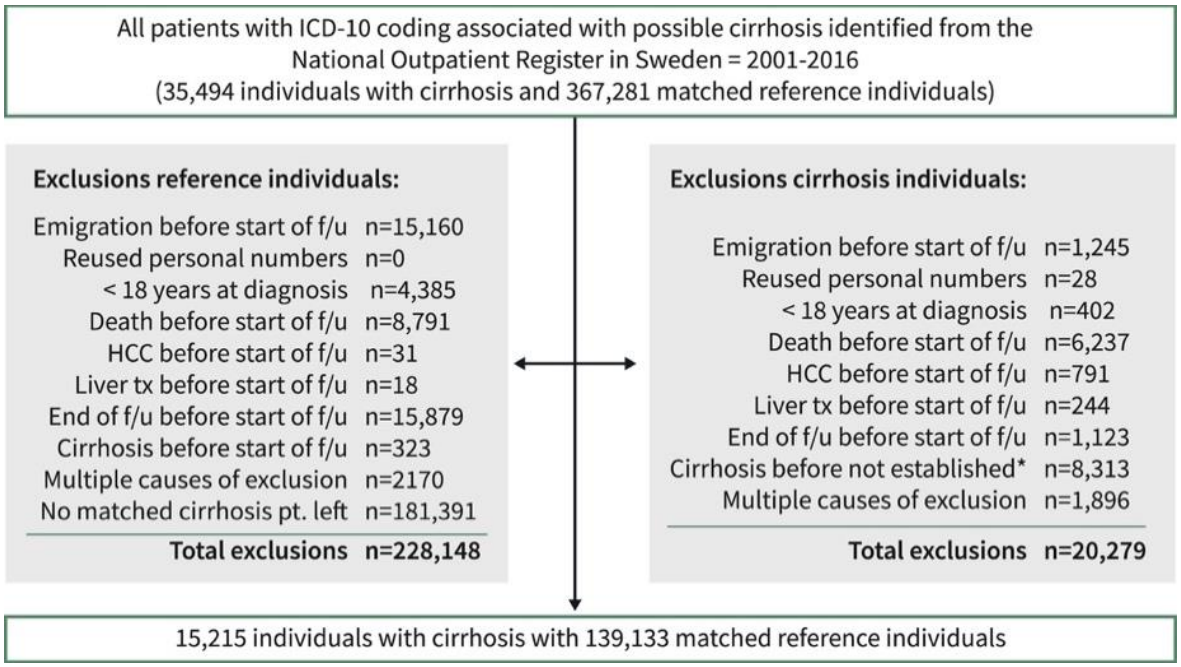


Figure 13. Flow chart study III. * Reused personal number refers to when the same personal number exists for several individuals over time. One example is when one individual who immigrates receive the same personal number as an individual that has emigrated. Abbreviations: f/u follow up, tx transplantation, pt patient, HCC hepatocellular carcinoma. Printed with permission

4.3.3.2 Definitions

ICD-coding was used to define the different chronic liver diseases. For individuals with several codes for etiology, a hierarchy was used in a descending order:
1) Viral hepatitis defined as coding for viral hepatitis with cirrhosis;
2) ARLD defined as coding for alcohol-related cirrhosis (K70.3) or for cirrhosis combined with coding for alcohol use disorders;
3) Metabolic liver disease (other than NAFLD) defined as coding associated with cirrhosis and a specific code for ∝-1-antitrypsin deficiency, hemochromatosis or Wilsons' disease;
4) Autoimmune liver disease: coding associated with cirrhosis and a code for AIH, PBC or PSC; and
5) NAFLD or other liver diseases defined as coding for cirrhosis (K74.6) and not meeting the criteria for the other definitions (1-4);

HCC was defined as the first recorded ICD-code for HCC registered in the NPR, the Swedish Cancer Register or the Causes of Death Register.

4.3.3.3 Statistics

Incidence rates of HCC were calculated as number of new cases per 1,000 person-years of follow-up. A competing risk regression was performed to calculate the cumulative incidence function and cumulative incidence of HCC in cirrhosis at five and ten years. Liver transplantation and death were considered competing events. For rate of time-to-event comparison with reference individuals, in this case rate of time to an HCC-diagnosis, a Cox proportional-hazard model was performed. All models were adjusted for sex, age, county, and year of diagnosis. An additional model with diabetes as a time-varying covariate was also performed.

4.3.4 Paper IV

4.3.4.1 Study population

We asked patients with cirrhosis seen at the hepatology clinic at the Karolinska University hospital between 2016-2019 to participate. Exclusion criteria were residency outside the Stockholm region, current treatment for infection and active or previous cancer with ongoing treatment or follow-up. Liver transplanted individuals were also excluded together with patients with HCV and HIV coinfection. A flowchart is presented in **Figure 14.** At inclusion, a blood sample was drawn from the patient. We also obtained blood samples from 35 healthy blood-donors matched on sex and age to serve as normal MAIT cell comparators.

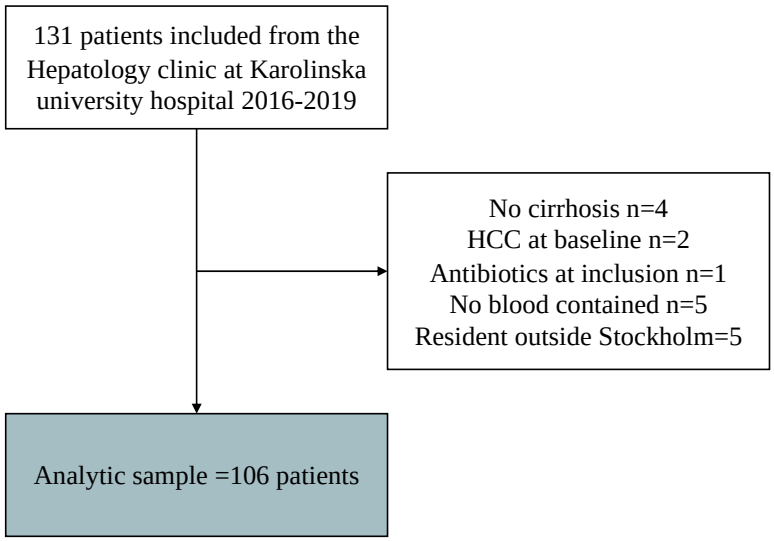


Figure 14. Flow chart study IV. Printed with permission

4.3.4.2 Definitions

MAIT cells were defined as $CD3^{+}CD4^{-}$ TcRV∝7.2 and CD161 which represents a majority of circulating MAIT cells.[195] MAIT cell levels in this study refer to the fraction (%) of MAIT cells out of $CD3^{+}CD4^{-}$ cells (cytotoxic T-cells). Cirrhosis diagnosis and etiology of liver disease were established according to standard diagnostic guidelines by a hepatologist. Decompensation events were defined as ascites, overt hepatic encephalopathy, and bleeding esophageal varices in patients free of decompensation at baseline. Bacterial infection was

defined as a bacterial infection that was diagnosed in a hospital (outpatient visit) or during hospitalization, and that required antibiotic treatment. The type of infection was defined as either:

1) SBP (ascitic fluid neutrophilic count >0.25x10^9/l);
2) Urinary tract infection (requiring a positive culture);
3) Respiratory tract infection (chest x-ray consistent with pneumonia in combination with typical symptoms);
4) Bacteremia (defined as a positive blood culture without a source of infection identified);
5) Others (clinically relevant such as endocarditis, wound infection, meningitis, and gastrointestinal tract infection. A positive culture from the site of infection was required).

