Title — Fibrosis nonalcoholic steatohepatitis index validation and applicability considering glycemic severity and T2D duration

Short Title— FNI in Type 2 diabetes

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Grant Support— Sociedade Portuguesa de Diabetologia, Fundação para a Ciência e a Tecnologia (PTDC/MEC-MET/29314/2017; UIDB/Multi/04462/2020), mtFoieGras (Marie Sklodowska-Curie Grant Agreement No. 734719).

Abbreviations

FNI, Fibrotic NASH Index;

NAFLD, Non-Alcoholic Fatty Liver Disease;

NASH, Non-Alcoholic Steatohepatitis;

HRQoL, Health-related quality of life

T2D, type 2 diabetes

Disclosures — The authors declare no conflict of interest.

Data Transparency Statement— Our data, analytic methods, and study materials are available upon request, by contacting our corresponding author.

Ethics statement

All participants gave written informed consent to the study, that has been approved by the APDP Ethics Committee and was conducted in accordance with the principles of the Declaration of Helsinki.

Abstract

Nonalcoholic fatty liver disease (NAFLD) diagnosis without using invasive methods is extremely challenging, highlighting the need of simple indexes for this end. Recently, fibrotic non-alcoholic steatohepatitis index (FNI) was developed and proposed as an affordable non-invasive score calculated with aspartate aminotransferase, high-density lipoprotein cholesterol, and hemoglobin A1c. Herein, and given the link between NAFLD and diabetes, we aimed at validating FNI in a population with type 2 diabetes (T2D), also considering diabetes duration and glycemic severity. The performance of FNI was higher than FIB-4 (AUROC = 0.89 vs 0.67, respectively). Additionally, using 0.1 as the rule-out cut-off of FNI, the sensitivity was 0.99, and the positive predictive value was 0.19. Both duration of diabetes and A1c did not impact FNI performance. In sum, FNI is a valuable score for predicting fibrotic non-alcoholic steatohepatitis not only for primary care units but also for diabetes specialized care.

Nonalcoholic fatty liver disease (NAFLD) encompasses a broad spectrum of conditions, including steatosis, fibrosis, and cirrhosis, whose diagnosis is extremely challenging. The consequent substantial healthcare costs, economic losses, and diminished health-related quality of life (HRQoL) bring into play the need for a global model of care.[1] As the prevalence of NAFLD is higher in people living with diabetes, it is crucial to have NAFLD diagnosis scores that can be easy to use and precise to be endorsed for this specific population.[2] Recently, Tavaglione et al. proposed the Fibrotic NASH Index (FNI), an affordable non-invasive score calculated with aspartate aminotransferase, high-density lipoprotein cholesterol, and hemoglobin A1c.[3] The authors emphasised that FNI validation in a population with type 2 diabetes (T2D) is highly relevant as the goal of the score is to be easily used not only in primary care units but also in diabetes clinical settings overcoming the difficulty in identifying silent pathologies in a patient-centred diabetes care.

For the validation of FNI in a population with T2D, 553 subjects were recruited at a Diabetes clinic (APDP). Besides routine blood sampling and biochemical analysis, fatty liver was evaluated by transient elastography (Fibroscan®). FAST™ score was calculated and individuals with FAST™ score >0.35 were considered at risk of fibrotic NASH[4]. FNI was calculated as previously described.[3]

Performance of FNI score was assessed by the area under the receiver operating characteristic curve (AUROC). AUROCs were compared using the DeLong test. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were computed considering the rule-out cut-off

of 0.1. Statistical analyses were performed using the R (R Foundation for Statistical Computing, Vienna, Austria).

For further application of FNI in an European/Portuguese population, PREVADIAB2 cohort, which has been described previously[5]; briefly, 1088 subjects that did not had T2D (IDF/WHO criteria[6]) 5 years before were recruited. After preprocessing the data and excluding missing values, 985 individuals were included and FNI was calculated.

All participants gave written informed consent to the study, that has been approved by the APDP Ethics Committee and was conducted in accordance with the principles of the Declaration of Helsinki.

Of the 553 well phenotyped subjects with diabetes enrolled in the validation cohort, 42% were women and the median age was 66 years with median duration of T2D of 13 years. 73% presented steatosis and 47% had fibrosis, detected by transient elastography (Supplementary Table 1). 17% of the subjects had FAST™ score >0.35 (96 subjects) and 13% of the individuals had FNI ≤0.10 (Fig. 1A). When comparing the performance of FNI with FIB-4, one of the most used scores for liver fibrosis, AUC for FNI was significantly higher than FIB-4 (0.89 vs 0.67; $p<0.001$) (Fig. 1B). Additionally, using 0.1 as the cut-off of FNI, the sensitivity was 0.99, and PPV=0.19 (Fig. 1C; Supplementary Table 2). We further accessed if FNI performance is affected by the duration of diabetes and glycemic severity/variability (low versus high HbA1c). Indeed, having T2D for more than 10 years, or for 10 years or less had a comparable AUC (0.881 vs. 0.897, respectively; Fig. 1D) and the same pattern was observed with higher versus lower than 8% HbA1c (0.918 vs. 0.908, respectively; Fig. 1E).

