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ORIGINAL ARTICLE

Prevalence of hepatic steatosis in patients with type 2 diabetes and response to glucose-lowering treatments. A multicenter retrospective study in Italian specialist care

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Abstract

Aim Type 2 diabetes (T2D) is a risk factor for metabolic dysfunction-associated fatty liver disease (MAFLD), which is becoming the commonest cause of chronic liver disease worldwide. We estimated MAFLD prevalence among patients with T2D using the hepatic steatosis index (HSI) and validated it against liver ultrasound. We also examined whether glucose-lowering medications (GLM) beneficially affected HSI.

Methods We collected data from 46 diabetes clinics ($n = 281{,}381$ T2D patients), extracted data to calculate HSI and validated it against ultrasound-detected hepatic steatosis. We then examined changes in HSI among patients with a follow-up visit within 1 year after initiating newer GLMs.

Results MAFLD (defined by HSI > 36, i.e., a high probability of steatosis) was present in 76.3% of the 78,895 included patients, while only 2.7% had HSI < 30 (low probability of steatosis). After age- and sex-adjusting, higher HSI was associated with higher prevalence of chronic kidney disease (odds ratio 1.35; 95%CI 1.22–1.51) and macroangiopathy (odds ratio 1.18; 95%CI 1.07–1.30). Among 2,179 subjects in the validation cohort, the prevalence of MAFLD was 67.8% and was greater in those with high HSI. Performance of HSI for ultrasound-detected MAFLD was moderate (AUROC 0.70), yet steatosis prevalence was > threefold higher among subjects with HSI > 36 than among those with HSI < 30. Notably, HSI declined significantly ~ 6 months after initiation of dapagliflozin or incretin-based therapies, but not gliclazide.

Conclusion About three quarters of patients with T2D have HSI values suggestive of MAFLD, a condition associated with macroangiopathy and nephropathy. Treatment with dapagliflozin or incretin therapies might improve MAFLD in T2D.

Keywords Ultrasonography · Validation · Biomarkers · SGLT2 · GLP-1RA · DPP4

Introduction

Type 2 diabetes (T2D) and obesity are strongly associated with the development of non-alcoholic fatty liver disease (NAFLD) [1], which has become the most common cause of chronic liver diseases worldwide in both individuals with and without T2D. Indeed, with the progressive global eradication of viral hepatitis [2], it is predicted that most cases of cirrhosis and hepatocellular carcinoma will be driven by NAFLD in the near future.

In addition, in people with T2D, the presence of NAFLD identifies those patients who are at higher risk for future adverse cardiovascular outcomes [3]. Microangiopathy, including diabetic retinopathy and chronic kidney disease (CKD), is also more prevalent amongst patients with T2D and NAFLD [4]. Conversely, growing evidence also suggest

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that NAFLD is associated with increased risk of developing new-onset T2D [5], supporting the existence of a strong, bidirectional relationship between these two conditions.

To date, a considerable scientific interest has been raised on the possibility that newer glucose-lowering medications (GLM) targeting various metabolic aspects of T2D [such as the sodium-glucose transport protein-2 (SGLT2) inhibitors or glucagon-like peptide-1 receptor agonists (GLP-1RA)] could exert beneficial effects on NAFLD [6].

In view of the extensive liver-related and cardio-metabolic consequences of NAFLD [7], recent international guidelines recommended that all patients with T2D should be screened for the presence of hepatic steatosis (mostly by using liver ultrasound) [8]. However, for large-scale screening, such as in the setting of T2D outpatient clinics, non-invasive biomarkers of liver steatosis and fibrosis might be the preferred first-line diagnostic tests, because the availability and costs of ultrasonography and other imaging methods could prevent mass screening [8]. Initial use of these biomarkers, even in place of liver ultrasound examination, could also aid identification of patients with T2D and NAFLD at risk for liver fibrosis, who will require specialist referral to monitor disease progression toward advanced liver disease [8, 9]. Most common and validated steatosis scores include the fatty liver index, the SteatoTest®, and the NAFLD liver fat score [10]. Recently, the Hepatic Steatosis Index (HSI) has been proposed as a simple tool for the screening of NAFLD in the general population [11]. This new index is calculated from sex, body mass index (BMI), serum aminotransferase levels, and pre-existing diabetes. To date, however, the performance and validity of HSI amongst patients with T2D has been assessed only in few studies with limited sample size [12].

In this study, we used a large database of outpatients with T2D followed under specialist care, for whom data to calculate HSI were available. We aimed to estimate the prevalence of hepatic steatosis using the HSI index and to validate this index against the presence of hepatic steatosis on ultrasonography. Finally, in a longitudinal assessment, we explored whether the use of newer GLMs (such as SGLT2 inhibitors or GLP-1RAs) may beneficially affect HSI in patients with T2D.

Material and methods

Study population

We used data from the DARWIN-T2D study, which is a retrospective, national, multicentre study conducted at 46 diabetes outpatient clinics in Italy. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Ethical Committees of all participating centers. The study used anonymous data and, based on National and International regulations, a waiver was applied to the requirement for the patient's informed consent.

The DARWIN-T2D study collected cross-sectional data of 281,381 outpatients with T2D, who were evaluated between 2016 and 2017. Details on the study design and the methods for data collection have been previously described in detail [13, 14]. For this study, we extracted information on outpatients with T2D aged 18–80 years, for whom data to estimate the HSI were available, i.e., sex, body mass index (BMI), and serum aminotransferase levels [11]; the HSI was calculated as specified in the statistical analysis section. To rule out that patients had acute liver injury, we excluded those with serum aminotransferase levels $\geq$ 500 IU/l. No other exclusion criteria were applied. We elected to use HSI, as a steatosis index, because data to calculate other steatosis biomarkers were not available in this database. In the DARWIN-T2D database, no information on daily alcohol intake and other causes of hepatic disease were recorded.

