Impact of statin treatment on non-invasive tests based prediction of fibrosis

by

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Abstract

Background and Rationale

The degree of fibrosis in nonalcoholic fatty liver disease (NAFLD) determines the risk of liver complications. Non-invasive tests (NITs) such as FIB-4, NFS and Hepamet, have been proposed to triage patients in primary care (PC) for further assessment due to their high negative predictive values (NPVs) for advanced fibrosis. These tests include AST±ALT in their calculations. Many patients with NAFLD take statins, and although these tests are used in the calculation of these NITs, it is unknown if NITs' performance is affected by statin use.

Purpose and Hypothesis

The purpose of this study was to determine whether statins modify NITs predictions of fibrosis as assessed by vibration controlled transient elastography (VCTE) in patients referred from PC for NAFLD.

Methods

We assessed 934 patients with suspected NAFLD from PC referred to a hepatology triage clinic and included those with a final NAFLD diagnosis (n=856). In this pilot pathway, all patients underwent VCTE, 832 with reliable measurements. We assessed with logistic regression the effects of being on a statin on the association between NITs and VCTE at different thresholds (8,10,12 and 16 kPa).

Results

129 patients were on a statin, and 138 additional patients fulfilled Canadian criteria for statin use but were not on a statin. Patients on a statin were older, more frequently diabetic and had higher BMI than patients not on a statin. In patients on a statin 25,16,13, and 7% had VCTE >=8,10,12 and 16 kPa respectively, while these figures were lower for patients not on a statin (10,7,4 and 2% respectively). For any given FIB-4 value, patients on a statin had higher probabilities of high VCTE than patients not on a statin. Adjusting for BMI, diabetes and age almost completely abrogated these differences, suggesting that these were related to patients' profile rather to a specific effect of statins. NPVs of a FIB-4 <1.3 for a VCTE >8,10,12 and 16 were, respectively, 89, 94, 96 and 100% in patients on a statin, and 92,95,98 and 99% in patients not on a statin. Statins' impact on the association between Hepamet and VCTE was similar to that for FIB-4, while statins did not affect NFS predictions of VCTE.

Conclusion

In patients with NAFLD referred from PC, those on statins had higher chances of a high VCTE for a given FIB-4 value. This was explained, in most part, by a different clinical profile of these patients and had a negligible impact on the NPV of the commonly used FIB-4 threshold (<1.3). A similar effect of statins was observed with Hepamet, but not with NFS. More than half of the patients with indications were not on a statin at the time of referral.

Preface

This thesis is an original work by Mustafa Thaier Salman Al-Karaghouli. The research project, of which this thesis is a part, received research ethics approval from the University of Alberta Research Ethics Board, Project Name “Evaluation of characteristics and outcomes of patients undergoing assessment at the Hepatology Triage Clinic: Q&I Initiative to assess and optimize screening protocols and resource allocation strategies for management of patients with chronic liver disease.”, ID No.Pro00076407, November 2017.

Dedication

I dedicate this thesis to my first hero, my father. You are the most wonderful mentor and the best friend a man could ever have. No words can express how much I love you and how much I respect you. I will keep working hard to make you proud. I inherited a lot from you, therefore, I feel very honoured when people acknowledge the resemblance between you and me. I will carry on to fulfil all what you wanted me to achieve. Although you are not with me anymore, but I always recall your precious encouragement and invaluable advices

Rest in peace, dear father. I will keep being the son you dreamed of.

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"Give a man a fish and you feed him for a day, teach a man to fish and you feed him for a lifetime" Chinese Philosopher, Lau Tzo. Therefore, I would like to start by thanking my supervisor Dr. Juan Gonzalez-Abraldes for teaching me and for pushing me to challenge myself, and for providing me with help and support during my master's study. The first day I met Dr. Gonzalez-Abraldes, my research experience was limited but, with his guidance, instructions and continuing education, I completed my first scientific research, which will certainly not be the last. I have continuously learned and gained invaluable research experience. I would like to thank him for showing me the right path and direction. I am honoured to be one of his students; I hope to work with him in future.

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represents 1.3 FIB-4 thresholds. The plots are shown for representative age 50 and 65, and for a statin, whether patients on a statin or not. Note that for a given value of FIB-4 patients with higher BMI have higher predicted mean VCTE. Diabetes but not prediabetes significantly affect this prediction with a higher mean predicted VCTE in diabetic patients than normal or prediabetes.

Figure 6: Effects of statin on Hepamet based prediction of different clinically relevant VCTE thresholds (8, 10, 12, and 16 kPa). Patients on statins had a higher risk of having higher VCTE than patients not on a statin, but this difference attenuated for higher thresholds. The vertical dashed line represents the 0.12 Hepamet threshold

Figure 7: Effects of statin on Hepamet based prediction of different clinically relevant VCTE thresholds (8, 10, 12, and 16 kPa) within patients with an indication for a statin. Despite patients on statins having a higher risk of having probabilities of high VCTE than patients not on a statin, the effect of statin is not significant. The vertical dashed line represents the 0.12 Hepamet threshold

Figure 8: Effect of statin on NFS based prediction of different clinically relevant VCTE thresholds (8, 10, 12, and 16 kPa). Statins did not affect NFS predictions of VCTE at any thresholds. The dashed line represents the threshold -1.455.

Figure 9: Effect of statin on NFS based prediction of different clinically relevant VCTE thresholds (8, 10, 12, and 16 kPa). The impact of statin is negligible and not significant. The dashed line represents the threshold -1.455.

List of Symbols and abbreviations

AHA/ACC: American Heart Association/American College of Cardiology

ALT: Alanine aminotransferase

AST: Aspartate aminotransferase

CAD: Coronary artery disease

CCS: Canadian Cardiovascular Society

CK: Creatine kinase

DILI: Drug induced liver injury

CVD: Cardiovascular diseases

HCC: Hepatocellular carcinoma

HDL: High-density lipoprotein

HMG-CoA: 3-hydroxy-3-methylglutaryl-CoA

LDL: Low-density lipoprotein

MetS: Metabolic Syndrome

NAFL: Nonalcoholic fatty liver

NAFLD: Nonalcoholic fatty liver disease

NAS: NAFLD activity score

NASH: Nonalcoholic steatohepatitis

NITs: Non-invasive tests

NPVs: Negative predictive values

PC: Primary care

PCP: Primary care provider

RCT: Randomized controlled trial

T2DM: Type 2 Diabetes Mellitus

UDCA: Ursodeoxycholic acid

ULN: Upper limit normal

VCTE: Vibration controlled transient elastograph

Chapter 1: Introduction

Background

NAFLD is considered the most common cause of chronic liver disease, with a prevalence of 25% worldwide.[1] The main determinant of adverse outcome in NAFLD is the level of fibrosis.[2] The higher the fibrosis stage, the worse outcome, whether it is liver related or cardiovascular related.[2] In addition, all-cause mortality and liver-related mortality are increasing exponentially with increasing levels of fibrosis.[3] Therefore, assessing the fibrosis stage in patients with NAFLD is essential to stratify them and to manage them appropriately. A two-step strategy to risk stratifying patients with advanced fibrosis is currently recommended.[4] The first step is by using a simple non-invasive test with high NPV and, therefore, to appropriately exclude advanced fibrosis in patients with low risk to avoid unnecessary investigations that can be costly and invasive, which carry additional risks on those patients. The second step is by using a test with a high positive predictive value so that patients with higher risk won't be missed.[5,6] The most commonly used tests with high NPVs that are used in the first step are FIB-4 and NFS.[4,7] Both tests include age, platelet and AST and/or ALT in their calculations. The difference between them is that NFS needs albumin and diabetes status (diabetes/ glucose intolerance), and therefore, it requires to include extra clinical information.[8,9] A new test called Hepamet has been proposed as a non-invasive test to exclude advanced fibrosis as it has high NPVs.[10]

NAFLD is an independent risk factor for cardiovascular diseases (CVD).[11,12] Patients with NAFLD share the same risk factor for CVD.[11,13] Statin or HMG-CoA reductase inhibitor is the

mainstay for primary and secondary prevention of CVD and since NAFLD and CVD are associated with metabolic syndrome.[14-16] Therefore, many patients with NAFLD have an indication for a statin. A number of studies have shown that statin could modify aminotransferase levels, though the clinical significance of this finding is unclear.[17] Since NITs depend on AST/ ALT and statin could modify aminotransferase levels, there is a possibility that statin could modify the association between NITs and the prediction of advanced fibrosis. Therefore, it is critical to assess the impact of statins on the performance of NITs as triage methods in referral pathways for patients with NAFLD.

Purpose

The purpose of this study was to determine the impact of statin on NITs prediction of liver advanced fibrosis as assessed by VCTE in patients referred from primary care for NAFLD. The second purpose was to assess the prevalence of statins indication and current use in patients with NAFLD from our referral base.

Hypothesis

It was hypothesized that statin can modify NITs prediction of liver advanced fibrosis as assessed by VCTE. Additionally, we hypothesized that the impact of statin on FIB-4 is similar to NFS and Hepamet.

Specific outcomes

The primary outcome of this study was the impact of statin on NITs based prediction of advanced fibrosis as assessed via VCTE.

Secondary outcome of this study was to determine whether the effect of statin among all NITs was similar to FIB-4 and to determine the prevalence of statin indication and the current use among patients with NAFLD from our referral pathway.

The significance of the study

This study addresses whether NITs threshold need to be modified when assessing fibrosis in patients with NAFLD on a statin as it might lead to over or underprediction of liver advanced fibrosis and to determine which non-invasive test has a superior effect when assessing advanced fibrosis in patients on statin treatment. Since these are routinely used in our referral pathways, the results would have a direct impact on how we manage referrals.

Chapter 2: literature review

2.1 The definition of NAFLD:

Non-alcoholic fatty liver disease is considered a spectrum of diseases ranging from simple steatosis or non-alcoholic fatty liver (NAFL) through a much more severe form named non-alcoholic steatohepatitis (NASH) to fibrosis and eventually liver cirrhosis.[18] Interestingly, the term NASH was first described in 1980 by Ludwig et al. to describe the clinical and pathological features of non-alcoholic diseases that resemble those seen in alcoholic liver disease.[19]

While different diagnostic tools could define liver steatosis, it could be defined more accurately by assessing liver fat content, which is ≥ 5% fat containing hepatocytes as evaluated by light microscopy or proton density fat fraction of ≥5 % as assessed by magnetic resonance imaging (MRI).[4] Histologically NAFL shows hepatic steatosis features without hepatocyte injury, and it is considered a benign condition. In contrast, the histological features of NASH demonstrate hepatic steatosis with hepatocellular injury, specifically hepatic ballooning and inflammation, and it is considered a serious disease that leads to cirrhosis in 9% to 22% of patients and eventually might progress to hepatocellular carcinoma (HCC) as a complication of cirrhosis.[18,20]

2.2 NAFLD epidemiology:

2.2.1 Incidence

There is limited data about the estimated incidence of NAFLD as there is no precise, reproducible non-invasive biomarker that can be used at ease in the general population to detect NAFLD. One study that conducted on 11,448 subjects who were followed for five years, the incidence of NAFLD was 12.3% as documented by U/S.[21] Another study that followed 213 participants for seven years, 147 of those were normal at baseline, reported an annual incidence rate of 3 per 100. [22] The reported incidence of NAFLD by using MRS on the Asian population in Hong Kong was 13.5 % throughout 3 to 5 years.[23]

2.2.2 Prevalence

There are many studies about NAFLD prevalence in the general population. One of the most recent meta-analyses reported that the overall pooled prevalence of NAFLD among the general population by using imaging techniques was around 25%. The highest prevalence reported was in the Middle East 31.8%, followed by South America 30.5%, while the lowest prevalence was in Africa 13.5%. The prevalence rate was also increased with increasing age, despite the fact that studies assess NAFLD in patients aged more than 70 are limited. The estimated pooled prevalence of NAFLD among patients by using blood tests was lower as compared with an imaging technique, as the prevalence of NAFLD in North America was around 24% using imaging whereas it was about 13% by using blood tests.[4,24]

When it comes to NASH, where liver biopsy is the gold standard for diagnosis, the estimated prevalence of NASH among the general population was assessed indirectly given that biopsy is not a feasible option in population studies. Studies in NASH reported a prevalence of 59% among NAFLD patients who had a liver biopsy for clinical indication while it was ranging from 7% to 30% among NAFLD patients who were biopsied in the context of a protocol. Given these estimates, the estimated prevalence rate of NASH in the general population was ranging from 1.5% to 6.5%.[24]

2.2.3 Gender differences

Until now there is the influence of sex is not well established and there are substantial differences in studies some demonstrated male predominance while others showed females predominance.[25]

2.2.4 NAFLD and Ethnic/racial disparities

Many studies evaluated NAFLD disparities among different racial/ethnic groups. These studies were in a recent meta-analysis of the racial and ethnic disparity. The pooled prevalence of NAFLD from population-based cohort studies was the highest in Hispanic 15.1%, intermediate in white 14.4% and the lowest in black 13%. The relative risk of NAFLD was the highest in Hispanic while it was the lowest in Black. In high-risk cohorts' studies, the prevalence of NAFLD was the highest in White 55.5%, intermediate in Hispanic 48.8% and the lowest in Black 47.6%. Although the relative risk of NAFLD in high-risk cohorts' studies was the highest in White while it was the lowest in Black, the difference was not significant.[26]

2.2.5 Diabetes mellitus

Type 2 Diabetes Mellitus (T2DM) is among the most crucial risk factors for NAFLD and NASH as well as it is among the most clinical predictors for the negative clinical outcome of NAFLD such as liver advanced fibrosis, HCC, and mortality.[27-29] One meta-analysis stated that the prevalence of T2DM among patients with NAFLD as assessed by imaging studies was 22.5%. [24] The same study reported a prevalence of T2DM in biopsy-proven NASH was 46.6%. Other studies reported the prevalence of NAFLD and NASH among T2DM patients as assessed by MRS or liver biopsy is 50% and 56%. [30] However, in the most recent meta-analysis that reviewed the global prevalence of NAFLD and NASH among T2DM patients reported the estimated NAFLD prevalence of 55.48% in T2DM patients as diagnosed by U/S or H-MRS, which mean that the prevalence rate of NAFLD in T2DM is twice that reported for the general population. The prevalence of NAFLD among T2DM by U/S 59% was not significantly different from the H-MRS prevalence 60%. Interestingly, the pooled prevalence of NAFLD as diagnosed by liver biopsy was 91.62%. Regarding NASH, the estimated global prevalence among patients with T2DM was 37.33%. [31] [25]

2.2.6 Obesity

Obesity is among the most well-established risk factors for NAFLD. The whole spectrum of obesity from overweight through obese and ultimately to severe and morbid obesity is associated with NAFLD. Almost all patients (>95%) with severe obesity who are undergoing bariatric

surgery have NAFLD. Also, NAFLD prevalence among T2DM patients increased dramatically with increasing BMI.[4,25]

2.2.7 Metabolic syndrome

According to the meta-analysis by Younossi et al, the pooled estimated prevalence of metabolic syndrome (MetS) in NAFLD patients was 42.54%, and the estimated pooled prevalence of MetS in NASH was 70.65%.[4]

2.2.8 Liver cirrhosis

The reported prevalence of NAFLD cirrhosis in the general population is 0.178% ,[32] whereas the reported incidence of NAFLD cirrhosis was 0.17%. [33] The prevalence of NAFLD cirrhosis in T2DM patients as assessed by transient elastography (TE) was 11.2%, whereas the prevalence of NAFLD cirrhosis in patients with severe obesity was 5.8%. Studies also suggested a higher incidence of NAFLD cirrhosis in T2DM and severe obesity.[34,35]

2.3 Consequences of NAFLD and clinical outcomes

2.3.1 Fibrosis

Fibrosis is a well-established adverse outcome of NAFLD. Many studies have investigated the progression from steatosis to fibrosis in NAFLD using liver biopsies. These studies reported that about one-third of patients with NAFL and NASH had progressive fibrosis, and about 20% had some regression during the follow-up.[36] In a meta-analysis evaluating 11 studies to estimate fibrosis progression in NAFLD, the results were similar, 33% with NAFLD had fibrosis progression, 43% had stable fibrosis, and about 22% had fibrosis regression. The estimated rate for fibrosis progression was slow. The annual fibrosis progression rate (FPR) in patients with baseline stage 0 fibrosis was 0.13 stages, which means an average of 7.7 years is needed to progress 1 stage. The FPR in NASH was twice that of the NAFL. An average of 14.3 years was estimated to progress 1 stage fibrosis in NAFL, while for NASH, it was 7.1 years.[37]

- **Risk factors associated with fibrosis progression** [36,37]
    1. Presence of hypertension
    2. High AST/ALT ratio at the time of baseline biopsy
    3. Higher steatosis grade
    4. Type 2 diabetes mellitus (T2DM)
    5. Higher BMI

2.3.2 NAFLD and hepatocellular carcinoma (HCC)

HCC incidence has been rapidly rising globally over the last 20 years. In 2012, HCC was considered the second most common cause of cancer-related death worldwide.[38-40] Since the prevalence of T2DM and obesity has increased significantly over the past decades which in turn led to rise the incidence of NAFLD that can progress to fibrosis, cirrhosis and ultimately HCC. The incidence of HCC related NAFLD is expected to increase in the future surpassing other etiologies [41-43] HCC related NAFLD and NASH, however, is underestimated. NAFLD can progress to liver fibrosis in some patients, which would lead to cirrhosis in 10% to 20% of cases. NAFLD cirrhosis can progress to HCC although some NAFLD related HCC cases happened without a history of cirrhosis. The magnitude of HCC risk in patients with NAFLD is unclear. In one study, the annual incidence rate of HCC in NAFLD patients was 0.21 per 1000 patients compared to 0.02 in control, and patients with NAFLD had 8.6 fold higher risk to acquire HCC than control. In patients with NAFLD without cirrhosis, the annual incidence rate of HCC was 0.08 per 1000 compared to 0.02 in the control group without cirrhosis. The annual incidence of HCC in NAFLD cirrhosis ranged from 1.6 to 23.7 per 1000 patients. HCC risk in NAFLD patients increases with age and with higher FIB-4 in patients with or without cirrhosis[44]

2.3.3 Liver cirrhosis

Cirrhosis is the end stage of various chronic liver conditions that have features of necroinflammation, fibrosis, and regenerative nodules that alter the liver structure and change its vascular architecture. Cirrhosis is considered major public problems that lead to morbidity and mortality.[45,46]

Chronic hepatitis B virus is the most common cause of cirrhosis in Asia and Africa, while alcoholic liver disease and hepatitis C virus are the most common cause of liver cirrhosis in developed countries.[47,48] NAFLD, however, has become a leading cause of cirrhosis in some countries, and it is projected to exceed that of hepatitis C and B as well as it is predicted that NAFLD will become the leading cause for liver transplantation.[49]

The rate of progression to cirrhosis is different among the histological subtype. The incidence of progression to cirrhosis of NASH is higher than that of NAFL. A longitudinal study with a mean follow-up of 15.6 years reported that 1% of NAFL advanced to cirrhosis, while 11% of NASH patients advanced to cirrhosis. Also, NASH progression to cirrhosis is more rapid than NAFL as the annual fibrosis progression rate is 0.14 in NASH compared to 0.07 in NAFL.[37,50]

Many studies showed that diabetes is the main metabolic risk factor that leads to progression in NAFLD to cirrhosis. One Canadian cohort study used the administrative health database in Ontario from 1994 to 2006 to evaluate whether newly diagnosed diabetes leads to serious liver disease. After a median follow-up of 6.4 years, the results showed that 3.71% of newly diagnosed diabetic patients develop cirrhosis compared to 1.34% in individuals without diabetes. Another study had consistent results and reported that high blood glucose was associated with cirrhosis independent of obesity. Metabolic factors such as hyperlipidemia, obesity, and hypertension were considered important risk factors for NAFLD progression to cirrhosis.[49,51,52]

In regards to age and cirrhosis, In a retrospective cohort study, patients with NAFLD were divided into three groups older (≥60), middle-age(50 to 60), and younger (≤50), and it showed that cirrhotic patients were significantly older than patients without cirrhosis. Compared to noncirrhotic patients, cirrhotic patients had more risk factors including diabetes, obesity, hypertension, and hyperlipidemia. In another study, NAFLD patients were classified into the

elderly (≥65) and nonelderly (18-65). The study showed that elderly patients had higher rates of advanced fibrosis. While there was no significant difference in the risk factors among these two groups, the study suggested that the association between age and NAFLD cirrhosis might be related to the disease's duration rather than the age itself.[52-54]

2.3.4 Mortality

Since the prevalence of NAFLD is high and predicted to further increase by 2030, determining the long-term outcome, especially mortality among patients with NAFLD, has important implications for decision-making in public health and clinical practice. NAFLD is a well-established risk factor for liver cirrhosis, liver cancer, chronic kidney disease, T2DM, and cardiovascular diseases (CVD), and therefore, NAFLD was suggested as a predictor of increased mortality. Whether NAFLD increases mortality compared to the general population or not remains controversial. Many studies reported that patients with NAFLD had a higher risk of all-cause mortality. NAFLD all causes mortality increases with age and advanced fibrosis stage. A recent cohort prospective study showed that female patients with NAFLD but not male patients had an increased risk of death from all-cause mortality, CVD, and HCC. Other studies found no association between NAFLD and all-cause mortality. These studies had reported that NAFLD patients had increased risk of CVD, liver cirrhosis, and HCC.[55-60] The most recent meta-analyses also showed solid evidence that NAFLD was associated with increased risk of all cause mortality.[61]

2.3.4.1 NAFLD and CVD mortality

While many studies showed a significant association between NAFLD and CVD mortality, the most recent meta-analysis reported no association between NAFLD and CVD mortality. These findings were consistent with the previous meta-analysis in 2016. The 2016 meta-analysis, however, showed that the severity of NAFLD predicted fatal and non-fatal CVD events, and therefore, whether the adverse outcome of NAFLD on mortality is restricted to NASH or it can also include those with simple steatosis is a challenging question since liver biopsy is required to diagnosis NASH. One study on patients with biopsy-proven NAFLD that used NAFLD activity score as an index to diagnose NASH showed that there is no significant difference between patients with or without NASH regarding the overall CVD mortality. Another prospective cohort study, which classified hepatic steatosis into normal, mild, moderate, and severe, showed that only patients with moderate to severe steatosis were predictors for increasing all-cause mortality and CVD mortality.[61-63]

2.3.4.2 NAFLD and Cancer mortality

The most recent meta-analysis reported no significant association between NAFLD and cancer mortality. In regards to NAFLD association with HCC and non-HCC , two studies had assessed the association between NAFLD and HCC, and they showed NAFLD increased the risk of HCC.[55,61,64] The meta-analysis reported that NAFLD patients had a 6.27 fold higher risk of death from HCC compared with patients without NAFLD.[61] The most common cause of non-HCC cancer death in patients with NAFLD was colorectal cancer.[64]

2.3.4.3 NAFLD and liver specific mortality

Liver diseases mortality rate is higher in patients with NAFLD.[61] The mortality rate ratio in NAFLD increased exponentially with increased fibrosis level. Mortality rate ratio increased significantly after fibrosis stage 2. Mortality related to liver causes accounts 18% of all-cause mortality in patients with NAFLD Stage 2 fibrosis while it increased to 24% and 59% with fibrosis stage 3 and 4, respectively.

