การศึกษารูปแบบสูตรการคำนวณคะแนนเพื่อประเมินความรุนแรงของการอักเสบของตับและพังผืดเพื่อใช้ทำนายภาวะแทรกซ้อนของโรคตับและการเสียชีวิตในผู้ป่วยโรคไขมันในตับที่ไม่ได้เกิดจากอัลกอฮอล์ร่วมกับอ้วนลงพุง

นายสมบัติ ตรีประเสริฐสุข

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
สาขาวิชาอายุรศาสตร์ ภาควิชาอายุรศาสตร์
คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2554

ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

บทคัดย่อและแฟ้มข้อมูลฉบับเต็มของวิทยานิพนธ์ตั้งแต่ปีการศึกษา 2554 ที่ให้บริการในคลังปัญญาจุฬาฯ (CUIR)
เป็นแฟ้มข้อมูลของนิสิตเจ้าของวิทยานิพนธ์ที่ส่งผ่านทางบัณฑิตวิทยาลัย
The abstract and full text of theses from the academic year 2011 in Chulalongkorn University Intellectual Repository(CUIR)
are the thesis authors' files submitted through the Graduate School.

THE NAFLD FIBROSIS SCORE: A PROGNOSTIC PREDICTOR FOR MORTALITY AND LIVER COMPLICATIONS AMONG NAFLD PATIENTS.

Mr. Sombat Treeprasertsuk

A Dissertation Submitted in Partial Fulfillment of the Requirements

for the Degree of Doctor of Philosophy Program in Medicine

Department of Medicine

Faculty of Medicine

Chulalongkorn University

Academic Year 2011

Copyright of Chulalongkorn University

Thesis Title	THE NAFLD FIBROSIS SCORE: A PROGNOSTIC PREDICTOR FOR MORTALITY AND LIVER COMPLICATIONS AMONG NAFLD PATIENTS.
By	Mr. Sombat Treeprasertsuk
Field of Study	Medicine
Thesis Advisor	Associate Professor Sompong Suwanvalaikorn, M.D.
Thesis Co-advisor	Associate Professor Varocha Mahachai, M.D. Associate Professor Pisit Tangkijvanich, M.D.

Accepted by the Faculty of Medicine, Chulalongkorn University in Partial Fulfillment of the Requirements for the Doctoral Degree

.....Dean of the Faculty of Medicine
(Associate Professor Sophon Napatorn, M.D.)

THESIS COMMITTEE

.....Chairman
(Assistant Professor Thanin Asawavichienjinda, M.D., Ph.D.)

.....Thesis Advisor
(Associate Professor Sompong Suwanvalaikorn, M.D.)

.....Thesis Co-advisor
(Associate Professor Varocha Mahachai, M.D.)

.....Thesis Co-advisor
(Associate Professor Pisit Tangkijvanich, M.D.)

.....Examiner
(Professor Prawit Asawanonth, M.D., Ph.D.)

.....External Examiner
(Professor Rungsun Tantrajit, Ph.D.)

สมบัติ ตรีประเสริฐสุข : การศึกษาการใช้รูปแบบสูตรการคำนวณคะแนนเพื่อประเมินความรุนแรงของการอักเสบของตับและพังผืดเพื่อใช้ทำนายภาวะแทรกซ้อนของโรคตับและการเสียชีวิตในผู้ป่วยโรคไขมันในตับที่ไม่ได้เกิดจากอัลกอฮอล์ร่วมกับอ้วนลงพุง (THE NAFLD FIBROSIS SCORE: A PROGNOSTIC PREDICTOR FOR MORTALITY AND LIVER COMPLICATIONS AMONG NAFLD PATIENTS.) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: รศ.นพ.สมพงษ์ สุวรรณวลัยกร, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: รศ.พญ.วโรชา มหาชัย, รศ.นพ.พิสิฐ ตั้งกิจวาณิชย์, 68 หน้า.

ที่มา การพยากรณ์โรคในระยะยาวของผู้ป่วยโรคไขมันในตับที่ไม่ได้เกิดจากอัลกอฮอล์ร่วมกับอ้วนลงพุงไม่มีข้อมูลการศึกษามาก่อน การศึกษานี้มุ่งศึกษาการใช้รูปแบบสูตรคำนวณคะแนนเพื่อประเมินความรุนแรงของการเกิดพังผืดในตับเพื่อใช้ทดสอบความแม่นยำในผู้ป่วยไทยและใช้ทำนายภาวะแทรกซ้อนของโรคตับและการเสียชีวิตในผู้ป่วยโรคไขมันในตับที่ไม่ได้เกิดจากอัลกอฮอล์ร่วมกับอ้วนลงพุง

วิธีการศึกษา ได้ทำการศึกษาเป็น 2 ระยะโดยระยะที่ 1 รวบรวมผู้ป่วยโรคไขมันในตับที่วินิจฉัยในช่วงเวลาปี พ.ศ. 2550 ถึง พ.ศ. 2553 จากโรงพยาบาลจุฬาลงกรณ์ จำนวน 115 รายเพื่อทดสอบความแม่นยำของรูปแบบสูตรคำนวณคะแนนเพื่อประเมินความรุนแรงของการเกิดพังผืดในตับ ส่วนการศึกษาในระยะที่ 2 เป็นการใช้ฐานข้อมูลที่มีอยู่เดิมของผู้ป่วยโรคไขมันในตับที่เมโยคลินิก สหรัฐอเมริกาจำนวน 302 รายจากทั้งหมด 479รายที่มีข้อมูลครบถ้วนเพื่อการติดตามในระยะยาวและคำนวณคะแนนเพื่อประเมินความรุนแรงของการเกิดพังผืดในตับได้

ผลการศึกษา ระยะที่ 1 ผู้ป่วยไทยโรคไขมันในตับจำนวน 115 รายมีอายุเฉลี่ย 50.5 $\pm$ 12.4 ปี และร้อยละ67 มีคะแนนเสี่ยงต่อการมีพังผืดในตับน้อยและมีค่าความไวและค่าความจำเพาะที่ร้อยละ 53 และ 70 ตามลำดับส่วนค่า NPV อยู่ที่ร้อยละ 91 ผลการศึกษาระยะที่ 2 กลุ่มผู้ป่วยโรคไขมันในตับที่เมโยคลินิกสหรัฐอเมริกาจำนวน 302 รายมีอายุเฉลี่ย 47.3 $\pm$12.9 ปี ติดตามการรักษาเป็นเวลานานเฉลี่ย 11.9 $\pm$ 3.9 ปี จากการคำนวณคะแนนเสี่ยงต่อการมีพังผืดในตับพบว่ามีผู้ป่วยที่อยู่ในกลุ่มที่มีคะแนนเสี่ยงต่อการมีพังผืดในตับน้อย (คะแนน <-1.5) ถึงร้อยละ 60 และมีผู้ป่วยที่อยู่ในกลุ่มที่มีคะแนนเสี่ยงต่อการมีพังผืดในตับมาก (คะแนน$\geq$-1.5) ร้อยละ 40 ที่สิ้นสุดการศึกษาพบว่ามีผู้ป่วย 55 รายหรือร้อยละ 18 มีจุดสิ้นสุดของการติดตามโดยมีผู้ป่วย 39 ราย (ร้อยละ 13) เสียชีวิต จากการวิเคราะห์ความแปรปรวนพหุคูณพบว่าผู้ป่วยที่อยู่ในกลุ่มที่มีคะแนนเสี่ยงต่อการมีพังผืดในตับมากและการพบภาวะหลอดเลือดหัวใจตีบที่เกิดขึ้นใหม่เพิ่มความเสี่ยงต่อโอกาสเสียชีวิต โดยมีค่า odd ratio เท่ากับ 2.6 and 9.2 ตามลำดับ; p<0.0001

สรุปผลการศึกษา: รูปแบบสูตรคำนวณคะแนนเพื่อประเมินความรุนแรงของการเกิดพังผืดในตับที่ทดสอบในผู้ป่วยไทยมีความแม่นยำเพียงพอและผู้ป่วยที่อยู่ในกลุ่มที่มีคะแนนเสี่ยงต่อการมีพังผืดในตับมากและการพบภาวะหลอดเลือดหัวใจตีบที่เกิดขึ้นใหม่เพิ่มความเสี่ยงต่อโอกาสเสียชีวิต

ภาควิชา อายุรศาสตร์ ลายมือชื่อนิสิต .....

สาขาวิชา อายุรศาสตร์ ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก .....

ปีการศึกษา 2554 ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม .....

ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม .....

4975251530: MAJOR MEDICINE
KEYWORDS: NAFLD FIBROSIS SCORE / PROGNOSTIC PREDICTOR / MORTALITY / LIVER COMPLICATIONS

SOMBAT TREEPRASERTSUK: THE NAFLD FIBROSIS SCORE: A PROGNOSTIC PREDICTOR FOR MORTALITY AND LIVER COMPLICATIONS AMONG NAFLD PATIENTS. ADVISOR : ASSOC. PROF. SOMPONG SUWANVALAIKORN, M.D., CO-ADVISOR : ASSOC. PROF. VAROCHA MAHACHAI, M.D., ASSOC. PROF. PISIT TANGKIJVANICH, M.D., 68 pp.

BACKGROUND: The prognostic Indicators for long-term outcomes of non alcoholic fatty liver disease (NAFLD) patients have not been well studied. We aimed to validate the NAFLD Fibrosis Score in the Thai NAFLD population and to assess whether the severity of liver fibrosis estimated by the NAFLD Fibrosis Score can predict the mortality of patients with NAFLD in long-term follow up..

METHODS: We divided our study into 2 phases; the first phase is a cross sectional study to collect 115 Thai NAFLD patients prospectively during 2007-2010 in King Chulalongkorn Memorial Hospital (KCMH) to validate the NAFLD Fibrosis Score. The second phase is a historical cohort design by using the existing data of NAFLD patients diagnosed during 1980 and 2000 drawn from the Rochester Epidemiology Project to analyze. Of 479 patients with NAFLD, 302 patients were included. We used the NAFLD Fibrosis Score for separating NAFLD patients with and without advanced liver fibrosis.

RESULTS: According to the first phase study, 115 Thai NAFLD patients with mean age of 50.5 $\pm$ 12.4 years were included. Seventy seven of the Thai NAFLD patients (67%) were in a group of low risk of advance fibrosis by using NAFLD Fibrosis Score. Advanced fibrosis was shown in 15 (13%) patients. Using the ROC curve, the NAFLD Fibrosis Score at baseline of >-1.5 was used for predicting significant liver fibrosis with a sensitivity of 53%, specificity of 70%, PPV of 21% and NPV of 91%. For phase 2 study, a total of 302 NAFLD patients (mean age 47.3 $\pm$12.9 years) were followed-up for an average of 11.9 $\pm$ 3.9 years. A low probability of advanced fibrosis (score <-1.5 at baseline) was found in 60 % while intermediate or high probability of advanced fibrosis (score $\geq$-1.5) was found in 40%. At the end of follow up, 55 patients (18%) developed primary endpoints including 39 patients (13%) who died during follow-up. In a multivariate analysis a higher NAFLD Fibrosis Score at baseline and presence of new onset of CHD were significantly predictive of death (OR = 2.6 and 9.2, respectively; p <0.0001).

CONCLUSIONS: The NAFLD Fibrosis Score has a high NPV in Thai NAFLD patients. A higher NAFLD Fibrosis Score at baseline and presence of new onset of CHD were significantly predictive of death.

Department: Medicine Student's Signature .....

Field of Study: Medicine Advisor's Signature .....

Academic Year: 2011 Co-advisor's Signature .....

Co-advisor's Signature .....

ACKNOWLEDGEMENTS

I would like to acknowledge my mentors and collaborators at Chulalongkorn University and Mayo Clinic, Rochester, MN, US; especially Prof. Pinit Kullavanijaya, Prof. Varocha Mahachai and Prof. Pisit Tangkijvanich from Faculty of Medicine, Chulalongkorn University for their support and suggestions during fellowship training in gastroenterology and hepatology in Thailand. I would like to thank Prof. Keith D Lindor, and Prof. Paul Angulo, my mentors, for their suggestion and guidance during my liver research fellow training in Mayo Clinic. I would like to acknowledge Dr. Felicity Enders from Biostatistics and Health Sciences Research; Mayo Clinic who teach and guide me in statistical knowledge. In addition, I would like to acknowledge all patients from King Chulalongkorn Memorial hospital, Bangkok, Thailand and Division of Gastroenterology and Hepatology, Mayo Clinic, Rochester, MN for their contribution and supporting medical research. Finally, I would like to acknowledge my family (Dr. Weeranuj, Sorawit and Cholatarn Treeprasertsuk) who always supported me. The project was made possible by Grant from Faculty of Medicine, Chulalongkorn University and I hope the new knowledge from our study will be applied for better care of patients. Its contents are solely the responsibility of the authors and do not represent the official view of the university.

CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

Page

LIST OF ABBREVIATIONS

ALP	Alkaline phosphatase;
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BMI	Body mass index
CAD	Coronary artery disease
CHD	Coronary heart disease
CI	Confidence interval
CRP	C-reactive protein
DM	Diabetes mellitus
FPG	Fasting plasma glucose
FRS	Framingham risk score
HCC	Hepatocellular carcinoma
HDL-C	High density lipoprotein cholesterol
HR	Hazard ratio
HT	Hypertension
IFG	Impaired fasting glucose
IR	Insulin resistance
LDL-C	Low density lipoprotein cholesterol
MetS	Metabolic syndrome
NAFLD	Nonalcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis
NCEP-ATPIII	National Cholesterol Education Program Adult Treatment Panel III
OR	Odd ratio
ROC	Receiver operating characteristics curve
VAT	Visceral adipose tissue
WC	Waist circumference

CHAPTER I
INTRODUCTION

1.1 Background and rationale

Nonalcoholic fatty liver disease (NAFLD) is one of the most common causes of chronic liver disease in Western countries (1). The prevalence of NAFLD is increasing and varies significantly with ethnicity; from 24% in blacks, 33% in whites, to 45 % in Hispanics (2). An increasing prevalence of NAFLD is associated with the increasing incidence of obesity and in 2004, about a third of the population aged 40 to 79 years-old was obese (3, 4). The mortality rate of NAFLD patients in the community was found to be higher than that in the general population in the United States (US) in 2000 (5). During the average 7.6 years of follow up of NAFLD patients, death occurred in 13% and coronary heart disease (CHD) was the second most common cause of death following malignancy (6). Another study also revealed that the survival outcome of patients with nonalcoholic steatohepatitis (NASH) was reduced significantly and they more often died from CHD ($p = 0.04$) and liver-related causes ($p = 0.04$) (5). Current evidence indicates that NAFLD, obesity and metabolic syndrome (MetS) have a strong association (7-9). Patients with more severity of liver fibrosis tend to have more liver complications than those without liver fibrosis.(5) Liver biopsy is a gold standard to diagnose liver fibrosis severity but it has several limitations for clinical practice including the expense and the invasive nature of the procedure which is associated with a number of complications (10). Previous reports showed that nonalcoholic steatohepatitis patients showed progression of fibrosis in about 5-32% during a 4.3 to 6 years follow up period (11-13). Recently, there were two important studies to identify the new and simple noninvasive tools by using the scoring system for liver fibrosis assessment (14, 15). Angulo P , et al developed a noninvasive and simple “NAFLD Fibrosis Score”, which is a composite score of age, hyperglycemia, body mass index, platelet count, albumin, and AST/ALT ratio.(14) These factors were found to be independent indicators of separating NAFLD patients with and without advanced fibrosis at the initial NAFLD diagnosis. Another study used the simple clinical score which composed of BMI $\geq$28 kg/m^2 (1

point), AST/ALT ratio $\geq$ 0.8 (2 point) and diabetes mellitus (1 point) and was called the BARD score. If the total score ranges from 2-4, the chance of liver fibrosis is high with OR of 17 called BARD score.(15) However, the BARD score had some limitations because it has no different predictive capacity for patients with higher BMI or higher ratio of AST/ALT while the NAFLD Fibrosis Score can apply to the different range of BMI or AST/ALT ratio. Currently, The NAFLD Fibrosis Score has not been validated in the Thai NAFLD population and has not been studied as a prognostic predictor for liver complications, cardiac complications and mortality in long-term follow up (primary end points).

Preliminary Studies

Study of the natural history of nonalcoholic fatty liver disease.

This published study enrolled 420 patients diagnosed with NAFLD in Olmsted County, Minnesota, between 1980 and 2000. The mean follow up was 7.6 years (range 0.1-23.5) culminating in 3192 person-years of follow up. The mortality rate was 12.6 % and NAFLD patients-survival was lower than the expected survival for the general population in 2000. Higher mortality was associated with age, impaired fasting glucose (IFG), and cirrhosis. Liver disease was the third leading cause of death occurring in seven of 420 NAFLD patients (1.7%). Twenty-one (5%) patients were diagnosed with cirrhosis, and 13 (3.1%) developed liver-related complications, including 1 requiring transplantation and 2 developing hepatocellular carcinoma (6). Our cohort study showed similar results for the leading causes of death in NAFLD patients as in the study published by Eksteadt, et al (5).

Study on liver histology changes during the follow up of NAFLD patients.

