# Gallstones in liver transplant recipients

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# Abbreviations

ALP: Alkaline phosphatase
ALT: Alanine transaminase
AST: Aspartate transaminase
BMI: Body mass index
BS: Biliary sludge
CC: Cryptogenic cirrhosis
CRP: C-reactive protein
CTP: Child-Turcotte-Pugh
DMII: Diabetes Mellitus type 2
ESLD: End-stage liver disease
FPG: Fasting plasma glucose
GD: Gallstone disease
GGT: Gamma-glutamyl transpeptidase
GHIH: Growth hormone-inhibiting hormone
GS: Gallstones
HBV: Hepatitis B virus
HCC: Hepatocellular carcinoma
HCV: Hepatitis C virus
Hgb: Haemoglobin
INR: International normalized ratio
IR: Insulin resistance
IRI: insulin resistance index
LC: Liver cirrhosis
LDL: Low-density lipoprotein
LT: Liver transplant
HDL: High-density lipoprotein
MELD: Model for end-stage liver disease
MSCT: Multi-slice computed tomography
NAFLD: Non-alcoholic fatty liver disease
NASH: Non-alcoholic steatohepatitis
RNA: Ribonucleic acid
TG: Triglycerides
Total-C: Total cholesterol
Trc: Thrombocytes

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# 1. Summary

**Background:** Gallstone disease (GD) is the most frequent pathology of the biliary system, and patients with end-stage liver disease (ESLD) are especially prone to develop it. It is difficult to treat such patients due to their poor general condition and decreased liver function. The aim of this study was to evaluate the prevalence of GD in liver transplant candidates and to investigate its associations with selected variables in the context of ESLD.

**Methods:** This cross-sectional study included 101 non-diabetic adult cirrhotic patients transplanted between 2014 and 2017 at University Hospital Merkur, Zagreb. GD was confirmed postoperatively on the explanted specimens. Clinical and laboratory parameters were collected from the medical records. The insulin resistance index (IRI) values >1.7 were considered as insulin resistance (IR).

**Results:** The prevalence of GD was 26.73%. The cohort consisted of 79 male and 22 female patients with a mean age of 57.39±8.79 years. Patients were divided into GS (gallstone)-present (n=27) and GS-absent groups (n=74). No statistically significant differences regarding age, gender, body mass index, aetiology or severity of liver disease (CTP or MELD score) between the two groups (p=ns) were noted. GS-present group had significantly higher alkaline phosphatase (ALP) 145 (range 44–306) IU/L and haemoglobin (Hgb) 113±25 g/L levels compared to the GS-absent group (ALP 109 (42–531) IU/L, p=0.031; Hgb 104±21 g/L, p=0.048). GD-present group had a significantly higher proportion of IR patients (IRI>1.7 31% vs IRI<1.7 5.9%) compared to the GS-absent group (IRI>1.7 69% vs IRI<1.7 94.1%) and higher fasting plasma glucose levels (5.44±0.74 mmol/L vs 5.22±0.62 mmol/L, p=0.029).

**Conclusion:** Liver transplant (LT) candidates have a high prevalence of GD (26.73%). IR is a hallmark of metabolic disturbances in cirrhosis, and may play an important role in the development of GD, which warrants further research.

Keywords: Gallstones, liver transplant, liver cirrhosis, lithogenesis

## 2. Sažetak

**Uvod:** Žučni kamenci (ŽK) su najčešća patologija bilijarnog sustava, a bolesnici s terminalnom bolesti jetre osobito su skloni njihovom nastanku. Liječenje ŽK u bolesnika s cirozom je otežano zbog njihovog općeg lošeg stanja i narušene funkcije jetre. Cilj ove studije bio je utvrditi prevalenciju ŽK u kandidata za transplantaciju jetre i istražiti njihovu povezanost s određenim kliničkim i biokemijskim varijablama u kontekstu terminalne bolesti jetre.

**Metode:** Ova presječna studija uključivala je 101 odraslog ne-dijabetičara transplantiranog zbog ciroze jetre u periodu od 2014-2017 u Kliničkoj bolnici Merkur, Zagreb. ŽK potvrđeni su na postoperativnim eksplantacijskim uzorcima. Klinički i laboratorijski parametri prikupljeni su iz medicinske dokumentacije. Inzulinska rezistencija (IR) definirana je vrijednostima indeksa inzulinske rezistencije (IRI) >1.7.

**Rezultati:** Prevalencija ŽK iznosila je 26.73%. Kohortu je sačinjavalo 79 muškaraca i 22 žene, prosječne starosti 57.39±8.79 godina. Bolesnici su podijeljeni u dvije skupine ŽK-prisutnu (n=27) i ŽK-odsutnu (n=74). Nisu utvrđene statistički značajne razlike između skupina vezano za dob, spol, indeks tjelesne težine, etiologiju ili težinu (CTP ili MELD) jetrene bolesti (p=ns). ŽK-prisutni imali su značajno više razine alkalne fosfataze (ALP) 145 (44–306) IU/L i hemoglobina (Hgb) 113±25 g/L u usporedbi sa skupinom bez ŽK (ALP 109 (42–531) IU/L, p=0.031; Hgb 104±21 g/L, p=0.048). Skupina sa ŽK imala je značajno višu proporciju bolesnika sa IR (IRI>1.7 31% vs IRI<1.7 5.9%) u odnosu na skupinu bez ŽK (IRI>1.7 69% vs IRI<1.7 94.1%) kao i više razine glukoze u serumu (5.44±0.74 mmol/L vs 5.22±0.62 mmol/L, p=0.029).