2.4 Cardiovascular risk factors and NAFLD

Many studies have proved that NAFLD is associated with higher risks of cardiovascular diseases (CVD), which are the most common cause of non-liver-related death in those patients. The higher the severity of NAFLD, the higher the risk of fatal and non-fatal CVD events. A large body of evidence has confirmed NAFLD association in different CVD manifestations, namely atherosclerotic CVD (Coronary artery syndrome and stroke), conduction abnormalities, and left ventricular dysfunction. These studies suggested that NAFLD might be an independent risk factor for CVD regardless of the presence of traditional CVD risk factors.[11,65-67]

2.4.1 The shared pathophysiology between NAFLD and CVD

In metabolic diseases NAFLD and metabolic syndrome, adipose tissue expansion, and dysfunction are the main common features among liver disease and cardiac metabolic complications. Excessive calorie intake is the main culprit of metabolic syndrome, NAFLD, and CVD, leading to increased free fatty acid serum level (FFA) that exceeds the adipose tissue storing capacity and ectopic and visceral fat deposition which involve the liver. This abnormal

expansion of adipose tissue causes adipocyte dysfunction and the release of inflammatory cytokines, which in turn can not only be associated with insulin resistance but also CVD.[68] In addition, enlarged fat mass causes lipogenesis, which leads to an increase in serum levels of FFA VLDL that cause atherosclerosis. NAFLD hyperlipidemia has higher atherogenic potential as serum LDL, and triglycerides are higher and HDL lower. There is a positive association between the histological severity of NAFLD and lipid parameters, suggesting a strong correlation between them.[69]

2.4.2 Association between NAFLD and CVD

NAFLD's role as a potential risk factor for CVD is controversial for a long time. Recent evidence, however, had reported an association between NAFLD and CVD. NAFLD is associated with an increase in the prevalence of CVD risk factors, especially T2DM and obesity.[24] Many studies had evaluated the long-term outcome in patients with NAFLD and showed that NAFLD is considered a risk factor for CVD. One of the most recent meta-analyses showed that patients with NAFLD are affected more by CVD rather than liver-specific complications, and patients with NAFLD are subjected to a higher risk of fatal and non-fatal cardiovascular events. The risk of cardiovascular events was higher, with more advanced liver disease.[62] Moreover, this meta-analysis showed a strong correlation between CV mortality and more histologically severe NAFLD. Patients with stage 3 and 4 fibrosis had a higher mortality rate regardless of the NAFLD activity score.[20]

2.4.3 Coronary vascular disease

Patients with NAFLD had a higher risk of coronary artery disease than the general population, and therefore, higher risk of CVD. NAFLD increases CVD regardless of the traditional risk factors. An analysis of the Framingham Heart study on 3529 patients reported that patients with NAFLD who underwent CT had subclinical markers of atherosclerosis even after metabolic risk factors adjustment.[70] Another study reported a higher prevalence of coronary artery stenosis on patients with fatty liver on U/S who underwent coronary angiography even after adjustment for other metabolic risk factors.[23] An observational study on 2103 diabetic patients with a mean follow-up of 6 years concluded that NAFLD gives additional CV risk more than that would be expected by metabolic risk factors as the study reported a higher frequency of non-fatal cardiovascular event (myocardial infarction(MI), and revascularization procedures), and death in patients with NAFLD.[71]

2.4.4 NAFLD and cardiovascular events

NAFLD showed to increase the risk of CVD. A meta-analysis found that patients with NAFLD had a higher risk of ischemic and hemorrhagic stroke, and the more the severity of the liver disease, the higher the risk.[72] There is a correlation between liver fibrosis as assessed by transient elastography and ischemic stroke. The higher the fibrosis stage, the more the risk for ischemic stroke.[73]

2.5 Diagnosis of NAFLD

2.5.1 Screening for NAFLD

Routine screening for NAFLD is not recommended by the American Association for the study of liver Diseases (AASLD),[4] or by the National Institute for Health and Care Excellence (NICE).[74] In contrast, the European Association for the Study of the Liver (EASL) recommends NAFLD screening in patients at risk (i.e. metabolic syndrome or obesity).[75]

2.5.1.1 The American Association of the Study of Liver Diseases (AASLD)

The AASLD does not recommend routine screening for NAFLD. Until now, there are uncertainties regarding the treatment options and the diagnostic tests besides the scarcity of evidence showing the cost-effectiveness and long-term benefits of screening. NAFLD screening among high-risk groups such as patients with diabetes or obesity is also not recommended by the AASLD. The AASLD, however, recommends using NAFLD fibrosis score (NFS), Fibrosis-4 (FIB-4), and transient elastography (TE) to risk-stratify patients with a high index of suspicion.[4]

2.5.1.2 National Institute for Health and Care Excellence (NICE)

Screening patients for NAFLD is not recommended by the NICE guideline. The NICE guideline does not recommend NAFLD screening in high-risk patients with metabolic syndrome and/or diabetes due to the lack of evidence that supports screening. The commercial ELF test is recommended by the NICE to risk-stratify patients and identify advanced fibrosis. Patients with

negative score should repeat testing after 3 years, while patients with positive score should be referred to a specialist.[74]

2.5.1.3 European Association for the Study of the Liver (EASL)

The EASL recommends screening for NAFLD with liver enzymes and/or U/S in all patients with metabolic syndrome and obesity. Also, patients with consistent elevation in liver enzymes should be screened for NAFLD. The EASL also recommends evaluating all patients with NAFLD for metabolic syndrome regardless of their liver enzymes. Assessment for advanced fibrosis in patients with NAFLD can be done by TE, or any of the clinical prediction rules such as NFS, FIB-4, and commercial ELF test.[75]

2.5.2 Diagnosis of NAFLD

A detailed history and physical examination, along with investigation, are needed to diagnose NAFLD. Therefore, to diagnose NAFLD, the following criteria need to be present:[4]

1-Evidence of hepatic steatosis by imaging or biopsy

2- No significant alcohol consumption, which is defined as >21 standard drinks per week in men and >14 standard drinks per week in women.

3- Absence of other etiology of hepatic steatosis (Table 1)

4- No coexisting etiology that causes chronic liver disease.

Table 1: Secondary causes of hepatic steatosis[4]

Macrovascular causes	Microvascular causes
Excessive alcohol consumption	Reye's syndrome
Hepatitis C virus	Acute fatty liver of pregnancy
Wilson disease	HELLP syndrome
Starvation	Inborn error of metabolism
Parenteral nutrition	Medication (e.g., Sodium valproate, antiretroviral medicine)
Abetalipoproteinemia	
Medication (e.g., amiodarone, methotrexate, tamoxifen, and corticosteroid)	

2.6 Non-invasive tests to assess fibrosis and hepatic steatosis

Fibrosis is the only histopathological feature that is used to predict the outcome for NAFLD. The major predictor of clinically significant liver outcome is advanced fibrosis. Therefore, The presence of advanced fibrosis is key in predicting the prognosis of patients with NAFLD. While patients without advanced fibrosis have a low risk of progression to cirrhosis or HCC, those with advanced fibrosis are more frequently prone to have severe liver-related outcomes and have a higher mortality rate. Liver biopsy is considered the gold standard to diagnose liver fibrosis. However, it is not reasonable to use liver biopsy as a screening tool in the general population or primary care. Furthermore, liver biopsy is an expensive procedure. Thus, many non-invasive scores have been developed to risk-stratify and identify patients with advanced fibrosis.

2.6.1 Elastography based techniques

2.6.1.1 External, mechanical vibration induced elastography

Transient elastography (TE)

This method generates a shear wave at low frequency (50 Hz) with volume measured 4X1 cm cylinder, with measured depth 25mm to 65 mm below the surface of the skin. The term shot used to define each shear wave generated. Successful shot gives measurements.[76,77]

Average of the measurement of ten valid shots are taken and considered failure if no shots valid, while it considered unreliable when less than ten measurements are taken, less than or equal 60% measurement are valid, and IQR/Med > 30% if VCTE Med > 7.1 kPa.[77]

Limitations of TE include:

1. It is difficult to perform in obese patients, and therefore, in obese patients XL probe is used to overcome obesity limitations but data are limited patients with ascites, and narrow intercostal space.[76]
2. Failure or unreliable results may be associated with[76]
    a. Operator experience, which is defined by less than 500 examinations
    b. BMI > 30 kg/m2
    c. Type 2 diabetes mellitus (T2DM)
    d. Age > 52 years
    e. Female sex
    f. Hypertension
3. Risk of overestimating liver stiffness and therefore fibrosis in patients with[76]
    a. Extrahepatic cholestasis
    b. Recent food intake
    c. Congestive heart failure
    d. Severe inflammation

A meta-analysis on 57 studies evaluated the use of transient elastography to detect advanced fibrosis. In these studies, liver biopsy was used as a reference standard diagnostic performance of TE to detect significant fibrosis ≥ F2 in patients with chronic liver disease. TE showed a good sensitivity and specificity to detect liver fibrosis with pooled sensitivity and specificity of TE 81%, and 88%, respectively.[78]

Magnetic resonance elastography (MRE)

It is an MRI based method that measures liver fibrosis but quantifies tissue stiffness. It has advantages over ultrasound-based elastography as it analyzes the liver almost entirely and is used in patients with obesity and ascites. However, it is a costly method and inaccurate in patients with iron overload.[79]

In a cross-sectional study which was conducted on 142 patients with liver biopsy proven NAFLD to compare the diagnostic accuracy of MRE and TE for detecting advanced fibrosis, the sensitivity and specificity of MRE for detecting advanced fibrosis ≥ F2 was higher than TE. Furthermore, MRE showed superior performance to TE in detecting significant advanced fibrosis ≥ F4 without failure due to obesity.[79]

2.6.1.2 US-induced shear wave

2-D Shear wave elastography

Two-dimensional shear wave elastography is an ultrasound elastography technique that uses the same principle as the transient elastography. It can measure liver stiffness based on the estimation of the shear wave's velocity. The difference between TE and SWE is that SWE can be conveniently performed using a conventional ultrasound scanner and can create a real-time, 2-D quantitative map of liver tissue stiffness, while TE needs an external mechanical vibrator. Two-dimensional SWE has shown to be a reliable non-invasive method to estimate liver stiffness and, therefore, fibrosis.[80,81]

One prospective cohort study compared the diagnostic accuracy of shear wave and transient elastography for the staging of fibrosis in NAFLD using liver biopsy as a reference. The cut-off

for shear wave value with a sensitivity of at least 90% to rule out advanced liver fibrosis F2, F3, and F4 was 6.3 kPa, 8.3 kPa, and 10.5 kPa, respectively, which was comparable to the the cut-off for transient elastography (TE) with the same sensitivity to rule out advanced fibrosis F2, F3, and F4 was 6.2 kPa, 8.2 kPa, and 9.5 kPa, respectively. The cut-off value for the specificity (where we can rule in advanced fibrosis) was lower in shear wave compared to TE. The cut-off with at least 90% specificity was 8.7 kPa, 10.7 kPa, 14.4 kPa for the shear wave, while it was 9.8 kPa, 12.5 kPa, and 16.1 kPa for the transient elastography (TE).[82]

Acoustic radial force imaging

This method is another ultrasound-based method used to estimate liver stiffness. It uses short-duration, high-intensity acoustic pulses to generate mechanical excitation in tissue. The velocity of these waves correlates with the degree of fibrosis. The optimal cut-off values vary among studies. A study conducted on 88 patients showed that by using a cut off 1.44m/s, the sensitivity and specificity of ARFI ≥ F2 was 85, 76, respectively. The sensitivity and specificity of ARFI imaging was 92%, 87% using a cut off 1.9m/s for a F4 fibrosis.[83,84]

A comparison was made between TE and ARFI in a study that includes 321 patients who underwent liver biopsy for the diagnosis of chronic liver diseases. There was no difference between TE and ARFI for the diagnosis of severe fibrosis. However, ARFI was better in obese patients.

2.6.2 Blood based tests

2.6.2.1 AST/ALT

ALT and AST are released from the damaged hepatocyte. Together, they yield much more information than each one alone. AST/ALT ratio was first proposed in 1957 by De Ritis et al.[85] The association between the AST/ALT ratio and liver cirrhosis was statistically significant. Liver cirrhosis increases the AST/ALT ratio to greater than 1.0 in patients with NAFLD.[85,86] McPherson reported that AST/ALT could avoid liver biopsy in 69% of patients with NAFLD, and the negative predictive value (NPV) to exclude advanced fibrosis at a cut off value of 0.8 was 95%.[87]

2.6.2.2 AST- Platelet ratio index (APRI)

APRI is calculated using the following formula, APRI = (AST (/ ULN)/Platelet counts (109/L)) × 100. APRI is one of the simplest marker panels that can be used to diagnose advanced fibrosis and cirrhosis with sufficient accuracy.[88]

Recent studies showed APRI acted similarly to other more sophisticated scores in exclusion advanced but not moderate fibrosis. For significant fibrosis, an APRI threshold of 0.5 was 81% sensitive, and 50% specific.[89] APRI value of more than 1 was the most significant predictor of advanced fibrosis in the study population.[90]

2.6.2.3 NFS

NAFLD fibrosis score (NFS) includes age, platelet count, albumin, ALT/AST, BMI and hyperglycemia status (diabetes and impared fasting glucose). Unlike the other scores which were created to evaluate the prognosis of other diseases, NFS were created specifically for NAFLD

and it was developed in 733 biopsy proven NAFLD patients.[91] The cutoff value to rule advanced fibrosis in NFS is -1.455 which has a high NPV between 88-93%. The cutoff to diagnose advanced fibrosis is 0.676 which has a high PPV between 82-90%. Further validation studies showed that NFS had even higher NPV to rule out advanced fibrosis F3, and F4 with NPVs up to 98%, while its performance to diagnose advanced fibrosis had some fluctuation.[92-94]

2.6.2.4 FIB-4

This index is a combination of four simple, readily available lab tests, AST, ALT, age, and platelet.

FIB-4 is calculated using the following formula[9] :

FIB-4 index = [age (years) × AST (IU/L)]/ [platelet count (109/L) × ALT (IU/L)]1/2.

This index was initially developed for use in HCV/HIV co-infection. The AUROC for FIB-4 was 0.765, with a sensitivity of 70% and specificity of 97%. FIB-4 reported good discrimination of cirrhosis (AUROC=0.91) and severe fibrosis (AUROC=0.85). FIB-4 has also been shown to be a reliable test in NAFLD.[9] This score offers dual cut-off values. The cut off <1.45 for had a NPV of 90% to rule out advanced fibrosis while the cut off >3.25 had a PPV of 65% to diagnose advanced fibrosis. [95,96] The cut off for FIB-4 to rule out advanced fibrosis was decreased to 1.3 which showed a NPV of 95%. The sensitivity and specificity for predicting advanced fibrosis (F3-F4) using a cut-off value of 1.3 were 85% and 65%, respectively.[87]

2.6.2.5 HEPAMET score

A new score used clinical and laboratory variables to predict advanced fibrosis. A study conducted on 2,452 showed that the ability of HFS to rule in or out advanced fibrosis was significantly superior to NFS and FIB-4. HFS cut-offs (0.12 and 0.47) showed better diagnostic performance than the other non-invasive score, and this score was not affected by age BMI or diabetes.[10]

2.7 Management of NAFLD according to current guidelines

Management of NAFLD includes treating liver diseases and managing the associated comorbidity such as diabetes, insulin resistance, obesity, and hyperlipidemia.[4]

2.7.1 Lifestyle modification

Lifestyle modification includes 3 main things:

2.7.1.1 Weight loss

Weight loss is the key to the histopathological improvement in patients with NAFLD.
Studies showed that 5% body weight reduction was associated with decrease hepatic steatosis while 7% body weight reduction was associated not only with hepatic steatosis reduction but also with decrease NAS. 10% body weight reduction, however, was associated with improvement of all NASH features such as hepatic ballooning, portal inflammation and fibrosis. Weight reduction, however, is challenging to achieve and in a trial only 50% of patients with NAFLD were able to achieve ≥ 7% weight reduction.[97,98]

2.7.1.2 Diet

While having a calorie- restricted diet helps to mobilize liver fat and improve cardiovascular risk. The effect of nutrient composition was less relevant than the primary goal which is weight loss.[4,99]
The AASLD guidelines states “decreasing caloric intake by at least 30% or by approximately 750-1,000 kcal/day results in improvement in IR and HS”[4]

2.7.1.3 Exercise

Patients with NAFLD engage with less exercise activity which is associated with increased risk of metabolic syndrome and CVD.[100,101]

Recent data showed improvements in hepatic steatosis with exercise and maintaining physical activity of more than 150 minutes per week or increased activity level by more than 60 minutes per week had shown to decrease aminotransferase levels.[102]

AASLD guidelines state "Exercise alone in adults with NAFLD may prevent or reduce HS, but its ability to improve other aspects of liver histology remains unknown"[4]

2.7.2 Pharmacological treatments

2.7.2.1 Metformin

Many studies evaluated the effect of metformin on patients with NAFLD.[4] Two meta-analyses had been shown that metformin did not significantly improve liver histology in NAFLD and NASH.[103,104] Metformin, however, improves aminotransferase levels and insulin resistance in NAFLD patients.[4,105] [3,85]

2.7.2.2 Thiazolidinediones

Pioglitazone showed to improve liver histology in patients with biopsy proven NASH with and without diabetes.[4] One RCT on pioglitazone 45 mg/day on patients with NASH and prediabetes and diabetes showed that pioglitazone improved aminotransferases, insulin sensitivity, steatosis, hepatic ballooning and inflammation. NAFLD Activity Score (NAS) improved in 73% of

patients in the treatment group compared with only 24% in the placebo group.[106] Another RCT by Cusi et al. concluded that 58% of patients with biopsy proven NASH with prediabetes and diabetes who were on pioglitazone 45 mg/day had a reduction of ≥ 2 points in NAS, and had improved fibrosis. [107] [87] [7] Pioglitazone proved to improve hepatic fibrosis and hepatocellular ballooning in patients with NASH without diabetes, hepatic steatosis, however, did not significantly improve.[107] Regarding the adverse outcome, weight gain was the most side effect associated with pioglitazone use.[108,109] Bladder cancer association with pioglitazone is inconclusive, some studies showed positive associations while others showed negative associations.[110,111]

2.7.2.3 Glucagon-like peptide-1 analogues

A RCT compared in 52 patients with biopsy-proven NASH liraglutide vs placebo for 48 weeks. Liraglutide was associated with less progression of fibrosis and weight loss.[112]

Guidelines states "It is premature to consider GLP-1 agonists to specifically treat liver disease in patients with NAFLD or NASH."[4]

2.7.2.4 Vitamin E

Vitamin E is an antioxidant and has been studied as a treatment for NASH.

Limitation of Vitamin E[4]:

a. Different doses of vitamin E
b. Different formulas that can affect the bioavailability
c. Use additional drug or antioxidant with Vitamin E
d. Limited histological data to assess outcomes
e. Potential prostate cancer and increase in CV outcomes

In many trials' vitamin E leads to decrease aminotransferases in NASH, improvement in steatosis, inflammation, ballooning. [4,108]

AASLD guideline states "Vitamin E (rrr a-tocopherol) administered at a daily dose of 800 IU/day improves liver histology in nondiabetic adults with biopsy-proven NASH and therefore may be considered for this patient population. Risks and benefits should be discussed with each patient before starting therapy."[4]

"Until further data supporting its effectiveness become available, vitamin E is not recommended to treat NASH in diabetic patients, NAFLD without liver biopsy, NASH cirrhosis, or cryptogenic cirrhosis."[4]

2.7.2.5 Ursodeoxycholic acid

Several studies have investigated ursodeoxycholic acid. However, a large RCT showed that UDCA had no histological benefit over placebo in patients with NASH.[113]

2.8 Role of statins in NAFLD

Statins are lipid-lowering agents that work by inhibiting HMG-CoA reductase enzyme, the rate-limiting step in hepatic cholesterol biosynthesis, and therefore, it significantly decreases total cholesterol level, and most importantly, LDL. [114] Therefore, statins are key in primary and secondary prevention of CAD and atherosclerotic disease equivalents.[115] Currently available statins include atorvastatin, fluvastatin, lovastatin, pitavastatin, pravastatin, rosuvastatin, simvastatin. Lipophilic statins include atorvastatin, fluvastatin, lovastatin, and simvastatin. Hydrophilic statins include pitavastatin, pravastatin, and rosuvastatin.[116]

Dyslipidemia plays a crucial role in the development of cardiovascular diseases, which is the most common cause of mortality in NAFLD patients. Since NAFLD is considered the hepatic manifestation of metabolic syndrome; therefore, dyslipidemia associated with NAFLD is defined by having a pattern of hypertriglyceridemia, increased LDL levels, and reduced high-density lipoprotein (HDL) levels. However, despite that, cardiovascular complications are the leading cause of mortality in those patients, neither NAFLD nor NASH are considered the traditional risk for cardiovascular diseases. As a result, cardiovascular risk should be assessed by either the AHA or CCS to determine whether the patient is a candidate to receive a lipid-lowering agent or not and to establish the LDL target for primary and secondary cardiovascular prevention.[117]

Table 2: Indications for Statin Treatment according to the Canadian Cardiovascular Society (CCS). [15]

Category	Consider Initiating pharmaco-therapy if:	Target
Primary Prevention	High Risk (FRS ≥20%)	LDL-C <2.0 mmol/L or >50%
	Intermediate Risk (FRS 10-19%) LDL-C ≥3.5 mmol/L or Non-HDL-C ≥4.3 mmol/L or Apo B ≥1.2 g/L or Men ≥50 and women ≥60 yrs and one additional CVD RF	Or Apo B <0.8 g/L
Statin Indicated Conditions	Clinical atherosclerosis	
	Abdominal aortic aneurysm	
	Diabetes mellitus ≥40 yrs 15 yrs Duration and Age ≥30 yrs Microvascular disease	Or
	Chronic kidney disease (age ≥50 y) eGFR <60 mL/min/1.73/m2 or ACR > 3 mg/mmol	non-HDL-C<2.6mmol/L
	LDL-C ≥5.0 mmol/L	>50% in LDL-C

Table 3: Indication of Statin according to the AHA/ACC.[118]

Presence of clinical atherosclerotic cardiovascular disease (coronary heart disease, symptomatic carotid artery disease, stroke/ transient ischemic attack, peripheral artery disease, abdominal aortic aneurysm)
Adults 40–77 years of age with diabetes mellitus and LDL levels of 70–189 mg/dL
Adults 40–75 years of age with a global 10-year risk of cardiovascular disease >=7.5% and an LDL level of 70–189 mg/dL
Adults with an LDL level >=190 mg/dL

2.8.1 Underprescription of statin

The hepatotoxicity of statins is one of the most well-known side effects of use.[119] However, there is an indication to use statin for patients with NAFLD as long as their aminotransferases remained three times below ULN.[58]

Although the risk of having hepatotoxicity is small, it is a major cause of the under prescription of this drug to NAFLD patients by the Primary care providers. One study which was conducted on 255 patients with NAFLD to evaluate statin use and discontinuation in patients with NAFLD. This study found that ~ 60% of patients were on appropriate statin therapy and of those ~ 38% received inappropriate statin dose reduction or discontinuation at the time of NAFLD detection by their PCP. Therefore, NAFLD diagnosis was the culprit for statin underprescription. This study also showed that PCPs were influenced more by the elevation of liver function tests LFTs in guiding their decision-making regarding statin use. [120]

In a survey which includes 937 primary care physicians, showed that only 50% of them would prescribe statins if the alanine aminotransferase (ALT) values were 1.5 times the upper limit of normal (ULN).[121]

Several factors could have contributed to suboptimal statin prescription, including lack of awareness of treatment targets according to cardiovascular risk, lack of proper assessment of cardiovascular risk, and assumption by gastroenterologists and hepatologists that dyslipidemia would be managed by physicians attending to the patients' other coexisting metabolic conditions. Secondly, physicians and patients may be concerned about the potential adverse effects of statins, which may include myopathy, increased liver enzymes and rhabdomyolysis.