4.3.4.3 Experimental work

Venous blood samples were collected in heparin tubes and peripheral blood mononuclear cells were isolated using Ficoll gradient centrifugation and thereafter cryopreserved. Flow cytometry staining of frozen samples were performed including an even fraction of control and patient samples using several antibodies for MAIT cell identification. After compensation, MAIT cells were defined by first removing dead cells, B cells (CD19) and monocytes (CD14) to avoid background and unspecific binding of antibodies. Thereafter, total CD3-expressing cells were identified, CD4-positive cells excluded and MAIT cells defined as CD161 and TcRVα7.2 double-expressing cells. A gating scheme to identify MAIT cells out of CD3+CD4- cells and plots for CD161 and Vα7.2 in individuals with and without bacterial infection is presented in **Figure 15.**

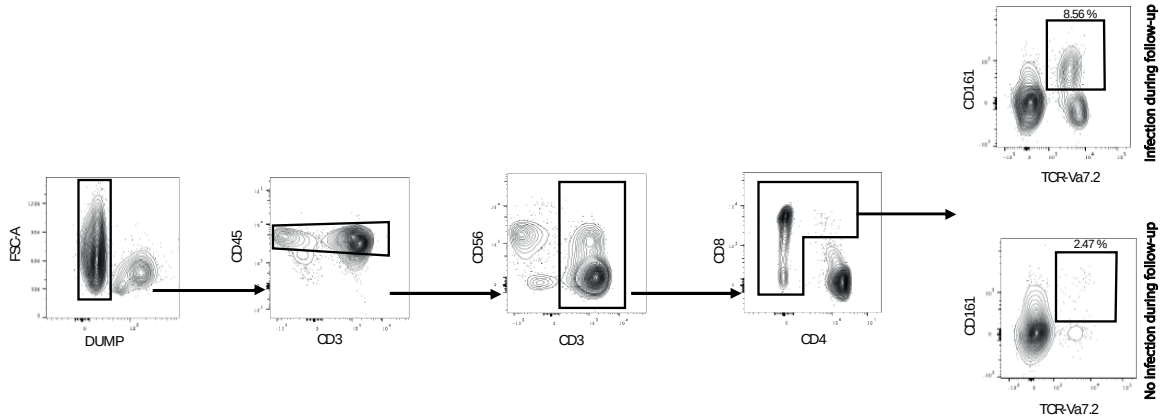


Figure 15. MAIT cells fraction in patients developing infection or not. (A) Gating scheme used to identify MAIT cells. (B) Representative plot showing the MAIT cells fraction in patients developing infection or not.

4.3.4.4 Statistics

Several methods were used to calculate the minimum number of patients to enroll in the study for adequate power. Given a HR of 0.5 of those with high vs low MAIT cells to develop an infection, a power at 80% and an alpha of 0.05, with a sigma at 0.5, the number of patients required to be included was estimated to 66. Based on this, the aim was to recruit 80 patients in the study to cover for potential losses to follow-up.

We compared differences between groups using the Chi^2 test and for comparison between several groups the Kruskal Wallis-test was used. Fractions of MAIT cells were divided into quartiles to assess if there were any threshold effect. The lowest 25% of MAIT cells were used as reference. To investigate the association between fraction of MAIT cells and the cumulative incidence of bacterial infection and hepatic decompensation, we performed a competing risk regression where liver transplantation and death from other causes than bacterial infection and decompensation in the respective model were considered competing events. To assess association between MAIT cell fraction and overall mortality, a Cox survival analysis was performed. All models were adjusted for age, sex and severity of liver disease assessed by MELD-Na.

5 RESULTS

5.1 PAPER I

In our cohort of 1,562 patients with HCC, we identified 225 patients (14.4% of the full cohort) with HCC and NAFLD. As the underlying cause of HCC, NAFLD became more common during the study period. In 2004, NAFLD was the sixth most common cause of HCC whereas in 2016 NAFLD was the second most common cause of HCC representing 15.8% cases, (p_{trend}=0.04). The underlying causes of HCC during the study period are shown in **Figure 16.** Median follow-up was 16.2 months (interquartile range (IQR) 5.9-36.3), 74% of the cohort died during follow-up.

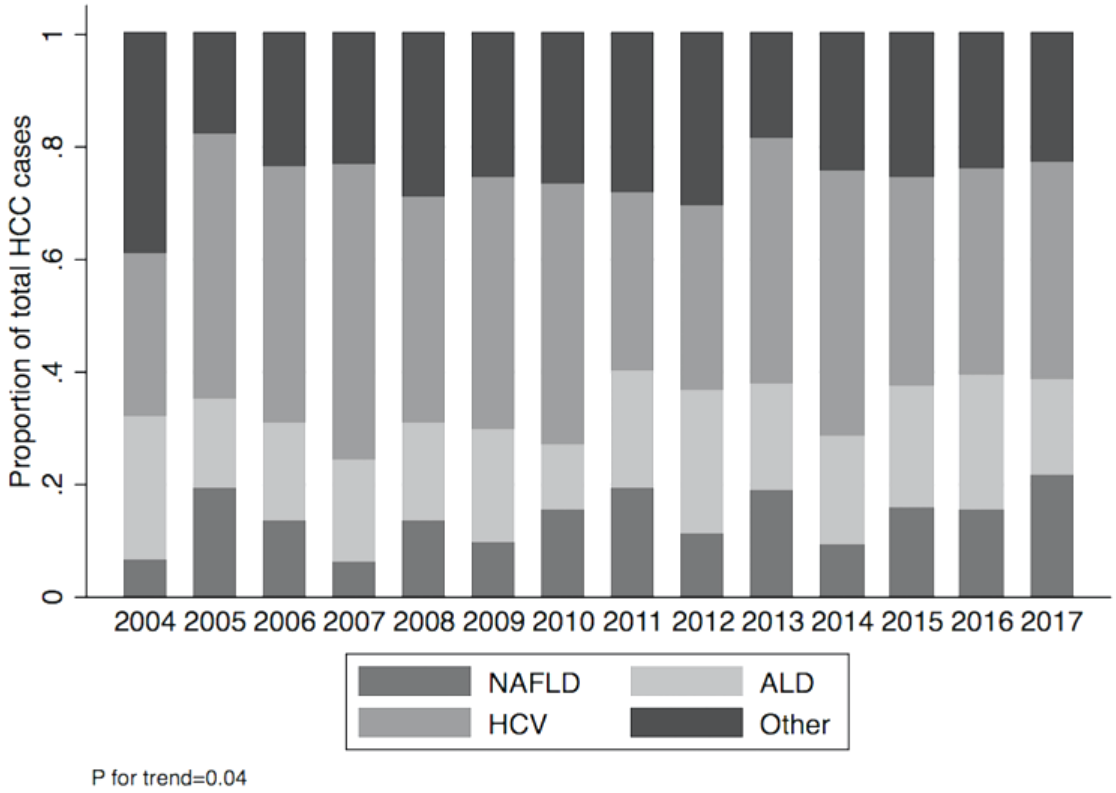


Figure 16. Underlying liver disease in patients with HCC. Printed with permission. Abbreviations: NAFLD non-alcoholic fatty liver disease, HCV hepatitis C, ALD alcoholic liver disease.