This published study enrolled 103 patients who underwent serial liver biopsies in the absence of effective treatment. They were reviewed, and biopsies scored in a blind fashion. The mean interval between liver biopsies was 3.2 years (range 0.7-21.3). Fibrosis stage progressed in 37%, remained stable in 34% and regressed in 29%. Aminotransferase decreased significantly between biopsies, paralleling improvement in

steatosis and inflammatory features but not fibrosis stage. The rate of fibrosis change ranged from -2.1 to 1.7 stages per year. By multivariate analysis, diabetes ($p = 0.007$) and low initial fibrosis stage ($p < 0.001$) were associated with higher rate of fibrosis progression, as was higher body mass index ($p = 0.008$). This study showed that liver fibrosis in NAFLD progresses slowly over time with considerable variability in the rate of change among patients. Changes of aminotransferase do not parallel changes in fibrosis stage. Diabetic patients with elevated BMI and low fibrosis stage are at risk for higher rates of fibrosis progression (16).

Studies related to noninvasive approaches for assessing the severity of liver fibrosis in NAFLD patients.

Liver biopsy has several limitations for clinical practice including the expense and the invasive nature of the procedure which is associated with a number of complications. Transient elastrography (FibroScan), which measures liver stiffness, based on ultrasonographic features is a noninvasive method to assess liver fibrosis. It showed a significant correlation between liver stiffness measurement and fibrosis stage in NAFLD patients but the cost of FibroScan is still expensive (17). Therefore, our research group with other colleagues constructed and validated a NAFLD fibrosis score consisting of routinely measured and readily available clinical and laboratory data to separate NAFLD patients with and without advanced fibrosis. This published study enrolled 733 patients with NAFLD confirmed by liver biopsy. We found that this scoring system with 6 variables including age, hyperglycemia, body mass index, platelet count, albumin, and AST/ALT ratio provided independent indicators of advanced liver fibrosis (14).By applying this model, a liver biopsy would have been avoided in 75%, with correct prediction in 496 (90%). By using the low cutoff score (-1.455), advanced liver fibrosis could be excluded with high accuracy (negative predictive value of 93% and 88% in the estimation and validation groups, respectively). By applying the high cutoff score (0.676), the presence of advanced liver fibrosis could be diagnosed with high accuracy (positive predictive value of 90% and 82% in the estimation and validation groups, respectively) (14). This study helps clinicians to separate NAFLD patients with

and without advanced fibrosis accurately. Moreover, the clinician can avoid unnecessary liver biopsy for identification of advanced fibrosis for three-quarters of patients (14). Recently, a study from Japan showing that at a new cutoff level of -0.876 which is modified from the original cut off level (-1.5), had the sensitivity, specificity, positive and negative predictive values for advanced liver fibrosis of 100%, 82.5%, 63.2%, and 100%, respectively (18)

1.2 Studies of the thesis

Overall Hypothesis: We aim to test the hypothesis that the NAFLD Fibrosis Score is a good scoring system to apply in Thai NAFLD patients and NAFLD Fibrosis Score is a good prognostic predictor for overall mortality, cardiac complications, and liver complications of NAFLD patients.

1.3 Objectives

Our specific aims are: We aim to evaluate the following:

Specific Aim 1: To validate the NAFLD Fibrosis Score in the Thai NAFLD population

Specific Aim 2: To assess whether severity of liver fibrosis estimated by the NAFLD fibrosis score can predict time to overall mortality, and/or cardiac complications, and/or liver complications among population of recently diagnosed NAFLD patients who have at least 5 years at follow up and adequate data for calculation of the NAFLD Fibrosis Score.

Specific Aim 3: To determine the rate of NAFLD fibrosis score change over the period of time from the baseline to the end of follow up among population of recently diagnosed NAFLD patients.

CHAPTER II
LITERATURE REVIEW

Nonalcoholic fatty liver disease (NAFLD) is one of the most common causes of chronic liver disease in Western countries. Natural history studies of NAFLD show that 1%-5% of patients with simple steatosis developed cirrhosis (19). The mortality rate of NAFLD patients in the community was higher than that in the general United States population (6). Death occurred in 12.6%-36% with mean follow up of 7.6-8.3 years (6, 20). Liver related causes were the second or third leading cause of death following malignancy and coronary heart disease (CHD) (6, 20). Another study revealed that the survival of patients with nonalcoholic steatohepatitis (NASH) was reduced significantly and they more often died from CHD ($p = 0.04$) and liver-related causes ($p = 0.04$) (5).

Previous reports showed that patients with NASH had progression of liver fibrosis in about 5%-32% during a 4 to 6 years follow-up (11-13). The liver fibrosis stage progression of patients with NAFLD who underwent serial liver biopsies was found in 37%, stability in 34% and regression in 29% (16). Diabetic patients with elevated body mass index (BMI) and low fibrosis stage had higher rates of fibrosis progression (16). Changes of aminotransferase do not closely parallel changes in fibrosis stage. Liver biopsy is the gold standard for evaluation of fibrosis severity; however it has several limitations for clinical practice including the expense and the invasive nature of the procedure which is associated with a number of complications (10). The NAFLD Fibrosis Score consisting of routinely measured and readily available clinical and laboratory data was constructed and validated to separate NAFLD patients with and without advanced fibrosis from 733 patients with NAFLD confirmed by liver biopsy (14). The scoring system has 6 variables including age, hyperglycemia, BMI, platelet count, albumin, and aspartate aminotransferase and alanine aminotransferase ratio (AST/ALT) as independent indicators of advanced liver fibrosis (14). The NAFLD Fibrosis Scores were classified into 2 categories for assessing advanced liver fibrosis (14). The NAFLD patients with a score less than -1.5 were classified as “low probability of advanced liver fibrosis” and those with a score of at least -1.5 were classified as “intermediate or high

probability of advanced liver fibrosis" (14). By applying this model, a liver biopsy would have been avoided in 75% of patients with correct prediction in 90%. Currently, the NAFLD Fibrosis Score is only used for the initial estimation of disease severity in US and Europe population but it has not been validated in Thai NAFLD patient and it was not used as a prognostic predictor for poor outcomes in patients with NAFLD. We aimed to validate the NAFLD Fibrosis Score in the Thai NAFLD population and to assess whether the severity of liver fibrosis estimated by the NAFLD Fibrosis Score can predict the mortality of patients with NAFLD.

2.1 NATURAL HISTORY OF NAFLD

NAFLD is one of the most common causes of chronic liver disease in Western countries and is becoming more prevalent worldwide. The diagnosis of NAFLD varies based on the intensity of investigations, from simple liver tests and abdominal ultrasonography to the more invasive test of liver biopsy for histological confirmation.(21, 22) In general, NAFLD is diagnosed based on the following criteria [1) liver biopsy showing steatosis in at least 5% of hepatocytes (23) or 2) imaging study confirmation; 3) exclusion of liver disease of other etiology including alcohol-induced (history of excessive alcohol consumption greater than 20 gm/day), drug-induced liver disease, autoimmune or viral hepatitis as well as cholestatic or metabolic/genetic liver disease.(1)

The disease spectrum of NAFLD includes varying severity of liver histology from simple steatosis to nonalcoholic steatohepatitis (NASH) to cirrhosis. Natural history studies of NAFLD showed that 1-5% of patients with simple steatosis developed cirrhosis (1, 24) while patients with NASH showed pathological progression of fibrosis in 15% to 39% within 10 years.(20, 25) The mortality rate of NAFLD patients in the community was higher than that in the general United States population.(6) Death occurred ranging from 13% to 45% with mean follow up of 8 to 11 years and coronary artery disease (CAD) was the leading cause of death (25% to 28% of mortality).(6, 26)

2.2 NAFLD AND RISK OF CAD EVENTS

Patients with NAFLD were associated with more prevalent CAD independent of other risk factors, including glycemic control and MetS components.(27, 28) This finding was despite factoring in the other risk factors for CAD and the components of metabolic syndrome (29). In patients with NAFLD, metabolic abnormalities are commonly found and vary from 33% to 100% depending on types of study and the selection criteria of NAFLD patients.(6, 27, 30) Central obesity, high triglyceride levels and hypertension are the major abnormal metabolic syndrome criteria in patients with NAFLD. Metabolic syndrome (MetS) components including central obesity, hypertension, hypertriglyceridemia, decreased high density lipoprotein cholesterol (HDL-C) and impaired glucose test or type 2 diabetes mellitus (DM) are commonly found in NAFLD.(31) MetS is defined by the presence of three or more of these metabolic abnormalities by the 2001 National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATPIII) criteria (32) and was modified by the International Diabetes Federation in 2005 (33) The criteria of MetS are not similarly worldwide as shown in table 1.

The prevalence of metabolic abnormalities such as diabetes and hypertension, were increased up to 15-fold in patients with NASH compared to steatosis independent of age or BMI (34) The impact of MetS or its components on CAD events is still controversy. Some studies showed that patients with MetS had 50% more rapid coronary artery stenosis progression than those without MetS and there was a strong relationship of the MetS and a higher mortality rate in patients with stable CAD.(35) However, other studies showed that diabetes and hypertension were better independent predictors of the progression and severity of CAD than MetS itself.(36, 37)

Previous cross sectional studies showed supporting evidence of higher CAD in NAFLD patients than in controls. The incidence of new CAD events in non cirrhotic patients with NAFLD varied from 2% to 11% with the overall mortality of 12% to 13% (Table 1).(26, 27, 38-41) The CAD related mortality ranged from 1 % to 3% in NAFLD (40, 41) and ranged from 12% to16% in patients with NASH.(5, 26) Patients with NASH

had more cardiovascular events than patients without NASH significantly.(5) The association of liver histological progression and the risk of CAD events is not linear and need more research studies.(42) The age of onset of CAD events in NAFLD patients ranged from 45 to 65 years. All had significantly higher estimated CAD risk at 10 years (17% vs10%) by the Framingham risk score (FRS) than NAFLD patients without new CAD events.(41)

2.3 POSSIBLE DISEASE MECHANISMS LINKING NAFLD AND ATHEROSCLEROSIS

The mechanism of liver injury in NAFLD is currently thought to be a "multiple-hit process" involving insulin resistance (IR), oxidative stress, apoptosis, and adipokines.(43) IR increases free fatty acid and lipogenesis. Apoptosis and oxidative stress contribute to the progression of NASH. These factors including hyperinsulinemia, hepatic iron, and lipid peroxidation, are potential mechanisms for producing the oxidative stress.(44)

The possible pathogenesis associations of CAD in NAFLD patients have been proposed.

1. Fatty liver may directly promote atherosclerosis.

This hypothesis was supported by evidence of a strong relationship between higher liver fat content and less hepatic insulin sensitivity. (45) In type 2 diabetic patients, the increased liver fat correlated significantly with impaired insulin clearance.(45) Endothelial dysfunction is an important component of IR. The primary factor which is linked to endothelial dysfunction and IR is the deficiency of endothelial-derived nitric oxide.(46) This direct atherogenic effect of NAFLD was evident regardless of other CAD risk factors such as DM status or MetS.(47) Recently, Gastaldelli, et al showed that the presence of fatty liver assessed by fatty liver index was significantly associated with increased CAD risk and reduced insulin sensitivity in nondiabetic subjects.(48) The strength of this study is the exclusion of patients with established cardiovascular risk factors at baseline. Recently another study performed by Alkhouri, et al(49) showed that the histologic severity of liver inflammation in NAFLD patients was

strongly associated with an increased cardiovascular risk and an also significantly associated with higher triglyceride level and lower HDL-cholesterol level (49).

2. The distribution of body fat

People with central obesity have large amounts of visceral adipose tissue (VAT). VAT is defined as intra-abdominal fat bounded by parietal peritoneum or transversalis fascia and it is a major source for free fatty acids, interleukin-6 and adipokines delivered to the liver.(50) Abdominal obesity correlates significantly with left ventricular dysfunction and all-cause mortality.(51) Another study showed a good correlation between VAT and the degree of severity of liver inflammation and fibrosis in NAFLD patients.(52) Waist circumference (WC) is highly correlated with VAT in both genders and is used as a clinical marker for abdominal obesity.(53) Recently, the increased VAT assessed by CT showed a significant association with CAD which was defined by the presence of plaque calcification (54); however, this pathophysiological mechanism which may contribute to the excess risk of CAD has not been studied in patients with NAFLD. Further studies to identify the association between NAFLD and CAD through VAT should be considered.

3. Role of adipokines and CAD

Adiponectin act as a protective adipokine by inhibiting liver gluconeogenesis and suppressing lipogenesis.(55) Patients with NAFLD have higher levels of oxidative stress and inflammation, hypoadiponectinemia and higher C-reactive protein (CRP) levels compared to those in controls.(55) Hypoadiponectinemia is associated with impaired glucose tolerance tests and CAD in non-diabetic persons.(56) Interestingly, patients with lower plasma adiponectin concentrations were associated with early CAD onset and multiple atherosclerotic lesions in coronary arteries and its concentration also correlated positively with age at onset of CAD.(57) However, adiponectin may not be a definite predictor of CAD but it may play an important role in the pathogenesis of IR. (58) Currently, there is no strong prospective data linking adiponectin and CAD in NAFLD patients.

DIAGNOSTIC MODALITIES TO PREDICT THE RISK OF CAD EVENTS

1. Cardiovascular risk scoring system

The Framingham Risk Score (FRS) which is a standard and accurate tool. FRS is based upon assessment of multiple variables such as gender, age, hypertension, serum LDL-C or total cholesterol, diabetes and smoking, to calculate the risk of CAD events over 10 years.(59) It has been used to predict CAD events in white and black subjects of both genders and the calculation of risk can be accessed from the web site, http://www.framinghamheartstudy.org/risk/coronary.html.(60) Based on FRS estimation of the 10-year risk for CAD events, it classifies people as low (<10%), intermediate (10% to 20%), or high risk (>20%).(61) Two studies reported the estimated 10-year coronary heart disease (CHD) risk derived from the FRS in patients with NAFLD and found that the overall calculated 10-year CHD risk was significantly higher in the NAFLD patients than in persons of the same age and sex.(41) Treeprasertsuk et al. (41) showed that new onset CHD occurred in 11% of NAFLD patients and this was not significantly different from the FRS estimated 10-year CHD risk at baseline of 10.9 $\pm$ 9.3%.

FRS may not be applicable worldwide, as in some populations, it overestimates cardiovascular risk such as Japanese, Hispanic men and Caribbean Indian patients (62, 63). Currently, there are several newly developed cardiovascular disease risk scoring systems; for example, the SCORE model which was recommended in 2007 by the European Society of Cardiology (64). This model differs from the FRS model in two aspects. First, it estimates the 10 year risk of any first fatal atherosclerotic event including stroke or CAD-related death and second, it estimates only CAD mortality, not all cardiovascular events (64). Currently, there is no data about the association of the SCORE model and NAFLD.

2. C-reactive protein; inflammatory biomarkers

C-reactive protein (CRP) is an index of inflammation synthesized by the liver and has proved to be a good predictor of CAD. Currently, the high-sensitivity C-reactive protein (hs-CRP) assay is a new marker of inflammation and may be superior to CRP. A

meta-analysis of prospective studies showed that subjects with upper tertile of hs-CRP had a relative odd of 2 (95% CI 1.6-2.5) for major coronary events which was higher than those subjects in the lower tertile of hs-CRP.(65) In 2003, the American Heart Association and Centers for Disease Control and Prevention recommended that hs-CRP may be used as part of a global coronary risk assessment in adults with intermediate cardiovascular risk (e.g. calculated CAD risk of 10% to 20% by using FRS).(66) An average of two sequential tests of CRP values, above a cut point of 3 mg/L was indicative of high risk of CAD.(66)

The association of hs-CRP and the prediction of CAD events in NAFLD patients have not been well studied. Targher, et al (67) found that plasma level of hs-CRP in patients with biopsy-proven NASH (2.7 mg/L) was significantly higher than those in non-obese healthy subjects (0.9 mg/L), and in overweight non-steatotic patients (1.8 mg/L). Another study showed that NAFLD patients had an increased concentration of ultrasensitive CRP (>3 ng/ml) independently of other metabolic factors. (68) Further research to address the association of hs-CRP and the prediction of CAD events in NAFLD patients is required.

2.4 TREATMENT OF NAFLD

PRIMARY PREVENTION FOR CAD RISK IN NAFLD PATIENTS

All NAFLD patients need an overall assessment of CAD risk and the comprehensive management of atherosclerotic risk factors. This is possible with a multidisciplinary approach to monitor and control related CAD risk factors. The combination of lifestyle modification with pharmacological treatment tailored to each individual's risk factors is also necessary. Asking for leisure-time and work-related physical activity can be helpful for evaluation of CAD risk. Risk-based algorithms based on FRS or other cardiovascular disease risk scoring systems, should be applied to all NAFLD patients. Waist circumference should be measured and it is a first approach to detect patients with excess VAT or abdominal obesity.(69) Recently, a study showed that physical inactivity and abdominal obesity were both independently associated with a higher risk of CAD.(70) The presence and magnitude of other risk factors, for example,

the levels of cholesterol, HDL-C and triglyceride are important to measure in screening programs. The frequency of screening is mainly dependent on the patients' risk factors. The National Cholesterol Education Program Adult Treatment Panel II (ATP II) recommendations suggested a 5-year interval of screening for people with previous normal results and more frequent screening for those who have borderline values.(71)

1. Lifestyle modification

Despite much research evidence that supports the association of NAFLD and cardiovascular risk, the treatment strategies for NAFLD are still limited. Lifestyle modifications including dietary restrictions and regular aerobic exercise should be the first line of management. A meta-analysis of 22 prospective cohort studies examined the effect of physical activity during leisure time on the primary prevention of CAD in 510,000 healthy individuals and found that a moderate-to-high level of physical activity during leisure time had a lower risk of CAD incidence of 12 % compared to patients with low levels of or no physical activity during leisure time.(72) Diet control with standardized nutritional counseling has been shown to reduce body weight in NASH patients.(73) The intense dietary intervention improved liver histology in 9 of 15 patients with a mean weight reduction of 3%.(73) Combined diet control and increasing physical activity was shown to reduce liver fat by 31% after 9 months of follow up.(74) Recently, a study of the benefit of exercise and diet control showed an improvement in anthropometric indices, total cholesterol, insulin sensitivity and liver tests after 10 weeks.(75)

In patients with NASH maintaining weight loss of at least 9% over 9 months also improved IR and liver histology.(76) The role of behavior treatment has been shown to effect weight reduction by decreasing excess nutrition and increasing exercise.(77) Behavioral intervention showed an important role in sustaining lifestyle modifications and improving blood pressure control in pre-hypertension patients.(78) A multidisciplinary approach by dietitian, internist and specialist may be needed for NAFLD patients to achieve the goal of CAD risk reduction.