We collected information on demographics, anthropometrics, laboratory exams and comorbidities (including ICD-9 code) as previously described [13]. Briefly, for what concerns vascular complications of diabetes, presence of ischemic heart disease (IHD) was defined as a history of angina or myocardial infarction or coronary revascularization procedures. Diabetic kidney disease (DKD) was defined by the presence of CKD stage $\geq$ 3 (defined as a CKD-EPI [15] estimated glomerular filtration rate [eGFR] < 60 ml/min/1.73 m^2) and/or albumin excretion rate > 30 mg/g of creatinine (or equivalent [16]). Microangiopathy was defined as the presence of DKD, diabetic eye disease (retinopathy or maculopathy) or neuropathy (either somatic or autonomic). Previous cardiovascular disease (CVD) was defined as a history of stroke or myocardial infarction or any site revascularization. Macroangiopathy was defined as previous CVD, cerebral, coronary or peripheral atherosclerosis, even if asymptomatic.

In the DARWIN-T2D study, 17,285 patients with established T2D were identified as those initiating selected new GLMs, i.e., a SGLT-2 inhibitor (dapagliflozin), a GLP-1RA (liraglutide or exenatide once weekly), a DPP-4 inhibitor (sitagliptin, alogliptin, vildagliptin or saxagliptin), or a sulphonylurea (gliclazide). These GLM were selected in the DARWIN-T2D study for being the most appropriate controls for dapagliflozin. While pioglitazone was previously shown to exert beneficial effects on NAFLD, it was not included among antihyperglycemic drugs evaluated in the longitudinal assessment of DARWIN-T2D, and it is also generally prescribed to a minority of patients with T2D in Italy. Overall, 6,751 of such new GLM users had a follow-up examination 3 to 12 months after baseline [13, 14]. For the present analysis, we retained only those patients with T2D for whom

data to compute HSI were available both at baseline and at follow-up. Overall, age, sex, duration of diabetes, BMI and HbA1c levels were clinically similar between participants with and without data available to compute the HSI (data not shown).

MAFLD diagnosis

Recently, based on insights gained from the past two decades, an international panel of experts has taken the initiative to propose a new name and definition for NAFLD in adult individuals—that is, metabolic dysfunction-associated fatty liver disease (MAFLD) [17]. The newly proposed definition of MAFLD is based on the evidence of hepatic steatosis (detected either by blood biomarkers/scores, such as HSI, or imaging methods or biopsy) in addition to one of the following three criteria (namely overweight/obesity, presence of established T2D, or evidence of metabolic dysregulation, regardless of daily alcohol consumption and other concomitant liver diseases [17]. In accord to this proposal, our T2D patients with HSI > 36 (high probability of hepatic steatosis) are more likely to have MAFLD.

Validation of HSI against liver ultrasound

We validated HSI against the diagnosis of hepatic steatosis on ultrasound that was performed by expert physicians in a single centre of the DARWIN-T2D study network (diabetes specialist outpatient clinic of the University Hospital of Padua). We extracted electronic healthcare records on liver ultrasound examination and diagnosis of hepatic steatosis. According to the recent definition of MAFLD, we have included also those patients with presence of other causes of liver disease. Since only few patients had information on the ultrasonographic severity of hepatic steatosis, such information was not collected, and hepatic steatosis on ultrasound was diagnosed only as present or absent. We retained only patients with available data to calculate HSI close to the hepatic ultrasound date (the median time between lab exams and ultrasound evaluation was 16 days; IQR 6 to 85 days).

Statistical analysis

Continuous variables are presented as mean and standard deviation (SD) if normally distributed or as median and interquartile range (IQR) if non-normally distributed. Normal distribution was checked using the Kolmogorov–Smirnov test. Categorical variables are presented as percentages. Comparison of characteristics between the groups was performed using Student's *t* test for continuous variables and chi-squared test for categorical variables. Non-normal variables were log-transformed before analysis with parametric tests.

HSI has been calculated according to the following equation: HSI = 8 × (ALT / AST ratio) + BMI + 2 (if female sex) + 2 (if diabetes) [11]. The originally validated cut-points for HSI were used to categorize hepatic steatosis probability as low (HSI < 30), intermediate (HSI between 30 and 36) and high (HSI > 36), respectively (11). Comparison between high *vs.* low HSI for all variables of interest was performed using logistic or linear regression analyses adjusted for age and sex.

The association between HSI, BMI, AST/ALT ratio and ultrasound diagnosis of steatosis was assessed by means of logistic regression. Discrimination was assessed with C-statistics, using ultrasound diagnosis of steatosis as the dependent variable and continuous HSI as the predictor. To identify optimal cut-off for presence of hepatic steatosis in our population we estimated the Jouden's J statistic, that is the cut-off point with the maximun "*J*" index, where J = sensitivity + specificity – 1.

A longitudinal assessment was also performed in a subset of T2D patients with a complete follow-up visit 3–12 months after initiating selected new GLMs. The changes from baseline in HSI after initiation of GLMs was evaluated according to the Student's *t*-test for paired data, or the McNemar test for categorical variables. A 2-tail p value < 0.05 was considered to be statistically significant. Statistical analyses were performed using SAS version 9.4 (TS1M4), graphs were produced with GraphPad Prism ver. 8

Results

Patient characteristics according to HSI values

The study included 78,895 patients with established T2D evaluated between 2016 and 2017 (Figure S1). This population is largely representative of T2D patients seen in specialist care in Italy. As shown in Table 1 and Fig. 1, a HSI > 36 was present in 60,233 subjects (76.3%), with only 2117 (2.7%) having a low HSI. Younger age and female sex were associated with higher HSI (both $p < 0.001$). After adjusting for age and sex, a HSI > 36 was associated with shorter duration of diabetes, higher HbA1c, higher plasma triglycerides, and lower HDL-cholesterol and LDL-cholesterol levels. HSI > 36 was also associated with lower e-GFR, higher prevalence of CKD (OR 1.35; 95% CI 1.22–1.51) and macroangiopathy (OR 1.18; 95% CI 1.07–1.30). Notably, the significant association of high HSI with CKD and macroangiopathy persisted even after further adjustment for diabetes duration and HbA1c levels (adjusted OR 1.38; 95% CI 1.23–1.53 for CKD, and 1.23; 95% CI 1.11–1.35 for macroangiopathy, both $p < 0.001$, respectively). This significant association was reflected by concomitant pharmacological treatments: patients with high HSI were more likely to

