PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Identification of patients with type 2 diabetes with non-alcoholic fatty liver disease who are at increased risk of progressing to advanced fibrosis: A cross-sectional study
AUTHORS	Mettananda, Chamila; Egodage, Thimira; Dantanarayana, Channaka; Fernando, Rumal; Ranaweera, Lakmali; Luke, Nathasha; Ranawaka, Chamila; Kottahachchi, Dulani; Pathmeswaran, Arunasalam; de Silva, Hithanadura; Dassanayake, Anuradha

VERSION 1 – REVIEW

REVIEWER	Ciardullo, Stefano University of Milan–Bicocca, Medicine and Surgery
REVIEW RETURNED	30-Jul-2022

GENERAL COMMENTS	In the present manuscript the authors report a protocol for a cross-sectional study aimed to investigate ways to identify advanced fibrosis related to NAFLD in patients with type 2 diabetes. The topic is certainly of interest given that the most effective way of identifying patients at higher risk of liver related events is debated in the literature. I have the following comments: 1. Please do not use the term “diabetics”, but change it to “patients with diabetes”, both in the title and within the manuscript 2. In order to set the stage for what data you expect to find, I suggest referring to a recent meta-analysis reporting the prevalence of elevated liver stiffness measured by VCTE in patients with type 2 diabetes (doi: 10.1016/j.diabres.2022.109981). 3. Arrows in Figure 1 should also go in the opposite direction as NASH resolution or regression of fibrosis is quite common in patients with NAFLD. 4. I would reconsider the cut-offs shown in figure 2, as the authors use a different cut-off (8.0 kPa) in their study 5. Please be aware that LSM>8.0 is not a surrogate marker of NASH, but it only estimates fibrosis. Please amend 6. Please describe whether presence of HBV and HCV will be checked in all patients with blood tests or whether exclusion will only be based on patients’ history 7. 10 parameters in the model, for a total of 60 patients with fibrosis seem too many and there is a risk of overfitting; a rule of thumb would be to include 1 parameter for every 10 patients with advanced fibrosis.

REVIEWER	Devaraj, Navin Kumar Universiti Putra Malaysia Fakulti Perubatan dan Sains Kesihatan, Family Medicine
REVIEW RETURNED	08-Oct-2022

GENERAL COMMENTS	Overall a good study design. Suggest a few additions: 1. In discussion can state how the authors plan to fund the study as it will use an expensive scan 2. is the calculated sample size of 398 pts include a possible 20% non response rate? 3. will any questionnaire be used to capture important data such as socio-demography, medical history and habits such as alcohol consumption 4.can mention in limitation, that study has started at recruitment stage

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Dr Stefano Ciardullo, University of Milan–Bicocca
Comments to the Author:
In the present manuscript the authors report a protocol for a cross-sectional study aimed to investigate ways to identify advanced fibrosis related to NAFLD in patients with type 2 diabetes. The topic is certainly of interest given that the most effective way of identifying patients at higher risk of liver related events is debated in the literature.
I have the following comments:
1. Please do not use the term “diabetics”, but change it to “patients with diabetes”, both in the title and within the manuscript
Revised.
2. In order to set the stage for what data you expect to find, I suggest referring to a recent meta-analysis reporting the prevalence of elevated liver stiffness measured by VCTE in patients with type 2 diabetes (doi: 10.1016/j.diabres.2022.109981).
Thank you for the information. We estimated the sample size considering the prevalence of liver fibrosis as 15% according to Lomonaco et al[1]. The reference you have suggested is of a very recent publication in August 2022 which is after we started the study. Since we had already started the study and obtained ethical approval for the protocol, we are not in a position to change this. Hope you would understand our position.

3. Arrows in Figure 1 should also go in the opposite direction as NASH resolution or regression of fibrosis is quite common in patients with NAFLD.
Revised
4. I would reconsider the cut-offs shown in figure 2, as the authors use a different cut-off (8.0 kPa) in their study. …..
An LSM value between 7.1-9.4 kPa is the stage of significant fibrosis (F2) which has the potential for progression to advanced fibrosis. The current consensus guideline in Nonalcoholic Fatty Liver Disease Screening in Type 2 Diabetes Mellitus Patients in the Primary Care Setting is to refer patients with a VCTE ≥8 kPa to a liver-specialized centre for further management. Therefore, we selected LSM 8 as the cut-off for differentiating having significant fibrosis[2-4].

5. Please be aware that LSM>8.0 is not a surrogate marker of NASH, but it only estimates fibrosis. Please amend
Thank you. We have corrected this in R1 (lines 90-91, 145)
.
6. Please describe whether presence of HBV and HCV will be checked in all patients with blood tests or whether exclusion will only be based on patients’ history
Hepatitis B and C will not be checked in all patients due to two reasons.
1. Sri Lanka has a very low prevalence of Hepatitis B and C. A study done on 81 patients with cirrhosis who were referred for liver transplantation, none had hepatitis B or C[5]. A single-centre retrospective study involving 696 patients with cirrhosis found only 13 (1.87%) patients had chronic hepatitis B infection6. The prevalence of hepatitis C infections in a high-risk sample of prison

inmates was only 0.5%[7] and B and therefore the chance of prevalence of those is almost zero in this sample
2. Furthermore we did not do Hep B and C tests due to financial constraints.