**Zaključak:** Prevalencija žučnih kamenaca u kandidata za transplantaciju jetre je visoka (26.73%). Inzulinska rezistencija je glavno obilježje metaboličkih promjena ciroze, te može imati ulogu u razvoju žučnih kamenaca, što usmjerava na daljnja istraživanja.

Ključne riječi: žučni kamenci, transplantacija jetre, ciroza jetre, litogeneza

# 3. Preface

The study aimed to evaluate the prevalence of gallstone disease in liver transplant candidates and to investigate its associations with selected clinical and biochemical variables in the context of end-stage liver disease. Finding new correlations or confirming already existing ones could help in the development of preventative measures such as screening, early detection, faster treatment and decreasing the morbidity and mortality for a large group of the population suffering from end-stage liver disease which eventually require liver transplantation.

This thesis was a prerequisite for the completion of the Medical Studies in English at the University of Zagreb, School of Medicine. The author of the thesis is Rafael Martin Astudillo, who wrote it with the assistance of his mentor, Assistant Professor Anna Mrzljak.

# 4. Hypothesis

The prevalence of gallstones in patients with end-stage liver disease is high and associated with metabolic disturbances in cirrhosis.

# 5. Introduction

Patients with end-stage liver disease (ESLD) are prone to gallstones disease (GD), however, treating complications in those patients is challenging because of the patient's reduced general health status, bleeding tendency and abdominal adhesions resulting from cholecystitis. Cholecystitis can cause life-threatening peritonitis and may be the sole reason to investigate the prevalence of GD in patients undergoing liver transplantation (LT).

Previous data show that the prevalence of gallstones (GS) in patients with liver cirrhosis (LC) ranges from 22.8% to 29.4% (1,2). A recent study by Shi *et al.* on 1640 patients with

ESLD showed that the incidence of GS is 5-10 times (32.56%) higher in LT recipients compared to the general population (3.5% - 7.3%) and that transplant recipient's age, Child-Turcotte-Pugh (CTP) score and the presence of cirrhosis are independent risk factors for development of GD (3).

Many risk factors for GD development in cirrhotic patients have been identified but there are still several aspects inadequately defined. Studies are continuously being published, trying to better explain the pathophysiological process and risk factors for lithogenesis in cirrhotic patients.

### 5.1 Gallstones

The most common disorder of the biliary tree is the formation of GS, and it is rare that the gallbladder is diseased in the absence of GS. GS are present in 10-20% of adults in developed countries, and it seems to be a 3:1 female predominance of GS in patients without chronic liver disease (4).

GS are divided based on their composition or based on the presence or absence of symptoms. Most GS are asymptomatic and remain so during the entire life of the patient. They are most commonly discovered accidentally during abdominal ultrasound examinations and are merely noted. Studies suggest that around 20% of asymptomatic patients will develop symptoms within 5-20 years from diagnosis. Nevertheless, 41.3% of cholecystectomies are performed in asymptomatic patients (5-7).

Roughly 75% of GD in the general population is asymptomatic, but when symptoms and complications occur it produces significant economic consequences (8).

Cirrhotic patients with GS are usually asymptomatic but have a higher chance of detecting them since they are regularly controlled by ultrasonographic. When biliary pain occurs, the

GS are said to be symptomatic. The pain is usually described as intense, continuous or colicky, radiating to the back and lasts more than 15-30 minutes (9). It is essential to recognize risk factors and symptoms early in able to treat the patient before complications occur, especially in patients with ESLD.

GS occur due to abnormal bile constitution and can be classified into three types according to their chemical composition: pigment, cholesterol and mixed stones. Although most are mixed, cholesterol stones are more frequent in developed countries (75% - 80%) while pigment stones occur more often in developing countries (8).

### 5.1.1 Cholesterol gallstones

Cholesterol is kept in a soluble state by associating with bile acids and phospholipids as micelles and vesicles. GS are formed when bile contains an excess of cholesterol which can be by either excessive cholesterol secretion or by a deficiency of bile salts (9).

Mucus, calcium, fatty acids and other proteins initiate crystallization of cholesterol and are known as “Nucleation Factors.” Nucleation factors cause cholesterol crystals to form more rapidly in patients with GS compared to patients without GS, even if the bile is equally saturated with cholesterol. Cholesterol stones are more likely to develop in the obese population because of increased biliary secretion, and in individuals who undergo rapid weight loss. Rapid weight loss mobilizes cholesterol in which causes the increased secretion. It is also associated with female sex hormones, pregnancy, oral contraceptives, estrogen replacement therapy, gallbladder hypomotility, decreased bile acid secretion and a high-fat diet (9,10).

### 5.1.2 Pigment gallstones

Brown pigment stones are almost always associated with bacterial or parasitic infection in the biliary tree. Infection of the biliary tree causes β-glucuronidase to hydrolyze conjugated bilirubin to its free form, which then precipitates as calcium bilirubinate crystals (9).

Black pigment stones mainly consist of polymerized calcium bilirubinate, the mechanism by which stones develop in developed countries is not adequately explained, but it is associated with chronic haemolytic disease, hepatic cirrhosis, cystic fibrosis, pernicious anaemia, increasing age and ileal resection/disease/bypass (9,10).

## 5.2 Biliary sludge

Biliary sludge (BS) is an important precursor to the formation of GS (9-11). It is described as gelatinous bile that mainly contains cholesterol microcrystals, calcium bilirubinate granules, and glycoproteins. BS is commonly formed under normal conditions but is typically dissolved and cleared. In approximately 15% of patients, BS persists to form cholesterol stones. Risk factors associated with BS formation include pregnancy, parenteral nutrition, and fasting.