Table 1 Clinical characteristics of the T2D outpatient population stratified by baseline HSI categories

	ALL		Low HSI (< 30)	Intermediate HSI (30–36)	High HSI (> 36)	Model 1	Model 2		
						P	Direction[§]	Effect ± SE or Odds Ratio (95% CI)	*p*
	Avail	Value	Value	Value	Value				
N		78,895	2,117	16,545	60,233				
Age, years	100%	68.8 ± 11.1	74.2 ± 11.6	72.2 ± 10.7	67.7 ± 11.0	< 0.001			
Male, *n* (%)	100%	45,281 (57.4%)	1403 (66.3%)	10,925 (66.0%)	32,953 (54.7%)	*			
Current smokers, %	71%	9048 (16.2%)	311 (20.7%)	2001 (17.0%)	6736 (15.8%)	< 0.001	↓	0.54 (0.48–0.62)	< 0.001
Diabetes duration, years	100%	12.0 ± 9.4	14.3 ± 10.8	13.6 ± 10.1	11.4 ± 9.0	< 0.001	↓	-0.90 ± 0.19	< 0.001
Body mass index, kg/m^2	100%	29.4 ± 5.5	20.6 ± 2.0	24.3 ± 2.2	31.2 ± 5.0	*			
Systolic blood pressure, mmHg	83%	137.2 ± 18.4	132.8 ± 19.8	135.6 ± 18.8	137.8 ± 18.1	< 0.001	↑	6.64 ± 0.44	< 0.001
Diastolic blood pressure, mmHg	82%	77.6 ± 9.5	73.2 ± 9.4	75.5 ± 9.2	78.3 ± 9.4	< 0.001	↑	4.07 ± 0.23	< 0.001
Fasting glucose, mg/dl	93%	144.1 ± 45.5	137.9 ± 51.8	138.3 ± 43.2	145.9 ± 45.7	< 0.001	↑	7.50 ± 1.06	< 0.001
Hemoglobin A1c, %	99%	7.2 ± 1.2	7.1 ± 1.2	7.1 ± 1.1	7.3 ± 1.3	< 0.001	↑	0.19 ± 0.03	< 0.001
Total cholesterol, mg/dl	95%	170.9 ± 39.2	167.4 ± 38.5	167.7 ± 38.4	171.9 ± 39.4	< 0.001		-0.99 ± 0.89	0.268
HDL cholesterol, mg/dl	92%	49.1 ± 14.2	55.1 ± 17.0	51.9 ± 15.3	48.1 ± 13.6	< 0.001	↓	-7.18 ± 0.31	< 0.001
Triglycerides, mg/dl	94%	138.3 ± 87.6	101.3 ± 64.6	114.7 ± 63.2	145.9 ± 92.3	< 0.001	↑	39.03 ± 2.14	< 0.001
LDL cholesterol, mg/dl	91%	94.3 ± 33.2	92.8 ± 32.3	93.0 ± 32.5	94.8 ± 33.4	0.023	↓	-2.26 ± 0.78	0.004
AST, U/l	100%	23.0 ± 15.0	25.8 ± 26.6	22.7 ± 17.0	23.0 ± 13.8	*			
ALT, U/l	100%	25.3 ± 19.4	15.1 ± 12.7	18.4 ± 13.6	27.5 ± 20.4	*			
HSI index	100%	41.2 ± 7.2	28.3 ± 1.5	33.6 ± 1.6	43.7 ± 6.4	ND			
e-GFR, ml/min/1.73 m^2	93%	75.3 ± 23.5	71.4 ± 24.6	72.5 ± 23.0	76.2 ± 23.5	< 0.001	↓	-2.74 ± 0.45	< 0.001
Albuminuria, mg/g	98%	24.9 ± 62.4	26.5 ± 41.2	23.8 ± 45.4	25.1 ± 66.9	0.338			
Glucose-lowering medications, n (%)	89%								
Insulin		24,487 (34.8%)	777 (41.8%)	5179 (35.6%)	18,531 (34.3%)	< 0.001	↓	0.81 (0.74–0.90)	< 0.001
Metformin		50,203 (71.3%)	945 (50.9%)	9305 (63.9%)	39,953 (73.9%)	< 0.001	↑	2.06 (1.87–2.27)	< 0.001
Sulfonylureas		16,548 (23.5%)	477 (25.7%)	3633 (24.9%)	12,438 (23.0%)	0.010		1.07 (0.96–1.20)	0.201
Pioglitazone		3345 (4.7%)	68 (3.7%)	600 (4.1%)	2677 (5.0%)	0.005	↑	1.28 (1.00–1.64)	0.049
DPP-4i		14,857 (21.1%)	454 (24.4%)	3463 (23.8%)	10,940 (20.2%)	< 0.001	↓	0.84 (0.75–0.93)	0.002
GLP-1RA		2887 (4.1%)	6 (0.3%)	89 (0.6%)	2792 (5.2%)	< 0.001	↑	12.6 (5.7–28.2)	< 0.001
SGLT2i		2781 (3.9%)	11 (0.6%)	219 (1.5%)	2551 (4.7%)	< 0.001	↑	6.35 (3.50–11.5)	< 0.001
Other concomitant therapies, n (%)	84%								
Antiplatelet agents		34,340 (51.8%)	978 (55.2%)	7689 (55.5%)	25,673 (50.7%)	< 0.001	↑	1.22 (1.11–1.35)	< 0.001

Table 1 (continued)

