Diploma Thesis

Evaluation of four simple noninvasive tests for prediction of fibrosis in chronic hepatitis C with persistently normal ALT (PNALT)

submitted by

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Graz, August 16, 2011 Thomas Markus Wroblewski

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ABSTRACT

Background: The prognosis of hepatitis C and the risk of developing cirrhosis are related to the stage of fibrosis. Liver biopsy is the gold standard for the assessment of histological activity and stage of disease, however, it has potential risks due to its invasive nature and histological assessment may suffer from sampling error and variability of results. Hence, accurate noninvasive tests to predict the stage of fibrosis are desired, especially in patients with persistently normal ALT who tend to show a slower progression of disease. Many noninvasive tests were proposed to predict fibrosis and cirrhosis, partially with high diagnostic accuracies, but only few studies are available for patients with persistently normal ALT. The aim of the current study was to evaluate the diagnostic performance of four noninvasive tests (AAR, APRI, FIB-4, Model 3) and platelet count per se in this particular group of patients.

Methods: Liver biopsy specimens of 103 patients enrolled in a previous study were examined applying the Ishak and METAVIR scoring systems for grading and staging. Descriptive data analysis and the evaluation of diagnostic accuracies of the noninvasive fibrosis tests and platelet count have been evaluated for the whole population as well as for subgroups of patients with persistently normal and elevated ALT.

Results: Receiver operating characteristic curve analysis revealed comparable diagnostic accuracies of all noninvasive tests and platelet count for prediction of significant fibrosis and cirrhosis in the total study population and in the subgroup of patients with elevated ALT. AAR was a poor predictor of fibrosis and cirrhosis in all our study groups. In patients with persistently normal ALT, areas under the curve were close to or under the reference line for all noninvasive fibrosis tests. The subgroup of patients with persistently normal ALT comprised patients with almost exclusively low fibrosis stages which hampered an adequate evaluation of the noninvasive tests.

Conclusion: Simple noninvasive tests to predict fibrosis and cirrhosis can substitute liver biopsy only in a fractional part of patients with hepatitis C. We could confirm the applicability of APRI, FIB-4, Model 3 and platelet count in patients with elevated ALT and in our whole population. Yet, we could not confirm their applicability in the subgroup of patients with persistently normal ALT.

ZUSAMMENFASSUNG

Hintergrund: Die Prognose von Patienten mit chronischer Hepatitis C und das Risiko, eine Zirrhose zu entwickeln, sind abhängig vom Ausmaß der Leberfibrose. Der Goldstandard zur Beurteilung der Fibrose und Entzündungsaktivität ist die Leberbiopsie, eine invasive Methode mit potentiellen Risiken. Außerdem kann die Histologie abhängig von vielen Faktoren variable Ergebnisse liefern. Deshalb wird nach nichtinvasiven Methoden zur Abschätzung der Leberfibrose gesucht, besonders für Hepatitis C Patienten mit persistierend normaler ALT, die gewöhnlich einen langsameren Krankheitsfortschritt zeigen. Viele nichtinvasive Fibrose-und Zirrhose-Tests wurden bereits evaluiert, teilweise mit guten Ergebnissen Bisher liegen für die Patientengruppe mit persistierend normaler ALT jedoch nur wenige Studien vor. Das Ziel dieser Studie ist die Evaluation von vier nichtinvasiven Fibrosetests (AAR, APRI, FIB-4, Model 3) und zusätzlich der Thrombozytenzahl als Fibrose- Prädiktoren bei Patienten mit persistierend normaler ALT.

Methoden: Leberbiopsien von 103 Patienten aus einer früheren Studie wurden herangezogen und anhand der Bewertungssysteme von Ishak et al. und METAVIR für das histologische Grading und Staging ausgewertet. Eine deskriptive Datenanalyse und Evaluierung der Aussagekraft der nichtinvasiven Fibrosetests und der Thrombozytenzahl erfolgte sowohl für die Gesamtpopulation auch als für Subgruppen mit persistierend normaler und erhöhter ALT.

Ergebnisse: ROC- Analysen zeigten eine vergleichbare Aussagekraft der Test und der Thrombozytenzahl in der Gesamtpopulation und der Subgruppe mit erhöhter ALT. AAR erwies sich in all unseren Gruppen als ungeeigneter Test zur Abschätzung einer Fibrose oder Zirrhose. AUROCs in der Subgruppe der Patienten mit persistierend normaler ALT waren nah oder sogar unter der Referenzlinie. In dieser Patientengruppe fanden sich fast ausschließlich niedrige Fibrosegrade, was die Evaluierbarkeit der Tests beeinträchtigte.

Diskussion: Simple nichtinvasive Tests können eine Leberbiopsie nur in einem Bruchteil von Patienten ersetzen. Wir konnten die Anwendbarkeit von APRI, FIB-4, Model 3 und der Thrombozytenzahl in der Gesamtpopulation und in der Gruppe mit erhöhter ALT bestätigen, jedoch nicht für Patienten mit persistierend normaler ALT.

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1 INTRODUCTION

1.1 HEPATITIS C INFECTION OF THE LIVER

Hepatitis C is an infection caused by the hepatotropic hepatitis C virus (HCV). Before identification of the hepatitis C virus in 1989 this infectious disease was classified as "non-A-non-B-Hepatitis". The hepatitis C virus is an enveloped, single stranded positive sense RNA virus consisting of 9400 nucleotides. It belongs to the family of F*laviviridae*. The gene encodes three structural proteins for the core and layer and four enzymes for virus replication. The virus shows a significant genetic instability and high mutation rate.[1] Eleven genotypes showing differences in treatment sensitivity have been identified.[2] Furthermore, many subtypes exist and different modifications of the virus, so called quasispecies may even be found in one single individual.[1] Using these mechanisms the virus efficiently escapes host immunologic detection and elimination causing chronic infection, severe inflammation and long-ranging complications. Given its heterogeneity the development of vaccines is difficult and not yet possible. Genotypes 1 to 3 are common worldwide. The most important subtypes are type 1a and 1b accounting for 60% of all infections and are predominant in Northern, Southern and Eastern Europe, North America and Japan. Type 2 is less frequent. Type 3 is endemic in South-East Asia, while genotype 4 is found in the Middle East including Egypt and also Central Africa: Type 5 is found in South Africa. Genotypes 3 to 11 are common in Asia. In terms of response to antiviral treatment, genotype 1 is commonly associated with a poor response to interferon monotherapy. Better responses are seen in the genotypes 2 and 3. The current gold standard therapy method is a combination of pegylated interferon and ribavirin with an improvement of response for all genotypes.[2]

1.2 EPIDEMIOLOGY OF HEPATITIS C

HCV is spread worldwide. In Europe there are almost 4 million HCV carriers, many of them with no symptoms. In total, 3 % of the world´s population is infected by HCV. Areas of increased prevalence are Mediterranean countries, the Far East and certain areas of Africa and Eastern Europe.[2]

1.3 TRANSMISSION OF HEPATITIS C

The main way of transmission is through percutaneous exposure to contaminated blood.[3] Transfusion of blood and blood products, dialysis as well as intravenous drug abuse with needle sharing contribute to high risk of transmission. Infections through blood transfusions decreased significantly after the establishment of virus contamination tests.[1] Also, infections through transplantation of organs were reported. Nosokomial inoculation through pin-pricks causes an infection in 3% of cases, if the needle was contaminated with Anti-HCV positive blood. 80-90% of all post-transfusional cases of hepatitis are caused by HCV. The development of hepatocellular carcinoma (HCC) is also highly associated with HCV infection. Non-percutaneous transmission is possible, however, the pathways of transmission are partly unknown. The risk of sexual transmission is low, but prostitutes and homosexual men have a higher risk. Perinatal transmission has been reported but is less frequent than in HBV-positive mothers. HIV and HCV coinfection, however, is related to a much higher risk of perinatal transmission. 40% of cases are caused by unknown risk factors.[3]

1.4 PATHOGENESIS OF HCV INFECTION

The early phases of infection are poorly understood. The presence of HCV-RNA in blood can be detected three days after inoculation by PCR or HCV core antigen detection and persists at least until elevated transaminase levels appear, a sign of liver cell damage. The infection of hepatocytes manifests with intraplasmatic antigen and the development of cytoplasmatic microtubular structures. Elevated expression of MHC-I molecules in the affected tissue and infiltration of HCV-peptide specific CD8-positive cytotoxic T-lymphocytes are symptoms of the virus specific immune response which is highly responsible for the liver cell damage. The cytopathogenity of the virus however may also play an important role in the pathogenesis.[3]

1.5 CLINICAL SYMPTOMS OF HEPATITIS C

After an average incubation time of seven to eight weeks most patients show flu-like symptoms like fever and fatigue, abdominal discomfort, nausea, emesis and a non-icteric course of disease.[2,3] 25% of patients show typical signs of a mild hepatitis with icterus and elevated transaminases whereas fulminant courses are very rare (<1%).[3] Marked elevations

in serum ALT levels are a characteristic feature of acute hepatitis C.[2] Spontaneous remissions can be seen in only 15-20% of cases.[1] A long lasting viraemia is typical.[3] Around 80% of patients with an acute HCV infection develop chronic hepatitis.[1] It may present shoves and turn into a chronic active hepatitis with marked elevation of transaminases and a bad prognosis.[3] Chronic liver disease with fluctuating or persistently elevated liver enzymes is seen in over 60% of patients. 20-30 years after infection up to 20% of patients develop cirrhosis. As a late complication, in 5% of patients with cirrhosis it leads to hepatocellular carcinoma (HCC).[2] Examples of extrahepatic manifestations of hepatitis C infection are arthritis, kryoglobulinemia and glomerulonephritis.[1]

1.6 DIAGNOSIS OF HEPATITIS C

Acute hepatitis C is often asymptomatic. Therefore diagnosis is often made rather lately, when the disease has become chronic.[4] Early diagnosis may increase the success of treatment and lifestyle changes. Furthermore it may limit the risk of cross-infection.[2]

1.6.1 SEROLOGICAL AND MICROBIOLOGICAL DIAGNOSIS

The diagnosis of hepatitis C is made by demonstration of Anti-HCV with the aid of an enzyme-linked immunoassay (EIA) or a recombinant immunoblot assay. It becomes detectable 2 to 8 weeks after evident liver injury and indicates a past or present infection. However, it cannot differentiate between acute and chronic disease. If the EIA is negative, the patient is very unlikely to have an HCV infection. If positive, it should be reconfirmed by application of the same diagnostic method or if necessary by detection of viral RNA through RT-PCR. This is a more sensitive method and gives information about the viral load and HCV genotype. Viremia can be verified within days. It is used to monitor interferon therapy in chronic hepatitis C.[2]

1.6.2 BIOCHEMICAL ASSESSMENT OF LIVER FUNCTION

1.6.2.1 The role of the transaminases in chronic hepatitis C

Liver function can be evaluated by the assessment of biochemical parameters.[2] The transaminases, aspartate aminotransferase (AST/GOT) and alanine aminotransferase (ALT/GPT) are useful indicators of hepatocellular damage.[5] Serum ALT is an indirect marker of HCV activity.[6] ALT can only be found in the cytoplasm of hepatocytes. AST is

present in both mitochondria and cytoplasm of hepatocytes. Mitochondrial injury may be a sign for severe liver disease.[7,8] AST is furthermore less liver specific than ALT and can also be found in the heart- und skeletal muscles, in the kidneys and brain.[5] The clearance of AST may decrease with progression of fibrosis causing elevated serum AST levels.[9] AST and ALT are elevated to a certain degree in all liver diseases. Highest measures are found in diseases causing liver cell necrosis like severe viral hepatitis or toxic liver damage.[5] Usually, the elevation of transaminases in acute hepatitis C is lower than in Hepatitis A or B. The peak values of transaminases in acute hepatitis C can reach a value 15 fold the upper limit of normal. After 5 to 12 weeks they tend to normalize if the disease ameliorates. If the disease becomes chronic, the levels of transaminases may remain elevated with values 2 to 5 fold the upper limit of normal, however, fluctuations are frequent. Yet in one third of patients with chronic hepatitis C the transaminases are persistently normal.[4] As for the histological image, patients with persistently normal ALT tend to show lower histological activity and fibrosis stage, a lower prevalence of cirrhosis and a slower progression of disease.[10] However, the correlation between serum ALT level and extent of damage or prognosis is insufficient. Even slight elevations of the transaminases can be associated with marked structural changes in the liver histology.[11]

1.6.2.2 Other biochemical parameters indicating liver damage in chronic hepatitis C

The liver plays an important role in hemostasis. It produces six different coagulation factors; fibrinogen (I), prothrombin (II) and the factors V, VII, IX and X. Except for factor V, all factors need vitamin K as a cofactor. Due to physiologically high concentrations, only severe liver diseases cause coagulation disturbances. These can be efficiently assessed by measuring the prothrombin time which describes the rate of conversion of prothrombin to thrombin in presence of thromboplastin and calcium. It requires almost all vitamin K dependent factors. Factor VII is the speed limiting factor and therefore has a strong influence on prothrombin concentration. Prothrombin time is depending on hepatic synthesis of coagulative factors and a sufficient resorption of vitamin K. Either in acute and chronic liver cell damage a prolonged prothrombin time is a sign for poor prognosis and should therefore be measured.[5]

Chronic liver disease, above all cirrhosis, is often associated with thrombocytopenia. The development of splenomegaly due to portal hypertension may lead to an enhanced pooling of platelets.[12]

Several parameters indicate biliary disturbances which are common in liver diseases. Spectrophotometric measurement of serum bilirubin distinguishes two fractions; the hydrophilic conjugated bilirubin which reacts directly with the reagent and the indirectly reacting lipophilic unconjugated bilirubin. Elevation of the conjugated fraction with elevation of direct and indirect bilirubin implicates a secretion problem into the bile, whereas an elevation of the unconjugated fraction is a sign for impeded conjugation.[5] In patients with chronic hepatitis C, levels of alkaline phosphatase (AP) and γ-glutamyl transpeptidase (γ-GT) can be elevated. Elevated levels of γ-GT are associated with bile duct lesions and steatosis.[4] γ-glutamyl transpeptidase can be found in all hepatobiliary tissues. In liver diseases, γ-GT correlates with alkaline phosphatase and is a sensitive parameter for bile duct lesions. Unspecific elevations are found in pancreatic, cardial, renal and pulmonary diseases as well as diabetes mellitus.[5]

Human serum contains several forms of alkaline phosphatase. Elevated activity of AP without the presence of a pregnancy or bone disease is a sign for biliary disturbance. Light elevations are found in diseases of the liver parenchyma like hepatitis and cirrhosis (1-2 folds of the norm). Highest elevations are found in extrahepatic mechanical biliary obstructions (3 to 10 folds of the norm) or intrahepatic functional cholestasis.[5]

Levels of cholinesterase and albumin fall only in very severe cases of chronic hepatitis C. Frequently an elevation of gammaglobulins without involvement of IgM is noticeable.[4]

1.6.3 LIVER BIOPSY

The percutaneous needle biopsy is an important diagnostic method in liver diseases. Diffuse parenchymal diseases like hepatitis, cirrhosis and toxic liver damage can be diagnosed with remarkable precision. Local anesthesia is used to numb the skin where a small incision is performed. A Menghini- or Trucut- needle is inserted percutaneously through a transpleural or subcostal access. Imaging techniques can be used to avoid injuring adjacent organs. A transvenous access or the performance during laparoscopic abdominal surgery is also possible. Side effects are rare except of pain and vasovagal reactions. Biopsies cannot be performed if the patient is unable to cooperate, suffers from a

coagulation disturbance, infection of the right pleura, septic cholangitis, ascites under pressure, biliary obstruction or vascular lesions.[5]

The need for biopsy in the diagnosis and management of patients with chronic hepatitis is not always unambiguous, as serological and virological parameters may be helpful to confirm the clinical diagnosis of chronic hepatitis C. Furthermore, liver biopsy is an invasive method with potential risks.[13-15] It may suffer from sampling error and inter- as well as intraobserver variability.[16,17] Nevertheless it is the gold standard for the assessment of histological activity and stage of disease and an important tool to exclude other overlapping liver diseases. Furthermore, histological assessment is being used in the evaluation of new treatment regimes.[18]

1.6.4 NON-INVASIVE DIAGNOSTIC TESTS FOR THE PREDICTION OF FIBROSIS AND CIRRHOSIS

Many indices have been proposed to predict fibrosis and cirrhosis in patients with hepatitis C. Common aims were to create simple, easy-to-use and accurate estimators of liver fibrosis. Most of the noninvasive fibrosis tests use ratios or more complex formulas of standard laboratory and demographic data.[19-24] In a part of patients these noninvasive markers may help predict the probability of significant fibrosis or cirrhosis and decrease the need of liver biopsy. Some patients, however, do not match with the extreme criteria of these scores and therefore cannot be classified clearly. These patients will still need a biopsy.[4]

1.6.4.1 AST/ALT Ratio (AAR)

The AST-ALT-ratio was proposed as a simple test to suggest the presence of cirrhosis.[19]

$$AAR = \frac{AST}{ALT}$$

Several investigators confirmed that an AAR value greater than 1 could predict cirrhosis with high diagnostic accuracy. Williams and Hoofnagle[19] observed a greater increase of AST in comparison to ALT with progression of liver disease. Therefore the AST-ALT-ratio was proposed as a predictor of cirrhosis. Sheth et al.[20] examined the

value of AAR in patients with chronic hepatitis C to distinguish cirrhotic and non-cirrhotic patients. This group reported a specificity and positive predictive value of 100% with a negative predictive value of 80.7% and sensitivity of 53.2%.

1.6.4.2 AST to platelet ratio index (APRI)

Wai et al.[21] constructed a simple model consisting of routine laboratory data to predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. They found that platelet count and AST levels were important predictors of both significant fibrosis and cirrhosis. While values of AST tended to increase with the stage of liver fibrosis, platelet count tended to decline. These effects were used to create a new index called APRI or AST to platelet ratio index:

$$APRI = \frac{\frac{AST}{ULN}}{Platelet\ count\ \left[\frac{10^9}{L}\right]} \times 100$$

Exclusion of fibrosis	< 0.5
Prediction of fibrosis	≥ 1.5
Exclusion of cirrhosis	< 1.0
Prediction of cirrhosis	≥ 2.0

Table 1-1 Proposed cutoff values for the AST to platelet ratio index by Wai et al.[21]

Different cutoff values were chosen for the prediction and exclusion of significant fibrosis and cirrhosis as shown in Table 1-1. Using the cutoff value of ≥ 1.5, the presence of significant fibrosis could be predicted with a positive predictive value of 88% and a specificity of 95%. The corresponding cutoff value of < 0.5 could exclude significant fibrosis with a negative predictive value of 86% and a sensitivity of 91%. The area under the receiver operating characteristic curve for prediction of significant fibrosis (F3-F6 of the Ishak fibrosis score) was 0.80.

Using the cutoff of > 2.0 cirrhosis could be predicted with a positive predictive value of 57% and a specificity of 93%. With the cutoff value of < 1.0 cirrhosis could be excluded with a negative predictive value of 98% and a sensitivity of 89%. The area under the receiver operating characteristic curve for prediction of cirrhosis (F5-F6 of the Ishak fibrosis score) was 0.89.[21]

1.6.4.3 FIB-4

FIB-4 was proposed by Sterling et al.[22] as a simple and inexpensive model containing routine laboratory tests to predict liver fibrosis in patients with HIV/HCV coinfection. It includes several factors that were proven to be associated with fibrosis: patient´s age, platelet count, ALT and AST. The proposed formula is as follows:

$$\frac{Age\ (years) \times AST[U/L]}{Platelet\ count[10^9/L]} \times ALT[U/L]^{1/2}$$

Two cutoff values were defined. To exclude significant fibrosis (Ishak F4-F6), this group proposed a cutoff value of < 1.45. The corresponding negative predictive value was 90% with a sensitivity of 70%. To confirm significant fibrosis (Ishak F4-F6), a cutoff of > 3.25 was defined. This cutoff value had a positive predictive value of 65% and a specificity of 97%. The area under the receiver operating curve was 0.77 for the prediction of significant fibrosis (F4-F6 in the Ishak fibrosis score).

FIB-4 has been validated by Vallet-Pichard et al.[23] in a population of HCV monoinfected patients. Using the same cutoff values, a FIB-4 index < 1.45 had a negative predictive value of 94.7% and a sensitivity of 74.3 % to exclude fibrosis. A FIB-4 index > 3.25 had a positive predictive value of 82.1% and a specificity of 98.2% to confirm significant fibrosis. The area under the receiver operating curve for the prediction of significant fibrosis (F3-F4 of the METAVIR fibrosis score) was 0.85 (95% CI 0.82-0.89) and 0.91 (95% CI 0.86-0.93) for the prediction of cirrhosis.

According to these studies, FIB-4 is an accurate method for assessing liver fibrosis for values < 1.45 and > 3.25.[22,23]

1.6.4.4 Model 3

Lok et al.[24] proposed a model to predict the probability of cirrhosis based on the following laboratory parameters: platelet count, AST/ALT ratio and INR.

The regression formula proposed by Lok et al. is the following:

$$Log\ odds\ (predicting\ cirrhosis) =$$

$$-5.56\ -0.0089\ \times Platelet\ count\ [10^3/mm^3]\ +1.26\ \times \frac{AST}{ALT} +\ 5.27 \times INR$$

To calculate predicted probability of cirrhosis the formula is:

$$\frac{\exp(\log\ odds)}{1 + \exp(\log\ odds)}$$

The defined cutoff values for Model 3 are < 0.2 to exclude and > 0.5 to predict cirrhosis. The area under the receiver operating curve for Model 3 was 0.81. In their study, a cutoff value of less than 0.2 misclassified 7.8% of patients with cirrhosis. The negative predictive value was 86%. A cutoff value > 0.5 to identify patients with cirrhosis misclassified 14.8% of patients without cirrhosis. The corresponding positive predictive value was 75%.

1.7 TREATMENT OF HEPATITIS C

Important rationales in the therapy of chronic hepatitis C are the reduction of inflammation, prevention of progression to fibrosis, cirrhosis and hepatocellular carcinoma through eradication of the virus as well a decrease of infectivity and the control of spread. An indicator of successful therapy is a sustained viral response defined by HCV RNA absence.[2] HCV generally shows sensitivity for interferon-α therapy, however, there are resistant and difficult-to-treat genotypes (Type 1). It is used for treatment of acute and chronic hepatitis C. Only 25% of treatments lead to permanent suppression of HCV-replication with a normalization of transaminase levels. After a primarily successful therapy and following therapy dismissal the viral load and transaminase levels may rise up again due to possible development of interferon antibodies. The nowadays used combined interferon-α and ribavirin treatment is twice as successful, leaving 45% of treated patients with no detectable viral RNA in their blood a year after therapy discontinuance. Polyethylene glycol complexed interferon has a longer half-life in blood and provides constant serum levels over one week after intake.[3] It has the highest response rates in combination with ribavirin. Side effects of interferon therapy are flu-like symptoms, hematologic as well as neuropsychiatric disturbances. Furthermore, weight loss, an ocular affection and autoimmune processes leading to hypothyroidism or diabetes can appear. The most important side effect of ribavirin is hemolytic anemia. Moreover, ribavirin is teratogenic and therefore contraindicated during pregnancy.[2]

1.8 HISTOPATHOLOGY OF HEPATITIS C INFECTION

1.8.1 THE PHYSIOLOGICAL STRUCTURE OF THE LIVER

The main structural element of the liver is the lobule. It consists of anastomosing plates of hepatocytes and vascular channels, called sinusoids that converge to the central vein. The portal tracts are situated between the lobules and contain vessels, nerves and bile ducts. The sinusoids are lined with fenestrated endothelial cells and phagocytic Kupffer cells. The space between hepatocytes and endothelium, called space of Disse, communicates with the sinusoids. It contains specialized stellate cells that accumulate lipids and vitamin A. These cells can transform into myofibroblasts when an induction takes place. Furthermore, collagen type 1 and fibronectin as well as other components of the liver matrix can be found in the space of Disse. Afferent blood from the portal vein and arteria hepatica reaches the liver through the vessels of the portal tracts and then passes the sinusoids to flow into the central vein in the centre of the lobule. The central veins finally end in the efferent venae hepaticae. Bile is collected in channels called canaliculi and flows reversely towards the portal tracts into bile ductules, interlobular bile ducts and finally into the hepatic duct and common bile duct. The hepatic acinus defined by Rappaport is a unit to describe hepatic function in a better way. The centre of the acinus consists of parts of the portal tract and small branches of the portal vein and arteria hepatica. The central veins are situated in the periphery of the acinus. Three zones have been defined by Rappaport. Zone 1 of liver parenchyma surrounds the portal tract and is supplied by blood that is richer in oxygen, hormons and nutrients than Zone 3. Zone 2 occupies an intermediate position.[1]

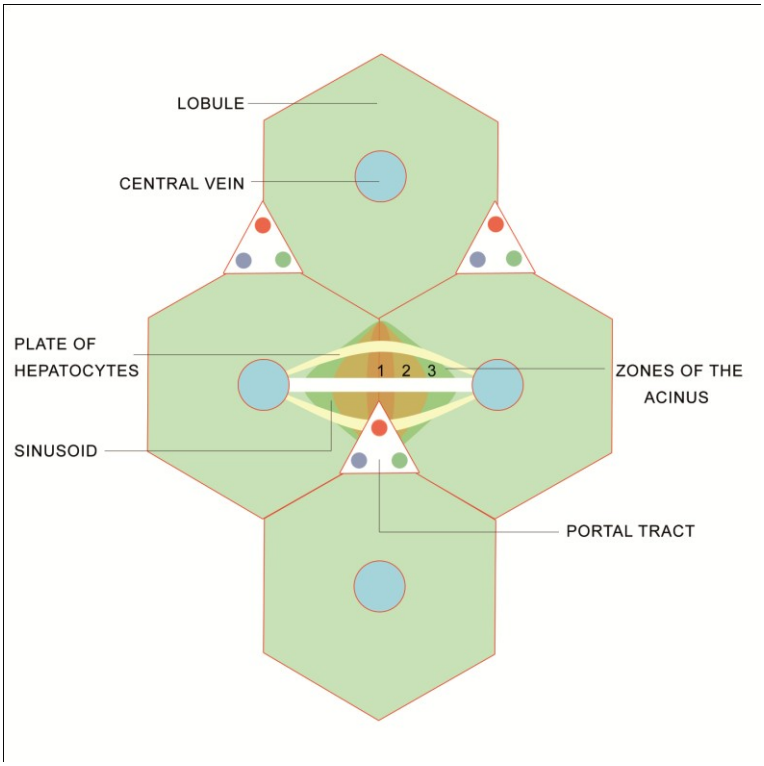


Figure 1-1: The acinus of Rappaport (modified version of the image given in Böcker W et al.[1]).

1.8.2 HISTOPATHOLOGY OF ACUTE HCV INFECTION OF THE LIVER

The histological changes can be separated into an early, acute phase and a later subacute phase. If elimination of the virus does not occur, the histological image changes into the one of chronic hepatitis. Differential diagnosis of subacute hepatitis C and a shove of chronic hepatitis C can be difficult. The total lack of fibrosis is the only definite symptom for an early infection.

The image of the active phase is given in Figure 1-2. It shows a dominant lobular inflammation with disseminated single or grouped apoptotic hepatocytes, so called Councilman-bodies. These degenerated hepatocytes usually have a pycnotic nucleus or no nucleus at all. In very early phases, hepatocytes are shrunk and show an angular shape with marked eosinophilic cytoplasma. Ballooned, hydropic hepatocytes can be seen in central areas of the lobule. Lymphoid infiltrations in the centre of the lobule and active Kupffer cells in areas of former hepatocytes are a sign for necrotic degeneration. The portal tracts show minimal infiltration and no fibrosis.

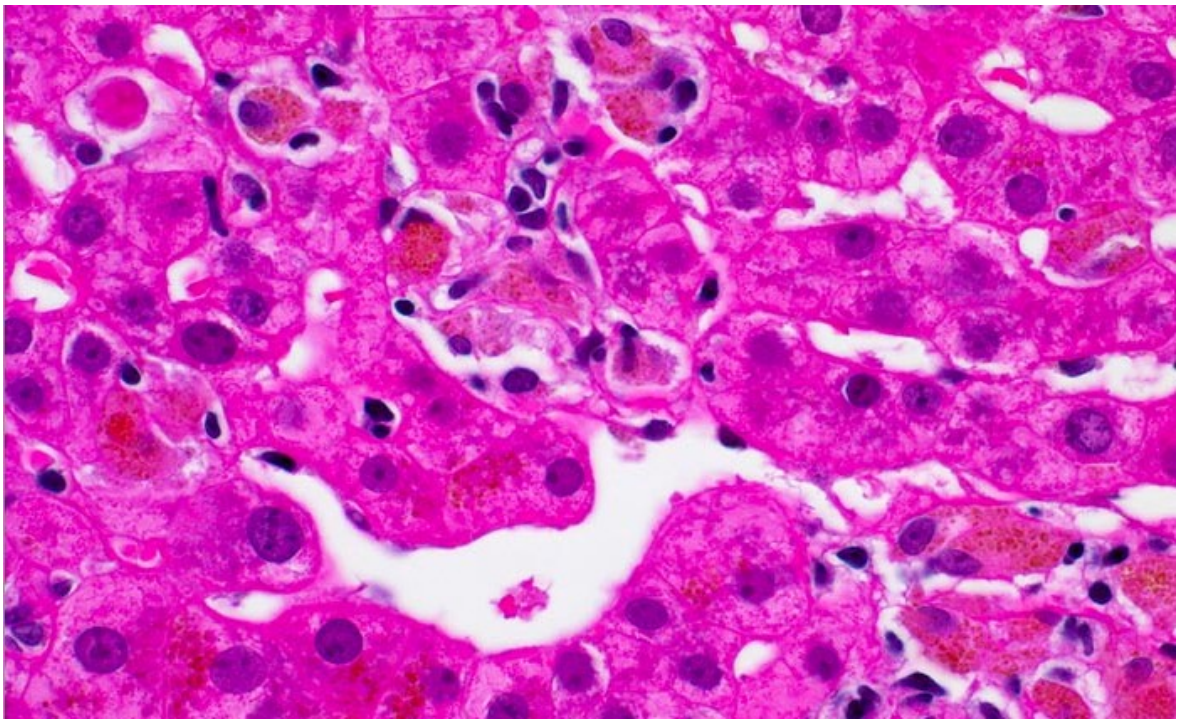


Figure 1-2: Acute hepatitis C: Lobular Hepatitis. Mild mononuclear lobular inflammation with Councilman-bodies and lipofuscin laden macrophages (H&E). Used with friendly permission from Glatz-Krieger K., Institute of Pathology, Basel.[25]

In cases of severe acute hepatitis C focal confluent necrosis, central lobular necrosis or even portal to portal bridging necrosis and bridging necrosis between portal veins and central arteries can appear. Severest cases show panacinar necrosis with a fulminant liver failure but are very rare and almost only seen in Asia.

Besides, a facultative hepatocellular or canalicular cholestasis is a typical finding. In 50% of cases a slight macrovesicular steatosis can be seen. Surviving hepatocytes can show reactive changes like a variability of shape and size, brightened or compressed cytoplasma and a hypertrophy of Kupffer cells. To estimate the localization and extent of liver cell degeneration a Gomori trichrome stain is useful as it shows the reticulin fiber collapse instead of the missing hepatocytes.

