Identification of fatty liver with advanced fibrosis in patients with type 2 diabetes using simple fibrosis scores and electronic reminder messages: A randomized controlled trial (Short title: NAFLD-NIT-RCT)

Clinical study protocol

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1. BACKGROUND

1.1 Importance of nonalcoholic fatty liver disease

Nonalcoholic fatty liver disease (NAFLD) is currently the most common chronic liver disease worldwide. In Hong Kong, NAFLD affects 27% of the local adult population [1], and the annual incidence is 3.4% [2]. Nonalcoholic steatohepatitis (NASH), the more active form of NAFLD with hepatic necroinflammation and accelerated fibrosis, has already become the second leading indication for liver transplantation and the third leading cause of liver cancer in the United States [3]. On the other hand, NAFLD is largely asymptomatic. Compared with other chronic liver diseases, patients with liver cancer from NAFLD/NASH often present at a late stage when curative treatment cannot be offered [4]. Besides, several drugs have now entered phase 3 development for fibrotic NASH [5]. If successful, the first NASH drugs are expected to be available at the clinic in 1 to 2 years. Therefore, it is important to identify patients with advanced NAFLD for future treatment and liver cancer surveillance.

1.2 High risk of severe NAFLD in patients with type 2 diabetes

NAFLD is strongly associated with metabolic syndrome and insulin resistance. In particular, NAFLD is present in more than 50% of patients with type 2 diabetes [6]. Diabetes is also associated with the histological severity of NAFLD [7]. In a study of 6508 NAFLD patients from Japan, diabetes was associated with a 3-fold increase in the risk of liver cancer [8]. Recently, our group conducted a screening study in 1918 patients with type 2 diabetes using transient elastography (FibroScan) and showed that 18% had increased liver stiffness suspicious of advanced liver fibrosis (i.e. bridging fibrosis [F3] or cirrhosis [F4]) [9]. All these suggest that patients with type 2 diabetes represent a high-risk group worthy of case identification. However, there has been no agreed policy on how to identify patients with advanced liver disease.

1.3 Non-invasive prediction of advanced fibrosis by prediction scores

The severity of liver fibrosis is a key determinant of liver-related morbidity and mortality in NAFLD patients. In a meta-analysis of 1495 biopsy-proven NAFLD patients from 5 studies, the incidence of all-cause mortality was increased by 3.5-fold and 6.4-fold in patients with bridging fibrosis (F3) and cirrhosis (F4), respectively, compared with those without fibrosis [10]. It is therefore important to identify patients with advanced fibrosis for further monitoring and management. Although we demonstrated the role of transient elastography screening in patients with type 2 diabetes [9], the number of diabetic patients exceeds the capacity of most medical centers. Besides, since diabetic patients can develop many other complications, it is important to keep the diagnostic and referral process simple.

Using liver histology as the reference standard, various groups have derived and validated

simple scores to predict advanced liver fibrosis [11]. These scores are calculated from routine clinical and laboratory parameters and have high applicability in different clinical settings. Although the scores are not as accurate as transient elastography or magnetic resonance elastography, they have high negative predictive values in excluding advanced fibrosis and are well suited for initial assessment [12, 13]. In our own study, the area under the receiver-operating characteristics curve for detecting advanced fibrosis (F3-4) and cirrhosis (F4) was 0.74 and 0.75 for the aspartate aminotransferase-to-platelet ratio index (APRI), 0.80 and 0.81 for the Fibrosis-4 (FIB-4) index, and 0.75 and 0.80 for the NAFLD fibrosis score, respectively [13]. Importantly, patients with low fibrosis scores have very low risk of developing liver-related morbidity and mortality during long-term follow-up [14, 15].

Among the published simple scores, the FIB-4 index and NAFLD fibrosis score have been consistently shown to be the most accurate in detecting advanced fibrosis by our group and others [12, 13]. We select the FIB-4 index and APRI as initial assessment in this study because we found that the NAFLD fibrosis score is increased in the majority of diabetic patients and is indiscriminating in this population (please see section on Preliminary Data for details).