	ALL		Low HSI (< 30)	Intermediate HSI (30–36)	High HSI (> 36)	Model 1	Model 2		
						P	Direction[§]	Effect ± SE or Odds Ratio (95% CI)	*p*
	Avail	Value	Value	Value	Value				
Statins		41,119 (62.0%)	920 (51.9%)	8533 (61.6%)	31,666 (62.5%)	< 0.001	↑	1.73 (1.57–1.90)	< 0.001
ACEi/ARBs		45,100 (68.0%)	927 (52.3%)	8526 (61.6%)	35,647 (70.3%)	< 0.001	↑	2.53 (2.29–2.78)	< 0.001
Calcium-channel blockers		17,077 (25.8%)	371 (20.9%)	3291 (23.8%)	13,415 (26.5%)	< 0.001	↑	1.66 (1.48–1.87)	< 0.001
Beta-blockers		21,443 (32.3%)	476 (26.8%)	4241 (30.6%)	16,726 (33.0%)	< 0.001	↑	1.48 (1.33–1.65)	< 0.001
Diuretics		13,071 (19.7%)	380 (21.4%)	2576 (18.6%)	10,115 (20.0%)	0.0945			
Kidney complications, n (%)									
CKD stage ≥ 3	93%	18,481 (25.1%)	615 (30.4%)	4368 (27.9%)	13,498 (24.1%)	< .0001	↑	1.35 (1.22–1.51)	< 0.001
Albuminuria > 30 mg/g	98%	11,909 (15.4%)	339 (16.2%)	2034 (12.5%)	9536 (16.2%)	0.642			
Eye disease, n (%)	70%								
Retinopathy		8875 (16.1%)	258 (17.1%)	2007 (16.6%)	6610 (15.9%)	0.246			
Macular oedema		1524 (2.8%)	50 (3.3%)	341 (2.8%)	1133 (2.7%)	0.200			
Neuropathy, n (%)	31%								
Peripheral		4251 (17.2%)	141 (18.5%)	946 (17.2%)	3164 (17.1%)	0.369			
Autonomic		578 (2.3%)	14 (1.8%)	111 (2.0%)	453 (2.4%)	0.242			
Lower limbs, n (%)	39%								
Atherosclerosis obliterans		5055 (16.6%)	205 (20.6%)	1394 (20.2%)	3456 (15.3%)	< 0.001		1.05 (0.89–1.23)	0.595
Revascularization		484 (1.6%)	21 (2.1%)	134 (1.9%)	329 (1.5%)	0.102			
Amputations		278 (0.9%)	14 (1.5%)	63 (1.0%)	201 (0.9%)	0.062			
Active foot lesions		873 (2.9%)	33 (3.5%)	231 (3.5%)	609 (2.7%)	0.143			
Cerebrovascular complications, n (%)	47%								
Stroke/TIA		1590 (4.2%)	67 (6.5%)	449 (5.4%)	1074 (3.8%)	< 0.001		0.79 (0.60–1.03)	0.079
Carotid atherosclerosis		16,073 (42.9%)	469 (45.2%)	3688 (44.8%)	11,916 (42.3%)	0.071			
Cardiac complications, n (%)	72%								
Left ventricular hypertrophy		3939 (6.9%)	103 (6.5%)	815 (6.5%)	3021 (7.0%)	0.297			
Ischemic heart disease		6706 (11.7%)	194 (12.2%)	1693 (13.6%)	4819 (11.2%)	0.312			
Revascularization		4523 (7.9%)	133 (8.3%)	1179 (9.5%)	3211 (7.5%)	0.277			
Microangiopathy, n (%)	100%	34,953 (44.3%)	1053 (49.7%)	7441 (45.0%)	26,459 (43.9%)	< 0.001		1.06 (0.97–1.16)	0.191
Macroangiopathy, n (%)	99%	22,885 (29.3%)	685 (32.5%)	5441 (33.1%)	16,759 (28.1%)	< 0.001	↑	1.18 (1.07–1.30)	0.001

Model 1: unadjusted comparison between low vs. high HSI. Model 2: adjusted for age and sex. e-GFR: estimated glomerular filtration rate, CKD, chronic kidney disease. *Variable included in the HSI equation. §Direction is shown only for variables significantly associated with HSI: "↑" and "↓" meaning that a high HSI is associated with higher or lower prevalence/values of the variable of interest, respectively. *ND* not determined

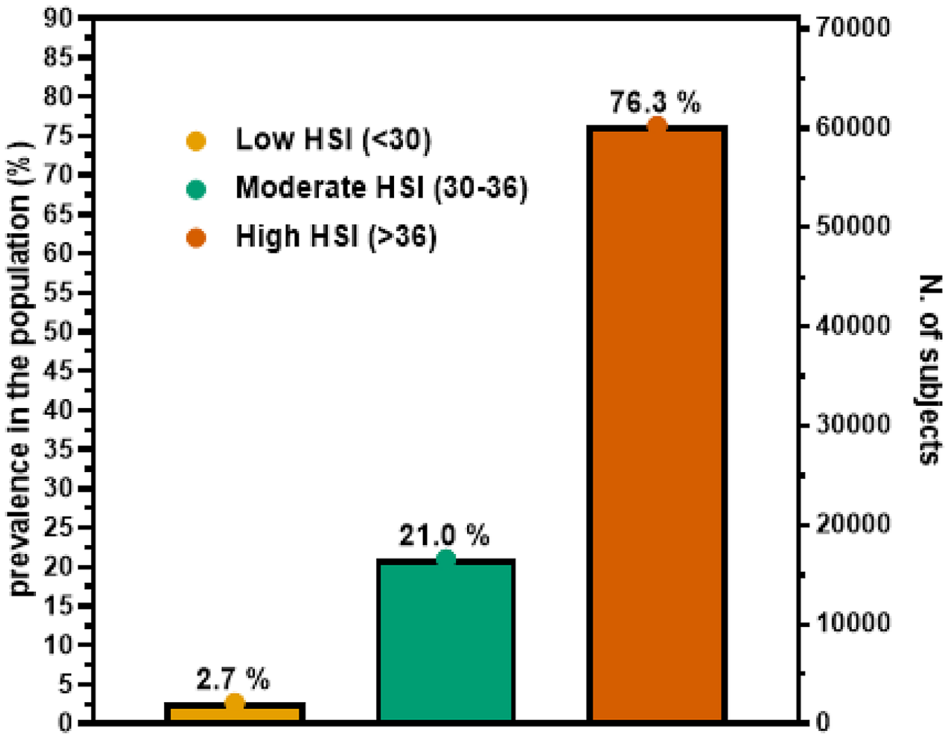

Fig. 1 Prevalence of different HSI categories among all T2D outpatients in the database ($n = 78,895$)

be treated with 'cardio-protective' medications, including SGLT-2 inhibitors, GLP-1RAs, statins, anti-hypertensive drugs, or antiplatelet agents.