2.8.2 Statin hepatotoxicity

Statins, in general, are well-tolerated, and the adverse effects of statins are rare. The most common reported adverse effects are muscle toxicity, which ranges from myalgia to rhabdomyolysis, a more severe and serious complication of statins. Other adverse effects are hepatic and renal toxicity.[122,123]

Statin-related liver toxicity is rare, and it is associated with self-limiting mild to moderate elevation in aminotransferases without need for discontinuation or dose adjustment.[124] Up to 3% of patients develop a mild increase in aminotransferases enzyme during their first year of statin therapy, but most are asymptomatic.[123,125,126] A meta-analysis by De Denus et al. showed that compared to placebo low to moderate dose of lovastatin, pravastatin, and simvastatin did not significantly cause an increase in aminotransferases level. The only statin cause increase in aminotransferase compared to placebo was fluvastatin. [127] Acute liver failure due to statin is extremely rare, with an estimated incidence of acute liver failure in patients exposed to statins (1:139,000) approximately similar to that of the general population (1:114,000).[128]

2.8.2.1 Drug-induced liver injury (DILI):

The criteria for DILI include the following[129] :

1. Elevations of either AST or ALT more than 3 times the upper limit of normal (ULN).
2. Elevations of the total serum bilirubin level more than 2 times the ULN
3. The elevations of liver aminotransferase and bilirubin not due to other identified causes.

Statins can cause DILI in 1 in 100,000 patients and can be presented in different histological presentations, some of them will have asymptomatic rises in the ALT level with less than 3 times ULN, and those can get improved with continuous statin use as this is called adaptation. However, some of those patients will develop symptoms, and their aminotransferase can be more than 3 times ULN.[130]

Four major studies evaluated the hepatotoxicity of statins. The Spanish Hepatotoxicity Registry study which was conducted to evaluate the potential hepatotoxic effect of statins in Spain compared to other drugs, 858 cases of drug-induced hepatotoxicity were collected from the Spanish registry over 18 years period from 1994 to 2012, and it had shown that only 47 case out of 858 (5.5%) were due to statin with atorvastatin having the greatest number of cases which means that statins hepatotoxicity is not common.[131]

The population-based prospective study in Iceland gathered data from all cases of DILI between 2010 and 2011. DILI was defined by having elevated alanine aminotransferase (ALT) more than 3 upper limit normal (ULN) and/ or increasing alkaline phosphatase (ALP) more than 2 times ULN. Ninety-eight patients were found to have DILI, while only 3 were due to statin. Based on the number of patients with prescribed statin, the population risk of statin-induced DILI was estimated to be 27 per 100,000 patients treated with atorvastatin.[132]

Björnsson et al. conducted a retrospective study. Data were collected from the Swedish Adverse Drug Reactions Advisory Committee between 1970 and 2004, aiming at finding the incidence of statin-induced DILI. They found only 8 out of 747 DILI was statin-induced. Following this study, Björnsson and his colleagues also conducted another study to evaluate statin associated

hepatotoxicity between 1988 and 2010. DILI was identified if patients had aminotransferases more than 5 ULN and/ or ALP was more than 2 times ULN. Seventy-three patients were diagnosed with statin-induced DILI, and statin-induced DILI was reported in 1.2 per 100,000 statin users and with the greatest probability of an event for atorvastatin.[133]

Another study, the US Drug-Induced Liver Injury Network (DILIN), aimed to describe statin DILI. Between 2004 and 2012, 1188 patients suspected to have statin-induced DILI, after further reviews, only 22 patients were most probably had statin-induced DILI. Liver injury, in most cases, was mild to moderate, 4 cases were severe, and only one fatal case was reported. More than 50% of the cases (12) were due to hepatocellular pattern. The study concluded that statin-induced DILI was rare 1.8%, mild to moderate in intensity, self-limited.[134]

2.8.3 Safety of Statins in Cirrhosis

Despite that the use of statins among patients with compensated cirrhosis is relatively common, the published data is not that common. Kumar et al studied the safety of long-term use of statin among patients with compensated cirrhosis. Eighty-one patients with biopsy-proven cirrhosis treated with statins for a 3 months period, while 162 of biopsy-proven cirrhosis did not receive statin with median follow-up duration of 36 and 30 months, respectively. Patients were evaluated for mortality and hepatic decompensation which was defined as development of ascites, bilirubin more than 2.5 mg/dL, encephalopathy, or variceal hemorrhage. Statin was significantly associated with a lower risk of decompensation ($p=0.04$) and mortality ($p=0.01$). This observational study results support the use of statin for primary and secondary prevention of CVD among patients with compensated cirrhosis.[135,136]

Another study which was conducted by Abraldes et al. on 59 patients with cirrhosis, and severe portal hypertension who were randomized to receive either simvastatin or placebo for 30 days had also proved the safety and the efficacy of the statin in cirrhosis patients. Simvastatin significantly decreased HVPG compared with placebo (−8.3% vs −1.6%, p=0.041), and there was no change in the hepatic blood flow, mean arterial pressure, and systemic vascular resistance between the groups which suggest that the change was mediated by reduction on the intrahepatic resistance. Individuals treated with simvastatin also had improved markers of effective hepatic metabolic capacity. The increase in the ALT was not more than two folds, and there was no difference in the adverse effect between the two groups. Only three patients had an increase in creatine kinase (CK) of more than twofold, one of them was in the placebo group, whereas the other two were in the simvastatin group.[136]

In addition to this, statins are considered contraindicated in patients with decompensated liver cirrhosis or acute liver failure.[137] However, data are very limited regarding the effect of statins on decompensated cirrhosis. A recent randomized controlled study compared the effect of high simvastatin 40 mg with rifaximin and simvastatin 20 mg with rifaximin, and placebo. The simvastatin 40mg with rifaximin group showed a significant increase in both ALT and AST compared to the placebo group, while there was no significant difference in the simvastatin 20 mg with rifaximin group compared to the placebo after 12 weeks. Therefore, they suggest the use of low dose simvastatin 20 mg for further studies.[138]

2.8.4 Review of statin studies in relation to NAFLD

2.8.4.1 RCTs in which NAFLD was the primary indication

Many studies have evaluated the use of statins in patients with NAFLD and dyslipidemia. Only one randomized placebo-controlled trial specifically assessed the efficacy of statins for the treatment of NASH. In that study, 16 biopsy-proven NASH patients were randomized to receive either simvastatin 40 mg (10 patients) or placebo (6 patients) for 12-months. There was no improvement in serum transaminases, hepatic steatosis, necro inflammatory activity, or fibrosis stage with simvastatin. Simvastatin group, however, showed a reduction in the LDL level by 26% compared to the placebo group. The study limitations were the small number of patients and the fact that all patients were low-risk patients and had grade 1 NASH, which provided a minimum window for improvement to happen.[139]

Additionally, a number of studies showed that statin is safe to use in NAFLD patients and result in Improvement in aminotransferase levels in those with elevated levels of ALT and AST. One of the earliest studies was by Rallidis et al., which was conducted in 2004 for 6 months on 5 patients with liver enzyme abnormalities and the diagnosis of steatohepatitis based on liver biopsy. Patients were given 20mg of pravastatin daily. At the end of the study, Improvement in the histological findings of NASH when pravastatin was given for approximately 6 months. In three cases, there was an improvement in the extent of the inflammation and in the fourth in the degree of steatosis.[140]

2.8.4.2 RCTs for cardiovascular indication: effect on NAFLD

Five post hoc analysis studies have been conducted to evaluate the potential effect of statins in NAFLD patients.

1. A study done by Athyros et al was conducted in 2006 on 189 patients with NAFLD over 54 weeks period, the primary endpoint was to assess the efficacy of multifactorial treatment of metabolic syndrome in reducing the prevalence of both biochemical and ultrasonographic evidence of NAFLD. The secondary endpoint was to assess the safety of this intervention. It compared the use of atorvastatin with fenofibrate versus atorvastatin alone versus fenofibrate alone. The results showed that about 70% of patients who used either atorvastatin and fenofibrate or atorvastatin alone had no longer biochemical and ultrasonographical features of NAFLD compared to about 40% of patients with fenofibrate alone. Four patients discontinued from this study, two of them were from the atorvastatin and fenofibrate group and those discontinued because of myalgia without elevated serum creatine kinase, one from the atorvastatin group alone due to increase ALT levels more than 3 ULN.[141]

2. Another post hoc analysis study was conducted in 2010 over 3.6 years on 80 patients with NAFLD diagnosed by the liver to spleen (LS) ratios, which was calculated by using computed tomography scans performed at baseline and follow-up to determine NAFLD prevalence. Patients were randomised to receive either a combination of atorvastatin 20 mg/day, Vitamin C 1g/day, and Vitamin E 1,000 IU/day or placebo. This study showed that atorvastatin in

combination with vitamin C and E significantly decreased the risk of moderate to severe steatosis (as assessed by CT scan) by 70%.[142]

3. The post hoc analysis of the prospective, randomized, open-label survival study, Greek Atorvastatin and Coronary Heart Disease Evaluation (GREACE), which was conducted in 2010 included 437 patients who had elevated liver aminotransferase enzymes due to NAFLD as assessed by ultrasound. Statin mainly atorvastatin 24mg/day (n=160) was administered to 227 of the patients (other statins include simvastatin 22mg/day, pravastatin 21mg/day, fluvastatin 40mg/day). The primary endpoint of this post hoc analysis was the first incident of any cardiovascular event or stroke in patients with elevated liver tests who were treated with a statin compared with the untreated group, while the Secondary endpoints were effects of statin treatment on LFTs in patients with elevated liver tests. Liver-related adverse effects of statin treatment were defined the same as DILI, which was an increase in aminotransferase levels more than three times the upper limit of normal. Atorvastatin was safe and substantially improved steatosis (assessed by liver ultrasonography and aminotransferase), while patients who were not treated with a statin had a further rise in liver ALT and AST. Cardiovascular events occurred in 10% of patients with abnormal liver tests who received statin, while they occurred in 30% of 210 patients with abnormal liver tests who did not receive a statin. The cardiovascular disease benefits of statin was more pronounced in patients with elevated aminotransferases than those with a normal level of ALT and AST.[143]

4. The post hoc analysis of the Incremental Decrease in End Points Through Aggressive Lipid Lowering (IDEAL) study published in 2013 which was from a prospective, randomized, open-label, blinded end-point trial conducted between 1999 and 2005 in Europe with median follow-up of 4.8 years; The study included 8888 patients with a history of acute myocardial infarction (MI). Patients were randomly assigned to a high dose of atorvastatin 80 mg/day to 4439 patients, or a usual-dose simvastatin 20–40 mg/day to 4449 patients. The occurrence of a major coronary event was the primary endpoint of the original study. This post hoc analysis compared intensive and moderate statin therapy for the prevention of CVD events in patients with a previous MI and normal or elevated baseline serum alanine aminotransferase (ALT) activity. There were 8863 patients with baseline ALT values, 7782 (87.8%) of them had normal Liver function tests, whereas 1081 patients (12.2%) had elevated ALT levels, due to NAFLD. Metabolic syndrome was common among those IDEAL patients with elevated ALT activity at baseline. This study showed that in those with elevated ALT levels, Atorvastatin 80 mg/day normalized ALT levels compared with moderate statin simvastatin 20mg/day or 40mg/day. In participants with elevated baseline ALT activity, major CVD event rates were 11.5% for simvastatin and 6.5% for atorvastatin, indicating a significant risk reduction with intensive statin therapy (p=0.0056). Atorvastatin at this high dose was safe. These findings suggest that any benefit from statin treatment on NAFLD may not be a drug class effect.[144,145]

5. The post hoc analysis for a prospective, randomized, open-label study named Assessing the Treatment Effect in Metabolic Syndrome Without Perceptible diabetes (ATTEMPT), which included 1123 metabolic syndrome patients without overt CVD over a 42 months period. Patients received intensive lifestyle intervention and drug therapy with atorvastatin in all

patients aimed for LDL <100 in group A and LDL <130 in group B, ACEI for hypertension, metformin for hyperglycemia and orlistat for obesity. In this primary CVD prevention study, 326 participants, who had modestly elevated liver function tests and ultrasonographic evidence of NAFLD, were treated with either atorvastatin 30 mg/day (group A) or atorvastatin 20 mg/day (group B). Patients on atorvastatin 30 mg/day had less than half CVD events than those on atorvastatin 20 mg/day. In both groups, liver enzymes and liver ultrasonography were normalized during the study.[146,147]

2.8.4.3 Observational *studies*

A prospective study by Hyogo et all included 31 patients with biopsy-proven NASH with hyperlipidemia. Those patients received atorvastatin 10 mg/day for 24 months. After treatment, ALT and AST levels were normalized in 23 (74.2%) patients. The NASH-related metabolic parameters improved with atorvastatin, including fibrosis in some patients. Liver steatosis and NAFLD activity score were significantly improved.[148]

In addition to this, Kargiotis et al. did an observational study on 20 patients with metabolic syndrome, biopsy-proven NASH and dyslipidemia with a follow up 12 months. They received rosuvastatin. A repeat biopsy was performed in all patients at the end of this study. In 19/20 patients complete NASH resolution was observed, liver function tests and ultrasonography were normalized.[149]

Table 4: Studies assessing the potential impact of statins on NAFLD

Randomized clinical trial specifically assess NAFLD						
Study	Patients' number	Study duration	Selection criteria	Intervention	Assessment method	Main outcome
Nelson 2009[139]	16	12 months	• Age 18 or older • Documented NASH with biopsy • Compensated liver disease	Participants were randomly assigned to receive simvastatin 40mg (n = 10) versus placebo (n = 6)	Liver Biopsy	26% reduction in LDL was seen in the simvastatin group compared with the placebo group, no significant improvement in serum aminotransferases, hepatic steatosis, necro-inflammatory activity, or stage of fibrosis was noted within or between groups
Post hoc Analysis of Randomized clinical trials for CV indication						
Athyros 2006[141]	189	54 weeks	Free of T2DM and cardiovascular diseases • Presence of metabolic syndrome • LDL >3.4 • Evidence of fatty liver by U/S • Elevated liver AST and/ or ALT levels	Participants were randomly assigned to atorvastatin 20 mg/day (n = 63) versus fenofibrate 200 mg/day (n = 62) versus both drugs (n = 61).	U/S	70% Of the patients who take either atorvastatin or atorvastatin with fenofibrate had no biochemical and ultrasonographic features of NAFLD in compare to 40% of patients who took fenofibrate alone
Foster 2010[142]	1,005 Just 80 where have NAFLD	3.6 Years	• Age between 50 and 70 • No history of CAD, T1DM, a bleeding diathesis, severe anemia, cancer within the past 5 years, any condition likely to lead to death within 5 years	Comparing atorvastatin plus antioxidants vs. placebo in 80 patients with NAFLD at baseline	CT (Liver to spleen ratio)	Atorvastatin 20 mg combined with vitamins C and E is effective in reducing the risk of having hepatic steatosis by 71 % in healthy individuals with NAFLD at baseline after 4 years of active therapy.

Athyros 2010[143,147] "GREACE"	1600 but the post hoc analysis included only the patients with NAFLD 437	3 years	• Patients had coronary heart disease • Aged younger than 75 years In the post hoc NAFLD patients assessed by U/S was additional criteria to the previously mentioned criteria.	Patients were randomly assigned to statin (n=227) versus untreatment (n=210)	U/S	Atorvastatin was safe and substantially improved steatosis (assessed by liver ultrasonography and aminotransferase), while patients who were not treated with a statin had a further rise in liver ALT and AST
Athyros 2011[146] "ATTEMPT"	1123 patients with MetS from those 326 had NAFLD	42 Months	• Age between 45-65 • Metabolic syndrome (MetS) • No diabetes or CVD	Patients divided into two groups A and B, all Patients received intensive lifestyle intervention and drug therapy with atorvastatin in all patients. However, the aim and the dose of atorvastatin was different. Group A aimed for LDL <100 and was given 30mg atorvastatin Group B aimed for LDL <130 and was given atorvastatin 20mg	U/S	Patients on 30 mg/day atorvastatin had less than half CVD events than those on 20 mg/day atorvastatin. In both groups, liver enzymes and liver ultrasonography were normalized during the study
Tikkanen 2013[145] "IDEAL"	8888 patients of those 1081 had NAFLD	4.8 Years	• Age less than 80 • Had definite MI • Eligible for statins	Patients were randomly assigned to a high dose of atorvastatin 80 mg, or a usual-dose simvastatin 20–40 mg	Biochemical	In participants with elevated baseline ALT activity, major CVD event rates were 11.5% for simvastatin and 6.5% for atorvastatin, indicating a

						significant risk reduction with intensive statin therapy (p=0.0056). Atorvastatin at this high dose was safe.
Randomized controlled clinical trials Not specific for NAFLD						
Lewis 2007[168]	326	36 Weeks	• Age greater than 18 years • At least 6 months history of compensated chronic liver disease • LDL level greater than or equal to 100 mg/dL • TG level lower than 400 mg/dL	Participant were assigned either to take pravastatin 80 mg vs. placebo	Radiological imaging and/ or Liver biopsy	Pravastatin reduced mean LDL cholesterol, total cholesterol, and triglyceride compared to placebo. ALT level was lower with the pravastatin group compared to the placebo group.
Hans 2012[169]	189	12 Weeks	• Age 25 to 75 years • Elevated ALT Levels between 1.25 times and 2.5 times the upper limit of the normal range • None had been treated with statins for more than 3 months • LDL concentration levels >= 3.36mmol	Two groups separately randomized to receive either pitavastatin 2 mg/day or atorvastatin 10 mg/day	CT	1. Patients with persistently increased ALT concentrations at screening and randomization showed significant reductions in ALT after 12 weeks of treatment with pitavastatin or atorvastatin . 2. Serial nonenhanced CT in 38 subjects (18 PITA, 20 ATOR) showed that both statins reduced the

						severity of hepatic steatosis.
Non-randomized studies						
Kiyici 2003[113]	44	6 Months	Biopsy proven NASH patients	Group (1) 17 patients received UDCA Group (2) 27 hyperlipide mic patients received atorvastatin	Liver Biopsy	In group 1 only GGT reduced significantly while in group 2 (the statin group) serum cholesterol, AST, ALT, ALP and GGT levels reduced significantly. ALT, AST normalization was also more prevalent in group 2
Rallidis 2004[140]	5	6 Months	• Patients with liver enzyme abnormalities and • Diagnosis of steatohepatitis based on liver biopsy	Patients were given 20 mg of pravastatin daily for 6 months.	Liver Biopsy	improvement in the histological findings of NASH when pravastatin was given for approximately 6 months.
Gomez 2006[170]	25	12 Months	Patient with dyslipidemia and NAFLD	Patients received atorvastatin (10-80 mg/daily) according to basal serum choleresterol levels; additionally, they were given standard weight-loss counselling and encouraged to follow a low fat diet.	U/S	Serum aminotransferase and lipid levels were reduced significantly in all patients with atorvastatin treatment. Therapy with atorvastatin in NAFLD patients with hyperlipidemia was found to be both effective and safe.
Hyogo 2006[148]	31	2 Years	All patient with biopsy proven NAFLD	Atorvastatin given to all of them	Liver Biopsy	ALT and AST levels were normalized in 23 (74.2%) patients. The NASH-related metabolic

						parameters improved with atorvastatin, including fibrosis in some patients. Liver steatosis and NAFLD activity score were significantly improved.
Kargiotis K 2014 [149]	20	12 Months	• Patients with metabolic syndrome (MetS), • NASH on liver biopsy • Dyslipidemia • No diabetes or arterial hypertension	Rosuvastatin given to all of them	Liver Biopsy	Resolution of NASH was observed in 19/20 patients who completed the study, liver function tests and ultrasonography were normalized

2.8.5 Conclusion

Many patients with NAFLD have an indication for a statin as the majority of them have diabetes or have shared risk factors for CVD. Statin use in patients with NAFLD is safe and the risk of hepatotoxicity due to statin is rare. Statin is safe in patients with compensated liver cirrhosis and there is low risk of adverse events to occur in patients with compensated liver cirrhosis while it is contraindicated in patients with decompensated cirrhosis and associated with higher risk of adverse events. However, statin is under-prescribed in patients with NAFLD. Studies assessed the effect of statin on patients with NAFLD showed that statin could decrease aminotransferases in patients and decrease hepatic steatosis. Statin should be prescribed to patients with NAFLD if they have indication even if they have elevated aminotransferases up to 3 ULN.

Chapter 3: Impact of statin treatment on NITs based prediction of advanced fibrosis.

Introduction

Non-alcoholic fatty liver disease (NAFLD) has become the most common etiology of chronic liver disease worldwide with a pooled estimated prevalence of 25%.[1] The main determinant of liver morbidity in NAFLD is the presence of advanced fibrosis.[2,3] Indeed, there is an exponential increase in the risk of liver related mortality with increased fibrosis stage. In patients with no or early fibrosis, the rate of liver related mortality is minimal, whereas this becomes as high as 24% and 59% of all-cause mortality in patients with F3 and F4 fibrosis respectively.[3] NITs are currently being used to risk stratify patients with NAFLD needing to see a liver specialist. The current recommendation is to use a two step strategy with an initial, simple test with high negative predictive value (NPV), such as FIB-4 or NFS, followed by a second test with more positive predictive value such as VCTE.[4,7] FIB-4, which includes in its calculation age, platelet count and AST/√ALT ratio[9], has some theoretical advantages since it can be computed directly from laboratory results,[5,6,87] whereas NFS will need albumin, BMI and the clinical information of diabetes/glucose intolerance,[8] which requires the introduction of clinical information.[87] Hepamet calculation requires similar variables, has recently been proposed as an additional non-invasive method for estimating liver fibrosis in NAFLD.[10]

The prevalence of risk factors for cardiovascular disease is very high in patients with NAFLD.[13,150] Furthermore, NAFLD has been suggested to be an independent risk factor for cardiovascular disease.[11,12,150] HMG-CoA Reductase Inhibitors or statins are the mainstay of primary and secondary prevention of cardiovascular disease.[14-16] Patients with suspected NAFLD have higher probability of having an indication for a statin than patients without NAFLD (~2-fold). Previous studies have shown that statins modify liver transaminases, demonstrating both increases or decreases, though the clinical significance of this finding is unclear.[17] Since most non-invasive tests for assessing liver fibrosis are based on either AST or the calculation of some form of AST to ALT ratio, there is the possibility that statins, by modifying transaminases, could modify the performance of these non-invasive tests in predicting liver fibrosis. In addition, more advanced NAFLD stages are associated with higher prevalence of metabolic risk factors, especially diabetes, and dyslipidemia, which is one of the main determinants of statin indication.[15,118] Thus, assessing the impact of statin treatment on non-invasive prediction of liver fibrosis is critical to understand the performance of these NITs as triage methods in referral pathways for patients with NAFLD.

The aim of the present study was to assess if statins modify NITs predictions of fibrosis (assessed by VCTE) in patients referred from primary care for NAFLD assessment. A secondary aim was to assess the prevalence of statins indication and current use in patients with NAFLD from our referral base.

Methods

Study Population selection.

We prospectively collected data from patients with suspected NAFLD referred from primary care (PC) to a RN-led hepatology triage clinic in Edmonton, Canada, from November 2016 to October 2019. Primary care providers received a short educational update on NAFLD and fibrosis assessment importance. Physicians were given information about the referral process for patients with suspected NAFLD to the RN assessment clinic. The combined estimated population of the participating networks was 850,000 adults.

We included patients with a final diagnosis confirmed to be NAFLD, aged ≥ 16. Patients who were found to have other chronic liver diseases were excluded from this analysis.

Clinical, Laboratory, and VCTE assessment.

All patients completed their lab tests according to the American College of Gastroenterology to rule out alternative liver diagnosis.[152] Medical history was obtained by the RN who then performed vibration controlled transient elastography (VCTE). A single operator performed All VCTE tests with a Fibroscan 502 touch (M Probe, XL Probe; KNS Inc., Scarborough, Canada) device.

We considered different thresholds for classifying patients as suspected advanced fibrosis based on VCTE: 8 kPa, 10 kPa, 12 kPa and 16 kPa. All have been proposed to define advanced fibrosis. The 16 kPa threshold, in addition, has been recently proposed as an indication to initiate hepatocellular carcinoma screening.[82,153-155] VCTE unreliable results were defined by having fewer

than 10 valid shots or interquartile range (IQR)/median value greater than 30% with a VCTE median of 7.1 kPa or higher.

Lab values, and patients' clinical history are clinically generated data, and these were collected from the electronic medical records (EMR).

Approval for the project was received from the University of Alberta Research Ethics Board.