## 5.3 Risk factors for lithogenesis

Patients with pigment stones are usually older than those with cholesterol stones. In a clinical study including 551 patients undergoing cholecystectomy is was found that those with pigments stones are generally older than 70 years and that pigment stones are strongly associated with LC (12).

Advanced age was shown to be an independent risk factor for symptomatic GD in patients with LC (3,13). Other studies and reviews also showed that the prevalence of GS in cirrhotic patients increased with age (2,9,14,15).

It is largely agreed that the lithogenic risk of cirrhotic patients is directly correlated to the cirrhotic change of the liver. However, some liver diseases have higher lithogenic risk than others. Alcohol abuse was shown to be an independent risk factor for GS formation in cirrhotic patients of all aetiologies (16). Chronic hepatitis C virus (HCV) infection represents a major cause of liver cirrhosis in developed countries, and some studies showed a higher incidence of GS in patients with chronic HCV (17,18). Another study showed a significantly higher prevalence of GD in patients with chronic HCV compared to patients with chronic hepatitis B virus (HBV) or alcohol-induced cirrhosis (15). Chronic HCV has been demonstrated to be an independent risk factor for the development of GS and patients with chronic HCV developed GS at a younger age.

The link between cholesterol GS and chronic HCV infection could be due to the increased IR, commonly found in obese patients and those with hepatic steatosis. Increased IR favours cholesterol GS formation by increasing biliary saturation in cholesterol. Central obesity, diabetes mellitus type 2 (DMII), and hypertriglyceridemia are all risk factors for the development of cholesterol GS in the general population, and as well in patients with LC (1,2,19).

There is also a significantly increased prevalence among patients with central obesity and hepatic steatosis (20).

Non-alcoholic fatty liver disease (NAFLD) is a cause of chronic liver disease in the absence of alcohol, arising from complications of metabolic syndrome. It is characterized by the accumulation of fat in the liver and includes a vast range of liver changes, from fatty liver

to non-alcoholic steatohepatitis (NASH) and LC. There are many common risk factors in cholesterol GD and NAFLD which explains the higher prevalence of cholesterol GD in patients with NAFLD.

Many studies have shown that the severity of liver disease seems to be the primary determinant for the development of GS, and when comparing CTP class C versus CTP class A in these studies, the prevalence was indeed higher (21-25).

**5.4 Gallstone disease in patients with cirrhosis**

The incidence of LC is increasing in the developed world. The highest risk factor for cirrhosis and cirrhosis-associated mortality are alcohol, viral hepatitis (HBV and HCV) and NAFLD.

The first studies demonstrating a higher prevalence of GS in LC originated from postmortem studies showing a prevalence of 24.8% and 29.4% (18,26). Later, these results were confirmed by prospective ultrasonography studies which showed a prevalence of 18.5% - 46% and incidence rate of 11.5% - 16.6% (1,14,16,22-24).

Conte *et al.* monitored 618 cirrhotic patients ultrasonographically for almost four years and found that 141 (22.8%) developed GS during this time. The estimated cumulative probability rates were 6.5%, 18.6%, 28.2% and 40,9% at 2, 4, 6 and 8 years respectively (2).

In cirrhotic patients, pigment stones are the most common while cholesterol stones occur in about 15% of cases (25). Multiple factors are contributing to the formation of GS and especially pigment stones in cirrhotic patients; gallbladder hypomotility, increased secretion of unconjugated bilirubin, increased hydrolysis of conjugated bilirubin, reduced secretion of bile acids and phospholipids all favour lithogenesis (3,7,28).

As in the general population, the presence of GS in cirrhotic patients is most commonly asymptomatic and conservatively managed. However, it is recommended that these patients

need closer monitoring to catch the symptoms early and to avoid complications. The patients with the highest surgical risk are those with a severe liver disease, and it is therefore preferred to treat symptomatic GS using non-invasive or minimally invasive methods until the patient is stabilized and suitable for surgery. (25)

# 6. Materials and methods

## 6.1 Study Population

One hundred and one consecutive non-diabetic adult cirrhotic patients transplanted between 2014 and 2017 at University Hospital Merkur in Zagreb were included in this study. Cirrhosis was diagnosed according to histological or clinical criteria, including the presence of complications such as variceal haemorrhage, ascites and/or hepatic encephalopathy and/or imaging and laboratory exams consistent with the diagnosis.

Patients who previously underwent LT or with a previously established diagnosis of diabetes with or without anti-diabetic therapy and/or fasting plasma glucose (FPG) > 7 mmol/L at the time of evaluation were excluded from the study.

## 6.2 Study Data

The patient's medical records were used to collect data about the past medical history, including the aetiology of liver disease, baseline characteristics, clinical evaluation and laboratory data. Baseline characteristics included age, gender, height, weight, and BMI.

Laboratory data before LT included lipid profile status (total-C, HDL cholesterol, LDL cholesterol and TG), liver biochemistry (AST, ALT, GGT, ALP), liver function test [total bilirubin, international normalised ratio (INR)]) albumin, kidney function (creatinine), fasting glucose, HbA1c and insulin. The degree of insulin resistance was calculated according to the HOMA-2 model (https://www.dtu.ox.ac.uk/homacalculator/). The insulin resistance index (IRI) values >1.7 were considered as insulin resistance. (29,30)

The CTP and Model for end-stage liver disease (MELD) scoring systems were used to determine the severity of liver disease. Before LT all patients were examined for the presence

of GS using ultrasound and/or multi-slice computed tomography (MSCT). The presence of GS for this study was confirmed postoperatively analyzing the explanted specimens.