Concordance between HSI and ultrasound diagnosis of liver steatosis

We calculated HSI in a subgroup of 2,179 patients with T2D undergoing liver ultrasound examination who attended a single diabetes outpatient clinic between 2003 and 2018. Clinical characteristics of this subgroup of patients are described in Table 2. As shown in Fig. 2, hepatic steatosis on ultrasound was diagnosed in 68.7% of these patients. Across the three categories of HSI, there was a progressive increase in the prevalence of ultrasound-detected hepatic steatosis (Fig. 2a). The ability of HSI as a continuous variable to discriminate T2D patients with hepatic steatosis on ultrasound was moderate (AUC 0.70, 95% CI 0.68–0.72; Fig. 2b), however it was significantly higher than AUC of BMI or ALT/AST ratio alone (AUC 0.67 and 0.64, respectively, both with $p < 0.001$ for difference from AUC of HSI). These results were confirmed when the population was stratified by age and sex, data not shown). The prevalence of hepatic steatosis was 3.5 times higher among patients with HSI > 36 compared to those with HSI < 30 (OR 3.5, 95% CI 2.0–6.3; $p < 0.0001$). In this patient population, the predefined HSI cut-off of 36 for detecting hepatic steatosis had a sensitivity and specificity of 84 and 40%, respectively, with a positive-predictive-value (PPV) of 75%. According to the Jouden's index, we found that the best HSI cut-off to discriminate patients with an ultrasound diagnosis of hepatic steatosis was 39.3, yielding a sensitivity of 64% and specificity of 67%, with a PPV of 81%. The negative-predictive value (NPV) of the predefined low HSI cut-off (< 30) was 75%, but a very small proportion of subjects (2.2%) had HSI below such cut-off. An alternative HSI cut-off of 29 would increase the NPV to 79% but would be applicable only to 1.3% of the population attending diabetic clinics.

Changes in HSI after initiation of selected new glucose-lowering medications

The temporal changes in HSI after initiating new GLMs were assessed in a subset of 1,090 patients with T2D, who initiated dapagliflozin ($n = 181$), GLP1-RA ($n = 128$), DPP-4i ($n = 460$) or gliclazide ($n = 321$) as second or more advanced line of treatment (Figure S1). As expected given the different prescription pattern of these drugs at the time of this study, the baseline characteristics of patients in the four groups were different. As reported in supplementary Table 1, patients initiating dapagliflozin had longer duration of diabetes, higher HbA1c and more frequent concomitant use of insulin despite being younger than patients treated with DPP4i or gliclazide. Dapagliflozin and GLP1-RAs were also used in patients with higher BMI. The median follow-up period in these patients was about 6 months (IQR 5.3–6.9 months), but it was shorter for those who initiated dapagliflozin or GLP1-RAs compared

Table 2 Clinical characteristics of patients with T2D, who underwent liver ultrasound examination at the validation center

