Nonalcoholic Fatty Liver Disease Is Associated With Ventricular Arrhythmias in Patients With Type 2 Diabetes Referred for Clinically Indicated 24-Hour Holter Monitoring

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OBJECTIVE

Recent studies have suggested that nonalcoholic fatty liver disease (NAFLD) is associated with an increased risk of heart rate–corrected QT interval prolongation and atrial fibrillation in patients with type 2 diabetes. Currently, no data exist regarding the relationship between NAFLD and ventricular arrhythmias in this patient population.

RESEARCH DESIGN AND METHODS

We retrospectively analyzed the data of 330 outpatients with type 2 diabetes without preexisting atrial fibrillation, end-stage renal disease, or known liver diseases who had undergone 24-h Holter monitoring for clinical reasons between 2013 and 2015. Ventricular arrhythmias were defined as the presence of nonsustained ventricular tachycardia (VT), >30 premature ventricular complexes (PVCs) per hour, or both. NAFLD was diagnosed by ultrasonography.

RESULTS

Compared with patients without NAFLD, those with NAFLD (n = 238, 72%) had a significantly higher prevalence of >30 PVCs/h (19.3% vs. 6.5%, $P < 0.005$), nonsustained VT (14.7% vs. 4.3%, $P < 0.005$), or both (27.3% vs. 9.8%, $P < 0.001$). NAFLD was associated with a 3.5-fold increased risk of ventricular arrhythmias (unadjusted odds ratio [OR] 3.47 [95% CI 1.65–7.30], $P < 0.001$). This association remained significant even after adjusting for age, sex, BMI, smoking, hypertension, ischemic heart disease, valvular heart disease, chronic kidney disease, chronic obstructive pulmonary disease, serum γ-glutamyltransferase levels, medication use, and left ventricular ejection fraction (adjusted OR 3.01 [95% CI 1.26–7.17], P = 0.013).

CONCLUSIONS

This is the first observational study to show that NAFLD is independently associated with an increased risk of prevalent ventricular arrhythmias in patients with type 2 diabetes.

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Nonalcoholic fatty liver disease (NAFLD) is increasingly diagnosed worldwide and is the most common chronic liver disease among patients with type 2 diabetes (occurring in up to 75% of these patients) (1,2). In addition, patients with type 2 diabetes are at higher risk for development of the most severe forms of NAFLD, such as nonalcoholic steatohepatitis, advanced fibrosis, and cirrhosis (3,4).

Over the past 10 years, it has become increasingly clear that NAFLD is not only associated with liver-related mortality or morbidity, but also that it is a multisystem disease that affects a variety of extrahepatic organ systems, including the vascular system and the heart (5,6). To date, there is clear evidence indicating that ischemic heart disease (IHD) is the leading cause of mortality in patients with NAFLD (5,6). Convincing evidence also substantiates the existence of a link between NAFLD and subclinical myocardial remodeling and dysfunction, valvular heart diseases (VHDs) (i.e., aortic valve sclerosis and mitral annulus calcification), and atrial fibrillation (7).

To date, although NAFLD is associated with a substantially increased risk of cardiac events and death (5,6), there is a paucity of published data regarding the association between NAFLD and the risk of ventricular arrhythmias, which are an established risk factor for sudden cardiac death. Preliminary evidence has suggested that NAFLD is associated with an increased prevalence of heart rate–corrected QT interval prolongation (i.e., an established risk factor for ventricular arrhythmias), independent of many cardiometabolic risk factors, both in patients with and without diabetes (8,9).

However, to our knowledge, no studies have assessed the association between NAFLD and the presence of ventricular arrhythmias seen on 24-h Holter monitoring. This issue could have important clinical implications because it might explain the increased risk of cardiac events and death observed among patients with NAFLD. In particular, an increased number of premature ventricular complexes (PVCs) (e.g., >30/h), nonsustained/sustained ventricular tachycardia (VT), or both are associated with adverse cardiac outcomes in patients both with and without established heart disease (10–15).

Therefore, the major aim of this study was to examine the association between NAFLD diagnosed on ultrasonography and the risk of prevalent ventricular arrhythmias (defined as the presence of VT, >30 PVCs/h, or both) in a sample of patients with type 2 diabetes.

RESEARCH DESIGN AND METHODS

Patients

For this study, we retrospectively analyzed the electronic records of all white outpatients with established type 2 diabetes (according to widely accepted diagnostic criteria) who regularly attended our diabetes clinic and who had undergone a first 24-h Holter monitoring for clinical reasons (e.g., palpitations, presyncope, syncope, chest pain, electrocardiographic abnormalities, or the presence of multiple cardiovascular risk factors) at our cardiology outpatient service over the last 2 years (September 2013 through October 2015).