### 6.3 Ethical Approval

The study was performed according to the principles of the declaration of Helsinki and approved by the Ethics Committee of the Merkur University Hospital in December 2013. Written informed consent was obtained from all patients.

### 6.4 Statistical Analysis

Normality of distribution was determined by using the Shapiro-Wilk test. The data values are expressed as means (± standard deviation) or median (range) as appropriate. Statistically significant differences between numerical variables were determined using student's t-tests for normally distributed data, and for variables with non-Gaussian distribution, the non-parametric Mann-Whitney U test was used. Statistically significant differences between categorical variables were determined by using the chi-squared test. IR was treated as a binary variable, with IRI values >1.7 considered as IR. All statistical analyses were done using the SPSS for Windows and a $p$-value of $<0.05$ was considered statistically significant.

# 7. Results

The study included one hundred and one non-diabetic adult cirrhotic patients transplanted at University Hospital Merkur, Zagreb. The cohort consisted of 79 male and 22 female patients with a mean age of 57.39±8.79. Majority of patients (90.1%) had decompensated liver disease, CTP class B+C, and only 9.9% had compensated cirrhosis, CTP class A (Figure 1).

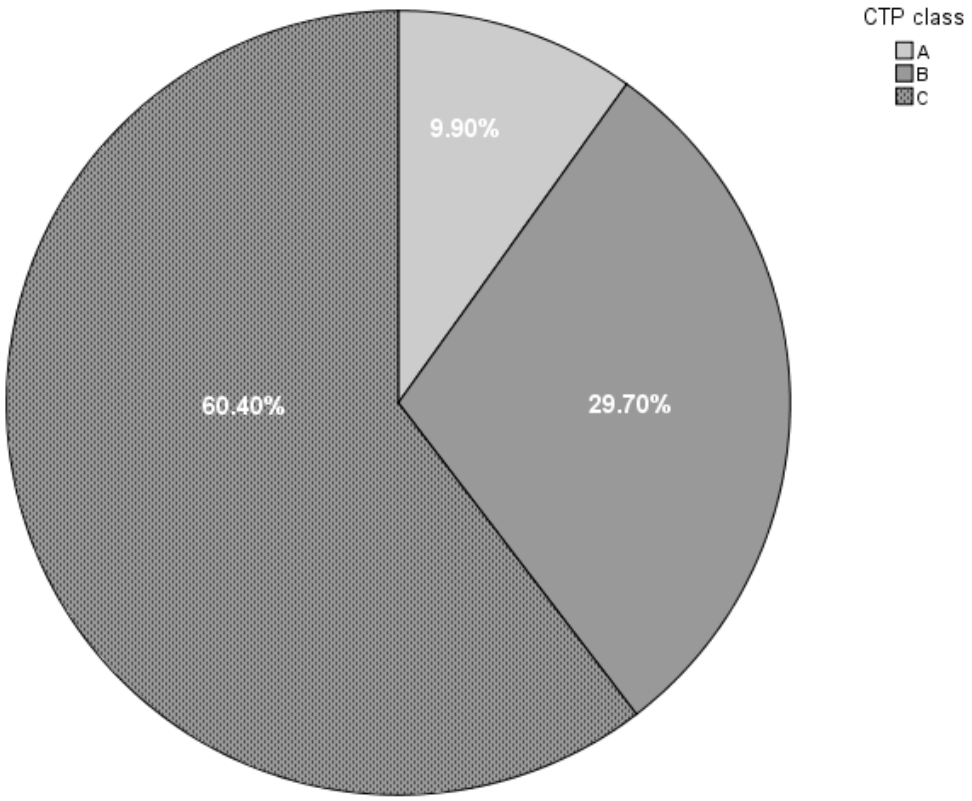


Figure 1. Distribution of CTP classes within a cohort.

### 7.1 Prevalence of GS in liver transplant candidates

GS were confirmed in 27 of the 101 LT candidates, and the prevalence was 26.73%.

The majority of patients had alcoholic liver disease (58.42%), followed by viral hepatitis (HBV+HCV) (21.78%), cryptogenic liver diseases (9.90%) and autoimmune liver disease (7.92%). Only two patients had other causes of liver disease: 1 patient had NASH and 1 had cirrhosis due to long-term antimalarial treatment accompanied by hepatocellular carcinoma (HCC). The distribution of GS among different liver diseases is presented in Table 1. The prevalence of GS according different liver disease aetiologies was as follow: cryptogenic cirrhosis (CC) 30%, viral hepatitis 27.27%, alcoholic 27.12% and autoimmune liver diseases 25%.

Table 1. The etiology of liver disease among 101 liver transplant candidates and the prevalence of gallstone disease.

| **Liver disease** | **Case (n=101)** | **Ratio (%)** | **GS present (n=27)** | **GS prevalence (%)** |
|---|---|---|---|---|
| Alcoholic liver disease | 59 | 58.42 | 16 | 27.12 |
| HBV, HCV | 22 | 21.78 | 6 | 27.27 |
| Autoimmune liver diseases | 8 | 7.92 | 2 | 25.00 |
| NAFLD | 1 | 0.99 | 0 | 0.00 |
| Cryptogenic cirrhosis | 10 | 9.90 | 3 | 30.00 |
| Other | 1 | 0.99 | 0 | 0.00 |

### 7.2 Data Analysis

Patients were divided into GS-present (n=27) and GS-absent groups (n=74).

Differences in socio-demographic, anthropometric and aetiological parameters are presented in Table 2.

No statistically significant differences regarding age, gender, body mass index (BMI), aetiology or severity of liver disease expressed as either CTP or MELD score were found between the two groups (p=ns) (Table 2).