	ALL		No steatosis on ultrasound	Steatosis on ultrasound	*P*
	Avail	*N* = 2179	*N* = 701	*N* = 1478	
Age, years	100%	67.6 ± 11.4	69.6 ± 11.6	66.7 ± 11.1	< 0.001
Male, *n* (%)	100%	1317 (60.4%)	417 (59.5%)	900 (60.9%)	0.531
Diabetes duration, years	100%	10.7 ± 9.2	11.8 ± 10.3	10.2 ± 8.5	< 0.001
Body mass index, kg/m^2	100%	29.1 ± 5.0	27.1 ± 4.5	30.0 ± 5.0	< 0.001
Systolic blood pressure, mmHg	100%	138.6 ± 19.9	137.5 ± 20.3	139.1 ± 19.7	0.082
Diastolic blood pressure, mmHg	100%	78.1 ± 10.4	76.3 ± 10.7	79.0 ± 10.2	< 0.001
Fasting glucose, mg/dl	74%	162.3 ± 57.5	153.2 ± 56.2	166.7 ± 57.6	< 0.001
Hemoglobin A1c, %	96%	7.6 ± 1.5	7.5 ± 1.5	7.7 ± 1.5	0.002
Total cholesterol, mg/dl	96%	178.0 ± 41.2	174.5 ± 38.8	179.7 ± 42.2	0.006
HDL cholesterol, mg/dl	93%	50.6 ± 15.4	53.0 ± 16.0	49.4 ± 14.9	< 0.001
Triglycerides, mg/dl	95%	143.5 ± 112.2	122.6 ± 89.1	153.2 ± 120.3	< 0.001
LDL cholesterol, mg/dl	90%	98.6 ± 32.4	97.0 ± 30.7	99.4 ± 33.1	0.111
AST, U/l	100%	26.3 ± 18.7	26.1 ± 23.5	26.4 ± 16.0	0.800
ALT, U/l	100%	30.1 ± 25.0	26.7 ± 26.3	31.8 ± 24.2	< 0.001
e-GFR, ml/min/1.73 m^2	93%	75.4 ± 21.7	71.2 ± 23.0	77.4 ± 20.8	< 0.001
Albuminuria (mg/g)	79%	15 (7–41)	17 (7–53)	14 (7–37)	0.068
HSI index	100%	41.1 ± 6.8	38.2 ± 6.6	42.4 ± 6.5	< 0.001
Low HSI (HSI < 30)	100%	48 (2.2%)	36 (5.1%)	12 (0.8%)	< 0.001
Intermediate HSI (HSI 30–36)	100%	443 (20.3%)	236 (33.7%)	207 (14.0%)	< 0.001
High HSI (HSI > 36)	100%	1688 (77.5%)	429 (61.2%)	1259 (85.2%)	< 0.001
Concomitant medications, *n* (%)	100%				
Metformin alone		485 (22.3%)	113 (16.1%)	372 (25.2%)	< 0.001
Insulin		713 (32.7%)	298 (42.5%)	415 (28.1%)	< 0.001
Sulfonylureas		421 (19.3%)	121 (17.3%)	300 (20.3%)	0.094
Pioglitazone		26 (1.2%)	1 (0.1%)	25 (1.7%)	0.002
DPP-4i		206 (9.5%)	40 (5.7%)	166 (11.2%)	< 0.001
GLP-1RA		36 (1.7%)	5 (0.7%)	31 (2.1%)	0.018
SGLT2i		14 (0.6%)	0 (0.0%)	14 (0.9%)	0.010
Anti-platelet agents		1016 (46.6%)	333 (47.5%)	683 (46.2%)	0.572
Statins		1202 (55.2%)	384 (54.8%)	818 (55.3%)	0.804
ACEi/ARBs		1414 (64.9%)	420 (59.9%)	994 (67.3%)	0.001
Beta-Blockers		568 (26.1%)	185 (26.4%)	383 (25.9%)	0.813
Calcium Channel Blockers		624 (28.6%)	169 (24.1%)	455 (30.8%)	0.001
Diuretics		983 (45.1%)	302 (43.1%)	681 (46.1%)	0.190
CKD stage ≥ 3, n (%)	93%	489 (24.2%)	208 (32.1%)	281 (20.5%)	< 0.001
Albuminuria > 30 mg/g, n (%)	82%	575 (32.2%)	196 (35.3%)	379 (30.8%)	0.063
Diabetic retinopathy, n (%)	56%	319 (26.0%)	129 (32.3%)	190 (23.0%)	0.001
Diabetic neuropathy, n (%)	18%	217 (54.0%)	73 (60.8%)	144 (51.1%)	0.072
Major CVD events, n (%)	100%	227 (10.4%)	86 (12.3%)	141 (9.5%)	0.052
Macroangiopathy, *n* (%)	100%	773 (35.5%)	278 (39.7%)	495 (33.5%)	0.005
Microangiopathy, *n* (%)	91%	1089 (55.1%)	397 (62.1%)	692 (51.7%)	< 0.001

to those who initiated DPP4i or gliclazide (follow-up duration: 5.5 ± 1.7, 5.8 ± 1.6, 6.3 ± 1.5, 6.5 ± 1.9 months, respectively; $p < 0.0001$ by one-way ANOVA). For each subgroup, the pre- to post-treatment changes in both HSI and variables that define HSI are reported in Table 3. HSI declined significantly after initiation of dapagliflozin, GLP-1RAs or DPP-4i, but not after initiation of gliclazide (Fig. 3), and the prevalence of subjects with high HSI (i.e. HSI > 36) was significantly reduced only in the dapagliflozin group (Table 3). Although a formal statistical comparison would be hampered by differences in baseline

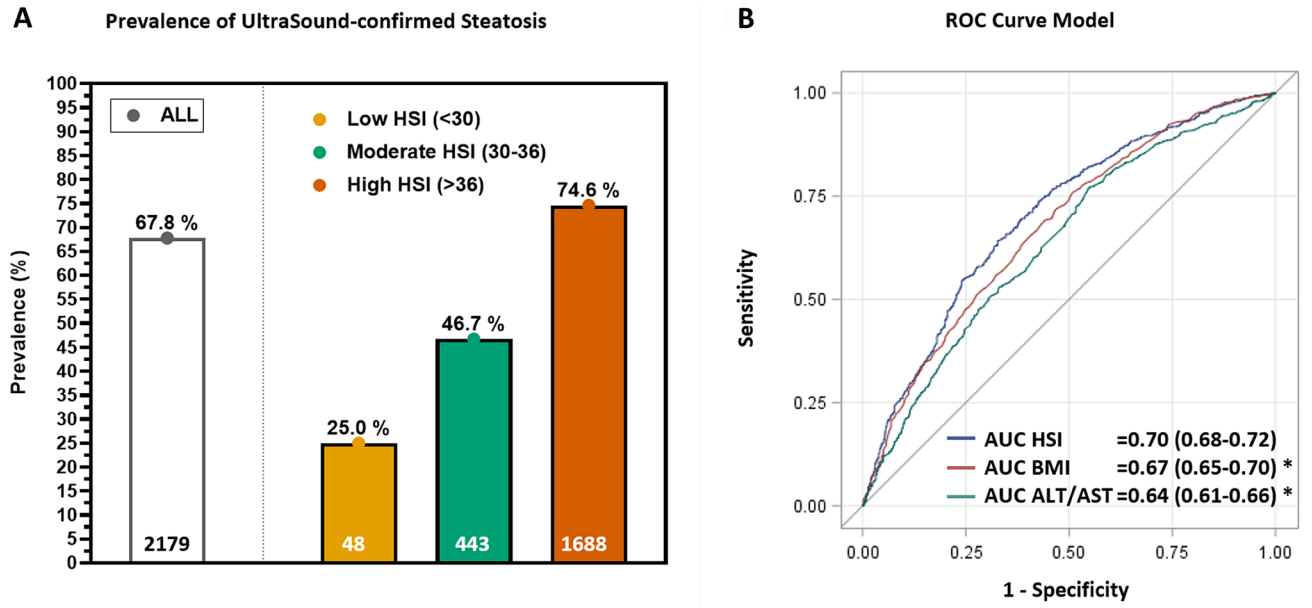

Fig. 2 Validation of HSI against liver ultrasound in patients with T2D. **a** Prevalence of ultrasound-detected hepatic steatosis across different HSI categories in 2,179 T2D patients, who underwent liver ultrasound examination (the number of individuals in each category is reported at the bottom of each column). **b** ROC curve for the prediction of ultrasound-detected hepatic steatosis according to the HSI index

clinical characteristics [14], the observed reductions in HSI appeared to be greater for dapagliflozin than for DPP-4i.