The subacute phase shows a movement of inflammation into the portal fields. Development of portal lymph follicles can appear early. Portal infiltration often exceeds the border into parenchyma, usually without necrosis. Active liver cell degeneration is rare.[1] Disseminated groups of hypertrophic Kupffer cells inside the lobule are noticeable and show a PAS-positive and Berlin-blue positive reaction. As they are involved in the clearance of degenerated hepatocytes, they accumulate ceroid and siderin. Several PAS-positive macrophages can be found inside the portal fields. The collapse of reticulin fibers

in areas of degenerated hepatocytes is marked and activated and proliferating stellate cells can be detected. Regeneration of parenchyma is visible through the broadening of parenchyma beams caused by small hepatocytes with elevated mitotic activity.[26]

1.8.3 HISTOPATHOLOGY OF CHRONIC HCV INFECTION OF THE LIVER

A typical histological sign of chronic hepatitis C is an infiltration of most small portal tracts by lymphocytes. Larger tracts are usually less affected. The lymphoid infiltration is associated with a smaller amount of plasma cells and segmented leukocytes. Few eosinophils may be present as well. Aggregates or entire follicles of lymphocytes in the small portal tracts are typical, but non exclusive for hepatitis C. Mild forms of chronic hepatitis C are characterized by infiltrates that are confined to portal tracts while the edges remain unaffected. In severer forms the extension of the infiltrate reaches the neighboring parenchyma. Mild chronic hepatitis is associated with enlarged tracts and short fibrous spurs. To assess these structural changes, reticulin or collagen stains are usually used. Interlobular bile duct lesions (Poulsen-lesions) are also typical. These lesions manifest by irregularity of the epithelial wall, vacuolation, stratification and crowding of epithelial cells, as well as infiltration of lymphocytes. Occasionally, bile duct damage can appear, clinically associated with a cholestatic course of the disease. Intralobular changes include acidophilic degeneration of hepatocytes and the development of acidophil bodies.

Except for the mildest forms, periportal lesions between connective tissue and parenchyma are typical for chronic hepatitis. The inflammatory infiltrate extends into the neighboring parenchyma and leads to hepatocellular damage as shown in Figure 2-2. Due to the visible irregularity of the limiting plates of hepatocytes around the portal tracts these periportal lesions are classified as ‘piecemeal necrosis’. Today, however, the term ‘interface hepatitis’ is being preferred because of the involvement of apoptotic rather than necrotic processes. In more severe cases, a trapping of surviving hepatocytes may be observed within the infiltrate as well as the development of short fibrous septa that originate in the portal tracts. With the extension of the interface hepatitis and hepatocellular damage, periportal progenitor cells tend to activate and produce a ductular reaction. The consequences are proliferated bile ductules besides periportal chronic inflammatory cells that blur the margin of the portal tracts. Scattered neutrophils near ductules are typical constituents of the ductular reaction.

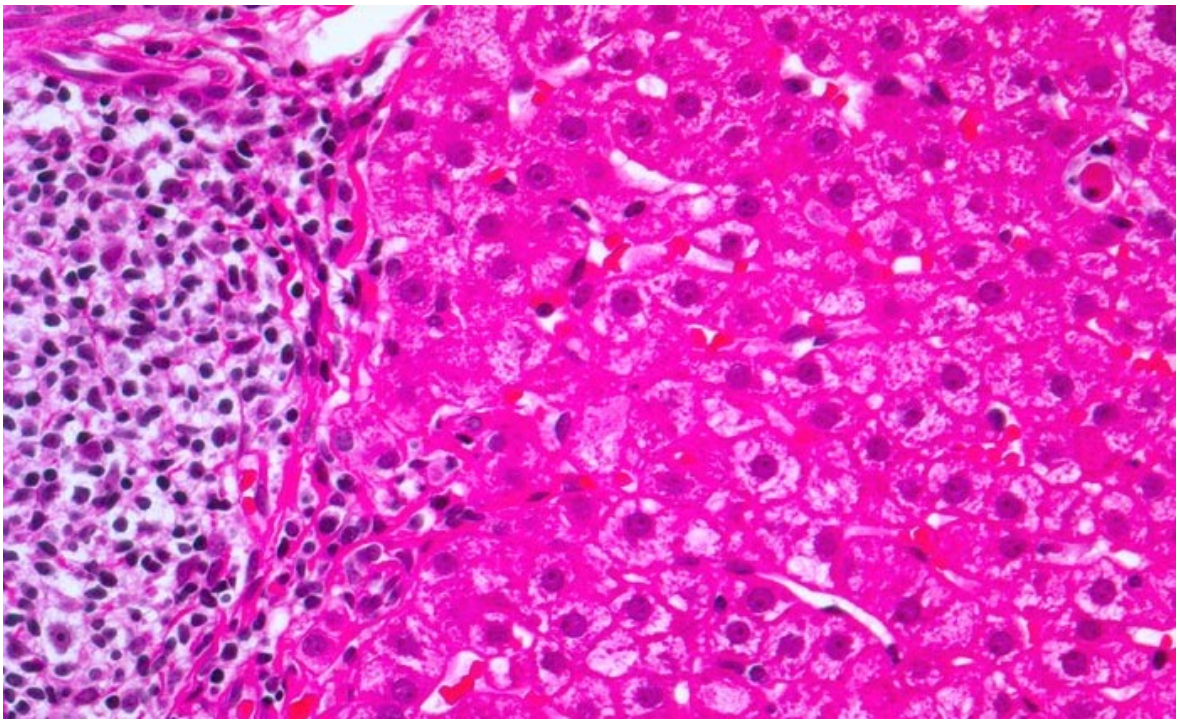


Figure 1-3: Chronic hepatitis C with mild activity. Left: Portal tract with mononuclear infiltration. The infiltration extends into the neighbouring parenchyma (Interface hepatitis). Right: Liver parenchyma with single Councilman-body (H&E). Used with friendly permission from Glatz-Krieger K., Institute of Pathology, Basel.[27]

Inside the lobuli, parenchymal lesions of varying degrees can be present. The finding of focal necrosis is typical. These areas represent a focal loss of hepatocytes with lymphoid infiltration, macrophages and other cells. The area of these lesions can be equal to about 5 hepatocytes. If larger, they are referred to as confluent or bridging necrosis, an uncommon finding in chronic hepatitis C. Lobular hepatitis without portal and periportal inflammation is rare. If lobular activity is severe, gland-like clusters of trapped hyperplastic hepatocytes can appear in a collapsed and inflamed area of parenchyma. An occasional feature in perivenular regions are multinucleated giant hepatocytes. Sinusoids can be focally or diffusely infiltrated by lymphocytes. Epitheloid-cell granulomas are an occasional finding in lobules and portal tracts. In periportal hepatocytes, clumped material and Mallory-bodies can be found.

Further typical changes in chronic hepatitis C include steatosis, iron deposition in hepatocytes, macrophages and endothelial cells as well as oncotic change of hepatocytes caused by mitochondrial hyperplasia. Sometimes, eosinophilic and diastase periodic acid-Schiff (PAS) positive inclusions can be found in the endothelial cells of the sinusoids. Steatosis, often associated with obesity, diabetes mellitus and alcohol consumption, is

more common in hepatitis C than in other types of chronic hepatitis and a risk factor for progression. Steatohepatitic features and pericellular fibrosis have been reported as well.

In early courses of the disease, the changes are usually mild with little interface hepatitis and fibrosis. With the progression of disease, fibrous septa develop emanating from enlarged portal tracts and linking vascular structures or other portal tracts as seen in Figure 1-4.[28] Porto-central septa correspond to bridging necrosis in acute exacerbation of chronic hepatitis. Ethyl-toxic or medical-toxic causes can be the reason for an exacerbation as well and can accelerate the development of fibrosis An active shove of chronic hepatitis C can imitate the histological image of an acute hepatitis. After elimination of the virus by antiviral therapy the infiltration disappears while portal or septal fibrosis tends to stay and decline only after many years.[26]

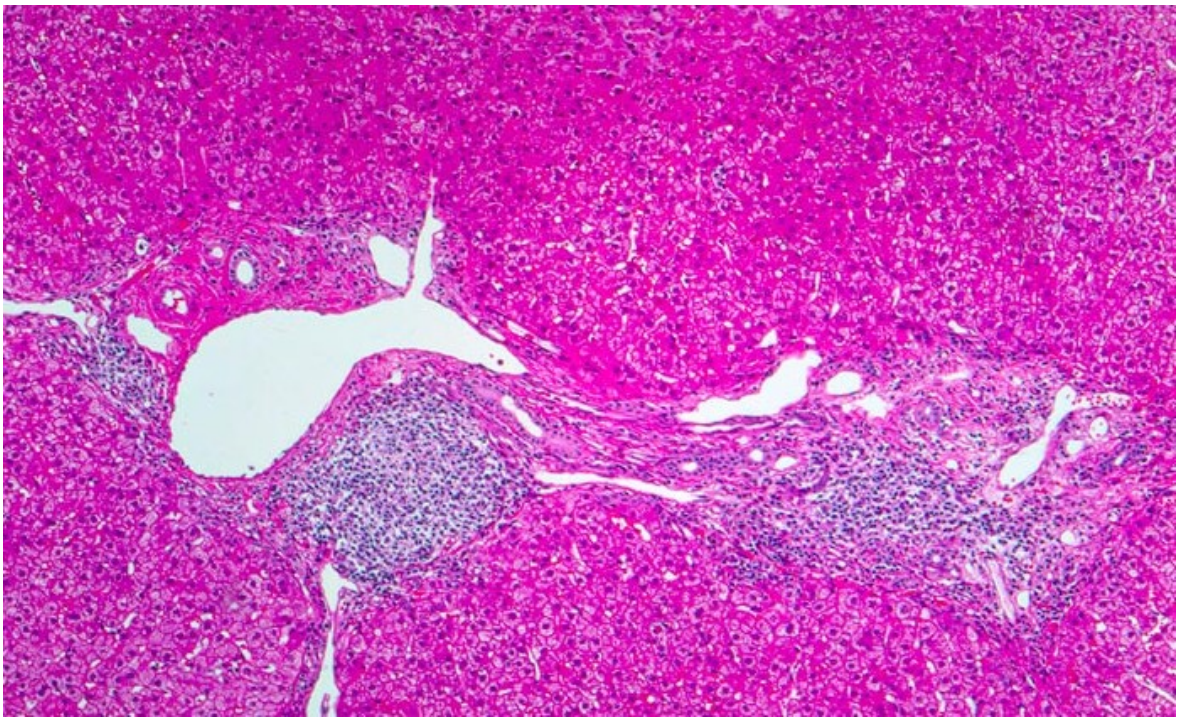


Figure 1-4: Chronic hepatitis C with mild activity and fibrosis. A dense lymphoid infiltration of portal tracts with development of follicles and fibrotic portal to portal septa is visible. In some places around the portal tracts, interface hepatitis is present. Used with friendly permission from Glatz-Krieger K., Institute of Pathology, Basel.[29]

1.8.4 HISTOPATHOLOGY OF CIRRHOSIS

Macronodular cirrhosis is the end stage of liver damage caused by hepatitis C. The development of fibrotic septa and irregularly shaped regenerative parenchymal nodes with a size of several centimeters leads to destruction of the liver´s lobular and vascular architecture. The nodes contain portal tracts and efferent veins and are surrounded by

broad and irregular fibrotic bands and scars as a consequence of the vast necrosis. hepatitis C related cirrhosis features inflammatory infiltration as well as necrosis of parenchyma as a sign for progress of the disease. A transition into a micronodular or mixed type is possible through fractionation of big nodes and reversely, a change into a mixed or macronodular type is possible through constant regeneration and enlargement of small nodules.[1]

1.8.5 HISTOPATHOLOGICAL ASSESSMENT OF INFLAMMATORY ACTIVITY AND FIBROSIS IN CHRONIC HCV INFECTION

Numerical grading and staging systems have been developed due to the need for critical evaluation of histological features in clinical trials. Low intra- and inter- observer variation, reasonable applicability and reproducibility and competent assessment of all crucial features of the disease are prerequisites for all these systems. Grading and staging algorithms however, are approximations and may suffer from potential sampling error depending on the size and quality of the biopsy used.[30] Intra and inter-observer variation can influence data acquired using scoring systems.[17]

Traditionally applied for neoplasms, grading describes the degree of differentiation and staging the extent of spread. With some modifications these concepts can also be used on histopathological assessment of chronic hepatitis C. Grading can be used as a measure of necroinflammatory activity and staging as a measure of structural progression of the disease, in particular architectural changes and fibrosis. These histological features are supposed to indicate severity and progression of chronic hepatitis C and may be of prognostic significance determining the treatment of a patient.[30]

1.8.5.1 The modified staging and the modified histological activity index (HAI) by Ishak et al.

According to Ishak et al.[30], a reasonable histological grading algorithm should include the assessment of periportal, portal and intraacinar inflammatory cell infiltration as well as various forms of liver cell damage and necrosis as they are crucial factors of severity, extent and prognosis of the disease. Their extent may predict an evolution toward fibrosis and cirrhosis.[31] All these factors have been included into the modified histological activity index by Ishak et al.[30] The categories and scores for the performance of grading according to Ishak et al.[30] are given in Table 1-2.

Necroinflammatory scores	
HAI A: Periportal or periseptal interface hepatitis (piecemeal necrosis)	**Score**
Absent	0
Mild: (focal, few portal areas)	1
Mild/moderate (focal, most portal areas)	2
Moderate (continuous around < 50% of tracts or septa)	3
Severe (continuous around > 50% of tracts or septa)	4
HAI B: Confluent necrosis	**Score**
Absent	0
Focal confluent necrosis	1
Zone 3 necrosis in some areas	2
Zone 3 necrosis in most areas	3
Zone 3 necrosis+ occasional portal-central (P-C) bridging	4
Zone 3 necrosis+ multiple P-C bridging	5
Panacinar or multiacinar necrosis	6
HAI C: Focal lytic necrosis, apoptosis and focal inflammation	**Score**
Absent	0
One focus or less per 10x objective	1
Two to four foci per 10x objective	2
Five to ten foci per 10x objective	3
More than ten foci per 10x objective	4
HAI D: Portal inflammation	**Score**
None	0
Mild, some or all portal areas	1
Moderate, some or all portal areas	2
Moderate/marked, all portal areas	3
Marked, all portal areas	4
Total HAI Score	Max. 18

Table 1-2 Modified histological activity index (HAI) by Ishak et al. [30]

According to Ishak et al.[30] the following additional features should be noted separately:

- Bile-duct inflammation and damage
- Lymphoid follicles
- Steatosis, mild, moderate or marked
- Hepatocellular dysplasia, large- or small-cell and adenomatous hyperplasia
- Iron or copper overload and intracellular inclusions
- Immunohistochemical findings and information on viral antigens, lymphocyte subsets or other features

The histological features to be considered for the staging of chronic hepatitis C are fibrosis, architectural changes and cirrhosis. The Ishak fibrosis score is given in Table 1-3. Figure 1-5 shows the corresponding histological image for each stage of fibrosis.

Change	Score
No fibrosis	0
Fibrous expansion of some portal areas, with or without short fibrous septa	1
Fibrous expansion of most portal areas, with or without short fibrous septa	2
Fibrous expansion of most portal areas with occasional portal to portal (P-P) bridging	3
Fibrous expansion of portal areas with marked bridging [portal to portal (P-P) as well as portal to central (P-C)]	4
Marked bridging (P-P and/or P-C) with occasional nodules (incomplete cirrhosis)	5
Cirrhosis, probable or definite	6
Maximum possible score	6

Table 1-3: Modified staging: architectural changes, fibrosis and cirrhosis (Ishak fibrosis score).[30]

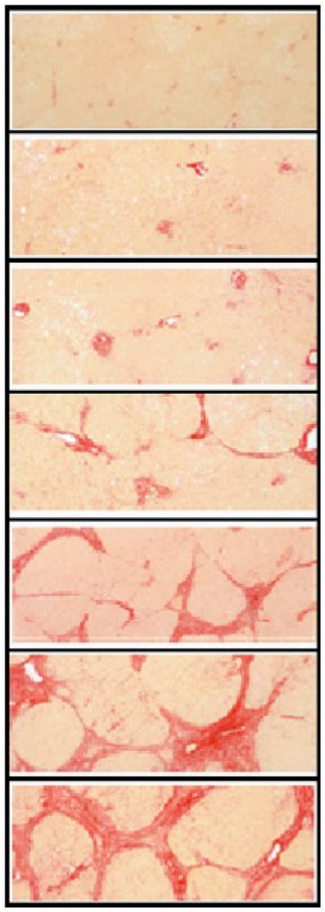


Figure 1-5: Architectural changes (Sirius red staining for collagen) according to stages of the Ishak fibrosis score.[32]

Additional features that should be noted but not scored according are intra-acinar fibrosis, perivenular fibrosis and phlebosclerosis of terminal hepatic venules.[30]

1.8.5.2 The METAVIR scoring system

Bedossa and Poynard[31] proposed a different algorithm for grading. This algorithm takes into account piecemeal necrosis and lobular necrosis. Portal inflammation has initially been included into a questionnaire during the phase of development, but this factor did not ameliorate the algorithm accuracy. Therefore it was taken out of the final algorithm. The coding values are globally assessed and integrated, taking into account that periportal and intralobular necroinflammatory lesions are related to the same mechanism of pathogenesis. The METAVIR grading questionnaire is shown in Table 1-4. The final algorithm is given in Table 1-5. A staging system has been proposed as well, which is exactly described in Table 1-6.[31]

Score	**Focal lobular necrosis**
0	Less than 1 necroinflammatory focus per lobule
1	At least one necroinflammatory focus per lobule
2	Several necroinflammatory foci per lobule or confluent or bridging necrosis
Score	**Portal inflammation**
0	Absent
1	Presence of mononuclear aggregates in some portal tracts
2	Mononuclear aggregates in all portal tracts
3	Large and dense mononuclear aggregates in all portal tracts
Score	**Piecemeal necrosis**
0	Absent
1	Focal alteration of the periportal plate in some portal tracts
2	Diffuse alteration of the periportal plate in some portal tracts or focal lesions around all portal tracts
3	Diffuse alteration of the periportal plate in all portal tracts
Score	**Bridging necrosis**
0	Absent
1	Present

Table 1- 4: METAVIR questionnaire for the performance of grading[31]

Score	Piecemeal necrosis
PMN 0	None
PMN 1	Mild
PMN 2	Moderate
PMN 3	Severe
Score	**Lobular necrosis**
LN 0	None or mild
LN 1	Moderate
LN 2	Severe
Score	**Histological activity**
A0	None
A1	Mild
A2	Moderate
A3	Severe

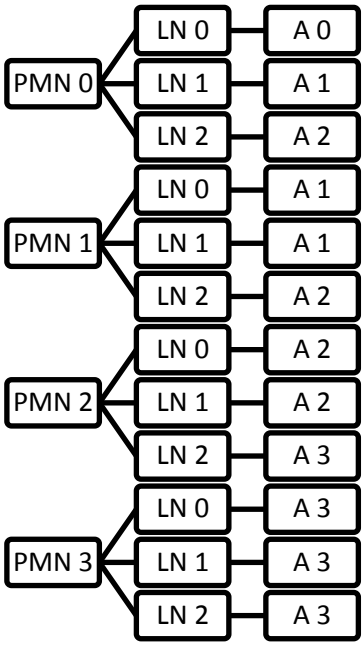


Table 1-5: METAVIR grading algorithm.[31]

Score	Description
0	No fibrosis
1	Stellate enlargement of portal tract but without septa formation
2	Enlargement of portal tract with rare septa formation
3	Numerous septa without cirrhosis
4	Cirrhosis

Table 1-6: METAVIR fibrosis score.[31]

2 OBJECTIVES

The stage of fibrosis has an impact on prognosis and therapeutic intervention in chronic hepatitis C. The risk of developing cirrhosis may increase with the expansion of fibrosis.[33] Patients with genotype 1 are usually difficult to treat. The decision for antiviral treatment depends on various factors, one of them the presence of significant fibrosis. In contrast, its absence may support a decision to defer antiviral treatment. Liver biopsy is the gold standard for the evaluation of activity and stage of fibrosis.[18] Nonetheless, it has limitations because of its invasive nature,[13-15] possible sampling error and intra- as well as interobserver variability.[16,17] Therefore noninvasive tests to estimate hepatic fibrosis are requested by clinicians and patients.[34] Several noninvasive scores containing routine clinical and biochemical parameters (age, AST, ALT, INR, platelet count) have been proposed for prediction of hepatic fibrosis and cirrhosis, such as the AST/ALT ratio (AAR), AST to platelet ratio index (APRI), FIB-4 and Model 3.[19-24] Platelet count per se was also proposed as a predictor of fibrosis and cirrhosis.[35] In previous studies, these noninvasive tests have been examined in populations with unselective or high ALT values partially obtaining high diagnostic accuracies for prediction of significant fibrosis and cirrhosis.[19-24,36] However, diagnostic performance of these tests in patients with chronic hepatitis C and persistently normal ALT is unknown yet. This subgroup of patients, however, would benefit most from accurate noninvasive fibrosis tests. They amount to 25-30% of patients with chronic hepatitis C[37] and often show a milder course of disease.[10.] Nonetheless some of them develop advanced fibrosis and cirrhosis.[38] Non-invasive fibrosis tests which could substitute liver biopsy in these patients would be appreciable. The ultimate goal of this study was to evaluate the diagnostic performance of four noninvasive tests for the prediction of fibrosis and cirrhosis in patients with chronic hepatitis C and persistently normal ALT. The applicability of platelet count per se as a predictor of fibrosis and cirrhosis has been tested additionally. Towards this end we assessed and compared patients with varied, elevated and persistently normal serum ALT levels with regard to:

1. The distribution of fibrosis stages and grades of histological activity.

2. The correlation of noninvasive fibrosis with histological stages of fibrosis.

3. The accuracy of the noninvasive fibrosis tests for prediction of fibrosis and cirrhosis.

3 MATERIALS AND METHODS

3.1 PATIENTS

This study is a secondary research that uses databases from a concluded Austrian retrospective multi-center cohort study (study number P03327). This multicenter study investigated efficacy and safety of antiviral treatment with PEG-interferon alpha-2b and ribavirin in patients with chronic hepatitis C and either elevated ALT (group A) or persistently normal ALT (group B). Liver biopsy was performed at baseline as part of the study protocol in order to assess fibrosis stage. The purpose of liver biopsy was explained in the informed consent form.[39]

A complete database comprising laboratory data, demographic data as initials, sex, date of birth, body height, body weight, HCV genotype, date of laboratory value and date of biopsy of a total number of 134 treatment-naïve patients, who had undergone percutaneous liver biopsy at the participating study centres, were available. Chronic hepatitis C was proven histologically. Laboratory data was accepted if retrieved less than 30 days from the day of liver biopsy. The obtained laboratory data included aspartate aminotransferase (AST), alanine aminotransferase (ALT), prothrombin time (PT) given by the International normalized ratio and platelet blood cell count.

In order to perform histological staging and grading, the archived liver biopsy specimens were sent to the Institute of Pathology of the University of Graz from all participating centres for examination. Table 3-1 shows the list of cooperating centers and the number of patients retrieved from each study centre. However, only selected patients with comprehensive data could be comprised in the current study.

103 patients had complete data for histological assessment and calculation of the noninvasive fibrosis tests. The data used in the previous study included a stratification for normal ALT (group B) and high ALT patients (group A). Patients were classified as normal ALT patients, if two measurements in 6 months showed normal ALT levels. However, reviewing this categorization and comparing it to the current ALT values retrieved within 30 days from liver biopsy we found patients with to some extent elevated ALT levels among group B. In order to apply a more rigorous categorization which takes into account the current histology and corresponding laboratory data, we applied a cutoff

value of ALT/ULN > 1 to newly stratify the study population of 103 patients into two groups. This way, we could exclude several patients with fluctuating ALT levels from our subgroup of patients with persistently normal ALT. Those with an ALT/ULN-level of > 1 were classified as patients with elevated ALT (45), those with an ALT/ULN-level of ≤ 1 as patients with persistently normal ALT (58).

Cooperating center	**Patients available**	**Patients included**
LKH Graz, Department of Internal Medicine, Institute of Pathology, Medical University of Graz	13	9
LKH Hörgas, Department of Internal Medicine	7	5
LKH Leoben, Department of Internal Medicine, Institute of Pathology	15	11
Elisabethinen Hospital Linz, Department of Internal Medicine	16	12
LKH Innsbruck, Department of Internal Medicine, Institute of Pathology, Medical University of Innsbruck	24	21
Hietzing Hospital, Lainz, Vienna, Department of Internal Medicine	40	30
Kaiser Franz-Josef Hospital Vienna, Department of Internal Medicine, Institute of Pathology and Bacteriology	10	8
Rudolfstiftung Hospital Vienna, Department of Internal Medicine Institute of Pathology and Microbiology	9	7
Total	134	103

Table 3-1 List of the cooperating centers.

3.2 LIVER BIOPSIES

The histological tissues were obtained by performance of a percutaneous needle biopsy with a Menghini needle. The specimens were fixed in formalin and embedded in paraffine. 4-µm sections were produced by using a microtome. Afterwards, one section was stained

with hematoxylin and eosin and another one with chromotrope aniline blue (CAB). The first staining was used to perform histological grading, the second one for the assessment of fibrosis stage. All necessary liver specimens were then were mailed to the Institute of Pathology of the Medical University of Graz for analysis. The histopathological examination of all liver biopsies was performed by an experienced single pathologist (L.C., Institute of Pathology, Medical University of Graz) in cooperation with a medical student (T.W.). The cooperative histological examination was performed without knowledge of the laboratory and clinical findings. Decision of grade and stage in the individual case has been made by consensus. To perform histological grading and staging the semi-quantitative scoring systems of Ishak et al.[30] as well as METAVIR[31] were applied simultaneously giving the possibility to compare the results with a larger number of studies. Rozario et al.[18] tested the relationship between the Ishak and METAVIR scoring systems. Concordance for necroinflammatory change was described as good, concordance between fibrosis as excellent. In addition we estimated the grade of steatosis using a four-step scale: (0- 5%, 5-33%, 34-66% and >66%). Only biopsy slides with 6 or more portal tracts have been considered representative. Slides with less than 6 portal tracts, missing stains, other overlapping liver disease or fragmentation were excluded.

3.3 LABORATORY DATA

Laboratory data was accepted if retrieved less than 30 days from the day of liver biopsy. Aspartate aminotransferase, alanine aminotransferase, prothrombin time (international normalized ratio) and platelet blood cell count were routinely determined in clinical laboratories of all participating centers. The various participating centers used different upper limits of normal for aminotransferases. Furthermore the upper limits of normal changed in course of the study in consequence of the introduction of the International Federation of Clinical Chemistry reference method for determination of aminotransferase activity at 37° C. Therefore, to achieve comparable values we used the ratios of transaminases and their upper limits of normal for analysis. The reference range for platelet blood cell count was 140 to 440 x 10^9/L.

3.4 NON-INVASIVE FIBROSIS TESTS

AAR, APRI, FIB-4 and Model 3 were calculated from clinical and laboratory variables as described in the original reports.[19-24]

3.5 STATISTICAL METHODS

All statistical analyses were performed using SPSS/PASW software version 18 (SPSS Inc., Chicago, IL). Patient characteristics and the distribution of laboratory data are given as mean ± standard deviation or median and interquartile range where appropriate. Frequency tables were used to present results of the histological scoring. The relationship between histological score and laboratory data as well as noninvasive tests was measured with the bivariate Spearman´s rank correlation coefficient. P-values were given as two-sided probabilities. The significance level was 0.05. Distributions of noninvasive fibrosis test values and platelet count among stages of fibrosis are given as minimum, maximum, median and interquartile range and were examined using nonparametric tests (Kruskal-Wallis H test and Mann Whitney U test) to find relevant differences. Bonferroni correction was applied for multiple testing to avoid an increase of type I error. Grades of activity and stages of fibrosis were compared between the subgroups with a Mann-Whitney U test. For each noninvasive fibrosis test and platelet count receiver operating characteristic curves were constructed. Diagnostic accuracies of the noninvasive tests were evaluated calculating sensitivity, specificity, positive predictive value, negative predictive value and the area under the receiver operating characteristic curve (AUROC) for each test. Individual optimized cutoff values for each test were obtained from coordinate points of each receiver operating characteristic curve and are specified by sensitivity, specificity and the Youden-index. All analyses were performed for the whole study group and separately in the groups of patients with persistently normal ALT and elevated ALT levels.

4 RESULTS

4.1 RESULTS IN THE WHOLE STUDY POPULATION

4.1.1 BASELINE CHARACTERISTICS OF THE STUDY POPULATION

First, we analyzed the data of the whole study population. 103 patients were studied. Table 4-1 summarizes the clinical and demographic characteristics of all patients at the time of liver biopsy. The sex ratio was almost equal in the whole study population. 64% of patients suffered from HCV genotype 1.

Characteristics of the study population		
Total population		103 patients
Sex	Female	49 (48.0 %)
	Male	54 (52.0 %)
Age		41 ± 11 a
BMI		23.8 ± 4.6
HCV genotype	1	66 (64.0 %)
	2	8 (7.8 %)
	3	21 (20.4 %)
	4	8 (7.8 %)
Subgroups	Persistently normal ALT	58 patients (56.3 %)
	Elevated ALT	45 patients (44.7 %)

Table 4-1 Clinical and demographic characteristics of the study population. Data is given as count and percentage, as well as mean and standard deviation where appropriate. For abbreviations see appendix.

4.1.2 ANALYSIS OF LABORATORY DATA

To calculate the noninvasive fibrosis tests AAR, APRI, FIB-4 and Model 3 the following laboratory parameters were gathered from all patients: levels of AST, ALT and the corresponding upper limits of normal, prothrombin time given as International normalized ratio and platelet blood cell count. Serum ALT levels ranged from 0.22 to 2.24 times the upper limit of normal in the whole study population. Relevant gender differences were not evident. Table 4-2 and Figure 4-1 give exact information about the distribution of laboratory data in male and female patients.

	Sex	Min	Max	Median	25% Quartile	75% Quartile
AST/ULN	Female	0.40	5.43	0.87	0.73	1.26
	Male	0.51	9.17	1.00	0.70	1.84
ALT/ULN	Female	0.29	8.49	0.88	0.71	1.34
	Male	0.22	19.60	1.00	0.68	2.24
PT/INR	Female	0.85	1.18	1.00	0.95	1.05
	Male	0.88	1.55	1.03	0.99	1.06
Platelet count	Female	109	441	255	214	301
	Male	98	314	203	175	272

Table 4-2: Distribution of laboratory data among female and male patients in the whole study population. Data is presented with minimum, maximum, median and quartiles for all laboratory parameters. The used unit of platelet count is x10^9/L.