1.4 Care model based on fibrosis scores

To facilitate efficient use of medical resource and identification of NAFLD patients at risk of future liver-related complications, we envision that the care model should be simple and convenient. Our center first promoted a care model to provide structured complications screening for patients with type 2 diabetes in the 1980s. At the diabetes center, patients undergo clinical assessment and laboratory tests to detect microvascular and macrovascular complications and check if they meet metabolic treatment targets. The care model has now extended to other hospitals in Hong Kong as well as other countries [16].

Since the calculation of liver fibrosis scores only require routine clinical and biochemical parameters, it would be easy to add them as one of the assessments for diabetic patients. We therefore propose including the fibrosis scores in diabetes complication screening and using electronic reminder message to suggest physicians to refer patients with elevated fibrosis scores for specialist care or further assessments. In the era of electronic health records, automatic reminder messages can improve identification of patients for appropriate screening and management [17].

2. AIMS

We aim to test the hypothesis that the use of simple fibrosis scores as part of a diabetes complications screening program followed by electronic reminder messages is more effective

than usual care in prompting physicians to correctly identify patients with suspected NAFLD and advanced liver fibrosis for specialist referral or further liver assessment. Our secondary aim is to test the hypothesis that the use of fibrosis scores and electronic reminder messages can increase the number of patients with confirmed diagnosis of advanced liver fibrosis.

3. STUDY DESIGN OVERVIEW

This will be a parallel group, randomized controlled trial (Figure 1). Patients fulfilling the inclusion and exclusion criteria above will be randomized 1:1 to two groups. Randomization will be carried out through the use of computer-generated list of random numbers in variable blocks of 4 to 10. Concealment of group allocation will be achieved through putting the group allocation cards in consecutively-numbered and sealed envelopes. The patients and physicians will know that the patients are in the intervention group if they see the reminder messages. When they do not see a reminder message, there will not be a specific indicator of whether the patient is in the control group or in the intervention group but having low fibrosis scores. Furthermore, the outcome assessors will be blinded to the group assignment.

For patients in the intervention group, we will calculate the fibrosis scores. For patients with increased fibrosis scores, we will type the following pop-up message in our electronic clinical management system:

"This patient has high Fibrosis-4 index (and/or AST-to-platelet ratio index) of xxx suggestive of significant liver fibrosis. Please consider referring the patient to the hepatology clinic or arranging further test such as FibroScan."

The reminder message will pop up when physicians see the patient at the clinic and use the electronic clinical management system. The message will remain active for one year. Although the message is entered manually at this stage, the arrangement mimics an automated computer system. If the study results are positive, the next step is to modify the system to automate the process.

Patients in the control group will undergo the same assessments as patients in the intervention group. Although physicians will have access to the raw liver biochemistry results and platelet count, the fibrosis score results will not be specifically shown, and there will be no electronic reminder messages regardless of the fibrosis scores. This is to mimic usual care when there is no dedicated care model for case identification.

4. PATIENTS

We will recruit consecutive subjects from the Diabetes Mellitus and Endocrine Centre, Prince of Wales Hospital when they attend for diabetes complications screening.

4.1 Inclusion criteria

All subjects should fulfill all of the following:

- Age 18-70 years
- Having type 2 diabetes
- Provided informed written consent

4.2 Exclusion criteria

Subjects having any of the following will be excluded from the study:

- Type 1 diabetes
- Already receiving specialist care by gastroenterologists or hepatologists
- Current or past history of hepatocellular carcinoma or liver decompensation
- Active malignancies other than hepatocellular carcinoma, unless in complete remission for more than 5 years

Notably, although our primary focus is to identify patients with NAFLD and advanced liver fibrosis, we will not exclude patients with other chronic liver diseases such as chronic viral hepatitis or alcohol-related liver disease. In real life, although NAFLD is the most common chronic liver disease among diabetic patients, they can also have other liver diseases. It would also be meaningful to detect those diseases, especially if advanced fibrosis has already developed.