Discussion

In this large Italian multicentre study, we found that nearly three out of four outpatients with T2D have high HSI values suggesting the presence of hepatic steatosis and would be thus classified as having MAFLD. In agreement with the knowledge that MAFLD is closely related to cardio-renal diseases [1, 4], we found that HSI was significantly associated with an increased prevalence of both CKD and macroangiopathy (defined as previous CVD, cerebral, coronary or peripheral atherosclerosis), thereby identifying T2D individuals with a heavier complication burden, despite having a relatively shorter diabetes duration.

Our results are in line to those obtained both in smaller Italian cohorts [18] and in the large database of T2D of the Cleveland Clinic, reporting that 87.9% of 121,513 United States patients with T2D had a HSI > 36 [19]. Similarly a study conducted on 2940 adults with T2D from the National Health and Nutrition Examination Survey (NHANES) reported an 84.7% of subjects with HSI > 36[9]. We believe that the slightly higher prevalence of HSI > 36 in this United States population of T2D individuals compared to the Italian one (76.3%) is likely related to the higher BMI of the former, taking into account that BMI concurs to the calculation of HSI.As in our study, also the cross-sectional analyses from the NHANES studies [9], found that patients with hepatic steatosis were younger and with shorter duration of diabetes. This finding supports (with caution given the cross-sectional design) the concept that subjects with hepatic steatosis are at higher risk of early onset of diabetes and of its complications.

To our knowledge, this is the first study testing the concordance between HSI and ultrasound diagnosis of hepatic steatosis in a large T2D outpatient population. Though HSI had a moderate predictive value for ultrasound-detected hepatic steatosis, our T2D patients with high HSI (> 36) were found to have a 3.5-fold higher likelihood of having ultrasound-detected steatosis, thus confirming that HSI is a simple screening tool also in patients with T2D, with better discrimination performance than BMI or AST/ALT alone. In addition, and most importantly, compared to other steatosis biomarkers, the HSI is based on clinical and biochemical data that are readily available in most primary care settings or secondary specialist clinics as well as in large databases. Indeed, the HSI does not require the measurement of waist circumference (as the fatty liver index) or fasting insulin concentrations (as the NAFLD liver fat score), which are not frequently measured in diabetes clinical practice [10].

However, whether steatosis biomarkers truly reflect liver fat content in people with T2D is currently uncertain. In a study conducted in 220 patients with T2D, who underwent

Table 3 Temporal changes in body mass index, serum aminotransferase levels and HSI after initiation of each new glucose-lowering treatment. *$p<0.05$ compared to pre-treatment levels. Value are shows as mean ± standard deviation

	Outcome(s)	Baseline	End-of-treatment	Change
Dapagliflozin ($N=181$)	BMI (kg/m^2)	33.1 ± 6.0	32.2 ± 6.0	− 0.9 ± 1.2*
	AST (UI/l)	27.4 ± 22.4	24.3 ± 13.5	− 3.0 ± 21.3
	ALT (UI/l)	35.1 ± 22.7	31.3 ± 23.8	− 3.8 ± 17.3*
	HSI index	46.5 ± 7.5	45.0 ± 7.6	− 1.5 ± 3.4*
	HSI > 36	92.3%	89.0%	− 3.3% *
	HSI < 30	0.6%	0.6%	0%
GLP1RAs ($N=128$)	BMI (kg/m^2)	35.6 ± 5.2	34.7 ± 5.2	− 0.9 ± 1.6*
	AST (UI/l)	27.4 ± 20.1	24.0 ± 12.3	− 3.4 ± 16.0*
	ALT (UI/l)	34.0 ± 23.5	30.7 ± 19.5	− 3.3 ± 17.3*
	HSI index	48.7 ± 6.8	47.8 ± 6.5	− 0.9 ± 3.3*
	HSI > 36	97.7%	96.9%	− 0.8%
	HSI < 30	0%	0%	0%
DPP4i ($N=460$)	BMI (kg/m^2)	29.4 ± 5.0	29.2 ± 4.9	− 0.2 ± 1.1*
	AST (UI/l)	21.9 ± 10.0	20.9 ± 8.8	− 1.0 ± 7.6*
	ALT (UI/l)	26.8 ± 16.1	25.0 ± 14.2	− 1.8 ± 12.6*
	HSI index	42.0 ± 6.7	41.5 ± 6.5	− 0.5 ± 3.3*
	HSI > 36	79.6%	79.6%	0.0%
	HSI < 30	1.7%	2.2%	+ 0.5%
Gliclazide ($N=321$)	BMI (kg/m^2)	29.9 ± 5.0	29.9 ± 5.0	0.0 ± 1.1
	AST (UI/l)	24.2 ± 13.8	22.7 ± 11.2	− 1.5 ± 10.2*
	ALT (UI/l)	30.0 ± 21.8	28.6 ± 18.3	− 1.4 ± 18.9
	HSI index	42.7 ± 7.8	42.7 ± 6.4	0.0 ± 5.4
	HSI > 36	84.1%	86.6%	+ 2.5%
	HSI < 30	0.9%	0.9%	0%

^{1}H-magnetic resonance spectroscopy, both HSI and fatty liver index had a similar intra-class correlation coefficient vs. liver fat content. Yet, both biomarkers had a low-to-moderate discrimination performance with an AUROC of 0.65–0.67 [12].