As detailed in Fig. 1, for the purpose of this study we excluded patients with type 2 diabetes with *1*) preexisting permanent/persistent atrial fibrillation; *2*) a pacemaker or implantable cardioverter defibrillator; *3*) missing ultrasound data of the liver; and *4*) a documented history of cancer, end-stage renal disease, cirrhosis of any etiology, or other known causes of chronic liver disease, including viral hepatitis and excessive alcohol intake (defined as intake of >30 g/day alcohol for men and >20 g/day for women). On the basis of these criteria, 330 outpatients (213 men and 117 women, mean age 70 ± 8 years) with type 2 diabetes were identified and included in the final analysis. It is important to note that in our diabetes clinic, an ultrasound examination of the liver is almost routinely performed among the outpatients with type 2 diabetes.

The local ethics committee approved the study protocol. The ethics committee exempted our research from the informed consent requirement because we only accessed retrospectively a deidentified database for the purpose of data analysis.

Clinical and Laboratory Data

BMI was calculated by dividing weight (in kilograms) by height (in square meters). Blood pressure was measured with a mercury sphygmomanometer at the right upper arm using an appropriate cuff size after the patient had been seated quietly for at least 5 min. Patients were considered as having hypertension if their blood pressure was ≥140/90 mmHg or if they were taking antihypertensive drugs. Information regarding alcohol consumption and medication use was obtained from all patients via medical visit interviews.

Venous blood samples were drawn in the morning after an overnight fast. Measurements of the levels of serum liver enzymes, lipids, creatinine (measured using a Jaffé rate blanked and compensated assay), electrolytes, thyroid-stimulating hormone, and other biochemical blood attributes were obtained using standard laboratory procedures. The normal ranges for serum aspartate aminotransferase, alanine aminotransferases (ALTs), and γ-glutamyltransferase (GGT) in our laboratory were 10–40 units/L for women and 10–50 units/L for men, respectively. LDL cholesterol was calculated

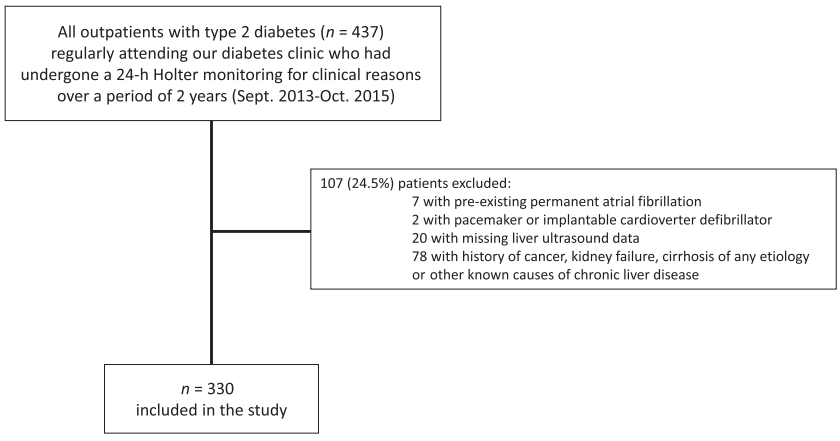

Figure 1—Details of the study design.

using the Friedewald equation. Hemoglobin A_{1c} (A1C) was measured using an automated high-performance liquid chromatography analyzer (HA-8140; Menarini Diagnostics, Florence, Italy); the upper normal limit for our laboratory was 5.6%. The estimated glomerular filtration rate (eGFR) was estimated using the four-variable Modification of Diet in Renal Disease (MDRD) study equation (16). Albuminuria was measured using an immunonephelometric method on a morning spot urine sample and expressed as the albumin-to-creatinine ratio. The presence of chronic kidney disease (CKD) was defined as an $eGFR_{MDRD}$ <60 mL/min/1.73 m^2, macroalbuminuria (defined as a urinary albumin-to-creatinine ratio of >300 mg/g), or both; as previously reported, patients with end-stage renal disease (defined as either $eGFR_{MDRD}$ <15 mL/min/1.73 m^2 or dialysis) were excluded from the study. The presence of IHD was defined as a documented history of myocardial infarction, angina, or coronary revascularizations. VHD was defined as described in the medical records, including diagnostic symptoms and echocardiogram results. The presence of chronic obstructive pulmonary disease (COPD) was confirmed by reviewing medical records, including diagnostic symptoms and the results of lung function tests. A preexisting history of hyperthyroidism (secondary to Graves disease, toxic multinodular goiter, toxic adenoma, or other less common etiologies) was confirmed by reviewing the medical records, and included hormone results from laboratory tests and thyroid imaging reports. All patients were euthyroid at the time of the medical examination. The presence of atherosclerotic plaques (i.e., stenosis $\geq$50%) at the level of either the internal or common carotid arteries was diagnosed via echo-Doppler scanning. Finally, the presence of lower-extremity sensory neuropathy was also recorded in all participants via clinical examination and biothesiometer.