Determination of statin indication

The indication for a statin was determined according to the Canadian cardiovascular society where statins are indicated to any patients with clinical atherosclerosis, abdominal aortic aneurysm, Chronic kidney disease (age ≥ 50 years) where eGFR <60 mL/min/1.73 m2 or albumin to creatinine ratio > 3 mg/mmol and diabetes mellitus when patients age is >= 40 or they have been diabetic for 15 years or more in case with type 1 diabetes mellitus.[15]

In addition, for primary prevention of cardiovascular disease, statin is indicated in all patients who have Framingham risk score (FRS) suggesting a 10-year cardiovascular disease risk of >20% and to some patients with intermediate 10 years cardiovascular risk (10-19%) (those with LDL-C >= 3.5 and for LDL-C <3.5 mmol/L, statin is indicated if Apo B ≥1.2 g/L or Non-HDL-C ≥4.3 mmol/L or men >=50 and women >=60 if they were smokers, had impaired fasting glucose, or hypertension, and low HDL).[15]

For our operational definition of "indication for a statin", all patients that were already on a statin were considered to have indication for a statin.

In 319 patients FRS could not be directly calculated due to missing values of systolic blood pressure. In these cases, we conducted a multiple imputation procedure with the aregimpute

command from the Hmisc package (FE Harrell).[156,157] Variables in the imputation model were age, BMI, total cholesterol, and diabetic status. We created 10 initial complete datasets with imputed systolic blood pressure where we determined the FRS (A). Since office blood pressure measurements were not conducted with on many occasions according to guideline recommendations, and recent data shows that blood pressure measurements in trials are lower (by ~5 mmHg) than same patient's out of trial office measurements,[158] we created 10 additional datasets by decreasing systolic blood pressure by 5 mmHg (B) and 10 additional datasets decreasing systolic blood pressure by 10 mmHg (C).

The median percentage of patients with indications for a statin and not on a statin was 53.26% in (A) datasets, 51.14% in (B) datasets and 49.41% in (C) datasets, suggesting that the impact of blood pressure variability or our imputation procedure in the classification of patients as with or without indication for a statin was negligible.

For all further analysis and operational classification according to indication for a statin, we selected at random one of the (B) datasets. Figure 1 shows the final numbers of patients with indications for a statin according to our algorithm.

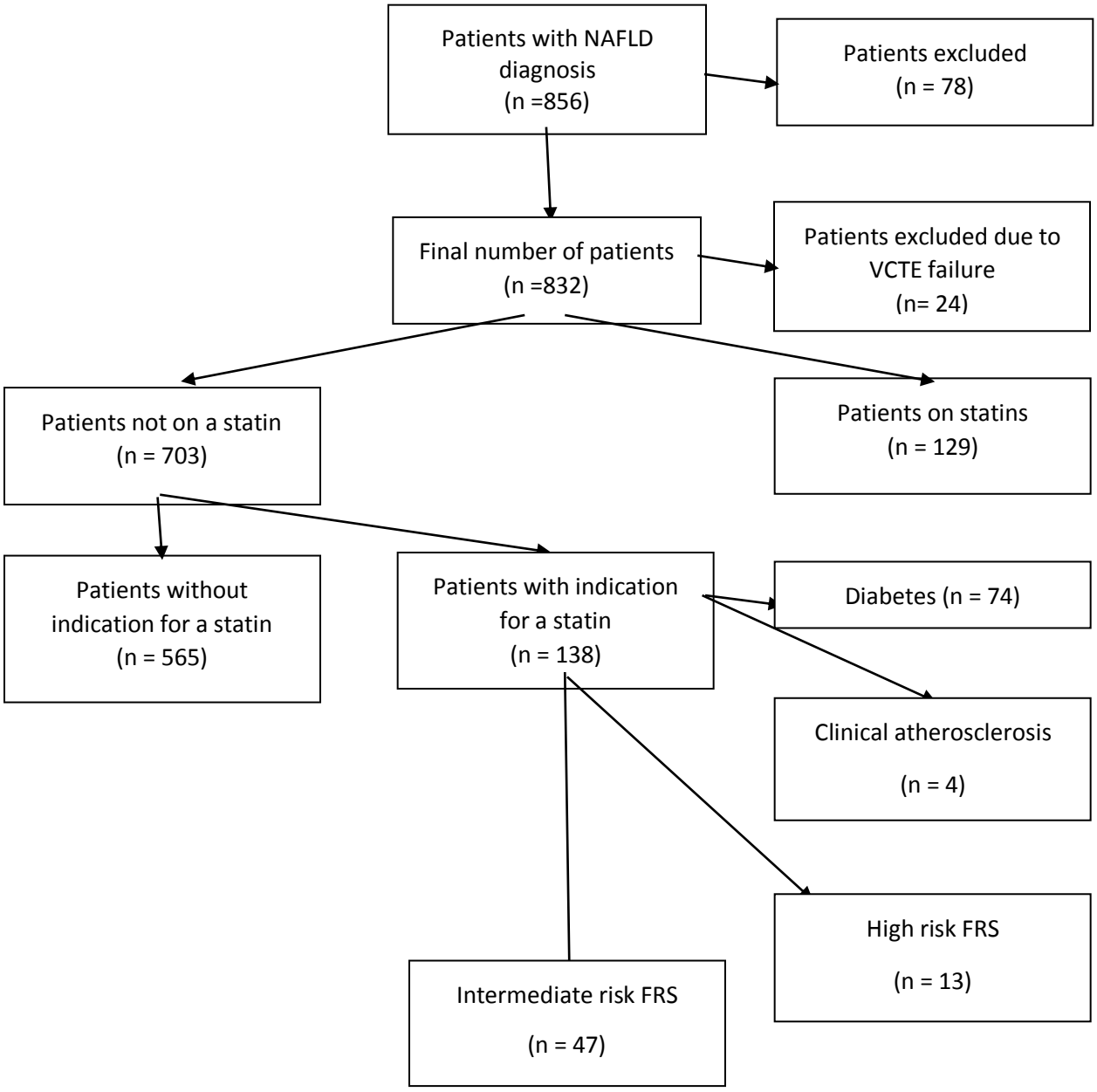


Figure 1: The final numbers of patients with indications for a statin.

Non-invasive tests

Non-invasive tests were used as continuous variables. However, we also tested the previously described low risk for fibrosis thresholds to assess their negative predictive values. FIB-4 was calculated as described in Sterling et al[9]. Patients with FIB-4 less than 1.3 were considered low risk for advanced fibrosis, while those with higher FIB-4 values were considered to be intermediate/high risk patients. Hepamet was calculated as described in Ampuero et al.[10], with a threshold 0.12 to define low risk. NFS was calculated as described in McPherson et al[87], with a threshold of -1.455 defining low risk.

Statistical analysis

Median (IQR) was used to describe the numerical variables, whereas absolute and relative frequencies were used to describe the categorical variables. The impact of statins on the association between NITs and and different thresholds of VCTE was modelled using logistic regression. To further assess whether the effect of statins was determined by the different clinical profile of patients with or without statin treatment, we conducted additional logistic regression models adjusting by age, diabetic status (classified as normal, prediabetes, and diabetes), and BMI. To further model the potential impact of statins on the association between FIB-4 and VCTE used linear regression (detailed in appendix A). R statistical software (R Foundation for Statistical Computing, Vienna, Austria) was used to conduct the analysis. We used rms[159] and ggplot2[160]

Results

Baseline characteristic of the patients

Out of 934 patients referred to the clinic, 856 were classified as having NAFLD. All patients underwent VCTE, and 832 patients had reliable VCTE measurements. Of the 129 patients who were on a statin, 82 were on rosuvastatin, 35 on atorvastatin, 9 on simvastatin, and 3 on pravastatin (Table 5). One hundred and thirty-eight additional patients had indications for statin treatment according to the Canadian Cardiovascular Society guideline for primary prevention from cardiovascular events but were not on a statin treatment (Table 5).

Table 5 shows the baseline characteristics of the patients, according to whether they were on a statin and whether they had an indication for a statin (vs no indication). Patients with indications for a statin were older with a median age of 51 years, and most were diabetic. There were no differences in liver enzymes or liver function tests between those with indication and those without indication. For every NIT (FIB-4, Hepamet, NFS, and VCTE), the median value for those with statin indication was higher than those without indication for a statin. In addition, the prevalence of advanced fibrosis according to VCTE (using different definitions as shown in table 1) was higher in patients with an indication for a statin than in patients without indication for a statin.

Patients with an indication for statin treatment but were not on a statin (52% of patients with an indication for statin treatment) had comparable characteristics to those that were actually on a statin, except for the lipid profile (Table 5).

Table 5: Baseline characteristics of patients with and without indications for a stain who were/ were not taking a statin.

Variable(unit)	Patients with No Indication for a Statin	Patients with Indication for a Statin	Patients with Indication for a statin and were on a Statin	Patients with Indication for a statin and were not on a Statin
Demographic data				
Number of patients (n)	565	267	129	138
Age (Year)	38.0(31.00-44.00)	51.0 (46.0-57.0)	51.00(46.00-58.00)	51.00(46.00-56.00)
Sex, n(%Male)	407 (72.10%)	197 (73.78%)	93(72.30%)	103(74.20%)
BMI (Kg/ M^2)	30.81(28.16-34.81)	31.93 (28.25-35.43)	32.43(28.70-35.18)	31.39(27.50-35.44)
Liver Function Test				
AST (U/L)	36.00(28.00-48.25)	35.5 (28.0-48.0)	37.00(28.00-49.00)	35.00(27.00-45.00)
ALT (U/L)	65.00(46.00-92.00)	59.0 (40.00-78.75)	60.00(44.00-83.00)	58.00(39.00-77.00)
Total Bilirubin (umol/L)	11(9-16)	11(9-15)	11 (9-15)	11(9-15)
Albumin (g/L)	46(44-47)	45.00(43.00-47.00)	45.00(43.00-47.00)	45(43-47)
GGT (U/L)	51.00(32.00-88.00)	58.0 (36.0-114)	66.0(36.0-141.0)	58.00(38.00-97.50)
Platelets(10^9/L)	243.0(207.0-282.8)	223.0 (192.0-267.0)	221.5 (188.2 -262.0)	223.0(192.0-270.00)
Diabetes profile				
HB^{A1C} (%)	5.50(5.30-5.80)	6.00 (5.30-7.1)	6.0 (5.78-6.75)	6.00(5.70-6.70)
Fasting Plasma Glucose (mmol/L)	5.20(4.90-5.60)	6.00 (5.60-6.70)	6.2 (5.45-7.10)	5.90(5.30-7.20)
Diabetic classification				
Diabetes (n)	40(7.1%)	135(50.6%)	61 (47.2%)	74(53.6%)
Prediabetes (n)	249(44.1%)	88(32.9%)	44 (31.1%)	44(31.9%)
Normal (n)	276(48.8%)	44(16.5%)	24 (18.6%)	20(14.5%)
Lipid profile				
Total Cholesterol (mmol/L)	5.01(4.43-5.65)	4.62(3.86-5.46)	4.05(3.52-4.68)	5.24(4.56-5.92)
HDL (mmol/L)	1.12(0.97-1.30)	1.12(0.96-1.26)	1.11(0.93-1.32)	1.12(0.99-1.22)
LDL (mmol/L)	3.04(2.57-3.56)	2.64(1.96-3.39)	2.00(1.64-2.56)	3.16(2.69-3.81)
Non-HDL Cholesterol (mmol/L)	3.87(3.30-4.51)	3.55(2.75-4.36)	2.84(2.36-3.46)	4.13(3.57-4.75)
Non-Invasive Tests				
AST/ALT	0.57(0.48-0.70)	0.62(0.52-0.77)	0.63 (0.53-0.80)	0.67(0.50-0.77)
AST/√ALT	4.50(3.95-5.32)	4.58(4.02-5.76)	4.77(4.03-5.89)	4.49(4.03-5.63)
FIB-4	0.69(0.52-0.93)	1.08 (0.80-1.49)	1.14(0.83-1.51)	1.02(0.80-1.47)
NFS	-2.89(-3.50- -2.22)	-1.35 (-2.22- -0.68)	-1.32(-2.09- -0.58)	-1.62(-2.38- -0.79)
HEPAMET	0.02(0.01-0.05)	0.10 (0.04-0.21)	0.10(0.04-0.21)	0.1(0.04-0.21)
VCTE (kPa)	5.20(4.30-6.10)	5.80 (4.55-7.35)	5.90(4.60-7.90)	5.45(4.50-6.80)
Fibrosis Prevalence				
VCTE ≥8	50 (9%)	54 (20%)	32 (25%)	22 (16%)
VCTE ≥10	31 (5%)	40 (15%)	21 (16%)	19 (14%)
VCTE ≥12	17 (3%)	27 (10%)	17 (13%)	10 (7%)
VCTE ≥16	8 (1%)	12 (4%)	9 (7%)	4 (3%)

* Results present in median (IQR) or in frequencies (%)

Table 6 shows the characteristics of those on lipophilic statins (atorvastatin or simvastatin) and those on hydrophilic statins (rosuvastatin or pravastatin). Patients on lipophilic statins were more diabetic and had higher BMI. There was no difference in liver enzymes or liver function test between those with lipophilic and hydrophilic statins. Also, differences in the median value of NITs (FIB-4, Hepamet, NFS, and VCTE) between patients on hydrophilic statins and those in lipophilic statins was negligible.

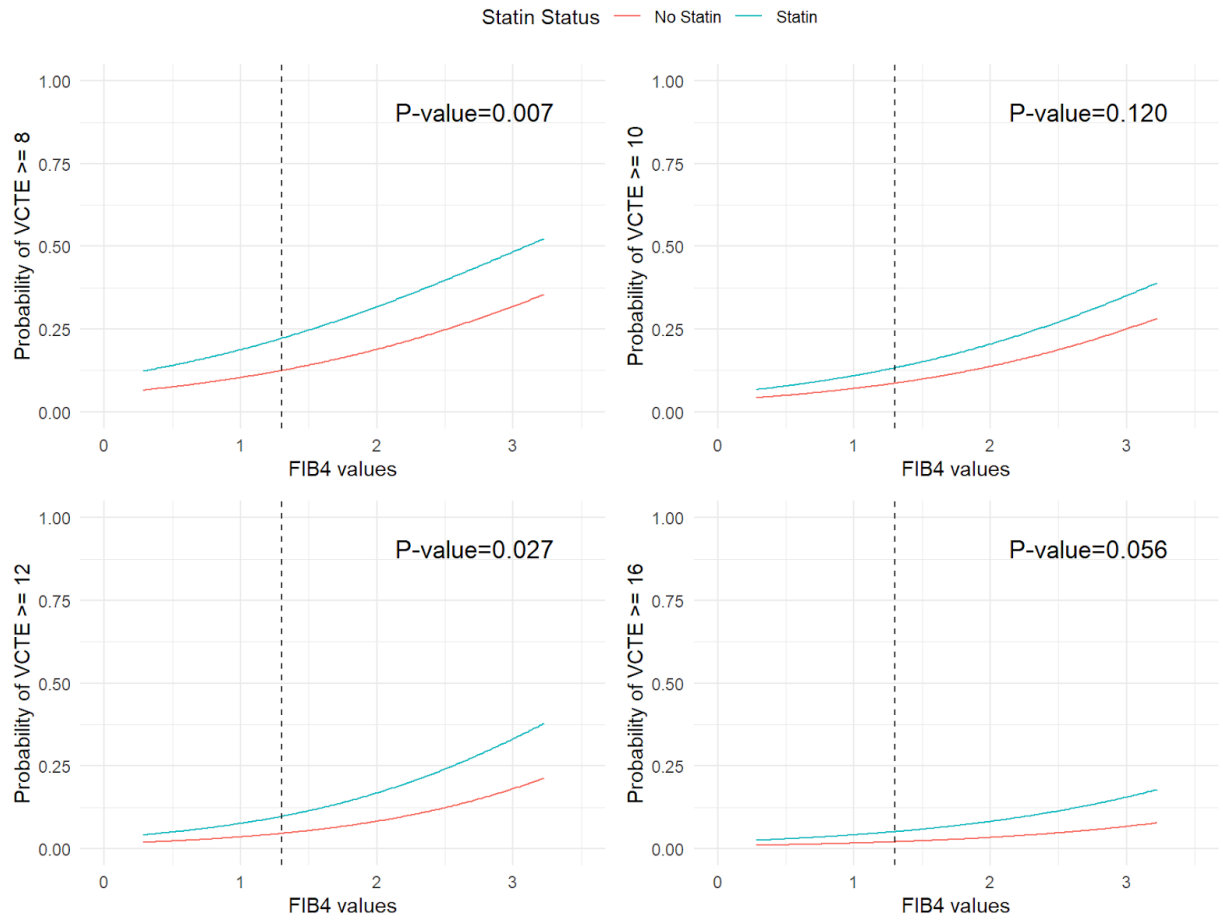
Table 6: Baseline characteristic of patients with hydrophilic and lipophilic statins

Variable(unit)	Patients on Hydrophilic Statin	Patients on Lipophilic Statin
Demographic data		
Number of patients (n)	85	44
Age (Year)	50(45-57)	53 (49- 59)
Sex, n(%Male)	61 (71.76%)	32 (72.70%)
BMI (Kg/ M^2)	31.97 (28.12-34.82)	33.37 (30.41-36.33)
Liver Function Test		
AST (U/L)	39 (31 -52)	32 (27-48)
ALT (U/L)	61(42 -64)	56 (44-76)
Total Bilirubin (umol/L)	11 (9-15)	11(8-15)
Albumin (g/L)	45 (44-47)	45 (43-46)
GGT (U/L)	67 (36-125)	56 (38-153)
Platelets (10^9/L)	218 (188 -262)	232 (195-256)
Diabetes profile		
HB^{A1C} (%)	5.9 (5.6-6.7)	6.4 (5.8-7.2)
Fasting Plasma Glucose (mmol/L)	6.1 (5.3-6.8)	6.6 (5.5-8.6)
Diabetic classification		
Diabetes (n)	35 (41.2%)	26(59.10%)
Prediabetes (n)	34 (40.0%)	10(22.70%)
Normal (n)	16 (18.8%)	8(18.20%)
Lipid profile		
Total Cholesterol (mmol/L)	4.05 (3.58-4.66)	4.03 (3.42-4.73)
HDL (mmol/L)	1.15 (0.95-1.32)	1.09 (0.91-1.33)
LDL (mmol/L)	2.02 (1.64-2.48)	1.95 (1.65-2.66)
Non-HDL Cholesterol (mmol/L)	2.84 (2.38-3.41)	2.79 (2.28-3.51)
Non-Invasive Tests		
AST/ALT	0.62 (0.53-0.80)	0.63 (0.55-0.77)
AST/√ALT	4.9 (4.10-5.83)	4.21 (3.91-6.06)
FIB-4	1.15 (0.91-1.51)	1.11 (0.78-1.50)
NFS	-1.44 (-2.24- -0.66)	-1.13 (-1.88- -0.25)
HEPAMET	0.10 (0.04-0.21)	0.10 (0.04-0.18)
VCTE (kPa)	5.90 (4.80-8.10)	6.15 (4.53-7.75)

Effect of statin use on FIB-4 based predictions of different VCTE thresholds

To assess if statins modify FIB-4 based predictions of VCTE we modeled with logistic regression the association between FIB-4 and VCTE adjusting for statin treatment. For any given value of FIB-4, patients on a statin had higher probabilities of having a VCTE value > 8 or 10 kPa (Figure 1). This difference was attenuated when higher thresholds of VCTE (12 and 16 kPa) were tested. Patients on statins still had higher probabilities of having VCTE > 12 or > 16 kPa, but the effect of statins, however, was not significant (Figure 2[161]). Of note, the interaction between FIB-4 and statins was not significant, indicating that the effect of statins on FIB-4 predictions was homogeneous across values of FIB-4.

Figure 2: Effect of statin treatment on FIB-4 based prediction of different clinically relevant VCTE thresholds (8, 10, 12, and 16 kPa). Patients on statins had a higher risk of having probabilities of high VCTE than patients not on a statin, but this difference attenuated for higher thresholds.

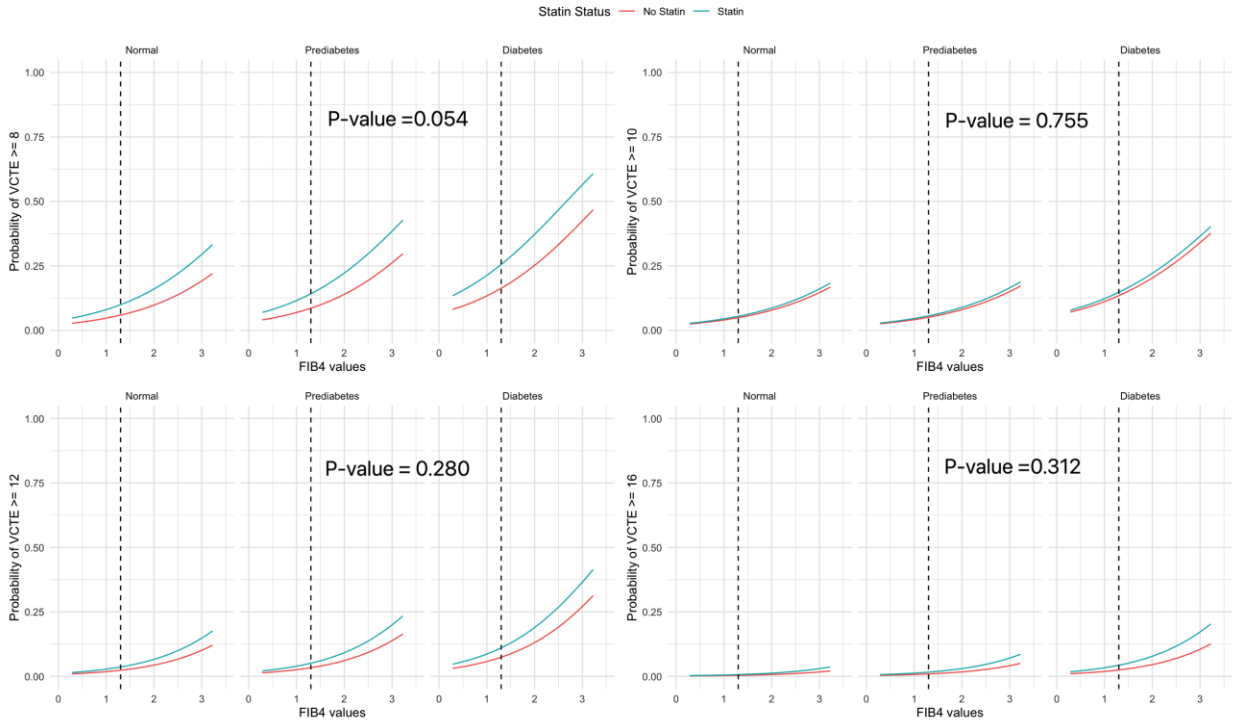


Effect of statin use on FIB-4 based predictions of different VCTE thresholds after adjusting for age, BMI, and diabetes status

We previously showed that age, BMI, and diabetes status modify the association between FIB-4 and VCTE[162]. In this study the patients on statins were older, had a higher BMI, and were more frequently diabetic (Table 1). Thus, to assess if the effect of statins of FIB-4 based predictions were the results of these differences in patient characteristics, we modeled again the effect of statins, in this case, adjusting by BMI, diabetes status, and age.

After adjusting for BMI, diabetes, and age, the effect of statins was markedly blunted. At VCTE of 8 kPa, patients on statins still had higher probabilities of having VCTE>8 kPa, but when higher VCTE thresholds were tested, the difference between patients on statins and not on statins was negligible and non-significant (Figure 3).