5. STUDY ASSESSMENTS

5.1 Clinical assessments

At study inclusion, all patients will undergo the following assessments:

- Record medical history including past medical history and drug history
- Record smoking and alcohol consumption using a standard questionnaire
- Measure blood pressure, body weight, body height and waist circumference; body mass index is calculated as body weight (kg) divided by body height (m) squared
- Blood tests including liver biochemistry (including alanine aminotransferase [ALT], aspartate aminotransferase [AST] and gamma-glutamyl transpeptidase [GGT]), renal function test, fasting plasma glucose, hemoglobin A1c and lipids
- Standard assessment for diabetic complications including spot urine for albumin-to-

creatinine ratio for albuminuria, retinal photography for retinopathy, foot examination including monofilament and tuning fork test for peripheral neuropathy

5.2 Fibrosis score

In this study, we will calculate the FIB-4 index using the following formula: age x AST (U/l) / platelet count (x109/l) / $\sqrt{}$ALT (U/l) [18]. APRI is calculated as AST (/upper limit of normal) / platelet count (x109/l) x 100 [19]. Table 1 lists the cutoffs for the interpretation of the fibrosis scores, with age-specific adjustments for the FIB-4 index and NAFLD fibrosis score [20].

5.3 Diagnostic workup at the hepatology clinic

Regardless of group assignment and fibrosis score results, diabetic patients referred to the hepatology clinic in this project will be seen within 12 weeks. We will perform history taking and physical examination to identify risk factors of chronic liver diseases. Viral hepatitis serology will be performed if not already done. The patients will then undergo transient elastography (FibroScan, Echosens, Paris, France) for liver stiffness measurement. Transient elastography will be performed by experienced operators under the training and instructions of the manufacturer [13]. The median of 10 liver stiffness measurements will be used to reflect the severity of liver fibrosis. Our center is equipped with the M probe and XL probe to cater for patients with different body built [21]; we will follow the machine's automatic probe selection tool. We will discuss the option of liver biopsy with patients whose diagnosis is uncertain or liver stiffness is >10 kPa suspicious of advanced fibrosis [22].

6. DATA ANALYSIS

6.1 Primary outcome

The primary outcome is the proportion of patients with high fibrosis scores who are referred for specialist care or further liver assessments within 1 year of the baseline visit. In this study, appropriate further liver assessments for advanced liver disease include transient elastography (FibroScan), shear wave elastography, acoustic radiation force impulse, magnetic resonance elastography, liver biopsy, and specific blood biomarkers of liver fibrosis such as FibroTest and FibroMeter. This primary outcome will address our primary aim to evaluate whether a care model comprising fibrosis score testing and electronic reminder messages can increase case identification and improve the diagnostic process.

6.2 Secondary outcomes

The following secondary outcomes will be analyzed:

- The proportion of patients referred for specialist care or further liver assessments, regardless of fibrosis score results.

- The proportion of patients with low fibrosis scores who are referred for specialist care. Because NAFLD is highly prevalent and only a minority of patients have advanced fibrosis, referral of patients who will unlikely develop liver-related complications to specialists represents inefficient use of precious healthcare resource and should be minimized. This notion has major resource implications, and the current study will provide important information to guide healthcare policy. We recognize that physicians may have other reasons to refer patients for specialist care (e.g. newly diagnosed viral hepatitis). The reasons for referral will be recorded and reported.
- The proportion of patients confirmed to have advanced fibrosis. In this study, a patient is considered to have confirmed advanced fibrosis if (1) liver stiffness measurement by transient elastography is >15 kPa [22], (2) a liver biopsy shows bridging fibrosis (F3) or cirrhosis (F4), (3) unequivocal radiological features of cirrhosis (cirrhosis with nodular appearance, splenomegaly, ascites or varices), or (4) clinical, radiological or endoscopic evidence of portal hypertension. In case of discrepant results, liver biopsy and unequivocal evidence of cirrhosis and/or portal hypertension will override the liver stiffness measurement results.