5.1.1 NAFLD-HCC compared to HCC with other chronic liver diseases

The cohort was first divided into two groups- patients with NAFLD-HCC and patients with HCC due to other chronic liver diseases. Patients with NAFLD-HCC had cirrhosis to a lower extent (63% vs. 81%, p<0.001) and were less likely to be men (71% vs. 78%, p=0.01) compared to HCC in patients with other liver diseases. Those with NAFLD were older (72 vs. 66 years, p<0.001), and underwent liver resection more often (18% vs. 11%, p=0.001) but liver transplantation was less common (7.1% vs. 13.2%, p=0.01). HCC surveillance was not performed as often in patients with NAFLD with known cirrhosis compared with other patients with known cirrhosis and HCC (27% vs. 41%, p=0.001). Comorbidities such as hypertension, hyperlipidemia, T2DM, and previous cardiovascular disease were more

common in patients with NAFLD-HCC. Despite this, patients with NAFLD-HCC had a similar survival to those with HCC and other liver diseases, as depicted in **Figure 15**.

5.1.2 Cirrhotic NAFLD-HCC compared with NAFLD-HCC without cirrhosis

The NAFLD group was further divided into NAFLD-HCC patients with and without cirrhosis (n=83, 37%). Patients with non-cirrhotic NAFLD-HCC were older than cirrhotic NAFLD-HCC patients (median 74 vs. 70 years, p<0.001), had a larger tumor size and were less likely to have diabetes (66% vs. 80%, p=0.02). Liver resections were more common, but no transplantation was performed in the non-cirrhotic NAFLD-group. We report BCLC class C-D, having ≥4 tumors, decreasing albumin and T2DM independently associated with a higher rate for overall mortality in patients with non-cirrhotic NAFLD-HCC compared to cirrhotic NAFLD-HCC. The total number of patients and outcomes were low, as reflected by the wide confidence intervals (CI) obtained from the model, and therefore these estimates must be interpreted cautiously.

Slightly more patients with non-cirrhotic NAFLD died of their HCC (43 cases, 93%) than patients with cirrhotic NAFLD-HCC (61 cases, 80%, p=0.051). A Kaplan-Meier curve for overall survival in patients with NAFLD-HCC compared to patients with HCC due to other causes is presented in **Figure 17A** and restricted to patients with NAFLD and stratified for the presence of cirrhosis, is displayed in **Figure 17B**.

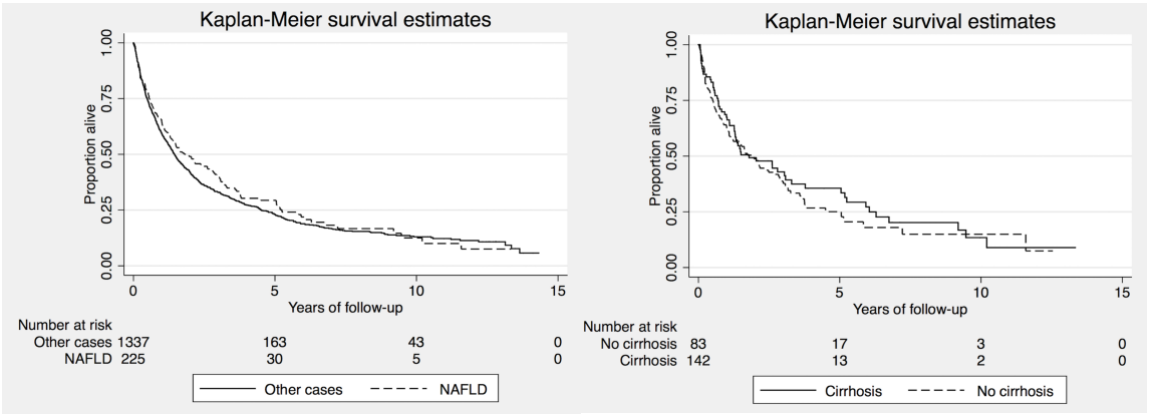


Figure 17. Mortality in **A**) NAFLD-HCC and other liver diseases and **B**) HCC in cirrhotic and non-cirrhotic NAFLD. Abbreviations: NAFLD non-alcoholic fatty liver disease.

5.2 PAPER II

In study II, the final cohort comprised 630 patients with sufficient data out of the requested 750 medical charts (84% of the full cohort). A majority (62%) had been diagnosed as inpatients and most (71%) had the defining ICD-10 code registered at a non-university hospital.

For alcohol-related cirrhosis, cirrhosis with unspecified etiology and esophageal varices we found PPVs above 90%. The PPV was slightly lower for HCC (PPV 84%). An additional code for chronic liver disease within two years was found in 53/109 patients with an ICD-code for HCC and for this group of patients PPV was 91%. An important disadvantage by

using this strategy is that 43 patients with a true HCC but without a code for chronic liver disease were not captured, leading to a decreased sensitivity.

Of the 129 cases of ascites validated, ascites was present in 99% but only 56/129 of the cases had ascites related to liver disease, corresponding to a PPV of 43%. Other causes for ascites were commonly malignancies in gynecologic or non-hepatic gastrointestinal organs (47/129) and more rarely heart failure, nephrotic syndrome, and other cancers. When combining the ICD-10 code for ascites with a code for chronic liver disease, the PPV increased to 93%. PPVs for validated ICD-codes are also presented in **Figure 18.**

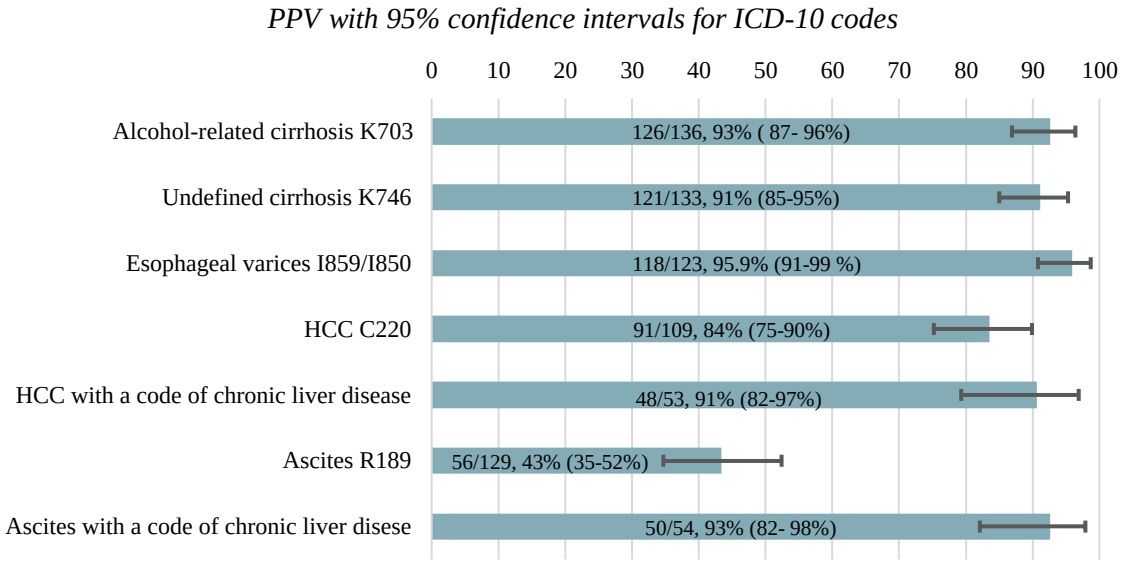


Figure 18. Positive predictive values for single ICD-codes and combinations of ICD-codes. Printed with permission. Abbreviations: PPV Positive predicted value, HCC hepatocellular carcinoma.

5.3 PAPER III

During the study period, 15,215 individuals with cirrhosis were included with a median age of 61 years. The distribution of the etiologies of cirrhosis and characteristics of the study population is further described in **Table 5**. Median follow-up for individuals with cirrhosis was 2.5 years compared to 5.6 in the reference population. During follow-up, 42.7% of individuals with cirrhosis died compared to 10.6% in the reference group.