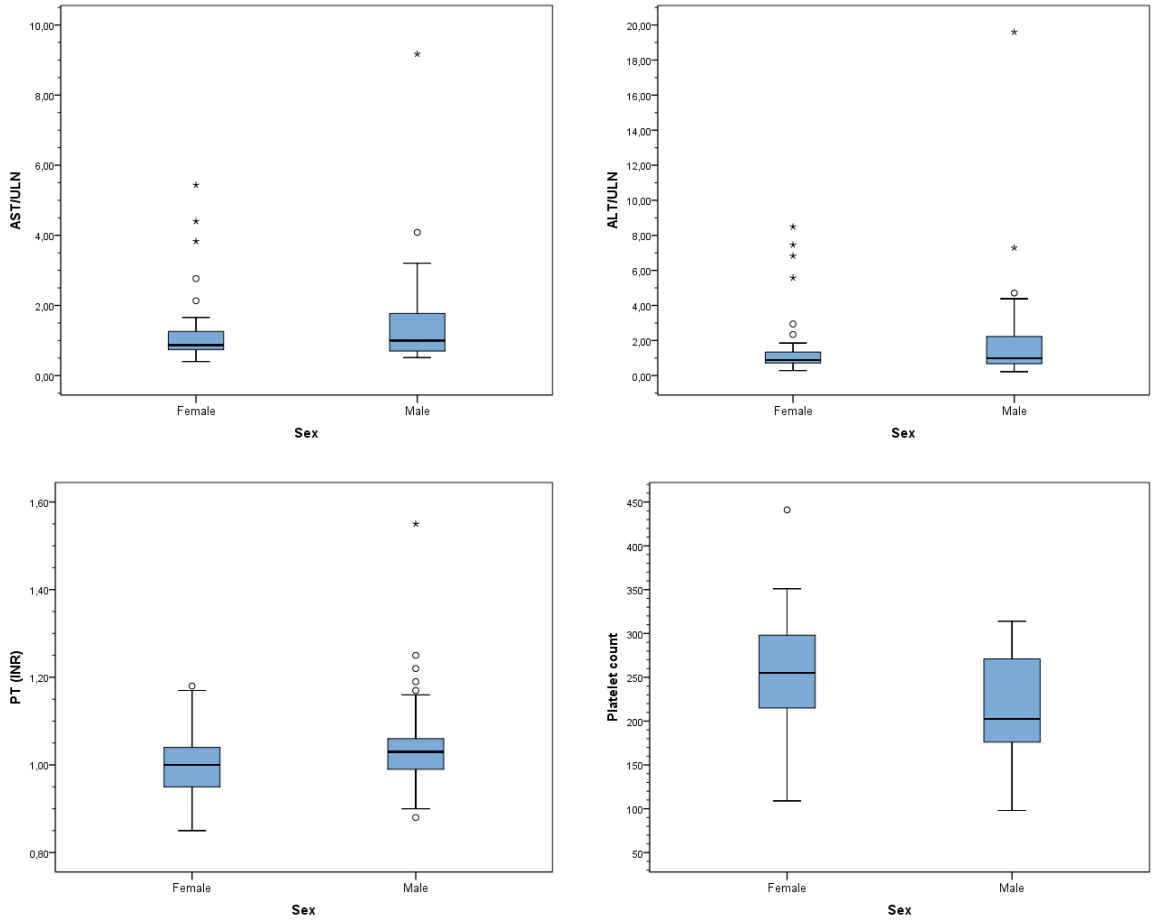


Figure 4-1: Boxplots of the distribution of laboratory parameters among male and female patients in the whole study population. The used unit of platelet count is x 10^9/L.

4.1.3 HISTOLOGICAL EVALUATION

The histological assessment comprised the performance of grading of histological activity and histological staging of fibrosis. The semi-quantitative scoring systems proposed by Ishak et al.[30] and the French METAVIR group[31] were applied simultaneously to enhance the comparability with other studies. Furthermore, grades of steatosis and iron deposition were estimated. General biopsy characteristics are given in Table 4-3.

Biopsy characterictics		
Total number of biopsy slides		103
Length of biopsy core (mm)		19 ± 9
Number of portal tracts, median (Q1, Q3)		11 (7, 15)
Histological grading		Ishak modified histological activity index
		METAVIR histological activity
Histological staging		Ishak fibrosis score
		METAVIR fibrosis score
Iron deposition		0 (0%)
Steatosis	0-5%	80 (77.7 %)
	6-33%	20 (19.4 %)
	34-66%	2 (1.9 %)
	> 66%	1 (1.0 %)

Table 4-3: Biopsy characteristics. Data is presented as mean and standard deviation, median and quartiles, count and percentage where appropriate.

4.1.3.1 Histological grading

The grading results for the Ishak histological activity index (HAI) are presented in Figure 4-2. HAI B, corresponding to confluent necrosis, has not been estimated as it is an uncommon finding in chronic hepatitis C. Total HAI results were grouped into three categories. A score reaching from 0 to 6 was defined as mild activity, a score between 7 and 12 as moderate activity, respectively. Scores between 13 and 18 would correspond to severe activity, however the highest score reached in our study population was 12. Low grades were predominant. 79.5% of all biopsies showed mild activity of inflammation. Male patients prevailed among higher grades of activity.

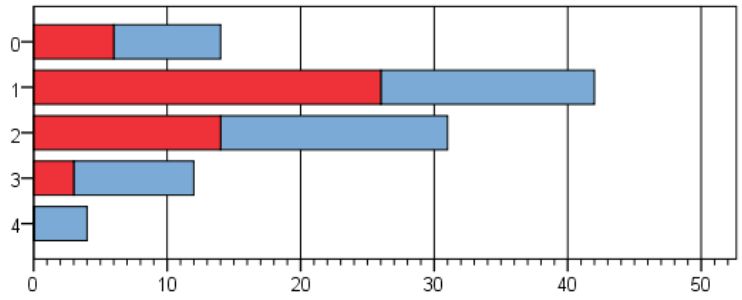
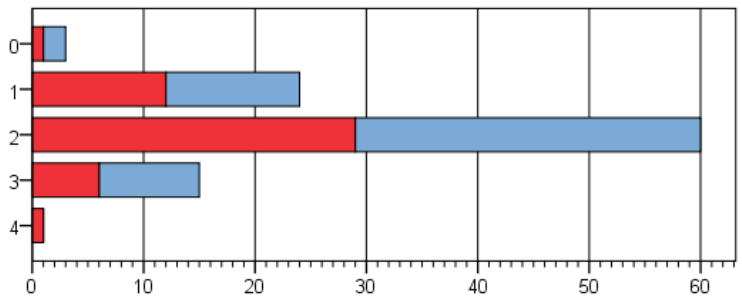
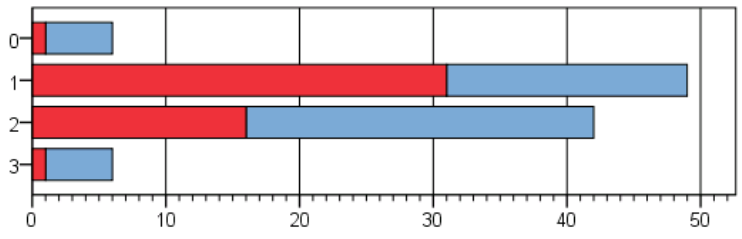
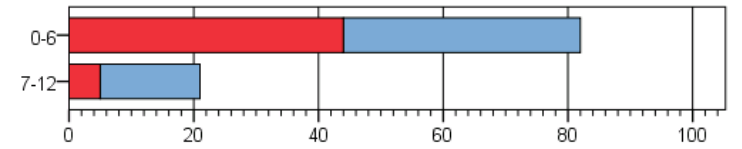
		Ishak modified histological activity index				
	Score	Count			%	Count Colors: red: female/ blue: male
		F	M	Total	Total	
HAI A	0	6	8	14	13.6	
	1	26	16	42	40.8	
	2	14	17	31	30.1	
	3	3	9	12	11.7	
	4	0	4	4	3.9	
HAI C	0	1	2	3	2.9	
	1	12	12	24	23.3	
	2	29	31	60	58.3	
	3	6	9	15	14.6	
	4	1	0	1	1.0	
HAI D	0	1	5	6	5.8	
	1	31	18	49	47.6	
	2	16	26	42	40.8	
	3	1	5	6	5.8	
Total HAI	0-6	44	38	82	79.5	
	7-12	5	16	21	20.4	

Figure 4-2: Application of the modified histological activity index by Ishak et al.[30] for the performance of grading in the whole study population. Results are given gender segregated as count, total percent and stacked bars for each category. Colors: red: female, blue: male. For abbreviations see appendix.

The METAVIR grading algorithm integrates the scores of the categories "piecemeal necrosis" and "lobular necrosis" to estimate the activity of inflammation, given by METAVIR A. The categories of METAVIR A differ from those used for the total modified histological activity index. This led to somewhat different results. 57.4% of patients showed no or mild activity. Interestingly, 16.5% of biopsies were classified as A3, corresponding to severe inflammatory activity. Figure 4-3 gives exact information about the results of the METAVIR algorithm. Similarly, male patients were predominant among higher grades of activity.

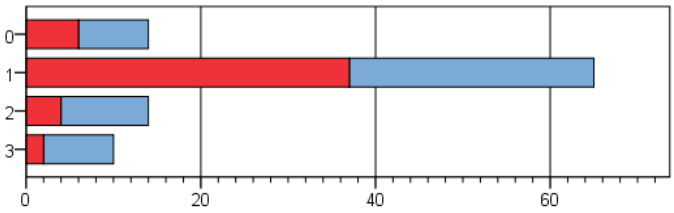
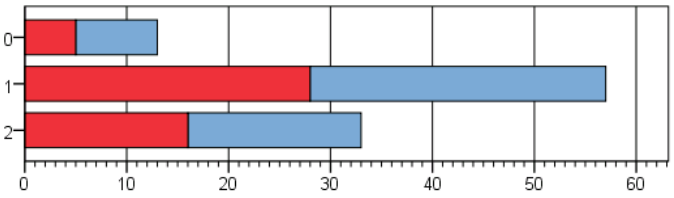
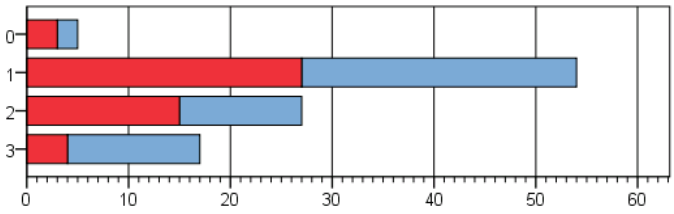
METAVIR activity						
	Score	Count			%	Count Colors: red: female/ blue: male
		F	M	Total	Total	
METAVIR PMN	0	6	8	14	13.6	
	1	37	28	65	63.1	
	2	4	10	14	13.6	
	3	2	8	10	9.7	
METAVIR LN	0	5	8	13	12.6	
	1	28	29	57	55.3	
	2	16	17	33	32.0	
METAVIR A	0	3	2	5	4.9	
	1	27	27	54	52.4	
	2	15	12	27	26.2	
	3	4	13	17	16.5	

Figure 4-3: Application of the METAVIR algorithm for the performance of grading in the whole study population. Results are given gender segregated as count, total percent and stacked bars for each category. Colors: red: female, blue: male. For abbreviations see appendix.

In order to measure the strength of correlation between histological activity and laboratory data, the bivariate Spearman´s rank correlation coefficient was used. Weak to moderate correlations were found for AST/ULN and ALT/ULN. The results are given in Table 4-4.

Correlation of grade with standard laboratory parameters		
	Modified histological activity index	METAVIR A
AST/ULN	0.33**	0.31**
ALT/ULN	0.41**	0.37**
PT/INR	0.16	0.13
Platelet count	-0.12	-0.08

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

Table 4-4: Correlation (Spearman´s rank correlation coefficient, two-tailed) of grade with standard laboratory parameters in the whole study population. Histological grades were estimated with the modified histological activity index by Ishak et al.[30] and the METAVIR grading algorithm.

4.1.3.2 Histological staging

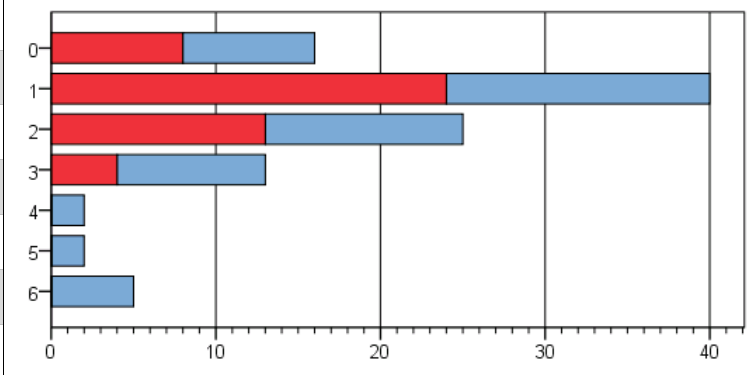
Stages of fibrosis were estimated by the Ishak and METAVIR fibrosis scores. Regarding the results of the Ishak fibrosis score, 78.6% of all patients showed low stages of fibrosis, i.e. in these patients the fibrotic expansion did not exceed stage F2. Stage F6, equivalent to cirrhosis was found in 5 patients. They were all male.

Using the METAVIR staging algorithm, 75.7% of patients showed low stages of fibrosis, which means the stage of fibrosis did not exceed F1. The number of cirrhotic patients did not differ between the scoring systems. However, stage F5 of the Ishak fibrosis score is defined as incomplete cirrhosis. Therefore, in many studies, patients with stage F5 are considered as cirrhotic patients as well. For ROC analysis which will be presented later, these two cases were added to the number of cirrhotic patients. Figure 4-4 shows the distribution of fibrosis stages for the Ishak fibrosis score and METAVIR fibrosis score, separated by gender. Male patients prevailed among cases with higher fibrosis stages.

Histological staging of fibrosis

Ishak fibrosis score

	Count			%	Count Colors: red: female/ blue: male
	F	M	Total	Total	
0	8	8	16	15.5	
1	24	16	40	38.8	
2	13	12	25	24.3	
3	4	9	13	12.6	
4	0	2	2	1.9	
5	0	2	2	1.9	
6	0	5	5	4.9	



METAVIR fibrosis score

	Count			%	Count Colors: red: female/ blue: male
	F	M	Total	Total	
0	8	8	16	15.5	
1	37	25	62	60.2	
2	4	12	16	15.5	
3	0	4	4	3.9	
4	0	5	5	4.9	

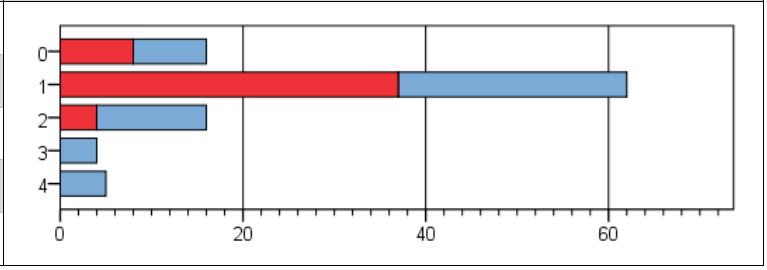


Figure 4-4: Application of the Ishak and METAVIR fibrosis score to estimate the stages of fibrosis in the whole study population. Results are given gender segregated as count, total percent and stacked bars for each category. Colors: red: female, blue: male. For abbreviations see appendix.

Equally, the Spearman´s rank correlation coefficient was used to estimate the correlation between stage of fibrosis and values of laboratory parameters. Weak positive correlations were found for AST/ULN and ALT/ULN, weak negative correlations for platelet count, respectively. The results were similar for both scoring systems. Exact information is given in Table 4-5.

Correlation of stage with standard laboratory parameters		
Laboratory data	**Ishak fibrosis score**	**METAVIR fibrosis score**
AST/ ULN	0.28**	0.26**
ALT/ ULN	0.34**	0.31**
PT/ INR	0.16	0.17
Platelet count	-0.22*	-0.21*

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

Table 4-5: Correlation (Spearman´s rank correlation coefficient, two-tailed) of stage with standard laboratory parameters in the whole study population. Histological stages of fibrosis were estimated with the Ishak and METAVIR fibrosis scores.

4.1.4 NON-INVASIVE TESTS FOR THE PREDICTION OF FIBROSIS AND CIRRHOSIS

AAR, APRI, FIB-4 and Model 3 were calculated exactly as described using the original formulas. The distribution of the test results among male and female patients is shown in Table 4-6 and Figure 4-5.

	Sex	**Min**	**Max**	**Median**	**25% Quartile**	**75% Quartile**
AAR	Female	0.33	1.75	0.87	0.65	1.15
	Male	0.36	3.00	0.75	0.60	0.85
APRI	Female	0.18	3.41	0.35	0.25	0.45
	Male	0.18	5.18	0.46	0.31	1.01
FIB-4	Female	0.22	2.58	0.80	0.57	1.12
	Male	0.28	3.65	1.11	0.69	1.75
Model 3	Female	0.05	0.46	0.20	0.11	0.28
	Male	0.85	0.98	0.24	0.15	0.39

Table 4-6: Distribution of the results of AAR, APRI, FIB-4 and Model 3 among female and male patients in the whole study population. Data is presented with minimum, maximum, median and quartiles for all noninvasive fibrosis tests.

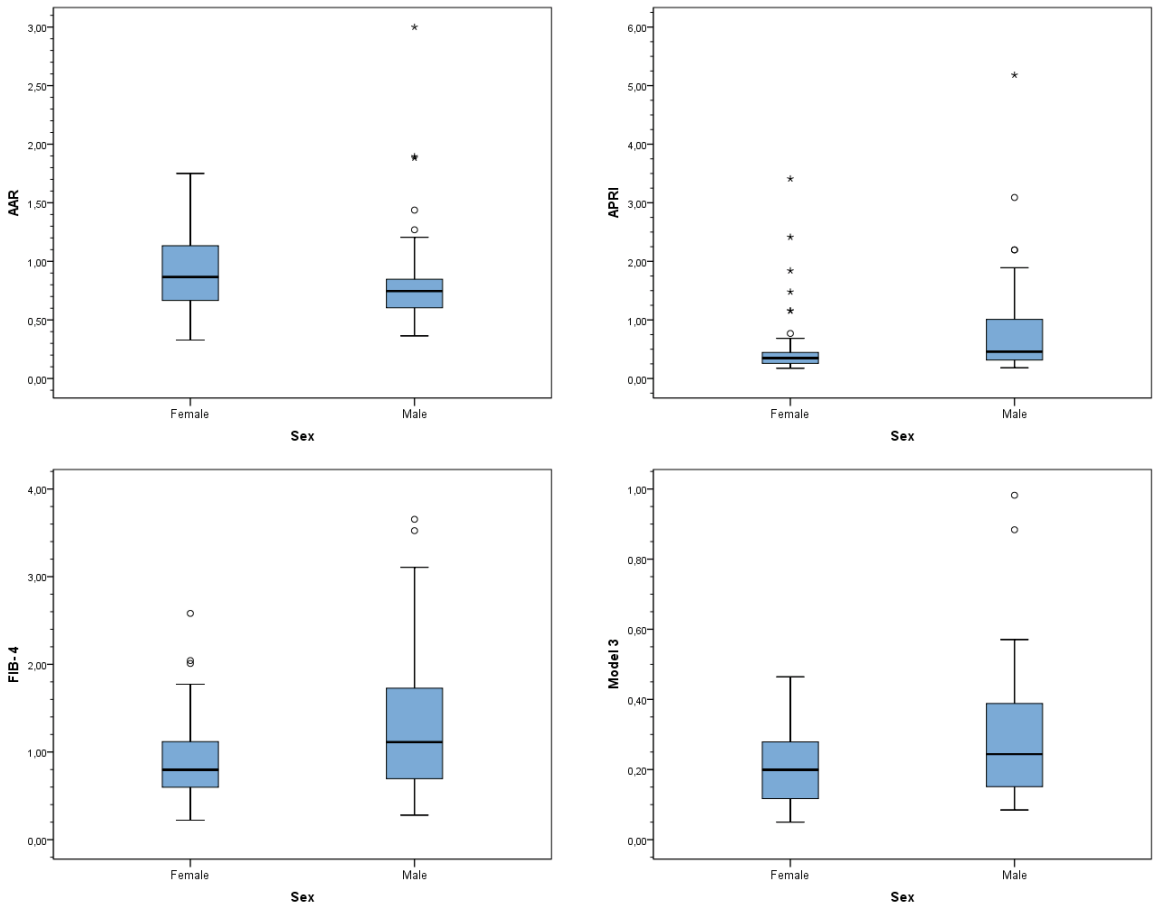


Figure 4-5: Boxplots of the distribution of noninvasive fibrosis tests among male and female patients in the whole study population.

In addition, the distributions of AAR, APRI, FIB-4 and Model 3 were examined for each stage of fibrosis. Platelet count was added to the four noninvasive fibrosis tests and examined equally, as it was proposed as a predictor of fibrosis itself.[35] The results are shown in Table 4-7 and Figure 4-6. A Kruskal-Wallis H test was performed to find significant differences in the distribution of the test results among stages of fibrosis. Significant differences among stages of the Ishak fibrosis score were found for APRI ($p = 0.002$) and FIB-4 ($p = 0.006$). For APRI and FIB-4, Mann Whitney U tests were applied for each pair of the Ishak fibrosis score. Bonferroni correction was applied to prevent an increase of type I error by multiple testing. For APRI, pairwise comparisons revealed significant differences between stage 0 and 6 ($p = 0.045$), as well as 1 and 6 ($p = 0.044$). For FIB-4, pairwise comparisons did not reveal significant differences when Bonferroni adjustment was applied.

	Ishak fibrosis score	Min	Max	Median	25% Quartile	75% Quartile	Kruskal-Wallis H test
AAR	0	0.48	3.00	0.93	0.77	1.25	n.s.
	1	0.33	1.75	0.84	0.64	1.14	
	2	0.45	1.18	0.76	0.60	1.00	
	3	0.39	1.21	0.63	0.53	0.84	
	4	0.59	0.75	0.67	0.59	--	
	5	0.60	0.61	0.60	0.60	--	
	6	0.57	1.88	0.69	0.58	1.31	
APRI	0	0.18	2.41	0.33	0.29	0.43	p = 0.002
	1	0.19	5.18	0.35	0.26	0.61	
	2	0.18	3.41	0.43	0.33	0.84	
	3	0.21	1.02	0.33	0.23	0.86	
	4	0.70	1.14	0.92	0.70	-	
	5	2.20	3.09	2.64	2.20	-	
	6	1.01	1.78	1.17	1.01	1.51	
FIB-4	0	0.22	2.02	0.80	0.65	1.19	p = 0.006
	1	0.28	2.04	0.87	0.53	1.17	
	2	0.51	3.65	0.96	0.71	1.41	
	3	0.24	2.29	0.85	0.61	1.37	
	4	1.56	2.34	1.95	1.56	-	
	5	2.42	3.11	2.76	2.42	-	
	6	1.20	3.52	1.87	1.22	3.17	
Model 3	0	0.05	0.88	0.25	0.17	0.31	n.s.
	1	0.05	0.46	0.20	0.14	0.28	
	2	0.07	0.48	0.21	0.12	0.30	
	3	0.08	0.39	0.20	0.12	0.30	
	4	0.21	0.56	0.38	0.21	-	
	5	0.39	0.42	0.40	0.39	-	
	6	0.12	0.98	0.39	0.21	0.78	
Platelet count	0	169	346	247	193	303	n.s.
	1	109	441	246	201	295	
	2	129	346	231	189	275	
	3	137	311	259	174	288	
	4	150	193	172	150	-	
	5	98	121	110	98	-	
	6	109	300	179	137	280	

Table 4-7: Distribution of the results of AAR, APRI, FIB-4, Model 3 and Platelet count among stages of the Ishak fibrosis score in the whole study population. Data is presented with minimum, maximum, median and quartiles for all tests and platelet count. P-values have been estimated with the Kruskal-Wallis H test. The used unit of platelet count is $x10^9/L$.

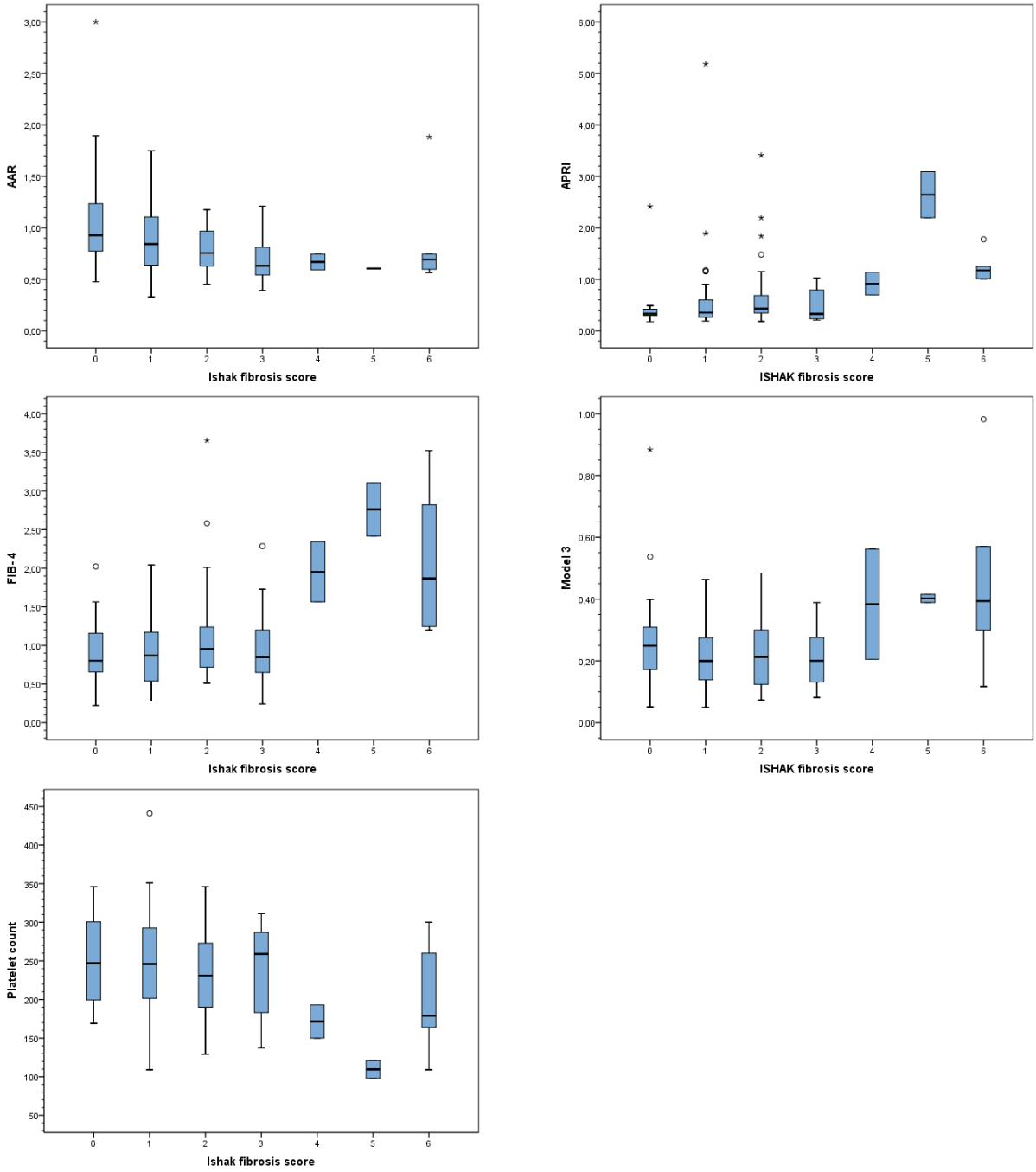


Figure 4-6: Boxplots demonstrating the distribution of the results of AAR, APRI, FIB-4, Model 3 and Platelet count among stages of the Ishak fibrosis score in the whole study population. The used unit of platelet count is $x10^9/L$.

The same tests have been performed for stages of the METAVIR fibrosis score. Detailed information is given in Table 4-8 and Figure 4-7. Significant differences were found in the distribution of APRI (p = 0.002), FIB-4 (p = 0.002) and platelet count (p = 0.042) among stages of METAVIR fibrosis score. For FIB-4, pairwise comparisons showed significant differences between stage 0 and 3 (p = 0.033) as well as stage 1 and 3 (p = 0.032) of the METAVIR fibrosis score. For APRI, pairwise comparisons showed significant differences between stage 0 and 4 (p = 0.021), 0 and 3 (p = 0.039), 2 and 4 (p = 0.050), and 1 and 4 (p

= 0.047). For platelet count, significant differences between stage 0 and 3 (p = 0.049) as well as 1 and 3 (p = 0.047) were found. The p-values were adjusted with Bonferroni correction for multiple testing.

	METAVIR fibrosis score	Min	Max	Median	25% Quartile	75% Quartile	Kruskal Wallis H-test
AAR	0	0.48	3.00	0.93	0.77	1.25	n.s.
	1	0.33	1.75	0.83	0.62	1.03	
	2	0.39	1.21	0.75	0.56	0.84	
	3	0.59	0.75	0.60	0.59	0.71	
	4	0.57	1.88	0.69	0.58	1.31	
APRI	0	0.18	2.41	0.33	0.29	0.43	p = 0.002
	1	0.18	5.18	0.39	0.27	0.62	
	2	0.21	1.15	0.34	0.24	0.89	
	3	0.70	3.09	1.67	0.81	2.87	
	4	1.01	1.78	1.17	1.01	1.51	
FIB-4	0	0.22	2.02	0.80	0.65	1.19	p = 0.002
	1	0.28	3.65	0.93	0.63	1.19	
	2	0.24	2.29	0.79	0.59	1.45	
	3	1.56	3.11	2.38	1.76	2.93	
	4	1.20	3.52	1.87	1.22	3.17	
Model 3	0	0.05	0.88	0.25	0.17	0.31	n.s.
	1	0.05	0.46	0.21	0.13	0.28	
	2	0.08	0.48	0.22	0.12	0.33	
	3	0.21	0.56	0.40	0.25	0.53	
	4	0.12	0.98	0.39	0.21	0.78	
Platelet count	0	169	346	247	193	303	p = 0.042
	1	109	441	242	192	288	
	2	137	311	252	178	282	
	3	98	193	136	104	182	
	4	109	300	179	137	280	

Table 4-8: Distribution of the results of AAR, APRI, FIB-4, Model 3 and Platelet count among stages of the METAVIR fibrosis score in the whole study population. Data is presented with minimum, maximum, median and quartiles for all noninvasive fibrosis tests. P-values have been estimated by the Kruskal-Wallis H test. The used unit of platelet count is $x10^9/L$.