Table 2. Comparing socio-demographic, anthropometric and aetiological parameters between GS-present and GS-absent groups.

| **Variables** | **GS-present (n=27)** | **GS-absent (n=74)** | **p** |
|---|---|---|---|
| Age, mean±SD, yrs | 56.58±7.8 | 57.70± 9.14 | 0.572 |
| Gender, M/F n (%) | 20 (74.1%)/7 (25.9%) | 59(79,7%)/15(20.3%) | 0.542 |
| Weight, mean±SD, kg | 87±20 | 81±17 | 0.133 |
| Height, mean±SD, m | 1.75±0.09 | 1.75±0.10 | 0.722 |
| BMI, median (range) | 27 (18-43) | 26 (19-39) | 0.164 |
| CTP score, median (range) | 10 (5-13) | 10 (5-14) | 0.125 |
| MELD score, median (range) | 16 (8-38) | 16 (8-37) | 0.416 |
| Alcoholic liver disease, n (%) | 16 (27.12) | 43(72.88) | 0.917 |
| HBV+HCV, n (%) | 6 (27.27) | 16 (72.73) | 0.948 |
| Autoimmune disease, n (%) | 2 (25.00) | 6 (75.00) | 0.908 |
| Cryptogenic cirrhosis, n (%) | 3 (30.00) | 7 (70.00) | 0.806 |

The prevalence of GD in patients with compensated liver disease (CP-A) (n=10) was 50% while patients with decompensated liver disease (Child-Pugh B+C) (n=91) had a prevalence

of 24.2%. However, there was no statistically significant difference between both groups, p=0.08 (Figure 2).

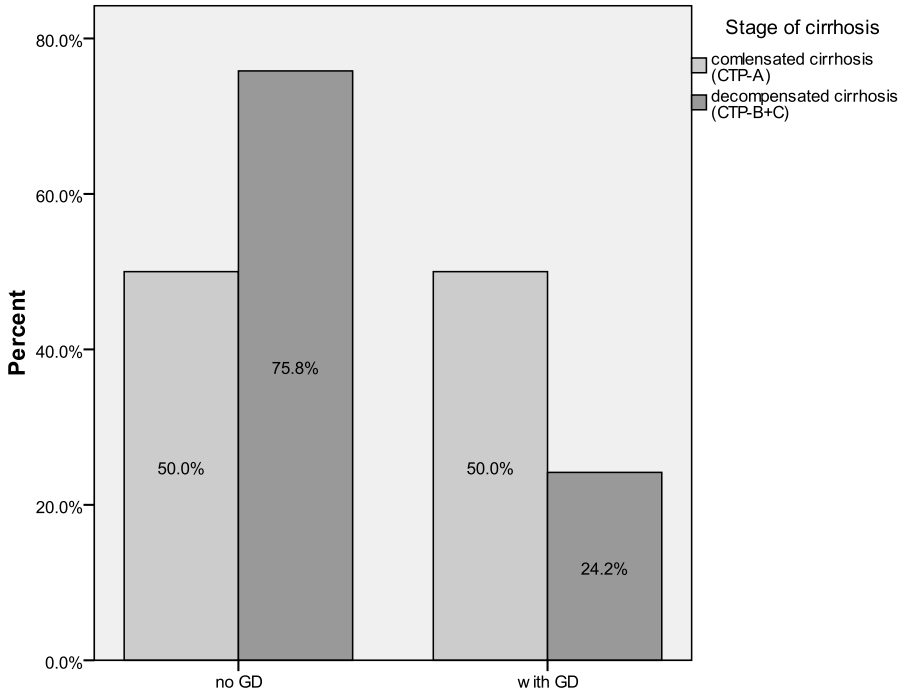


Figure 2. The prevalence of GD disease in compensated and decompensated patients.

Fasting plasma glucose (FPG), haemoglobin (Hgb) and alkaline phosphatase (ALP) levels differed significantly between the two groups (Table 3).

The mean FPG level was significantly higher in the GS-present group (5.44±0.74 mmol/L) compared to the GS-absent group (5.22±0.62 mmol/L), p=0.029.

The mean Hgb concentration was 113±25 g/L in patients with GS, significantly higher than in patients without GS, (104±21 g/L) p=0.048.

Patients with GS also had a significantly higher median value of ALP levels, 145 (range 44–306) IU/L compared to 109 (42–531) IU/L in patients without GS, p=0.031.

Table 3. Comparing laboratory variables in GS-present group vs GS-absent group.

| Laboratory variables | GS-present (n=27) | GS-absent (n=74) | P |
|---|---|---|---|
| Lipid status | | | |
| Total-C, median (range), mmol/L | 3.3 (0.4–8.8) | 3.0 (0.3–12.7) | 0.827 |
| HDL median (range), mmol/L | 0.92 (0.24–2.14) | 0.84 (0.16–2.38) | 0.658 |
| LDL median (range), mmol/L | 2.0 (0.2–5.9) | 1.9 (0.6–10.8) | 0.930 |
| TG median (range), mmol/L | 0.84 (0.47–1.87) | 0.68 (0.41–3.22) | 0.062 |
| Liver function tests | | | |
| AST median (range), IU/L | 63 (23–160) | 64 (22–903) | 0.982 |
| ALT median (range), IU/L | 41 (14-154) | 31 (10-236) | 0.514 |
| GGT median (range), IU/L | 53 (17-351) | 51 (13-728) | 0.262 |
| ALP median (range), IU/L | 145 (44–306) | 109 (42–531) | **0.031** |
| Total bilirubin (μmol/L) | 57 (11–605) | 57 (7-647) | 0.618 |
| INR median (range) | 1.4 (1.0–2.7) | 1.6 (1.0-3.0) | 0.145 |
| Albumin, mean±SD, g/L | 32.70±5.6 | 30.99±6.7 | 0.239 |
| Kidney function | | | |
| Creatinine median (range), μmol/L | 76 (41–382) | 74 (40–362) | 0.596 |
| Glycemic parameters | | | |
| Fasting glucose, mean±SD, mmol/L | 5.44±0.74 | 5.22±0.62 | **0.029** |
| HbA1c median (range), IU/L % | 4.9 (3.8-6.0) | 4.7 (3.4-54.9) | 0.183 |
| Insulin median (range), pmol/L | 115.3 (57.5–505.0) | 113.8 (42.2–731.5) | 0.642 |
| Hematological and inflammatory parameters | | | |
| Hgb, mean±SD, g/L | 113±25 | 104±21 | **0.048** |
| Trc median (range), $10^3/mm^3$ | 94 (26–250) | 84 (40–464) | 0.794 |
| CRP median (range), mg/L | 6.2 (0.4–62.1) | 8.1 (0.4–78.4) | 0.468 |