5.3.1 Incidence of HCC in patients with cirrhosis

The incidence rate for HCC in all individuals with cirrhosis was 23/1,000 person-years, ranging from 15/1,000 person-years in ARLD to 41/1,000 person-years in viral hepatitis. The incidence rate of HCC in the reference population was 0.16/1,000 person-years. Incidence differed depending on sex, in women incidence rate was reported 14/1,000 person-years and in men with cirrhosis 29/1,000 person-years. The incidence rate also differed depending on age.

At five years, the *cumulative* incidence of HCC in cirrhosis in the full cohort was 8.3% and 12.2% at ten years. At ten years, men with viral hepatitis had the highest cumulative incidence (26.6%) and the lowest was seen in women with ARLD (4.3%). Additional cumulative incidence numbers at five and ten years is presented in **Table 6.** The cumulative incidence of HCC for different liver diseases compared to the reference population is also presented in **Figure 19.**

	Individuals with cirrhosis						*Reference individuals*
	All	*Alcohol*	*Viral hepatitis*	*NAFLD/ Other*	*Auto-immune*	*Metabolic*	
Included individuals, n (% of all)	15,215 (100%)	7,485 (49 %)	4,084 (27%)	2,446 (16%)	1,010 (7%)	190 (1%)	139,133 (100%)
Follow-up years, sum	55,781	28,366	13,963	9,077	3,675	700	885,767
Median (IQR) follow-up year/person	2.5 (4.3)	2.6 (4.6)	2.4 (3.8)	2.5 (4.3)	2.4 (4.3)	2.2 (4.9)	5.6 (7.5)
Sex, men n (%)	9,564 (62.9%)	5,165 (69.0%)	2,750 (67.3%)	1,193 (48.8%)	330 (32.7%)	126 (66.3%)	86,989 (62.5%)
Age at diagnosis, years median (IQR)	61 (15)	61 (13)	55 (13)	68 (15)	65 (19)	65 (13)	60 (15)
Country of birth							
Nordic (n/%)	13,383 (88.0%)	6,987 (93.3%)	3,147 (77.1%)	2,138 (87.4%)	935 (92.6%)	176 (92.6%)	124,608 (89.6%)
Other (n/%)	1,832 (12.0%)	498 (6.7%)	937 (22.9%)	308 (12.6%)	75 (7.4%)	14 (7.4%)	14,525 (10.4%)
Comorbidity at /before cirrhosis diagnosis							
Decompensation†, n (%)	7,664 (50.4%)	4,421 (59.1%)	1,561 (38.2%)	931 (38.1%)	667 (66.0%)	84 (44.2%)	22 (0.02%)
Diabetes, n (%)	3,218 (21.2%)	1,488 (19.9%)	683 (16.7%)	844 (34.5%)	160 (15.8%)	43 (22.6%)	7,653 (5.5%)

Table 5. Baseline characteristics study III. Abbreviations: IQR interquartile range, NAFLD nonalcoholic fatty liver disease. Printed with permission.

5.3.2 Rate of HCC compared to reference individuals

In the reference group 0.1% were diagnosed with HCC compared to 8.4% of persons with cirrhosis during follow-up. The rate of HCC in individuals with cirrhosis was as expected higher compared to the reference group as seen in a HR of 162. Younger age and female sex were consistently associated with a lower rate of HCC compared to older individuals and male sex. In a model adjusted for diabetes type I and II as a time-varying covariate, the rate of HCC was attenuated (HR 145, (95%CI=113-185) compared to HR 162 (95%CI=127-207) and diabetes was also found to be an independent risk factor of HCC development (HR 3.1, 95%CI=2.1-4.4).

	Number of exposed	*Cumulative incidence at 5 years (95%CI)*	*Cumulative incidence at 10 years (95%CI)*
All individuals with cirrhosis	15,215 (100%)	8.3 (7.8-8.8)	12.2 (11.6-13.0)
Decompensation before cirrhosis	7,664 (50.4%)	7.5 (6.8-8.2)	10.8 (10.0-11.7)
No decompensation before cirrhosis	7,551 (49.6%)	9.1 (8.4-9.9)	13.9 (12.8-15.0)
Women	5,651 (37.1%)	5.3 (4.6-6.0)	8.2 (7.3-9.2)
Men	9,564 (62.9%)	10.0 (9.4-10.7)	14.7 (13.8-15.7)
Age <50	2,497 (16.4%)	5.1 (4.1-6.2)	9.7 (8.1-11.5)
Age 50-65	7,784 (51.2%)	8.9 (8.2-9.7)	13.3 (12.3-14.3)
Age >65	4,934 (32.4%)	8.9 (8.0-9.8)	11.9 (10.8-13.1)
Viral	4,084 (26.8%)	15.6 (14.3-17.0)	23.1 (21.1-25.0)
Women	1,334 (32.7%)	11.0 (9.0-13.2)	15.9 (13.1-18.8)
Men	2,750 (67.3%)	17.9 (16.2-19.7)	26.6 (24.1-29.2)
Age <50	1,046 (25.6%)	9.6 (7.6-11.9)	17.6 (14.3-21.3)
Age 50-65	2,446 (59.9%)	17.7 (15.9-19.6)	25.2 (22.6-28.0)
Age >65	592 (14.5%)	18.8 (15.0-23.0)	24.6 (19.8-29.6)
Alcohol	7,485 (49.2%)	4.9 (4.3-5.4)	7.9 (7.1-8.7)
Women	2,320 (31.0%)	2.4 (1.8-3.2)	4.3 (3.3-5.4)
Men	5,165 (69.0%)	5.9 (5.3-6.7)	9.6 (8.6-10.6)
Age <50	970 (13.0%)	2.2 (1.3-3.4)	4.6 (3.0-6.8)
Age 50-65	4,147 (55.4%)	4.4 (3.7-5.2)	7.7 (6.7-8.8)
Age >65	2,368 (31.6%)	6.9 (5.8-8.2)	9.9 (8.4-11.5)
NAFLD/Other	2,446 (16.1%)	8.4 (7.2-9.7)	11.3 (9.8-13.0)
Women	1,253 (51.2%)	5.3 (4.0-6.8)	8.1 (6.3-10.2)
Men	1,193 (48.8%)	11.7 (9.7-13.9)	14.7 (12.3-17.4)
Age <50	245 (10.0%)	1.8 (0.5-4.8)	--
Age 50-65	787 (32.2%)	9.3 (7.1-11.8)	13.7 (10.6-17.1)
Age >65	1,414 (57.8%)	9.2 (7.6-11.0)	--
Autoimmune	1,010 (6.6%)	6.4 (4.7-8.4)	10.3 (7.8-13.2)
Women	680 (67.3%)	5.4 (3.7-7.7)	9.6 (6.8-13.0)
Men	330 (32.7%)	9.4 (5.8-14.0)	---
Age <50	214 (21.2%)	5.3 (1.9-11.5)	---
Age 50-65	326 (32.3%)	7.6 (4.6-11.6)	---
Age >65	470 (46.5%)	6.9 (4.6-9.8)	9.4 (6.2-13.3)
Metabolic	190 (1.2%)	12.2 (7.6-18.0)	---
Women	64 (33.7%)	11.0 (3.6-23.0)	---
Men	126 (66.3%)	14.3 (8.3-21.8)	---
Age <50	22 (11.6%)	---	---
Age 50-65	78 (41.1%)	15.2 (7.3-25.7)	---
Age >65	90 (47.4%)	12.8 (6.5-21.3)	---

Table 6. Cumulative incidence of HCC at five and ten years in different subgroups. Abbreviations: NAFLD non-alcoholic fatty liver disease, CI confidence intervals. Printed with permission.