2. Bariatric surgery

Weight loss by bariatric surgery in obese patients improved hypertension, DM and hypercholesterolemia after 1 year of follow up.(79) Interestingly, remission of type 2 DM in obese patients with BMI >30 kg/m^2 was achieved by 73% in the surgical group compared to 13% in the conventional-therapy group with a relative risk of remission for the surgical group of 5.5 (95% CI, 2.2-14).(80) A meta-analysis including 15 studies with 766 paired liver biopsies with mean age at the time of surgery ranging from 36 to 49 years, found that the mean BMI reduction ranged from 19 to 42 kg/m^2.(81) The proportions of patients with improvement of liver fibrosis on histology in NASH patients were 66%.(81) Currently, no study of bariatric surgery in NAFLD patients has shown improvement of CAD outcome. However, a cohort study of 2010 Swedish patients who underwent bariatric surgery showed that the overall mortality of patients with bariatric surgery (5%) was significant lower than that in controls (6%).(82)

3. Pharmacological therapy

3.1 Insulin sensitizing agents

The role of IR and oxidative stress are major factors in the pathogenesis of NAFLD.(83, 84) Several studies testing the role of insulin sensitizing agents in patients with NAFLD have been reported. Metformin improves IR in NAFLD patients with or without diabetes (85) Three randomized clinical trials found that metformin was associated with more normalization of serum ALT versus diet (OR = 2.8) or versus vitamin E (OR = 7.7). The improvement of liver histology was inconclusive due to the small number of patients.(85) Recently, a study of 48 weeks of metformin (2000 mg/day) therapy in 28 NAFLD patients found that 30% showed a histologic response and improved insulin sensitivity (86);however, the degree of insulin sensitivity change did not correlate with histologic improvement. With limited data of the efficacy of metformin in patients with NAFLD and CAD, we could not draw firm conclusion about its therapeutic benefit and further studies are required.

Pioglitazone is a thiazolidinedione (TZDs) which activates the peroxisome proliferator-activated receptor-γ and decrease the hepatic supply of fatty acids from

adipose tissue. Several studies provided some evidence that the TZDs may be beneficial in the short term. A randomized, controlled trial of pioglitazone (30 mg/day) was conducted for 1 year in nondiabetic patients with NASH and showed significant improvement in MetS components and liver histology.(87) After stopping therapy with pioglitazone, significant worsening of parenchymal inflammation and steatosis were found.(88) For safety profile of TZDs, a meta-analysis study reported that rosiglitazone increased the risk of myocardial infarction and the overall cardiovascular death in patients with type 2 DM (89) whereas pioglitazone showed a significantly lower risk of death and myocardial infarction but it increased serious heart failure relating to fluid retention in patients with type 2 DM.(90) Thus, the long-term benefit and the safety profiles of TZDs need more study.

3.2 Statins

Statins used to treat hypercholesterolemia are safe and can be used in patients with NAFLD.(91) Chalasani, et al. (92) conducted a study in hyperlipidemic patients with statins and showed that 1437 patients with normal transaminase had a significantly lower incidence of mild-moderate elevations (2%) than those 342 patients with elevated baseline enzymes (5%). Severe elevations in liver biochemistries were not different between both groups. Another study was conducted in hypercholesterolemic subjects with a history of compensated chronic liver disease of at least 6 months duration and 64% of patients had NAFLD. They showed that pravastatin (80 mg/day) lowered LDL-C and total cholesterol compared to a placebo and was safe and well tolerated.(93) The potential effect of statins on liver histological change was studied in a small number of NAFLD patients and showed no improvement in liver fibrosis.(94) Currently, no study has assessed the efficacy of statins to reduce CAD mortality in NAFLD patients although the benefit are well recognized for both primary and secondary prevention for CAD and reduction of the overall mortality in the general populations (95, 96). Thus, statins should be considered only in NAFLD patients with dyslipidemia and/ or high calculated risk of coronary artery disease by the Framingham risk score.

3.3 Antiplatelet agents

Antiplatelet agents have not been studied in NAFLD patients. Only one study was performed in rats with fatty liver with or without 3 kinds of antiplatelet agents including aspirin, ticlopidine or cilostazol for 16 weeks.(97) This study showed that all antiplatelet agents especially cilostazol significantly improved liver steatosis, inflammation and fibrosis by suppressing mitogen-activated protein kinase activation induced by oxidative stress.(97)

Table 1 Definition of Metabolic Syndrome (MetS); by IDF 2005 criteria

1. Central obesity as measured by waist circumference (WC*) (WC for USA men >40 inches [>102 cm]; WC, women >35 inches [>88 cm]) or BMI >30 kg/m^2
2. Hypertriglyceridemia; Fasting blood triglycerides (TG) >150 mg/dL [>1.7 mmol/L].
3. Low level of HDL-C, men <40 mg/dL [<1.03 mmol/L], HDL-C, and women <50 mg/dL [<1.29 mmol/L].
4. Elevated systolic blood pressure (BP) ≥130 mmHg or diastolic BP ≥85 mmHg or treatment of previously diagnosed hypertension (HT).
5. Elevated fasting plasma glucose (FPG) ≥100 mg/dL (≥5.6 mmol/L) or previously diagnosed type2 DM. (If FPG above 100 mg/dL, oral glucose tolerance test is highly recommended.)

Note. * different cut-off of WC is based on ethnicity; For south Asian and chinese; central obesity use WC ≥90 cm (men), ≥80 cm (women), for people of European origin; central obesity use WC ≥94 cm (men), ≥80 cm (women).(33)

Table 2. Studies of CAD events in NAFLD patients.

Author (year)/ Country	NAFLD criteria	Number of patients	Age; years (mean ± SD)	Diagnostic modalities of CAD	CAD Prevalence (%)	Overall deaths (%)	Average time to follow-up (years)
Targher, et al. (2007)/ Italy	- Abnormal abdominal ultrasound	NAFLD (1974) vs Non NAFLD (418) - All diabetic patients	65 + 6 (NAFLD)	Medical records, ECG, echo-Doppler	NAFLD (26.6) vs. Non NAFLD (18.3); OR = 1.8	No data	No data
Kadayifci, et al.[21] (2008)/ United States	- Abnormal ALT, liver ultrasound and / or liver histology	NASH cirrhosis (60) vs.. Cirrhosis from other causes(60)	55 + 9 (NASH)	Medical records, stress echo-Doppler and/or coronary angiography	NASH cirrhosis (21.6) vs. cirrhosis from other causes (3.3)	No data	No data
Hamaguchi , et al.[22] (2007)/ Japan	- Abnormal abdominal ultrasound	- NAFLD (231) vs. Healthy controls (990)	49 + 9 (NAFLD)	Self-reported questionnaire	NAFLD (2.2) vs. Non NAFLD (0.3)	No death due to CAD	7115 person-years
Schindhelm, et al.[23] (2007)/ Netherlands	- Divided into ALT tertiles	- The third ALT tertile (468) vs. the first ALT tertile (551)	59.7 vs. 62.9	Symptoms / signs of CAD followed by angioplasty (presence of >50 % stenosis) or ECG changes	Age and sex-adjusted risk for CAD= 2.0 (1.3-3.1)	12.1	10

Table 2. Studies of CAD events in NAFLD patients.

Author (year)/ Country	NAFLD criteria	Number of patients	Age; years (mean ± SD)	Diagnostic modalities of CAD	CAD Prevalence (%)	Overall deaths (%)	Average time to follow-up (years)
Treeprasertsuk et al.[24] (2009)/ United States	- Abnormal ALT, liver ultrasonography and / or liver histology	309 with the overall calculated 10-year CHD risk =10.9 ± 9.3% (using FRS)	49 + 11	Medical records, ECG, and echo Doppler	11	13.3	11.5 + 4.1 (3554 person-years)
Rafiq, et al[9] (2009)/ United States	- Liver biopsy proven	173 NASH (72) and Steatosis (101)	50 + 14.5	Medical records, data from National Death Index Plus	CAD death =12.2%	59.5	18.5

CHAPTER III
METHODOLOGY

3.1 Study population

Study design: We divided our study into 2 phases; the first phase is a cross sectional study to collect 115 Thai NAFLD patients prospectively during 2007-2010 in King Chulalongkorn Memorial Hospital (KCMH) to validate the NAFLD Fibrosis Score. The second phase is a historical cohort design by using the existing data of NAFLD patients diagnosed during 1980 and 2000 drawn from the Rochester Epidemiology Project to analyze.

Phase 1: Patients residing in Thailand who had been diagnosed with NAFLD in King Chulalongkorn Memorial Hospital (KCMH) were included prospectively during 2007-2010. The inclusion criteria were the patients with age of at least 18 years old and NAFLD was diagnosed base on liver biopsy with standard criteria. Exclusion criteria were the incomplete data needed for the NAFLD Fibrosis Score calculation. Patients who fit in the inclusion and exclusion criteria were admitted for liver biopsy under standard procedure with ultrasonography guidance. Data of all patients were recorded including demographic data, anthropometric data and biochemical tests on the day of liver biopsy or within 2 weeks of procedure. All patients were followed-up for the results of liver histological findings within the next 2 weeks. We used the NAFLD Fibrosis Score to calculate in all included patients and validate with the histological findings of liver fibrosis. The liver fibrosis staging is divided into stage 0 to stage 4 as shown in figure 1. One hundred and fifteen Thai NAFLD patients were included during the three years of study period.

Phase 2: Patients residing in Olmsted County, Rochester, Minnesota, US who had been diagnosed with NAFLD-Fatty liver (HICDA Code 05710420) Fatty Liver (HICDA Code 05710421) Hypertrophy, Liver, Fatty (HICDA Code 05710422) Cirrhosis, Liver, Fatty (HICDA Code 05710423) Steatohepatitis or NASH (HICDA Code 05710431) Fatty liver,

Steatohepatitis (HICDA Code05710-42-43) or Steatosis (HICDA Code 02790-44-1) over a 20-year period, between January 1, 1980, and January 1, 2000, were drawn from the Rochester Epidemiology Project (REP) master diagnostic index. The REP index is a unique database system of medical diagnoses of the population living in Olmsted County, Minnesota (98). Although fatty liver was recognized prior to 1980, this liver condition was better characterized in 1980 (99), therefore, we chose to identify patients after this date.

3.2 Inclusion Criteria

From the 479 patients with NAFLD assessed, three-hundred and two patients (63%) aged greater than 18 years old were included. All of them were followed-up and medical charts were reviewed until August 31, 2009 or the date when the first primary end point occurred. By using a standardized case record form, we recorded detailed history and physical examinations and use of pharmacologic agents of unproven efficacy for NAFLD during the follow up period for further analysis and adjusted for these variables in the regression model.

3.3 Exclusion criteria

We excluded NAFLD patients who lacked data needed for the NAFLD Fibrosis Score calculation, patients with pre-existing poor outcomes including overt CHD or overt liver complications at the time of NAFLD diagnosis or patients with duration of follow up less than 5 years. One hundred and seventy seven NAFLD patients were excluded due to missing data needed for the NAFLD Fibrosis Score calculation at baseline or at the end of follow up (N = 95), overt CHD confirmed at baseline (N = 63), liver cirrhosis with complications confirmed at baseline (N = 11) and patients who had duration of follow-up of less than 5 years (N = 8).

3.4 Definition

The diagnosis of NAFLD was based on the following criteria 1) liver biopsy showing steatosis in at least 5% of hepatocytes or 2) fatty infiltration of the liver was confirmed on imaging studies (ultrasound, computed tomography, or magnetic resonance imaging) (23) and 3) exclusion of liver disease of other etiology including alcohol-induced (history of excessive alcohol consumption greater than 20 gm/day), drug-induced liver disease, autoimmune or viral hepatitis as well as cholestatic or metabolic/genetic liver disease (1).

The staging of liver inflammation and fibrosis in patients with NAFLD is based on the Kleiner D, et al.(23) and was summarized in the figure 1.

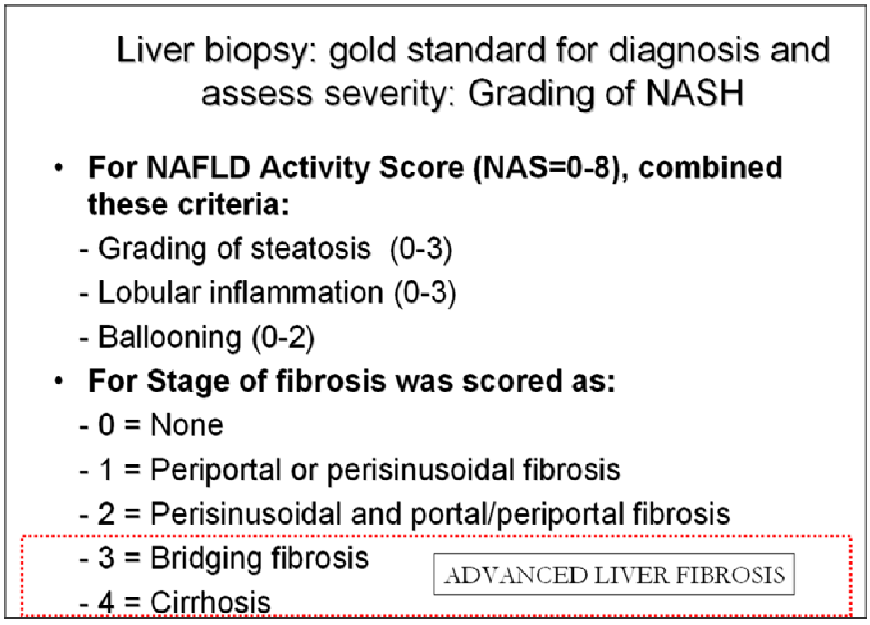


Figure 1 Staging of liver inflammation and fibrosis in patients with NAFLD is based on the Kleiner D, et al.(23)

- Cirrhosis was defined (100, 101) based on the pathological term for the chronic liver diseases which grossly showed the irregular surface of the liver. By histopathological findings, it showed a diffuse fibrotic change and the distortion of normal liver architecture into liver nodules. The progression of liver cirrhosis commonly takes long term in several years but it may occur within few weeks or months in some etiologies. The staging of liver fibrosis usually divided into fibrosis stage 0 to stage 4 depending on the etiologies for example using Brunt criteria in NAFLD patients.(21)

- The primary endpoint was defined by the presence of all-cause mortality, and/or cardiac complications, and/or liver complications.

- The cardiac complications included new onset of CHD events which were based on the medical records with designation as validated congestive heart failure (CHF), unstable angina or myocardial infarction and/or a documented flow-limiting stenosis from angiography or angina requiring revascularization during follow up and need for hospitalization (59).

- The liver complications were diagnosed by clinical signs and symptoms (100) included the presence of ascites, variceal bleeding, severe grade of hepatic encephalopathy, liver failure or hepatocellular carcinoma (HCC) which occurred during follow up and need for hospitalization with/or without death (102). However, mild grades of hepatic encephalopathy may be difficult to detect and some of these patients may be included in the study. All causes of death listed on the death certificates or pathological findings (underlying, intermediate, immediate and other major conditions) using International Classification of Diseases (ICD)-10 revision were recorded.

- The presence of metabolic syndrome (MetS) was defined by using the 2001 National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATPIII) criteria and the new definition which required the presence of at least three of the five features (33, 103). BMI was calculated as weight divided by height squared (kg/m^2). As the majority of patients did not have waist circumference (WC) measurements, we defined obesity using BMI >30 kg/m^2 in accordance with the World Health Organization (WHO) definition of the insulin resistance syndrome (104, 105).

- The NAFLD patients with a histological liver fibrosis stage of 1-2 were classified as “mild liver fibrosis” and those with a histological fibrosis stage of 3-4 were classified as “advanced liver fibrosis” according to Brunt, et al (21, 23).

- Baseline NAFLD Fibrosis Scores calculation

The NAFLD fibrosis score composed of 6 variables including age, hyperglycemia, body mass index (BMI), platelet count, albumin, and aspartate aminotransferase and alanine aminotransferase ratio (AST/ALT) ratio as independent indicators of advanced liver fibrosis (14). In this study, the NAFLD Fibrosis Scores were classified into 2 categories for assessing advanced liver fibrosis (14). The NAFLD patients with a score less than -1.5 were classified as “low probability of advanced liver fibrosis” and those with a score of at least -1.5 were classified as “intermediate or high probability of advanced liver fibrosis” (14). Angulo, et al proposed that if applying this model, a liver biopsy would have been avoided in 75% of patients with correct prediction in 90%.(14) The range of the NAFLD Fibrosis Score proposed by Angulo P, et al(14) was classified into 3 subgroups including the low probability of fibrosis (NAFLD Fibrosis Score<-1.5), the intermediate probability of advanced liver fibrosis (NAFLD Fibrosis Score≥-1.5 and <0.67) and the high probability of advanced liver fibrosis (NAFLD Fibrosis Score≥ 0.67),

Since the information required for the NAFLD Fibrosis Score may not all be available at the same day scores were calculated at the time of NAFLD diagnosis and were used data from the medical records for visits within 3 months of the “true” NAFLD diagnosis date and the last follow up date. If more than one assessment for a given variable was available in the medical record during this time period the value closest to the “true” follow up date was used for the NAFLD Fibrosis Score calculation (14).