To understand the impact of the use of the FNI in the general population we used an European/Portuguese population-based cohort, of which 60% were women. After performing a 2h OGTT and according to IDF/WHO guidelines for dysglycemia, 72% of the individuals had normoglycemia, 22% prediabetes and 6% had T2D (Supplementary Table 1). Regarding the overall population, the FNI ruled out 566 individuals (Fig. 1F). From these, 454 had normoglycemia, 93 had prediabetes and 19 had T2D (Fig. 1F).

Non-alcoholic fatty liver disease (NAFLD) affects approximately 25% of the worldwide population and its prevalence is even higher in individuals within metabolic diseases such as T2D.[7] The current gold standard for the diagnosis and staging of NAFLD is liver biopsy. However, considering its costs, possible complications and invasiveness, liver biopsy is not usually considered for screening thus the development of precise and simple ways of screening is highly relevant. Indeed, the non-invasive assessment of liver fibrosis overcomes some of the limitations of the biopsy. Several scores have been developed in the last few years and are now being used in the clinical practice (e.g., FIB-4 and FAST™ score[4,8]). Recently, Tavaglione et al. developed the FNI score, that differently from FIB-4, was specifically developed to identify fibrotic NASH in people at high risk for NAFLD within a dysmetabolic profile.[3] Moreover, it only needs AST, HDL and HbA1c, it is an unexpensive and easy index to be used both in primary care and specialized clinics for a better diagnosis of fibrotic NASH.

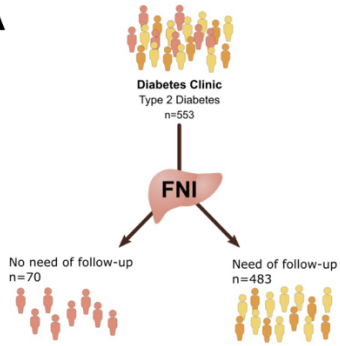
Herein, we aimed at validating the FNI in a population with T2D taking in account disease duration and glycemic severity. 13% of the individuals in the validation T2D cohort had a FNI ≤0.1, inferior to what was described in the

derivation and external validation cohorts of the previous study.[3] This highlights the relevance to screening individuals with T2D at the primary healthcare for NASH. On the contrary, in the general population cohort, 57% had a FNI ≤0.1 which is in accordance with the previous study and highlights the importance and relevance of this index. In fact, the use of FNI in the general population would allow to identify individuals that need to pursue further exams (e.g., Fibroscan and liver biopsy). The decrease to 13% when the population was recruited in a specialized diabetes clinic, identify FNI as highly relevant as it performed better than FIB-4. These results parallel the FNI performance found in Tavaglione et al. when they compared it to Fibroscan and to liver biopsy. In sum, we validated that FNI is an affordable and easy score for fibrotic NASH in individuals with T2D independently of diabetes duration and severe hyperglycemia.

References

1. Lazarus JV, et al. Nat Rev Gastroenterol Hepatol 2022; 19: 60-78.
2. Younossi ZM, et al. J Hepatol 2019; 71: 793-801.
3. Tavaglione F, et al. Clin Gastroenterol Hepatol 2022.
4. Newsome PN, et al. The Lancet Gastroenterology & Hepatology 2020; 5: 362-373.
5. Pina AF, et al. Journal of Clinical Medicine 2020; 9: 2588.
6. World Health O, et al.; World Health Organization: Geneva, 2006.
7. Vanni E, et al. Semin Liver Dis 2015; 35: 236-249.
8. Abdelmalek MF. Nat Rev Gastroenterol Hepatol 2021; 18: 85-86.