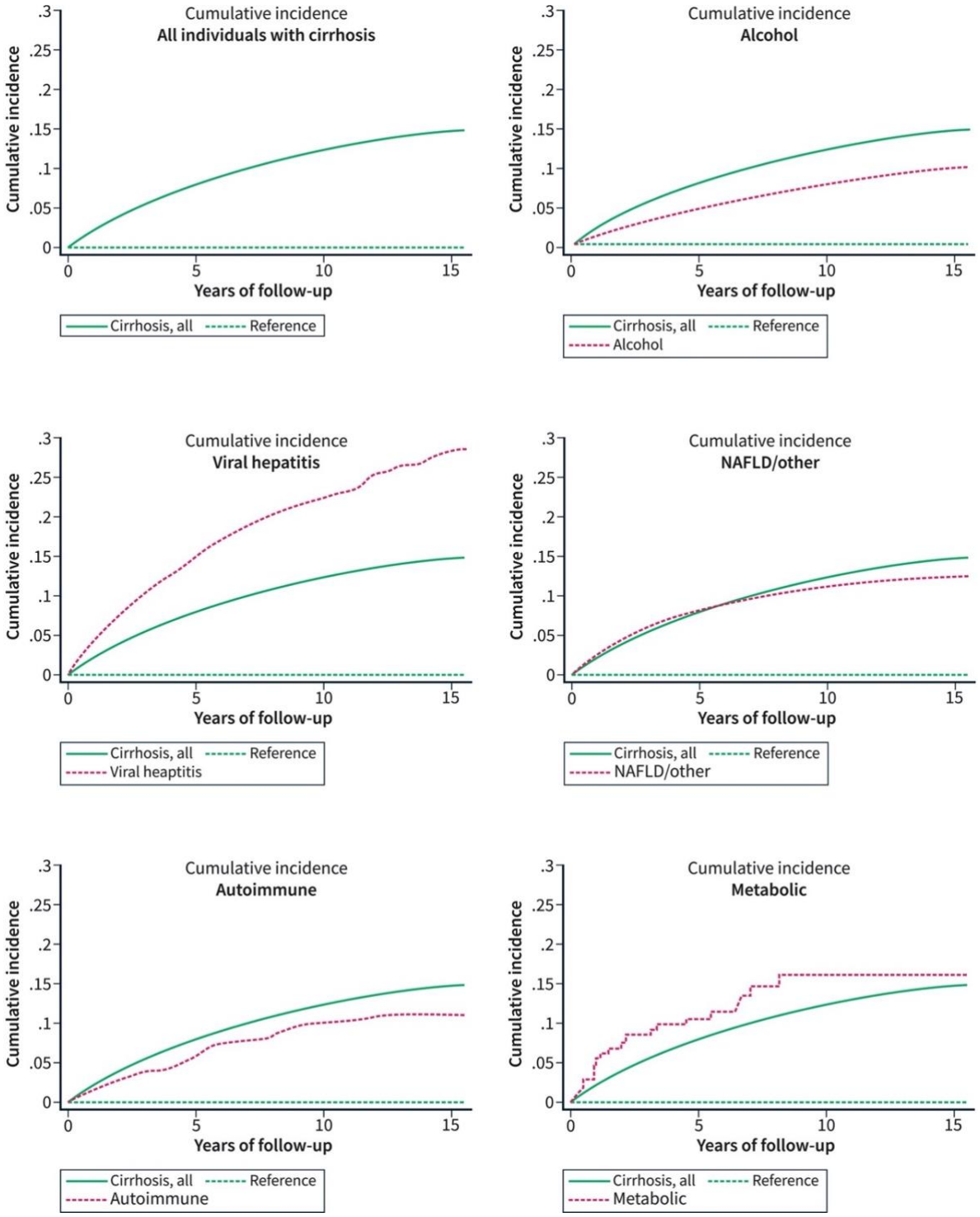


Figure 19. Cumulative incidence of HCC in cirrhosis stratified on liver diseases. Abbreviations: NAFLD non-alcoholic fatty liver disease. Printed with permission.

5.4 PAPER IV

In study IV, 106 patients with cirrhosis were included in the analysis, median age was 63 years and 64% were men. Liver disease etiology is presented in **Figure 20.** In healthy

individuals, the median MAIT cell fraction was 6.1% out of $CD3^{+}CD4^{-}$ cells compared to a median MAIT cell fraction of 0.8% ($p<0.01$) in all individuals with cirrhosis.

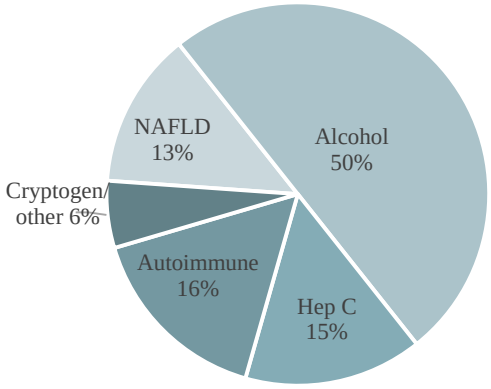


Figure 20. Etiologies of cirrhosis.

Stratified on disease severity assessed by Child-Pugh class, no significant difference in the fraction of MAIT cells was seen (Child-Pugh class A=0.9%, B=0.6% and C=0.9%, p=0.72). The highest median MAIT cell fraction was seen in patients with HCV, but there was no significant difference between disease etiologies (p=0.12) (**Figure 21)**. The risk for infection increased for every MAIT cell quartile, where the first quartile (lowest MAIT cell level) was used as the reference group, aSHR for quartile 2 vs quartile 1 1.24 (95%CI=0.43-3.55), for quartile 3 1.41(95%CI=0.50-3.94) and for quartile 4 1.81 (95%CI=0.65-5.07) indicating a dose-response relationship although this was not statistically significant (p_{trend}=0.69).