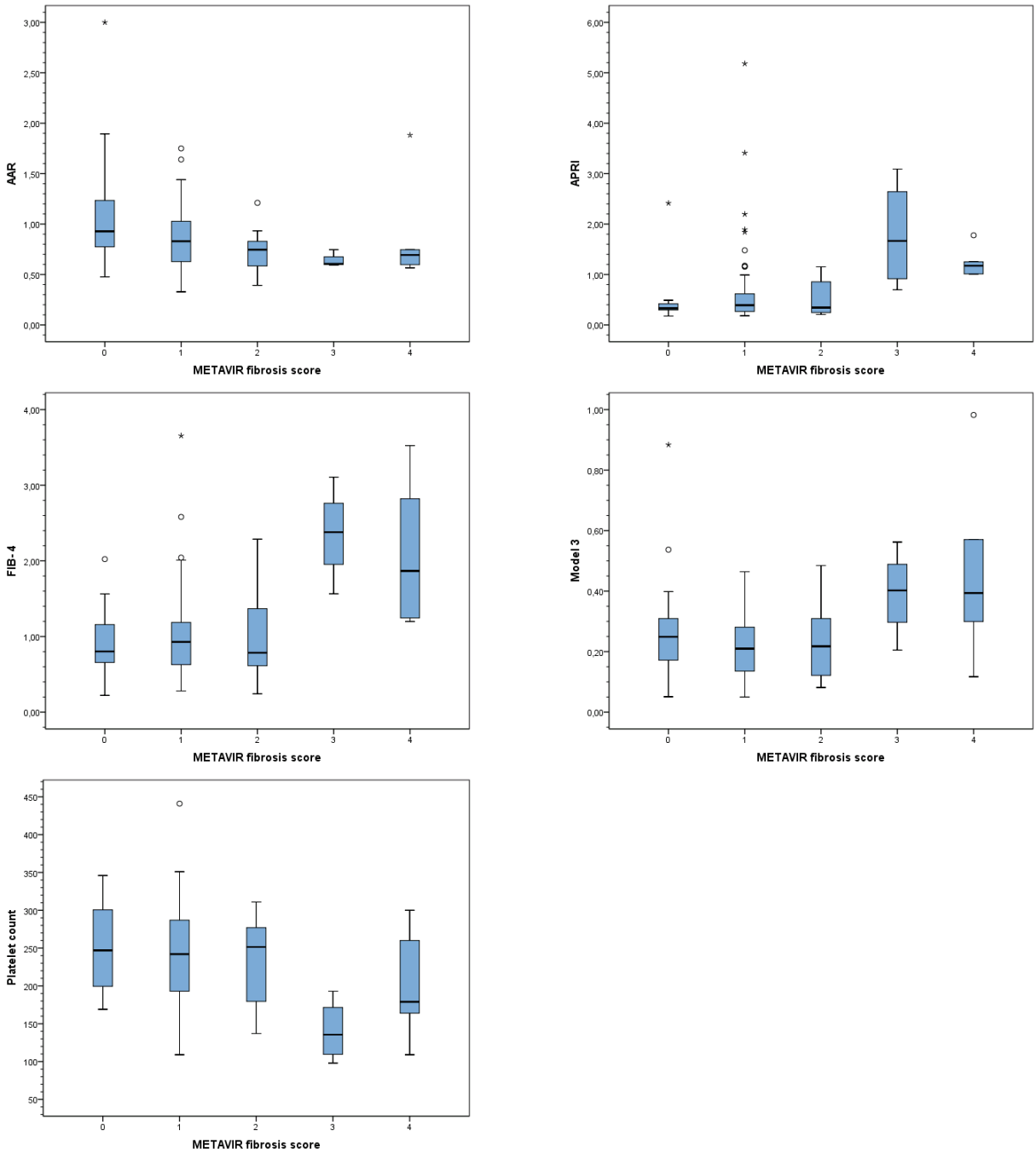


Figure 4-7: Boxplots demonstrating the distribution of the results of AAR, APRI, FIB-4, Model 3 and Platelet count among stages of the METAVIR fibrosis score in the whole study population. The used unit of platelet count is x10^9/L.

We used the bivariate Spearman´s rank correlation coefficient to evaluate the correlation between stage of fibrosis and noninvasive fibrosis test. Weak positive correlations were found for APRI and FIB-4. Surprisingly, values of AAR showed a weak but significant negative correlation with stages of fibrosis. The results are given in Table 4-9. Platelets, as mentioned earlier, correlated negatively as well.

Correlation of stage with values of noninvasive fibrosis tests		
Laboratory data	**Ishak fibrosis score**	**METAVIR fibrosis score**
AAR	-0.30**	-0.29**
APRI	0.29**	0.25**
FIB-4	0.28**	0.24*
Model 3	0.09	0.10

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

Table 4-9: Correlation (Spearman´s rank correlation coefficient, two-tailed) of stage with values of noninvasive fibrosis tests (AAR, APRI, FIB-4, Model 3) in the whole study population. Histological stages of fibrosis were estimated with the Ishak and METAVIR fibrosis scores.

Receiver operating characteristic curves were constructed for each test. Furthermore diagnostic accuracies for all noninvasive fibrosis tests and platelet count were evaluated by calculation of sensitivity, specificity, positive and negative predictive value. To construct ROC curves, two different states were defined; one corresponding to the actual state of disease, the other one to the state of disease absence. Fibrosis stages were clustered in several ways to give a nuanced response. First, the actual state of disease was defined as significant fibrosis comprising all patients with fibrosis stages F3, F4, F5 and F6 according to the Ishak fibrosis score. This cluster was compared with the remaining group of patients comprising stages F0, F1, F2, corresponding to the absence of significant fibrosis. An analogous cutoff value was defined for stages of the METAVIR fibrosis score. Subsequently, we chose a higher cutoff value to distinct patients with bridging fibrosis from all other stages. The highest chosen cutoff value separated all cases of cirrhosis from the non-cirrhotic cases. Exact information and the proportions of patients are given in Table 4-10.

Clustering of fibrosis stages for ROC analysis		
State of disease	**Defined cutoff**	**Proportion of cases**
Significant fibrosis	Ishak F3-F6 vs. F0-F2	22 vs. 81
Bridging fibrosis	Ishak F4-F6 vs. F0-F3	9 vs. 94
Cirrhosis	Ishak F5-F6 vs. F0-F4	7 vs. 96
Significant fibrosis	METAVIR F2-F4 vs. F0-F1	25 vs. 78
Bridging fibrosis	METAVIR F3-F4 vs. F0-F2	9 vs. 94
Cirrhosis	METAVIR F4 vs. F0-F3	5 vs. 98

Table 4-10: Clustering of fibrosis stages for ROC analysis in the whole study population. Data is shown for the Ishak and METAVIR scoring system.

Figure 4-8 shows the receiver operating characteristic curves of all noninvasive tests and platelet count for the prediction of significant fibrosis (Ishak F3-F6 and METAVIR F2-F4). The ROC analysis of platelet count which showed a negative correlation to stages of fibrosis was performed separately, because of the inverse direction of the test. As mentioned before, also AAR correlated negatively with stages of fibrosis. Nevertheless, the direction of the test was not changed, because it was originally proposed as a test with a positive direction.[14,15,19,31] The resulting ROC curve is situated under the reference line.

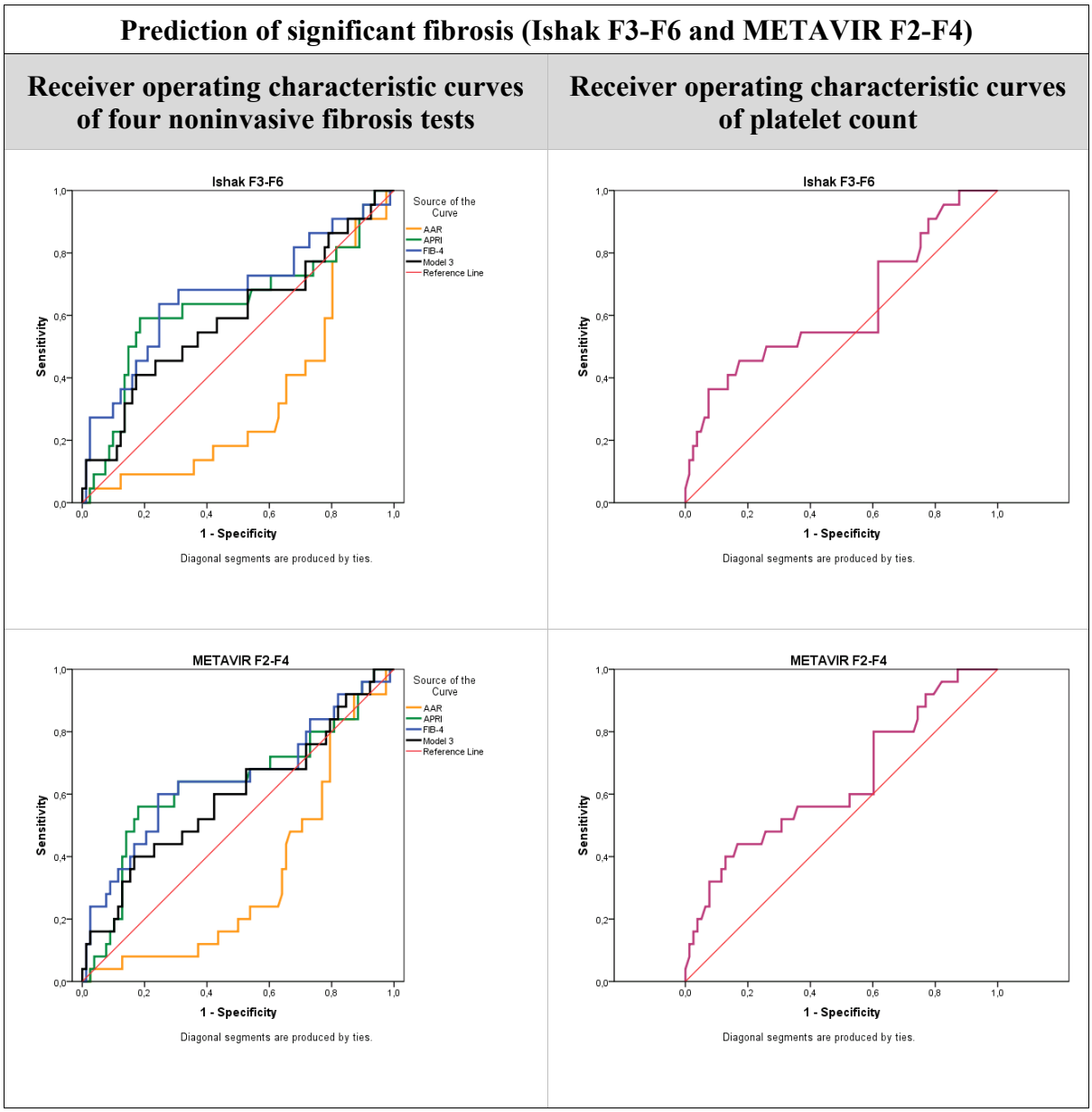


Figure 4-8: Receiver operating characteristic curves of all noninvasive fibrosis tests (AAR, APRI, FIB-4, Model 3 and Platelet count for distinction of the presence or absence of significant fibrosis (Ishak F3-F6 and METAVIR F2-F4). Larger values of the noninvasive fibrosis test results indicate stronger evidence for a positive actual state. Smaller values of platelet count indicate stronger evidence for a positive actual state.

Figure 4-9 shows the ROC curves for the distinction of patients with bridging fibrosis stages from all other patients.

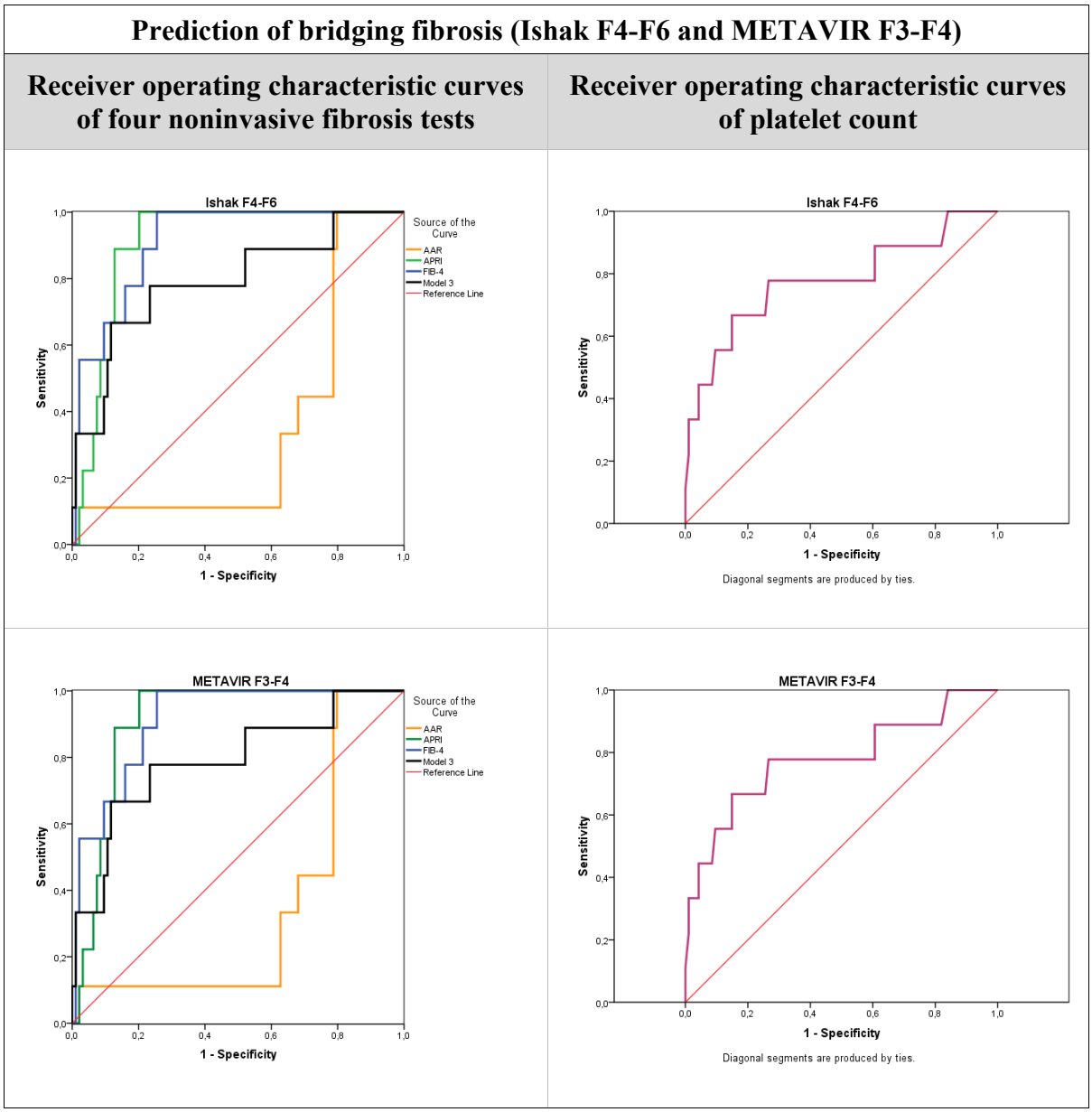


Figure 4-9: Receiver operating characteristic curves of all noninvasive fibrosis tests (AAR, APRI, FIB-4, Model 3 and Platelet count for distinction of the presence or absence of bridging fibrosis (Ishak F4-F6 and METAVIR F3-F4). Larger values of the noninvasive fibrosis test results indicate stronger evidence for a positive actual state. Smaller values of platelet count indicate stronger evidence for a positive actual state.

Figure 4-10 shows the ROC curves for the distinction of patients with cirrhosis from all non-cirrhotic patients.

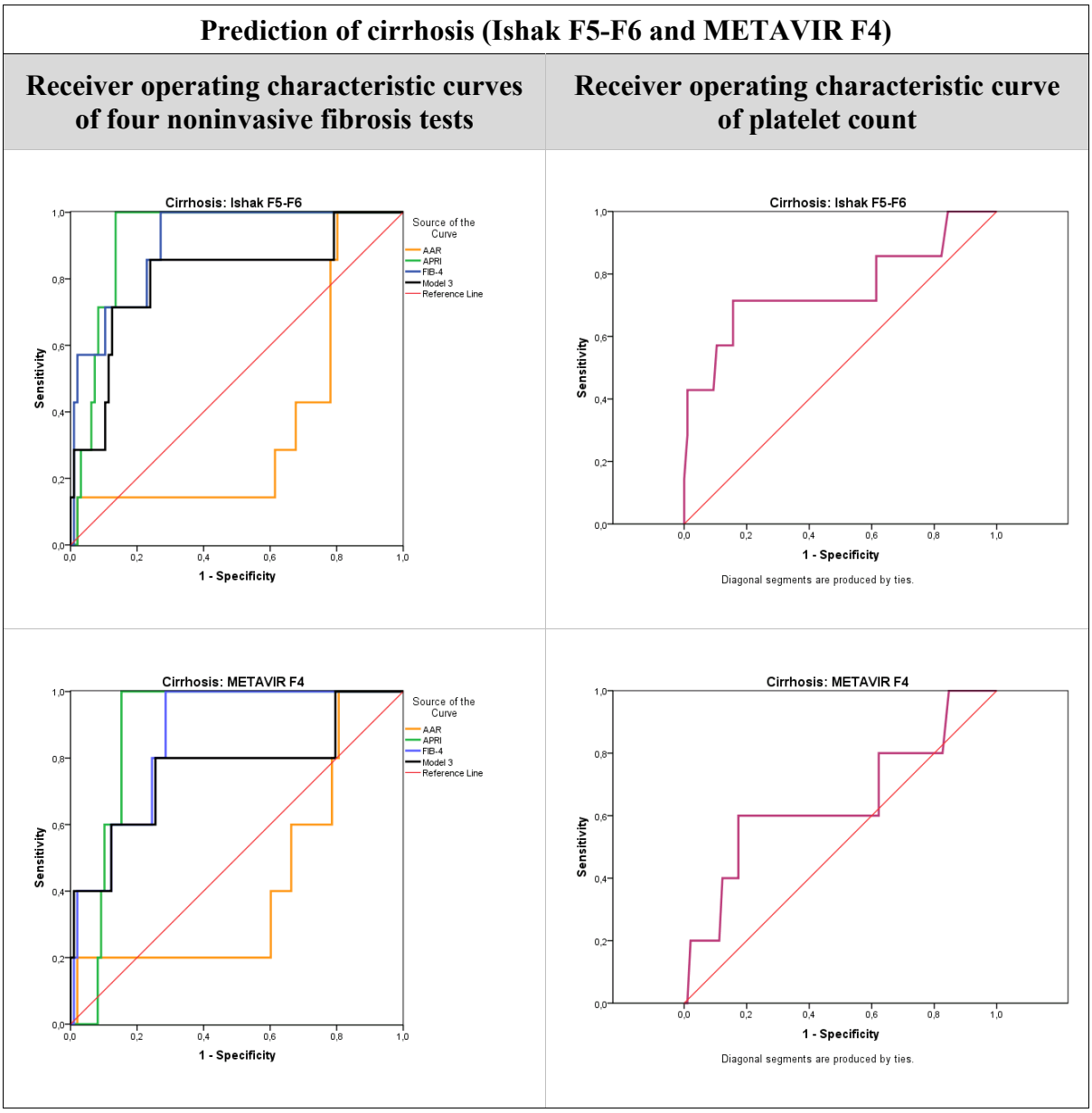


Figure 4-10: Receiver operating characteristic curves of all noninvasive fibrosis tests (AAR, APRI, FIB-4, Model 3 and platelet count for distinction of the presence or absence of cirrhosis (Ishak F5-F6 and METAVIR F4). Larger values of the noninvasive fibrosis test results indicate stronger evidence for a positive actual state. Smaller values of platelet count indicate stronger evidence for a positive actual state.

Furthermore, the areas under the receiver operating characteristic curves for prediction of significant fibrosis (Ishak F3-F6 and METAVIR F2-F4), bridging fibrosis (Ishak F4-F6 and METAVIR F3-F4) and cirrhosis (Ishak F5-F6 and METAVIR F4) were calculated for each test. The results are shown in Table 4-11. The AUROCs for the prediction of significant fibrosis were rather low for all noninvasive tests and platelet count. Large AUROCs were found for the prediction of bridging fibrosis and cirrhosis. APRI and FIB-4 showed the highest AUROCs for the prediction of bridging fibrosis and cirrhosis. Model 3 and AAR were originally proposed as markers to predict exclusively cirrhosis. AAR showed an AUROC under the reference line and can therefore not be seen an adequate

predictor neither of cirrhosis nor of fibrosis in our population. Model 3 showed an AUROC of 0.80 for the prediction of cirrhosis.

		AUROC F3-F6	AUROC F4-F6	AUROC F5-F6
Ishak fibrosis score vs.	AAR	0.33 (0.21-0.45)*	0.34 (0.17-0.51)	0.36 (0.16-0.57)
	APRI	0.63 (0.48-0.78)	0.91 (0.85-0.97)*	0.92 (0.87-0.98)*
	FIB- 4	0.67 (0.53-0.81)*	0.91 (0.84-0.98)*	0.91 (0.82-0.99)*
	Model 3	0.59 (0.45-0.74)	0.79 (0.62-0.96)*	0.80 (0.61-1.00)*
	Platelet count	0.63 (0.48-0.77)	0.78 (0.59-0.97)*	0.75 (0.52-0.99)*
		AUROC F2-F4	AUROC F3-F4	AUROC F4
METAVIR fibrosis score vs.	AAR	0.34 (0.22-0.45)*	0.34 (0.17-0.51)	0.42 (0.16-0.69)
	APRI	0.63 (0.50-0.77)*	0.91 (0.85-0.97)*	0.88 (0.82-0.95)*
	FIB-4	0.65 (0.51-0.78)*	0.91 (0.84-0.98)*	0.86 (0.75-0.97)*
	Model 3	0.58 (0.45-0.72)	0.79 (0.62-0.96)*	0.76 (0.46-1.00)*
	Platelet count	0.64 (0.51-0.77)*	0.78 (0.59-0.97)*	0.65 (0.36-0.93)

* $P < 0.05$ vs. AUROC

Table 4-11: Areas under the receiver operating characteristic curves of AAR, APRI, FIB-4, Model 3 and Platelet count for the distinction of significant fibrosis, bridging fibrosis and cirrhosis using cutoff values of the Ishak and METAVIR fibrosis score.

In order to assess the diagnostic accuracies of each simple fibrosis test for prediction of significant fibrosis (Ishak F3-F6 and METAVIR F2-F4), bridging fibrosis (F4-F6 and METAVIR F3-F4) and cirrhosis (F5-F6 and METAVIR F4) sensitivity, specificity, positive and negative predictive values were calculated. The originally proposed cutoff values for noninvasive test results were used for the calculations.[19-24,36] Table 4-12 shows all results for the Ishak and METAVIR fibrosis score. Best results were achieved for the distinction of high disease stages, i.e. bridging fibrosis and cirrhosis, especially for their exclusion. Having a population with predominantly low fibrosis stages, the noninvasive test results tended to be rather low as well. As a consequence, only few patients reached the high cutoff values of APRI, FIB-4, Model 3 and Platelet count for the prediction of significant fibrosis or cirrhosis as shown in Table 4-12. Many patients had test results within the cutoff values proposed for exclusion of significant fibrosis and cirrhosis. APRI values < 0.5 excluded bridging fibrosis with a negative predictive value of 100% and a sensitivity of 100%. Values < 1 excluded cirrhosis with a similar diagnostic accuracy. A FIB-4 index < 1.45 could exclude bridging fibrosis (Ishak F4-F6) with a negative predictive value of 98% and a sensitivity of 78%. Model 3 values <0.2 excluded cirrhosis (Ishak F5-F6) with a negative predictive value of 98% and a sensitivity of 86%. Platelet

count values <130 x 10^9/L excluded cirrhosis (Ishak F5-F6) with a negative predictive value of 96% and a sensitivity of 43%. The cutoff values for prediction of fibrosis and cirrhosis revealed lower diagnostic accuracies. Platelet count <130 x 10^9/L showed the highest positive predictive value of 60% for the prediction of cirrhosis (Ishak F5-F6) with a specificity of 98%. The Model 3 cutoff value > 0.5 showed the highest PPV for the prediction of significant and bridging fibrosis (Ishak F3-F6 and F4-F6) of 60% with a specificity of 98%.

	Cutoff value	Patients within cutoff value (%)	Diagnostic accuracy (%)	Ishak fibrosis score			METAVIR fibrosis score		
				F3-F6	F4-F6	F5-F6	F2-F4	F3-F4	F4
AAR	≥ 1.0	28 (27%)	Sensitivity	9	11	14	8	11	20
			Specificity	68	71	72	67	71	72
			PPV	7	4	4	7.1	4	4
			NPV	73	89	92	69	89	95
APRI	< 0.5	67 (65%)	Sensitivity	59	100	100	56	100	100
			Specificity	72	71	70	72	71	68
			PPV	36	25	19	39	25	14
			NPV	87	100	100	84	100	100
	≥1.5	9 (9%)	Sensitivity	14	33	43	12	33	20
			Specificity	93	94	94	92	94	92
			PPV	33	33	33	33	33	11
			NPV	80	94	96	77	94	96
	<1.0	83 (81%)	Sensitivity	41	89	100	40	89	100
			Specificity	86	87	87	87	87	85
			PPV	45	40	35	50	40	25
			NPV	84	99	100	82	99	100
	≥2.0	6 (6%)	Sensitivity	9	22	29	8	22	0
			Specificity	95	96	96	95	96	94
			PPV	33	33	33	33	33	0
			NPV	79	93	95	76	93	95
FIB-4	<1.45	79 (77%)	Sensitivity	46	78	71	44	78	60
			Specificity	83	82	80	83	82	79
			PPV	42	29	21	46	29	13
			NPV	85	98	98	82	98	98
	>3.25	2 (2%)	Sensitivity	5	11	14	4	11	20
			Specificity	99	99	99	99	99	99
			PPV	50	50	50	50	50	50
			NPV	79	92	94	76	92	96
Model 3	<0.2	45 (44%)	Sensitivity	68	89	86	68	89	80
			Specificity	47	47	46	47	47	45
			PPV	26	14	10	29	14	7
			NPV	84	98	98	82	98	98
	>0.5	5 (5%)	Sensitivity	14	33	29	12	33	40
			Specificity	98	98	97	97	98	97
			PPV	60	60	40	60	60	40
			NPV	81	94	95	77	94	97
Platelet count	<130 x 10^9/L	5 (5%)	Sensitivity	14	33	43	12	33	20
			Specificity	98	98	98	97	98	96
			PPV	60	60	60	60	60	20
			NPV	81	94	96	78	94	96
	<150 x 10^9/L	7 (%)	Sensitivity	18	33	43	16	33	20
			Specificity	96	96	96	96	96	94
			PPV	57	43	43	57	43	14
			NPV	81	94	96	78	94	96

Table 4-12: Diagnostic accuracies of the noninvasive fibrosis tests AAR, APRI, FIB-4, Model 3 and Platelet count for prediction of significant fibrosis (Ishak F3-F6), bridging fibrosis (Ishak F4-F6) and cirrhosis (F5-F6). Only original cutoff values proposed by the authors of the noninvasive fibrosis tests were applied.[19-24,36] For abbreviations see appendix.

Table 4-13 shows the number and percentage of reliably classified patients of our study population for each test. High percentages were found for APRI, FIB-4 and Platelet count and AAR.

Reliably classified patients: Correct prediction and exclusion of fibrosis and cirrhosis			
	Ishak fibrosis score		
	F0-F2 vs. F3-F6	F0-F3 vs. F4-F6	F0-F4 vs. F5-F6
AAR < 1.0 vs. > 1.0	57 (55.3 %)	68 (66.0%)	70 (68.0 %)
APRI < 0.5 vs. ≥ 1.5	61 (59.2 %)	70 (68.0 %)	70 (68.0 %)
APRI < 1 vs. ≥ 2	72 (69.9 %)	84 (81.6 %)	85 (82.5 %)
FIB-4 < 1.45 vs. > 3.25	68 (66.0%)	78 (75.7 %)	78 (75.7%)
Model 3 < 0.2 vs. > 0.5	41 (39.8%)	47 (45.6%)	46 (44.7 %)
Platelet count < 130 x 10^9/L vs. > 130 x 10^9/L	82 (79.6 %)	95 (92.2 %)	97 (94.2 %)
Platelet count < 150 x 10^9/L vs. > 150 x 10^9/L	82 (79.6 %)	93 (90.3 %)	95 (92.2%)
	METAVIR fibrosis score		
	F0-F1 vs. F2-F4	F0-F2 vs. F3-F4	F0-F3 vs. F4
AAR < 1.0 vs. > 1.0	54 (52.4 %)	68 (66.0%)	72 (69.9%)
APRI < 0.5 vs. ≥ 1.5	58 (56.3%)	70 (68.0%)	68 (66.0%)
APRI < 1 vs. ≥ 2	70 (68.0%)	84 (81.6 %)	78 (75.7%)
FIB-4 < 1.45 vs. > 3.25	66 (64.0 %)	78 (75.7 %)	83 (80.6%)
Model 3 < 0.2 vs. > 0.5	40 (38.8 %)	47 (45.6 %)	46 (44.7%)
Platelet count < 130 x 10^9/L vs. > 130 x 10^9/L	79 (76.7 %)	95 (92.2 %)	95 (92.2%)
Platelet count < 150 x 10^9/L vs. > 150 x 10^9/L	79 (76.7%)	93 (90.3 %)	93 (90.3%)

Table 4-13: Number and percentage of correctly classified patients for each noninvasive fibrosis test. Results are given for fibrosis stage clusters within Ishak fibrosis score and METAVIR fibrosis score.

In addition to the evaluation of the noninvasive fibrosis test cutoff values proposed in the original studies, new cutoff values using the coordinate points of the receiver operating characteristic curves were calculated. Coordinate points with the highest Youden index values were selected as optimized cutoff values for the noninvasive fibrosis tests and platelet count. The results are given in Table 4-14.

		Ishak fibrosis score			METAVIR fibrosis score		
		F3-F6	F4-F6	F5-F6	F2-F4	F3-F4	F4
AAR	Youden-Index	0.03	0.20	0.20	0.05	0.20	0.19
	Best cutoff value	0.52	0.57	0.57	0.52	0.57	0.57
	Sensitivity	91%	100%	100%	92%	100%	100%
	Specificity	12%	20%	20%	13%	20%	19%
	PPV	22%	11%	8%	25%	11%	6%
	NPV	83%	100%	100%	83%	100%	100%
APRI	Youden-Index	0.41	0.80	0.87	0.38	0.80	0.85
	Best cutoff value	0.69	0.69	1.00	0.69	0.69	1.00
	Sensitivity	59%	100%	100%	56%	100%	100%
	Specificity	82%	80%	87%	82%	80%	85%
	PPV	46%	32%	35%	50%	32%	25%
	NPV	88%	100%	100%	85%	100%	100%
FIB-4	Youden-Index	0.39	0.75	0.73	0.36	0.75	0.71
	Best cutoff value	1.19	1.19	1.19	1.19	1.19	1.19
	Sensitivity	64%	100%	100%	60%	100%	100%
	Specificity	75%	75%	73%	76%	75%	71%
	PPV	41%	27%	21%	44%	27%	15%
	NPV	88%	100%	100%	86%	100%	100%
Model 3	Youden-Index	0.24	0.55	0.62	0.23	0.55	0.55
	Best cutoff value	0.34	0.39	0.29	0.34	0.39	0.29
	Sensitivity	41%	67%	86%	40%	67%	80%
	Specificity	83%	88%	76%	83%	88%	75%
	PPV	39%	35%	21%	44%	35%	14%
	NPV	84%	97%	99%	81%	97%	99%
Platelet count	Youden-Index	0.29	0.52	0.56	0.27	0.52	0.43
	Best cutoff value	165	180	180	184	180	180
	Sensitivity	36%	67%	71%	44%	67%	60%
	Specificity	93%	85%	84%	83%	85%	83%
	PPV	57%	29%	24%	44%	29%	15%
	NPV	84%	96%	98%	82%	96%	98%

Table 4-14: Diagnostic accuracies of the noninvasive fibrosis tests AAR, APRI, FIB-4, Model 3 and platelet count for prediction of significant fibrosis (Ishak F3-F6, and METAVIR F2-F4), bridging fibrosis (Ishak F4-F6 and METAVIR F3-F4) and cirrhosis (Ishak F5-F6 and METAVIR F4). Youden- indices were calculated for all coordinate points of the ROC curves to select optimized cutoff values. For abbreviation see appendix.