Twenty-Four-Hour Holter Monitoring, Echocardiography, and Liver Ultrasonography

The 24-h Holter monitoring, echocardiography, and liver ultrasonography findings for all participants were read before enrollment into the study. All 24-h Holter monitoring (Seer Light-DC3V Compact Digital Holter; GE Healthcare) were performed at our institution. Experienced cardiologists analyzed the results of Holter monitoring. The number of PVCs, the mean hourly PVC number, and the number of sustained or nonsustained VT episodes were recorded for all patients. VT was defined as three or more PVC beats with a mean R-R interval length of <600 ms, and was considered to be sustained if it lasted >30 s, produced syncope or cardiac arrest, or required cardioversion (17). The number of atrial premature complexes (APCs), mean hourly APC number, and the number of episodes of paroxysmal supraventricular tachycardia (SVT) or paroxysmal atrial fibrillation were also recorded.

Experienced cardiologists performed a transthoracic conventional echocardiography (Vivid 7; GE Vingmed, Horten, Norway) at our institution in a subset of patients (n = 265, 80.3% of total). Conventional echocardiography was used to measure the left ventricular (LV) diameter, wall thickness and ejection fraction according to international standard criteria (18). No significant differences were observed in demographic variables and the prevalence of NAFLD or ventricular arrhythmias between those with (n = 265) and those without (n = 65) echocardiographic data (data not shown).

Experienced radiologists performed liver ultrasonography for all patients. Hepatic steatosis was diagnosed based on characteristic ultrasonographic features, such as diffuse hyperechogenicity of the liver relative to the kidneys, ultrasound beam attenuation, and poor visualization of the intrahepatic vessel borders and diaphragm (19). Liver ultrasonography has high sensitivity and specificity for detecting moderate and severe hepatic steatosis. However, the sensitivity is reduced when the hepatic fat infiltration identified via liver biopsy is <30% (19). In this study, a semiquantitative ultrasonographic scoring of the degree of hepatic steatosis was not available.

Statistical Analysis

Data are presented as the mean $\pm$ SD, median and interquartile range, or percentages. Based on previous studies (12,13,17), ventricular tachyarrhythmia was defined as the presence of nonsustained VT, >30 PVCs/h, or both. Differences in clinical and biochemical characteristics as well as in 24-h ambulatory Holter monitoring data among patients stratified by their NAFLD (Table 1) or ventricular arrhythmia (Supplementary Table 1) statutes were assessed using the unpaired t test (for normally distributed variables) and the Mann-Whitney U test (for non-normally distributed variables (i.e., diabetes duration, levels of serum triglycerides and liver enzymes, as well as the number of PVCs, APCs, and VT episodes seen over 24 h on Holter monitoring). The χ^2 test was used to test for between-group differences among the categorical variables. A binary logistic regression analysis was used to examine the association between NAFLD and the presence of ventricular arrhythmias, which was included as the dependent variable. The following four forced-entry multivariate logistic regression models (Table 2) were performed: an unadjusted model; a model adjusted for age and sex (model 1); a model adjusted for age, sex, BMI, hypertension, smoking, IHD, VHD, CKD, COPD, and serum GGT levels, and antiarrhythmic drug use (model 2); and, finally, a model adjusted for the same variables included in model 2 plus LV ejection fraction (model 3). The covariates included in these forced-entry multivariate regression models were chosen as potential confounding factors based on their significance in univariate analyses or their biological plausibility (i.e., age, smoking, hypertension, IHD, VHD, CKD, and antiarrhythmic drug use). A stepwise logistic regression analysis with a forward selection of covariates was also performed (using the likelihood ratio method). A P value of <0.05 was considered to be statistically significant.