A



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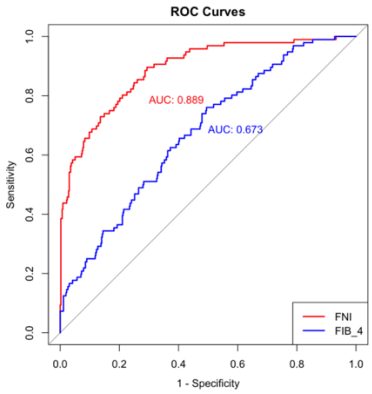

C

	FAST score >0.35	FAST score ≤0.35		
FNI >0.1	95	388	Sen = 0.99 Spe = 0.15	PPV = 0.19 NPV = 0.99
FNI ≤0.1	1	69		
FIB-4 ≥1.3	63	188	Sen = 0.66 Spe = 0.59	PPV = 0.25 NPV = 0.89
FIB-4 <1.3	33	269		

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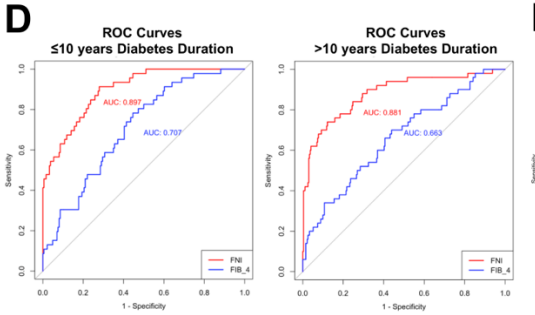

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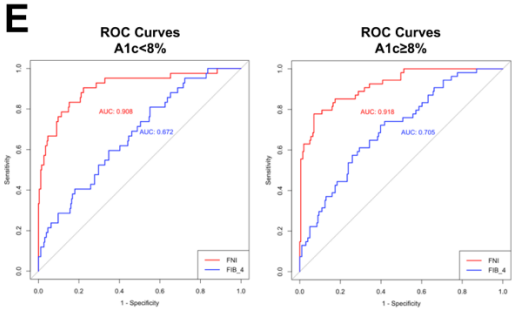

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	Total Population	FNI ≤ 0.1	FNI > 0.1
Normoglycemia	713 (72%)	454 (46%)	259 (26%)
Prediabetes	214 (22%)	93 (10%)	121 (12%)
Diabetes	58 (6%)	19 (2%)	39 (4%)

Figure 1. A – Use of fibrotic NASH index (FNI) in a population with type 2 diabetes; **B** – ROC curves for fibrotic NASH by FNI and FIB-4 in Type 2 Diabetes cohort (n=553); **C** – Diagnostic Performance of FNI and FIB-4 for Fibrotic NASH; **D** – ROC curves for fibrotic NASH by FNI and FIB-4 in Type 2 Diabetes cohort (n=553) divided by diabetes duration; **E** – ROC curves for fibrotic NASH by FNI and FIB-4 in Type 2 Diabetes cohort (n=553) divided by A1c levels; **F** – Use of FNI in a European/Portuguese population.

Supplementary Data

Supplementary Table 1. Clinical Characteristics of the Cohorts

Clinical Data	Type 2 Diabetes (Validation Cohort)	PREVADIAB2 (General Population Cohort)
n	553	985
Women, n (%)	232 (42)	590 (60)
Age (years)	66 [59, 72]	62 [53, 70]
BMI (Kg/m^2)	29 [27, 32]	27 [24, 30]
Waist circumference (cm)	99 [92, 108]	96 [89, 102]
Type 2 Diabetes		
Normoglycemia, n (%)	0 (0)	713 (72)
Prediabetes, n (%)	0 (0)	214 (22)
Diabetes, n (%)	985 (100)	58 (6)
Diabetes duration (years)	13 [6,20]	NA
Diabetes complications, n (%)	174 (31)	NA
Metabolic profile		
HbA1c, %	7.9 [6.9, 9.2]	5.5 [5.2, 5.8]
Cholesterol, mg/dL HDL	45 [38, 53]	52 [44, 61]
Cholesterol, mg/dL LDL	114 [92, 139]	135 [116, 158]
Cholesterol, mg/dL	169 [146, 199]	198 [176, 225]
Triglvcerides, mg/dL	156 [111, 219]	103 [78, 138]
Liver function		
ALT, U/L	21 [16, 32]	21 [16, 27]
AST, U/L	20 [17, 26]	23 [20, 27]
GGT, U/L	28 [19, 43]	22 [16, 33]
Platelets, 10^3/uL	230 [190, 271]	NA
Fibroscan		**NA**
Steatosis, n (%)	401 (73)	
F0, n (%)	291 (53)	

F1, n (%)	128 (23)	
F2, n (%)	46 (8)	
F3, n (%)	45 (8)	
F4, n (%)	43 (8)	
FAST Score	0.097 [0.043, 0.242]	**NA**
Rule in (>0.67), n (%)	30 (5)	
Rule out (<0.35), n (%)	457 (82) (96 - 17)	
FIB-4 score	1.23 [0.95, 1.69]	**NA**
Rule in (>1.3), n (%)	251 (45)	

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