4.2 RESULTS IN THE SUBGROUPS

4.2.1 BASELINE CHARACTERISTICS OF THE STUDY POPULATION

Subsequently, the study population was divided into two groups for separate examination. Patients with an ALT/ULN level ≤ 1 in the most recent blood sample were classified as patients with persistently normal ALT (PNALT) and patients with an ALT/ULN level > 1 as patients with elevated ALT levels (EALT), respectively. All statistics have been performed separately for each group. The clinical and demographic characteristics of both subgroups at the time of liver biopsy are shown in Table 4-15. Patients with persistently normal ALT were slightly leaner and younger than patients with elevated ALT. The sex ratio was almost equal in patients with persistently normal ALT, whereas there were more males than females among patients with elevated ALT. Genotype 1 was predominant in both groups with a likewise percentage.

		Persistently normal ALT	**Elevated ALT**
Total number of patients		58	45
Sex	Female	30 (51.7 %)	19 (42.2 %)
	Male	28 (48.3 %)	26 (57.8 %)
Age		41 ± 13 a	43 ± 10 a
BMI		23.6 ± 3.5	24.2 ± 5.7
HCV genotype	1	35 (60.3 %)	31 (68.8 %)
	2	6 (10.3 %)	2 (4.4 %)
	3	11 (18.9 %)	10 (22.3 %)
	4	6 (10.3 %)	2 (4.4 %)

Table 4-15: Clinical and demographic characteristics of the two subgroups (Patients with persistently normal ALT vs. patients with elevated ALT). Data is given as count and percentage, as well as mean and standard deviation where appropriate. For abbreviations see appendix.

4.2.2 ANALYSIS OF LABORATORY DATA

The distribution of laboratory values was analyzed separately for each subgroup. Table 4-16 gives exact information about the distribution in the group of patients with persistently normal ALT, Table 4-17 in the group of patients with elevated ALT, respectively. Figure 4-11 gives an overview of the distributions of laboratory parameters in both subgroups. The stratification of patients based on ALT levels influenced the distribution of other laboratory parameters in the subgroups. Most

obviously, the distribution of AST/ULN values was influenced by the stratification based on ALT/ULN. Patients with normal ALT tended to have low or only slightly elevated AST/ULN levels. Patients with elevated ALT showed, in contrast, a large range of AST/ULN levels. This fact is important, as the values of either one or both transaminases are included in all formulas of the noninvasive fibrosis tests evaluated in this study. Levels of platelet blood cell count and PT/INR showed more or less similar ranges in both subgroups.

Distribution of laboratory values in patients with persistently normal ALT						
	Sex	**Min**	**Max**	**Median**	**25% Quartile**	**75% Quartile**
AST/ULN	Female	0.40	1.37	0.76	0.66	0.94
	Male	0.51	2.35	0.75	0.61	0.90
ALT/ULN	Female	0.29	1.00	0.75	0.58	0.85
	Male	0.22	1.00	0.68	0.56	0.87
PT (INR)	Female	0.85	1.18	0.99	0.94	1.01
	Male	0.88	1.55	1.01	0.96	1.06
Platelet count	Female	152	351	258.00	221.75	291.75
	Male	109	314	228.50	187.75	282.25

Table 4-16: Distribution of laboratory data among female and male patients in the subgroup of patients with persistently normal ALT. Data is presented with minimum, maximum, median and quartiles for all laboratory parameters. The used unit of platelet count is x10⁹/L.

Distribution of laboratory values in patients with elevated ALT						
	Sex	**Min**	**Max**	**Median**	**25% Quartile**	**75% Quartile**
AST/ULN	Female	0.72	5.43	1.27	0.97	2.13
	Male	0.64	9.17	1.75	1.25	2.79
ALT/ULN	Female	1.12	8.49	1.60	1.26	2.94
	Male	1.04	19.60	2.24	1.42	3.67
PT (INR)	Female	0.88	1.17	1.00	0.98	1.06
	Male	0.91	1.25	1.03	1.00	1.07
Platelet count	Female	109	441	255	203	303
	Male	98	300	183	160	264

Table 4-17: Distribution of laboratory data among female and male patients in the subgroup of patients with elevated ALT levels. Data is presented with minimum, maximum, median and quartiles for all laboratory parameters. The used unit of platelet count is x10⁹/L.

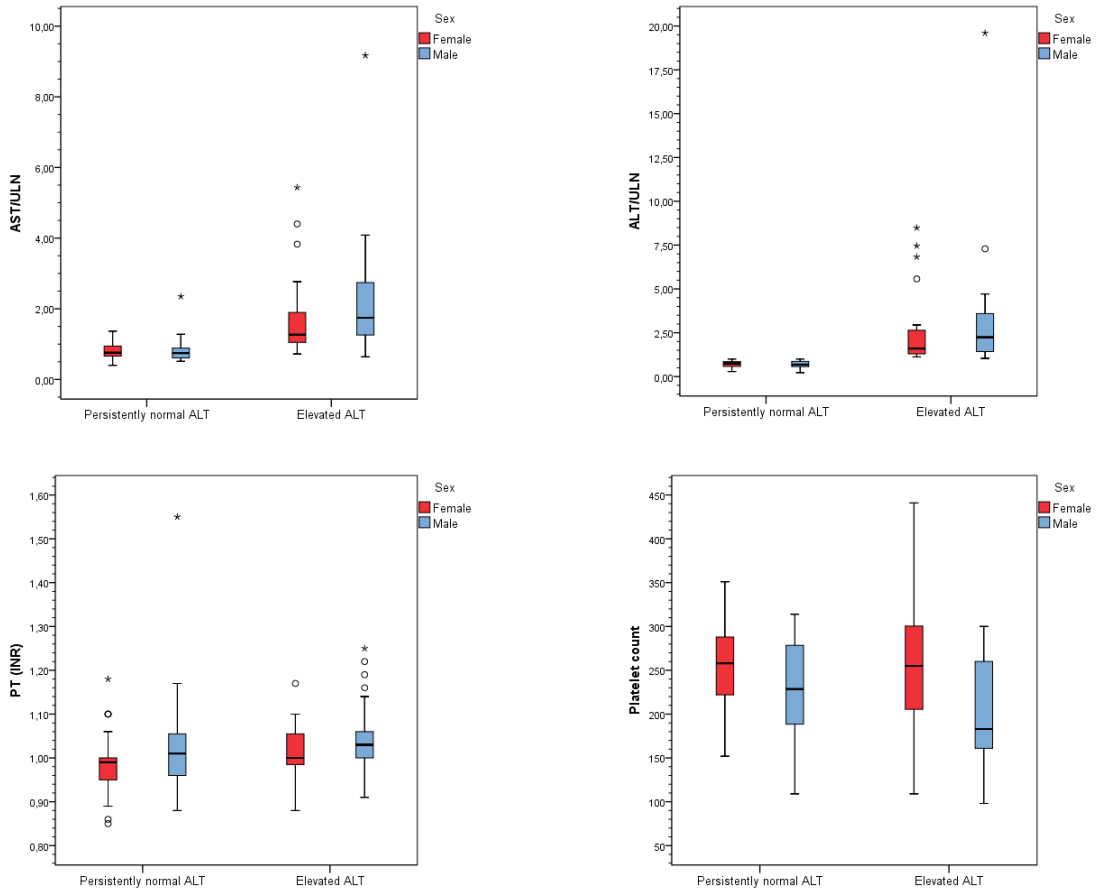


Figure 4-11: Boxplots of the distribution of laboratory parameters among male and female patients in both subgroups (Patients with persistently normal ALT versus patients with elevated ALT). The used unit of platelet blood count is x109/L. Colors: red female/ blue: male.

4.2.3 HISTOLOGICAL EVALUATION

4.2.3.1 Histological grading

Histological grading has been performed separately for each group using the Ishak histological activity index and the METAVIR grading algorithm analogously as for the whole study population. The grading results in both subgroups for the Ishak histological activity index categories A, C and D are shown in Figure 4-12. The results showing the total HAI score for both subgroups are given in Figure 4-13.

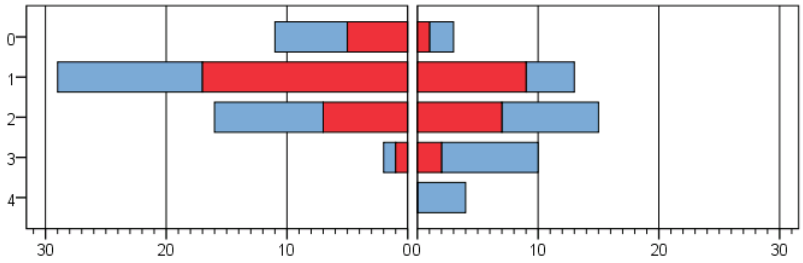
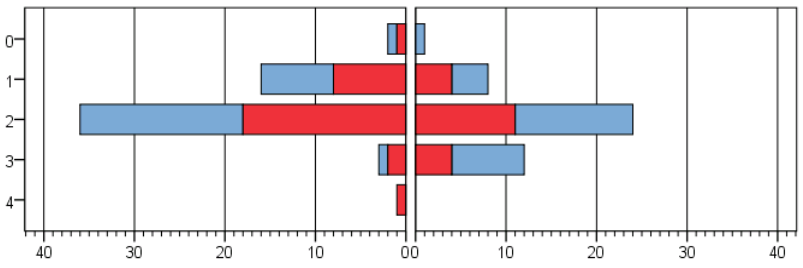
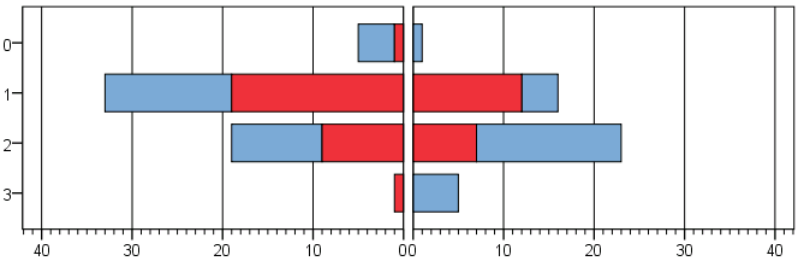
Ishak modified histological activity index							
		PNALT			EALT		
Histological activity index A	Score	n, F	n, M	n, Total (%)	n, F	n. M	n, Total (%)
	0	5	6	11 (19.0)	1	2	3 (6.7)
	1	17	12	29 (50.0)	9	4	13 (28.9)
	2	7	9	16 (27.6)	7	8	15 (33.3)
	3	1	1	2 (3.4)	2	8	10 (22.2)
	4	0	0	0 (0.0)	0	4	4 (8.9)
							
Histological activity index C	Score	n, F	n, M	n, Total (%)	n, F	n. M	n, Total (%)
	0	1	1	2 (3.4)	0	1	1 (2.2)
	1	8	8	16 (27.6)	4	4	8 (17.8)
	2	18	18	36 (62.1)	11	13	24 (53.3)
	3	2	1	3 (5.2)	4	8	12 (26.7)
	4	1	0	1 (1.7)	0	0	0 (0.0)
							
Histological activity index D	Score	n, F	n, M	n, Total (%)	n, F	n. M	n, Total (%)
	0	1	4	5 (8.6)	0	1	1 (2.2)
	1	19	14	33 (56.9)	12	4	16 (35.6)
	2	9	10	19 (32.8)	7	16	23 (51.1)
	3	1	0	1 (1.7)	0	5	5 (11.1)
							

Figure 4-12: Application of the modified histological activity index A, C and D by Ishak et al.[30] for the performance of grading in both subgroups. Results are given gender segregated as count, total percent and stacked bars for each category. The stacked bars of each subgroup are mirrored horizontally for direct comparison. Colors: red: female, blue: male. For abbreviations see appendix.

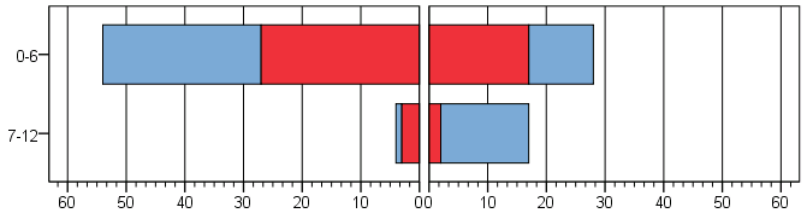
Ishak modified histological activity index							
		PNALT			EALT		
Total HAI	**Score**	n, F	n, M	n, Total (%)	n, F	n. M	n, Total (%)
	0-6	27	27	54 (93.1)	17	11	28 (62.2)
	7-12	3	1	4 (6.9)	2	15	17 (37.8)
							

Figure 4-13: Calculation of the total modified histological activity index by Ishak et al.[30] for the performance of grading in both subgroups. Results are given gender segregated as count, total percent and stacked bars for each category. The stacked bars of each subgroup are mirrored horizontally for direct comparison. Colors: red: female, blue: male. For abbreviations see appendix.

The results for the METAVIR grading algorithm are shown in Figure 4-14. The total grades of activity in both subgroups were compared applying a Mann-Whitney U test. Regarding the modified histological activity index the grades of inflammatory activity were significantly higher in patients with elevated ALT (p-value < 0.001). Similar results were found for the METAVIR grading algorithm (p-value < 0.001). Males were predominant among higher grades of activity.

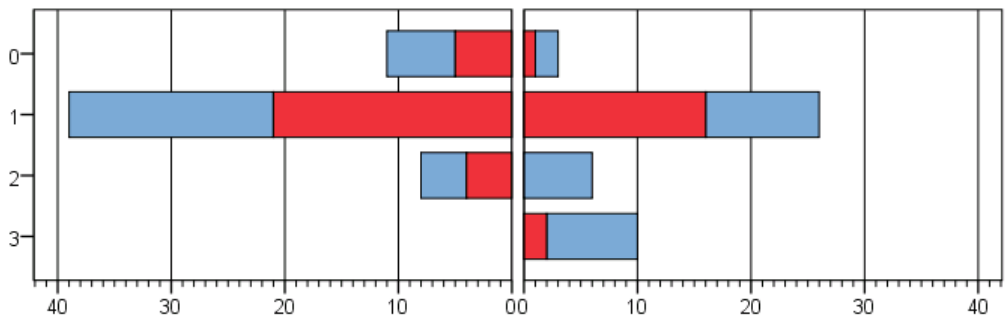
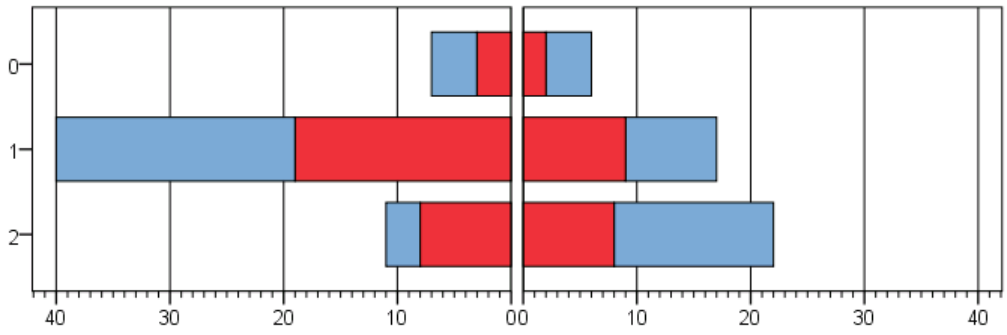
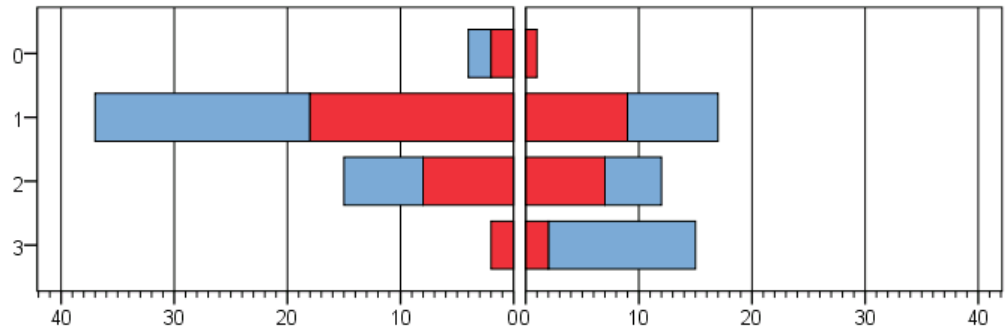
METAVIR histological activity							
		PNALT			EALT		
METAVIR PMN	Score	n, F	n, M	n, Total (%)	n, F	n. M	n, Total (%)
	0	5	6	11 (19.0)	1	2	3 (6.7)
	1	21	18	39 (67.2)	16	10	26 (57.8)
	2	4	4	8 (13.8)	0	6	6 (13.3)
	3	0	0	0 (0.0)	2	8	10 (22.2)
							
METAVIR LN	Score	n, F	n, M	n, Total (%)	n, F	n. M	n, Total (%)
	0	3	4	7 (12.1)	2	4	6 (13.3)
	1	19	21	40 (69.0)	9	8	17 (37.8)
	2	8	3	11 (19.0)	8	14	22 (48.9)
							
METAVIR A	Score	n, F	n, M	n, Total (%)	n, F	n. M	n, Total (%)
	0	2	2	4 (6.9)	1	0	1 (2.2)
	1	18	19	37 (63.8)	9	8	17 (37.8)
	2	8	7	15 (25.9)	7	5	12 (26.7)
	3	2	0	2 (3.4)	2	13	15 (33.3)
							

Figure 4-14: Application of the METAVIR algorithm for the performance of grading in both subgroups. Results are given gender segregated as count, total percent and stacked bars for each category. The stacked bars of each subgroup are mirrored horizontally for direct comparison. Colors: red: female, blue: male. For abbreviations see appendix.

Correlations between laboratory values and grades of activity have been examined with the bivariate Spearman´s rank Correlation coefficient. Table 4-18 shows the results for patients with persistently normal ALT and for patients with elevated ALT. Weak significant negative correlations were found for platelet count in both groups. The significant correlations of AST/ULN and ALT/ULN that were found in the whole study population were no longer evident after the stratification of patients based on ALT/ULN levels.

Correlation of grade with standard laboratory parameters			
	Laboratory data	**Modified histological activity index**	**METAVIR A**
Patients with persistently normal ALT	**AST/ULN**	-0.12	-0.14
	ALT/ULN	-0.01	-0.09
	INR	0.00	-0.06
	Platelet count	0.16	0.27*
Patients with elevated ALT	**AST/ULN**	0.16	0.13
	ALT/ULN	0.15	0.13
	INR	0.27	0.22
	Platelet count	-0.30*	-0.28

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

Table 4-18: Correlation (Spearman´s rank correlation coefficient, two-tailed) of grade with standard laboratory parameters in both subgroups (Patients with persistently normal ALT vs. patients with elevated ALT). Histological grades were estimated with the modified histological activity index by Ishak et al.[30] and the METAVIR grading algorithm.

4.2.3.2 Histological staging

The distributions of fibrosis stages within the METAVIR and Ishak fibrosis score were evaluated for each subgroup. Exact information is given in Figure 4-15. Four cases of cirrhosis were found among patients with elevated ALT levels, only one case among patients with persistently normal ALT. Stage 4 and 5 of the Ishak fibrosis score were only seen in patients with elevated ALT. All patients with stages higher than F3 were male. A Mann-Whitney U test was applied to compare the distribution of fibrosis stages in both subgroups. The group of patients with persistently normal ALT had significantly lower fibrosis stages than the group of patients with elevated ALT. The p-value for the Ishak fibrosis score was 0.001, the p-value for the METAVIR fibrosis score was 0.002 at a significance level of 0.05.

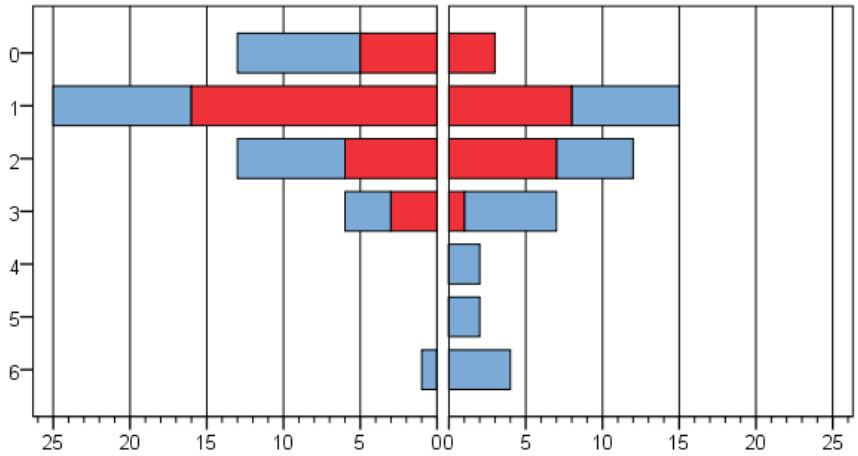
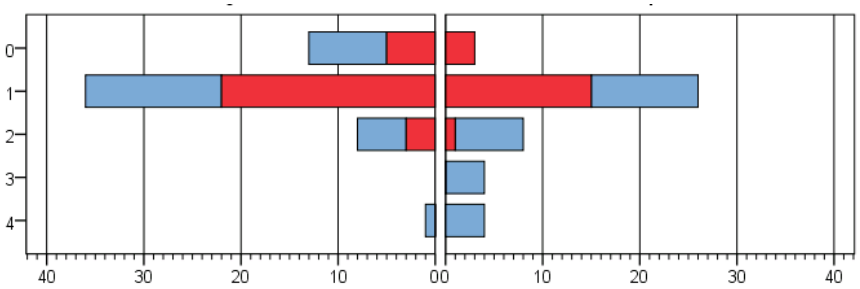
Histological staging							
		PNALT			EALT		
Ishak fibrosis score		n, F	n, M	n, Total (%)	n, F	n. M	n, Total (%)
	0	5	8	13 (22.4)	3	0	3 (6.7)
	1	16	9	25 (43.1)	8	7	15 (33.3)
	2	6	7	13 (22.4)	7	5	12 (26.7)
	3	3	3	6 (10.3)	1	6	7 (15.6)
	4	0	0	0 (0.0)	0	2	2 (4.4)
	5	0	0	0 (0.0)	0	2	2 (4.4)
	6	0	1	1 (1.7)	0	4	4 (8.9)
							
METAVIR fibrosis score		n, F	n, M	n, Total (%)	n, F	n. M	n, Total (%)
	0	5	8	13 (22.4)	3	0	3 (6.7)
	1	22	14	36 (62.1)	15	11	26 (57.8)
	2	3	5	8 (13.8)	1	7	8 (17.8)
	3	0	0	0 (0.0)	0	4	4 (8.9)
	4	0	1	1 (1.7)	0	4	4 (8.9)
							

Figure 4-15: Application of the Ishak and METAVIR fibrosis score to estimate the stages of fibrosis in both subgroups (Patients with persistently normal ALT versus patients with elevated ALT). Results are given gender segregated as count, total percent and stacked bars for each category. The stacked bars of each subgroup are mirrored horizontally for direct comparison. Colors: red = female, blue = male. For abbreviations see appendix.

To evaluate correlations between fibrosis stage and standard laboratory values the bivariate Spearman´s rank correlation coefficient was again calculated separately for each group. Results are given in Table 4-19 for patients with persistently normal ALT and elevated ALT. Weak significant correlations were found for PT/INR and platelet count among patients with elevated ALT. No significant correlation was found for patients with

persistently normal ALT. The correlations seen for values of ALT/ULN and AST/ULN in the whole population disappeared with the stratification of patients based on ALT.

Correlation of stage with standard laboratory parameters			
	Laboratory data	**Ishak fibrosis score**	**METAVIR fibrosis score**
Patients with persistently normal ALT	**AST/ULN**	0.05	0.04
	ALT/ULN	0.12	0.12
	PT/INR	-0.09	-0.07
	Platelet count	-0.02	0.03
Patients with elevated ALT	**AST/ULN**	0.16	0.10
	ALT/ULN	0.18	0.12
	INR	0.33*	0.33*
	Platelet count	-0.32*	-0.35*

Table 4-19: Correlation (Spearman´s rank correlation coefficient, two-tailed) of stage with standard laboratory parameters in both subgroups (patients with persistently normal ALT versus patients with elevated ALT). Histological stages of fibrosis were estimated with the Ishak and METAVIR fibrosis scores.

4.2.4 NON-INVASIVE TESTS FOR THE PREDICTION OF FIBROSIS AND CIRRHOSIS

The distribution of results of the noninvasive fibrosis tests for patients with persistently normal ALT is shown in Table 4-20, for patients with elevated ALT in Table 4-21, respectively. Clustered boxplots for both groups are given in Figure 4-16. The data emphasizes the influence of the transaminases on the test results. Most evidently, the values of APRI (AST to platelet ratio index) differed in both subgroups. As seen earlier, platelet count values did not differ much between the subgroups. AST/ULN values, however, did, being strongly influenced by the stratification based on ALT/ULN. The range and median of AAR values was lower in patients with elevated ALT than in patients with persistently normal ALT.

	Sex	Min	Max	Median	25% Quartile	75% Quartile
AAR	Female	0.48	1.75	1.00	0.85	1.20
	Male	0.46	3.00	0.84	0.75	1.17
APRI	Female	0.18	0.63	0.29	0.23	0.36
	Male	0.18	1.18	0.32	0.26	0.42
FIB-4	Female	0.22	1.77	0.79	0.60	1.14
	Male	0.28	3.52	0.77	0.58	1.21
Model 3	Female	0.05	0.45	0.21	0.13	0.28
	Male	0.08	0.98	0.24	0.15	0.37

Table 4-20: Distribution of the results of AAR, APRI, FIB-4 and Model 3 among female and male patients in the subgroup of patients with persistently normal ALT. Data is presented with minimum, maximum, median and quartiles for all noninvasive fibrosis tests.

	Sex	Min	Max	Median	25% Quartile	75% Quartile
AAR	Female	0.33	1.03	0.67	0.55	0.86
	Male	0.36	0.85	0.62	0.56	0.75
APRI	Female	0.23	3.41	0.42	0.38	1.16
	Male	0.33	5.18	0.97	0.59	1.38
FIB-4	Female	0.35	2.58	0.90	0.55	1.12
	Male	0.65	3.65	1.55	1.02	2.00
Model 3	Female	0.05	0.46	0.18	0.09	0.23
	Male	0.09	0.57	0.24	0.15	0.39

Table 4-21: Distribution of the results of AAR, APRI, FIB-4 and Model 3 among female and male patients in the subgroup of patients with elevated ALT. Data is presented with minimum, maximum, median and quartiles for all noninvasive fibrosis tests.

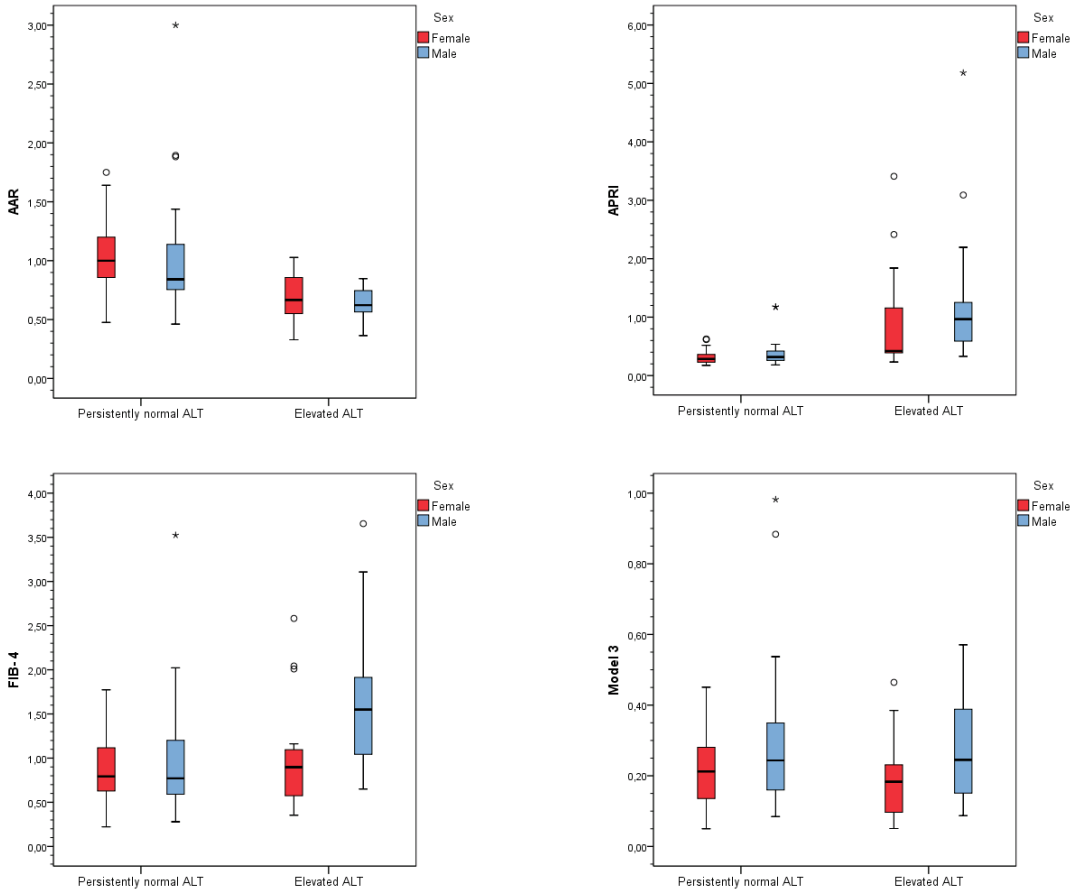


Figure 4-16: Boxplots of the distribution of the results of AAR, APRI, FIB-4 and Model 3 among female and male patients in both subgroups (patients with persistently normal ALT vs. patients with elevated ALT). Colors: red: female, blue: male.

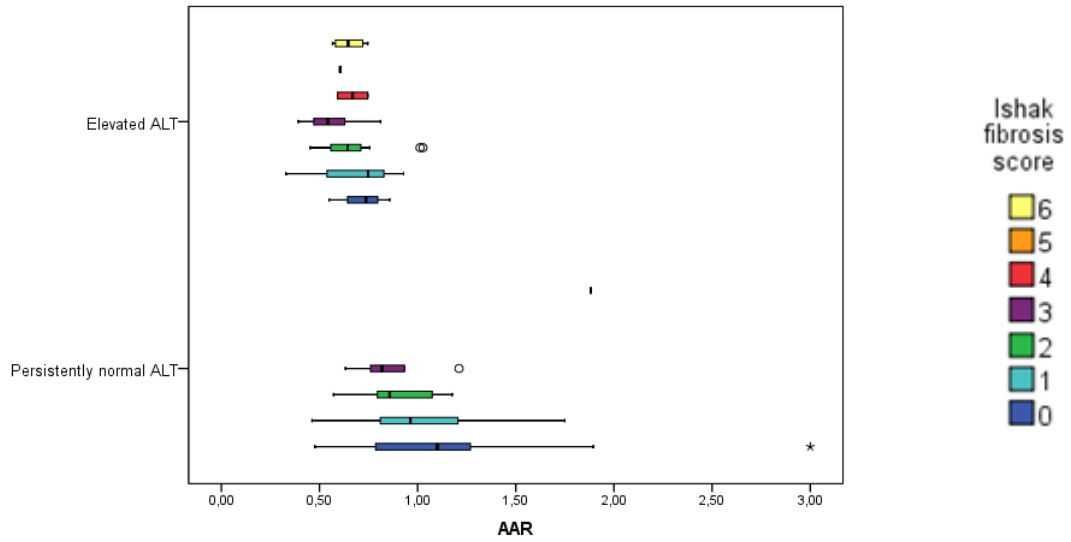
Furthermore, the distributions of results of AAR, APRI, FIB-4, Model 3 and platelet count among stages of fibrosis were examined in both subgroups. Exact information is given in Table 4-22 for the Ishak fibrosis score and patients with persistently normal ALT, in Table 4-23 for patients with elevated ALT, respectively. Figure 4-17 shows the results for both groups among stages of the Ishak fibrosis score. Differences in the distributions among stages of fibrosis were examined with a Kruskal-Wallis H test for each group. Significant differences were found neither in patients with persistently normal ALT nor in patients with elevated ALT.