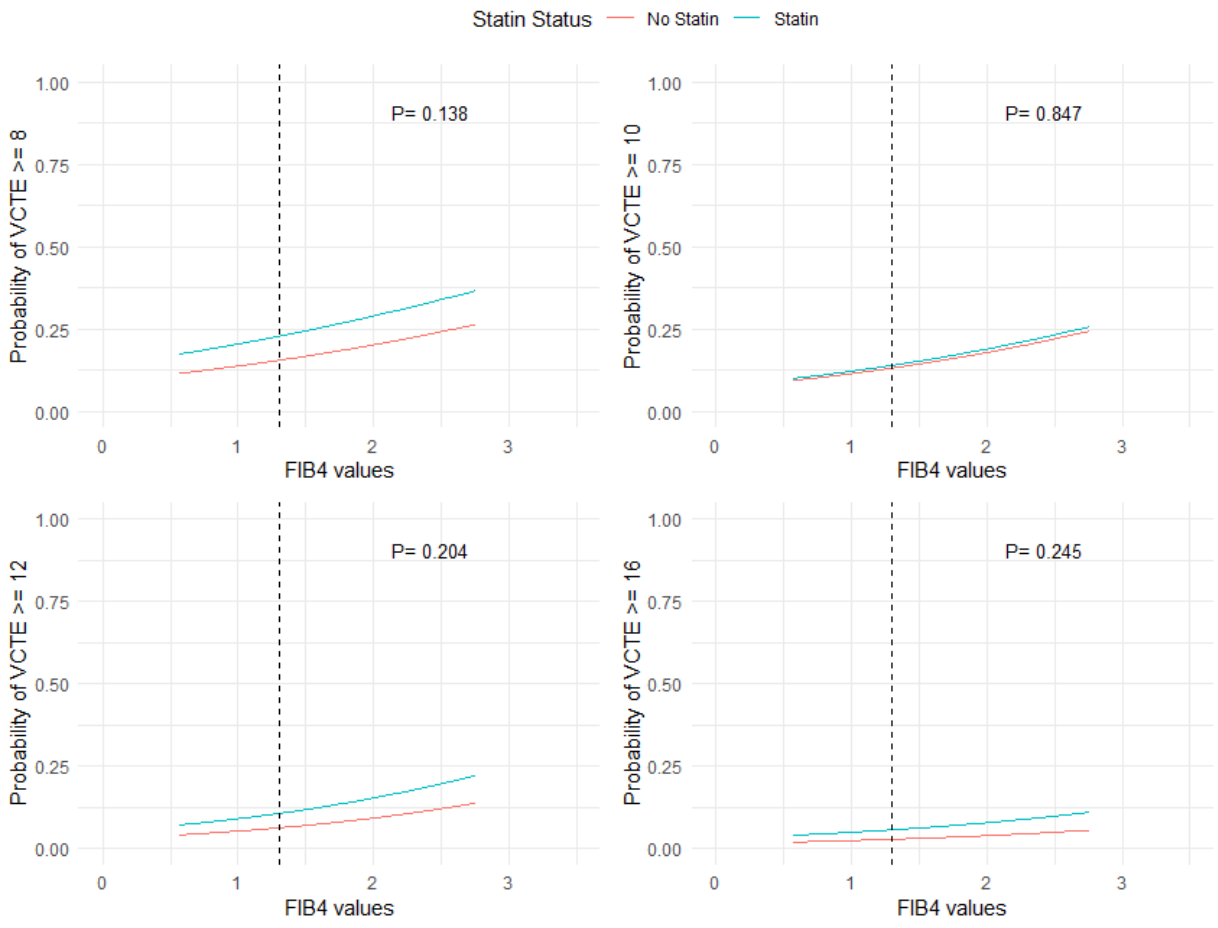
Figure 3: Effect of statin on FIB-4 based prediction of different clinically relevant VCTE thresholds (8, 10, 12, and 16 kPa) after adjusting for age, BMI, and diabetes. Adjusting for BMI, diabetes and age almost completely abrogated these differences, suggesting that these were related to patients' profile rather to a specific effect of statins.



To further analyze the confounding by indication, we assessed if statins still altered FIB-4 predictions in the subset of patients with indications for statins (that were comparable in every patient characteristic except for the lipid profile). In this subgroup of patients, the effect of statins on FIB-4 predictions behaved similarly as in the adjusted analysis with the full sample, showing some effect only with the VCTE threshold of 8 kPa. The difference was progressively attenuated with higher thresholds and became not significant (Figure 4) Which shows that the probability of having higher VCTE in patients with a statin was slightly higher than those with indication for a

statin but were not taking statins. The effect of statin in this subgroup, however, was negligible and not significant.

Figure 4: Effect of statin treatment on FIB-4 based prediction of different clinically relevant VCTE thresholds (8, 10, 12, and 16 kPa) within patients with an indication for a statin. Patients on statins had a higher risk of having probabilities of high VCTE than patients not on a statin, but this difference attenuated for higher thresholds. The effect of statin, however, is not significant. The vertical dashed line represents the 1.3 FIB-4 threshold



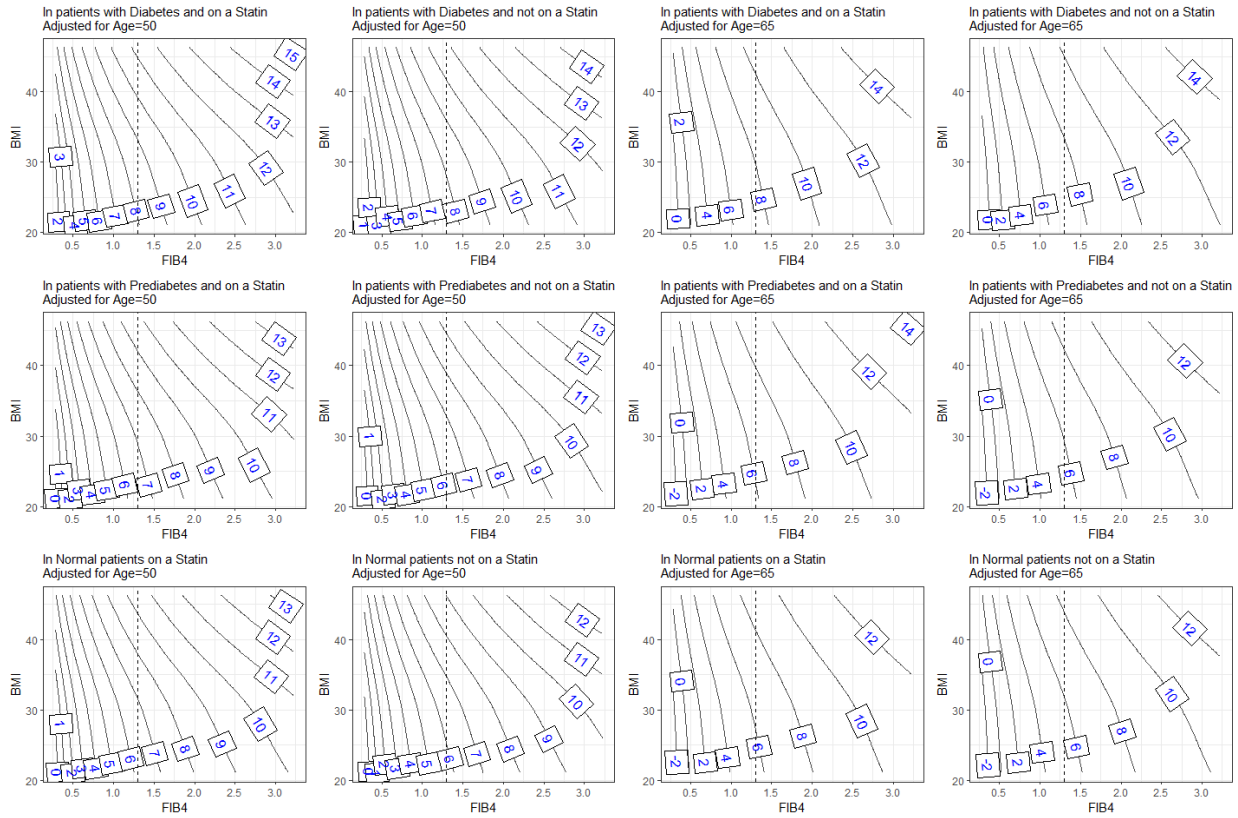
Finally, we assessed the impact of statin treatment on the NPVs of a FIB-4 <1.3 for different thresholds of VCTE. FIB-4 classified 85% of the patients not on statins and 64% of patients on statins as low-risk. Table 7 shows the NPVs of a FIB-4 <1.3 on different VCTE thresholds for significant liver fibrosis. Statins had a small impact on low thresholds of liver fibrosis as according to VCTE and this decrease in NPV disappeared for higher thresholds of VCTE.

Table 7: The difference between NPV between patients with or without a statin among different NITs.

Statin status	VCTE>8	VCTE>10	VCTE>12	VCTE>16
FIB-4<1.3 NPVs				
Statin	89%	94%	96%	100%
No Statin	92%	95%	98%	99%
HEPAMET<0.12 NPVs				
Statin	85%	93%	94%	99%
No Statin	92%	95%	98%	99%
NFS<-1.455 NPVs				
Statin	90%	91%	95%	99%
No Statin	93%	96%	98%	99.6%

Finally, to further understand how statins, BMI age and diabetes interplay to modify the association between FIB-4 and VCTE, we conducted a multiple linear regression analysis as detailed in Appendix A. For a given value of FIB-4, patients on a statin had only a slightly higher mean VCTE value than those not on a statin but the difference was small and non-significant (p-value =0.339). BMI had a significant and major impact on the FIB-4-based predictions of VCTE, the higher the BMI, the higher the predicted mean VCTE. In the case of diabetic status, only the diagnosis of diabetes but not prediabetes had significant effects on the predicted mean VCTE as compared to normal patients, being the predicted mean VCTE was higher in diabetes patients than in patients with prediabetes or eugkycemia for a given FIB-4 value. Figure 5

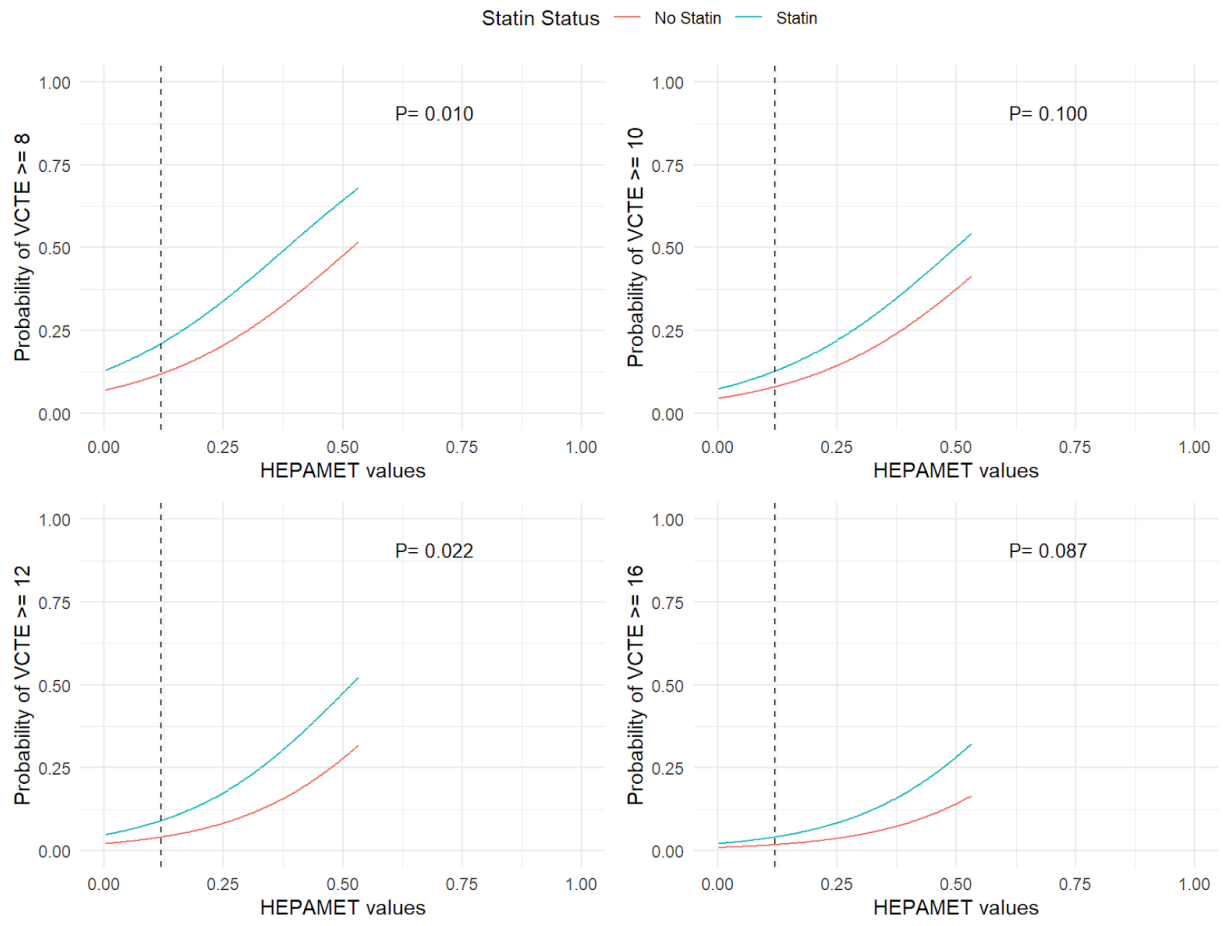
Figure 5: Predicted mean values of VCTE based on FIB-4 and BMI and adjusted for age, statin whether patients taking or not taking a statin and diabetes status (Normal, prediabetes and diabetes). The lines within the plots show the mean predicted VCTE and the dashed line represents 1.3 FIB-4 thresholds. The plots are shown for representative age 50 and 65, and for a statin, whether patients on a statin or not. Note that for a given value of FIB-4 patients with higher BMI have higher predicted mean VCTE. Diabetes but not prediabetes significantly affects this prediction with a higher mean predicted VCTE in diabetic patients than normal or prediabetes.



Effect of statin use on Hepamet and NFS-based predictions of VCTE.

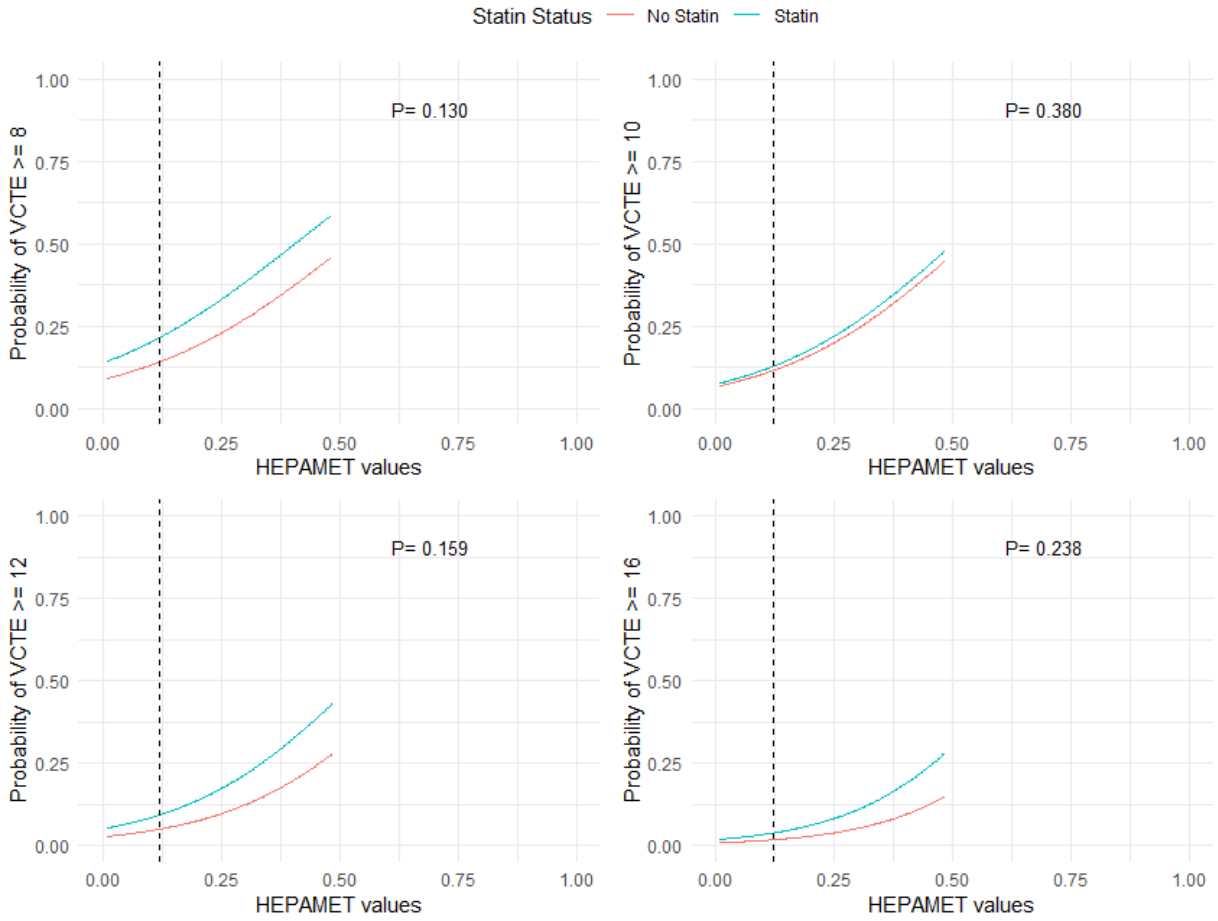
We additionally tested whether statins impacted the other most common NITs. NAFLD Fibrosis Score (NFS) and a newly proposed test that has shown high accuracy (Hepamet). The effect of statins on the association between Hepamet and VCTE was similar to FIB-4. For a given value of Hepamet, patients with statins had higher probabilities of higher VCTE>8 compared with patients not on statins. The effect of statins attenuated for higher clinically relevant VCTE values, 10, 12, and 16 kPa (Figure 5). 91% and 73% were classified as low risk by Hepamet (<0.12). Table 7 shows the NPVs of Hepamet <0.12 for different VCTE thresholds for liver fibrosis and the predictions were minimally impacted by statins.

Figure 6: Effects of statin on Hepamet based prediction of different clinically relevant VCTE thresholds (8, 10, 12, and 16 kPa). Patients on statins had a higher risk of having probabilities of high VCTE than patients not on a statin, but this difference attenuated for higher thresholds. Vertical dashed line represents the 0.12 threshold



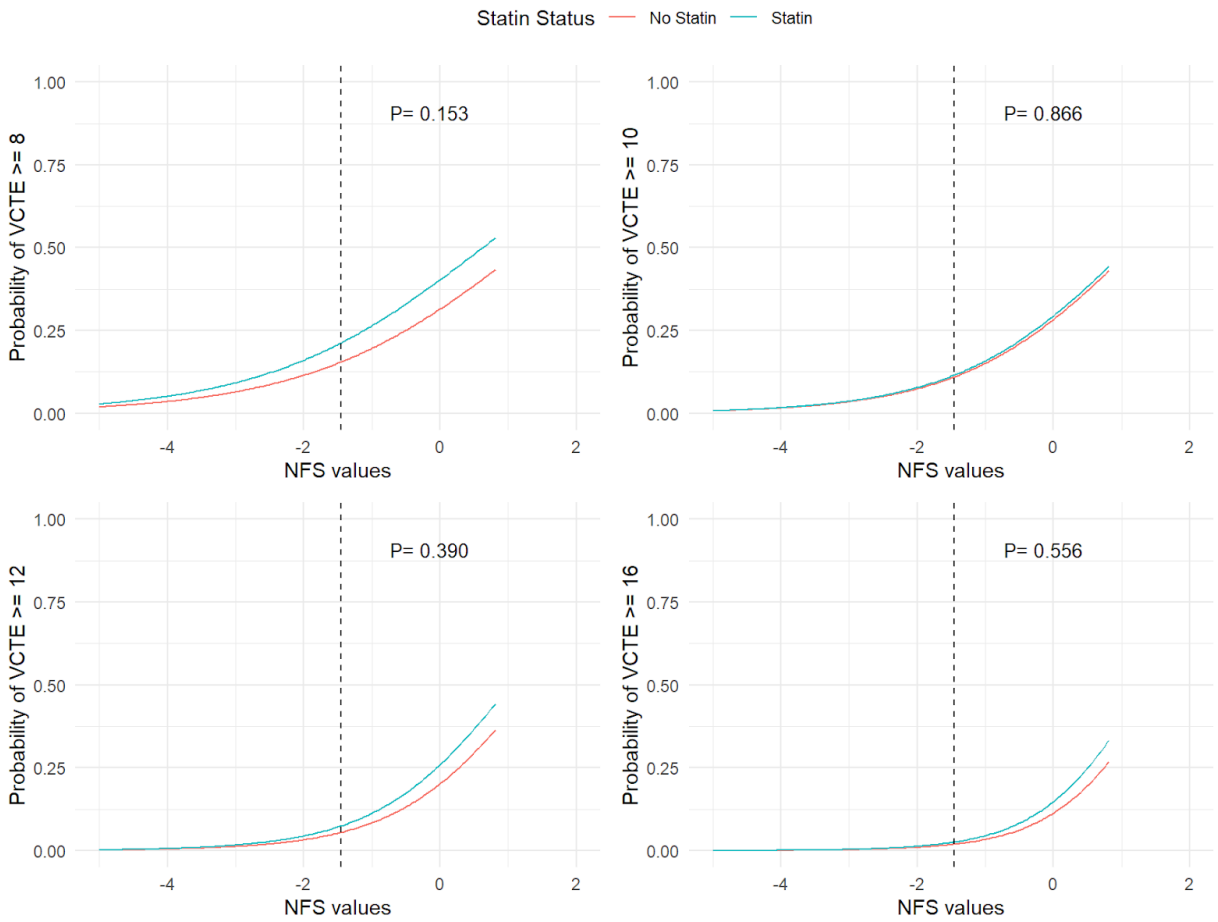
The effect of statins on Hepamet based prediction of advanced fibrosis on subset of patients with indication for a statin (those with similar baseline characteristic) showed that statin effect on Hepamet based prediction of advanced fibrosis is negligible and not significant and it further abrogated with higher VCTE thresholds. (Figure 7)

Figure 7: Effects of statin on Hepamet based prediction of different clinically relevant VCTE thresholds (8, 10, 12, and 16 kPa) within patients with an indication for a statin. Despite patients on statins had a higher risk of having probabilities of high VCTE than patients not on a statin, the effect of statin is not significant. The vertical dashed line represents the 0.12 Hepamet threshold



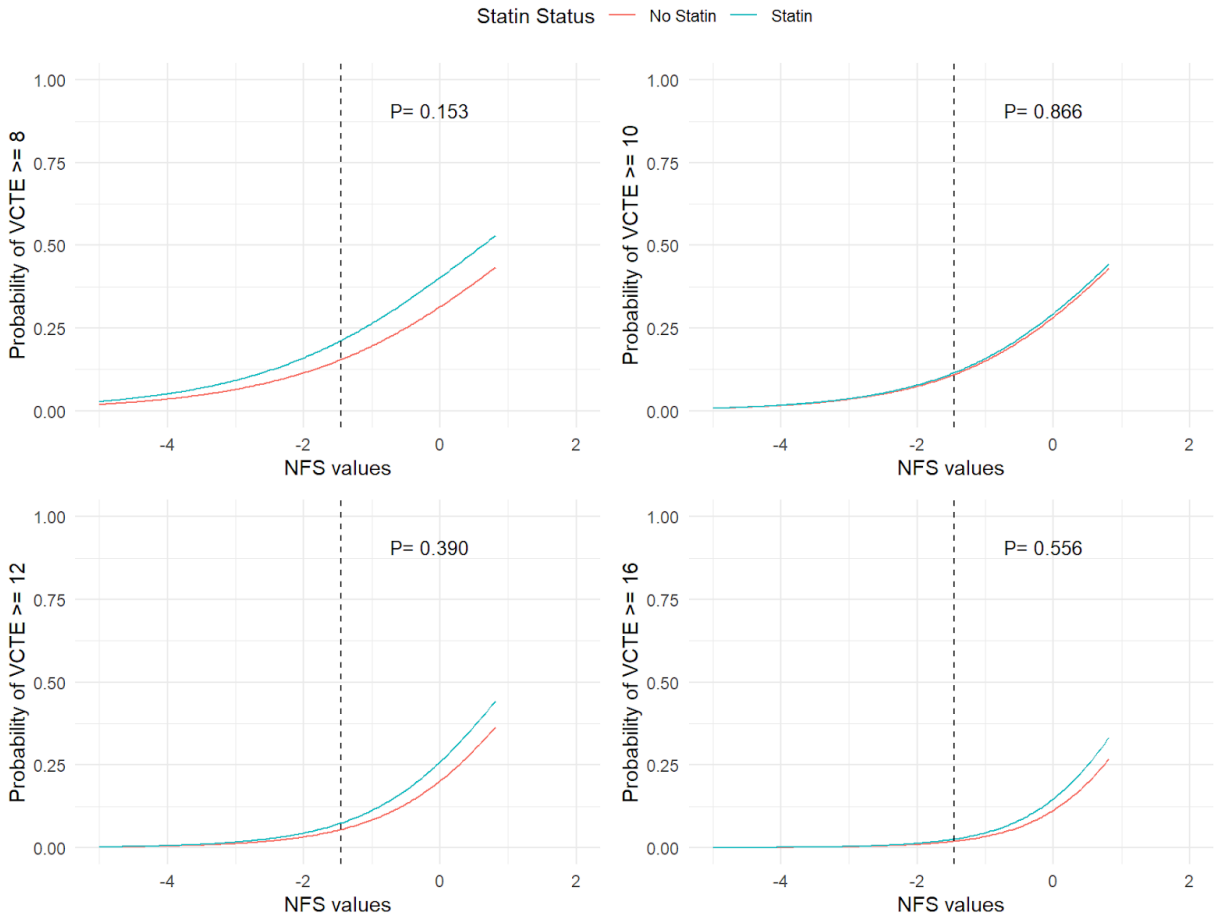
The effect of statins on NFS-based prediction of VCTE was distinct from the effect of FIB-4 and Hepamet predictions. Indeed, statins did not alter the association between NFS and VCTE even at a low VCTE threshold (8 kPa) (Figure 8). However, NFS classified a smaller proportion of patients as low risk (83% on statins and 49% not on statins) than FIB-4. NPVs were comparable to those of FIB-4 and Hepamet (Table 7)

Figure 8: Effect of statin on NFS based prediction of different clinically relevant VCTE thresholds (8, 10, 12, and 16 kPa). Statins did not affect NFS predictions of VCTE at any thresholds. The dashed line represents the threshold -1.455.