No statistically significant differences between GS-present and –absent groups were noted in other laboratory parameters including: lipid status (p=ns), other liver function tests (p=ns), kidney function (p=ns), HbA1c (p=ns), insulin levels (p=ns), platelets (p=ns) or an inflammatory marker (p=ns) (Table 3.)

The median value of the IRI, calculated according to the HOMA-2 model was 3.195 (0.9-15.9) for the entire cohort. 83% of transplant candidates had IR defined by an IRI value of >1.7.

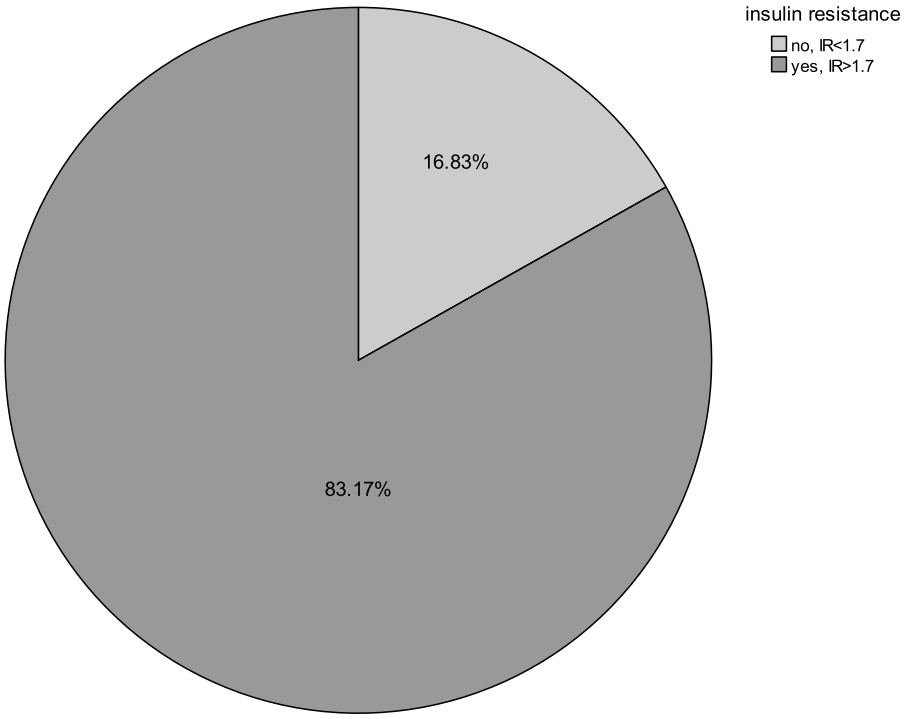


Figure 3. The distribution of insulin resistance according to HOMA-2 model for the entire cohort.

There was a significant statistical difference regarding IR between both groups, (p=0.03). Significantly more patients with GS had IR, (IRI>1.7 31% vs. IRI<1.7 5.9%) compared to the patients without GS (IRI>1.7 69% vs. IRI<1.7 was 94.1%) (Figure 4).

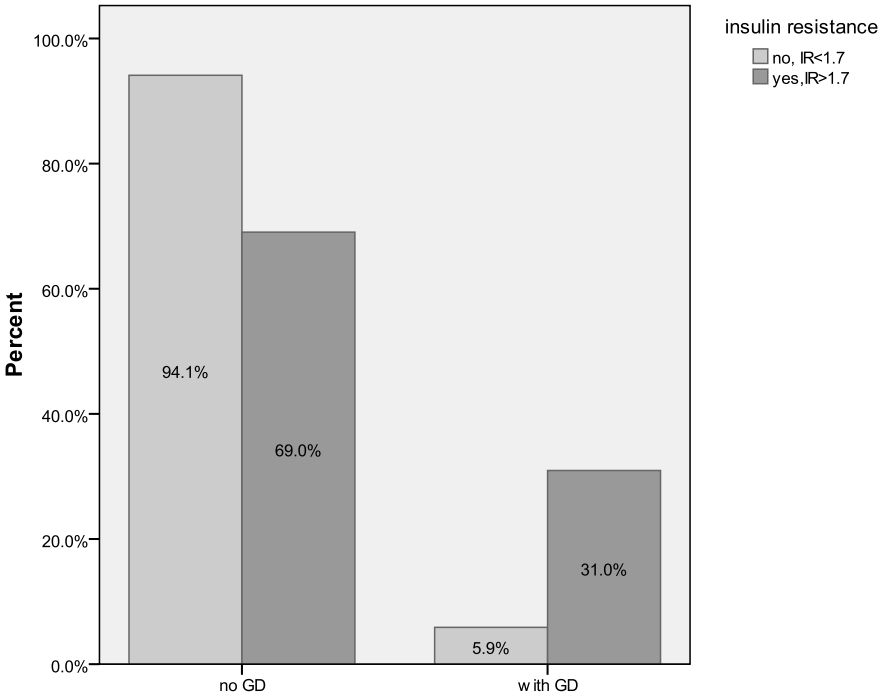


Figure 4. The insulin resistance between the GD-present and the GD-absent groups (p=0.03).