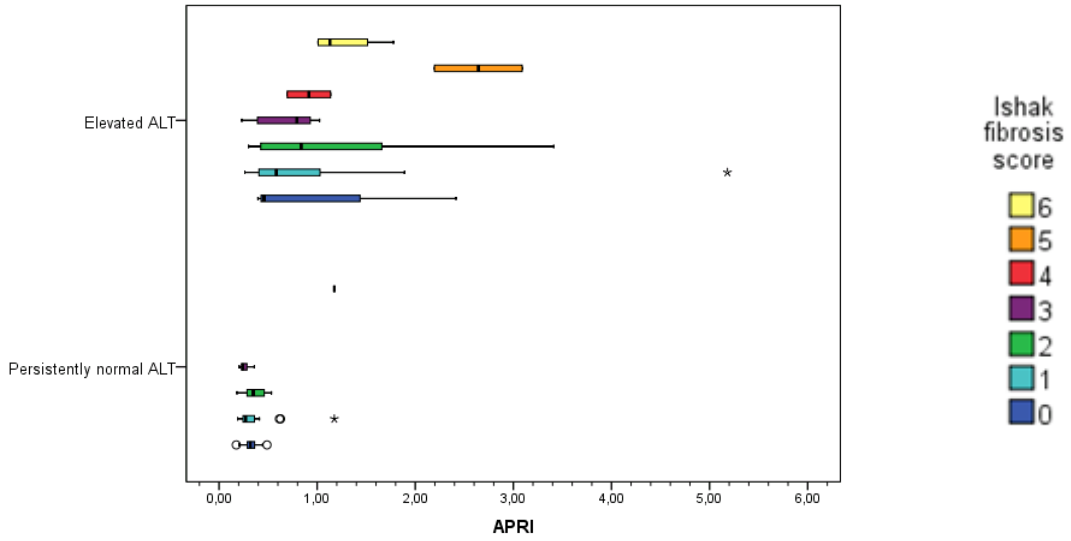
	Ishak fibrosis score	Min	Max	Median	25% Quartile	75% Quartile	Kruskal-Wallis H Test
AAR	0	0.48	3.00	1.10	0.78	1.35	n.s.
	1	0.46	1.75	0.96	0.76	1.21	
	2	0.57	1.18	0.86	0.77	1.08	
	3	0.63	1.21	0.82	0.73	1.00	
APRI	0	0.18	0.49	0.32	0.26	0.47	n.s.
	1	0.19	1.18	0.27	0.24	0.62	
	2	0.18	0.53	0.35	0.25	0.53	
	3	0.21	0.36	0.25	0.22	xx	
FIB-4	0	0.22	2.02	0.80	0.49	1.84	n.s.
	1	0.28	1.77	0.80	0.46	1.48	
	2	0.51	1.94	0.74	0.61	1.80	
	3	0.24	1.19	0.78	0.49	xx	
Model 3	0	8.04	88.37	28.17	17.83	35.51	n.s.
	1	5.00	45.08	24.62	13.86	28.81	
	2	7.33	46.06	21.16	12.79	25.94	
	3	8.46	27.53	17.42	10.25	24.49	
Platelet count	0	169	330	241	185	296	n.s.
	1	152	351	249	218	294	
	2	155	346	231	192	271	
	3	215	296	265	247	290	

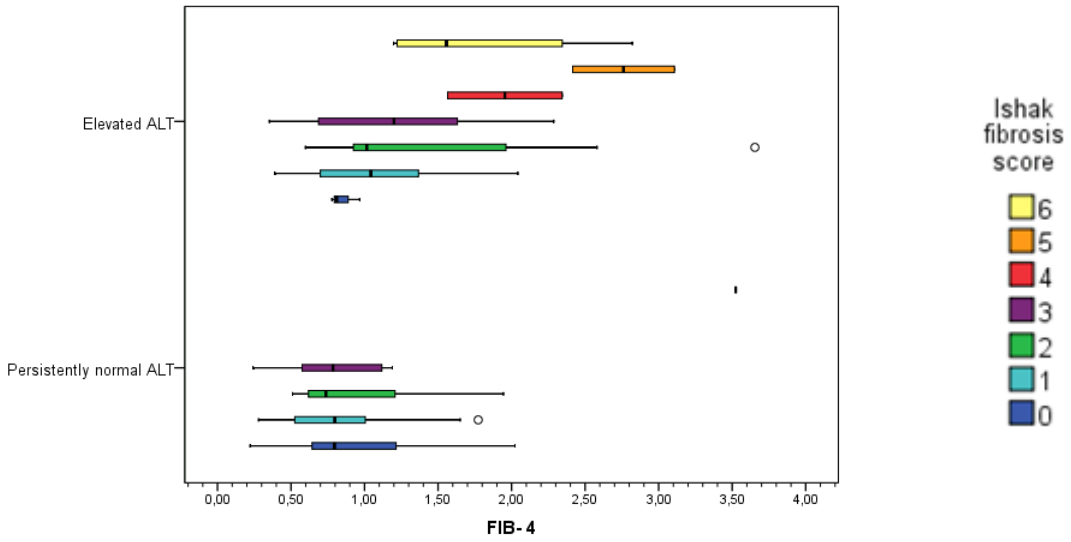
Table 4- 22: Distribution of the results of AAR, APRI, FIB-4, Model 3 and Platelet count among stages of the Ishak fibrosis score in patients with persistently normal ALT. Data is presented with minimum, maximum, median and quartiles for all noninvasive fibrosis tests. P-values were estimated with the Kruskal-Wallis H test. The used unit of platelet count is $x10^9/L$.

	Ishak fibrosis score	Min	Max	Median	25% Quartile	75% Quartile	Kruskal-Wallis H test
AAR	0	0.55	0.86	0.74	0.55		n.s.
	1	0.33	0.93	0.75	0.46	0.85	
	2	0.45	1.03	0.64	0.56	0.73	
	3	0.39	0.81	0.54	0.42	0.63	
	4	0.59	0.75	0.67	0.59		
	5	0.60	0.61	0.60	0.60		
	6	0.57	0.75	0.65	0.57	0.73	
APRI	0	0.40	2.41	0.46	0.40		n.s.
	1	0.26	5.18	0.58	0.40	1.15	
	2	0.30	3.41	0.84	0.42	1.75	
	3	0.23	1.02	0.79	0.33	0.94	
	4	0.70	1.14	0.92	0.70		
	5	2.20	3.09	2.64	2.20		
	6	1.01	1.77	1.13	1.01	1.65	
FIB-4	0	0.78	0.97	0.81	0.78		n.s.
	1	0.39	2.04	1.04	0.55	1.57	
	2	0.60	3.65	1.02	0.91	1.99	
	3	0.35	2.29	1.20	0.65	1.73	
	4	1.56	2.34	1.95	1.56		
	5	2.42	3.11	2.76	2.42		
	6	1.20	2.82	1.56	1.21	2.58	
Model 3	0	0.05	0.20	0.17	0.05	-	n.s.
	1	0.05	0.46	0.19	0.10	0.23	
	2	0.09	0.48	0.24	0.11	0.35	
	3	0.08	0.39	0.25	0.13	0.37	
	4	0.21	0.56	0.38	0.21	-	
	5	0.39	0.42	0.40	0.39	-	
	6	0.12	0.57	0.35	0.16	0.53	
Platelet count	0	225	346	295	225	-	n.s.
	1	109	441	216	183	298	
	2	129	303	225	179	286	
	3	137	311	183	155	287	
	4	150	193	172	150	-	
	5	98	121	110	98	-	
	6	164	300	220	168	290	

Table 4-23: Distribution of the results of AAR, APRI, FIB-4, Model 3 and Platelet count among stages of the Ishak fibrosis score in patients with elevated ALT. Data is presented with minimum, maximum, median and quartiles for all noninvasive fibrosis tests. P-values were estimated with the Kruskal-Wallis H test. The used unit of platelet count is x10^9/L.


Elevated ALT
Persistently normal ALT
0,00
0,50
1,00
1,50
2,00
2,50
3,00
AAR
Ishak fibrosis score
6
5
4
3
2
1
0


Elevated ALT
Persistently normal ALT
0,00
1,00
2,00
3,00
4,00
5,00
6,00
APRI
Ishak fibrosis score
6
5
4
3
2
1
0


Elevated ALT
Persistently normal ALT
0,00
0,50
1,00
1,50
2,00
2,50
3,00
3,50
4,00
FIB-4
Ishak fibrosis score
6
5
4
3
2
1
0

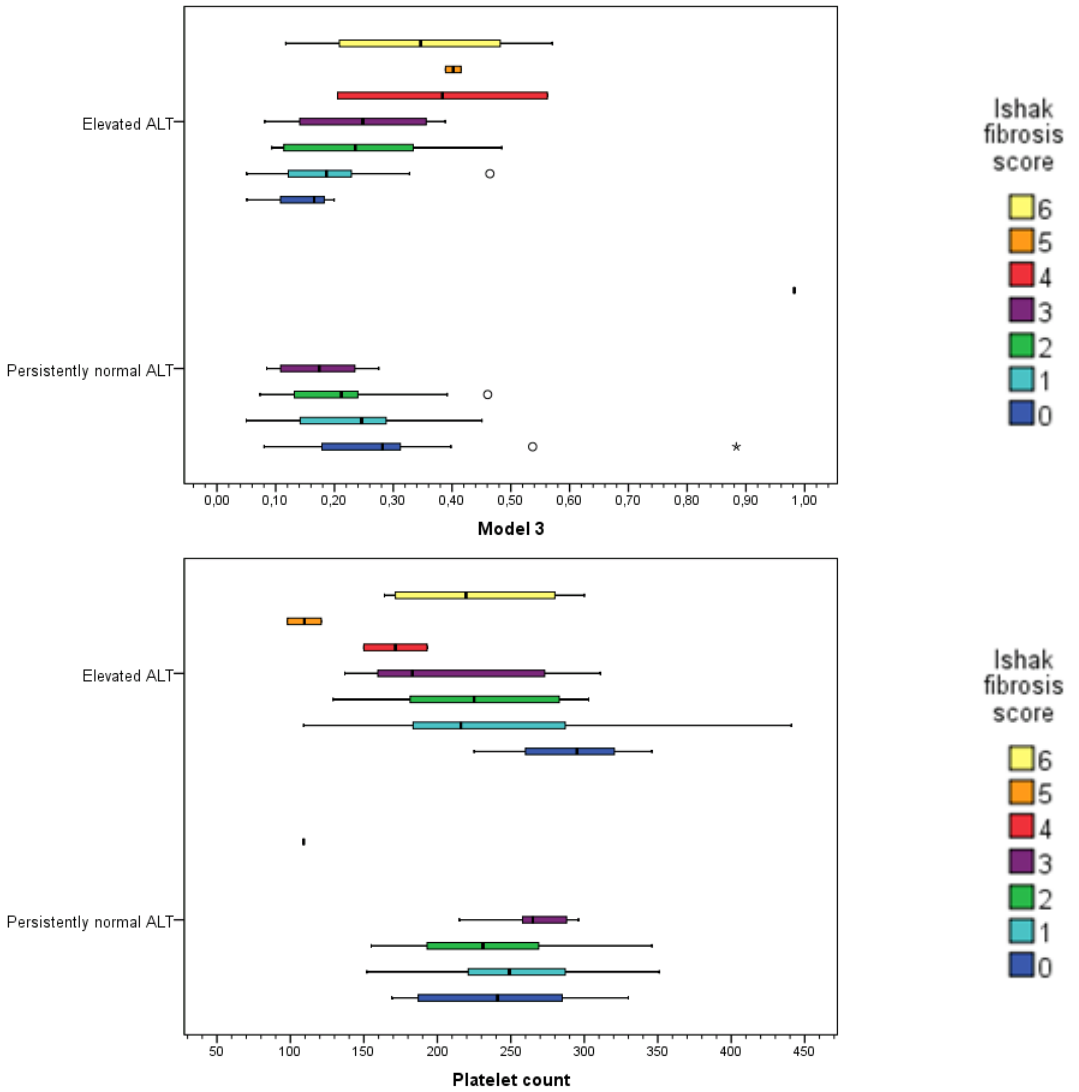


Figure 4-17: Boxplots demonstrating the distribution of the results of AAR, APRI, FIB-4, Model 3 and Platelet count among stages of the Ishak fibrosis score in both subgroups (patients with persistently normal ALT vs. patients with elevated ALT). The used unit of platelet count is x10⁹/L.

The same procedure has been performed using the METAVIR staging algorithm. Exact information for patients with persistently normal ALT is given in Table 4-24, for patients with elevated ALT in Table 4-25. Clustered boxplots for both groups are shown in Figure 4-18. Significant differences among stages of fibrosis in each group were not found. The boxplots for the Ishak and METAVIR fibrosis score, however, reveal that the distribution of the noninvasive test results was more heterogeneous among fibrosis stages in patients with elevated ALT except for AAR. The boxplots of FIB-4 and Model 3 show an elevation, the boxplot of platelet count a decline of the medians with higher stages of fibrosis in patients with elevated ALT. This tendency was not evident in the results of patients with normal ALT. In patients with elevated ALT, additionally, stages of cirrhosis (F6 of the Ishak or F4 of the METAVIR fibrosis

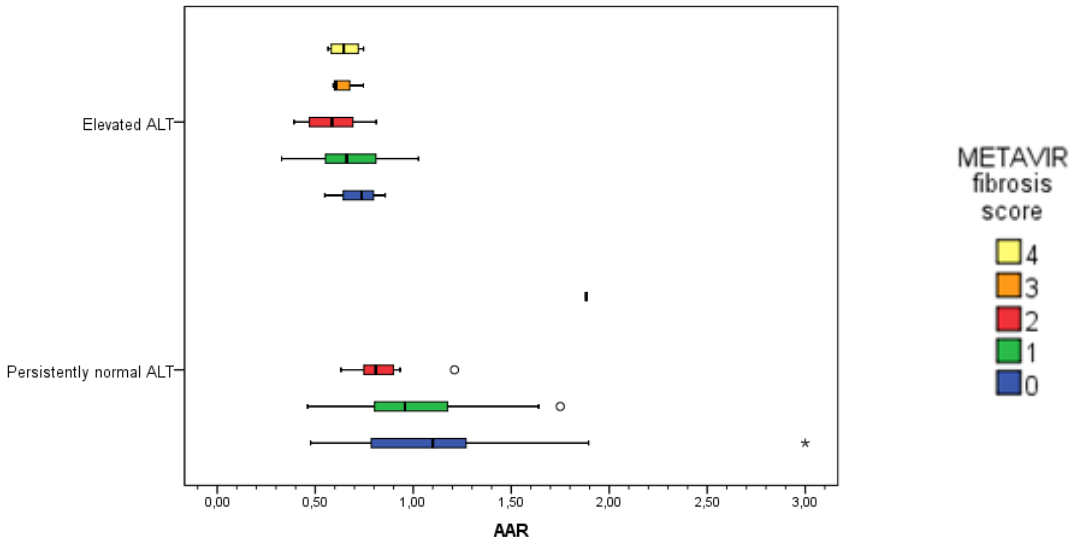
score) had lower medians of APRI, FIB-4, Model 3 and a higher median of platelet count than the previous stage of fibrosis (F5 of the Ishak fibrosis score and F4 of the METAVIR fibrosis score).

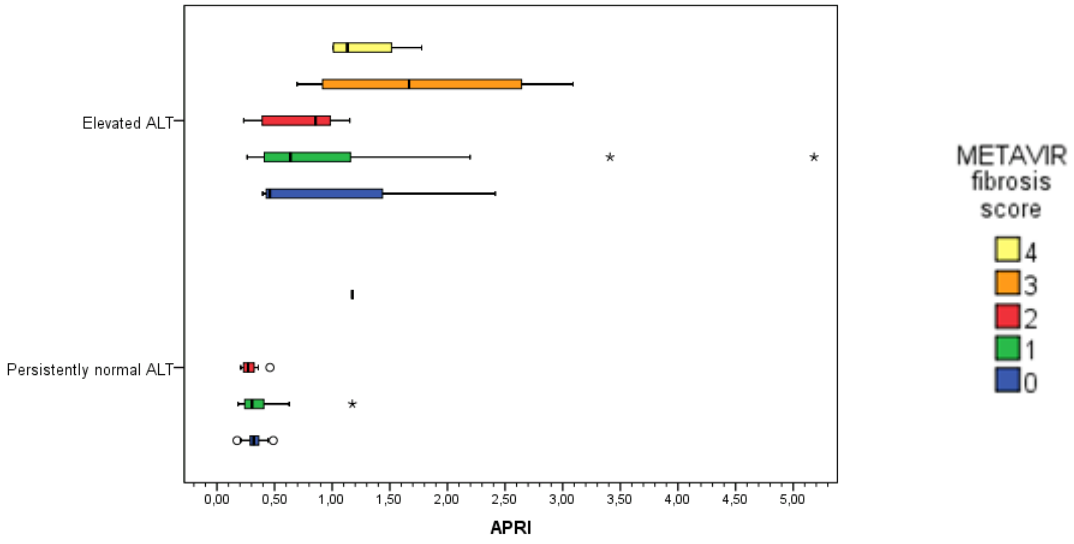
	METAVIR fibrosis score	Min	Max	Median	25% Quartile	75% Quartile	Kruskal-Wallis H test
AAR	0	0.48	3.00	1.10	0.78	1.35	n.s.
	1	0.46	1.75	0.96	0.80	1.18	
	2	0.63	1.21	0.81	0.74	0.92	
APRI	0	0.18	0.49	0.32	0.26	0.37	n.s.
	1	0.18	1.18	0.30	0.24	0.41	
	2	0.21	0.46	0.27	0.23	0.34	
FIB-4	0	0.22	2.02	0.80	0.49	1.29	n.s.
	1	0.28	1.94	0.79	0.61	1.19	
	2	0.24	1.19	0.71	0.55	1.05	
Model 3	0	8.04	88.37	28.17	17.83	35.51	n.s.
	1	5.00	46.06	21.83	13.71	28.61	
	2	8.46	27.53	17.42	10.94	23.85	
Platelet count	0	169	330	241	185	296	n.s.
	1	152	351	243	199	287	
	2	205	296	261	223	283	

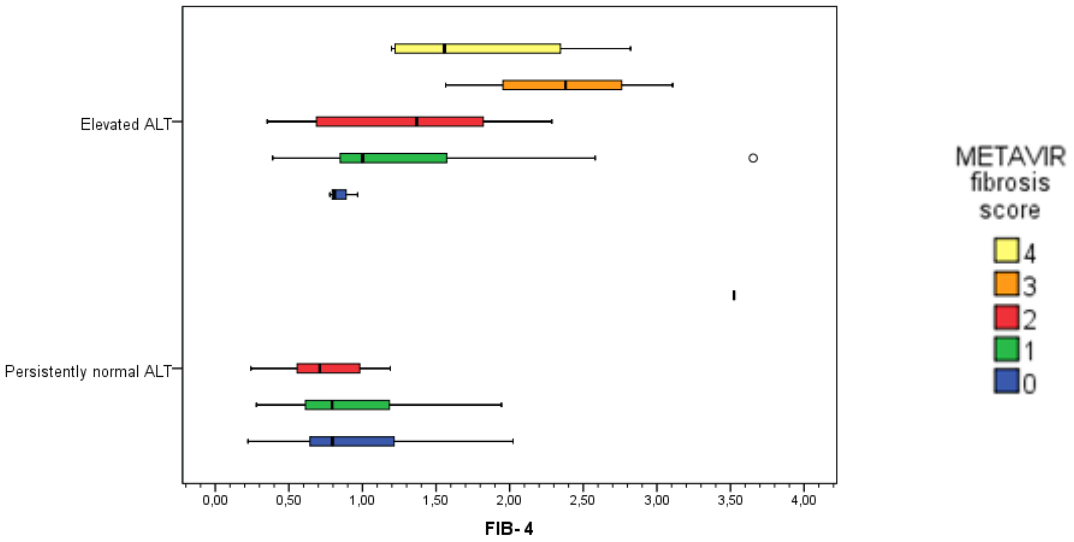
Table 4-24: Distribution of the results of AAR, APRI, FIB-4, Model 3 and Platelet count among stages of the METAVIR fibrosis score in patients with persistently normal ALT. Data is presented with minimum, maximum, median and quartiles for all noninvasive fibrosis tests. P-values were estimated with the Kruskal-Wallis H test. The used unit of platelet count is $x10^9/L$.

	METAVIR fibrosis score	Min	Max	Median	25% Quartile	75% Quartile	Kruskal-Wallis H test
AAR	0	0.55	0.86	0.74	0.55	-	n.s.
	1	0.33	1.03	0.66	0.54	0.82	
	2	0.39	0.81	0.59	0.45	0.72	
	3	0.59	0.75	0.60	0.59	0.71	
	4	0.57	0.75	0.65	0.57	0.73	
APRI	0	0.40	2.41	0.46	0.40	-	n.s.
	1	0.26	5.18	0.64	0.41	1.24	
	2	0.23	1.15	0.86	0.36	1.00	
	3	0.70	3.09	1.67	0.81	2.87	
	4	1.01	1.78	1.13	1.01	1.65	
FIB-4	0	0.78	0.97	0.81	0.78	-	n.s.
	1	0.39	3.65	1.00	0.82	1.64	
	2	0.35	2.29	1.37	0.67	1.87	
	3	1.56	3.11	2.38	1.76	2.93	
	4	1.20	2.82	1.56	1.21	2.58	
Model 3	0	0.05	0.20	0.17	0.05	-	n.s.
	1	0.05	0.46	0.19	0.11	0.26	
	2	0.08	0.48	0.30	0.14	0.38	
	3	0.21	0.56	0.40	0.25	0.53	
	4	0.12	0.57	0.35	0.16	0.53	
Platelet count	0	225	346	295	225	-	n.s.
	1	109	441	229	184	291	
	2	137	311	180	157	280	
	3	98	193	136	104	182	
	4	164	300	220	168	290	

Table 4-25: Distribution of the results of AAR, APRI, FIB-4, Model 3 and Platelet count among stages of the METAVIR fibrosis score in patients with elevated ALT. Data is presented with minimum, maximum, median and quartiles for all noninvasive fibrosis tests. P-values were estimated with the Kruskal-Wallis H test. The used unit of platelet count is $x10^9/L$.


Elevated ALT
Persistently normal ALT
0,00
0,50
1,00
1,50
2,00
2,50
3,00
AAR
METAVIR fibrosis score
4
3
2
1
0


Elevated ALT
Persistently normal ALT
0,00
0,50
1,00
1,50
2,00
2,50
3,00
3,50
4,00
4,50
5,00
APRI
METAVIR fibrosis score
4
3
2
1
0


Elevated ALT
Persistently normal ALT
0,00
0,50
1,00
1,50
2,00
2,50
3,00
3,50
4,00
FIB-4
METAVIR fibrosis score
4
3
2
1
0

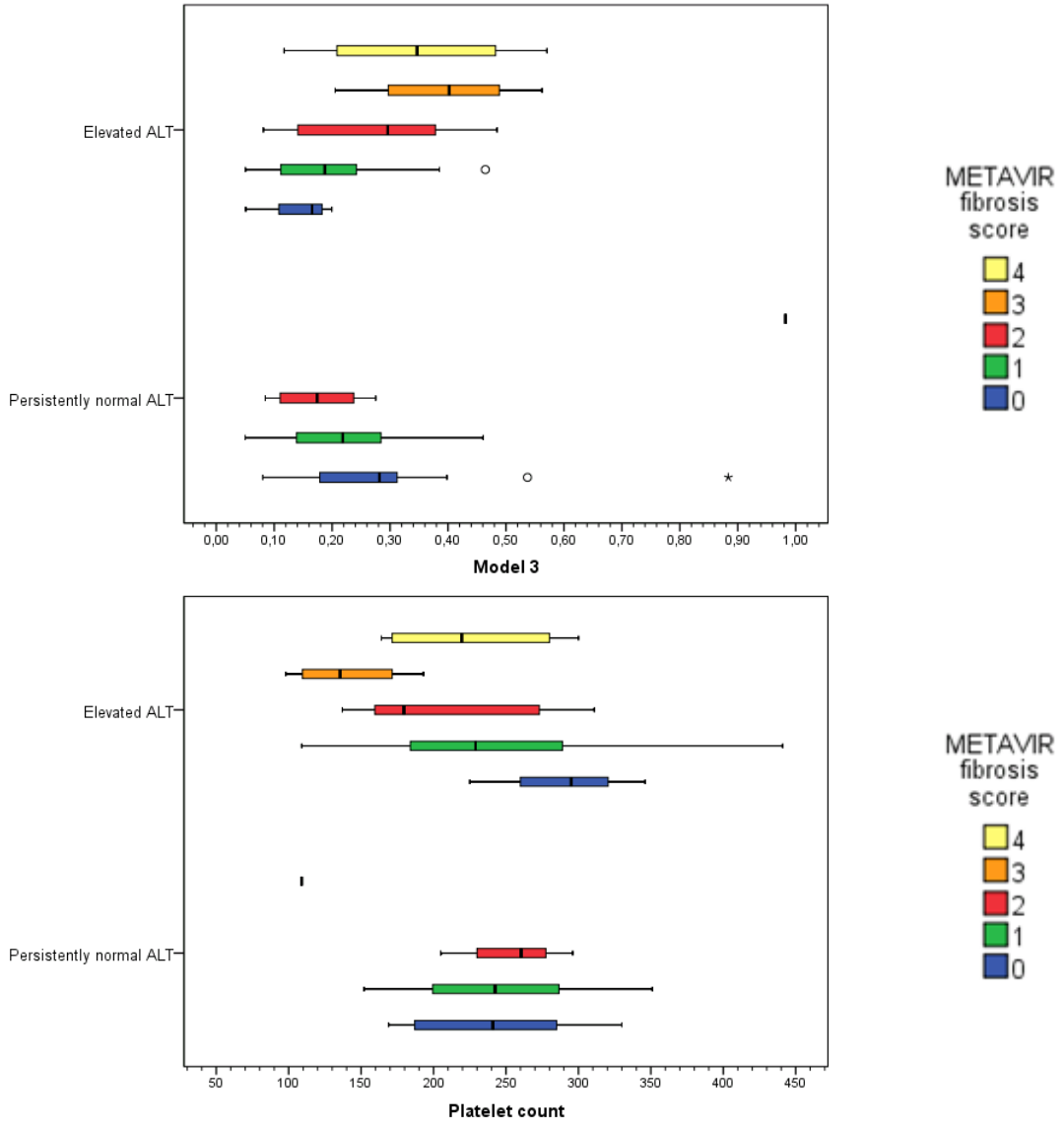


Figure 4-18: Boxplots demonstrating the distribution of the results of AAR, APRI, FIB-4, Model 3 and Platelet count among stages of the Ishak fibrosis score in both subgroups (patients with persistently normal ALT vs. patients with elevated ALT). The used unit of platelet count is x10^9/L.

Similarly as done for the whole study population, we calculated the bivariate Spearman´s rank correlation coefficient for each test to evaluate the correlation between stage of fibrosis and noninvasive fibrosis test. The results for patients with persistently normal ALT as well as for patients with elevated ALT are given in Table 4-26. As already seen in the boxplots, Model 3 and FIB-4 showed a weak positive correlation with stages of fibrosis in patients with elevated ALT. Also platelet count showed a weak, but negative correlation as seen in Table 4-19. The noninvasive fibrosis tests showed no significant correlations in patients with persistently normal ALT.

Correlation of stage with the results of noninvasive fibrosis tests			
	Laboratory data	**Ishak fibrosis score**	**METAVIR fibrosis score**
Patients with persistently normal ALT	**AAR**	-0.16	-0.16
	APRI	0.00	-0.05
	FIB-4	0.02	-0.04
	Model 3	-0.20	-0.20
Patients with elevated ALT	**AAR**	-0.21	-0.16
	APRI	0.25	0.23
	FIB-4	0.41**	0.42**
	Model 3	0.43**	0.45**

Table 4-26: Correlation (Spearman´s rank correlation coefficient, two-tailed) of stage with values of noninvasive fibrosis tests (AAR, APRI, FIB-4, Model 3) in both subgroups (patients with persistently normal ALT vs. patients with elevated ALT). Histological stages of fibrosis were estimated with the Ishak and METAVIR fibrosis scores.

For each group we constructed receiver operating characteristic curves of all noninvasive fibrosis tests and platelet count and evaluated the diagnostic accuracies by calculating sensitivity, specificity, PPV and NPV. We used the same cutoff points for the Ishak and METAVIR fibrosis score as in the whole group. However, the group of patients with persistently normal ALT showed almost exclusively low fibrosis stages. Therefore, higher cutoffs for the distinction of bridging fibrosis stages and cirrhosis (Ishak F4-F6 and Ishak F6, METAVIR F3-F4 and METAVIR F4) could not be applied meaningfully. The exact distributions of cases are shown in Table 4-27.

Clustering of fibrosis stages for ROC analysis			
State of disease	**Defined cutoff**	**Proportion of cases**	
		PNALT	**EALT**
Significant fibrosis	Ishak F3-F6 vs. F0-F2	7 vs. 51	15 vs. 30
Bridging fibrosis	Ishak F4-F6 vs. F0-F3	1 vs. 57	8 vs. 37
Cirrhosis	Ishak F5-F6 vs. F0-F4	1 vs. 57	6 vs. 39
Significant fibrosis	METAVIR F2-F4 vs. F0-F1	9 vs. 49	16 vs. 29
Bridging fibrosis	METAVIR F3-F4 vs. F0-F2	1 vs. 57	8 vs. 37
Cirrhosis	METAVIR F4 vs. F0-F3	1 vs. 57	4 vs. 41

Table 4-27: Clustering of fibrosis stages for ROC analysis in both subgroups. Data is shown for the Ishak and METAVIR scoring system.

Figure 4-19 shows the ROC curves for all noninvasive tests and platelet count in patients with persistently normal ALT. ROC curves for the distinction of cirrhosis and bridging

fibrosis could not be constructed. Only one patient had the positive actual state of bridging fibrosis or cirrhosis.

The ROC curves for the prediction of significant fibrosis in patients with persistently normal ALT were close to or even below the reference line. The corresponding AUROCs are given Table 4-28.