Further analysis on the effect of the statins on patients with indications for a statin was similar to the whole sample and proved that statin effects was due to the difference in the baseline characteristics between patients taking statin and those not taking statin rather than the effect of statins themselves.

Figure 9: Effect of statin on NFS based prediction of different clinically relevant VCTE thresholds (8, 10, 12, and 16 kPa). Statins did not affect NFS predictions of VCTE at any thresholds. The dashed line represents the threshold -1.455.



Chapter 4: discussion

Study purpose

The purpose of this study was to determine the impact of statin treatment on the predictive performance of NITs based prediction of advanced liver fibrosis in patients with NAFLD, and we hypothesized that since statins modify aminotransferases levels, it could affect NITs based prediction that are used in first step pathway to diagnose advanced fibrosis in NAFLD. The second aim was to assess the prevalence of statins use in patients with indication for a statin according to the Canadian Cardiovascular Society.

Key findings

In this study we assessed the potential impact of statin treatment on a NAFLD referral pathway utilizing NITs (FIB-4, Hepamet and NFS) to triage NAFLD patients for fibrosis. In our study patients who were taking statins had a higher probability of having VCTE>8 for a given value of FIB-4. This difference, however, was attenuated for higher VCTE thresholds for liver fibrosis. This difference was mainly related to the higher baseline risk (and hence, pre-test probability) of liver fibrosis. The patients on a stain were older, more frequently diabetic, and had a higher BMI. Second, the effects of a statin on the association between Hepamet and VCTE was similar to FIB-4, while it was much less marked for NFS. Overall statin treatment, however, had a negligible impact on NPVs of these non-invasive tests, which would be the relevant metric to assess their value as a first step in the referral process. Finally, we show that more than half of patients with a theoretical indication for a statin were not on a statin at the time of referral.

General discussion

Our study shows that patients with an indication for a statin had higher probabilities of having VCTE >8 kPa. Also, it shows that patients with an indication for a statin were older, higher BMI, more frequently diabetic, and had higher median NITs compared to those with no indication for a statin, which is consistent with previous data[163] According to the Canadian Cardiovascular Society, statins are indicated to any patients aged more than 40 with diabetes, which explains why patients with an indication for a statin mostly have diabetes and older.[15] Adjusting for age, BMI, and diabetes abrogated the effects of statins on FIB-4 predictions of liver fibrosis. The higher probability of VCTE>8 kPa can mostly be explained by the difference in baseline characteristics rather than the effects of statin itself. When we assessed the effects of statins on FIB-4 based prediction of VCTE in patients with an indication for statins, the prediction difference was not significantly different between those with an indication for a statin and were taking a stain compared to those with an indication for a statin that were not taking a statin. The above analysis provided further supportive evidence that statin treatment has a minimal impact on FIB 4 prediction of liver fibrosis.

The statins' effects on Hepamet based predictions were comparable to that of FIB-4 based predictions, whereas NFS predictions were minimally altered. This can be explained by the fact that neither Hepamet nor FIB-4 include BMI in their calculation, while this is included in NFS. We have shown before [162] and we show again here that BMI has a pronounced effect on NITs based prediction of advanced fibrosis. Since BMI was different in patients with and without statins, this differential effect between the three NITs is not unexpected. However, in all three NITs, differences markedly attenuated for higher thresholds of VCTE (10, 12, and 16 kPa),

which are probably more in keeping with current trends in the stratification in patients with NAFLD. Indeed, recent guidelines for HCC screening in patients with NAFLD suggest a VCTE measurement of 16.1 kPa as the threshold for initiating screening.

Previous studies have shown that statins were under prescribed in patients with NAFLD, with 44%-74% of patients with indication for a statin not receiving a statin.[120,163,164]. Several explanations might account for this. The most important one is the concern that patients with NAFLD or NASH and dyslipidemia might have baseline serum aminotransferases elevation, and therefore potential statin liver toxicity is a matter of concern.[165] another explanation is that, at the time of referral, primary care providers could be awaiting hepatologist assessment to decide whether patients had contraindications or not for statins. Finally, in a subset of the patients, our definition of statin indication was based on thorough modelling with the data that was available to us, though we show that the impact of this modeling on the calculation of statin indication was minimal.

We did not explore the causes of non-prescription or suspension of statins, which is a limitation of the study. Moreover, no information about any previous therapy with statins or any potential adverse reactions resulting in statin discontinuation was collected. Additionally, the indication of statin in our study was based on the Canadian Cardiovascular Society, which has some differences from the American Heart Association guidelines and the European guidelines.[118,166]

Conclusion

This study provides evidence that NITs can be used safely as a first step in the referral pathway to rule out advanced fibrosis in patients with NAFLD who are taking statins as we have found that statin treatment had only a minor effect on the ability of NITs FIB-4, Hepamet and NFS to predict advanced liver fibrosis. This effect was relevant only when a low threshold of VCTE (8 kPa) was used to classify patients with advanced fibrosis, but not with higher thresholds of 10. 12 or 16 kPa. Our data demonstrates that performance of FIB-4, Hepamet and NFS as a first step in a referral pathway for NAFLD is not significantly affected by statin use. Furthermore, only half of the patients referred through the pathway with an indication for a statin were taking a statin, which calls for a further understanding on the factors determining statins under prescription and sub-optimal treatments for cardiovascular prevention in patients with NAFLD and strategies to optimize cardiovascular prevention, which is still the main cause of morbidity and mortality in NAFLD

Future directions

Statin is a key in primary and secondary prevention of CVD, and there is substantial evidence that it decreases mortality and morbidity. Despite that, there is a high percentage of patients not receiving statin treatment.

Several reasons account for statin prescription variation influenced by physicians and patients' factors. Patients' factors include patients' characteristics such as lipid profile, patients' comorbidities such as hypertension, diabetes, kidney and liver diseases, statin side effects and contraindication. Physicians' factors which include the reluctance of physicians to prescribe statin when developing side effects is a matter of concern, and the lack of evidence base to prescribe statin at lower prevention thresholds. Since patients with NAFLD have increased liver aminotransferase, physicians are concerned about prescribing a statin to patients with NAFLD. Also, in general practice, physicians tend not to describe or discontinue statin when there is an increase in aminotransferases level, or any minor side effects happen. There are also challenges when it comes to reintroducing statin to those patients with side effects despite education and physician advice. Lack of risk stratification could be another potential cause of statin under prescriptions. A limited number of randomized controlled trials assess statin treatment in NAFLD patients, and most of these studies are of less than 5 years duration.

In order to solve these problems studies with longer follow up periods are needed to assess statin benefits and side effects in those subsets of patients and whether the benefits of statin treatment outweigh the risks even to those with high levels of aminotransferases. Longer follow up period

is needed as statin treatment in the majority of cases is prescribed for life, and most current studies are of less than 5 years duration. Large, randomized trials are needed to assess the effectiveness of statin in NAFLD patients as well as the safety of using statin on patients with higher levels of aminotransferases and different fibrosis stages. Furthermore, studies investigating patients and physicians' factors that influence statin treatment to identify the most common contributing cause on a regional level and worldwide and address these factors.

Bibliography

1. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease—Meta- analytic assessment of prevalence, incidence, and outcomes. *Hepatology*. 2016;64(1):73-84. https://onlinelibrary.wiley.com/doi/abs/10.1002/hep.28431. doi: 10.1002/hep.28431.

2. Ekstedt M, Hagström H, Nasr P, et al. Fibrosis stage is the strongest predictor for disease-specific mortality in NAFLD after up to 33 years of follow- up. *Hepatology*. 2015;61(5):1547-1554. https://onlinelibrary.wiley.com/doi/abs/10.1002/hep.27368. doi: 10.1002/hep.27368.

3. Dulai PS, Singh S, Patel J, et al. Increased risk of mortality by fibrosis stage in non-alcoholic fatty liver disease. *Hepatology*. 2017;65(5):1557. http://urn.kb.se/resolve?urn=urn:nbn:se:liu:diva-134875.

4. Chalasani N, Younossi Z, Lavine JE, et al. The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the american association for the study of liver diseases. *Hepatology (Baltimore, Md.)*. 2018;67(1):328-357. https://search.datacite.org/works/10.1002/hep.29367. doi: 10.1002/hep.29367.

5. Tapper EB, Loomba R. Noninvasive imaging biomarker assessment of liver fibrosis by elastography in NAFLD. *Nature reviews. Gastroenterology & hepatology*. 2018;15(5):274-282. https://search.datacite.org/works/10.1038/nrgastro.2018.10. doi: 10.1038/nrgastro.2018.10.

6. Tsochatzis EA NP. Non-alcoholic fatty liver disease and the interface between primary and secondary care. . 2018;3:510-517. https://doi.org/10.1016/S2468-1253(18)30077-3.

7. Leoni S, Tovoli F, Napoli L, Serio I, Ferri S, Bolondi L. Current guidelines for the management of non-alcoholic fatty liver disease: A systematic review with comparative analysis. *World journal of gastroenterology : WJG*. 2018;24(30):3361-3373. https://www.ncbi.nlm.nih.gov/pubmed/30122876. doi: 10.3748/wjg.v24.i30.3361.

8. Angulo P, Hui JM, Marchesini G, et al. The NAFLD fibrosis score: A noninvasive system that identifies liver fibrosis in patients with NAFLD. *Hepatology*. 2007;45(4):846-854. https://onlinelibrary.wiley.com/doi/abs/10.1002/hep.21496. doi: 10.1002/hep.21496.

9. Sterling RK, Lissen E, Clumeck N, et al. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. *Hepatology (Baltimore, Md.)*. 2006;43(6):1317-1325. https://search.datacite.org/works/10.1002/hep.21178. doi: 10.1002/hep.21178.

10. Ampuero J, Pais R, Aller R, et al. Development and validation of hepamet fibrosis scoring System–A simple, noninvasive test to identify patients with nonalcoholic fatty liver disease with advanced fibrosis. *Clinical gastroenterology and hepatology*. 2020;18(1):216-225.e5. https://search.datacite.org/works/10.1016/j.cgh.2019.05.051. doi: 10.1016/j.cgh.2019.05.051.

11. Targher G, Day CP, Bonora E. Risk of cardiovascular disease in patients with nonalcoholic fatty liver disease. *The New England journal of medicine*. 2010;363(14):1341-1350. https://search.datacite.org/works/10.1056/nejmra0912063. doi: 10.1056/nejmra0912063.

12. Martins E, Oliveira A. NAFLD and cardiovascular disease. *Porto biomedical journal*. 2018;3(2):e2. https://search.proquest.com/docview/2303202211. doi: 10.1016/j.pbj.0000000000000002.

13. Targher G, Marra F, Marchesini G. Increased risk of cardiovascular disease in non-alcoholic fatty liver disease: Causal effect or epiphenomenon? *Diabetologia*. 2008;51(11):1947-1953. https://www.ncbi.nlm.nih.gov/pubmed/18762907. doi: 10.1007/s00125-008-1135-4.

14. Hadjiphilippou S, Ray K. Cholesterol-lowering agents: Statins—For everyone? *Circulation research*. 2019;124(3):354-363. http://ovidsp.ovid.com/ovidweb.cgi?T=JS&NEWS=n&CSC=Y&PAGE=fulltext&D=ovft&AN=00003012-201902010-00002. doi: 10.1161/CIRCRESAHA.118.313245.

15. Anderson TJ, Grégoire J, Pearson GJ, et al. 2016 canadian cardiovascular society guidelines for the management of dyslipidemia for the prevention of cardiovascular disease in the adult. *Canadian journal of cardiology*. 2016;32(11):1263-1282. https://search.datacite.org/works/10.1016/j.cjca.2016.07.510. doi: 10.1016/j.cjca.2016.07.510.

16. Stone NJ, Robinson JG, Lichtenstein AH, et al. 2013 ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults: A report of the american college of cardiology/american heart association task force on practice guidelines. *Journal of the American College of Cardiology*. 2014;63(25 Pt B):2889-2934. https://www.ncbi.nlm.nih.gov/pubmed/24239923. doi: 10.1016/j.jacc.2013.11.002.

17. Sigler MA, Congdon L, Edwards KL. An evidence-based review of statin use in patients with nonalcoholic fatty liver disease. *Clin Med Insights Gastroenterol*. 2018;11:1179552218787502. https://doi.org/10.1177/1179552218787502. doi: 10.1177/1179552218787502.

18. Cobbina E, Akhlaghi F. Non-alcoholic fatty liver disease (NAFLD) – pathogenesis, classification, and effect on drug metabolizing enzymes and transporters. *Drug Metab Rev*. 2017;49(2):197-211. https://doi.org/10.1080/03602532.2017.1293683. doi: 10.1080/03602532.2017.1293683.

19. Jurgen Ludwig, Thomas R. Viggiano, Douglas B. Mcgill, Beverly J. Ott. Nonalcoholic steatohepatitis: Mayo clinic experiences with a hitherto unnamed disease. *Mayo Clinic Proceeding*. 1980;55:434-438.

20. Ekstedt M, Franzén LE, Mathiesen UL, et al. Long-term follow-up of patients with NAFLD and elevated liver enzymes. *Hepatology*. 2006;44(4):865-873. https://doi.org/10.1002/hep.21327. doi: 10.1002/hep.21327.

21. Sung K, Wild SH, Byrne CD. Development of new fatty liver, or resolution of existing fatty liver, over five years of follow-up, and risk of incident hypertension. *Journal of hepatology*. 2014;60(5):1040-1045. https://search.datacite.org/works/10.1016/j.jhep.2014.01.009. doi: 10.1016/j.jhep.2014.01.009.

22. Zelber-Sagi S, Lotan R, Shlomai A, et al. Predictors for incidence and remission of NAFLD in the general population during a seven-year prospective follow-up. *Journal of hepatology*. 2012;56(5):1145-1151. https://search.datacite.org/works/10.1016/j.jhep.2011.12.011. doi: 10.1016/j.jhep.2011.12.011.

23. Wong VW, Wong GL, Yeung DK, et al. Incidence of non-alcoholic fatty liver disease in hong kong: A population study with paired proton-magnetic resonance spectroscopy. *Journal of hepatology*. 2015;62(1):182-189. https://search.datacite.org/works/10.1016/j.jhep.2014.08.041. doi: 10.1016/j.jhep.2014.08.041.

24. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology (Baltimore, Md.)*. 2016;64(1):73-84. https://search.datacite.org/works/10.1002/hep.28431. doi: 10.1002/hep.28431.

25. Mitra S, De A, Chowdhury A. Epidemiology of non-alcoholic and alcoholic fatty liver diseases. *Translational gastroenterology and hepatology*. 2020;5:16. https://www.ncbi.nlm.nih.gov/pubmed/32258520. doi: 10.21037/tgh.2019.09.08.

26. Rich NE, Oji S, Mufti AR, et al. Racial and ethnic disparities in nonalcoholic fatty liver disease prevalence, severity, and outcomes in the united states: A systematic review and meta-analysis. *Clinical gastroenterology and hepatology*. 2018;16(2):198-210.e2. https://search.datacite.org/works/10.1016/j.cgh.2017.09.041. doi: 10.1016/j.cgh.2017.09.041.

27. Younossi ZM, Gramlich T, Matteoni CA, Boparai N, McCullough AJ. Nonalcoholic fatty liver disease in patients with type 2 diabetes. *Clinical gastroenterology and hepatology*. 2004;2(3):262-265. http://dx.doi.org/10.1016/S1542-3565(04)00014-X. doi: 10.1016/S1542-3565(04)00014-X.

28. Stepanova M, Rafiq N, Makhlouf H, et al. Predictors of all-cause mortality and liver-related mortality in patients with non-alcoholic fatty liver disease (NAFLD). *Dig Dis Sci*. 2013;58(10):3017-3023. https://www.ncbi.nlm.nih.gov/pubmed/23775317. doi: 10.1007/s10620-013-2743-5.

29. Fracanzani AL, Valenti L, Bugianesi E, et al. Risk of severe liver disease in nonalcoholic fatty liver disease with normal aminotransferase levels: A role for insulin resistance and diabetes. *Hepatology (Baltimore, Md.)*. 2008;48(3):792-798. https://search.datacite.org/works/10.1002/hep.22429. doi: 10.1002/hep.22429.

30. Portillo-Sanchez P, Bril F, Maximos M, et al. High prevalence of nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus and normal plasma aminotransferase levels. *The journal of clinical endocrinology and metabolism*. 2015;100(6):2231-2238. https://search.datacite.org/works/10.1210/jc.2015-1966. doi: 10.1210/jc.2015-1966.

31. Younossi ZM, Golabi P, de Avila L, et al. The global epidemiology of NAFLD and NASH in patients with type 2 diabetes: A systematic review and meta-analysis. *Journal of hepatology*. 2019;71(4):793-801. https://search.datacite.org/works/10.1016/j.jhep.2019.06.021. doi: 10.1016/j.jhep.2019.06.021.

32. Kabbany MN, Selvakumar PKC, Watt K, et al. Prevalence of nonalcoholic steatohepatitis-associated cirrhosis in the united states: An analysis of national health and nutrition examination survey data. *The American journal of gastroenterology*. 2017;112(4):581-587. https://search.datacite.org/works/10.1038/ajg.2017.5. doi: 10.1038/ajg.2017.5.

33. Fung J, Lee C, Chan M, et al. High prevalence of non-alcoholic fatty liver disease in the chinese – results from the hong kong liver health census. *Liver Int*. 2015;35(2):542-549. https://doi-org.login.ezproxy.library.ualberta.ca/10.1111/liv.12619. doi: 10.1111/liv.12619.

34. Wree A, Broderick L, Canbay A, Hoffman HM, Feldstein AE. From NAFLD to NASH to cirrhosis—new insights into disease mechanisms. *Nature reviews. Gastroenterology & hepatology*. 2013;10(11):627-636. https://www.ncbi.nlm.nih.gov/pubmed/23958599. doi: 10.1038/nrgastro.2013.149.

35. Kwok R, Choi KC, Wong GL, et al. Screening diabetic patients for non-alcoholic fatty liver disease with controlled attenuation parameter and liver stiffness measurements: A prospective cohort study. *Gut*. 2016;65(8):1359-1368. http://dx.doi.org/10.1136/gutjnl-2015-309265. doi: 10.1136/gutjnl-2015-309265.

36. Calzadilla Bertot L, Adams L. The natural course of non-alcoholic fatty liver disease. *International journal of molecular sciences*. 2016;17(5):774. https://search.datacite.org/works/10.3390/ijms17050774. doi: 10.3390/ijms17050774.

37. Singh S, Allen AM, Wang Z, Prokop LJ, Murad MH, Loomba R. Fibrosis progression in nonalcoholic fatty liver versus nonalcoholic steatohepatitis: A systematic review and meta-analysis of paired-biopsy studies. *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association*. 2014;13(4):643-654.e9. https://www.openaire.eu/search/publication?articleId=od_____267::8a709abb97728420e6dfd243148c0507. doi: 10.1016/j.cgh.2014.04.014.

38. Petrick JL, Kelly SP, Altekruse SF, McGlynn KA, Rosenberg PS. Future of hepatocellular carcinoma incidence in the united states forecast through 2030. *Journal of clinical oncology*. 2016;34(15):1787-1794. https://search.datacite.org/works/10.1200/jco.2015.64.7412. doi: 10.1200/jco.2015.64.7412.

39. White DL, Thrift AP, Kanwal F, Davila J, El-Serag HB. Incidence of hepatocellular carcinoma in all 50 united states, from 2000 through 2012. *Gastroenterology (New York, N.Y. 1943)*. 2017;152(4):812-820.e5. https://search.datacite.org/works/10.1053/j.gastro.2016.11.020. doi: 10.1053/j.gastro.2016.11.020.

40. Vogel A, Cervantes A, Chau I, et al. Hepatocellular carcinoma: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. *Annals of oncology*. 2018;29(Supplement_4):iv238-iv255. https://search.datacite.org/works/10.1093/annonc/mdy308. doi: 10.1093/annonc/mdy308.

41. Yatsuji S, Hashimoto E, Tobari M, Taniai M, Tokushige K, Shiratori K. Clinical features and outcomes of cirrhosis due to non-alcoholic steatohepatitis compared with cirrhosis caused by chronic hepatitis C. *Journal of gastroenterology and hepatology*. 2009;24(2):248-254. https://search.datacite.org/works/10.1111/j.1440-1746.2008.05640.x. doi: 10.1111/j.1440-1746.2008.05640.x.

42. Bugianesi E, Leone N, Vanni E, et al. Expanding the natural history of nonalcoholic steatohepatitis: From cryptogenic cirrhosis to hepatocellular carcinoma. *Gastroenterology (New York, N.Y. 1943)*. 2002;123(1):134-140. https://search.datacite.org/works/10.1053/gast.2002.34168. doi: 10.1053/gast.2002.34168.

43. Noureddin M, Rinella ME. Nonalcoholic fatty liver disease, diabetes, obesity, and hepatocellular carcinoma. *Clinics in liver disease*. 2015;19(2):361-379. https://search.datacite.org/works/10.1016/j.cld.2015.01.012. doi: 10.1016/j.cld.2015.01.012.

44. Kanwal F, Kramer JR, Mapakshi S, et al. Risk of hepatocellular cancer in patients with non-alcoholic fatty liver disease. *Gastroenterology (New York, N.Y. 1943)*. 2018;155(6):1828-1837.e2. https://search.datacite.org/works/10.1053/j.gastro.2018.08.024. doi: 10.1053/j.gastro.2018.08.024.