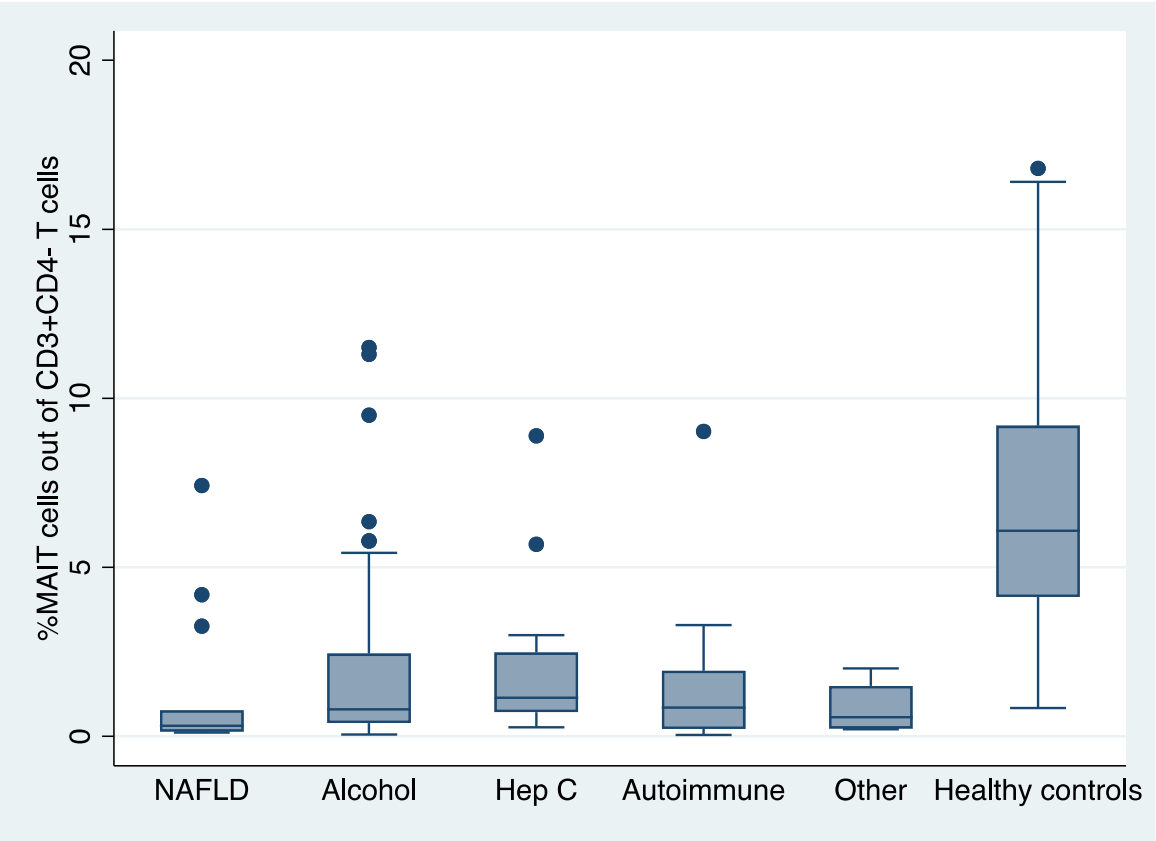


Figure 21. Boxplot of median MAIT cell proportions in different liver diseases and controls. No significant difference in median MAIT cell proportions between different liver diseases were seen (p=0.12). Abbreviations: NAFLD non-alcoholic fatty liver disease, Hep C hepatitis C, MAIT mucosal associated invariant T-cells

5.4.1 Risk of infection

One third of the patients (32/106) developed an infection during follow-up. Surprisingly, *higher* fractions of MAIT cells were associated with an increased risk of bacterial infection

(aSHR=1.15 (95%CI=1.01-1.31)). Estimates from the crude and adjusted competing risk model stratified on quartiles of MAIT cells are presented in **Table 7** and the cumulative incidence of infections stratified on MAIT cell quartiles is presented in **Figure 22**. The distribution of MAIT cell fractions for controls and cirrhosis patients with and without infection is presented in **Figure 23.**

Competing risk regression-bacterial infection	*Number exposed*	*Number of events*	*SHR*	*95%CI*	*aSHR*	*95%CI*
% MAIT cells/ T-cells	106	32	1.10	0.97-1.25	1.15	1.01-1.31
Age	106	32	1.00	0.97-1.03	1.01	0.97-1.04
MELD-Na	104	30	1.05	0.98-1.12	1.05	0.99-1.12
Quartile 1 % MAIT cells/T-cells	27	7	reference		reference	
Quartile 2 % MAIT cells/T-cells	26	8	1.05	0.38-2.88	1.16	0.35-3.80
Quartile 3 % MAIT cells/T-cells	27	8	1.17	0.43-3.16	1.46	0.52-4.13
Quartile 4 % MAIT cells/T-cells	26	9	1.51	0.55-4.06	2.25	0.71-7.13

Table 7. Crude and adjusted competing risk ratios stratified on quartiles of MAIT cells. Abbreviations: SHR subdistribution hazard ratio, aSHR adjusted subdistribution hazard, MELD model of end-stage liver disease.

5.4.2 Risk of decompensating event and death

During follow-up, 11 of 71 (15%) patients free of decompensation at baseline developed a decompensating event. We found no association between higher MAIT cell fraction and a higher risk of hepatic decompensation or with risk of death.

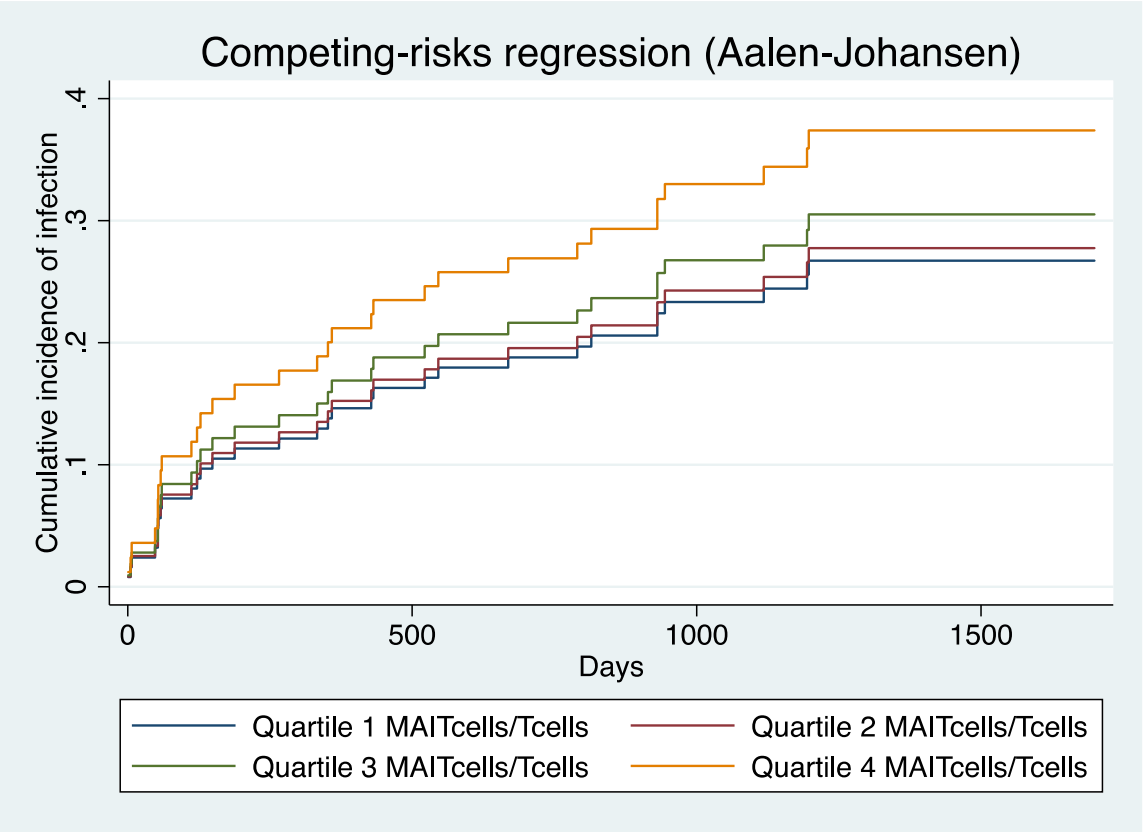


Figure 22. Cumulative incidence of infection by quartiles of MAIT cells. Abbreviations: MAIT mucosal associated invariant T-cells.