# 8. Discussion

The purpose of this study was to evaluate the prevalence of gallstone disease among non-diabetic LT candidates and to investigate it's associated socio-demographic, anthropometric and laboratory parameters in the context of advanced liver disease.

According to European data on almost 30.000 participants, the overall prevalence rate of GS in the general population is 18.8% for women and 9.5% for men (31). However, patients with advanced liver disease are at greater risk of developing GD (1,2,14, 32).

Previous studies have shown that the prevalence of GS in patients with liver cirrhosis ranges from 22.8% to 32.6 % (1-3), results which are several times higher than in general population. In our study, the prevalence of GD among LT candidates was 27.73%, which is in line with the previously mentioned data. And when comparing this data with statistics from the general population (31), our study demonstrated that females and males with liver cirrhosis are 1.69 and 2.66 times more likely to develop GS than patients without LC.

Several factors have been suggested as contributors for the higher prevalence of GD in cirrhotic patients. Cirrhotic patients have a weak gallbladder that cannot contract properly, consequently, bile emptying is decreased and copious amounts of unconjugated bilirubin deposits in the gallbladder which contributes to GS formation (3,27,28). In end-stage liver disease, liver function is decreased by the inactivation of various substances such as oestrogen, glucagon, vasoactive intestinal peptide, histamine, and somatostatin. These substances relax the gallbladder smooth muscle, which contributes to the formation of gallstones (3,32).

Previous studies have shown that the severity of cirrhosis positively correlates with the incidence of gallstone disease (3,33-35). CTP and MELD scores are commonly used indicators for evaluating the severity or stage of liver disease. Our study did not find any statistical difference between the severity of liver disease (expressed as MELD score or CTP

score/stages) and the presence of GS (Figure 2), which is not consistent with other studies. The prevalence of GS in compensated Child-A LC was 50%, while in decompensated Child class B and C, was 26.6% and 22.95% respectively. Among the transplant candidates in our study, only 9.9% had compensated Child A LC, this result should, therefore, be interpreted with caution.

We did not find any statistically significant differences in terms of GD and the aetiology of liver disease. In fact, the prevalence of GS was equally distributed among four major liver disease aetiologies; cryptogenic cirrhosis, viral hepatitis, alcoholic and autoimmune liver diseases, 30%, 27.27%, 27.12% and 25%, respectively. The remaining aetiologies included drug-related cirrhosis with HCC and NAFLD-related cirrhosis, but none of the patients had GD. Interestingly, only one patient in our study had NAFLD-related cirrhosis. Nevertheless, the data concerning cryptogenic cirrhosis should be interpreted with caution given the fact that the underlying disease often is an under-diagnosed NAFLD (36, 37). 9.9% of our transplant candidates had cryptogenic cirrhosis, and those patients had the highest GS prevalence rate of 30 % (Table 1).

NAFLD, represents the liver manifestation of the metabolic syndrome characterised by the morbidity cluster of obesity, DMII, hypertension and dyslipidemia. The prevalence of metabolic syndrome in the west is rapidly increasing, given the changes in eating habits and inclination towards a sedentary lifestyle. Metabolic syndrome is now becoming a growing epidemic. Consequently, NAFLD has become the most common cause of chronic liver disease in developed countries.

There are many common risk factors in cholesterol GD and NAFLD which explains the higher prevalence of cholesterol GD in patients with NAFLD. Central obesity, DMII, and

hypertriglyceridemia are all risk factors for the development of cholesterol GD in the general population, and as well in patients with LC (1,2, 19).

Previous studies have shown a higher incidence of GS in patients with chronic HCV infection (3,17,18), and particularly in cirrhotic patients with HCV compared to patients with HBV or alcohol-induced cirrhosis (15). HCV RNA has been found in gallbladder specimens of hepatitis C patients (15), directly infecting the gallbladder which may change its function and gallbladder mucosa, contributing to the development of gallstone disease (38).
Alcohol is the most frequent cause of liver disease in western countries (39), and according to a prospective study an independent risk factor for GS formation (16). Chronic alcohol abuse increases the formation of GS by its hepatotoxic effect and by reducing bile salt synthesis.

Most studies from the general population suggest that women are more prone to develop GS (40). Women are 2-3 times more likely to acquire GD but the gap narrows after menopause and male incidence start to catch up (4,32). Female sex hormones, parity, oral contraception and hormone replacement therapy are well-recognized risk factors for GS formation (41,42). Female sex hormones influence the bile secretion and gallbladder function. Estrogens increase cholesterol secretion and reduce bile salt excretion, while progestins decrease bile salt secretion and therefore impairs gallbladder emptying (32). Our study has shown no statistically significant difference regarding the prevalence between the genders (Table 2).

In the general population, the prevalence of GS increase with age, and some studies have also shown a positive association between the prevalence of GS in patients with ESLD and increasing age (2,9,14,15,43,44) Our study, did not find a relationship between the prevalence

of GS in patients with ESLD and age, which in is line with a similar research by Coelho *et. al* on 400 cirrhotic patients in Brazil, who underwent LT between 1991 to 2009 (45).