Prediction of significant fibrosis (Ishak F3-F6 and METAVIR F2-F4) in patients with persistently normal ALT

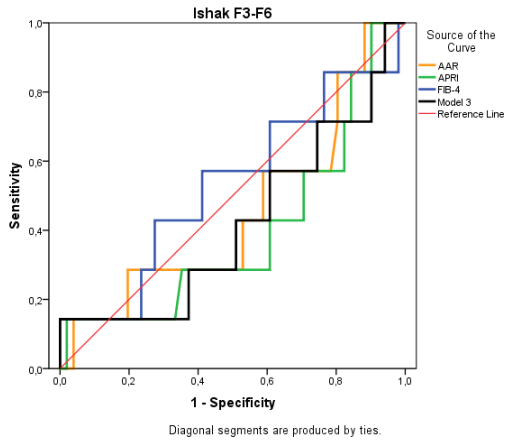
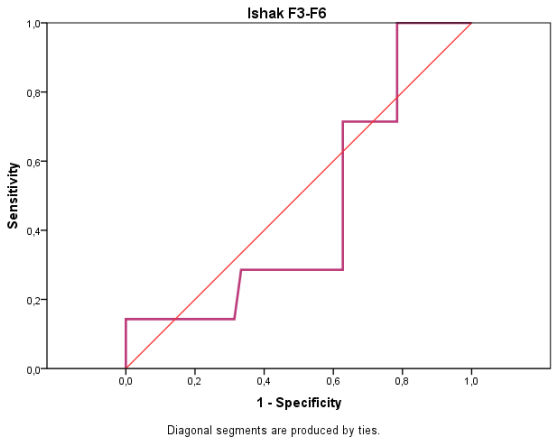
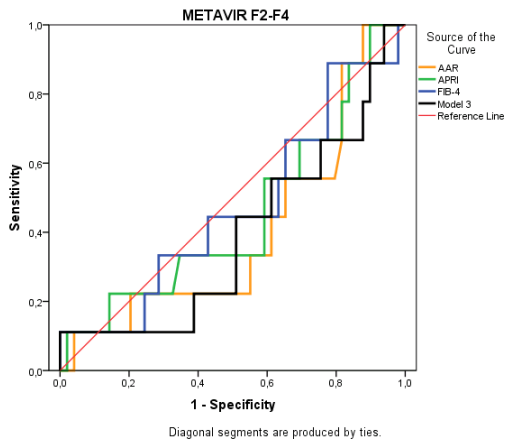
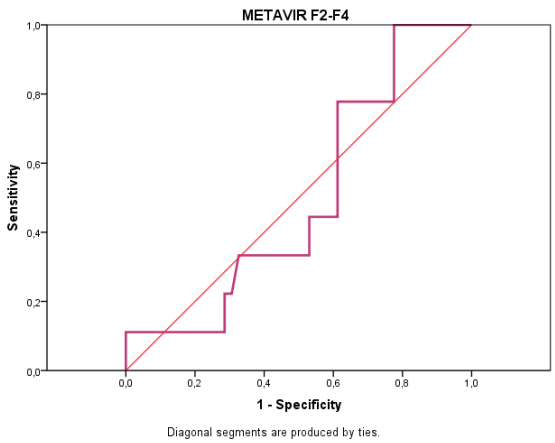
Receiver operating characteristic curves of four noninvasive fibrosis tests	Receiver operating characteristic curves of platelet count
	
	

Figure 4-19: Receiver operating characteristic curves of all noninvasive fibrosis tests (AAR, APRI, FIB-4, Model 3 and Platelet count for distinction of the presence or absence of significant fibrosis (Ishak F3-F6 and METAVIR F2-F4) in patients with persistently normal ALT. Larger values of the noninvasive fibrosis test results indicate stronger evidence for a positive actual state. Smaller values of platelet count indicate stronger evidence for a positive actual state.

Staging algorithm	Noninvasive test	AUROC F3-F6
Ishak fibrosis score vs.	AAR	0.45 (0.22-0.69)
	APRI	0.39 (0.16-0.63)
	FIB- 4	0.53 (0.29-0.78)
	Model 3	0.42 (0.18-0.66)
	Platelet count	0.46 (0.24-0.68)
		AUROC F2-F4
METAVIR fibrosis score vs.	AAR	0.40 (0.20-0.60)
	APRI	0.45 (0.24-0.66)
	FIB-4	0.47 (0.26-0.68)
	Model 3	0.39 (0.19-0.59)
	Platelet count	0.50 (0.31-0.68)

* $P < 0.05$ vs. AUROC

Table 4-28: Area under the receiver operating characteristic curve of AAR, APRI, FIB-4, Model 3 and Platelet count for the distinction of significant fibrosis using cutoff values of the Ishak fibrosis score and METAVIR fibrosis score in patients with persistently normal ALT.

The ROC curves for patients with elevated ALT are given in Figures 4-20, 4-21 and 4-22. The results are similar to the ones found in the whole population. Highest ROC-curves were achieved for the distinction of bridging fibrosis and cirrhosis.

Prediction of significant fibrosis (Ishak F3-F6 and METAVIR F2-F4) in patients with elevated ALT

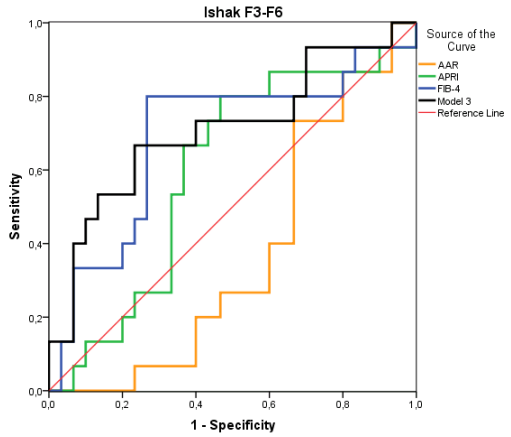
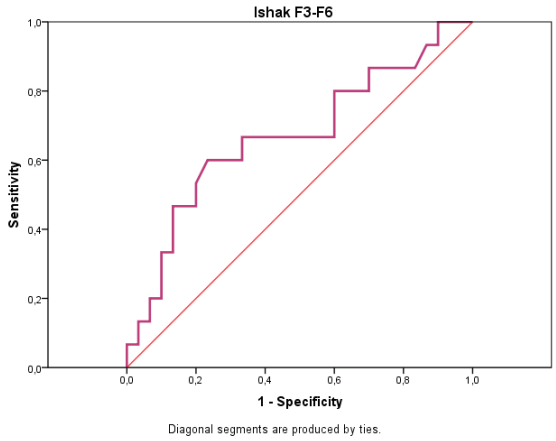
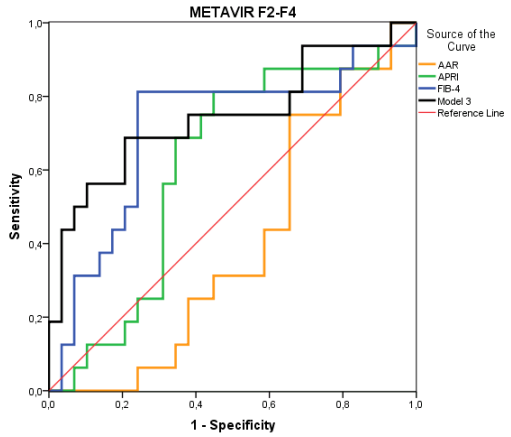
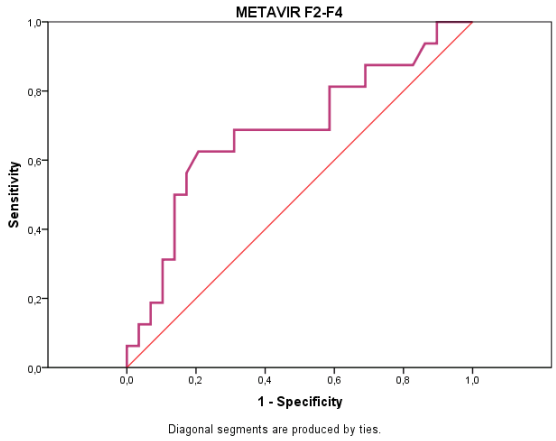
Receiver operating characteristic curves of four noninvasive fibrosis tests	Receiver operating characteristic curves of platelet count
	
	

Figure 4-20: Receiver operating characteristic curves of all noninvasive fibrosis tests (AAR, APRI, FIB-4, Model 3 and Platelet count for distinction of the presence or absence of significant fibrosis (Ishak F3-F6 and METAVIR F2-F4) in patients with elevated ALT. Larger values of the noninvasive fibrosis test results indicate stronger evidence for a positive actual state. Smaller values of platelet count indicate stronger evidence for a positive actual state.

Prediction of bridging fibrosis (Ishak F4-F6 and METAVIR F3-F4) in patients with elevated ALT

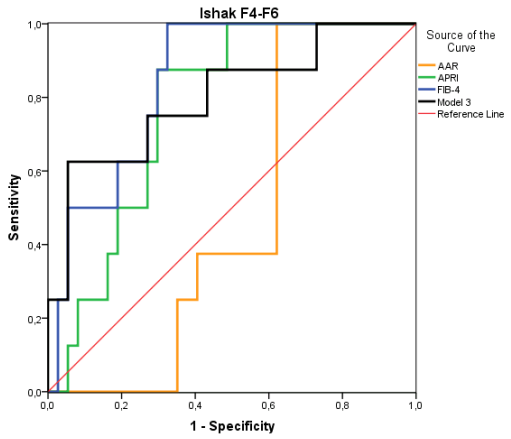
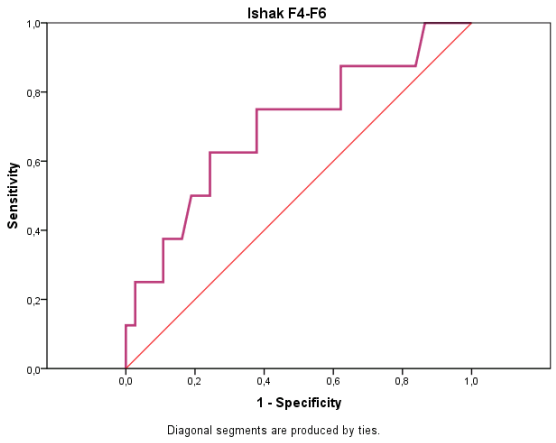
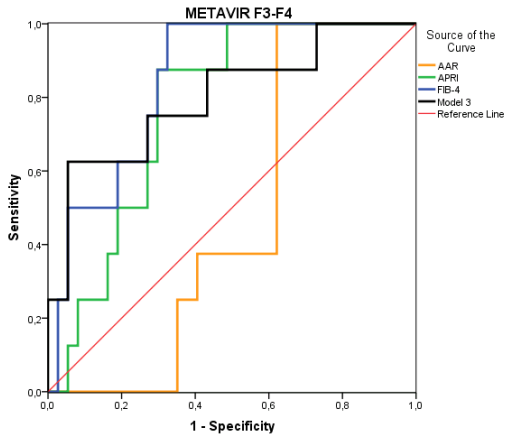
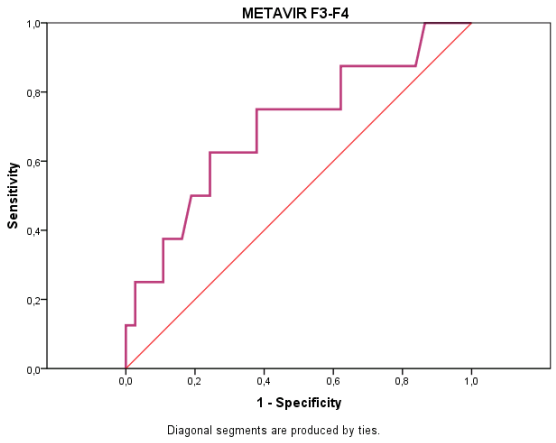
Receiver operating characteristic curves of four noninvasive fibrosis tests	Receiver operating characteristic curves of platelet count
	
	

Figure 4-21: Receiver operating characteristic curves of all noninvasive fibrosis tests (AAR, APRI, FIB-4, Model 3 and Platelet count for distinction of the presence or absence of bridging fibrosis (Ishak F4-F6 and METAVIR F3-F4) in patients with elevated ALT. Larger values of the noninvasive fibrosis test results indicate stronger evidence for a positive actual state. Smaller values of platelet count indicate stronger evidence for a positive actual state

Prediction of cirrhosis (Ishak F5-F6 and METAVIR F4) in patients with elevated ALT

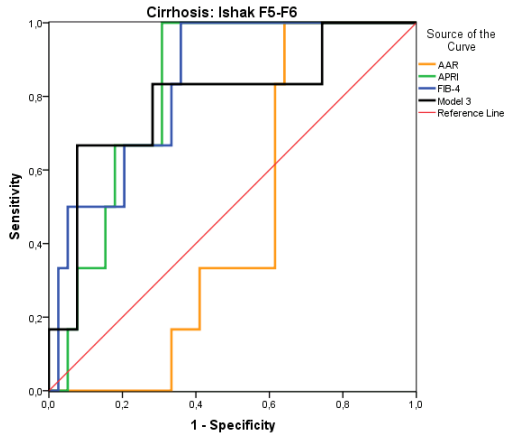
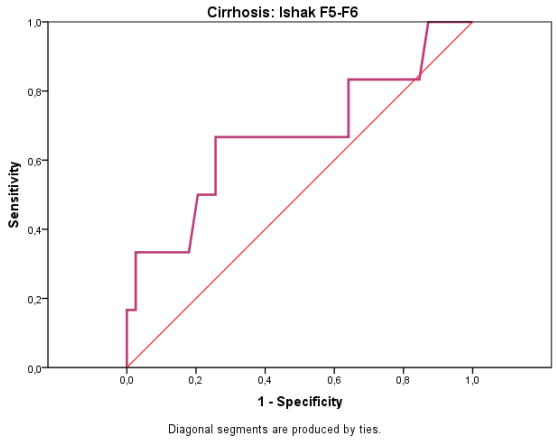
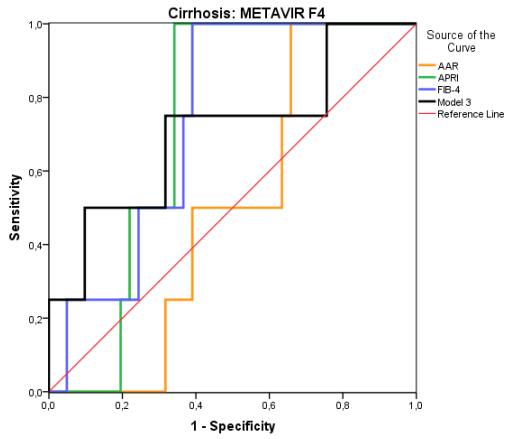
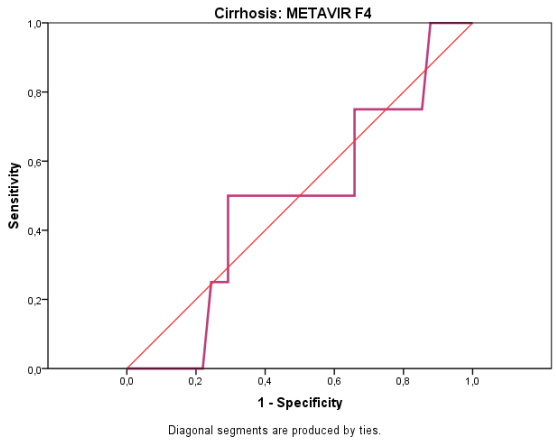
Receiver operating characteristic curves of 4 noninvasive fibrosis tests	Receiver operating characteristic curve of platelet count
	
	

Figure 4-22: Receiver operating characteristic curves of all noninvasive fibrosis tests (AAR, APRI, FIB-4, Model 3 and Platelet count for distinction of the presence or absence of cirrhosis (Ishak F5-F6 and METAVIR F4) in patients with elevated ALT. Larger values of the noninvasive fibrosis test results indicate stronger evidence for a positive actual state. Smaller values of platelet count indicate stronger evidence for a positive actual state.

The AUROCs for the noninvasive fibrosis tests in patients with elevated ALT are given in Table 4-29. High AUROCs were found for FIB-4, APRI and Model 3 for the prediction of bridging fibrosis and cirrhosis. AAR showed a ROC curve under the reference line in patients with elevated ALT as in the whole study population.

Staging algorithm	Noninvasive test	AUROC F3-F6	AUROC F4-F6	AUROC F5-F6
Ishak fibrosis score vs.	**AAR**	0.37 (0.21-0.53)	0.47 (0.30-0.64)	0.46 (0.29-0.63)
	APRI	0.60 (0.42-0.77)	0.77 (0.63-0.91)*	0.82 (0.69-0.95)*
	FIB- 4	0.69 (0.51-0.87)*	0.85 (0.72-0.97)*	0.83 (0.69-0.97)*
	Model 3	0.71 (0.54-0.88)*	0.80 (0.62-0.98)*	0.79 (0.57-1.00)*
	Platelet count	0.67 (0.49-0.85)	0.70 (0.49-0.91)	0.67 (0.41-0.94)
		AUROC F2-F4	**AUROC F3-F4**	**AUROC F4**
METAVIR fibrosis score vs.	**AAR**	0.39 (0.23-0.56)	0.47 (0.31-0.64)	0.50 (0.30-0.70)
	APRI	0.61 (0.44-0.79)	0.77 (0.63-0.91)*	0.73 (0.58-0.87)
	FIB-4	0.71 (0.54-0.88)*	0.85 (0.72-0.97)*	0.74 (0.56-0.91)
	Model 3	0.75 (0.58-0.91)*	0.80 (0.62-0.98)*	0.70 (0.41-1.00)
	Platelet count	0.69 (0.52-0.86)*	0.70 (0.49-0.91)	0.49 (0.21-0.77)

* $P < 0.05$ vs. AUROC

Table 4-29: Area under the receiver operating characteristic curve of AAR, APRI, FIB-4, Model 3 and Platelet count for the distinction of significant fibrosis, bridging fibrosis and cirrhosis in patients with elevated ALT using cutoff values of the Ishak fibrosis score.

In order to assess the diagnostic accuracies of each simple fibrosis test for the prediction of significant fibrosis, bridging fibrosis and cirrhosis, sensitivity, specificity, PPV and NPV have been calculated separately for both groups. We used the originally proposed cutoff values for our calculations. Table 4-30 shows all results for patients with persistently normal ALT. As mentioned before, ROC curves for the distinction of bridging fibrosis and cirrhosis could not be constructed in patients with persistently normal, having only one case of cirrhosis and neither one case of Ishak F4, F5 nor METAVIR F3. The resulting ROC curve for high fibrosis stages would be a straight vertical line. It must be therefore emphasized that the high diagnostic accuracies that are shown in Table 4-30 for the distinction of bridging fibrosis and cirrhosis in patients with persistently normal ALT have limited validity. Both cutoff values of platelet count and the high cutoff value of FIB-4 were reached by the same single patient. This patient had cirrhosis (Ishak F6, METAVIR F4). The cutoff values of platelet count and FIB-4 could predict cirrhosis with perfect accuracy in this patient. The high cutoff values of APRI for the prediction of significant fibrosis and cirrhosis were not reached by any patient. The low cutoff values of APRI, FIB-4 and Model 3 could exclude bridging fibrosis and cirrhosis with a NPV of 100% and a sensitivity of 100%. Similarly, AAR values < 1 and platelet count values above both defined cutoff values excluded bridging fibrosis and cirrhosis with a NPV of 100%

and a sensitivity of 100%. Best proportions of cases were found the distinction of significant fibrosis (Ishak F3-F6 and METAVIR F2-F4) in patients with persistently normal ALT. as shown in Table 4-27. The diagnostic accuracies for the prediction of significant fibrosis, however, were low. The cutoff value of FIB-4 > 3.25 and both platelet count cutoff values reached PPVs of 100% and NPVs of 90% for the prediction and exclusion of significant fibrosis (Ishak F3-F6). Specificity was 100%, but sensitivity only 14% as and mentioned, only one patient was classified by the cutoff values of the tests.

	Cutoff value	Patients within cutoff value (%)	Diagnostic accuracy (%)	Ishak fibrosis score			METAVIR fibrosis score		
				F3-F6	F4-F6	F5-F6	F2-F4	F3-F4	F4
AAR	≥ 1.0	26 (45%)	Sensitivity	29	100	100	22	100	100
			Specificity	53	56	56	51	56	56
			PPV	8	4	4	8	4	4
			NPV	84	100	100	78	100	100
APRI	< 0.5	51 (88%)	Sensitivity	14	100	100	11	100	100
			Specificity	88	90	90	88	90	90
			PPV	14	14	14	14	14	14
			NPV	88	100	100	84	100	100
	≥1.5	0 (0%)	Sensitivity Specificity PPV NPV	-	-	-	-	-	-
	<1.0	56 (97%)	Sensitivity	14	100	100	11	100	100
			Specificity	98	98	98	98	98	98
			PPV	50	50	50	50	50	50
			NPV	89	100	100	86	100	100
	≥2.0	0 (0%)	Sensitivity Specificity PPV NPV	-	-	-	-	-	-
FIB-4	<1.45	51 (88%)	Sensitivity	14	100	100	11	100	100
			Specificity	88	90	90	88	90	90
			PPV	14	14	14	14	14	14
			NPV	88	100	100	84	100	100
	>3.25	1 (2%)	Sensitivity	14	100	100	11	100	100
			Specificity	100	100	100	100	100	100
			PPV	100	100	100	100	100	100
			NPV	90	100	100	86	100	100
Model 3	<0.2	23 (40%)	Sensitivity	57	100	100	56	100	100
			Specificity	39	40	40	39	40	40
			PPV	11	3	3	14	3	3
			NPV	87	100	100	83	100	100
	>0.5	3 (5%)	Sensitivity	14	100	100	11	100	100
			Specificity	96	97	97	96	97	97
			PPV	33	33	33	33	33	33
			NPV	89	100	100	86	10	100
Platelet count	<130 x 10^9/L	1 (2%)	Sensitivity	14	100	100	11	100	100
			Specificity	100	100	100	100	100	100
			PPV	100	100	100	100	100	100
			NPV	90	100	100	86	100	100
	<150 x 10^9/L	1 (2%)	Sensitivity	14	100	100	11	100	100
			Specificity	100	100	100	100	100	100
			PPV	100	100	100	100	100	100
			NPV	90	100	100	86	100	100

Table 4-30: Diagnostic accuracies of the noninvasive fibrosis tests AAR, APRI, FIB-4, Model 3 and Platelet count for prediction of significant fibrosis, bridging fibrosis and cirrhosis in patients with persistently normal ALT. Original cutoff values proposed by the authors of the noninvasive fibrosis tests were applied.[19-24,36] For abbreviations see appendix.

Table 4-31 shows the number and percentage of patients that were classified correctly by the cutoff values of the noninvasive fibrosis tests and platelet count. The cutoff values of platelet count reached the highest percentages of correctly classified patients.

	Ishak fibrosis score		
	F0-F2 vs. F3-F6	F0-F3 vs. F4-F6	F0-F4 vs. F5-F6
AAR < 1.0 vs. > 1.0	29 (50.0%)	33 (56.9%)	33 (56.9%)
APRI < 0.5 vs. ≥ 1.5	45 (77.6%)	56 (96.6%)	56 (96.6%)
APRI < 1 vs. ≥ 2	50 (86.2%)	51 (87.9%)	51 (87.9%)
FIB-4 < 1.45 vs. > 3.25	46 (79.3%)	52 (89.7%)	52 (89.7%)
Model 3 < 0.2 vs. > 0.5	21(36.2%)	24 (41.4%)	24 (41.4%)
Platelet count < 130 x 10^9/L vs. > 130 x 10^9/L	52 (89.7%)	58 (100.0%)	58 (100.0%)
Platelet count < 150 x 10^9/L vs. > 150 x 10^9/L	52 (89.7%)	58 (100.0%)	58 (100.0%)
	METAVIR fibrosis score		
	F0-F1 vs. F2-F4	F0-F2 vs. F3-F4	F0-F3 vs. F4
AAR < 1.0 vs. > 1.0	27 (46.6%)	33 (56.9%)	33 (56.9%)
APRI < 0.5 vs. ≥ 1.5	43 (74.1%)	51 (87.9%)	51 (87.9%)
APRI < 1 vs. . ≥ 2	48 (82.8%)	56 (96.6%)	56 (96.6%)
FIB-4 < 1.45 vs. > 3.25	44 (75.9%)	52 (89.7%)	52 (89.7%)
Model 3 < 0.2 vs. > 0.5	20 (34.5 %)	24 (41.4%)	24 (41.4%)
Platelet count < 130 x 10^9/L vs. > 130 x 10^9/L	50 (86.2%)	58 (100.0%)	58 (100.0%)
Platelet count < 150 x 10^9/L vs. > 150 x 10^9/L	50 (86.2%)	58 (100.0%)	58 (100.0%)

Table 4-31: Number and percentage of correctly classified patients in the subgroup with persistentely normal ALT for each noninvasive fibrosis test. Results are given for fibrosis stage clusters within Ishak fibrosis score and METAVIR fibrosis score.

Table 4-32 shows the diagnostic accuracies of noninvasive fibrosis tests and platelet count in patients with elevated ALT. APRI values of < 0.5 showed high diagnostic accuracies for the exclusion of significant fibrosis, bridging fibrosis and cirrhosis. An APRI value ≥ 1.5 for the prediction of fibrosis showed low PPVs with high specificity for the prediction of significant fibrosis, bridging fibrosis and cirrhosis. The results for the APRI cirrhosis cutoff values of < 1 and > 2 showed likewise results. A FIB-4 index < 1.45 showed good diagnostic accuracy for the exclusion of bridging fibrosis (Ishak F4-F6) with a negative predictive value of 93% and a sensitivity of 75%, whereas the cutoff value of > 3.25 showed poor results for the prediction of bridging fibrosis (Ishak F4-F6). Only one patient

reached a FIB-4 index value > 3.25, therefore these results have limited validity. Best diagnostic accuracies in patients with elevated ALT were found for Model 3. A cutoff > 0.5 predicted bridging fibrosis (Ishak F4-F6 and METAVIR F3-F4) with a positive predictive value of 100% with a specificity of 100%. A cutoff value < 0.2 excluded bridging fibrosis with a negative predictive value of 96 % and a sensitivity of 88%. The results for the prediction of bridging fibrosis were better than those for the prediction of cirrhosis. AAR and platelet count showed low diagnostic accuracies.

	Cutoff value	Patients within cutoff value (%)	Diagnostic accuracy (%)	Ishak fibrosis score			METAVIR fibrosis score		
				F3-F6	F4-F6	F5-F6	F2-F4	F3-F4	F4
AAR	≥ 1.0	3 (7%)	Sensitivity	0	0	0	0	0	0
			Specificity	93	95	95	93	95	95
			PPV	0	0	0	0	0	0
			NPV	65	81	86	63	81	91
APRI	< 0.5	17 (29%)	Sensitivity	80	100	100	81	100	100
			Specificity	43	43	41	45	43	39
			PPV	41	28	21	45	28	14
			NPV	81	100	100	81	100	100
	≥1.5	9 (20%)	Sensitivity	20	38	50	18	38	25
			Specificity	80	84	85	79	84	81
			PPV	33	33	33	33	33	11
			NPV	67	86	92	64	86	92
	<1.0	28 (62%)	Sensitivity	53	88	100	56	88	100
			Specificity	67	70	69	69	70	66
			PPV	44	39	33	50	39	22
			NPV	74	96	100	74	96	100
	≥2.0	6 (10%)	Sensitivity	13	25	33	13	25	0
			Specificity	87	89	90	86	89	85
			PPV	33	33	33	33	33	0
			NPV	67	85	90	64	85	90
FIB-4	<1.45	29 (64%)	Sensitivity	60	75	67	63	75	50
			Specificity	73	70	67	76	71	63
			PPV	53	35	24	59	35	12
			NPV	79	93	93	79	93	93
	>3.25	1 (2%)	Sensitivity	0	0	0	0	0	0
			Specificity	97	97	98	97	97	98
			PPV	0	0	0	0	0	0
			NPV	66	82	86	64	82	91
Model 3	<0.2	23(51%)	Sensitivity	73	88	83	75	88	75
			Specificity	60	57	54	62	57	51
			PPV	48	30	22	52	30	13
			NPV	82	96	96	82	96	96
	>0.5	2 (4%)	Sensitivity	13	25	17	13	25	25
			Specificity	100	100	97	100	100	98
			PPV	100	100	50	100	100	50
			NPV	70	86	88	67	86	93
Platelet count	<130 x 10^9/L	4 (9%)	Sensitivity	13	25	33	13	25	0
			Specificity	93	95	95	93	95	90
			PPV	50	50	50	50	50	0
			NPV	68	85	90	66	85	90
	<150 x 10^9/L	6 (13%)	Sensitivity	20	25	33	19	25	0
			Specificity	90	89	90	90	89	85
			PPV	50	33	33	50	33	0
			NPV	69	85	90	67	85	90

Table 4-32: Diagnostic accuracies of the noninvasive fibrosis tests AAR, APRI, FIB-4, Model 3 and Platelet count for prediction of significant fibrosis, bridging fibrosis and cirrhosis in patients with elevated ALT. Original cutoff values proposed by the authors of the noninvasive fibrosis tests were applied.[19-24,36] For abbreviations see appendix.

Table 4-33 shows patients with elevated ALT that were correctly classified by the defined cutoff values of noninvasive fibrosis tests and platelet count. Highest results were found for tests using only a single cutoff value. It must be considered that most patients had AAR values lower than 1 and platelet count greater than $150x10^9$/L. Though being a good predictor of high stages of fibrosis, Model 3 classified only 51.1% of patients correctly. The rest of patients had values between the two cutoffs. Likewise results were found for APRI and FIB-4.

	Ishak fibrosis score		
	F0-F2 vs. F3-F6	F0-F3 vs. F4-F6	F0-F4 vs. F5-F6
AAR < 1.0 vs. > 1.0	28 (62.2%)	35 (77.8%)	37 (82.2%)
APRI < 0.5 vs. ≥ 1.5	16 (35.6%)	19 (42.2%)	19 (42.2%)
APRI < 1 vs. ≥ 2	22 (48.9%)	28 (62.2%)	29 (64.4%)
FIB-4 < 1.45 vs. > 3.25	22 (48.9%)	26 (57.8%)	26 (57.8%)
Model 3 < 0.2 vs. > 0.5	20 (44.4%)	23 (51.1%)	22 (48.9%)
Platelet count < 130 x 10^9/L vs. > 130 x 10^9/L	30 (66.7%)	37 (82.2%)	39 (86.7%)
Platelet count < 150 x 10^9/L vs. > 150 x 10^9/L	30 (66.7%)	35 (77.8%)	37 (82.2%)
	METAVIR fibrosis score		
	F0-F1 vs. F2-F4	F0-F2 vs. F3-F4	F0-F3 vs. F4
AAR < 1.0 vs. > 1.0	27 (60.0%)	35 (77.8%)	39 (86.7%)
APRI < 0.5 vs. ≥ 1.5	16 (35.6%)	19 (42.2%)	17 (37.8%)
APRI < 1 vs. ≥ 2	22 (48.9%)	28 (62.2%)	27 (60.0%)
FIB-4 < 1.45 vs. > 3.25	22 (48.9%)	26 (57.8%)	26 (57.8%)
Model 3 < 0.2 vs. > 0.5	20 (44.4%)	23 (51.1%)	22 (48.9%)
Platelet count < 130 x 10^9/L vs. > 130 x 10^9/L	29 (64.4%)	37 (82.2%)	37 (82.2%)
Platelet count < 150 x 10^9/L vs. > 150 x 10^9/L	29 (64.4%)	35 (77.8%)	35 (77.8%)

Table 4-33: Number and percentage of correctly classified patients for each noninvasive fibrosis test in the subgroup with elevated ALT. Results are given for fibrosis stage clusters within Ishak fibrosis score and METAVIR fibrosis score.