RESULTS

Of the 330 outpatients with type 2 diabetes included in this study, 238 (72.1%) met the diagnostic criteria for NAFLD (i.e., hepatic steatosis on ultrasonography among patients with no history of excessive alcohol intake or other known causes of chronic liver disease), and 92 (27.9%) did not. Of the entire sample, 74 patients (22.4%) had ventricular arrhythmias (i.e., nonsustained VT, >30 PVCs/h, or both), whereas the remaining 256 patients (77.6%) did not. No patients had sustained VT, serum electrolyte disturbances, or hyperthyroidism.

Table 1 shows the clinical, biochemical, and 24-h Holter monitoring data of

patients stratified by NAFLD status. Compared with patients without NAFLD, those with NAFLD had higher values of body weight, BMI, and serum triglyceride and GGT levels. The prevalence of COPD and the percentage of patients using diuretic or β-blocker agents was also higher among patients with NAFLD, whereas the percentage of those treated with insulin was lower. In addition, patients with NAFLD tended to have higher systolic blood pressure and a higher prevalence of diabetic lower-extremity sensory neuropathy. The two groups of patients did not significantly differ in terms of sex, age, diabetes duration, diastolic blood pressure, A1C, HDL cholesterol, LDL cholesterol, electrolytes, eGFR, hypertension, IHD, and other comorbidities (e.g., VHD, CKD, prior hyperthyroidism, or carotid artery stenoses), or in terms of the use of oral hypoglycemic, lipid-lowering, antiplatelet/anticoagulant, and antihypertensive drugs (except for β-blockers) or antiarrhythmic agents (which were used by only a few patients). Table 1 also shows that patients with NAFLD had a lower LV ejection fraction and higher 24-h PVC values, mean hourly PVC values, 24-h APC values, and mean hourly APC values compared with those without NAFLD. They also showed a higher prevalence of both paroxysmal SVT and paroxysmal atrial fibrillation.

Notably, as shown in Fig. 2, patients with NAFLD had a markedly higher prevalence of nonsustained VT, >30 PVCs/h, or both compared with those without NAFLD (P values <0.005–0.001 for between-group differences).

Supplementary Table 1 shows the clinical, biochemical, and 24-h Holter monitoring data of the patients stratified by ventricular arrhythmia status. Compared with patients without ventricular arrhythmias, those with ventricular arrhythmias were more likely to be male and have a lower LV ejection fraction as well as higher serum GGT levels, CKD rates, and lower-extremity sensory neuropathy. Notably, they also showed a higher prevalence of NAFLD. Regarding the 24-h Holter monitoring data, patients with ventricular arrhythmias had higher 24-h APC values, and, as expected, higher 24-h PVC values, higher mean hourly PVC values, and more VT episodes. None of the other clinical and biochemical parameters

Table 1—Clinical, biochemical, and 24-h Holter monitoring data of patients with type 2 diabetes stratified by NAFLD status