So far, many studies have evaluated biochemical markers associated with liver and gallbladder disease to analyze their relationship with GS (46,47). Nevertheless, no single biochemical marker has been linked with the development of GS. In this study, we chose to investigate multiple biochemical variables in patients with advanced liver disease and the prevalence of GS.

The only liver function test that differed between GD-present and –absent groups was ALP concentration (Table 3). GS patients had significantly higher ALP levels (145 (range 44–306) IU/L) compared to the ones without GS (109 (42–531) IU/L), p=0.031. In the context of GD, this is not surprising since elevated ALP and GGT are the biochemical markers of a biliary disease and especially cholestasis (48, 49).

These enzymes are located in the plasma membrane of hepatocytes, and as bile acids accumulate it acts as a detergent causing these enzymes to be released. Bile acid itself also increases the synthesis of ALP (50). ALP has a half-life of 7 days, (48) and elevated ALP levels may be present for some time after bile duct obstruction is resolved.

On the other hand, alanine transaminase (ALT) and aspartate transaminase (AST) are biochemical markers of intracytoplasmic hepatocellular damage. Thus, the concentrations of these enzymes are not increased to the same extent as in biliary diseases.

IR is the inability of insulin to exert its peripheral tissue actions, resulting in an impaired ability to suppress glucose production and stimulate peripheral glucose uptake (51). Insulin secretion is increased to overcome IR and maintain normal metabolic functions (52). This metabolic dysfunction leads to a cluster of abnormalities including DMII and cardiovascular disease (51).

IR is a hallmark of metabolic disturbances in cirrhosis. It is an early event in the course of the disease, explained by insufficient clearance of insulin due to reduced liver function, subsequent hyperinsulinemia and downregulation of insulin receptors that ultimately lead to diabetes mellitus (53-57). This also has been proven in our cohort of non-diabetic cirrhotic patients, where IR was 83%. Independently of disease aetiology, IR in cirrhosis identifies a subgroup of patients with worse prognosis. Nkontchou et al. showed that IR was independently associated with increased mortality or LT in a cohort of 249 chronic HCV patients with compensated cirrhosis (58).

Furthermore, previous studies have shown that increased IR favours cholesterol GS formation by increasing biliary saturation in cholesterol (25). Our data support this, namely, GS-present group had a significantly higher proportion of increased IR patients (IRI>1.7 31% vs IRI<1.7 5.9%) compared to the GS-absent group (IRI>1.7 69% vs IRI<1.7 94.1%) (Figure 3) and consequently higher FPG levels (5.44±0.74 mmol/L vs 5.22±0.62 mmol/L, p=0.029) (Table 3).

Patients with GD had significantly higher (113±25 g/L) Hgb levels compared with patients without GS (104±21 g/L), p=0.048. This may be explained by the association between haematological markers and insulin resistance (59,60). Erythropoietin is an important factor for the differentiation of erythropoietic precursors (61), and insulin itself also seems to stimulate the growth of human erythroid progenitors (60,62-65).

GS are formed when bile contains an excess of cholesterol which can be by either excessive cholesterol secretion or by a deficiency of bile salts. (9) Cholesterol stones are more likely to develop in obese population and in patients with the metabolic syndrome because of increased biliary secretion, but also in various conditions associated with a gallbladder hypomotility (9,10) In patients with ESLD, cholesterol synthesis, uptake, and secretion are all decreased (65). Previous studies have shown that the severity of LC is associated with

abnormal lipid profiles of cirrhotic patients (66-69. Between the two groups in our study, no differences were noted in the serum levels of lipid profile (total-C, HDL cholesterol, LDL cholesterol and TG).

Treating GD in patients with end-stage liver disease is challenging mainly due to their poor general health, portal hypertension, ascites and poor haemostasis. GD is likely to cause serious clinical consequences such as obstructive jaundice, cholangitis, peritonitis or sepsis which can be life-threatening or abdominal adhesion which could complicate the transplant procedure. Thus, investigating GD in end-stage liver disease is essential and could help in the development of preventative measures such as screening, early detection and faster treatment to decrease morbidity and mortality for patients who eventually require liver transplantation.

# 9. Conclusion

The prevalence of GD in our cohort of liver transplant candidates was 26.73%. The prevalence of GS was equally distributed among four different liver aetiologies. Although previous studies have shown that the GD prevalence seems to increase with age and the severity of liver cirrhosis, our results do not support that. Our study confirms that insulin resistance is a hallmark of metabolic disturbances in cirrhosis, and shows that IR may play an important role in the development of GD, which warrants further research.

Understanding the pathophysiology and associated risk factors for the development of GD enables creating better guidelines for screening and treatment in population at risk with the aim to prevent or decrease GD morbidity and mortality.

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## 12. Biography

Rafael Astudillo is a soon to be a doctor that studied a 6-year long medical program at the University of Zagreb School of Medicine in Croatia. He was born on the 19th of November 1990 in Örebro and was later raised in Stockholm, Sweden. He has a somewhat broad inheritance with his father originating from Ecuador and mother from Sweden. His passion for becoming a medical doctor started in early years of childhood and adolescence since both parents worked in the health sector and inspired him to study medicine. During his studies, Rafael participated in several extracurricular activities such as *SLF Utland Zagreb* (Swedish Medical Association in Zagreb), where he was president for the last 2 years organizing more than 10 extracurricular courses for all international students in Zagreb. After spending so many years in a Croatia and working very hard to finish his education, he is ready to move back to Sweden to start his career. His interests in the medical field are very broad but mostly ranges between internal medicine and surgery. He enjoyed many moments during his studies in Croatia and has grown and matured immensely.