3.5 Conceptual frameworks

The disease spectrum of NAFLD includes varying severity of liver histology from simple steatosis to nonalcoholic steatohepatitis (NASH) to cirrhosis. Natural history studies of NAFLD showed that 1-5% of patients with simple steatosis developed cirrhosis (1, 24) while patients with NASH showed pathological progression of fibrosis in 15% to 39% within 10 years.(20, 25)

We summarized the possible related factors which aggravated the progression of liver fibrosis including older age, female gender, higher body mass index, and presence of diabetes as shown in figure 2 (5, 6, 14, 30).

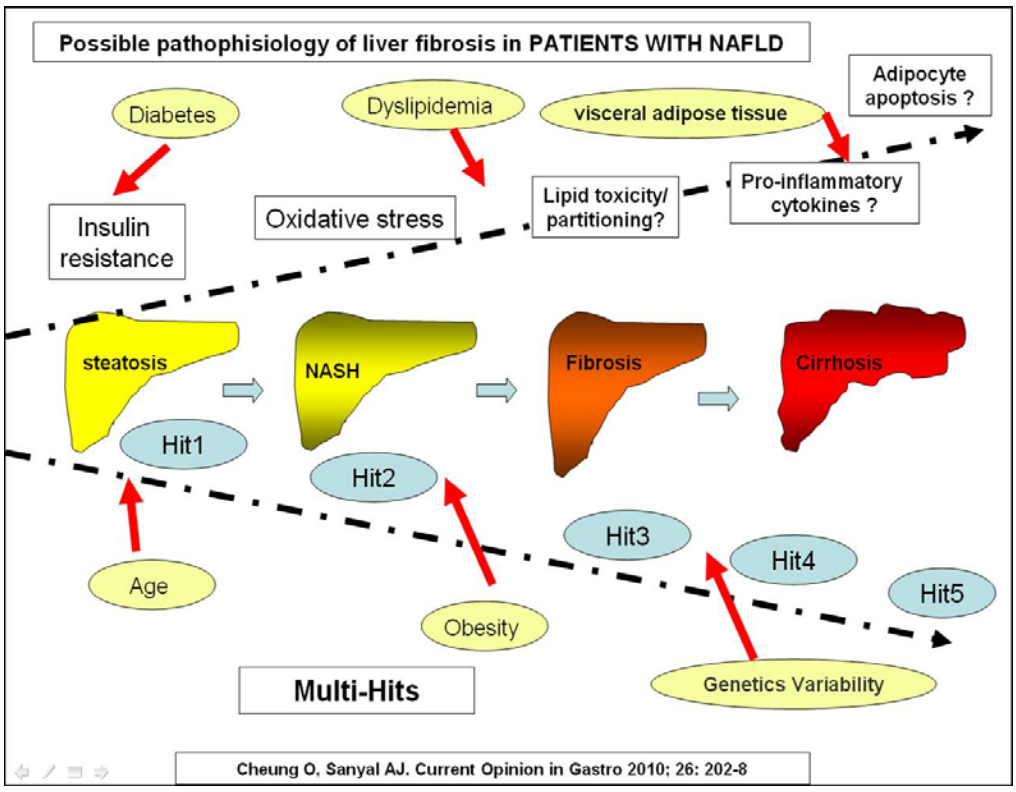


Figure 2 Related factors which aggravated the progression of liver fibrosis in patients with NAFLD

3.6 Sample-size/statistical power considerations

Phase 1: We assumed that NAFLD patients with mild liver or fibrosis FO-F2 were classified as controls whereas the patients with advanced liver fibrosis or F3-F4 were classified as cases. We use the sample size calculation formula by the analytic case-control study and the number of patients in each group was calculated as the followings;

Number per group (group1 = F0-F2 and group 2 = F3-F4)

$= (z\alpha_{/2} \sqrt{2PQ} + zB \sqrt{p1q1+p0q0})^2 / (p1-p0)^2$

if $z\alpha$=1.645, zB = 1.28 , p0=0.4, q0 = 0.6

OR= 0.56, p1 = 0.78, q1 =0.22,

Thus, the number of patients in each group was 52 cases and the total number of enrolled patients is 104 cases.

Phase 2: We assumed that the overall mortality rate in NAFLD would be 12% and liver-related complications may occur in at least 3%, and cardiac complications may occur in 11% from the previous study (6). We anticipated that at least 150 patients or 50% of total cohort would be classified as “low probability of advanced liver fibrosis” using the NAFLD Fibrosis Score and at least 150 would be classified as “intermediate or high probability of advanced liver fibrosis”. (16) However, some patients may develop new CHD events, liver complications and death. Therefore, we anticipated that 15% of patients would be identified as having experienced a primary endpoint. We assumed that at most 10% of the patients with low probability of advanced liver fibrosis would experience events within 5 years and at least 20% of the patients with intermediate or high probability of advanced liver fibrosis would experience events within 5 years, to get a statistical power of at least 82%.

3.7 Statistical Analyses

Phase 1: Thai NAFLD patients were categorized by the severity or staging of liver fibrosis into 2 groups of the mild and advanced liver fibrosis. Continuous outcomes were presented as mean ± standard deviation (SD) and categorical data were presented as numbers (percentage).Differences between both group were tested by independent t tests for continuous variables and were tested by the Chi-square test for proportions. P value less than 0.05 was taken to be statistically significant. The overall accuracy of the NAFLD Fibrosis Score in identifying the mild or advanced stage of liver fibrosis was analyzed using the area under the receiver operating characteristics curve (ROC). The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated.

Phase 2: Patients were categorized by the NAFLD Fibrosis Score into 2 groups of the probability of advanced liver fibrosis. Differences between the primary endpoint including the overall mortality, new onset CHD events or liver complications of those two groups of low and high NAFLD Fibrosis Scores were tested using the Chi-square test. Differences between NAFLD patients with and without primary endpoints were tested by

independent t tests for continuous variables and were tested by the Chi-square test for proportions. Continuous outcomes were presented as mean ± standard deviation (SD) and categorical data were presented as numbers (percentage). Logistic regression analysis was used to identify the factors significantly associated with death among NAFLD patients. Only those variables with a p value <0.1 by univariate analysis were included in multivariate analysis. In order to avoid overestimation of the model, we excluded those variables used as a part of the NAFLD Fibrosis Score calculation. We estimated receiver operating characteristics (ROC) of related variables for predicting of death to maximize the area under the curve (AUC). Two-sided *P* values <0.05 were considered to indicate statistical significance. The rate of NAFLD Fibrosis Score change in each patient was calculated by the difference of NAFLD Fibrosis Score at the end of follow-up and at baseline divided by the duration of follow up time (Δ NAFLD Fibrosis Score / Δ time).

Both phases of study used the SPSS statistical software package (SPSS Version 15.0.1.1, Windows VISTA, July 3, 2007) for analysis. The study was approved by the Institutional Review Board (IRB) of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand (phase 1) and the IRB of the Mayo Clinic, Rochester, MN, US and all participants provided permission for their medical information to be used for research.

CHAPTER IV
RESULTS

Phase 1 study

4.1 Validation of the NAFLD Fibrosis Score in Thai NAFLD patients

According to the first phase study, 115 Thai NAFLD patients with mean age of 50.5 ± 12.4 years were included during the three years of the study period. Male and female were 1:1 Fifty percent of Thai NAFLD patients had BMI above 28 kg/m^2 and 50% had type 2 diabetes or impaired fasting glucose. Fifty-four percent had hypertension whereas 80% had dyslipidemia. Baseline characteristics of 115 Thai patients with NAFLD were shown in table 3. Seventy seven of the Thai NAFLD patients (67%) were in a group of low risk of advance fibrosis by using NAFLD Fibrosis Score. By histological findings, the advanced fibrosis was found in 15 patients (13%). Patients with advanced fibrosis were significantly older and had higher blood glucose level than those with mild liver fibrosis. Using the ROC curve with the cut-off level of NAFLD Fibrosis Score at baseline >-1.5, the sensitivity, specificity, PPV and NPV for identifying the Thai NAFLD patients with advance liver fibrosis (F3-F4) were 53%, 70%, 21% and 91% respectively with AUC of 0.64 (95% CI 0.49-.78; P = 0.09) (Figure 3).

Phase 2 study

4.2 Baseline Characteristic data of 302 patients with NAFLD

Three hundred and two NAFLD patients were predominantly middle-aged (47.3 ± 12.9 years; range 21-86 years). Most patients were white (95%) and 44% were male. Obesity was present in 73% of the population. History of diabetes and hypertension were found in 16% and 41% respectively. At baseline, patients with NAFLD included in this study were significantly younger, less often male, had less diabetes, lower plasma glucose levels, lower levels of AST and ALT and were more often white than those excluded from the study. The average NAFLD Fibrosis Score at baseline of included and excluded patients was not significantly different (P = 0.7). The characteristics of 302 patients based on degree of advanced liver fibrosis estimating by the NAFLD Fibrosis

Score at baseline were shown in Table 4. A low probability of advanced liver fibrosis (score <-1.5) was found in 60 % while intermediate or high probability of advanced liver fibrosis (score ≥-1.5) was found in 40%. The mean (± SD) values of NAFLD Fibrosis Score in patients with a low probability of advanced liver fibrosis was -2.6 ± 0.8 and was lower than those in patients with an intermediate or high probability of advanced liver fibrosis (-0.4 ± 0.9; P <0.0001). The proportion of male patients in a low probability of advanced liver fibrosis was higher than those in an intermediate or high probability of advanced liver fibrosis (51% versus 33%; P = 0.002). Patients with a low probability of advanced liver fibrosis had lower CHD risk at baseline estimating by the Framingham risk score calculation than those with an intermediate or high probability of advanced liver fibrosis (14% versus 19%; P = 0.003).

Liver biopsy was performed in 46 patients (15% of 302 patients). Mild liver fibrosis (stage F0-2) was found in 34 patients (74%) while advanced fibrosis (stage F3-F4) was found in 12 patients (26%). NAFLD patients with liver biopsy had significantly lower diastolic blood pressure, lower BMI and higher AST level than those without liver biopsy (P<0.05). The average of NAFLD Fibrosis Score at baseline of patients with and without liver biopsy was not significantly different (-1.8 + 1.6 versus -1.7 + 1.4; P = 0.6). The NAFLD Fibrosis Score at baseline of patients with histological advanced liver fibrosis (n = 12) was significantly higher than those with histological mild liver fibrosis (n = 34) (-0.7 versus -2.4, respectively, P <0.0001). By using the ROC curves, the NAFLD Fibrosis Score at baseline of -1.8 was the best cut off value for the detection of histological advanced liver fibrosis based on a sensitivity of 92%, specificity of 65%, positive predictive value (PPV) of 48%, and negative predictive value (NPV) of 96% with AUC of 0.8

4.3 Clinical Outcomes of Long-Term Follow-Up

The mean follow-up of the total cohort was 11.9 ± 3.9 years for a total of 3594 person-years. About 47% of patients with a low probability of advanced liver fibrosis at baseline progressed to an intermediate or high probability of advanced liver fibrosis at

the end of follow up while 94% of patients with an intermediate or high probability of advanced liver fibrosis remained in the same group.

Table 5 shows that at the end of follow up, patients with an intermediate or high probability of advanced liver fibrosis had significantly higher BMI, more frequent diabetes, more frequent patients with history of hypothyroidism, history of cholecystectomy and history of obstructive sleep apnea, more use of metformin, glitazones and aspirin than those with a low probability of advanced liver fibrosis. For laboratory findings at the end of follow up, patients with an intermediate or high probability of advanced liver fibrosis had significantly higher glucose, and higher AST/ALT ratio than those with a low probability of advanced liver fibrosis. Hematocrit, platelet counts, AST, ALT, albumin, cholesterol and LDL-cholesterol levels were significantly lower in patients with an intermediate or high probability of advanced liver fibrosis compared to those with a low probability of advanced liver fibrosis.

Table 2 shows that 55 patients (18%) developed primary endpoints including 39 patients (13%) who died during follow-up, 30 patients (10%) with new onset of CHD and 6 patients (2%) with liver complications. Patients with an intermediate or high probability of advanced liver fibrosis had primary endpoints, all-cause death, and liver complications more often than those with a low probability of advanced liver fibrosis ($P < 0.05$).

The leading causes of death were non liver cancer (n = 13; 33.3%), CHD (n = 8; 20.5%), and liver related mortality (n = 5; 12.8%). The other 13 patients (33.3%) died from various causes as shown in table 6. The primary location of cancers were gastric cancer (n = 2), colon cancer (n = 2), pancreatic cancer (n = 2), breast cancer (n = 2), leiomyosarcoma of uterus (n = 1), diffuse B cell lymphoma (n = 1), endometrial cancer (n = 1), lung cancer (n = 1) and unknown primary cancer with liver metastasis (n = 1).
Of 30 patients with new onset CHD, 8 of them (27%) died during follow up. Patients with new CHD events (n = 30) were significantly older, had higher SBP, higher Framingham risk score at baseline, higher calculated %CHD risk at baseline and higher NAFLD Fibrosis Score at the end of follow up and lower ALT than those without new CHD events

(n = 272) (P <0.05).The NAFLD Fibrosis Score at baseline was not significantly different between patients with and without new CHD events (-1.2 ± 1.6 versus -1.8 ± 1.4; P = 0.07).

Liver complications occurred in 6 patients and 5 of them (83%) died during follow-up. The liver complications included massive ascites requiring abdominal paracentesis (n = 3), hepatopulmonary syndrome (n = 1) and hepatocellular carcinoma (n = 1).

4.4 Predicting of mortality

Table 7 shows that at baseline, patients who died (n = 39) were significantly older, had higher SBP, higher NAFLD Fibrosis Score (Figure 4), higher FRS, higher glucose, more frequent diabetes, lower diastolic blood pressure, lower ALT and lower albumin than those who survived (n = 263) (P <0.05). At the end of follow up, patients who died had significantly higher NAFLD Fibrosis Scores, greater NAFLD Fibrosis score changes per year, higher creatinine, higher AST/ALT ratio, more frequent patients with CHD or liver complications and more frequent patients with an intermediate or high probability of advanced liver fibrosis, lower hematocrit, lower albumin and less use of metformin and simvastatin than those who survived (P <0.05). Figure 5 showed that patients with primary endpoint (n = 55) had higher NAFLD Fibrosis Score than those without primary endpoint (n = 247).

Table 8 shows results of 3 models of the multivariate analysis to identify the best fit model for predictors of death. We analyzed by using 3 models as shown in table5. In model 1, we added 9 variables including gender, systolic blood pressure, diastolic blood pressure, NAFLD Fibrosis Score at baseline, use of metformin, use of simvastatin, use of aspirin, presence of new onset of CHD and new onset of liver complications without interaction among these variables. Model 2 used 10 included variables; gender, systolic blood pressure, diastolic blood pressure, NAFLD Fibrosis Score at baseline, NAFLD Fibrosis Score changes per year, use of metformin, use of simvastatin, use of aspirin, presence of new onset of CHD and new onset of liver complications without

interaction among variables. Finally, model 3 added the interaction between NAFLD Fibrosis Score at baseline and NAFLD Fibrosis Score changes per year, the interaction among use of aspirin, metformin, aspirin and simvastatin into model 2. We did not add the FRS into these models due to the repetition of several variables in the NAFLD Fibrosis Score and the FRS.

Model 3 was the best fit model which found that a higher NAFLD Fibrosis Score at baseline and more frequent new onset of CHD were significantly predictive of death (OR = 2.6 and 9.2, respectively; P <0.0001). Use of metformin or simvastatin were significantly associated with fewer death in patients with NAFLD (OR = 0.2 and 0.03 respectively; P<0.05).

Using the ROC curves for the detection of death, we found that the NAFLD Fibrosis Score at baseline of -0.9 was the best cut off value based on a sensitivity of 62%, specificity of 76%, PPV of 28%, and NPV of 93% and AUC of 0.7 (Figure 6).

4.5 The rate of NAFLD Fibrosis Score change

The median rate of NAFLD Fibrosis Score change per year of 302 patients was 0.1 with IQR of 0.02, 0.13 and it was not normally distributed. The rate of NAFLD Fibrosis Score change per year in patients with an intermediate or high probability of advanced liver fibrosis predicted by the NAFLD Fibrosis Score was significantly lower than those in a low probability of advanced liver fibrosis (0.06 versus 0.09; P = 0.004). The NAFLD Fibrosis Score change per year in patients who died was significantly higher than those in survived patients (0.14 versus 0.07; P = 0.03). By linear regression, the NAFLD Fibrosis Score at baseline had a small relationship with the FRS (R^2 = 0.13; P <0.0001). Additionally, the NAFLD Fibrosis Score at the end of follow-up had a significant relationship with Child-Pugh score (R^2 = 0.15; P <0.0001), and Model for End-Stage Liver Disease score (MELD) (R^2 = 0.03; P <0.04).

According to the three subgroup of low, intermediate and high probability of fibrosis, classified by the NAFLD Fibrosis Score by using cutoff level of <-1.5 for low, $\geq$-1.5 and <0.67 for intermediate and $\geq$ 0.67 for high probability of fibrosis, we defined the patients into 3 subgroups at the end of the follow up by using the progression pattern of

the NAFLD Fibrosis Score at baseline and at the end of follow up. The first group of stable fibrosis defines as the stable of the subgroup of fibrosis using the NAFLD Fibrosis Score during the follow up period.