	Without NAFLD (n = 92)	With NAFLD (n = 238)	P value
Male sex/female sex (n)	58/34	155/83	0.723
Age (years)	69.5 ± 8.4	70.6 ± 7.9	0.250
Weight (kg)	74.0 ± 13.4	83.1 ± 14.9	<0.001
BMI (kg/m^2)	26.1 ± 3.6	29.7 ± 4.7	<0.001
Diabetes duration (years)	7.3 (6–11)	7.3 (5–11)	0.289
Smoking	16.3	23.6	0.082
Systolic blood pressure (mmHg)	136.5 ± 16.4	140.5 ± 17.5	0.060
Diastolic blood pressure (mmHg)	76.3 ± 8.9	77 ± 9.3	0.542
Fasting glucose (mmol/L)	8.0 ± 2.6	7.8 ± 2.2	0.445
A1C (%)	7.3 ± 1.2	7.3 ± 1.3	0.884
A1C (mmol/mol)	56 ± 6	56 ± 7	0.884
Total cholesterol (mmol/L)	4.1 ± 1.0	4.3 ± 1.0	0.146
HDL cholesterol (mmol/L)	1.4 ± 0.4	1.3 ± 0.3	0.099
LDL cholesterol (mmol/L)	2.2 ± 0.9	2.3 ± 0.9	0.354
Triglycerides (mmol/L)	1.3 (0.8–1.8)	1.4 (1.1–1.9)	0.010
AST (units/L)	19 (14–23)	19 (15–24)	0.203
ALT (units/L)	20 (15–28)	23 (17–29)	0.082
GGT (units/L)	28 (19–39)	30 (20–47)	0.031
$eGFR_{MDRD}$ (mL/min/1.73 m^2)	71.7 ± 21.0	71.5 ± 23.7	0.948
Na (mmol/L)	139.7 ± 2.8	139.5 ± 2.5	0.987
K (mmol/L)	4.2 ± 0.4	4.1 ± 0.3	0.621
Hypertension	85.8	91.2	0.164
IHD	21.7	23.9	0.658
VHD	2.2	2.9	0.694
COPD	2.2	7.9	0.032
CKD	22.8	31.1	0.131
Prior hyperthyroidism	1.1	0.8	0.835
Diabetic sensory neuropathy (n = 270)	15.5	24.9	0.079
Carotid artery stenosis ≥50% (n = 325)	4.3	6.0	0.663
Oral hypoglycemia drug users	44.5	52.9	0.197
Insulin users	47.8	35.7	0.044
ACE inhibitors/ARB users	56.5	63.0	0.280
Calcium-channel blocker users	22.8	28.5	0.288
Diuretic users	38.0	50.4	0.042
β-Blocker users	22.8	39.0	0.006
Nitroderivate users	5.4	6.3	0.763
Antiarrhythmic drug users	3.2	5.4	0.386
Statin users	76.1	71.4	0.390
Antiplatelet drug users (n = 202)	53.0	67.9	0.079
Anticoagulant drug users	7.6	7.9	0.908
LV ejection fraction (%) (n = 265)	61.6 ± 9.9	58.2 ± 11.5	0.024
24-h Holter monitoring			
Heart rate (bpm)	72.9 ± 10.2	70.0 ± 10.4	0.498
24-h PVC count (n)	16.5 (3–107)	42.5 (5–254)	0.004
Hourly PVC count (n)	1 (0–5)	2 (0–11)	0.010
Number of VT episodes (n)	0 (0–0)	0 (0–0)	0.092
24-h APC count (n)	35 (10–146)	51 (15–232)	0.046
Hourly APC count (n)	1.6 (0.3–6)	2 (1–10)	0.049

Continued on p. 1420

Table 1—Continued

	Without NAFLD (n = 92)	With NAFLD (n = 238)	P value
Paroxysmal SVT	33.6	47.4	0.023
Paroxysmal atrial fibrillation	2.2	7.6	0.044

Sample size, n = 330 except where indicated. Data are expressed as the mean ± SD, median (interquartile range), or percentages, unless otherwise indicated. Differences between the two groups were tested by the χ^2 test for categorical variables, the unpaired Student t test for normally distributed continuous variables, and the Mann-Whitney test for non-normally distributed continuous variables (i.e., diabetes duration, triglycerides, liver enzymes as well as 24-h PVCs, APCs, and VT episodes on Holter monitoring). ARB, angiotensin II receptor blockers; AST, aspartate aminotransferase; K, potassium; Na, sodium.

or medication use significantly differed between the two groups of patients.

Supplementary Table 2 shows the main clinical and biochemical characteristics of the patients stratified simultaneously by NAFLD and ventricular arrhythmia statutes. The four subgroups of patients significantly differed only in terms of gender distribution, weight, BMI, serum triglyceride levels, serum GGT levels, the presence of CKD and sensory neuropathy, LV ejection, and use of β-blockers (P values for the trends). Borderline significant trends were also found in serum ALT levels, COPD, use of antiplatelet drugs, and LV ejection fraction among the four subgroups of patients. Conversely, no significant differences were found in the duration of diabetes; A1C levels; serum electrolyte levels; smoking; hypertension; IHD; VHD; and the use of antiarrhythmic drugs and medications for diabetes, dyslipidemia, and hypertension (except for β-blockers).

Table 2 shows the results after adjusting for several potential confounding factors regarding the association between NAFLD and the presence of ventricular arrhythmias on 24-h Holter monitoring. In univariable regression analysis, NAFLD was associated with an ~3.5-fold increased rate of ventricular arrhythmias (unadjusted odds ratio [OR] 3.47 [95% CI 1.65–7.30], $P < 0.001$). After adjusting for age and sex (model 1), NAFLD maintained a significant association with ventricular tachyarrhythmias. The strength of this association was not attenuated after additional adjustment for BMI, hypertension, smoking status, IHD, VHD, CKD, COPD, serum GGT levels, and antiarrhythmic drug use (model 2). Finally, additional adjustment for the LV ejection fraction did not appreciably weaken the association between NAFLD and ventricular arrhythmias (model 3, n = 265). The other independent predictors of ventricular arrhythmias in model 3 were male sex, a lower LV ejection fraction, and higher serum GGT levels.