6.3 Outcome ascertainment

Two researchers will review the clinical records of all patients for evidence of specialist referral or arrangement of further liver assessments. They will also use the electronic clinical management system to look for new specialist clinic appointments and radiological test arrangements. The researchers will be blinded to the group assignment. In case of discrepant interpretation, a third assessor will study the case and discuss with the other two assessors to come up with a consensus on whether a patient has specialist referral, further liver assessments, and advanced fibrosis.

6.4 Statistical analysis

We will perform intention-to-treat analysis for all primary and secondary outcomes. All randomized patients will be analyzed for the outcomes. Patients who are lost to follow-up will be considered not to have specialist referral, further liver assessments, and confirmed advanced fibrosis. Statistical significance is taken as a two-sided p value of <0.05.

Primary outcome

The primary outcome includes patients with high fibrosis scores in both groups and is calculated as the number of patients with specialist referral or further liver assessments divided by the total number of patients. The proportions in the two study groups will be compared by chi-square test.

Secondary outcomes

The following secondary outcomes will all be compared by chi-square test.

- The proportion of patients with specialist referral or further liver assessments, regardless of fibrosis score results, is calculated as the number of patients with specialist referral or further liver assessments divided by the total number of patients.
- Among patients with low fibrosis scores, the proportion of patients with inappropriate specialist referrals is calculated as the number of patients referred for specialist care divided by the total number of patients.
- The proportion of patients with confirmed advanced fibrosis is calculated as the number of patients with confirmed advanced fibrosis divided by the total number of patients.

6.5 Sample size calculation

According to our preliminary data (see below), 33% of patients with type 2 diabetes had increased FIB-4 index suspicious of advanced fibrosis, and 10% had increased APRI suspicious of significant fibrosis. In our previous screening study using transient elastography, only around 10% of diabetic patients with advanced liver fibrosis were under specialist care [9]. To be conservative, we assume that 20% of patients with high fibrosis scores in the control group will be referred for specialist care or further liver assessments. We also decide that the care model would be clinically meaningful if it can at least increase the referral rate to 37% in the intervention group. To detect this difference at a 5% significance level and 80% power, we need 107 patients with high fibrosis scores per group. Using a more conservative estimation of 25% of patients having increased fibrosis scores, a total of 856 patients is needed for this study. Assuming a dropout rate of 10%, the final sample size is 952 patients (476 per arm). The assumption on dropout rate is realistic as all patients should have been on regular follow-up and we will only be observing the referral pattern and linkage to care in 1 year.

6.6 Preliminary data

To facilitate sample size calculation and study design, we prospectively evaluated 612 patients who underwent diabetic complications screening at our center, of whom 599 had complete laboratory data for fibrosis score calculation. Elevated FIB-4 index, APRI and NAFLD fibrosis score was observed in 33%, 10% and 68% of patients, respectively. In previous studies by our group and others, the FIB-4 index and NAFLD fibrosis score had higher accuracy for advanced fibrosis than other simple scores when liver histology was used as the reference standard [12, 13]. On the other hand, hyperglycemia is an important component of the NAFLD fibrosis score [14]. As a result, the majority of diabetic patients would have increased NAFLD fibrosis score, rendering this score indiscriminating and not suitable for the triage of diabetic patients. Therefore, the FIB-4 index and APRI are chosen as the initial assessment in this study.

7. ETHICS

7.1 Ethics review

The final study protocol, including the final version of the Informed Consent Form, must be approved in writing by the Joint The Chinese University of Hong Kong – New Territories East Cluster Clinical Research Ethics Committee (CUHK-NTEC CREC). The protocol must be re-approved by the CREC annually. All subsequent protocol amendments must be approved by the CREC.

7.2 Ethical conduct of the study

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with ICH/GCP, applicable regulatory requirements and the CREC.