For each group, we calculated optimized cutoff values using the individual receiver operating characteristic curves. We chose values with the highest Youden index. The results for patients with persistently normal ALT are given in Table 4-34. Most of the cutoff values showed high accuracies for the exclusion of fibrosis and low accuracies for its prediction, except for Model 3. Youden-indices were generally low.

		Ishak 3-6	METAVIR 2-4
AAR	Youden-Index	0.12	0.12
	Best cut off point	0.60	0.60
	Sensitivity	100%	100%
	Specificity	12%	12%
	PPV	14%	17%
	NPV	100%	100%
APRI	Youden-Index	0.12	0.10
	Best cut off point	0.90	0.21
	Sensitivity	14%	100%
	Specificity	98%	10%
	PPV	50%	17%
	NPV	89%	100%
FIB-4	Youden-Index	0.16	0.11
	Best cut off point	0.85	0.53
	Sensitivity	57%	89%
	Specificity	59%	22%
	PPV	16%	17%
	NPV	91%	92%
Model 3	Youden-Index	0.14	0.11
	Best cut off point	0.93	0.93
	Sensitivity	14%	11%
	Specificity	100%	100%
	PPV	100%	100%
	NPV	90%	86%
Platelet count	Youden-Index	0.22	0.22
	Best cut off point	298	298
	Sensitivity	100%	100%
	Specificity	22%	22%
	PPV	15%	19%
	NPV	100%	100%

Table 4-34: Diagnostic accuracies of the noninvasive fibrosis tests AAR, APRI, FIB-4, Model 3 and Platelet count for prediction of significant fibrosis (Ishak F3-F6, and METAVIR F2-F4), bridging fibrosis (Ishak F4-F6 and METAVIR F3-F4) and cirrhosis (Ishak F5-F6 and METAVIR F4) in patients with persistently normal ALT. Youden- indices were calculated for all coordinate points of the ROC curves to select optimized cutoff values. For abbreviation see appendix.

Optimized cutoff values for all noninvasive fibrosis tests and platelet count in patients with elevated ALT are given in Table 4-35. High Youden-indices were found for APRI and FIB-4.

		Ishak fibrosis score			METAVIR fibrosis score		
		F3-F6	F4-F6	F5-F6	F2-F4	F3-F4	F4
AAR	Youden-Index	0.07	0.38	0.36	0.10	0.38	0.34
	Best cutoff value	0.38	0.57	0.57	0.57	0.57	0.57
	Sensitivity	100%	100%	100%	75%	100%	100%
	Specificity	7%	38%	36%	35%	38%	34%
	PPV	35%	26%	19%	39%	26%	13%
	NPV	100%	100%	100%	71%	100%	100%
APRI	Youden-Index	0.33	0.58	0.69	0.37	0.58	0.66
	Best cutoff value	0.69	1.00	1.00	0.69	1.00	1.00
	Sensitivity	80%	88%	100%	81%	88%	100%
	Specificity	53%	70%	69%	55%	70%	66%
	PPV	46%	39%	33%	50%	39%	22%
	NPV	84%	96%	100%	84%	96%	100%
FIB-4	Youden-Index	0.53	0.68	0.64	0.57	0.68	0.61
	Best cutoff value	1.18	1.18	1.18	1.18	1.18	1.18
	Sensitivity	80%	100%	100%	81%	100%	100%
	Specificity	73%	68%	64%	76%	68%	61%
	PPV	60%	40%	30%	65%	40%	20%
	NPV	88%	100%	100%	88%	100%	100%
Model 3	Youden-Index	0.43	0.57	0.59	0.48	0.57	0.43
	Best cutoff value	0.24	0.39	0.39	0.24	0.39	0.27
	Sensitivity	67%	63%	67%	69%	63%	75%
	Specificity	77%	95%	92%	79%	95%	68%
	PPV	59%	71%	57%	65%	71%	19%
	NPV	82%	92%	95%	82%	92%	97%
Platelet count	Youden-Index	0.37	0.38	0.41	0.42	0.38	0.21
	Best cutoff value	184	181	181	184	181	181
	Sensitivity	60%	63%	67%	63%	63%	50%
	Specificity	77%	76%	74%	79%	76%	71%
	PPV	56%	36%	29%	63%	36%	14%
	NPV	79%	90%	94%	79%	90%	94%

Table 4-35: Diagnostic accuracies of the noninvasive fibrosis tests AAR, APRI, FIB-4, Model 3 and platelet count for prediction of significant fibrosis, bridging fibrosis and cirrhosis in patients with elevated ALT. Youden- indices were calculated for all coordinate points of the ROC curves to select optimized cutoff values. For abbreviation see appendix.

5 DISCUSSION

Our major aim was to evaluate the applicability of noninvasive fibrosis tests in patients with persistently normal ALT. To give an appropriate answer we examined three different groups of patients with chronic hepatitis C. Firstly, we examined the ALT-unselective total population and secondly the two subgroups with either persistently normal ALT or elevated ALT. In the unselective whole population, we could confirm most of the reported AUROCs and diagnostic accuracies for noninvasive fibrosis tests.[19-24,36] Likewise results were found in a subgroup of patients with elevated ALT values. However, the results in this subgroup were slightly lower which may be due to the small number of patients included. In our population, AUROCs for the distinction of significant fibrosis tended to be generally lower than for the distinction of higher stages as bridging fibrosis and cirrhosis. Furthermore, our population had primarily patients with low stages of fibrosis and only few cases with advanced stages and cirrhosis. This imbalance may have caused better results for exclusion of advanced disease than for its prediction. It manifested for instance in smaller positive predictive values than reported by other authors, while other diagnostic accuracies were in good concordance at the same time. Yet only in patients with persistently normal ALT, ROC curves for the prediction and exclusion of significant fibrosis were situated very close to or under the reference line of 0.5, implicating a generally worse applicability of these noninvasive fibrosis tests. Prediction of higher stages like bridging fibrosis and cirrhosis could not be evaluated in patients with normal ALT because of the lack of a sufficient count of cases with high disease stages. In the following we will discuss the results of every fibrosis test; AAR had very low diagnostic accuracies in predicting significant fibrosis and cirrhosis in our study population and the two subgroups with AUROCs under the reference line. It could not predict fibrosis and cirrhosis with sufficient accuracy in any of our groups. AAR has originally been proposed as a predictor of cirrhosis[19,20] and until now, there is no evidence of its use as a predictor of significant fibrosis.[21] Wai et al.[21] also reported that AAR was insufficient in the prediction of cirrhosis. However, there also studies who confirm its accuracy.[19,20]

APRI has been proposed as a simple model to predict both fibrosis and cirrhosis. Wai et al.[21] reported an AUROC of 0.88 (95% CI 0.80-0.96) for the prediction of

significant fibrosis (Ishak F3-F6) and an AUROC of 0.94 (0.89-1.00) for prediction of cirrhosis (Ishak F5-F6). Our findings in the whole study population showed a lower AUROC for prediction of significant fibrosis (Ishak F3-F6) of 0.63 (95% CI 0.48-0.78) and a similar AUROC of 0.92 (95% CI 0.87-0.98) for the prediction of cirrhosis. In patients with elevated ALT the corresponding AUROCs were 0.60 (95% CI 0.42-0.77) and 0.82 (0.69-0.95). In contrast, in patients with normal ALT APRI showed a ROC curve under the reference line (0.39, 95% CI 0.16-0.63). ROC curves for the prediction of cirrhosis could not be constructed. Table 5-1 shows the diagnostic accuracies for APRI in our whole population, the PNALT and EALT group. For comparison, reported diagnostic accuracies by Wai et al. are shown in a separate column.

APRI cutoff value	Accuracy	All patients	EALT	PNALT	Wai et al.[21]
			Prediction of significant fibrosis (Ishak F3-F6)		
< 0.5	Sensitivity. Specificity PPV NPV	59 72 36 87	80 43 41 81	14 88 14 88	91 47 61 86
≥1.5	Sensitivity. Specificity PPV NPV	14 93 33 80	20 80 33 67	-	41 95 88 64
			Prediction of cirrhosis (Ishak F5-F6)		
<1.0	Sensitivity. Specificity PPV NPV	100 87 35 100	100 69 33 100	100 98 50 100	89 75 38 98
≥2.0	Sensitivity. Specificity PPV NPV	29 96 33 95	33 90 33 90	-	57 93 57 93

Table 5-1: Diagnostic accuracies of APRI in the whole study population, patients with elevated ALT and persistently normal ALT. The reported accuracies by Wai et al.[21] have been added for comparison. For abbreviations see appendix.

The diagnostic accuracies found for APRI in the whole study population and EALT group were comparable to those reported by Wai et al[21]with slight differences that may be related to the different population characteristics mentioned before. E.g. positive predictive values were lower in our populations which may be due to the small number of patients who reached the high cutoff values as ≥1.5 and ≥2. However,

patients with persistently normal ALT showed remarkably lower accuracies. A cutoff value of <0.5 had a very low sensitivity and PPV of 14%. The accuracies found for the exclusion of cirrhosis may be biased because there was only one cirrhotic patient. An adequate answer to the question, if APRI can predict or exclude cirrhosis in patients with normal ALT cannot be given. A population with more cirrhotic patients with persistently normal ALT is needed to prove our findings.

Vallet-Pichard et al.[23] proposed that FIB-4 could correctly identify patients with severe fibrosis (METAVIR F3-F4) with an AUROC of 0.85 (95% CI 0.82-0.89) and cirrhosis with an AUROC of 0.91 (95% CI 0.86-0.93).We found an AUROC of 0.91 (95% CI 0.84-0.98) for the identification of patients with severe fibrosis (METAVIR F3-F4) in our whole study population. The AUROC for the prediction of cirrhosis (METAVIR F4) was 0.86 (95% CI 0.75-0.97). Applying the categorization of Ishak to define cirrhosis we found an AUROC of 0.91 (95% CI 0.82-0.99). The corresponding AUROCs for patients with elevated ALT were 0.85 (95% CI 0.72-0.97) for the prediction of METAVIR F3-F4-fibrosis and 0.74 (95% CI 0.56-0.91) for the prediction of METAVIR F4-cirrhosis. These AUROCs could not be calculated for patients with persistently normal ALT. Evaluation of FIB-4 for a lower fibrosis cutoff value, METAVIR F2-F4, showed a ROC-curve under the reference line.

In our whole population a FIB-4 index <1.45 had a negative predictive value of 98% (in comparison to Vallet-Pichard et al.[23]: 94.7%) and a sensitivity of 78% (Vallet-Pichard et al.[23]: 74.3%) to exclude severe fibrosis. For a FIB-4 index higher than 3.25 we found a positive predictive value to confirm the existence of significant fibrosis (METAVIR F3-F4) of 50% (Vallet-Pichard et al.[23]: 82.1%) with a specificity of 99% (Vallet-Pichard et al.[23]: 98.2%). The results were in good concordance with the ones reported by Vallet-Pichard et al.[23] except for the positive predictive value corresponding to the cutoff value of > 3.25. Differences in the results may be related to the fact that our population included predominantly low disease stages. Only two patients had FIB-4 values greater than 3.25.

In patients with persistently normal ALT a FIB-4 index < 1.45 had a negative predictive value of 100 % to exclude significant fibrosis (METAVIR F3-F4) and a sensitivity of 100%. However, these results are hampered by the fact of having only one patient within the stages F3 to F4 of the METAVIR fibrosis score.

In patients with elevated ALT a FIB-4 index < 1.45 had a negative predictive value of 93% and a sensitivity of 75%. Diagnostic accuracy for the cutoff value > 3.25 to predict significant fibrosis in the subgroups could not be evaluated meaningfully as there was only one patient classified in each of them.

Model 3 has been proposed by Lok et al.[24] as a model for prediction of cirrhosis in patients with chronic hepatitis C. This group reported that a cutoff of less than 0.2 to exclude cirrhosis would misclassify only 7.8 % of patients with cirrhosis. Applying this cutoff value for our total study population it would misclassify 1 patient, corresponding to 14 % of all cirrhotic patients. In the group of patients with elevated ALT it would misclassify 17% of all cirrhotic patients. The other proposed cutoff value of greater than 0.5 to predict cirrhosis misclassified 14.8% of non-cirrhotic patients in the HALT-C Cohort.[24] In our total population and in the group of patients with elevated ALT this cutoff value would misclassify 3% of patients without cirrhosis. However, it has to be taken into account that there were only 7 cirrhotic patients in our total study population.

The AUROC in our study was 0.80 (95% CI 0.61-1.00) for the prediction of cirrhosis (Ishak F5-F6) in the total study population and 0.79 (95% CI 0.57-1.00) in the group of patients with elevated ALT. Lok et al.[24] reported an AUROC of 0.78 and 0.81 in their training and validation sets. We could confirm the applicability of the test to predict cirrhosis in our total study population and the group of patients with elevated ALT. In the group of patients with persistently normal ALT, however, we could not confirm its applicability having only 1 patient with cirrhosis. The distinction of lower fibrosis stages (Ishak F3-F6) revealed a ROC-curve under the reference line exclusively in patients with persistently normal ALT. Diagnostic accuracies for all groups including the original accuracies reported by Lok et al.[24] are given in Table 5-2. The results are in good concordance to the ones reported except for the positive predictive values which were lower in all our study groups. In our study, patients with elevated ALT showed the best concordance with the results of Lok et al.[24] This may be due to the fact that Model 3 was developed in the HALT-C cohort using laboratory data of patients with high ALT levels. Differences in the results may be related to our different patient characteristics, especially because of having much less cirrhotic patients included than the original report. Lok et al.[24] reported that 50% of their

patients were distinguished correctly regarding the presence or absence of cirrhosis which is in good concordance to our results. 41.4% of patients with persistently normal ALT, 51% of patients with elevated ALT and 44.7% of all patients were classified correctly.

Model 3 cutoff value	Accuracy	All patients	EALT	PNALT	Lok et al.[24]
Prediction of cirrhosis (Ishak F5-F6)					
< 0.2	Sensitivity.	86	83	100	98
	Specificity	46	54	40	53
	PPV	10	22	3	27
	NPV	98	96	100	99
>0.5	Sensitivity.	29	17	100	40
	Specificity	97	97	97	99
	PPV	40	50	33	84
	NPV	95	88	100	90

Table 5-2: Diagnostic accuracies of Model 3 in the whole study population, patients with elevated ALT and persistently normal ALT. The reported accuracies from the validation cohort by Lok et al.[24] have been added for comparison. For abbreviations see appendix.

We assessed the diagnostic value of platelet count, a routine laboratory parameter, for prediction of hepatic fibrosis and cirrhosis in addition to the four fibrosis tests. As noted by other investigators we found an inverse correlation between platelet count and degree of hepatic fibrosis.[35,36,40,41] This fact may be due to enhanced pooling of platelets in an enlarged spleen in patients with advanced liver disease and portal hypertension.[12] Furthermore, production of thrombopoietin by hepatocytes decreases in the worsening of fibrosis.[42,43]

Using a cutoff value of < 130 x 10^9/L for prediction of cirrhosis Giannini et al.[44] reported high diagnostic accuracies. We found a low sensitivity and PPV of both 43% with a specificity of 96% for the prediction of cirrhosis (Ishak F5-F6) in our whole population and a sensitivity and PPV of both 33% with a specificity of 90% in patients with elevated ALT. These results are similar to the ones found in our previous study.[36] In patients with normal ALT, diagnostic accuracies were not significant because only one patient had cirrhosis.

Myers et al.[45] reported an AUROC of 0.67 for platelet count for prediction of significant fibrosis (METAVIR F2-F4). In our whole study population the corresponding AUROC was 0.64 (95% CI 0.51-0.77) and 0.69 (95% CI 0.52-0.86) in

patients with elevated ALT. In patients with persistently normal ALT, ROC curves for the prediction of significant fibrosis (METAVIR F2-F4) were under the reference line.

We acknowledge that our study has limitations. This study included patients from various hospitals in Austria. Many different investigators acquired the laboratory data that was finally brought together for this study. There may have been differences in the elicitation of data and analysis of blood samples by each clinical laboratory. Similarly, the quality of biopsy specimens and stains differed from centre to centre. Furthermore, these patients have initially been selected for a different study which evaluated the efficacy and safety of antiviral treatment with PEG-interferon alpha-2b and ribavirin in patients with chronic hepatitis C and either elevated ALT or persistently normal ALT.[39] Our aim was to evaluate the quality of four non invasive tests and platelet count for prediction of fibrosis and cirrhosis in patients with persistently normal ALT. This subgroup had only 58 patients. It showed significantly lower stages of fibrosis than the group of patients with elevated ALT. This condition has been reported by other investigators.[6,10]. Furthermore, only one cirrhotic patient was classified as a case with persistently normal ALT. Many noninvasive fibrosis tests were proposed to predict exclusively cirrhosis, like AAR and Model 3.[19,24] We were unable to evaluate their accuracy in this subgroup because of the lack of cases. However, this problem may reappear in other studies as patients with persistently normal ALT generally have a lower prevalence of cirrhosis.[10] To give an appropriate answer if these tests are applicable in patients with persistently normal ALT or not, a study population with a more or less equal distribution of all possible fibrosis stages including a sufficient amount of cirrhotic patients is absolutely necessary. Furthermore, a uniform definition of persistently normal ALT is needed to make results of different investigators comparable. Jamal et al.[10] used a definition of at least 4 consecutive normal ALT values within a period of 12 months and at least 3 values within 6 months. However, the decision if one single value of ALT is considered elevated or not depends on several factors. Applied upper limits of normal differ from one clinical laboratory to another. Usage of an ALT/ULN ratio can solve the problem of incomparable ALT values. However, varying cutoff values can be found in different studies. Our database has already been categorized during the previous study. Patients were classified as members of the persistently normal ALT group if two measurements in 6 months showed normal ALT values. However, after reviewing the data, we

decided to apply a more rigorous cutoff value and did not include any patient with an ALT/ULN value greater than 1 in the most recent blood sample into the group of patients with persistently normal ALT. Several patients with fluctuating ALT levels could be detected this way.

New methods for a noninvasive assessment of liver fibrosis have been evaluated, among these Fibroscan, a morphological, painless method to measure the stiffness of the liver trough the skin.[6,46] Routine of this test, unfortunately, may be hampered by its relatively high costs.[23] Liver elasticity was said to be highly correlated with fibrosis.[6] Colletta et al.[6] found high diagnostic accuracies for the prediction of significant or extensive fibrosis in patients with normal ALT. In their study, HCV carriers with normal ALT were frequently leaner than those with elevated ALT which favored the applicability of Fibroscan. In our study, patients with persistently normal ALT were also slightly leaner than patients with elevated ALT (BMI: 23.6 ± 3.5 vs. 24.2 ± 5.7). Morphological tests may have important advantages in this group of patients. They are independent from laboratory data which is the basis for the calculation of the described noninvasive fibrosis tests. The tests results in our population were unspecific for patients with persistently normal ALT

Concluding our results, liver biopsy for the estimation of fibrosis cannot be substituted by AAR, APRI, FIB-4 and Model 3 in patients with persistently normal ALT.

6 REFERENCES

1. Denk H, Dienes HP, Trauner M. Entzündliche Lebererkrankungen. In: Böcker W, Denk H, Heitz, editors. Pathologie. 3rd ed. München: Urban&Fischer, Elsevier; 2004. p. 764-767.
2. World Health Organization. Hepatitis C [Internet]. 2002 [cited 2011 June 30]. Available from: http://www.who.int/csr/disease/hepatitis/whocdscsrlyo2003/en/index.html.
3. Hof H, Dörries R. Medizinische Mikrobiologie. 3rd ed. Stuttgart: Georg Thieme Verlag; 2005. p. 203-205.
4. Manns MP, Wedemeyer H, Wiegand J. Handbuch Hepatitis C: Diagnostik, Verlauf, Therapie. 3rd ed. Bremen: UNI-MED Verlag AG; 2009.
5. Podolsky DK, Isselbacher KJ. Diagnostische Methoden bei Lebererkrankungen [A. Holstege, trans]. In: Isselbacher KJ, Braunwald E, Wilson JD, Martin JB, Fauci AS, Kasper DL. Harrisons Innere Medizin 2. 13th ed. Milano: McGraw-Hill Libri Italia srl; 1995. p. 1693-1697.
6. Colletta C, Smirne C, Fabris C, Toniutto P, Rapetti R, Minisini R, Pirisi M. Value of two noninvasive methods to detect progression of fibrosis among HCV carriers with normal aminotransferases. HEPATOLOGY 2005; 42:838-845.
7. Okuda M, Li K, Beard MR, Showalter LA, Scholle F, Lemon SM, Weinman SA. Mitochondrial injury, oxidative stress, and antioxidant gene expression are induced by hepatitis C virus core protein. Gastroenterology 2002;122:366-375.
8. Nalpas B, Vassault A, Le Guillou A, Lesgourgues B, Ferry N, Lacour B, Berthelot P. Serum activity of mitochondrial aspartate aminotransferase: a sensitive marker of alcoholism with or without alcoholic hepatitis. HEPATOLOGY 1984;4:893-896.
9. Kamimoto Y, Horiuchi S, Tanase S, Morino Y. Plasma clearance of intravenously injected aspartate aminotransferase isozymes: evidence for preferential uptake by sinusoidal liver cells. HEPATOLOGY 1985;5:367-375.
10. Jamal MM, Soni A, Quinn PG, Wheeler DE, Arora S, Johnston DE. Clinical features of hepatitis C-infected patients with persistently normal alanine transaminase levels in the Southwestern United States. Hepatology. 1999;30:1307–1311.
11. Maier KP. Akute und chronische Hepatitis C. Epidemiologie, Diagnostik, Therapie. 2nd ed. Stuttgart: Georg Thieme Verlag; 2002.

12. Aster R. Pooling of platelets in the spleen: role in the pathogenesis of “hypersplenic” thrombocytopenia. J Clin Invest 1996;45:645-657.
13. Cadranel JF, Rufat P, Degos F. Practices of liver biopsy in France: results of a prospective nationwide survey. For the Group of Epidemiology of the French Association for the Study of the Liver (AFEF).HEPATOLOGY 2000;32:477-481.
14. Castera L, Negre I, Samii K, Buffet C. Pain experienced during percutaneous liver biopsy. HEPATOLOGY 1999;30:1529-1530.
15. Bravo AA, Sheth SG, Chopra S. Liver biopsy. N Engl J Med 2001;344:495-500.
16. Regev A, Berho M, Jeffers LJ, Milikowski C, Molina EG, Pyrsopoulos NT, Feng ZZ, et al. Sampling error and intraobserver variation in liver biopsy in patients with chronic HCV infection. Am J Gastroenterol 2002;97:2614-2618.
17. Intraobserver and interobserver variations in liver biopsy interpretation in patients with chronic hepatitis C. The French METAVIR Cooperative Study Group. HEPATOLOGY 1994;20:15-20.
18. Rozario R, Ramakrishna B. Histopathological study of chronic hepatitis B and C: a comparison of two scoring systems. J Hepatol 2003; 38: 223-229
19. Williams AL, Hoofnagle JH. Ratio of serum aspartate to alanine aminotransferase in chronic hepatitis. Relationship to cirrhosis. Gastroenterology 1988;95:734-739.
20. Sheth SG, Flamm SL, Gordon FD, Chopra S. AST/ALT ratio predicts cirrhosis in patients with chronic hepatitis C virus infection. Am J Gastroenterol 1998;93:44-48.
21. Wai CT, Greenson JK, Fontana RJ, Kalbfleisch JD, Marrero JA, Conjeevaram HS, Lok ASF. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. Hepatology 2003;38:518-526.
22. Sterling RK, Lissen E, Clumeck N, Sola R, Correa MC, Montaner J, et al. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. HEPATOLOGY 2006;43:1317- 1325.
23. Vallet-Pichard A, Mallet V, Nalpas B, Verkarre V, Nalpas A, Dhalluin-Venier V, Fontaine H, Pol S. FIB-4: an inexpensive and accurate marker of fibrosis in HCV infection. comparison with liver biopsy and fibrotest. Hepatology 2007;46:32-6.
24. Lok AS, Ghany MG, Goodman ZD, Wright EC, Everson GT, Sterling RK, Everhart JE, Lindsay KL, Bonkovsky HL, Di Bisceglie AM, Lee WM, Morgan TR, Dienstag JL, Morishima C. Predicting cirrhosis in patients with hepatitis C based on standard laboratory tests: results of the HALT-C cohort. Hepatology 2005;42:282-92.

25. Glatz-Krieger K. Unresolved viral hepatitis (hepatitis C): lobular hepatitis [image on the internet]. 2002 [cited 2011 Jul 27]. Available from: http://alf3.urz.unibas.ch/pathopic/e/getpic-fra.cfm?id=004325
26. Dries V, Dienes HP. Roche Pharma AG. Die bioptische Diagnostik der Hepatitis C. [Internet]. 2010 [updated 2010 Dec 10; cited 2011 June 30]. Available from: http://www.hepatitis-care.de/hcv/diagnostik/histologie/histo_4_1.htm
27. Glatz-Krieger K. Active chronic hepatitis C and portal tract fibrosis [image on the internet]. 2002 [cited 2011 Jul 27]. Available from: http://alf3.urz.unibas.ch/pathopic/e/getpic-fra.cfm?id=004962
28. Lefkowitch JH. Scheuer's Liver Biopsy Interpretation. 8th ed. New York: Saunders, Elsevier; 2010. Chapter 9, Chronic Hepatitis; p. 133-162.
29. Glatz-Krieger K. Active chronic hepatitis C and fibrosis [image on the internet]. 2002 [cited 2011 Jul 27]. Available from: http://alf3.urz.unibas.ch/pathopic/e/getpic-fra.cfm?id=004963
30. Ishak K, Baptista A, Bianchi L, Callea F, De Groote J, Gudat F, Denk H et al. Histological grading and staging of chronic hepatitis. J Hepatol 1995;22:696-699.
31. Bedossa P, Poynard T. An algorithm for the grading of activity in chronic hepatitis C. The METAVIR Cooperative Study Group. Hepatology 1996;24:289–293.
32. Standish RA, Cholongitas E, Dhillon A, Burroughs AK, Dhillon AP. An appraisal of the histopathological assessment of liver fibrosis. Gut 2006;55:569-578.
33. Yano M, Kumada H, Kage M, Ikeda K, Shimamatsu K, Inoue O, Hashimoto E et al. The long-term pathological evolution of chronic hepatitis C. Hepatology 1996;23:1334-1340.
34. Stauber RE, Lackner C. Noninvasive diagnosis of hepatic fibrosis in chronic hepatitis C. World J Gastroenterol 2007;13:4287-4294.
35. Ono E, Shiratori Y, Okudaira T, Imamura M, Teratani T, Kanai F, Kato N, Yoshida H, Shiina S, Omata M. Platelet count reflects stage of chronic hepatitis C. Hepatol Res 1999; 15:192-200.
36. Lackner C, Struber G, Liegl B, Leibl S, Ofner P, Bankuti C, Bauer B, Stauber RE. Comparison and validation of simple noninvasive tests for prediction of fibrosis in chronic hepatitis C. Hepatology 2005;41:1376-82.
37. Marcellin P, Levy S, Erlinger S. Therapy of hepatitis C: patients with normal aminotransferase levels. HEPATOLOGY 1997;26:133S-136S.

38. Puoti C, Castellacci R, Montagnese F, Zaltron S, Stornaiuolo G, Bergami N, et al. Histological and virological features and follow-up of hepatitis C virus carriers with normal aminotransferase levels: the Italian prospective study of the asymptomatic C carriers (ISACC). J Hepatol 2002;37:117-123.
39. Vogel W, Brunner H, Maieron A, Graziadei I, Rosenberger D, Jilek K, Stauber RE, Wolkersdorfer D. Peginterferon alfa-2b and ribavirin treatment of patients with chronic hepatitis C and normal versus elevated aminotransferase levels –final results of a prospective open trial. HEPATOLOGY 2007;46:385A.
40. Pohl A, Behling C, Oliver D, Kilani M, Monson P, Hassanein T. Serum aminotransferase levels and platelet counts as predictors of degree of fibrosis in chronic hepatitis C virus infection. Am J Gastroenterol 2001;96:3142-3146.
41. Poynard T, Bedossa P, METAVIR and CLINIVIR cooperative study groups. Age and platelet count: a simple index for predicting the presence of histological lesions in patients with antibodies to hepatitis C virus. JViral Hepat 1997;4:199-208.
42. Kawasaki T, Takeshita A, Souda K, Kobayashi Y, Kikuyama M, Suzuki F, Kageyama F, et al. Serum thrombopoietin levels in patients with chronic hepatitis and liver cirrhosis. Am J Gastroenterol 1999;94:1918-1922.
43. Adinolfi LE, Giordano MG, Andreana A, Tripodi MF, Utili R, Cesaro G, Ragone E, et al. Hepatic fibrosis plays a central role in the pathogenesis of thrombocytopenia in patients with chronic viral hepatitis. Br J Haematol 2001;113:590-595.
44. Giannini EG, Zaman A, Ceppa P, Mastracci L, Risso D, Testa R. A simple approach to noninvasively identifying significant fibrosis in chronic hepatitis C patients in clinical practice. J Clin Gastroenterol 2006; 40: 521-527
45. Myers RP, Ratziu V, Imbert-Bismut F, Charlotte F, Poynard T. Biochemical markers of liver fibrosis: a comparison with historical features in patients with chronic hepatitis C. Am J Gastroenterol 2002;97:2419-2425.
46. Castera L, Vergniol J, Foucher J, Le Bail B, Chanteloup E, Haaser M, et al. Prospective comparison of transient elastography, FibroTest, APRI, and liver biopsy for the assessment of fibrosis in chronic hepatitis C. Gastroenterology 2005;128:343-350.

APPENDIX

ABBREVIATIONS AND SYMBOLS

A	“anni”, Latin: years
A.	“arteria” Latin: artery
AAR	AST-ALT –ratio
ALT	Alanine aminotransferase
Anti-HCV	Antibodies against hepatitis C virus
AP	Alkaline phosphatase
APRI	AST-to-Platelet-Ratio Index
AST	Aspartate aminotransferase
AUROC	Area under the receiver operating Characteristics
BMI	Body mass index
CAB	Chromotrop-anilinblue
CD-8 (positive-T-lymphocyte)	Cluster of differentiation 8 (positive-T-lymphocyte)
95% CI	95% Confidence interval
e.g.	“exempli gratia” Latin: for example
EIA	Enzyme immunoassay
ELISA	Enzyme-linked immunosorbent assay
F	Female
γ-GT	Gamma-glutamyl transferase
HAI	Histological activity index
HALT-C trial	High alanine aminotransferase cohort trial
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HIV	Human immunodeficiency virus
i.e.	“id est”, Latin: that is
IgM	Immunoglobulin M
INR	International normalized ratio

LN	Lobular necrosis
M	Male
MHC-I	Major histocompatibility complex I
N	“numerus” Latin: number of counts
NPV	Negative predictive value
PAS (reaction)	Periodic acid-Schiff (reaction)
PCR	Polymerase chain reaction
PEG-interferon	Polyethylene-glycosylated interferon
PMN	Piecemeal necrosis
PPV	Positive predictive value
PT	Prothrombin time
P	Statistical p-value
Q1	25% Quartile
Q3	75% Quartile
ROC curve	Receiver-operating characteristic curve
RT-PCR	Real time polymerase chain reaction
ULN	Upper limit of normal

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Language competence	
German	fluent, mother tongue
Polish	fluent, mother tongue
English	fluent, Medical English
Italian	fluent, Medical Italian
Latin	Latin proficiency certificate, Medical Latin
French	Fluent
Spanish	Fluent
Japanese	basic knowledge, Hiragana alphabet