The second group of patients with regression of fibrosis defines as the presence of reduction of the NAFLD Fibrosis Score with changing the subgroup of probability of fibrosis during the follow up period. Last, the group of patients with progression of fibrosis defines as the presence of increasing of the NAFLD Fibrosis Score with changing the subgroup of probability of fibrosis during the follow up period. Most patients were in the stable fibrosis (60%) and progression fibrosis (37%) whereas only 3% were in the regression fibrosis (Table 6-7). The annual rate of liver fibrosis progression in the group of progression fibrosis and the stable diseases was 0.19 $\pm$0.02 and 0.05 $\pm$0.08 respectively (Table 9-10).

Table 11 showed the association between the subgroups of progression pattern of liver fibrosis and the primary outcomes. We found that there was no association of the progression pattern of liver fibrosis during the follow up period and the proportion of patients with new onset of CHD (P = 0.80). The primary outcomes including death or liver complications associated with the progression pattern of liver fibrosis significantly (P = 0.001). Table 12 showed the comparison of NAFLD patients with and without new CHD events, we found that the NAFLD Fibrosis Score at baseline in patients with and without new CHD events had no statistical significant difference (-1.2 $\pm$ 1.6 vs. -1.8 $\pm$ 1.4, P=0.07).

4.6 The association between use of metformin or simvastatin and death in patients with NAFLD

As results shown in table 13 that use of metformin or simvastatin were significantly associated with fewer death in patients with NAFLD (OR = 0.2 and 0.03 respectively; P<0.05). We found the significant association between use of metformin or simvastatin and death in patients with NAFLD as shown in Table 13. In our study, the

definition of use of metformin or simvastatin was the presence of prescription for at least 3 months at any time during the follow-up.

Table 3 Baseline characteristics of 115 Thai patients with NAFLD

Variable at baseline; Mean ± SD or Number (%)	Patients with advanced liver fibrosis (N = 15)	Patients without advanced liver fibrosis (N = 100)	P value*
Age	58 ± 7	49 ± 13	0.01*
Male	5 (33%)	52%	0.18
Diabetes/ IFG	8 (53%)	41%	0.36
Hypertension	9 (60%)	53%	0.61
Dyslipidemia	12 (80%)	80%	1.0
BMI (kg/m^2)	29 ± 4	30 ± 8	0.14
Obesity (BMI>28 kg/m^2)	10 (67%)	47%	0.16
AST (U/L)	71 ± 29	56 ± 31	0.84
ALT (U/L)	103 ± 37	104 ± 49	0.36
AST/ALT ratio	0.7 ± 0.2	0.6 ± 0.3	0.66
NAFLD Fibrosis Score baseline	-1.7 ± 1.1	-2.2± 1.4	0.31
Platelet ($X\ 10^9$ /L)	270 ± 74	270 ± 58	0.47
Albumin (g/dL)	4.2± 0.4	4.3± 0.4	0.81
Cholesterol (mg/dL)	218 ± 68	207 ± 48	0.09
Triglyceride (mg/dL)	154 ± 70	162 ± 69	0.79
Glucose (mg/dL)	151 ± 60	116 ± 33	<0.001*

Table 4 Demographic data of 302 patients with NAFLD by NAFLD Fibrosis Score at baseline.

Variable at baseline; Mean ± SD or Number (%)	Total (N = 302)	Patients with a low probability of advanced liver fibrosis (score <-1.5) (N = 181)	Patients with an intermediate or high probability of advanced liver fibrosis (score ≥-1.5) (N = 121)	P value*
Age (years)	47.3 ± 12.9	42.9 ± 11.1	53.8 ± 12.8	<0.0001
Sex (% male)	132 (44)	92 (51)	40 (33)	0.002
Race, number (%White)	288 (95)	170 (94)	119 (97.5)	0.15
History of diabetes	48 (16)	5 (2.8)	43 (35.5)	<0.0001
History of hypertension	125 (41)	55 (30.4)	70 (58)	<0.0001
BMI (kg/m^2)	33.6 ± 6.2	32 ± 5.2	36 ± 6.9	<0.0001
Presence of obesity (BMI >30 kg/m^2)	221 (73)	121 (67)	100 (82.6)	0.002
Systolic blood pressure (mmHg)	136 ± 18	133 ± 17	139 ± 18	0.003
Diastolic blood pressure (mmHg)	83 ± 9	84 ± 8	81 ± 9	0.01
Cholesterol (mg/dL)	214 ± 48	215 ± 46	214 ± 50	0.78
Triglycerides (mg/dL)	221 ± 167	208 ± 123	242 ± 218	0.15
Glucose (mg/dL)	115 ± 41	103 ± 25	132 ± 54	<0.0001
ALT (U/L)	61.5 ± 43.3	69.7 ± 46	49.4 ± 35.7	<0.0001
AST/ALT ratio	0.8 ± 0.4	0.7 ± 0.3	1.0 ± 0.6	<0.0001
Platelet (X 10^9/L)	240 ± 62	259 ± 60	212 ± 53	<0.0001
Albumin (g/dL)	4.3 ± 0.4	4.4 ± 0.3	4.1 ± 0.3	<0.0001
Alkaline phosphatase (U/L)	196 ± 88	186 ± 68	211 ± 111	0.03
Framingham Risk Score	8.4 ± 6.2	6.9 ± 6.4	10.5 ± 5.2	<0.0001
Calculated CHD risk (%)	16.2 ± 14.6	14.1 ± 13.8	19.3 ± 15.2	0.003
NAFLD Fibrosis Score	-1.7 ± 1.4	-2.6 ± 0.8	-0.4 ± 0.9	<0.0001

Note: * P <0.05 means significant difference between patients in a low probability of advanced liver fibrosis groups versus those in an intermediate or high probability of advanced liver fibrosis group.

Table 5 Clinical parameters, laboratory features and clinical outcomes at the end of follow-up by NAFLD Fibrosis Score at baseline.

Variable at the end of follow-up; Mean ± SD or Number (%)	Patients with a low probability of advanced liver fibrosis (score <-1.5) (N = 181)	Patients with an intermediate or high probability of advanced liver fibrosis (score ≥-1.5) (N = 121)	P value*
A. Clinical findings			
BMI (kg/m^2)	32.9 ± 6.6	34.9 ± 7.6	0.02
Obesity (BMI >30 kg/m^2)	119 (65.8)	91 (75.2)	0.08
NAFLD Fibrosis Score	-1.4 ± 1.3	0.4 ± 1.4	<0.0001
NAFLD Fibrosis Score of intermediate or high probability of advanced liver fibrosis (%)	85 (47)	114 (94)	<0.0001
History of diabetes	54 (29.8)	83 (68.6)	<0.0001
Use of metformin	32 (17.7)	48 (39.7)	<0.0001
Use of glitazones	10 (5.5)	19 (15.7)	0.003
Use of aspirin	84 (46)	83 (69)	0.0001
History of hypothyroidism	19 (10.5)	31 (25.6)	0.0005
History of cholecystectomy	27 (15)	33 (27.3)	0.008
History of obstructive sleep apnea	33 (18.2)	34 (28.1)	0.04
B. Laboratory findings			
AST (U/L)	38.9 ± 30.6	33.2 ± 17.8	0.04
ALT (U/L)	53.9 ± 49.7	38.9 ± 21	0.0004
AST/ALT ratio	0.8 ± 0.5	1.0 ± 0.8	0.03
Hematocrit (%)	40.4 ± 4.4	38.6 ± 5.3	0.003
Platelet (X 10^9/L)	259 ± 67	217 ± 74	<0.0001
Albumin (g/dL)	4.1 ± 0.4	3.9 ± 0.6	<0.0001
Cholesterol (mg/dL)	193 ± 40	178 ± 43	0.005

Table 5 Clinical parameters, laboratory features and clinical outcomes at the end of follow-up by NAFLD Fibrosis Score at baseline.

Variable at the end of follow-up; Mean ± SD or Number (%)	Patients with a low probability of advanced liver fibrosis (score <-1.5) (N = 181)	Patients with an intermediate or high probability of advanced liver fibrosis (score ≥-1.5) (N = 121)	P value*
LDL-cholesterol (mg/dL)	109 ± 34	92 ± 30	<0.0001
Glucose (mg/dL)	119 ± 42	131 ± 42	0.02
C. Clinical outcomes at the end of follow-up			
- Lost to follow up	27 (15)	8 (7)	
- Alive with continued follow-up	131 (72)	81 (67)	
- Presence of primary endpoints	23 (13)	32 (26)	0.002
- All-cause death	12 (6.6)	27 (22.3)	<0.0001
- New events of coronary heart disease	15 (8.3)	15 (12.4)	0.24
- Liver complications	1 (0.6)	5 (4.1)	0.03

Note: ** P <0.05 means significant difference between patients in a low probability of advanced liver fibrosis groups versus those in an intermediate or high probability of advanced liver fibrosis group.

Table 6 Causes of Mortality in 39 Patients with NAFLD

Causes of death; Number (%)	All causes mortality (% of death)	All causes mortality (% of 302 patients)
Non-liver cancer	13 (33.3)	4.3
Coronary heart disease	8 (20.5)	2.6
Liver-related mortality (including hepatocellular carcinoma)	5 (12.8)	1.7
Infection (including sepsis)	4 (10.3)	1.3
Stroke	3 (7.7)	1.0
Cardiac arrhythmia	2 (5.1)	0.7
COPD and/ or respiratory failure	2 (5.1)	0.7
Other causes of death (GI bleeding, renal failure)	2 (5.1)	0.7
Total	39 (100)	12.9

Table 7 Comparison of NAFLD patients with versus those without death

Variables Mean ± SD, Number (%)	NAFLD patients without death (N=263)	NAFLD patients with death (N=39)	P value
A. At baseline			
Age (years)	45.2 ± 11.5	61.1 ± 13.8	<0.0001*
Sex (% male)	120 (45.6)	12 (30.8)	0.08
History of diabetes	37 (14.1)	11 (28.2)	0.02*
Systolic blood pressure (mmHg)	134 ± 17	143 ± 21	0.02*
Diastolic blood pressure (mmHg)	83 ± 8	79 ± 10	0.03*
Glucose (mg/dL)	112 ± 38.6	132.7 ± 54.3	0.03*
AST (U/L)	42.2 ± 25.5	35.5 ± 20.0	0.06
ALT (U/L)	64.2 ± 44.6	43.6 ± 27.4	0.0002*
AST/ALT ratio	0.8 ± 0.4	1.0 ± 0.7	0.06
Albumin (g/dL)	4.3 ± 0.3	4.0 ± 0.4	<0.0001*
Framingham Risk Score (FRS)	7.9 ± 6.2	11.4 ± 5.2	0.0003*
Calculated CHD risk (%)	15.3 ± 14.0	22.2 ± 17.1	0.02*
NAFLD Fibrosis Score	-1.9 ± 1.3	-0.8 ± 1.7	0.0004*
NAFLD Fibrosis Score of intermediate or high probability of advanced liver fibrosis (%)	94 (35.7)	27 (69.2)	<0.0001*
Presence of histological advanced liver fibrosis**	7/35 (20.0)	5/11 (45.5)	0.09
B. During the follow-up periods			
Use of metformin	77 (29.3)	3 (7.7)	0.004*
Use of aspirin	151 (57.4)	16 (41.0)	0.05*
Use of simvastatin	107 (40.7)	3 (7.9)	<0.0001*
New events of coronary heart disease	16 (6.1)	14 (35.9)	<0.0001*
Liver complications	1 (0.4)	5 (12.8)	<0.0001*
C. At the end of follow-up			
BMI (kg/m2)	33.9 ± 6.9	31.8 ± 8.2	0.1

Table 7 Comparison of NAFLD patients with versus those without death

Variables Mean ± SD, Number (%)	NAFLD patients without death (N=263)	NAFLD patients with death (N=39)	P value
Hematocrit (%)	40.4 ± 4.1	34.5 ± 6.3	<0.0001*
Glucose (mg/dL)	122 ± 38.6	139 ± 62.3	0.12
AST/ALT ratio	0.9 ± 0.5	1.3 ± 1.0	0.01*
Albumin (g/dL)	4.1 ± 0.3	3.3 ± 0.7	<0.0001*
Creatinine (mg/dL)	1.0 ± 0.5	1.7 ± 1.3	0.004*
NAFLD Fibrosis Score	-0.9 ± 1.4	0.7 ± 2.3	<0.0001*
NAFLD Fibrosis Score change per year (Median; IQR)	0.07 (0.02, 0.12)	0.14 (0.01, 0.31)	0.03*
NAFLD Fibrosis Score of intermediate to high probability of advanced liver fibrosis (%)	168 (63.9)	31 (79.5)	0.05*

Note:* P <0.05, all variables with *P* <0.1 by univariate analysis were included in multivariate analysis model. ****P* <0.05 for NAFLD patients with available results of liver biopsy (n = 46). In order to avoid overestimation of the model, we excluded those variables used as a part of NAFLD Fibrosis Score calculation (age, history of diabetes, AST/ALT ratio, platelet counts, albumin, BMI and Framingham risk score).

Table 8 Multivariate Logistic Regression Model showing OR (95% CI) of predictors for death in 302 patients with NAFLD.

Multivariate analysis;	P value	OR	95% CI
Model 1*			
- Presence of new onset of CHD	<0.0001	9.0	2.9-28.4
- NAFLD Fibrosis Score at baseline	<0.0001	1.9	1.4-2.6
- Use of metformin	0.02	0.2	0.04-0.8
- Use of simvastatin	0.001	0.05	0.01-0.3
Model 2**			
- NAFLD Fibrosis Score changes per year	0.04	14.9	1.1-206.4
- Presence of new onset of CHD	0.001	8.0	2.4-26.1
- NAFLD Fibrosis Score at baseline	<0.0001	2.1	1.5-2.9
- Use of metformin	0.02	0.2	0.04-0.7
- Use of simvastatin	0.001	0.06	0.01-0.3
Model 3***			
- Presence of new onset of CHD	<0.0001	9.2	2.6-32.2
- NAFLD Fibrosis Score at baseline	<0.0001	2.6	1.7-3.9
- Use of metformin	0.03	0.2	0.04-0.8
- Use of simvastatin	0.001	0.03	0.003-0.2
- Interaction between NAFLD Fibrosis Score at baseline and NAFLD Fibrosis Score change per year	0.004	0.06	0.008-0.4
- NAFLD Fibrosis Score changes per year	0.6	2.2	0.07-67.8

Note: Model 1* without interaction among 9 included variables; gender, systolic blood pressure, diastolic blood pressure, NAFLD Fibrosis Score at baseline, use of metformin, use of simvastatin, use of aspirin, presence of new onset of CHD and new onset of liver complications.

Model 2** without interaction among 10 included variables; gender, systolic blood pressure, diastolic blood pressure, NAFLD Fibrosis Score at baseline, NAFLD Fibrosis Score changes per year, use of metformin, use of simvastatin, use of aspirin, presence of new onset of CHD and new onset of liver complications.

Model 3*** added variables of interaction between NAFLD Fibrosis Score at baseline and NAFLD Fibrosis Score changes per year, interaction among use of aspirin, metformin, aspirin and simvastatin into model 2.