Table 2—Independent predictors of ventricular arrhythmias in patients with type 2 diabetes

Logistic regression models	ORs	95% CIs	P value
NAFLD (yes vs. no)			
Unadjusted model	3.47	1.65–7.30	<0.001
Adjusted model 1	3.39	1.60–7.20	0.001
Adjusted model 2	3.26	1.44–7.37	0.005
Adjusted model 3	3.01	1.26–7.17	0.013
Other independent predictors of ventricular arrhythmias in model 3			
Male sex	3.03	1.31–7.01	0.008
Serum GGT (units/L)	1.02	1.01–1.03	0.009
LV ejection fraction (%)	0.96	0.93–0.99	0.028

Sample size, n = 330, except for the model 3 where 265 patients were included. Data are expressed as the OR and 95% CI, as assessed by either univariate (unadjusted) or multivariate forced-entry logistic regression analyses. The presence of nonsustained VT, >30 PVCs/h, or both was included as the dependent variable. Other covariates included in these forced-entry multivariate regression models, along with NAFLD, were as follows: model 1, adjusted for age, sex; model 2, adjusted for age, sex, BMI, hypertension (i.e., blood pressure ≥140/90 mmHg and/or use of any antihypertensive agents, including β-blockers), smoking history, CKD, COPD, IHD, VHD, antiarrhythmic drug use, or serum GGT levels; model 3, adjustment for the same variables included in model 2 plus LV ejection fraction.

Similar results were observed when we excluded the patients who were treated with antiarrhythmic drugs from the analysis (n = 15, data not shown), or when we additionally adjusted the model 3 also for presence of diabetic sensory neuropathy (as reported in Table 1, these data were available only in 270 patients); also in this latter fully adjusted regression model (which included 219 patients in total), NAFLD remained significantly associated with ventricular arrhythmias (adjusted OR 2.46 [95% CI 1.01–6.15], $P < 0.05$).

We also performed a stepwise logistic regression analysis with a forward selection of covariates (Supplementary Table 3). Also in this case, male sex (adjusted OR 2.81 [95% CI 1.29–6.13]), higher serum GGT levels (adjusted OR 1.01 [95% CI 1.002–1.017]), lower LV ejection fraction (adjusted OR 0.96 [95% CI 0.94–0.99]), and NAFLD (adjusted OR 2.76 [95% CI 1.21–6.27]) were the only four variables significantly included in the final stepwise regression model.

CONCLUSIONS

This study is the first to examine the association between NAFLD and ventricular arrhythmias, defined as the presence of nonsustained VT, >30 PVCs/h, or both in a large sample of outpatients with type 2 diabetes referred for clinically indicated 24-h ambulatory Holter monitoring.

The major findings of our study are as follows: *1*) patients with NAFLD had a markedly increased prevalence of nonsustained VT, >30 PVCs/h, or both compared with those without NAFLD; *2*) a complementary electrocardiographic finding revealed that patients with NAFLD also had a higher prevalence of paroxysmal atrial fibrillation (although the frequency of this abnormality was low) and a higher burden of APCs (i.e., higher mean hourly APC values and more paroxysmal SVT episodes); *3*) NAFLD was associated with an approximately threefold increased risk of nonsustained VT, >30 PVCs/h, or both even after adjusting for a wide range of cardiovascular risk factors and potential confounding factors, such as age, sex, BMI, hypertension, smoking, LV ejection fraction, medication use, prior IHD, microvascular complication status (diabetic