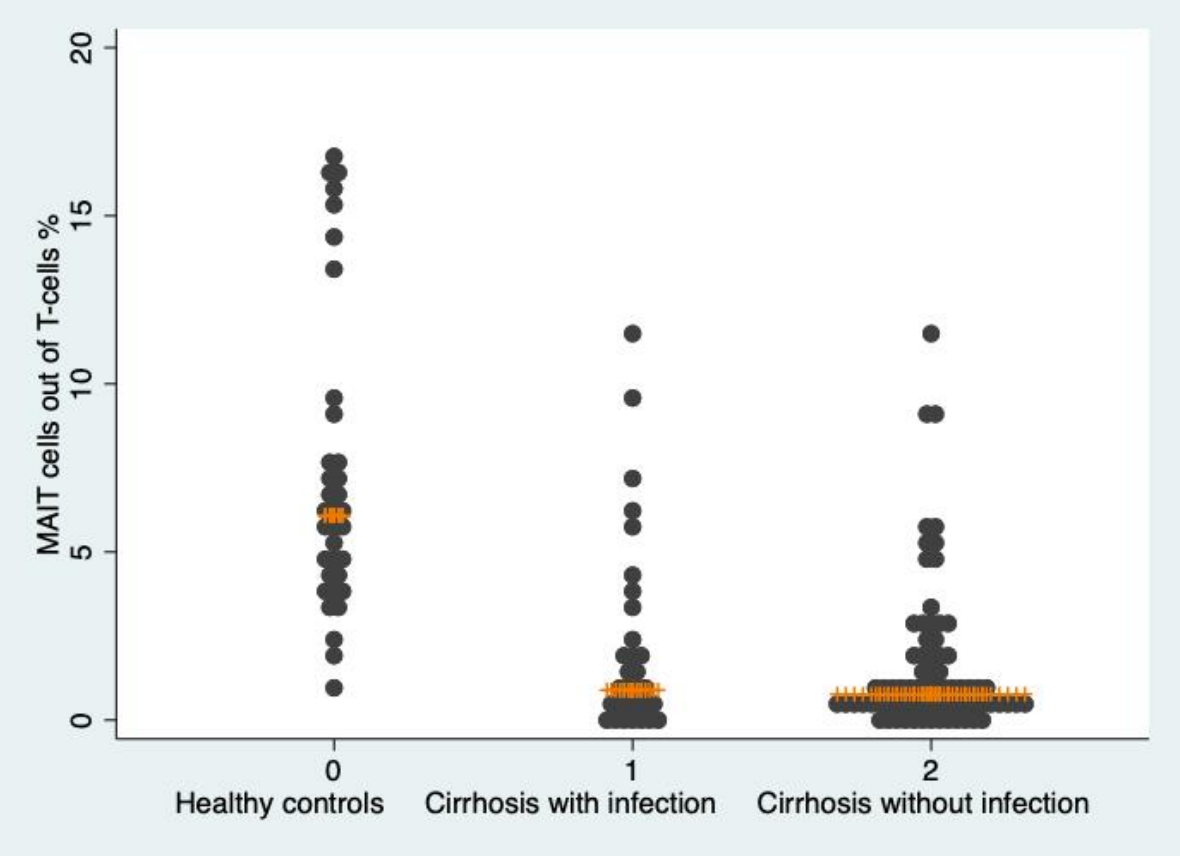


Figure 23. Dot plot of MAIT cells for controls and cirrhosis patients with and without infection. The orange line indicates the median.

6 DISCUSSION

6.1 PAPER I

In this study we report that NAFLD is an increasing cause of HCC in Sweden, a finding consistent with previous studies from other countries. NAFLD was a quite new disease entity in the early inclusion period, and that has implications when interpreting the increasing incidence. A few patients were classified as cryptogenic in the patient chart but according to our definition of etiologies, they were reclassified as NAFLD. We may still not have captured all NAFLD cases in the beginning of the inclusion period.

We confirm previous findings that HCC in the absence of cirrhosis is more common in NAFLD and that patients with NAFLD-HCC are older at diagnosis compared to patients with HCC and other chronic liver diseases,[138, 196] a finding that was accentuated in non-cirrhotic NAFLD-HCC patients. In our cohort, 75% of the patients with non-cirrhotic NAFLD-HCC were ≥70 years. This is an important finding as it highlights that the risk in younger individuals is very low. We suggest that any future surveillance strategies should take age into account as surveillance is probably unwarranted in younger individuals.

There was a clear male dominance in our HCC cohort. This is consistent with previous research. One reason for higher rates of HCC in men might be explained by sex-specific differences in exposure to risk factors such as alcohol and HCV. However, we also report a clear male dominance in NAFLD-HCC, where risk factors such as T2DM and obesity affect men and women equally.[197, 198] In this study, we did not investigate this further as it was not the scope of the research project. There is still much to learn about what pathophysiological mechanisms in HCC that might explain the difference in risk for men and women.

Some studies report that patients with NAFLD-HCC are less likely to be candidates for curative treatment.[199] We found that patients with NAFLD-HCC were less likely to be transplanted but more often underwent liver resection. Our interpretation is that the lower prevalence of cirrhosis in the NAFLD-HCC group contribute to more patients being eligible for resection.

Conflicting results regarding whether survival in NAFLD-HCC and non-NAFLD-HCC is similar or not have been reported previously.[196, 200, 201] In our study, survival was similar, and was similar also for patients with cirrhotic NAFLD-HCC and non-cirrhotic NAFLD-HCC. One would think that if patients with NAFLD are older, have more comorbidities, are less often candidates for transplantation and have larger tumor at diagnosis, survival would be shorter. These adverse factors, or the poor prognosis of HCC in general, might mask protective survival factors in tumor biology or other unknown factors for patients with NAFLD-HCC.

The strengths of this study include the size of the cohort and the fact that our institution covers the entire HCC care for the Stockholm County area, decreasing the risk of selection bias. Another strength is that all patient charts were manually reviewed, ensuring low risk for

misclassification bias. The retrospective design is a limitation of this study with possible inconsistencies in data recording by clinicians in the patient charts. There is also risk of referral bias given that patients with advanced cancer at presentation might not be referred from secondary care hospitals to the same extent as younger patients with localized disease. Additionally, the NAFLD diagnosis was not based solely on histology but also clinical parameters increasing the risk of misclassification bias, although biopsy is not part of the routine diagnosis of NAFLD.[202]

6.2 PAPER II

We report a high validity of ICD-10 codes associated with cirrhosis in the Swedish NPR, supporting their use for register-based research. However, the ICD-10 code for ascites alone had an unsatisfactory low PPV, but when combined with a code for chronic liver disease, the PPV increased to acceptable levels. The PPV for HCC was 84% and when an additional ICD-code for chronic liver disease was required, PPV increased to 91% but requiring two codes had the consequence that nearly half (47%) of the accurately coded HCC cases with only one code, were excluded. One part of the explanation why patients with accurately coded HCC lack an ICD-10 code for chronic liver disease could be that 10–20% of HCC cases occur in patients without cirrhosis. An additional explanation could be that cirrhosis and HCC are sometimes discovered at the same time and the ICD-10 code for an underlying chronic condition as cirrhosis, despite being un-diagnosed before, might not be registered in these patients with a newly diagnosed malignancy. We also found that most of the HCC cases registered as having "insufficient data" were diagnosed in a late palliative stage when ascertaining the liver cancer diagnosis was not prioritized. These patients did not meet our pre-set definition of HCC. When these cases were included in the analysis, the PPV for HCC increased to 87%.

Algorithms that include several ICD-codes to identify cirrhosis and decompensation events have been presented in previous studies.[185, 187, 203] A problem with relying on some algorithms to identify cases, such as requiring the code to be present at several timepoints, is that you will not capture patients only seen once in hospital or who die after receiving only one ICD-code. Therefore, an important finding in our study is that when using the Swedish NPR, it is sufficient to use a single ICD code for cirrhosis, varices, and HCC.