Figure 3 Using the ROC curve with the cut-off level of NAFLD Fibrosis Score at baseline >-1.5 to identify the Thai NAFLD patients with advance liver fibrosis (F3-4)

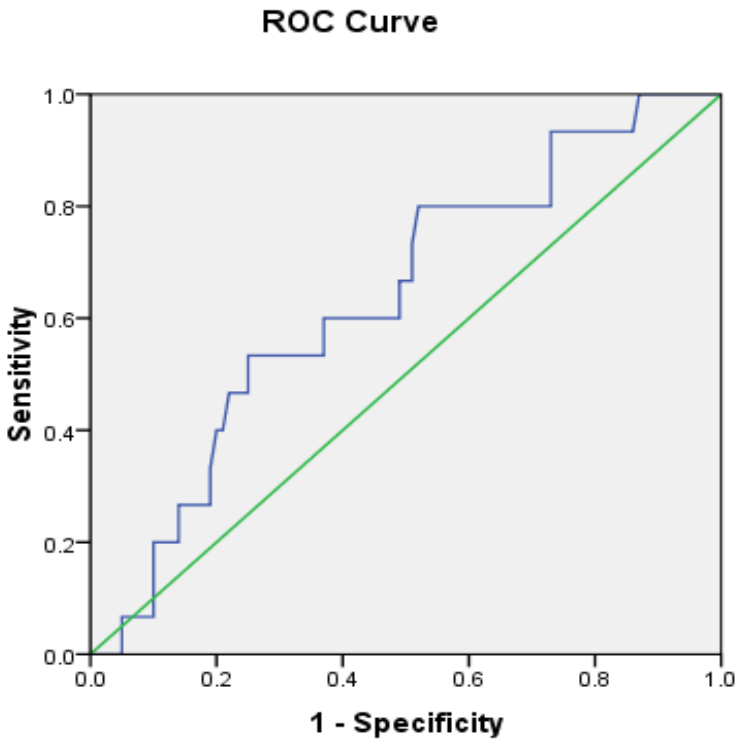


Figure 4 Comparison of the NAFLD Fibrosis Score at baseline of patients with (n = 39) versus those without death (n = 263)

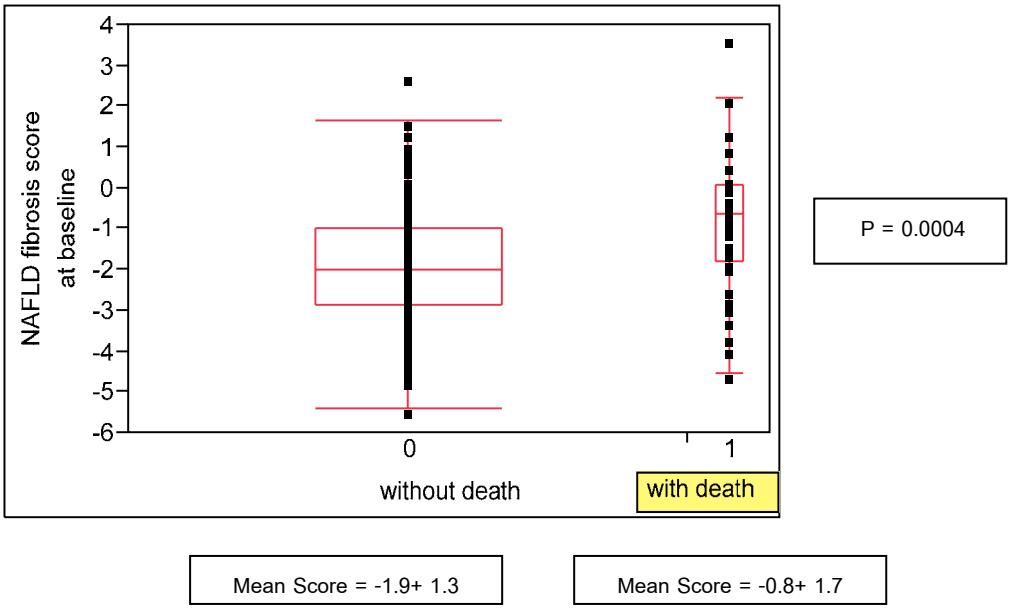


Figure 5 Comparison of the NAFLD Fibrosis Score at baseline of patients with (n = 55) versus those without primary endpoint (n = 247)

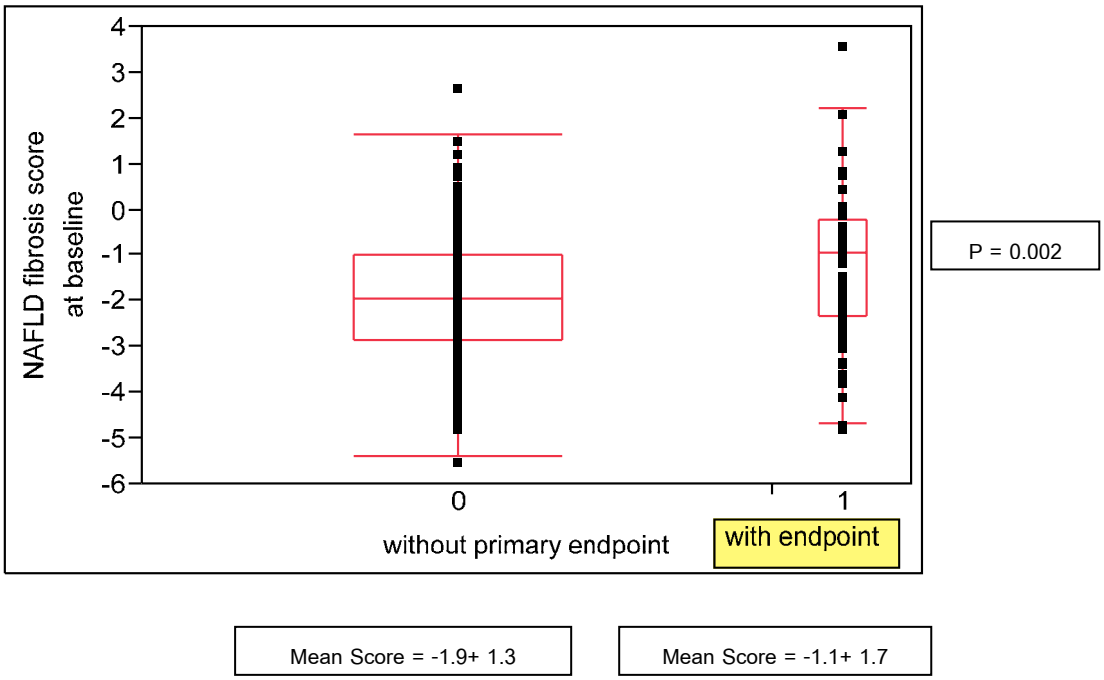
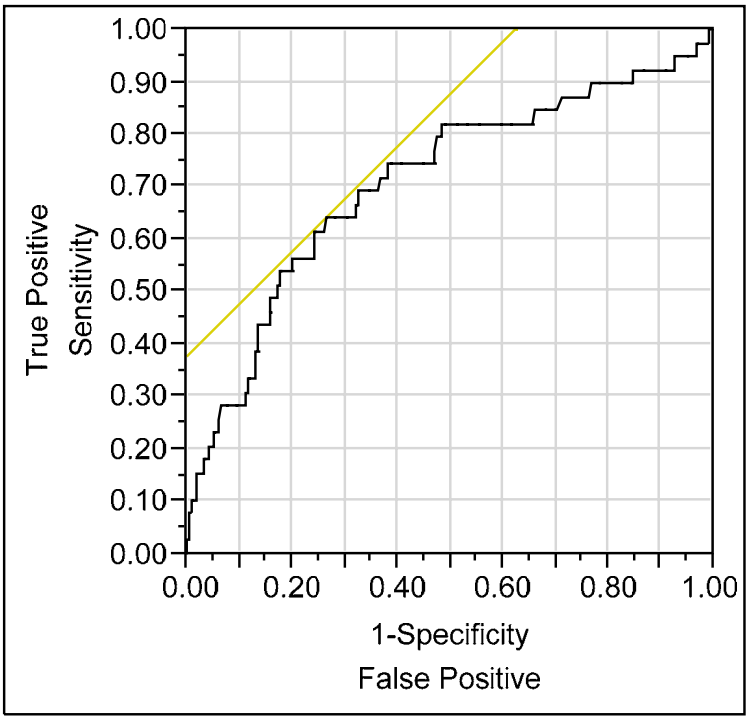


Figure 6 Presence of death estimated by the NAFLD Fibrosis Score at baseline



Note: Using the ROC curve, the NAFLD Fibrosis Score at baseline of -0.9 was the best cut off value for predicting death based on a sensitivity of 62%, specificity of 76%, positive predictive value (PPV) of 28%, and negative predictive value (NPV) of 93% and AUC of 0.7

Table 9 Association of the NAFLD Fibrosis Score at baseline and at the end of follow up categorized by the probability of advanced liver fibrosis in 302 patients with NAFLD

Grading of the NAFLD Fibrosis Score	Low prob. of advanced liver fibrosis (at the end)	intermediate prob. of advanced liver fibrosis (at the end)	High prob. of advanced liver fibrosis (at the end)	Total
Low prob. of advanced liver fibrosis (at baseline)	96 (52.5%)	76(42%)	10 (5.5%)	181
Intermediate prob. of advanced liver fibrosis (at baseline)	7 (6.5%)	73(67.6%)	28 (25.9%)	108
High prob. of advanced liver fibrosis (at baseline)	0 (0%)	3(23%)	10 (77%)	13
Total	102	152	48	302

Table 10 Comparison of the NAFLD Fibrosis Score change per year in 3 subgroups of patients categorized by the progression of the NAFLD Fibrosis Score at the end of follow-up and at baseline.

The progression of the NAFLD Fibrosis Score at the end of follow up and at baseline*	Number of patients	NAFLD Fibrosis Score change per year (Mean±SD)
Stable fibrosis	178 (60%)	0.05 ±0.08
Regression of fibrosis	10 (3%)	-0.2 ±0.25
Progression of fibrosis	114 (37%)	0.2 ±0.02

Note: *P <0.0001 among the group by ANOVA,

According to the three subgroup of low, intermediate and high probability of fibrosis, patients with stable fibrosis defines as the stable of the subgroup of fibrosis using the NAFLD Fibrosis Score during the follow up period.

- Patients with regression of fibrosis define as the presence of reduction of the NAFLD Fibrosis Score with changing the subgroup of probability of fibrosis during the follow up period.

- Patients with progression of fibrosis define as the presence of increasing of the NAFLD Fibrosis Score with changing the subgroup of probability of fibrosis during the follow up period.

Table 11 Primary outcomes among the NAFLD patients categorized by the progression of the NAFLD Fibrosis Score at the end of follow up and at baseline.

The progression of the NAFLD fibrosis score at the end of follow up and at baseline*	**Number of patients**	**Presence of new onset of CHD***	**Presence of new onset of primary endpoints****
Stable fibrosis (60%of total)	178	16 (9%)	17 (9.6%)
Regression of fibrosis (3% of total)	10	1 (10%)	5 (50%)
Progression of fibrosis (37% of total)	114	13 (11.4%)	18 (15.8%)
total	302	30	40

Note: * P= 0.80, **P = 0.001 among the group by chi-square,

According to the three subgroup of low, intermediate and high probability of fibrosis, patients with stable fibrosis defines as the stable of the subgroup of fibrosis using the NAFLD fibrosis score during the follow up period.

- Patients with regression of fibrosis define as the presence of reduction of the NAFLD fibrosis score with changing the subgroup of probability of fibrosis during the follow up period.

- Patients with progression of fibrosis define as the presence of increasing of the NAFLD fibrosis score with changing the subgroup of probability of fibrosis during the follow up period

Table 12 Comparison of NAFLD patients with and without new CHD events.

Variables Mean ± SD, Number (%)	NAFLD patients without CHD events (N = 272)	NAFLD patients with new CHD events (N = 30)	P value
Age (years)	46.2 ± 12.4	57.1± 13.8	0.0002*
Sex (% male)	116(42.7)	16(53.3)	0.26
History of current smoking	33(12.1)	6(20)	0.22
History of hypertension	109(40.1)	15(50)	0.29
BMI at baseline (kg/m^2)	33.7 ± 6.3	32.8 ± 5.4	0.39
Systolic blood pressure (mm Hg)	134.6 ± 16.7	144.5 ± 23.2	0.03*
Diastolic blood pressure (mm Hg)	82.9 ± 8.3	79.9 ± 11.9	0.18
Cholesterol (mg/dL)	212.9 ± 47.3	228.8 ± 48.5	0.10
ALT (U/L)	63.5 ± 44.6	44.2 ± 23.1	0.0003*
Framingham risk score at baseline	8.1 ± 6.2	11.1 ± 5.3	0.006*
Calculated CHD risk (%) at baseline	15.1 ± 13.9	25.5 ± 18.0	0.005*
NAFLD Fibrosis Score at baseline	-1.8 ± 1.4	-1.2 ± 1.6	0.07
NAFLD Fibrosis Score at the end of follow up	-0.8 ± 1.6	-0.05 ± 1.8	0.04

Table 13 Association between use of metformin or simvastatin and death in patients with NAFLD

Variables Mean ± SD, Number (%)	Survived NAFLD patients (N=263)	NAFLD patients with death (N=39)	P value
- Use of metformin	77 (29.3)	3 (7.7)	0.004*
- Use of simvastatin	107 (40.7)	3 (7.7)	<0.0001*

Table 14 Data of three studies of the all cause mortality of patients with NAFLD

Study Year, Type	Authors,	No. of NAFLD patients, Age (mean ± SD) years	Proportion of NASH NAFLD (%)	All causes mortality
2006, Cohort study	Ekstedt M, et al. Liver biopsy (from Sweden)	N = 129, Age 51 ± 12.9 - FU. for an average of 13.7 ± 1.3 years	55% - DM = 53%	20% (26/129) Most common cause of death was CVD (42%), extrahepatic malignancy (15%), liver-related causes (8%).
2009, data from National Death Index Plus.	Rafiq N, et al, Liver biopsy (from US)	N = 173 Age at biopsy = 50.2 ± 14.5 - NASH group had a median follow-up of 10.5 yr.	42% - DM = 29%	45% (78/173) Most common cause of death was CAD (28%), malignancy (18%) and liver- related death (15%).
2011 Present study	Treeprasertsuk S, et al. Ultrasound (from US)	N = 302, Age 47.3.1±12.9 - Mean follow-up of 11.9 ± 3.9 years	Intermediate or high prob. of advanced fibrosis (score ≥-1.5) = 40 %, - DM = 16%	13% (39/302) Most common cause of death was non liver malignancy (33%), CHD (21%), and liver-related death (13%)

4.7 DISCUSSION

In the phase 1 study, we demonstrate that the NAFLD Fibrosis Score with the cut-off value of less than 1.5 had high NPV of 91% and low PPV of 21%, for excluding advanced liver fibrosis in Thai NAFLD patients which is similar to the previous report from China(106). The low prevalence of advanced liver fibrosis in Asian NAFLD patients which was 11% in China and 13% in current study were much lower than those found in

the original study (27%) by Angulo P, et al. and another study in Argentina (27%) (14, 107) With the limited sensitivity and specificity of the accuracy of this scoring system in Thai NAFD patients, we can use the NAFLD Fibrosis Score for excluding the patients with severe disease because of the high NPV of 91%. In this current study, we can avoid liver biopsy in 67% of patients if we used the cut-off level of less than -1.5. Our phase 1 study has some limitations. First, we had small number of NAFLD patients with advanced liver fibrosis however this study design was a prospective cohort which is clearly better than the results from retrospective study. Second, we had limited information of long-term follow up whereas the natural history of liver complications or the presence of new onset of CHD took longer duration for detection. Last, we included the patients from hospital based which may be more severe than the community based patients. Thus, our results may not be applicable in the community based NAFLD patients.

In the phase 2 study, we demonstrate that a higher NAFLD Fibrosis Score at baseline and the presence of new onset of CHD were significantly predictive of death (OR = 2.6 and 9.2, respectively). Currently, there is limited information about noninvasive methods used to predict the poor clinical outcomes in NAFLD patients during follow-up. Recently, Vuppalanchi, et al. (108) suggested that studies of noninvasive markers to identify steatohepatitis patients from NAFLD require validation before being widely used. Even though liver biopsy is the recommended current practice for identifying liver fibrosis in NAFLD patients with risk factors including older age, diabetes, severe obesity and metabolic syndrome, but serial liver biopsies are invasive and are not applicable in clinical practice. Most of these risk factors are the components of NAFLD Fibrosis Score (14) and thus, the benefit of our current study is to extend the clinical use of the NAFLD Fibrosis Score system for predicting death in patients with NAFLD.

Our study found that the annual NAFLD Fibrosis Score change in patients who died were two times higher than those in survived patients. Thus, the NAFLD Fibrosis Score should be calculated in newly diagnosed patients. More recent data by Rafiq, et

al.(26) showed that at least 3 risk factors including type 2 diabetes, older age and low albumin level were predictors for mortality and liver related mortality which was similar to our result.

The annual rate of NAFLD Fibrosis Score change in the group of progression fibrosis was about 4 times higher than the progression rate in the group of stable diseases (0.2 $\pm$0.02 vs. 0.05 $\pm$0.08, respectively) whereas the median (IQR) rate of NAFLD Fibrosis Score change was 0.1 per year. Thus, the median value of the rate of NAFLD Fibrosis Score change may be used as a surrogate marker for progression of liver fibrosis and it needs further study to confirm.

The long-term outcome of patients with NAFLD is not uniform across the spectrum of disease (5, 20, 26). Poor outcomes are more frequent in patients with nonalcoholic steatohepatitis (NASH). This finding was confirmed by our results showing that patients with an intermediate or high probability of advanced liver fibrosis had primary endpoints, all-cause death, and liver complications more often than those with a low probability of advanced liver fibrosis. Differences in study patient populations and their components of metabolic syndromes may explain the different results among studies. For instance, the prevalence of diabetes or impaired glucose tolerance, a well-known risk factor for increased mortality was three times higher in the Swedish study than in our study (53% versus 16%) (5). In addition, previous studies showed a higher mortality rate than our study (13%) which varied from 30% to 45% and may be explained by the different patient selection. One of the most important reasons is that previous studies did not exclude patients with known CHD or known liver complications at baseline as shown in table14 (5, 20, 26).

Obese patients or high BMI (>30(kg/m^2) are found in both groups of low and high probability of liver fibrosis by using the NAFLD Fibrosis Score which was not changed significantly from baseline and at the end of follow up. This finding may be explained by the fact that there was no standard pharmacological therapy for NAFLD during the study period. Currently, the obesity is a common complex problem worldwide(109) and new information of the linkage between the obesity and NAFLD are

published continuously. (110, 111) The role of visceral adipose tissue is important and plays roles in secreting of free fatty acids and adipokines which effects on the pathophysiology of NAFLD.(110, 111)

Our study showed that use of metformin or simvastatin are the protective factor for death which can not be explained easily due to the limitation of historical cohort design study however, it may relate to the effect of improving insulin resistance. Moreover, the definition of use of metformin or simvastatin in our study was the presence of prescription for at least 3 months at any time during the follow-up which may had some improvement effects on the insulin resistance. Recent data showed that diabetes mellitus was one of the important predictors for developing moderate to severe liver fibrosis (OR = 1.6) (112) and Loomba, et al (86) found that treatment with metformin improved liver histology and ALT levels in one-third of patients with NASH. The limitation of this study is the presence of the confounding factors of significant weight loss (6 kg) in these patients (86). The other two studies suggested that metformin improved only the insulin sensitivity but it did not improve liver histology in NASH patients (85, 113). With limited data about the efficacy of metformin in patients with NAFLD, we could not draw firm conclusions about its therapeutic benefit and further studies are required.