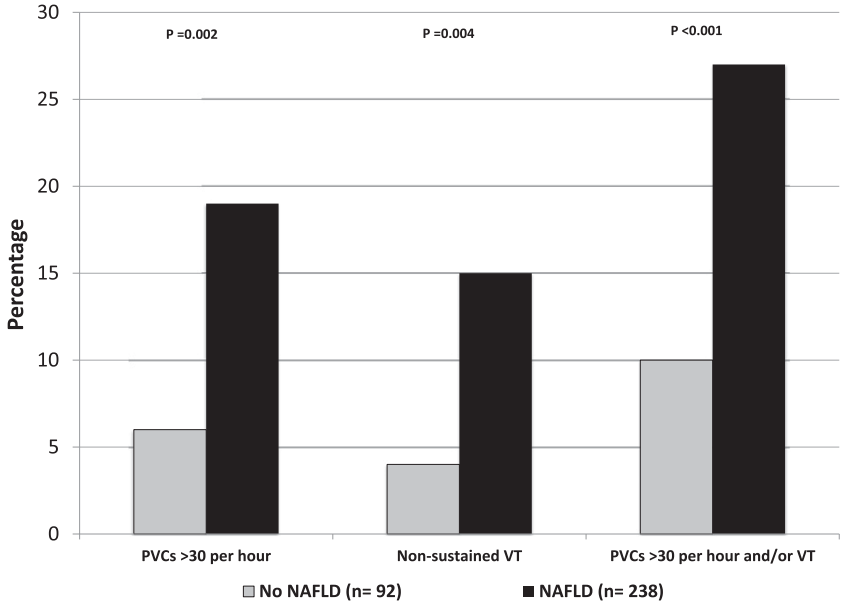

Figure 2—Prevalence of >30 PVCs/h, nonsustained VT, or both diagnosed on 24-h ambulatory Holter monitoring in patients with type 2 diabetes stratified by NAFLD status.

neuropathy and nephropathy), and other comorbidities; and *4*) the other variables that were independently associated with an increased risk of ventricular arrhythmias (besides NAFLD) were male sex, a lower LV ejection fraction and higher serum GGT levels.

Our findings might have important clinical implications. The NAFLD-related increases in PVCs to >30/h, nonsustained VT, or both might partly explain the increased risk of cardiac events and mortality observed in patients with NAFLD, and further highlights the importance of assessing the global cardiovascular and arrhythmic risk among this group of patients.

To date, the putative biological mechanisms that account for the observed association between NAFLD and ventricular arrhythmias have not been fully elucidated. The most obvious explanation for our findings is that the association between NAFLD and ventricular arrhythmias is simply an epiphenomenon of coexisting cardiometabolic risk factors and comorbidities. However, it is important to emphasize that the association between NAFLD and ventricular arrhythmias remained significant in our study even after adjusting for many cardiometabolic risk factors, LV ejection fraction, preexisting IHD, and comorbid conditions. This finding corroborates previous observations reporting a strong association between NAFLD and an increased risk of QTc interval prolongation, which is a powerful risk factor for ventricular arrhythmias (8,9). In particular, we recently demonstrated that NAFLD was associated with an increased QTc interval among patients with type 2 diabetes without known liver diseases, and this association remained significant after adjusting for age, sex, hypertension, diabetes-related variables, LV hypertrophy, and comorbid conditions (8). Similarly, Hung et al. (9) reported that the ultrasonographic severity of NAFLD was associated with a higher risk for QTc interval prolongation in a large population-based study, independently of age, sex, hypertension, diabetes, total cholesterol, eGFR, BMI, LV hypertrophy, history of IHD, or hypokalemia.

The results of our study suggest that NAFLD is not simply an epiphenomenon of ventricular arrhythmia and its coexisting risk factors and comorbidities (also including IHD, lower LV ejection fraction, and diabetic neuropathy); rather, it might also partly contribute to their pathogenesis. Although more research is needed to identify mechanistic clues, some putative pathophysiological mechanisms may be proposed to explain the strong association between NAFLD and ventricular arrhythmias. Growing evidence suggests that NAFLD is not only associated with an increased risk of cardiovascular events and death, but is also associated with subclinical myocardial remodeling, an increase in LV filling pressures, and enlarged left atrial volume (5,7,20–23). Previous studies (24–28) have also shown that patients with NAFLD have an increased risk of atrial fibrillation compared with those without NAFLD. Moreover, experimental evidence indicates that NAFLD is associated with insulin resistance, myocardial lipid toxicity, and the systemic release of a myriad of proinflammatory, procoagulant, pro-oxidant, and profibrogenic mediators that play important roles in the pathogenesis of the functional, structural, and arrhythmic abnormalities of the heart (3,5–7,29,30). NAFLD-related derangements in myocardial function and structure might induce alterations in fiber continuity and potential circuit re-entry, which might produce electrophysiological changes. Finally, preliminary evidence also suggests that noncirrhotic NAFLD is associated with cardiac autonomic dysfunction, which is a risk factor for ventricular arrhythmias and sudden cardiac death (31).