The main strength of this study is the use of a nationwide register to identify patients, that were randomly included from different healthcare facilities in Sweden. A limitation is the different amount and quality of data sent from different healthcare institutions, which might have had an impact on how often a diagnosis of chronic liver disease could be found or not. Other test characteristics than the PPV could not be estimated in this design.

6.3 PAPER III

In study III we report the incidence rate of HCC in Swedish patients with cirrhosis to be 23/1,000 person-years and a corresponding lower-than-expected cumulative HCC incidence of 12% at ten years, due to the high competing risk of dying from other causes than HCC. We

report higher estimates than two recently published Swedish studies with individuals with biopsy-proven ARLD and NAFLD-cirrhosis.[143, 204] We hypothesize that the higher estimates found here can be explained by the selection bias introduced when including only biopsy-proven patients. Patients with cirrhosis and comorbidities might not be eligible for biopsy, and in patients with symptoms from cirrhosis a biopsy may not add any useful information. We also found higher risks for men, individuals with viral hepatitis, older individuals and in those with diabetes.

An important strength of paper III is the nationwide inclusion of all individuals in Sweden with cirrhosis and the large sample size, enabling meaningful subgroup analyses (e.g., age, etiology, and sex) of important risk factors for HCC. In previous research the focus has often been the risk of HCC within a specific disease etiology making comparison of HCC risk between different subgroups difficult as the setting might differ between different studies. Similar findings have been reported before.[121, 127] However, we believe that this study's most important implication is that it can be used to put the risk of HCC into context between etiologies and subgroups of cirrhosis. Another strength is that the registers are of high quality and, as we know from study II, the PPV for ICD-coding for cirrhosis and HCC in this setting is over 90%.

There are several limitations. First, relying on ICD-codes, especially those not validated, comes with a remaining risk of selection bias and misclassification bias introduced by incorrect coding. To create an algorithm that can identify all individuals with cirrhosis and then place them in the right group of liver diseases was a complicated work. No matter how you twist and turn the algorithm, the main limitation is the compromises made to identify and distinguish individuals with different diagnoses of cirrhosis. Cirrhosis cases with a certain etiology are identified by our algorithm but also cases where a definite etiology could not be ascertained. For example, an individual with undefined cirrhosis but with no code for etiology was defined as having NAFLD/other causes, which has implications for how to interpret the estimates for HCC in this group. We did not include patients with an uncertain cirrhosis diagnosis. For example- an individual with a code for ascites but no code for cirrhosis or a chronic liver disease, was not included in our cohort.

Patients are often diagnosed with HCC and cirrhosis at the same time. These individuals were excluded from the study as we could not establish the date of cirrhosis diagnosis. The risk estimates should be interpreted cautiously for individuals with autoimmune and metabolic liver disease and for the reference population due to the relatively low numbers of outcomes in these groups.

6.4 PAPER IV

We found that MAIT cell fractions closer to those found in healthy controls were associated with a higher risk to develop bacterial infections. The difference in MAIT cells between those with cirrhosis with and without an infection was discrete as pointed out in **Figure 23**. We did not find a significant association between fractions of MAIT cells and the risk of future

decompensation or death, nor with disease severity. This is in line with most previous research conducted. Hegde et al. report no association between MAIT cell levels and liver disease severity in a study of 74 patients with biopsy-proven cirrhosis.[205] Niehaus et al. found similar levels of peripheral MAIT cells in patients with compensated and decompensated cirrhosis[160] and von Seth et al. found no correlation to disease severity when investigating MAIT cells in blood in patients with PSC.[165] The pathophysiology explanation for this is not clarified.

A strength of this study is the prospectively included patients reducing the risk of selection bias compared to previous retrospective studies. Bacterial infections were ascertained by thorough chart review, increasing internal validity. The definition of bacterial infection was constructed to catch as many clinically relevant infections as possible. There are of course other ways to define infection such as ICD-codes and to include viral infections as well. The method to determine the fraction of MAIT cells is considered of high standard allowing for robust results.

One limitation with study IV is that the electronic patient chart used cover seven out of eight hospitals in the Stockholm region, but not hospitals outside the Stockholm region. All patients resided in the Stockholm region, but we cannot rule out missing capture of events that occurred outside this setting. Another limitation is that patients treated more often in hospital such as patients with comorbidities or more severe liver disease might be diagnosed with more infections in hospital, as they are more carefully followed than less sick patients (surveillance bias). The patients were followed for in median 2.5 years but despite the long follow-up period, there were few outcomes. Our estimates should be interpreted in the light of that.

7 CONCLUSIONS

Paper I

NAFLD is a growing cause of HCC in Sweden and is now the second most common underlying liver disease in patients with HCC in our cohort. HCC in absence of cirrhosis is common in NAFLD, one third of patients with NAFLD-HCC had no cirrhosis. HCC in non-cirrhotic patients is associated with higher age, 75% are ≥70 years old. Survival was similar for NAFLD and other chronic liver diseases as well as for patients with cirrhotic- and non-cirrhotic NAFLD HCC.

Paper II

The validity of administrative ICD-10 coding for cirrhosis and esophageal varices is high in the Swedish NPR. The PPV for HCC was 84% and over 90% when an additional ICD-code for chronic liver disease was required. The PPV for ascites due to liver disease was low and adding a code for chronic liver disease is recommended to achieve a PPV over 90%.

Paper III

The cumulative incidence of HCC in Swedish outpatients with cirrhosis is approximately 12% ten years after diagnosis, but varies greatly according to cirrhosis etiology, sex, and age. The annual rate of HCC development in cirrhosis was 2.3% but this also figure varied and the threshold for when HCC surveillance is considered cost-effective was not reached in several subgroups. Our findings highlight the large variation in risk of HCC in cirrhosis.

Paper IV

In cirrhosis, relatively preserved MAIT cell levels in peripheral blood was associated with a higher risk of bacterial infection. No significant association between fractions of MAIT cells and decompensation or death was found.

8 POINTS OF PERSPECTIVE

Paper I

The objective of this study was to learn more about the natural history of NAFLD-HCC. The growing burden of NAFLD is becoming a major challenge. It is unclear how to best perform surveillance in patients with NAFLD. More than 75% of patients with non-cirrhotic NAFLD HCC were ≥70 years. In the light of that and of previous findings, we suggest that age should be considered when discussing future surveillance attempts in patients with non-cirrhotic NAFLD. There is also a need for studies exploring if surveillance of older patients with non-cirrhotic NAFLD would be cost-effective.

Paper II

We believe that our findings in study II are important for how to design register-based research in hepatology, at least in a Swedish setting. The most important finding is that when using the Swedish NPR, it is sufficient to use a single ICD-code for cirrhosis, varices, and HCC but not for ascites. We suggest adding an ICD-10 code for chronic liver disease when using ICD-code for ascites to examine liver-related outcomes.

Paper III

The annual incidence of HCC was 2.3% in our cohort. For HCC surveillance to be cost-effective an annual HCC-incidence of at least 1.5% is required. For several subgroups this requirement was not met. Surveillance might be most effective in the groups where we found a particularly high risk of HCC. It is important to not refer patients for unnecessary examinations that might have a negative psychological impact on the individuals and a negative effect on healthcare costs. Our results support an individualized HCC-risk evaluation where risk factors such as age, sex, etiology, and diabetes should be considered when deciding whether to initiate HCC surveillance or not.

Paper IV

Our findings are not demonstrative but indicate that blood levels of MAIT cells may help to assess the risk of future infection in patients with cirrhosis. Our findings need to be validated in larger studies. Future research should also focus on the understanding of the mechanism by which MAIT cells might influence their environment to be able to explain the association between relatively preserved fractions of MAIT cells and risk of infections.

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