Our study also demonstrated that use of simvastatin was also a preventive factor for death in patients with NAFLD. It may relate to the effect of the prevention of the new onset of CHD. CHD was the second leading cause of death and accounted for 20% of death in our study. Recent data showed that the statin is safe and well tolerated in patients with NAFLD (91, 92, 114). No study has assessed the efficacy of statins to reduce CHD mortality in NAFLD patients although the benefits are well recognized for both primary and secondary prevention for CHD and reduction of the overall mortality in the general population (95, 96). A meta-analysis study found that for every 10% reduction in serum cholesterol, the risk of CHD was reduced by 15% (95) and another study showed that an average reduction in total cholesterol by 22% can reduce the overall mortality of 22% (96). Thus, statins should be considered in NAFLD patients with dyslipidemia and/ or high calculated risk of coronary heart disease by the Framingham

Risk Score. There are data to confirm its safety and efficacy in NAFLD patients for reduction of the hepatic steatosis.(115, 116)

The main strengths of our phase 2 study are the inclusion of NAFLD patients from the community along with the long-term follow up. All patients had complete data for calculation of the NAFLD Fibrosis Score at the time of NAFLD diagnosis and at the end of follow-up. The exclusion of known CHD or liver cirrhosis with complications at baseline is important to reduce the overestimation of the incidence of primary endpoints or mortality rate during the follow-up period.

Our phase 2 study has some limitations. First, only 6.6% of the patients with a low probability of advanced liver fibrosis died which was less than expected (10%) in sample size calculation and may affect the power of the study. Second, only 63% of our patients with NAFLD were included and excluded patients were significantly older, were more often male, and had diabetes more often than those included in the study. This may be explained by the exclusion criteria of known CHD (N = 63) or known liver cirrhosis with complications at baseline (N = 11). Therefore, extrapolation of these results to all patients with NAFLD has to be done with some caution. Finally, most of our patients in Olmsted County are white, and recent data showed that non-Caucasian race was an important predictor of decreased survival (26). Thus, our results may not be entirely applicable in other ethnic groups.

Our current study is important because it is not only to validate the accuracy of the NAFLD Fibrosis Score in Thai patients with a high NPV (91%) but also to extend the clinical use of the NAFLD Fibrosis Score system for predicting death or liver complications in NAFLD patients. A higher NAFLD Fibrosis Score and higher creatinine at the end of follow-up can be used as prognostic predictors for mortality and liver complications among NAFLD patients. Currently, there is limited information about noninvasive methods used to predict the poor clinical outcomes in NAFLD patients during follow-up. Moreover, the prognosis of patients with NAFLD varies across the spectrum of disease. Further research is needed to validate the benefit of the NAFLD

Fibrosis Score for predicting death or liver complications in NAFLD patients; however some conclusions from our study should be used in clinical practice.

1. The NAFLD Fibrosis Score seem to be simpler and less invasive than liver biopsy for initial evaluation of degree of liver fibrosis in Thai patients with NAFLD. It should be calculated for all patients with NAFLD at initial consultation to estimate the probability of advanced liver fibrosis. According to our results, we can avoid liver biopsy in 67% of patients if we used the cut-off level of less than -1.5.

2. Our phase 2 study showed that 40% of patients with NAFLD were in an intermediate or high probability of advanced liver fibrosis at baseline and 94% of them were still in advanced liver fibrosis group at the end of follow up. Only 6% of these patients improved the degree of liver fibrosis. Thus, further studies of clinical trials are needed to identify the treatment options to improve the degree of liver fibrosis and should focus on patients with an intermediate or high probability of advanced liver fibrosis. Using the NAFLD Fibrosis Score as a marker of the severity of liver fibrosis for follows up after treatment will be another important area to study.

3. Our phase 2 study showed that 60% of patients with NAFLD were in a low probability of advanced liver fibrosis at baseline and 47% of them turned to be patients in an intermediate or high probability of advanced liver fibrosis group at the end of follow up. Further studies are needed to identify the appropriate treatment to slow down the progression rate of liver fibrosis.

4. We found that use of simvastatin or metformin appeared protection in patients with NAFLD. Thus, further prospective cohort study with long-term follow up is necessary to evaluate the efficacy of simvastatin or metformin for reduction of the overall mortality and liver complications in patients with NAFLD.

5. NAFLD have a higher 10-year CHD risk than predicted in the general population.

6. One of the limitations of our study is that we are unable to extract an accurate dates of primary events especially date of CHD event or liver complications event which were not recorded clearly.

The larger cohort, prospective and multicenter study with liver biopsy–proven NAFLD is necessary to validate the diagnostic accuracy of the NAFLD Fibrosis Scoring for separating patients with and without poor outcomes. The future study may focus on subgroups of population with high risk for example NAFLD patients with diabetes or metabolic syndrome. The subsequent validated studies may show lower accuracy of this scoring system for predicting of mortality but it is useful for clinical application. Therefore, extrapolation of our current results to all patients with NAFLD has to be done with some caution.

SUMMARY

Our study aims to validate the NAFLD Fibrosis Score in Thai NAFLD patients and to assess whether the severity of liver fibrosis estimated by the NAFLD Fibrosis Score can predict time to death or liver complications among NAFLD patients. We divided our study into 2 phases; the first phase is a cross sectional study to collect 115 Thai NAFLD patients prospectively during 2007-2010 in King Chulalongkorn Memorial Hospital (KCMH) to validate the NAFLD Fibrosis Score. The second phase is a historical cohort design by using the existing data of NAFLD patients diagnosed during 1980 and 2000 drawn from the Rochester Epidemiology Project to analyze. Of 479 patients with NAFLD, 302 patients were included. We used the NAFLD Fibrosis Score for separating NAFLD patients with and without advanced liver fibrosis.

According to the first phase study, 115 Thai NAFLD patients with mean age of 50.5 $\pm$ 12.4 years were included. Seventy seven of the Thai NAFLD patients (67%) were in a group of low risk of advance fibrosis by using NAFLD Fibrosis Score. Advanced fibrosis was shown in 15 (13%) patients. Using the ROC curve, the NAFLD Fibrosis Score at baseline of >-1.5 was used for predicting significant liver fibrosis with a sensitivity of 53%, specificity of 70%, PPV of 21% and NPV of 91%. For the second phase study, a total of 302 NAFLD patients (mean age 47.3 $\pm$12.9 years) were followed-up for an average of 11.9 $\pm$ 3.9 years. A low probability of advanced fibrosis (score <-1.5 at baseline) was found in 60 % while intermediate or high probability of advanced

fibrosis (score ≥-1.5) was found in 40%. At the end of follow up, 55 patients (18%) developed primary endpoints including 39 patients (13%) who died during follow-up. In a multivariate analysis a higher NAFLD Fibrosis Score at baseline and presence of new onset of CHD were significantly predictive of death (OR = 2.6 and 9.2, respectively; p <0.0001). The NAFLD Fibrosis Score seem to be simpler and less invasive than liver biopsy for initial evaluation of degree of liver fibrosis in Thai patients with NAFLD. Additionally, the NAFLD Fibrosis Score should be calculated for all patients with NAFLD to predict the poor outcomes and liver complications. Further studies of clinical trials are needed to identify the treatment options to improve the degree of liver fibrosis. Using the NAFLD Fibrosis Score as a marker of the severity of liver fibrosis for follows up after treatment will be another important area to study.

STUDY HIGHLIGHTS

1. **What is known knowledge?**

The NAFLD Fibrosis Score was constructed and validated to separate NAFLD patients with and without advanced fibrosis in Caucasian patients. The mortality rate of NAFLD patients in the community was higher than that in the general United States population

2. **What is current knowledge from our study?**

- The NAFLD Fibrosis Score (<-1.5) can be applied in Thai NAFLD patients with a high negative predictive value of 91% for excluding patients with advanced liver disease
- The NAFLD Fibrosis Score change per year in patients who died or developed liver complications was significantly higher than those in survived NAFLD patients.
- A higher NAFLD Fibrosis Score at baseline and presence of new onset of CHD were significantly predictive of death (OR = 2.6 and 9.2, respectively).

REFERENCES

1. Adams LA, Lindor KD. Nonalcoholic fatty liver disease. **Ann Epidemiol**. 2007 Nov;17(11):863-9.
2. Browning JD, Szczepaniak LS, Dobbins R, Nuremberg P, Horton JD, Cohen JC, et al. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. **Hepatology**. 2004 Dec;40(6):1387-95.
3. Ogden CL, Yanovski SZ, Carroll MD, Flegal KM. The epidemiology of obesity. **Gastroenterology**. 2007 May;132(6):2087-102.
4. Angulo P. Obesity and nonalcoholic fatty liver disease. **Nutr Rev**. 2007 Jun;65(6 Pt 2):S57-63.
5. Ekstedt M, Franzen LE, Mathiesen UL, Thorelius L, Holmqvist M, Bodemar G, et al. Long-term follow-up of patients with NAFLD and elevated liver enzymes. **Hepatology**. 2006 Oct;44(4):865-73.
6. Adams LA, Lymp JF, St Sauver J, Sanderson SO, Lindor KD, Feldstein A, et al. The natural history of nonalcoholic fatty liver disease: a population-based cohort study. **Gastroenterology**. 2005 Jul;129(1):113-21.
7. Watanabe S, Yaginuma R, Ikejima K, Miyazaki A. Liver diseases and metabolic syndrome. **J Gastroenterol**. 2008;43(7):509-18.
8. Sung KC, Ryan MC, Kim BS, Cho YK, Kim BI, Reaven GM. Relationships between estimates of adiposity, insulin resistance, and nonalcoholic fatty liver disease in a large group of nondiabetic Korean adults. **Diabetes Care**. 2007 Aug;30(8):2113-8.
9. Cheung O, Kapoor A, Puri P, Sistrun S, Luketic VA, Sargeant CC, et al. The impact of fat distribution on the severity of nonalcoholic fatty liver disease and metabolic syndrome. **Hepatology**. 2007 Oct;46(4):1091-100.
10. Oh MK, Winn J, Poordad F. Review article: diagnosis and treatment of non-alcoholic fatty liver disease. **Aliment Pharmacol Ther**. 2008 Sep 1;28(5):503-22.

11. Powell EE, Cooksley WG, Hanson R, Searle J, Halliday JW, Powell LW. The natural history of nonalcoholic steatohepatitis: a follow-up study of forty-two patients for up to 21 years. **Hepatology**. 1990 Jan;11(1):74-80.

12. Harrison SA, Torgerson S, Hayashi PH. The natural history of nonalcoholic fatty liver disease: a clinical histopathological study. **Am J Gastroenterol**. 2003 Sep;98(9):2042-7.

13. Fassio E, Alvarez E, Dominguez N, Landeira G, Longo C. Natural history of nonalcoholic steatohepatitis: a longitudinal study of repeat liver biopsies. **Hepatology**. 2004 Oct;40(4):820-6.

14. Angulo P, Hui JM, Marchesini G, Bugianesi E, George J, Farrell GC, et al. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. **Hepatology**. 2007 Apr;45(4):846-54.

15. Harrison SA, Oliver D, Arnold HL, Gogia S, Neuschwander-Tetri BA. Development and validation of a simple NAFLD clinical scoring system for identifying patients without advanced disease. **Gut**. 2008 Oct;57(10):1441-7.

16. Adams LA, Sanderson S, Lindor KD, Angulo P. The histological course of nonalcoholic fatty liver disease: a longitudinal study of 103 patients with sequential liver biopsies. **J Hepatol**. 2005 Jan;42(1):132-8.

17. Yoneda M, Yoneda M, Mawatari H, Fujita K, Endo H, Iida H, et al. Noninvasive assessment of liver fibrosis by measurement of stiffness in patients with nonalcoholic fatty liver disease (NAFLD). Dig Liver Dis. 2008 May;40(5):371-8.

18. Nakano M, Murohisa T, Imai Y, Hiraishi H. Validity of the NAFLD fibrosis score in a Japanese population. **Nihon Shokakibyo Gakkai Zasshi**. 2012 May;109(5):751-9.

19. Schreuder TC, Verwer BJ, van Nieuwkerk CM, Mulder CJ. Nonalcoholic fatty liver disease: An overview of current insights in pathogenesis, diagnosis and treatment. **World J Gastroenterol**. 2008 Apr 28;14(16):2474-86.

20. Matteoni CA, Younossi ZM, Gramlich T, Boparai N, Liu YC, McCullough AJ. Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. **Gastroenterology**. 1999 Jun;116(6):1413-9.

21. Brunt EM. Pathology of nonalcoholic steatohepatitis. **Hepatol Res**. 2005 Oct;33(2):68-71.

22. Farrell GC, Larter CZ. Nonalcoholic fatty liver disease: from steatosis to cirrhosis. **Hepatology**. 2006 Feb;43(2 Suppl 1):S99-S112.

23. Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. **Hepatology**. 2005 Jun;41(6):1313-21.

24. Fan JG, Farrell GC. Epidemiology of non-alcoholic fatty liver disease in China. **J Hepatol**. 2009 Jan;50(1):204-10.

25. Bacon BR, Farahvash MJ, Janney CG, Neuschwander-Tetri BA. Nonalcoholic steatohepatitis: an expanded clinical entity. **Gastroenterology**. 1994 Oct;107(4):1103-9.

26. Rafiq N, Bai C, Fang Y, Srishord M, McCullough A, Gramlich T, et al. Long-term follow-up of patients with nonalcoholic fatty liver. Clin Gastroenterol Hepatol. 2009 Feb;7(2):234-8.

27. Targher G, Bertolini L, Padovani R, Rodella S, Tessari R, Zenari L, et al. Prevalence of nonalcoholic fatty liver disease and its association with cardiovascular disease among type 2 diabetic patients. **Diabetes Care**. 2007 May;30(5):1212-8.

28. Chen CH, Nien CK, Yang CC, Yeh YH. Association Between Nonalcoholic Fatty Liver Disease and Coronary Artery Calcification. Dig Dis Sci. 2009 Aug 18.

29. Arslan U, Turkoglu S, Balcioglu S, Tavil Y, Karakan T, Cengel A. Association between nonalcoholic fatty liver disease and coronary artery disease. **Coron Artery Dis**. 2007 Sep;18(6):433-6.

30. Ong JP, Pitts A, Younossi ZM. Increased overall mortality and liver-related mortality in non-alcoholic fatty liver disease. **J Hepatol**. 2008 Oct;49(4):608-12.

31. Khashab MA, Liangpunsakul S, Chalasani N. Nonalcoholic fatty liver disease as a component of the metabolic syndrome. **Curr Gastroenterol Rep**. 2008 Feb;10(1):73-80.

32. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). **JAMA**. 2001 May 16;285(19):2486-97.

33. Alberti KG, Zimmet P, Shaw J. The metabolic syndrome--a new worldwide definition. **Lancet**. 2005 Sep 24-30;366(9491):1059-62.

34. Sung KC, Ryan MC, Wilson AM. The severity of nonalcoholic fatty liver disease is associated with increased cardiovascular risk in a large cohort of non-obese Asian subjects. **Atherosclerosis** 2009 Apr;203(2):581-6.

35. Zhao XQ, Krasuski RA, Baer J, Whitney EJ, Neradilek B, Chait A, et al. Effects of combination lipid therapy on coronary stenosis progression and clinical cardiovascular events in coronary disease patients with metabolic syndrome: a combined analysis of the Familial Atherosclerosis Treatment Study (FATS), the HDL-Atherosclerosis Treatment Study (HATS), and the Armed Forces Regression Study (AFREGS). **Am J Cardiol** 2009 Dec 1;104(11):1457-64.

36. Kramer CK, von Muhlen D, Gross JL, Laughlin GA, Barrett-Connor E. Blood pressure and fasting plasma glucose rather than metabolic syndrome predict coronary artery calcium progression: the Rancho Bernardo Study. **Diabetes Care** 2009 Jan;32(1):141-6.

37. Ebrahimi M, Kazemi-Bajestani SM, Ghayour-Mobarhan M, Moohebati M, Paydar R, Azimi-Nezhad M, et al. Metabolic syndrome may not be a good predictor

of coronary artery disease in the Iranian population: population-specific definitions are required. **ScientificWorldJournal** 2009;9:86-96.

38. 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. **J Hepatol** 2008 Oct;49(4):595-9.
39. Hamaguchi M, Kojima T, Takeda N, Nagata C, Takeda J, Sarui H, et al. Nonalcoholic fatty liver disease is a novel predictor of cardiovascular disease. **World J Gastroenterol** 2007 Mar 14;13(10):1579-84.
40. Schindhelm RK, Dekker JM, Nijpels G, Bouter LM, Stehouwer CD, Heine RJ, et al. Alanine aminotransferase predicts coronary heart disease events: a 10-year follow-up of the Hoorn Study. **Atherosclerosis** 2007 Apr;191(2):391-6.
41. Treeprasertsuk S, Angulo P, Adams LA, Lindor KD. The Framingham risk score accurately predicts the higher risk of coronary heart disease in patients with nonalcoholic fatty liver disease. **Gastroenterology** [abstract]. 2009;136((Suppl1)):97(Suppl1, abstract no. 645).
42. Rubinstein E, Lavine JE, Schwimmer JB. Hepatic, cardiovascular, and endocrine outcomes of the histological subphenotypes of nonalcoholic fatty liver disease. **Semin Liver Dis** 2008 Nov;28(4):380-5.
43. Kim CH, Younossi ZM. Nonalcoholic fatty liver disease: a manifestation of the metabolic syndrome. **Cleve Clin J Med** 2008 Oct;75(10):721-8.
44. Edmison J, McCullough AJ. Pathogenesis of non-alcoholic steatohepatitis: human data. **Clin Liver Dis** 2007 Feb;11(1):75-104, ix.
45. Kotronen A, Juurinen L, Tiikkainen M, Vehkavaara S, Yki-Jarvinen H. Increased liver fat, impaired insulin clearance, and hepatic and adipose tissue insulin resistance in type 2 diabetes. **Gastroenterology** 2008 Jul;135(1):122-30.
46. Cersosimo E, DeFronzo RA. Insulin resistance and endothelial dysfunction: the road map to cardiovascular diseases. **Diabetes Metab Res Rev** 2006 Nov-Dec;22(6):423-36.