7. 10 parameters in the model, for a total of 60 patients with fibrosis seem too many and there is a risk of overfitting; a rule of thumb would be to include 1 parameter for every 10 patients with advanced fibrosis.
We followed Riley et al. in sample size calculation and predictor identification [8] . According to Riley et al. the rule of thumb of 10 events per variable need not be strictly followed. Since we are looking at several possible risk factors for liver fibrosis in our questionnaire, we decided to use 10 as the number of risk associations. However, as you pointed out these risk factors could be overlapping with each other and could even be condensed to fewer variables depending on the weightage of associations we find at the final analysis.

Reviewer: 2
Dr Navin Kumar Devaraj, University Putra Malaysia Fakulti Perubatan dan Sains Kesihatan
Comments to the Author:
Overall a good study design. Suggest a few additions:
1. In discussion can state how the authors plan to fund the study as it will use an expensive scan
There is no funding for this study. Patients will undergo fibr0scanning free of charge at the North Colombo teaching hospital, which is the only state hospital with a fibro scanner in Sri Lanka.
2. is the calculated sample size of 398 pts include a possible 20% non-response rate? No, this is the total sample size. We expect the non-response rate to be negligible. We planned to collect data from all the patients at enrolment and therefore the only instance of possible non-response rate is when they are asked to come for fiber scanning. However, since this is a very expensive scan done free of charge and the fact that this is the only place in Sri Lanka to have it free of charge we expected all patients to attend for FibroScanning without fail and the same was observed up to now.

3. will any questionnaire be used to capture important data such as socio-demography, medical history, and habits such as alcohol consumption
Yes all these are included in our questionnaire
4. can mention in the limitation, that study has started at the recruitment stage
Thank you. We may consider it

Reviewer: 1
Competing interests of Reviewer: None

Reviewer: 2
Competing interests of Reviewer: None

1 Lomonaco, R. *et al.* Advanced Liver Fibrosis Is Common in Patients With Type 2 Diabetes Followed in the Outpatient Setting: The Need for Systematic Screening. *Diabetes care* **44**, 399-406 (2021). https://doi.org:10.2337/dc20-1997
2 Vieira Barbosa, J. & Lai, M. Nonalcoholic Fatty Liver Disease Screening in Type 2 Diabetes Mellitus Patients in the Primary Care Setting. *Hepatology Communications* **5**, 158-167 (2021). https://doi.org:https://doi.org/10.1002/hep4.1618
3 Cusi, K. *et al.* American Association of Clinical Endocrinology Clinical Practice Guideline for the Diagnosis and Management of Nonalcoholic Fatty Liver Disease in Primary Care and Endocrinology Clinical Settings: Co-Sponsored by the American Association for the Study of Liver Diseases (AASLD). *Endocrine Practice* **28**, 528-562 (2022). https://doi.org:https://doi.org/10.1016/j.eprac.2022.03.010
4 Mózes, F. E. *et al.* Diagnostic accuracy of non-invasive tests for advanced fibrosis in patients with NAFLD: an individual patient data meta-analysis. *Gut* **71**, 1006-1019 (2022). https://doi.org:10.1136/gutjnl-2021-324243
5 Wijewantha, H. S. Liver Disease in Sri Lanka. *Euroasian J Hepatogastroenterol* **7**, 78-81 (2017). https://doi.org:10.5005/jp-journals-10018-1217
6 Senanayake, S. M. *et al.* Survival of patients with alcoholic and cryptogenic cirrhosis without liver transplantation: a single center retrospective study. *BMC Res Notes* **5**, 663 (2012). https://doi.org:10.1186/1756-0500-5-663

7 Niriella, M. A. *et al.* Prevalence of hepatitis B and hepatitis C infections and their relationship to injectable drug use in a cohort of Sri Lankan prison inmates. *Ceylon Med J* **60**, 18-20 (2015). https://doi.org:10.4038/cmj.v60i1.7288
8 Riley, R. D. *et al.* Calculating the sample size required for developing a clinical prediction model. *BMJ (Clinical research ed.)* **368**, m441 (2020). https://doi.org:10.1136/bmj.m441

VERSION 2 – REVIEW

REVIEWER	Ciardullo, Stefano University of Milan–Bicocca, Medicine and Surgery
REVIEW RETURNED	24-Nov-2022

GENERAL COMMENTS	Thank you for the thorough revision. I have no further comments.

REVIEWER	Devaraj, Navin Kumar Universiti Putra Malaysia Fakulti Perubatan dan Sains Kesihatan, Family Medicine
REVIEW RETURNED	21-Nov-2022

GENERAL COMMENTS	Tq for the hard work in addressing all the comments well. The article is much polished now.