7.3 Informed consent

The investigator(s) will ensure that the subject is given full and adequate oral and written information about the nature, purpose, possible risk and benefit of the study. The informed consent forms are available in traditional Chinese. Subjects must also be notified that they are free to discontinue from the study at any time. The subject should be given the opportunity to ask questions and allow time to consider the information provided. The subject's signed and dated informed consent must be obtained before conducting any procedure specific for the study. The Principal Investigator must store the original, signed Informed Consent Forms. One copy of the signed Informed Consent Form must be given to the subject. If modifications are made according to local requirements, the new version has to be approved by the CREC.

8. ADVERSE EVENT REPORTING

The adverse event reporting period for this study is from signing the informed consent through 1 year of follow-up.

8.1 Adverse events

An adverse event is any undesirable medical event occurring in the subject within the trial period, whether or not it is related to the study intervention.

The severity of an adverse event is defined as:

Mild: Transient symptoms, no interference with the subject's daily activities

Moderate: Marked symptoms, moderate interference with the subject's daily activities

Severe: Severe interference with the subject's daily activities.

The relationship of an adverse event to the study intervention is defined as:

Probable: Good reasons and sufficient documentation to assume a causal relationship

Possible: A causal relationship is conceivable and cannot be dismissed

Unlikely: The event is unlikely related to the study intervention

A telephone enquiry hotline for reporting subject's adverse events is available. The investigator will record all relevant information including signs and symptoms of the event, the onset time, the date of the event, the laboratory findings, concomitant drugs, and the final outcome. All adverse events will be followed up until we have reached a defined outcome of the event, which can be one of the followings: (1) recovered with sequelae (for chronic conditions), (2) recovered, or (3) the management of the adverse event is taken over by another physician when the study ends.

A clinical laboratory adverse event is any clinical laboratory abnormality that suggests a disease and/or organ toxicity is of sufficient severity that requires active intervention (i.e. change of dose, discontinuation of drug, more frequent follow-up or further investigation).

8.2 Serious adverse events

A serious adverse event is an adverse event that results in one of the following outcomes:

- Death
- Life-threatening
- In-patient hospitalization or prolongation of existing hospitalization
- A persistent or significant disability or incapacity
- A congenital anomaly or birth defect

The definitions of causal relationship to study intervention are the same as those for adverse events. We have a 24-hour on-call system to handle serious adverse events. The investigator will assess and treat the subjects as soon as possible. A standard serious adverse event form will be used (provided by the CREC at http://intranet.ccter.cuhk.edu.hk/sae/) to report the events within 24 hours after acknowledgement. We will arrange unscheduled follow-up visits immediately or within 24 hours on receiving the subject's self-report of serious adverse events after the subjects have been discharged or if the subject has already been admitted to the hospital. Our investigators will assess the subject within 24 hours. The study team will record all relevant information including signs and symptoms of the event, the onset time, the date of the event, the laboratory findings, concomitant drugs, and the final outcome. A follow-up serious adverse event form will be sent within 14 days after submitting the initial serious adverse event form. Serious adverse events related to the study drug will be followed up until the subject has "recovered", "recovered with sequelae" or "died". SAE reports will be sent to

our ethics committee. An unscheduled visit will be performed if subjects have to withdraw early from the study.