47. Targher G, Bertolini L, Padovani R, Rodella S, Zoppini G, Zenari L, et al. Relations between carotid artery wall thickness and liver histology in subjects with nonalcoholic fatty liver disease. **Diabetes Care** 2006 Jun;29(6):1325-30.

48. Gastaldelli A, Kozakova M, Hojlund K, Flyvbjerg A, Favuzzi A, Mitrakou A, et al. Fatty liver is associated with insulin resistance, risk of coronary heart disease, and early atherosclerosis in a large European population. **Hepatology** 2009 Jan 23.

49. Alkhouri N, Tamimi TA, Yerian L, Lopez R, Zein NN, Feldstein AE. The Inflamed Liver and Atherosclerosis: A Link Between Histologic Severity of Nonalcoholic Fatty Liver Disease and Increased Cardiovascular Risk. **Dig Dis Sci** 2009 Dec 5.

50. Jensen MD. Role of body fat distribution and the metabolic complications of obesity. **J Clin Endocrinol Metab** 2008 Nov;93(11 Suppl 1):S57-63.

51. Ammar KA, Redfield MM, Mahoney DW, Johnson M, Jacobsen SJ, Rodeheffer RJ. Central obesity: association with left ventricular dysfunction and mortality in the community. **Am Heart J** 2008 Nov;156(5):975-81.

52. van der Poorten D, Milner KL, Hui J, Hodge A, Trenell MI, Kench JG, et al. Visceral fat: a key mediator of steatohepatitis in metabolic liver disease. **Hepatology** 2008 Aug;48(2):449-57.

53. Han TS, van Leer EM, Seidell JC, Lean ME. Waist circumference as a screening tool for cardiovascular risk factors: evaluation of receiver operating characteristics (ROC). **Obes Res** 1996 Nov;4(6):533-47.

54. Marques MD, Santos RD, Parga JR, Rocha-Filho JA, Quaglia LA, Miname MH, et al. Relation between visceral fat and coronary artery disease evaluated by multidetector computed tomography. **Atherosclerosis** 2009 Oct 29.

55. McKimmie RL, Daniel KR, Carr JJ, Bowden DW, Freedman BI, Register TC, et al. Hepatic steatosis and subclinical cardiovascular disease in a cohort enriched for type 2 diabetes: the diabetes heart study. **Am J Gastroenterol** 2008 Dec;103(12):3029-35.

56. Otsuka F, Sugiyama S, Kojima S, Maruyoshi H, Funahashi T, Sakamoto T, et al. Hypoadiponectinemia is associated with impaired glucose tolerance and coronary artery disease in non-diabetic men. **Circ J** 2007 Nov;71(11):1703-9.

57. Hashimoto N, Kanda J, Nakamura T, Horie A, Kurosawa H, Hashimoto T, et al. Association of hypoadiponectinemia in men with early onset of coronary heart disease and multiple coronary artery stenoses. **Metabolism** 2006 Dec;55(12):1653-7.

58. Wannamethee SG. Adiponectin and cardiovascular risk prediction: can the ambiguities be resolved? **Nutr Metab Cardiovasc Dis** 2008 Nov;18(9):581-4.

59. Wilson PW, D'Agostino RB, Levy D, Belanger AM, Silbershatz H, Kannel WB. Prediction of coronary heart disease using risk factor categories. **Circulation** 1998 May 12;97(18):1837-47.

60. Cosin Aguilar J, Hernandiz Martinez A, Rodriguez Padial L, Zamorano Gomez JL, Aristegui Urrestarazu R, Armada Pelaez B, et al. [Assessment of cardiovascular risk in population groups. Comparison of Score system and Framingham in hypertensive patients]. **Rev Clin Esp** 2006 Apr;206(4):182-7.

61. Ford ES, Giles WH, Mokdad AH. The distribution of 10-Year risk for coronary heart disease among US adults: findings from the National Health and Nutrition Examination Survey III. **J Am Coll Cardiol** 2004 May 19;43(10):1791-6.

62. Angulo P. NAFLD, obesity, and bariatric surgery. **Gastroenterology** 2006 May;130(6):1848-52.

63. Jaquet A, Deloumeaux J, Dumoulin M, Bangou J, Donnet JP, Foucan L. Metabolic syndrome and Framingham risk score for prediction of cardiovascular events in Caribbean Indian patients with blood glucose abnormalities. **Diabetes Metab** 2008 Mar 17.

64. Graham I, Atar, D, Borch-Johnsen, K, et al. European guidelines on cardiovascular disease prevention in clinical practice: executive summary. **Eur Heart J** 2007;28:2375.

65. Danesh J, Whincup P, Walker M, Lennon L, Thomson A, Appleby P, et al. Low grade inflammation and coronary heart disease: prospective study and updated meta-analyses. **BMJ** 2000 Jul 22;321(7255):199-204.

66. Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon RO, 3rd, Criqui M, et al. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. **Circulation** 2003 Jan 28;107(3):499-511.

67. Targher G, Bertolini L, Rodella S, Lippi G, Franchini M, Zoppini G, et al. NASH predicts plasma inflammatory biomarkers independently of visceral fat in men. **Obesity (Silver Spring)** 2008 Jun;16(6):1394-9.

68. Lizardi-Cervera J, Chavez-Tapia NC, Perez-Bautista O, Ramos MH, Uribe M. Association among C-reactive protein, Fatty liver disease, and cardiovascular risk. **Dig Dis Sci** 2007 Sep;52(9):2375-9.

69. Despres JP. Cardiovascular disease under the influence of excess visceral fat. **Crit Pathw Cardiol** 2007 Jun;6(2):51-9.

70. Arsenault BJ, Rana JS, Lemieux I, Despres JP, Kastelein JJ, Boekholdt SM, et al. Physical inactivity, abdominal obesity and risk of coronary heart disease in apparently healthy men and women. **Int J Obes (Lond)** 2009 Nov 17.

71. Pignone MP, Phillips CJ, Atkins D, Teutsch SM, Mulrow CD, Lohr KN. Screening and treating adults for lipid disorders. **Am J Prev Med** 2001 Apr;20(3 Suppl):77-89.

72. Sofi F, Capalbo A, Cesari F, Abbate R, Gensini GF. Physical activity during leisure time and primary prevention of coronary heart disease: an updated meta-analysis of cohort studies. **Eur J Cardiovasc Prev Rehabil** 2008 Jun;15(3):247-57.

73. Huang MA, Greenson JK, Chao C, Anderson L, Peterman D, Jacobson J, et al. One-year intense nutritional counseling results in histological improvement in patients with non-alcoholic steatohepatitis: a pilot study. **Am J Gastroenterol** 2005 May;100(5):1072-81.

74. Kantartzis K, Thamer C, Peter A, Machann J, Schick F, Schraml C, et al. High Cardiorespiratory Fitness is an independent Predictor of the Reduction in Liver Fat during a Lifestyle Intervention in Non-Alcoholic Fatty Liver Disease. **Gut** 2008 Dec 11:PMID: 19074179 [Epub ahead of print].

75. Chen SM, Liu CY, Li SR, Huang HT, Tsai CY, Jou HJ. Effects of therapeutic lifestyle program on ultrasound-diagnosed nonalcoholic fatty liver disease. **J Chin Med Assoc** 2008 Nov;71(11):551-8.

76. Harrison SA, Fecht W, Brunt EM, Neuschwander-Tetri BA. Orlistat for overweight subjects with nonalcoholic steatohepatitis: A randomized, prospective trial. **Hepatology** 2009 Jan;49(1):80-6.

77. Bellentani S, Dalle Grave R, Suppini A, Marchesini G. Behavior therapy for nonalcoholic fatty liver disease: The need for a multidisciplinary approach. **Hepatology** 2008 Feb;47(2):746-54.

78. Elmer PJ, Obarzanek E, Vollmer WM, Simons-Morton D, Stevens VJ, Young DR, et al. Effects of comprehensive lifestyle modification on diet, weight, physical fitness, and blood pressure control: 18-month results of a randomized trial. **Ann Intern Med** 2006 Apr 4;144(7):485-95.

79. Pontiroli AE, Pizzocri P, Librenti MC, Vedani P, Marchi M, Cucchi E, et al. Laparoscopic adjustable gastric banding for the treatment of morbid (grade 3) obesity and its metabolic complications: a three-year study. **J Clin Endocrinol Metab** 2002 Aug;87(8):3555-61.

80. Dixon JB, O'Brien PE, Playfair J, Chapman L, Schachter LM, Skinner S, et al. Adjustable gastric banding and conventional therapy for type 2 diabetes: a randomized controlled trial. **JAMA** 2008 Jan 23;299(3):316-23.

81. Mummadi RR, Kasturi KS, Chennareddygari S, Sood GK. Effect of bariatric surgery on nonalcoholic fatty liver disease: systematic review and meta-analysis. **Clin Gastroenterol Hepatol** 2008 Dec;6(12):1396-402.

82. Sjostrom L. Bariatric surgery and reduction in morbidity and mortality: experiences from the SOS study. **Int J Obes (Lond)** 2008 Dec;32 Suppl 7:S93-7.

83. Machado M, Cortez-Pinto H. Non-alcoholic fatty liver disease and insulin resistance. **Eur J Gastroenterol Hepatol** 2005 Aug;17(8):823-6.

84. Ibdah JA, Perlegas P, Zhao Y, Angdisen J, Borgerink H, Shadoan MK, et al. Mice heterozygous for a defect in mitochondrial trifunctional protein develop hepatic steatosis and insulin resistance. **Gastroenterology** 2005 May;128(5):1381-90.

85. Angelico F, Burattin M, Alessandri C, Del Ben M, Lirussi F. Drugs improving insulin resistance for non-alcoholic fatty liver disease and/or non-alcoholic steatohepatitis. **Cochrane Database Syst Rev** 2007(1):CD005166.

86. Loomba R, Lutchman G, Kleiner DE, Ricks M, Feld JJ, Borg BB, et al. Clinical trial: Pilot study of metformin for the treatment of nonalcoholic steatohepatitis. **Aliment Pharmacol Ther** 2008 Oct 9;29:172-82.

87. Aithal GP, Thomas JA, Kaye PV, Lawson A, Ryder SD, Spendlove I, et al. Randomized, placebo-controlled trial of pioglitazone in nondiabetic subjects with nonalcoholic steatohepatitis. **Gastroenterology** 2008 Oct;135(4):1176-84.

88. Lutchman G, Modi A, Kleiner DE, Promrat K, Heller T, Ghany M, et al. The effects of discontinuing pioglitazone in patients with nonalcoholic steatohepatitis. **Hepatology** 2007 Aug;46(2):424-9.

89. Nissen SE, Wolski K. Effect of rosiglitazone on the risk of myocardial infarction and death from cardiovascular causes. **N Engl J Med** 2007 Jun 14;356(24):2457-71.

90. Lincoff AM, Wolski K, Nicholls SJ, Nissen SE. Pioglitazone and risk of cardiovascular events in patients with type 2 diabetes mellitus: a meta-analysis of randomized trials. **JAMA** 2007 Sep 12;298(10):1180-8.

91. Chalasani N. Statins and hepatotoxicity: focus on patients with fatty liver. **Hepatology** 2005 Apr;41(4):690-5.

92. Chalasani N, Aljadhey H, Kesterson J, Murray MD, Hall SD. Patients with elevated liver enzymes are not at higher risk for statin hepatotoxicity. **Gastroenterology** 2004 May;126(5):1287-92.

93. 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 Nov;46(5):1453-63.

94. Rallidis LS, Drakoulis CK, Parasi AS. Pravastatin in patients with nonalcoholic steatohepatitis: results of a pilot study. **Atherosclerosis** 2004 May;174(1):193-6.

95. Gould AL, Rossouw JE, Santanello NC, Heyse JF, Furberg CD. Cholesterol reduction yields clinical benefit: impact of statin trials. **Circulation** 1998 Mar 17;97(10):946-52.

96. Hebert PR, Gaziano JM, Chan KS, Hennekens CH. Cholesterol lowering with statin drugs, risk of stroke, and total mortality. An overview of randomized trials. **JAMA** 1997 Jul 23-30;278(4):313-21.

97. Fujita K, Nozaki Y, Wada K, Yoneda M, Endo H, Takahashi H, et al. Effectiveness of antiplatelet drugs against experimental non-alcoholic fatty liver disease. **Gut** 2008 Nov;57(11):1583-91.

98. Melton LJ, 3rd. History of the Rochester Epidemiology Project. **Mayo Clin Proc** 1996 Mar;71(3):266-74.

99. Ludwig J VT, McGill DB, Oh BJ. . Nonalcoholic steatohepatitis: Mayo Clinic experiences with a hitherto unnamed disease. **Mayo Clin Proc** 1980;55:434-8.

100. EASL clinical practice guidelines on the management of ascites, spontaneous bacterial peritonitis, and hepatorenal syndrome in cirrhosis. **J Hepatol** 2010 Sep;53(3):397-417.

101. Gines P, Quintero E, Arroyo V, Teres J, Bruguera M, Rimola A, et al. Compensated cirrhosis: natural history and prognostic factors. **Hepatology** 1987 Jan-Feb;7(1):122-8.

102. Lebrec D, Vinel JP, Dupas JL. Complications of portal hypertension in adults: a French consensus. **Eur J Gastroenterol Hepatol** 2005 Apr;17(4):403-10.

103. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. **Circulation** 2002 Dec 17;106(25):3143-421.

104. Janssen I, Katzmarzyk PT, Ross R. Body mass index, waist circumference, and health risk: evidence in support of current National Institutes of Health guidelines. **Arch Intern Med** 2002 Oct 14;162(18):2074-9.

105. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. **Diabet Med** 1998 Jul;15(7):539-53.

106. Wong VW, Wong GL, Chim AM, Tse AM, Tsang SW, Hui AY, et al. Validation of the NAFLD fibrosis score in a Chinese population with low prevalence of advanced fibrosis. **Am J Gastroenterol** 2008 Jul;103(7):1682-8.

107. Ruffillo G, Fassio E, Alvarez E, Landeira G, Longo C, Dominguez N, et al. Comparison of NAFLD fibrosis score and BARD score in predicting fibrosis in nonalcoholic fatty liver disease. **J Hepatol** 2011 Jan;54(1):160-3.

108. Vuppalanchi R, Chalasani N. Nonalcoholic fatty liver disease and nonalcoholic steatohepatitis: Selected practical issues in their evaluation and management. **Hepatology** 2009 Jan;49(1):306-17.

109. McCullough AJ. Epidemiology of the metabolic syndrome in the USA. **J Dig Dis** 2011 Oct;12(5):333-40.

110. Wree A, Kahraman A, Gerken G, Canbay A. Obesity affects the liver - the link between adipocytes and hepatocytes. **Digestion** 2011;83(1-2):124-33.

111. Barr J, Caballeria J, Martinez-Arranz I, Dominguez-Diez A, Alonso C, Muntane J, et al. Obesity-dependent metabolic signatures associated with nonalcoholic fatty liver disease progression. **J Proteome Res** 2012 Apr 6;11(4):2521-32.

112. Hossain N, Afendy A, Stepanova M, Nader F, Srishord M, Rafiq N, et al. Independent predictors of fibrosis in patients with nonalcoholic fatty liver disease. **Clin Gastroenterol Hepatol** 2009 Nov;7(11):1224-9, 9 e1-2.

113. Uygun A, Kadayifci A, Isik AT, Ozgurtas T, Deveci S, Tuzun A, et al. Metformin in the treatment of patients with non-alcoholic steatohepatitis. **Aliment Pharmacol Ther** 2004 Mar 1;19(5):537-44.

114. Tandra S, Vuppalanchi R. Use of statins in patients with liver disease. **Curr Treat Options Cardiovasc Med** 2009 Aug;11(4):272-8.

115. Ajamieh H, Farrell G, Wong HJ, Yu J, Chu E, Chen J, et al. Atorvastatin protects obese mice against hepatic ischemia-reperfusion injury by Toll-like receptor-4 suppression and endothelial nitric oxide synthase activation. **J Gastroenterol Hepatol** 2012 Aug;27(8):1353-61.

116. Nseir W, Mograbi J, Ghali M. Lipid-lowering agents in nonalcoholic Fatty liver disease and steatohepatitis: human studies. **Dig Dis Sci** 2012 Jul;57(7):1773-81.

BIOGRAPHY

NAME Sombat Treeprasertsuk M.D., M.Sc

BIRTH DATE APRIL 5, 1967

Education and training

1991 M.D. Second class honor, Mahidol University, Faculty of Medicine, Thailand.

1997 Internal Medicine, Mahidol University, Faculty of Medicine, Thailand.

2000 Gastroenterology, Chulalongkorn University, Faculty of Medicine

2000 M.SC., Chulalongkorn University, Faculty of Medicine: Thesis title "The Prevalence of Nonalcoholic Steatohepatitis in Thai Patients with Non-HBV, Non-HCV Chronic Hepatitis"

Professional Memberships

1994 Thai Medical Council

1997 Royal College of Physicians, Thailand

1998 Gastroenterological Association of Thailand (GAT)

2005 Thai Association for Gastrointestinal Endoscopy (TAGE)

2006 American Association for the Study of Liver Diseases (AASLD; International member)

2008 American Gastroenterology Association (AGA; International member)