45. de la Garza, Rocio G, Morales-Garza LA, Martin-Estal I, Castilla-Cortazar I. Insulin-like growth factor-1 deficiency and cirrhosis establishment. *Journal of clinical medicine research*. 2017;9(4):233-247. https://www.ncbi.nlm.nih.gov/pubmed/28270882. doi: 10.14740/jocmr2761w.

46. Stasi C, Silvestri C, Voller F, Cipriani F. Epidemiology of liver cirrhosis. *Journal of Clinical and Experimental Hepatology*. 2015;5(3):272. http://www.sciencedirect.com.login.ezproxy.library.ualberta.ca/science/article/pii/S097368831500081X. doi: https://doi-org.login.ezproxy.library.ualberta.ca/10.1016/j.jceh.2015.06.002.

47. Mokdad AA, Lopez AD, Shahraz S, et al. Liver cirrhosis mortality in 187 countries between 1980 and 2010: A systematic analysis. *BMC medicine*. 2014;12(1):145. https://search.datacite.org/works/10.1186/s12916-014-0145-y. doi: 10.1186/s12916-014-0145-y.

48. Kadayifci A, Tan V, Ursell PC, Merriman RB, Bass NM. Clinical and pathologic risk factors for atherosclerosis in cirrhosis: A comparison between NASH-related cirrhosis and cirrhosis due to other aetiologies. *Journal of hepatology*. 2008;49(4):595-599. https://search.datacite.org/works/10.1016/j.jhep.2008.05.024. doi: 10.1016/j.jhep.2008.05.024.

49. van den Berg, Eline H, Douwes RM, de Meijer VE, Schreuder TCMA, Blokzijl H. Liver transplantation for NASH cirrhosis is not performed at the expense of major post-operative morbidity. *Digestive and liver disease*. 2018;50(1):68-75. http://dx.doi.org/10.1016/j.dld.2017.08.022. doi: 10.1016/j.dld.2017.08.022.

50. Mariana Lazo, Ruben Hernaez , Susanne Bonekamp , Ihab R Kamel, Frederick L Brancati, Eliseo Guallar , Jeanne M Clark. Non-alcoholic fatty liver disease and mortality among US adults: Prospective cohort study. . 2011. https://doi-org.login.ezproxy.library.ualberta.ca/10.1136/bmj.d6891.

51. Porepa L, Ray JG, Sanchez-Romeu P, Booth GL. Newly diagnosed diabetes mellitus as a risk factor for serious liver disease. *Canadian Medical Association journal (CMAJ)*. 2010;182(11):E526-E531. https://search.datacite.org/works/10.1503/cmaj.092144. doi: 10.1503/cmaj.092144.

52. Nderitu P, Bosco C, Garmo H, et al. The association between individual metabolic syndrome components, primary liver cancer and cirrhosis. *International Journal of Cancer*. 2017;141(6):1148. http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-333059.

53. Frith J, Day CP, Henderson E, Burt AD, Newton JL. Non-alcoholic fatty liver disease in older people. *Gerontology (Basel)*. 2009;55(6):607-613. https://search.datacite.org/works/10.1159/000235677. doi: 10.1159/000235677.

54. Vernon G, Baranova A, Younossi ZM. Systematic review: The epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. *Aliment Pharmacol Ther*. 2011;34(3):274-285. https://doi-org.login.ezproxy.library.ualberta.ca/10.1111/j.1365-2036.2011.04724.x. doi: 10.1111/j.1365-2036.2011.04724.x.

55. Ekstedt M, Hagström H, Nasr P, et al. Fibrosis stage is the strongest predictor for disease-specific mortality in NAFLD after up to 33 years of follow-up. *Hepatology*. 2015;61(5):1547-1554. https://doi.org/10.1002/hep.27368. doi: 10.1002/hep.27368.

56. Adams LA, Lymp JF, St. Sauver J, et al. The natural history of nonalcoholic fatty liver disease: A population-based cohort study. *Gastroenterology (New York, N.Y. 1943)*. 2005;129(1):113-121. https://search.datacite.org/works/10.1053/j.gastro.2005.04.014. doi: 10.1053/j.gastro.2005.04.014.

57. Hwang Y, Ahn H, Park S, Park C. Nonalcoholic fatty liver disease associates with increased overall mortality and death from cancer, cardiovascular disease, and liver disease in women but not men. *Clinical gastroenterology and hepatology*. 2018;16(7):1131-1137.e5. http://dx.doi.org/10.1016/j.cgh.2017.11.026. doi: 10.1016/j.cgh.2017.11.026.

58. Chinnadurai R, Ritchie J, Green D, Kalra PA. Non-alcoholic fatty liver disease and clinical outcomes in chronic kidney disease. *Nephrology, dialysis, transplantation*. 2019;34(3):449-457. https://search.datacite.org/works/10.1093/ndt/gfx381. doi: 10.1093/ndt/gfx381.

59. Le MH, Devaki P, Ha NB, et al. Prevalence of non-alcoholic fatty liver disease and risk factors for advanced fibrosis and mortality in the united states. *PloS one*. 2017;12(3):e0173499. https://search.datacite.org/works/10.1371/journal.pone.0173499. doi: 10.1371/journal.pone.0173499.

60. Rose JB. *Diseases of the biliary tract.* Vol 99, number 2 (April 2019). Philadelphia, Pennsylvania: Elsevier; 2019. http://digitale-objekte.hbz-nrw.de/storage2/2019/05/05/file_36/8459278.pdf.

61. Liu Y, Zhong G, Tan H, Hao F, Hu J. Nonalcoholic fatty liver disease and mortality from all causes, cardiovascular disease, and cancer: A meta-analysis. *Scientific reports*. 2019;9(1):11124-10. https://www.ncbi.nlm.nih.gov/pubmed/31366982. doi: 10.1038/s41598-019-47687-3.

62. Targher G, Byrne CD, Lonardo A, Zoppini G, Barbui C. Non-alcoholic fatty liver disease and risk of incident cardiovascular disease: A meta-analysis. *J Hepatol*. 2016;65(3):589-600. http://www.sciencedirect.com.login.ezproxy.library.ualberta.ca/science/article/pii/S0168827816301994. doi: https://doi-org.login.ezproxy.library.ualberta.ca/10.1016/j.jhep.2016.05.013.

63. Unalp-Arida A, Ruhl CE. Noninvasive fatty liver markers predict liver disease mortality in the U.S. population. *Hepatology*. 2016;63(4):1170-1183. https://doi-org.login.ezproxy.library.ualberta.ca/10.1002/hep.28390. doi: 10.1002/hep.28390.

64. Wild SH, Walker JJ, Morling JR, et al. Cardiovascular disease, cancer, and mortality among people with type 2 diabetes and alcoholic or nonalcoholic fatty liver disease hospital admission. *Diabetes care*. 2018;41(2):341-347. https://search.datacite.org/works/10.2337/dc17-1590. doi: 10.2337/dc17-1590.

65. Ma J, Hwang S, Pedley A, et al. Bi-directional analysis between fatty liver and cardiovascular disease risk factors. *Journal of hepatology*. 2017;66(2):390-397. https://search.datacite.org/works/10.1016/j.jhep.2016.09.022. doi: 10.1016/j.jhep.2016.09.022.

66. Lonardo A, Ballestri S, Marchesini G, Angulo P, Loria P. Nonalcoholic fatty liver disease: A precursor of the metabolic syndrome. *Digestive and liver disease*. 2015;47(3):181-190. https://search.datacite.org/works/10.1016/j.dld.2014.09.020. doi: 10.1016/j.dld.2014.09.020.

67. Stefano Ballestri Amedeo Lonardo Stefano Bonapace Christopher D Byrne Paola Loria Giovanni Targher. Risk of cardiovascular,cardiac and arrhythmic complications in patients with non-alcoholic fatty liver disease. *World journal of gastroenterology : WJG*. 2014;20(7):1724-1745. http://lib.cqvip.com/qk/84123X/201407/90888889504849524855484948.html. doi: 10.3748/wjg.v20.i7.1724.

68. Alkhouri N, Alkhouri N, Tamimi T, et al. The inflamed liver and atherosclerosis: A link between histologic severity of nonalcoholic fatty liver disease and increased cardiovascular risk. *Dig Dis Sci*. 2010;55(9):2644-2650. https://www.ncbi.nlm.nih.gov/pubmed/19960252. doi: 10.1007/s10620-009-1075-y.

69. Loria P, Marchesini G, Nascimbeni F, et al. Cardiovascular risk, lipidemic phenotype and steatosis. A comparative analysis of cirrhotic and non-cirrhotic liver disease due to varying etiology. *Atherosclerosis*. 2014;232(1):99-109. https://search.datacite.org/works/10.1016/j.atherosclerosis.2013.10.030. doi: 10.1016/j.atherosclerosis.2013.10.030.

70. Mellinger JL, Pencina KM, Massaro JM, et al. Hepatic steatosis and cardiovascular disease outcomes: An analysis of the framingham heart study. *Journal of Hepatology*. 2015;63(2):470-476. https://www.clinicalkey.es/playcontent/1-s2.0-S0168827815001671. doi: 10.1016/j.jhep.2015.02.045.

71. Targher G, Bertolini L, Rodella S, et al. Nonalcoholic fatty liver disease is independently associated with an increased incidence of cardiovascular events in type 2 diabetic patients. *Diabetes care*. 2007;30(8):2119-2121. https://search.datacite.org/works/10.2337/dc07-0349. doi: 10.2337/dc07-0349.

72. Hu J, Xu Y, He Z, et al. Increased risk of cerebrovascular accident related to non-alcoholic fatty liver disease: A meta-analysis. *Oncotarget*. 2018;9(2):2752-2760. https://www.ncbi.nlm.nih.gov/pubmed/29416808. doi: 10.18632/oncotarget.22755.

73. Kim SU, Song D, Heo JH, et al. Liver fibrosis assessed with transient elastography is an independent risk factor for ischemic stroke. *Atherosclerosis*. 2017;260:156-162. https://www.clinicalkey.es/playcontent/1-s2.0-S0021915017300576. doi: 10.1016/j.atherosclerosis.2017.02.005.

74. Glen J, Floros L, Day C, Pryke R. Non-alcoholic fatty liver disease (NAFLD): Summary of NICE guidance. *BMJ*. 2016;354:i4428. http://www.bmj.com/content/354/bmj.i4428.abstract. doi: 10.1136/bmj.i4428.

75. EASL–EASD–EASO clinical practice guidelines for the management of non-alcoholic fatty liver disease. *Journal of hepatology*. 2016;64(6):1388-1402. https://search.datacite.org/works/10.1016/j.jhep.2015.11.004. doi: 10.1016/j.jhep.2015.11.004.

76. Castera L. Noninvasive evaluation of nonalcoholic fatty liver disease. *Seminars in Liver Disease*. 2015;35(3):291-303. http://dx.doi.org/10.1055/s-0035-1562948. doi: 10.1055/s-0035-1562948.

77. Paolo Del Poggio Silvia Colombo. Is transient elastography a useful tool for screening liver disease. *World journal of gastroenterology : WJG*. 2009;15(12):1409-1414. http://lib.cqvip.com/qk/84123X/200912/30021673.html. doi: 10.3748/wjg.15.1409.

78. Geng X, Huang R, Lin J, Jiang N, Yang X. Transient elastography in clinical detection of liver cirrhosis: A systematic review and meta-analysis. *Saudi Journal of Gastroenterology*. 2016;22(4):294-303. http://www.saudijgastro.com/article.asp?issn=1319-

3767;year=2016;volume=22;issue=4;spage=294;epage=303;aulast=Geng;type=0. doi: 10.4103/1319-3767.187603.

79. Imajo K, Kessoku T, Honda Y, et al. Magnetic resonance imaging more accurately classifies steatosis and fibrosis in patients with nonalcoholic fatty liver disease than transient elastography. *Gastroenterology (New York, N.Y. 1943)*. 2016;150(3):626-637.e7. https://search.datacite.org/works/10.1053/j.gastro.2015.11.048. doi: 10.1053/j.gastro.2015.11.048.

80. Shiina T, Nightingale KR, Palmeri ML, et al. WFUMB guidelines and recommendations for clinical use of ultrasound elastography: Part 1: Basic principles and terminology. *Ultrasound in medicine & biology*. 2015;41(5):1126-1147. https://search.datacite.org/works/10.1016/j.ultrasmedbio.2015.03.009. doi: 10.1016/j.ultrasmedbio.2015.03.009.

81. Bamber J, Cosgrove D, Dietrich CF, et al. EFSUMB guidelines and recommendations on the clinical use of ultrasound elastography. part 1: Basic principles and technology. *Ultraschall in der Medizin - European Journal of Ultrasound*. 2013;34(2):169-184. http://dx.doi.org/10.1055/s-0033-1335205. doi: 10.1055/s-0033-1335205.

82. Cassinotto C, Boursier J, de Lédinghen V, et al. Liver stiffness in nonalcoholic fatty liver disease: A comparison of supersonic shear imaging, FibroScan, and ARFI with liver biopsy. *Hepatology (Baltimore, Md.)*. 2016;63(6):1817-1827. https://search.datacite.org/works/10.1002/hep.28394. doi: 10.1002/hep.28394.

83. Boursier J, Isselin G, Fouchard-Hubert I, et al. Acoustic radiation force impulse: A new ultrasonographic technology for the widespread noninvasive diagnosis of liver fibrosis.

European journal of gastroenterology & hepatology. 2010;22(9):1074-1084. https://www.ncbi.nlm.nih.gov/pubmed/20440210. doi: 10.1097/MEG.0b013e328339e0a1.

84. Ferraioli G, Tinelli C, Dal Bello B, Zicchetti M, Filice G, Filice C. Accuracy of real-time shear wave elastography for assessing liver fibrosis in chronic hepatitis C: A pilot study. *Hepatology (Baltimore, Md.)*. 2012;56(6):2125-2133. https://search.datacite.org/works/10.1002/hep.25936. doi: 10.1002/hep.25936.

85. De Ritis F, Coltorti M, Giusti G. An enzymic test for the diagnosis of viral hepatitis: The transaminase serum activities. *Clinica chimica acta*. 2006;369(2):148-152. https://search.datacite.org/works/10.1016/j.cca.2006.05.001. doi: 10.1016/j.cca.2006.05.001.

86. Yoav Lurie Muriel Webb Ruth Cytter-Kuint Shimon Shteingart Gerardo Z Lederkremer. Non-invasive diagnosis of liver fibrosis and cirrhosis. *World journal of gastroenterology : WJG*. 2015;21(41):11567-11583. http://lib.cqvip.com/qk/84123X/201541/90888889504849535249484856.html. doi: 10.3748/wjg.v21.i41.11567.

87. McPherson S, Stewart SF, Henderson E, Burt AD, Day CP. Simple non-invasive fibrosis scoring systems can reliably exclude advanced fibrosis in patients with non-alcoholic fatty liver disease. *Gut*. 2010;59(9):1265-1269. http://dx.doi.org/10.1136/gut.2010.216077. doi: 10.1136/gut.2010.216077.

88. Wai C, Greenson JK, Fontana RJ, et al. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. http://hdl.handle.net/2027.42/34794. Updated 2003.

89. Shaheen AAM, Myers RP. Diagnostic accuracy of the aspartate aminotransferase-to-platelet ratio index for the prediction of hepatitis c–related fibrosis: A systematic review. *Hepatology*

(Baltimore, Md.). 2007;46(3):912-921. https://search.datacite.org/works/10.1002/hep.21835. doi: 10.1002/hep.21835.

90. Tapper EB, Krajewski K, Lai M, et al. Simple non-invasive biomarkers of advanced fibrosis in the evaluation of non-alcoholic fatty liver disease. *Gastroenterology report*. 2014;2(4):276-280. https://www.ncbi.nlm.nih.gov/pubmed/25002154. doi: 10.1093/gastro/gou034.

91. Angulo P, Hui JM, Marchesini G, et al. The NAFLD fibrosis score: A noninvasive system that identifies liver fibrosis in patients with NAFLD. *Hepatology*. 2007;45(4):846-854. https://doi.org/10.1002/hep.21496. doi: https://doi.org/10.1002/hep.21496.

92. Sun W, Cui H, Li N, et al. Comparison of FIB-4 index, NAFLD fibrosis score and BARD score for prediction of advanced fibrosis in adult patients with non-alcoholic fatty liver disease: A meta-analysis study. *Hepatol Res*. 2016;46(9):862-870. https://doi.org/10.1111/hepr.12647. doi: https://doi.org/10.1111/hepr.12647.

93. Jun DW, Kim SG, Park SH, et al. External validation of the non-alcoholic fatty liver disease fibrosis score for assessing advanced fibrosis in korean patients. *Journal of Gastroenterology and Hepatology*. 2017;32(5):1094-1099. https://doi.org/10.1111/jgh.13648. doi: https://doi.org/10.1111/jgh.13648.

94. Ruffillo G, Fassio E, Alvarez E, et al. Comparison of NAFLD fibrosis score and BARD score in predicting fibrosis in nonalcoholic fatty liver disease. *Journal of hepatology*. 2010;54(1):160-163. https://www.clinicalkey.es/playcontent/1-s2.0-S016882781000718X. doi: 10.1016/j.jhep.2010.06.028.

95. Shah AG, Lydecker A, Murray K, Tetri BN, Contos MJ, Sanyal AJ. Comparison of noninvasive markers of fibrosis in patients with nonalcoholic fatty liver disease. *Clinical*

gastroenterology and hepatology. 2009;7(10):1104-1112. https://search.datacite.org/works/10.1016/j.cgh.2009.05.033. doi: 10.1016/j.cgh.2009.05.033.

96. Kobayashi N, Kumada T, Toyoda H, et al. Ability of cytokeratin-18 fragments and FIB-4 index to diagnose overall and mild fibrosis nonalcoholic steatohepatitis in japanese nonalcoholic fatty liver disease patients. *Digestive diseases (Basel)*. 2017;35(6):521-530. https://www.ncbi.nlm.nih.gov/pubmed/29040984. doi: 10.1159/000480142.

97. Musso G, Cassader M, Rosina F, Gambino R. Impact of current treatments on liver disease, glucose metabolism and cardiovascular risk in non-alcoholic fatty liver disease (NAFLD): A systematic review and meta-analysis of randomised trials. *Diabetologia*. 2012;55(4):885-904. https://www.ncbi.nlm.nih.gov/pubmed/22278337. doi: 10.1007/s00125-011-2446-4.

98. Eduardo Vilar-Gomez,1,2 Yadina Martinez-Perez,1 Luis Calzadilla-Bertot,1 Ana Torres-Gonzalez,1 Bienvenido Gra-Oramas,3 Licet Gonzalez-Fabian,3 Scott L. Friedman,4 Moises Diago,5 and Manuel Romero-Gomez2. Weight loss through lifestyle modification significantly reduces features of nonalcoholic steatohepatitis. *Clinical liver disease*. 2015;5(1):24-25. https://www.gastrojournal.org/article/S0016-5085(15)00496-5/abstract. doi: 10.1002/cld.467.

99. Fontana L, Meyer TE, Klein S, Holloszy JO. Long-term calorie restriction is highly effective in reducing the risk for atherosclerosis in humans. *Proceedings of the National Academy of Sciences - PNAS*. 2004;101(17):6659-6663. https://search.datacite.org/works/10.1073/pnas.0308291101. doi: 10.1073/pnas.0308291101.

100. Kistler KD, Brunt EM, Clark JM, Diehl AM, Sallis JF, Schwimmer JB. Physical activity recommendations, exercise intensity, and histological severity of nonalcoholic fatty liver disease. *The American Journal of Gastroenterology*. 2011;106(3):460-468. http://dx.doi.org/10.1038/ajg.2010.488. doi: 10.1038/ajg.2010.488.

101. Church TS, Kuk JL, Ross R, Priest EL, Biltoff E, Blair SN. Association of cardiorespiratory fitness, body mass index, and waist circumference to nonalcoholic fatty liver disease. *Gastroenterology*. 2006;130(7):2023-2030. https://www.sciencedirect.com/science/article/pii/S0016508506005683. doi: 10.1053/j.gastro.2006.03.019.

102. St. George A, Bauman A, Johnston A, Farrell G, Chey T, George J. Independent effects of physical activity in patients with nonalcoholic fatty liver disease. *Hepatology*. 2009;50(1):68-76. https://onlinelibrary.wiley.com/doi/abs/10.1002/hep.22940. doi: 10.1002/hep.22940.

103. LI Y, LIU L, WANG B, WANG J, CHEN D. Metformin in non-alcoholic fatty liver disease: A systematic review and meta-analysis. *Biomedical Reports*. 2013;1(1):57-64. https://www.ncbi.nlm.nih.gov/pubmed/24648894. doi: 10.3892/br.2012.18.

104. Musso G, Gambino R, Cassader M, Pagano G. A meta- analysis of randomized trials for the treatment of nonalcoholic fatty liver disease. *Hepatology*. 2010;52(1):79-104. https://onlinelibrary.wiley.com/doi/abs/10.1002/hep.23623. doi: 10.1002/hep.23623.

105. Omer Z, Cetinkalp S, Akyildiz M, et al. Efficacy of insulin-sensitizing agents in nonalcoholic fatty liver disease. *European Journal of Gastroenterology & Hepatology*. 2010;22(1):18-23. https://www.ncbi.nlm.nih.gov/pubmed/19667999. doi: 10.1097/MEG.0b013e32832e2baf.

106. Belfort R, Harrison SA, Brown K, et al. A placebo-controlled trial of pioglitazone in subjects with nonalcoholic steatohepatitis. *The New England Journal of Medicine*. 2006;355(22):2297-2307. http://content.nejm.org/cgi/content/abstract/355/22/2297. doi: 10.1056/NEJMoa060326.

107. Cusi K, Orsak B, Bril F, et al. Long-term pioglitazone treatment for patients with nonalcoholic steatohepatitis and prediabetes or type 2 diabetes mellitus: A randomized trial. *Annals of internal medicine*. 2016;165(5):305-315. https://www.ncbi.nlm.nih.gov/pubmed/27322798. doi: 10.7326/M15-1774.

108. Sanyal AJ, Chalasani N, Kowdley KV, et al. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. *The New England Journal of Medicine*. 2010;362(18):1675-1685. http://dx.doi.org/10.1056/NEJMoa0907929. doi: 10.1056/NEJMoa0907929.

109. Aithal GP, Thomas JA, Kaye PV, et al. Randomized, placebo-controlled trial of pioglitazone in nondiabetic subjects with nonalcoholic steatohepatitis. *Gastroenterology*. 2008;135(4):1176-1184. https://www.clinicalkey.es/playcontent/1-s2.0-S0016508508011013. doi: 10.1053/j.gastro.2008.06.047.

110. Yau H, Rivera K, Lomonaco R, Cusi K. The future of thiazolidinedione therapy in the management of type 2 diabetes mellitus. *Curr Diab Rep*. 2013;13(3):329-341. https://www.ncbi.nlm.nih.gov/pubmed/23625197. doi: 10.1007/s11892-013-0378-8.

111. Lewis JD, Habel LA, Quesenberry CP, et al. Pioglitazone use and risk of bladder cancer and other common cancers in persons with diabetes. *JAMA*. 2015;314(3):265-277. https://www.ncbi.nlm.nih.gov/pubmed/26197187. doi: 10.1001/jama.2015.7996.

112. Armstrong MJ, PhD, Gaunt P, MSc, Aithal GP, PhD, et al. Liraglutide safety and efficacy in patients with non-alcoholic steatohepatitis (LEAN): A multicentre, double-blind, randomised, placebo-controlled phase 2 study. *Lancet, The*. 2016;387(10019):679-690. https://www.clinicalkey.es/playcontent/1-s2.0-S014067361500803X. doi: 10.1016/S0140-6736(15)00803-X.

113. Murat Kiyici, Macit Gulten, Selim Gurel, et al. Ursodeoxycholic acid and atorvastatin in the treatment of nonalcoholic steatohepatitis. *Canadian journal of gastroenterology = Journal canadien de gastroenterologie*. 2003;17(12):713-718. http://dx.doi.org/10.1155/2003/857869. doi: 10.1155/2003/857869.