9. References

1. Farrell GC, Larter CZ. Nonalcoholic fatty liver disease: from steatosis to cirrhosis. Hepatology 2006;43:S99-S112.
2. Wong VW, Chan HL, Hui AY, et al. Clinical and histological features of non-alcoholic fatty liver disease in Hong Kong Chinese. Aliment Pharmacol Ther 2004;20:45-49.
3. Wong VW, Wong GL, Chim AM, et al. Validation of the NAFLD fibrosis score in a Chinese population with low prevalence of advanced fibrosis. Am J Gastroenterol 2008;103:1682-1688.
4. Hui AY, Wong VW, Chan HL, et al. Histological progression of non-alcoholic fatty liver disease in Chinese patients. Aliment Pharmacol Ther 2005;21:407-413.
5. Caldwell SH, Oelsner DH, Iezzoni JC, Hespenheide EE, Battle EH, Driscoll CJ. Cryptogenic cirrhosis: clinical characterization and risk factors for underlying disease. Hepatology 1999;29:664-669.
6. Wong VW, Hui AY, Tsang SW, et al. Metabolic and adipokine profile of Chinese patients with nonalcoholic fatty liver disease. Clin Gastroenterol Hepatol 2006;4:1154-1161.
7. Targher G, Bertolini L, Rodella S, et al. Nonalcoholic fatty liver disease is independently associated with an increased incidence of cardiovascular events in type 2 diabetic patients. Diabetes Care 2007;30:2119-2121.
8. Wong VW, Hui AY, Tsang SW, et al. Prevalence of undiagnosed diabetes and postchallenge hyperglycaemia in Chinese patients with non-alcoholic fatty liver disease. Aliment Pharmacol Ther 2006;24:1215-1222.
9. Belfort R, Harrison SA, Brown K, et al. A placebo-controlled trial of pioglitazone in subjects with nonalcoholic steatohepatitis. N Engl J Med 2006;355:2297-2307.
10. Nissen SE, Wolski K. Effect of rosiglitazone on the risk of myocardial infarction and death from cardiovascular causes. N Engl J Med 2007;356:2457-2471.
11. Chan HL, de Silva HJ, Leung NW, et al. How should we manage patients with non-alcoholic fatty liver disease in 2007? J Gastroenterol Hepatol 2007;22:801-808.
12. Chan HL, Wong VW. Can dietetic intervention for obesity ever succeed in real life? J Gastroenterol Hepatol 2007;22:459-460.
13. Ludwig DS. Clinical update: the low-glycaemic-index diet. Lancet 2007;369:890-892.
14. Woo J, Sea MM, Tong P, et al. Effectiveness of a lifestyle modification programme in weight maintenance in obese subjects after cessation of treatment with Orlistat. J Eval Clin Pract 2007;13:853-859.
15. Johnson NA, Walton DW, Sachinwalla T, et al. Noninvasive assessment of hepatic lipid

composition: Advancing understanding and management of fatty liver disorders. Hepatology 2008;47:1513-1523.

16. Chu WC, Lam WW, Lee KH, Yeung DK, Sihoe J, Yeung CK. Phosphorus-31 MR spectroscopy in pediatric liver transplant recipients: a noninvasive assessment of graft status with correlation with liver function tests and liver biopsy. AJR Am J Roentgenol 2005;184:1624-1629.
17. Wong VW, Wong GL, Chim AM, et al. Combination of the median and variability of liver stiffness measurements by transient elastography improves the accuracy of diagnosing advanced fibrosis in nonalcoholic fatty liver disease. J Hepatol 2008;48(Suppl 2):S367.
18. Prati D, Taioli E, Zanella A, et al. Updated definitions of healthy ranges for serum alanine aminotransferase levels. Ann Intern Med 2002;137:1-10.
19. Woo J, Leung SS, Ho SC, Lam TH, Janus ED. A food frequency questionnaire for use in the Chinese population in Hong Kong: Description and examination of validity. Nutr Res 1997;17:1633-1641.
20. Hamaguchi M, Kojima T, Takeda N, et al. The metabolic syndrome as a predictor of nonalcoholic fatty liver disease. Ann Intern Med 2005;143:722-728.
21. Wong VW, Irles M, Wong GL, et al. Unified interpretation of liver stiffness measurement by M and XL probes in non-alcoholic fatty liver disease. Gut 2019;68:2057-2064.
22. de Franchis R, Baveno VI Faculty. Expanding consensus in portal hypertension: Report of the Baveno VI Consensus Workshop: Stratifying risk and individualizing care for portal hypertension. J Hepatol 2015;63:743-52.