Nor it is definitely clear if steatosis biomarkers predict the future development of advanced liver disease, which takes many years to develop. In the Edinburgh T2D study, among 1,059 participants with no baseline cirrhosis, non-invasive markers of both steatosis (fatty liver index) and fibrosis (NAFLD fibrosis score or fibrosis-4) exhibited poor performance in identifying those who developed cirrhosis or hepatocellular carcinoma during a follow-up of 11 years [20]. Therefore, more studies are needed to establish whether simple biomarkers can substitute imaging techniques for identifying hepatic steatosis and patients at risk of liver disease progression, especially in the T2D population. Notwithstanding these limitations and current knowledge gaps, the literature now supports the first-line use of these non-invasive biomarkers for specialist referral [18].

Given the close inter-connections between T2D, MAFLD and chronic vascular complications, a considerable scientific interest has been raised on the possible beneficial effects of newer GLMs on liver steatosis and markers of disease progression. Indeed, various GLMs may have different effects on liver fat content in people with T2D and certain GLMs might even be used to counter steatosis in patients without T2D [21]. Taking advantage of the longitudinal data collected in the DARWIN-T2D study [13], we examined the effects of selected new GLMs on the changes of HSI over time. We found that the greatest reductions in HSI could be observed during treatment with the SGLT-2 inhibitor dapagliflozin and, to a lesser extent, with the GLP-1RA liraglutide or exenatide once weekly. A modest HSI reduction was also observed during treatment with DPP-4 inhibitors, but not with a sulphonylurea (gliclazide). It is reasonable to hypothesize that the beneficial effects of newer GLMs on MAFLD are driven not only by reductions in body weight, but also by improvements in serum aminotransferase levels. However, it remains to be elucidated whether this is a simple reflection of concomitant visceral fat reduction or other ancillary mechanisms (e.g., anti-inflammatory effects) may also be involved. As a notable limitation, it is still unknown whether the reduction in continuous HSI can be translated into a net clinical benefit. In our study, only a small proportion of patients shifted class, and only those receiving

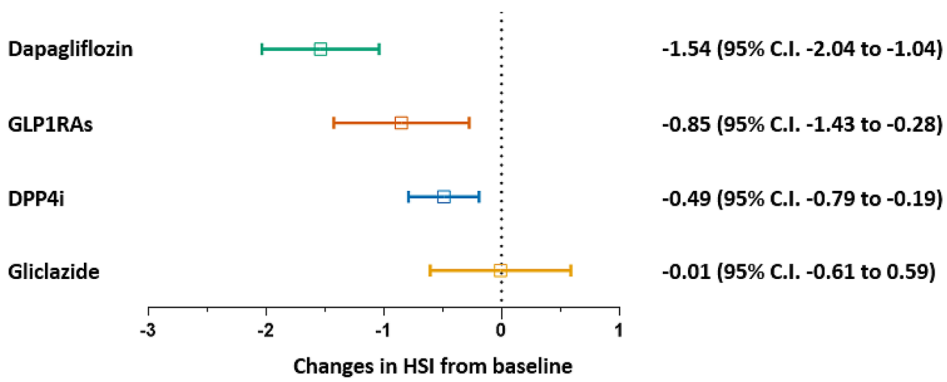

Fig. 3 Changes in HSI between pre-treatment to first-follow-up visit after initiation of new glucose lowering medication

dapagliflozin group experienced a significant reduction in the prevalence of high HSI after a relatively short treatment. Therefore, further studies with longer duration and larger sample size are needed to evaluate the clinical benefit of HSI reduction. Yet, our findings are consistent with available data from some clinical trials and observational studies [22]. In dedicated trials, both empagliflozin and dapagliflozin reduced significantly liver fat content examined by magnetic resonance in patients with T2D [23, 24]. In a three-arm controlled trial conducted in patients with T2D, dapagliflozin and pioglitazone, but not glimepiride, reduced liver steatosis [25]. Greater efficacy of dapagliflozin than sulphonylurea in reducing liver fat content was also demonstrated in combination with the DPP-4 inhibitor saxagliptin [26]. In a recent systematic review, GLP-1RAs and SGLT-2 inhibitors improved hepatic steatosis in patients with NAFLD, whereas DPP-4 inhibitors was less effective [6]. Interestingly, the combination of a SGLT-2 inhibitor (dapagliflozin) and a GLP-1RA (exenatide once weekly) resulted in greater improvements in biomarkers of hepatic steatosis and fibrosis than the use of these drugs alone [27]. Finally, in a small case series, T2D patients with ultrasound-detected hepatic steatosis who received either a SGLT-2 inhibitor or a GLP-1RA showed a significant improvement of liver fat content [28]. Therefore, our study provides further support to the notion that SGLT-2 inhibitors and GLP-1RAs may exert beneficial effects on MAFLD in patients with T2D, and adds an indirect validation to the routine use of HSI to non-invasively monitor hepatic steatosis during diabetes treatment.

Conclusions

In summary, we provide a consistent estimate of the prevalence of MAFLD in a large population of T2D outpatients, based on the use of the HSI that we have validated against an ultrasound diagnosis of hepatic steatosis. In addition, a ~ 6-month treatment with selected new GLMs, especially SGLT-2 inhibitors (dapaglifozin) and GLP-1RAs, is significantly associated with improvement of such biomarker of hepatic steatosis.

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Data availability Original data are available from the corresponding author at a reasonable request.

Compliance with ethical standards

Conflict of interest MLM received lecture or advisory fees or grant support from Mylan, SlaPharma, Amryt Pharma and Servier. AA received research grants, lecture or advisory board fees from Merck Sharp & Dome, AstraZeneca, Novartis, Boeringher-Ingelheim, Sanofi, Mediolanum, Janssen, Novo Nordisk, Lilly, Servier, and Takeda. GPF received lecture fees or grant support from Abbott, AstraZeneca, Boehringer, Lilly, Merck-Sharp-Dome, Mundipharma, Novartis, Novo Nordisk, Sanofi, Servier. GT does not have any competing interest to declare.

Ethical approval The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Ethical Committees of all participating centers.

Informed consent The study used anonymous data and, based on National and International regulations, a waiver was applied to the requirement for the patient's informed consent.

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