114. Blaha MJ, Martin SS. How do statins work?: Changing paradigms with implications for statin allocation. *Journal of the American College of Cardiology*. 2013;62(25):2392-2394. http://dx.doi.org/10.1016/j.jacc.2013.08.1626. doi: 10.1016/j.jacc.2013.08.1626.

115. Taylor F, Huffman MD, Macedo AF, et al. Statins for the primary prevention of cardiovascular disease. *Cochrane Database of Systematic Reviews*. 2013(1). https://doi.org//10.1002/14651858.CD004816.pub5. doi: 10.1002/14651858.CD004816.pub5.

116. Sirtori CR. The pharmacology of statins. *Pharmacological Research*. 2014;88:3-11. http://www.sciencedirect.com.login.ezproxy.library.ualberta.ca/science/article/pii/S1043661814000255. doi: https://doi-org.login.ezproxy.library.ualberta.ca/10.1016/j.phrs.2014.03.002.

117. Pais R, Giral P, Khan J, et al. Fatty liver is an independent predictor of early carotid atherosclerosis. *Journal of hepatology*. 2016;65(1):95-102. https://search.datacite.org/works/10.1016/j.jhep.2016.02.023. doi: 10.1016/j.jhep.2016.02.023.

118. Arnett DK, Blumenthal RS, Albert MA, et al. 2019 ACC/AHA guideline on the primary prevention of cardiovascular disease. *Journal of the American College of Cardiology*. 2019;74(10):e177-e232. https://search.datacite.org/works/10.1016/j.jacc.2019.03.010. doi: 10.1016/j.jacc.2019.03.010.

119. BRUEHL W. Medical evaluation of patients with chronic liver disease. *Munchener medizinische Wochenschrift (1950)*. 1964;106:885. https://www.ncbi.nlm.nih.gov/pubmed/14233339.

120. Blais P, Lin M, Kramer JR, El-Serag HB, Kanwal F. Statins are underutilized in patients with nonalcoholic fatty liver disease and dyslipidemia. *Dig Dis Sci*. 2015;61(6):1714-1720. https://search.datacite.org/works/10.1007/s10620-015-4000-6. doi: 10.1007/s10620-015-4000-6.

121. Rzouq FS, Volk ML, Hatoum HH, Talluri SK, Mummadi RR, Sood GK. Hepatotoxicity fears contribute to underutilization of statin medications by primary care physicians. *The American journal of the medical sciences*. 2010;340(2):89-93. https://search.datacite.org/works/10.1097/maj.0b013e3181e15da8. doi: 10.1097/maj.0b013e3181e15da8.

122. Guyton JR, Bays HE, Grundy SM, Jacobson TA. An assessment by the statin intolerance panel: 2014 update. *Journal of Clinical Lipidology*. 2014;8(3, Supplement):S72-S81. http://www.sciencedirect.com.login.ezproxy.library.ualberta.ca/science/article/pii/S1933287414001585. doi: https://doi-org.login.ezproxy.library.ualberta.ca/10.1016/j.jacl.2014.03.002.

123. Be J, Yna Wójcicka G, Jamroz-Wi A. Adverse effects of statins -mechanisms and consequences. *Current Drug Safety*. 2009;4:209.

124. Björnsson ES. Hepatotoxicity of statins and other lipid-lowering agents. *Liver international*. 2017;37(2):173-178. https://search.datacite.org/works/10.1111/liv.13308. doi: 10.1111/liv.13308.

125. Chalasani N. Statins and hepatotoxicity: Focus on patients with fatty liver. *Hepatology*. 2005;41(4):690-695. https://doi.org/10.1002/hep.20671. doi: https://doi.org/10.1002/hep.20671.

126. Russo MW, Hoofnagle JH, Gu J, et al. Spectrum of statin hepatotoxicity: Experience of the drug-induced liver injury network. *Hepatology*. 2014;60(2):679-686. https://doi.org/10.1002/hep.27157. doi: https://doi.org/10.1002/hep.27157.

127. de Denus S, Spinler SA, Miller K, Peterson AM. Statins and liver toxicity: A meta-analysis. *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy*. 2004;24(5):584-591. https://doi-org.login.ezproxy.library.ualberta.ca/10.1592/phco.24.6.584.34738. doi: https://doi-org.login.ezproxy.library.ualberta.ca/10.1592/phco.24.6.584.34738.

128. CHANG CY, SCHIANO TD. Review article: Drug hepatotoxicity. *Alimentary Pharmacology & Therapeutics*. 2007;25(10):1135-1151. http://www.ingentaconnect.com/content/bsc/apt/2007/00000025/00000010/art00001. doi: 10.1111/j.1365-2036.2007.03307.x.

129. Drug-Induced Liver Injury:. Guidance for industry. .

130. Russo MW, Hoofnagle JH, Gu J, et al. Spectrum of statin hepatotoxicity: Experience of the drug-induced liver injury network. *Hepatology (Baltimore, Md.)*. 2014;60(2):679-686. https://search.datacite.org/works/10.1002/hep.27157. doi: 10.1002/hep.27157.

131. Perdices EV, Medina-Cáliz I, Hernando S, et al. Hepatotoxicity associated with statin use: Analysis of the cases included in the spanish hepatotoxicity registry. *Revista española de enfermedades digestivas*. 2014;106(4):246-254. https://www.ncbi.nlm.nih.gov/pubmed/25075655.

132. Björnsson ES, Bergmann OM, Björnsson HK, Kvaran RB, Olafsson S. Incidence, presentation, and outcomes in patients with drug-induced liver injury in the general population of iceland. *Gastroenterology (New York, N.Y. 1943)*. 2013;144(7):1419-1425.e3. https://search.datacite.org/works/10.1053/j.gastro.2013.02.006. doi: 10.1053/j.gastro.2013.02.006.

133. Björnsson E, Jacobsen EI, Kalaitzakis E. Hepatotoxicity associated with statins: Reports of idiosyncratic liver injury post-marketing. *Journal of Hepatology*. 2011;56(2):374-380.

https://www.clinicalkey.es/playcontent/1-s2.0-S0168827811006581. doi: 10.1016/j.jhep.2011.07.023.

134. Hoofnagle JH. Drug-induced liver injury network (DILIN). *Hepatology (Baltimore, Md.)*. 2004;40(4):773. https://www.ncbi.nlm.nih.gov/pubmed/15382161. doi: 10.1002/hep.20445.

135. Kumar S, Grace N, Qamar A. Statin use in patients with cirrhosis: A retrospective cohort study. *Dig Dis Sci*. 2014;59(8):1958-1965. https://www.ncbi.nlm.nih.gov/pubmed/24838495. doi: 10.1007/s10620-014-3179-2.

136. Abraldes JG, Albillos A, Bañares R, et al. Simvastatin lowers portal pressure in patients with cirrhosis and portal hypertension: A randomized controlled trial. *Gastroenterology*. 2009;136(5):1651-1658. https://www.clinicalkey.es/playcontent/1-s2.0-S0016508509001449. doi: 10.1053/j.gastro.2009.01.043.

137. Jacobson, Terry A., MD, FNLA. NLA task force on statin safety - 2014 update. *Journal of Clinical Lipidology*. 2014;8(3):S1-S4. https://www.clinicalkey.es/playcontent/1-s2.0-S1933287414001597. doi: 10.1016/j.jacl.2014.03.003.

138. Pose E, Napoleone L, Amin A, et al. Safety of two different doses of simvastatin plus rifaximin in decompensated cirrhosis (LIVERHOPE-SAFETY): A randomised, double-blind, placebo-controlled, phase 2 trial. *The Lancet Gastroenterology & Hepatology*. 2020;5(1):31-41. https://search.proquest.com/docview/2305474879. doi: 10.1016/S2468-1253(19)30320-6.

139. Nelson A, Torres D, Morgan A, Fincke C, Harrison S. A pilot study using simvastatin in the treatment of nonalcoholic steatohepatitis: A randomized placebo-controlled trial. *Journal of Clinical Gastroenterology*. 2009;43(10):990-994. http://ovidsp.ovid.com/ovidweb.cgi?T=JS&NEWS=n&CSC=Y&PAGE=fulltext&D=ovft&AN=00004836-200911000-00019. doi: 10.1097/MCG.0b013e31819c392e.

140. Rallidis LS, Drakoulis CK, Parasi AS. Pravastatin in patients with nonalcoholic steatohepatitis: Results of a pilot study. *Atherosclerosis*. 2004;174(1):193-196. https://www.sciencedirect.com/science/article/pii/S0021915004000644. doi: 10.1016/j.atherosclerosis.2004.01.008.

141. Athyros VG, Mikhailidis DP, Didangelos TP, et al. Effect of multifactorial treatment on non-alcoholic fatty liver disease in metabolic syndrome: A randomised study. *Current Medical Research and Opinion*. 2006;22(5):873-883. http://www.tandfonline.com/doi/abs/10.1185/030079906X104696. doi: 10.1185/030079906X104696.

142. Foster T, Budoff MJ, Saab S, Ahmadi N, Gordon C, Guerci AD. Atorvastatin and antioxidants for the treatment of nonalcoholic fatty liver disease: The st francis heart study randomized clinical trial. *The American Journal of Gastroenterology*. 2011;106(1):71-77. http://dx.doi.org/10.1038/ajg.2010.299. doi: 10.1038/ajg.2010.299.

143. Vasilios G Athyros, Konstantinos Tziomalos, Thomas D Gossios, Theodora Griva, Panagiotis Anagnostis, Konstantinos Kargiotis, Efstathios D Pagourelias, Eleni Theocharidou, Asterios Karagiannis, Dimitri P Mikhailidis,. The efficacy and safety of statins for the treatment of non-alcoholic fatty liver disease. *Digestive and Liver Disease*. 2014;47(1):4-11. https://www.clinicalkey.es/playcontent/1-s2.0-S1590865814005970. doi: 10.1016/j.dld.2014.07.170.

144. Athyros VG, Katsiki N, Karagiannis A, Mikhailidis DP. Are statins 'IDEAL' for non-alcoholic fatty liver disease? *Current Medical Research & Opinion*. 2014;30(2):229-231. http://www.tandfonline.com/doi/abs/10.1185/03007995.2013.855192. doi: 10.1185/03007995.2013.855192.

145. Tikkanen MJ, Fayyad R, Faergeman O, et al. Effect of intensive lipid lowering with atorvastatin on cardiovascular outcomes in coronary heart disease patients with mild-to-moderate baseline elevations in alanine aminotransferase levels. *International Journal of Cardiology*. 2013;168(4):3846-3852. https://www.clinicalkey.es/playcontent/1-s2.0-S0167527313010784. doi: 10.1016/j.ijcard.2013.06.024.

146. Athyros VG, Ganotakis E, Kolovou GD, et al. Assessing the treatment effect in metabolic syndrome without perceptible diabetes (ATTEMPT): A prospective-randomized study in middle aged men and women. *Current vascular pharmacology*. 2011;9(6):647. https://www.ncbi.nlm.nih.gov/pubmed/21476961.

147. Athyros, Vasilios G, MD|Tziomalos, Konstantinos, MD|Gossios, Thomas D, MD|Griva, Theodora, MD|Anagnostis, Panagiotis, MD|Kargiotis, Konstantinos, MD|Pagourelias, Efstathios D, MD|Theocharidou, Eleni, MD|Karagiannis, Asterios, Prof|Mikhailidis, Dimitri P, Dr. Safety and efficacy of long-term statin treatment for cardiovascular events in patients with coronary heart disease and abnormal liver tests in the greek atorvastatin and coronary heart disease evaluation (GREACE) study: A post-hoc analysis. *Lancet, The*. 2010;376(9756):1916-1922. https://www.clinicalkey.es/playcontent/1-s2.0-S014067361061272X. doi: 10.1016/S0140-6736(10)61272-X.

148. Hyogo H, Tazuma S, Arihiro K, et al. Efficacy of atorvastatin for the treatment of nonalcoholic steatohepatitis with dyslipidemia. *Metabolism*. 2008;57(12):1711-1718. https://www.clinicalkey.es/playcontent/1-s2.0-S0026049508002874. doi: 10.1016/j.metabol.2008.07.030.

149. Konstantinos Kargiotis, Niki Katsiki, Vasilios G. Athyros, Olga Giouleme, Kalliopi Patsiaoura, Evangelia Katsiki, Dimitri P. Mikhailidis, Asterios Karagiannis. Effect of

rosuvastatin on non-alcoholic steatohepatitis in patients with metabolic syndrome and hypercholesterolaemia: A preliminary report. . ;12.

150. Loomba R, Sanyal AJ. The global NAFLD epidemic. *Nature reviews. Gastroenterology & hepatology*. 2013;10(11):686-690. https://search.datacite.org/works/10.1038/nrgastro.2013.171. doi: 10.1038/nrgastro.2013.171.

151. National institute on alcohol abuse and alcoholism. drinking levels defined. https://www.niaaa.nih.gov/alcohol-health/overview-alcohol-consumption/moderate-binge-drinking Web site. https://www.niaaa.nih.gov/alcohol-health/overview-alcohol-consumption/moderate-binge-drinking.

152. Kwo PY, Cohen SM, Lim JK. ACG clinical guideline: Evaluation of abnormal liver chemistries. *The American journal of gastroenterology*. 2017;112(1):18-35. https://search.datacite.org/works/10.1038/ajg.2016.517. doi: 10.1038/ajg.2016.517.

153. Caballería L, Pera G, Arteaga I, et al. High prevalence of liver fibrosis among european adults with unknown liver disease: A population-based study. *Clinical gastroenterology and hepatology*. 2018;16(7):1138-1145.e5. https://search.datacite.org/works/10.1016/j.cgh.2017.12.048. doi: 10.1016/j.cgh.2017.12.048.

154. Crespo G, Fernández-Varo G, Mariño Z, et al. ARFI, FibroScan®, ELF, and their combinations in the assessment of liver fibrosis: A prospective study. *Journal of hepatology*. 2012;57(2):281-287. https://search.datacite.org/works/10.1016/j.jhep.2012.03.016. doi: 10.1016/j.jhep.2012.03.016.

155. de Ledinghen V. Fibrosis assessment in patients with NAFLD. In: *Liver elastography*. Cham: Springer International Publishing; 2020:123-139. http://link.springer.com/10.1007/978-3-030-40542-7_10. 10.1007/978-3-030-40542-7_10.

156. Fienberg SE. Springer series in statistics. *Springer Series in Statistics*.

157. Frank E Harrell Jr, with contributions from Charles Dupont and many others. *Hmisc: Harrell miscellaneous.* ; 2020. https://CRAN.R-project.org/package=Hmisc.

158. Drawz PE, Agarwal A, Dwyer JP, et al. Concordance between blood pressure in the systolic blood pressure intervention trial and in routine clinical practice. *JAMA internal medicine*. 2020. http://dx.doi.org/10.1001/jamainternmed.2020.5028. doi: 10.1001/jamainternmed.2020.5028.

159. Frank E Harrell Jr. *Rms: Regression modeling strategies.* ; 2019. https://CRAN.R-project.org/package=rms.

160. H. Wickham. *Ggplot2: Elegant graphics for data analysis.* Springer-Verlag New York; 2016. https://ggplot2.tidyverse.org.

161. Davyduke T, Tandon P, Al- Karaghouli M, Abraldes JG, Ma MM. Impact of implementing a "FIB- 4 first" strategy on a pathway for patients with NAFLD referred from primary care. *Hepatology communications*. 2019;3(10):1322-1333. https://search.datacite.org/works/10.1002/hep4.1411. doi: 10.1002/hep4.1411.

162. Davyduke T, Tandon P, Al- Karaghouli M, Abraldes JG, Ma MM. Impact of implementing a "FIB- 4 first" strategy on a pathway for patients with NAFLD referred from primary care. *Hepatology communications*. 2019;3(10):1322-1333. https://search.datacite.org/works/10.1002/hep4.1411. doi: 10.1002/hep4.1411.

163. Del Ben M, Baratta F, Polimeni L, et al. Under-prescription of statins in patients with non-alcoholic fatty liver disease. *Nutrition, metabolism, and cardiovascular diseases*. 2017;27(2):161-167. https://search.datacite.org/works/10.1016/j.numecd.2016.09.011. doi: 10.1016/j.numecd.2016.09.011.

164. Khoo S, Wong VW, Goh GB, et al. Suboptimal treatment of dyslipidemia in patients with nonalcoholic fatty liver disease. *Journal of gastroenterology and hepatology*. 2019;35(2):320-325. https://search.datacite.org/works/10.1111/jgh.14794. doi: 10.1111/jgh.14794.

165. Barrios V, Escobar C, Calderón A, Echarri R, González-Pedel V, Ruilope LM. Cardiovascular risk profile and risk stratification of the hypertensive population attended by general practitioners and specialists in spain. the CONTROLRISK study. *Journal of Human Hypertension*. 2007;21(6):479-485. http://dx.doi.org/10.1038/sj.jhh.1002167. doi: 10.1038/sj.jhh.1002167.

166. Mach F, Baigent C, Catapano AL, et al. 2019 ESC/EAS guidelines for the management of dyslipidaemias: Lipid modification to reduce cardiovascular risk. *European heart journal*. 2020;41(1):111-188. https://search.datacite.org/works/10.1093/eurheartj/ehz455. doi: 10.1093/eurheartj/ehz455.

167. Arnett D, Blumenthal R, Albert M, et al. 2019 ACC/AHA guideline on the primary prevention of cardiovascular disease: A report of the american college of cardiology/american heart association task force on clinical practice guidelines. *Circulation*. 2019;140(11):e596-e646. https://www.ncbi.nlm.nih.gov/pubmed/30879355. doi: 10.1161/CIR.0000000000000678.

168. Lewis JH, Mortensen ME, Zweig S, Fusco MJ, Medoff JR, Belder R. Efficacy and safety of high- dose pravastatin in hypercholesterolemic patients with well- compensated chronic liver disease: Results of a prospective, randomized, double- blind, placebo- controlled, multicenter trial. Hepatology. 2007;46(5):1453-1463. https://onlinelibrary.wiley.com/doi/abs/10.1002/hep.21848. doi: 10.1002/hep.21848.

169. Han KH, MD, Rha SW, MD, Kang H, MD, et al. Evaluation of short-term safety and efficacy of HMG-CoA reductase inhibitors in hypercholesterolemic patients with elevated serum alanine

transaminase concentrations: PITCH study (PITavastatin versus atorvastatin to evaluate the effect on patients with hypercholesterolemia and mild to moderate hepatic damage). *Journal of Clinical Lipidology*. 2012;6(4):340-351. https://www.clinicalkey.es/playcontent/1-s2.0-S1933287412000232. doi: 10.1016/j.jacl.2012.01.009.

170. GOMEZ-DOMINGUEZ E, GISBERT JP, MORENO-MONTEAGUDO JA, GARCIA-BUEY L, MORENO-OTERO R. A pilot study of atorvastatin treatment in dyslipemid, non-alcoholic fatty liver patients. *Alimentary Pharmacology & Therapeutics*. 2006;23(11):1643-1647. http://www.ingentaconnect.com/content/bsc/apt/2006/00000023/00000011/art00018. doi: 10.1111/j.1365-2036.2006.02926.x.

Appendix A

Examining the association between VCTE and FIB-4, adjusted for age, BMI, statin, and presence of prediabetes/diabetes.

To assess the association between FIB-4, BMI, statin, and diabetic status (prediabetes/ diabetes), we conducted multiple linear regression analysis. Since Age in FIB-4 is introduced as a multiplicative term, and its weight has been shown to be overestimated, we allowed interaction between FIB-4 and Age to correct for this fact. We used restricted cubic splines to model non-linear terms.

BMI and diabetes, but not prediabetes, significantly impacted the association between VCTE and FIB. Statin effect was not significant (p-value 0.339).

The figure shows a graphical representation of the equation model.

Significance testing of the terms in the model

	P
FIB-4	<.001
Statin	0.339
Prediabetes	0.416
Diabetes	<0.001
BMI	<0.001
FIB-4 * Age	<0.001

Model Equation

```
VCTE = 6.1746267 + 0.10661988 * FIB-4 - 0.061988652 * Age + 0.43942359 *

Statin + 0.27940647 * Prediabetes + 1.7883265 * Diabetes 0.071072554 * BMI +

0.00043274812 * pmax(BMI - 25.264, 0)^3 - 0.00076237092 * pmax(BMI - 31.19,

0)^3 + 0.0003296228 * pmax(BMI - 38.97, 0)^3 + 0.084670346 * FIB-4 * Age



VCTE: vibration controlled transient elastography
BMI: Body mass index
Note: pmax is a function of R base that returns the maximum value of the two
terms separated by the comma. For example, pmax(3,0) would return a 3,
whereas pmax(-1,0) would return a 0.
```

Appendix B

Logistic regression model assessing the association between FIB-4 and Probability of advanced fibrosis as assessed by VCTE on four clinically relevant thresholds.

Effects of statins on FIB-4 based prediction of advanced fibrosis at different VCTE thresholds.

	Coef	P
VCTE 8	0.6989	0.007
VCTE 10	0.4812	0.120
VCTE 12	0.8080	0.027
VCTE 16	0.9446	0.056

Logistic regression model of the association between FIB-4 and Probability of advanced fibrosis as assessed by VCTE on four clinically relevant thresholds. (Adjusted for AGE, BMI, Diabetic status)

Adjusted effect of statins on FIB-4 based prediction of advanced fibrosis VCTE 8 kPa.

Significance testing and coefficients of the terms in the model (VCTE 8)

	Coef	P
FIB-4	1.2672	<.001
Statin	0.5658	0.054
Prediabetes	0.4043	0.185
Diabetes	1.1335	<0.001
BMI	0.0943	<0.001
Age	-0.0080	0.0757
FIB-4 * Age	-0.0097	0.4637

Adjusted effect of statins on FIB-4 based prediction of advanced fibrosis VCTE 10 kPa.

Significance testing and coefficients of the terms in the model (VCTE 10)

	Coef	P
FIB-4	1.2269	0.078
Statin	0.1082	0.755
Prediabetes	0.0328	0.931
Diabetes	1.1005	0.003
BMI	0.1185	<0.001
Age	0.0221	0.282
FIB-4 * Age	-0.0104	0.413

Adjusted effect of statins on FIB-4 based prediction of advanced fibrosis VCTE 12 kPa.

Significance testing and coefficients of the terms in the model (VCTE 12)

	Coef	P
FIB-4	2.1928	0.010
Statin	0.4389	0.280
Prediabetes	0.3588	0.497
Diabetes	1.1972	0.021
BMI	0.1080	<0.001
Age	0.0467	0.089
FIB-4 * Age	- 0.0258	0.087

Adjusted effect of statins on FIB-4 based prediction of advanced fibrosis VCTE 16 kPa.

Significance testing and coefficients of the terms in the model (VCTE 16)

	Coef	P
FIB-4	2.4577	0.004
Statin	0.5723	0.312
Prediabetes	0.9123	0.368
Diabetes	1.9133	0.049
BMI	0.1802	<0.001
Age	0.0760	0.051
FIB-4 * Age	- 0.0311	0.029

Effects of statins on the association between Hepamet and Probability of advanced fibrosis as assessed by VCTE at four clinically relevant VCTE thresholds.

Unadjusted effects of statins on Hepamet based prediction of advanced fibrosis at different VCTE thresholds.

	Coef	P
VCTE 8	0.6848	0.010
VCTE 10	0.5175	0.100
VCTE 12	0.8543	0.022
VCTE 16	0.8726	0.087

Effects of statins on the association between NFS and Probability of advanced fibrosis as assessed by VCTE at four clinically relevant VCTE thresholds.

Unadjusted effects of statins on NFS based prediction of advanced fibrosis at different VCTE thresholds.

	Coef	P
VCTE 8	0.3846	0.153
VCTE 10	0.0542	0.866
VCTE 12	0.3265	0.390
VCTE 16	0.3078	0.556