Interestingly, in our study elevated serum GGT levels were also independently associated with an increased risk of ventricular tachyarrhythmias. Serum GGT levels have been commonly used as a marker for excessive alcohol consumption or liver disease. However, because of the key role played by GGT in the synthesis and metabolism of glutathione, elevated serum GGT levels are also a marker of increased oxidative stress (32). Many population-based studies have shown that moderately elevated GGT levels are independent, long-term predictors of all-cause and cardiovascular mortality (33). Recently, Alonso et al. (25) reported that moderately elevated GGT levels are also independently associated with an increased incidence of atrial fibrillation in a community-based study. Similarly, we found that increased GGT levels and hepatic steatosis had additive and independent effects on the prevalence of permanent/persistent atrial fibrillation in a hospital-based sample of patients with type 2 diabetes (24). Interestingly, Wang et al. (34) reported that increased GGT levels were associated with longer QTc interval duration and higher QT interval dispersion in patients with newly diagnosed type 2 diabetes. In line with these findings, our results suggest

that oxidative stress may play a role in the development of cardiac arrhythmias (35,36).

Although the arrhythmogenic potential of NAFLD requires further confirmation in future studies, we believe that this field of research is promising. The pathophysiological pathways through which NAFLD contributes to chronic inflammation, hypercoagulation, and insulin resistance might represent potential therapeutic targets for the prevention and treatment of myocardial remodeling and the electrophysiological abnormalities of the myocardium in patients with NAFLD.

The major limitations of this study are its retrospective, cross-sectional design, which limits our ability to establish the temporality and causality of the observed associations. Moreover, a selection bias might have occurred because patients with missing 24-h Holter monitoring data were excluded from analysis (e.g., patients with diabetes with a more favorable cardiovascular risk profile may be less likely to be included). Although we believe that the patients in this cohort were representative of the whole sample of outpatients with type 2 diabetes of similar age, who regularly attended our diabetes clinic, further investigations involving this population are desirable. In addition, although our statistical models were extensive, we cannot exclude the possibility of residual confounding by some unmeasured factors (e.g., cardiac autonomic neuropathy) that might partly explain the observed associations. Furthermore, the diagnosis of NAFLD was based on ultrasonography and the exclusion of known causes of chronic liver diseases; this diagnosis was not confirmed by liver biopsy, which is considered to be the "gold standard" of NAFLD diagnosis and its more progressive forms (e.g., nonalcoholic steatohepatitis). However, we believe that it would have been hazardous to perform liver biopsies in these patients with normal or only moderately elevated serum liver enzyme levels. Indeed, ultrasonography enables the reliable and accurate detection of moderate hepatic steatosis compared with histology. A recent meta-analysis by Hernaez et al. (19) reported that the overall sensitivity, specificity, positive likelihood ratio, and negative likelihood ratio of ultrasound for the detection of moderate-to-severe fatty liver, compared with histology (gold standard), were 84.8% (95% CI 79.5–88.9), 93.6% (87.2–97.0), 13.3 (6.4–27.6), and 0.16 (0.12–0.22), respectively. Thus, although some nondifferential misclassification of NAFLD based on ultrasonography is likely, this limitation would attenuate the magnitude of our effect measures toward null; therefore, our data are most likely a conservative estimate of the relationship between NAFLD and ventricular arrhythmias. However, future studies in larger cohorts of well-characterized patients with NAFLD (as diagnosed either by biopsy or by magnetic resonance-proton density fat fraction and magnetic resonance elastography, which are rapidly being recognized as being as good as liver biopsies) (37,38) are needed to better elucidate whether the severity of NAFLD may differentially affect the risk of ventricular arrhythmias. Finally, because our sample was limited to white individuals with type 2 diabetes who were assessed at an outpatient diabetes clinic and referred for 24-h Holter monitoring for clinical reasons, our results might not necessarily be generalizable to other populations of individuals with diabetes.

Despite these limitations, our study has important strengths, including its relatively large sample size, the completeness of the dataset, the ability to adjust for multiple clinical risk factors and potential confounding factors, and the exclusion of patients with pacemakers or implantable cardioverter defibrillators; and those with permanent atrial fibrillation, kidney failure, or cirrhosis. We believe that including patients with these complications might have confounded our interpretations of the data.

In conclusion, this study is the first to demonstrate a positive and independent association between NAFLD and ventricular arrhythmias among patients with type 2 diabetes referred for clinically indicated 24-h Holter monitoring. Further larger studies are needed to corroborate these findings and to better elucidate the underlying mechanisms responsible for this association.

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

Author Contributions. A.M. and G.T. conceived and designed the study, analyzed the data, and wrote the draft of the manuscript. A.R., S.B., B.B., M.P., G.M., L.F., C.B., L.B., F.V., R.R., and I.P. researched the data and reviewed and edited the manuscript. G.Z., E.B., and F.V. contributed to discussion and reviewed and edited the manuscript. G.T. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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