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ORIGINAL ARTICLE

# Early Cardiac Dysfunction in Biopsy-proven Nonalcoholic Fatty Liver Disease

Peter C. Johnson[1,2], Anthony A. Cochet[3], Rosco S. Gore[2,3], Stephen A. Harrison[4], John P. Magulick[5], James K. Aden[6] and Angelo H. Paredes[2,5]

Department of Medicine, Brooke Army Medical Center[1], San Antonio, TX; Department of Medicine, Uniformed Services University of Health Sciences[2], Bethesda, MD; Department of Medicine, Cardiology Service, Brooke Army Medical Center[3], San Antonio, TX; Pinnacle Clinical Research[4], San Antonio, TX; Department of Medicine, Gastroenterology and Hepatology Service, Brooke Army Medical Center[5], San Antonio, TX; US Army Institute for Surgical Research[6], San Antonio, TX, USA

**Background/Aims:** Nonalcoholic fatty liver disease (NAFLD) encompasses a range of diseases from nonalcoholic fatty liver (NAFL) to nonalcoholic steatohepatitis (NASH) and has been linked to cardiovascular disease and sub-clinical cardiac remodeling. This paper presents a retrospective study of biopsy-proven NAFL and NASH to examine the differences in subclinical cardiac remodeling.
**Methods:** Patients were recruited from an institutional repository of patients with liver-biopsy-confirmed NAFLD. Patients with a transthoracic echocardiogram (TTE) within 12 months of the liver biopsy were included. The parameters of the diastolic dysfunction were reviewed for the differences between NAFL and NASH as well as between the stages and grades of NASH.
**Results:** Thirty-three patients were included in the study, 17 with NAFL and 16 with NASH. The NASH patients were more likely to have lower platelets, higher AST, higher ALT, and higher rates of type 2 diabetes mellitus, coronary artery disease, and hypertension than the NAFL patients. The E/e' ratio on transthoracic echocardiogram was significantly higher in NASH compared to NAFL, advanced-stage NASH compared to early stage, and high-grade NASH compared to low-grade. The E/e' ratio was also significantly higher in NASH than NAFL in patients without diabetes mellitus. The presence of diastolic dysfunction trended toward significance. The other markers of diastolic dysfunction were similar. Logistic regression revealed a statistical association with E/e' and NASH.
**Conclusions:** NASH patients had evidence of a higher E/e' ratio than NAFL, and there was a trend towards a significant diastolic dysfunction. Patients with NASH compared to NAFL should be closely monitored for signs and symptoms of cardiac dysfunction. (**Korean J Gastroenterol 2021;78:161-167**)

**Key Words:** Non-alcoholic fatty liver disease; Steatohepatitis; Diastolic heart failure; Cardiac diseases

## INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) encompasses a spectrum of liver diseases ranging from nonalcoholic fatty liver (NAFL) to nonalcoholic steatohepatitis (NASH), which may progress to cirrhosis and hepatocellular carcinoma. NAFLD has become a public health problem in the United States and worldwide, affecting almost a quarter of the world's adult population and imposing a considerable economic burden on the health care systems of many countries.[1,2] The prevalence of NAFLD is rising in concert with the rising rates of obesity and type 2 diabetes mellitus (T2DM), with an estimated 33.8% of the population meeting the criteria for obesity and 10.6% for T2DM.[3] NAFLD entails a systemic derangement of multiple

organ systems, the best characterized of which are the coexistence of cardiovascular disease (CVD), T2DM, and other features of metabolic syndrome. CVD is the leading cause of death among those with hepatic steatosis because of the exacerbated hepatic and systemic insulin resistance, predisposing the individual to atherogenic dyslipidemia and causing the release of several proinflammatory and vasoactive mediators that may promote the development of obesity-related cardiometabolic complications.[4-7] Previous studies suggested that NAFLD is associated with subclinical echocardiogram findings indicating a diastolic dysfunction, including increased left ventricular wall thickening, lower early diastolic relaxation tissue velocity (e'), low E/A ratio, and a higher estimated left ventricular filling pressures (E/e').[8-10] These studies are limited by the use of imaging findings of hepatic steatosis alone and do not differentiate histologically between NAFL and NASH. The pathogenesis of cardiac dysfunction in NAFLD is related to the release of inflammatory cytokines among those with NASH.[11] However, it is unclear if NASH is associated with a worsened diastolic dysfunction compared to NAFL. Previous studies with histological diagnoses have reported conflicting data, with one study showing no significant difference in cardiac dysfunction between biopsy-proven NAFLD groups.[12] On the other hand, more recent studies have shown a possible link between biopsy-proven NAFLD and cardiac dysfunction. An increased cardiac dysfunction is observed in more severe liver disease.[13-15] This paper presents a retrospective study of patients with biopsy-proven NAFL and NASH with an assessment of the cardiac structure and function using transthoracic echocardiograms to show that the severity of NAFLD affects the level of diastolic dysfunction.

## SUBJECTS AND METHODS

The test population included subjects enrolled in an ongoing tissue and serum repository at Brooke Army Medical Center in San Antonio, Texas, USA. The study protocol for the repository was approved by an ethics review board and conducted in accordance with the principles of the Declaration of Helsinki. All subjects gave their informed consent to participate in the repository. This study underwent expedited IRB approval with a waiver of informed consent to use patients already enrolled in this repository (Brooke Army Medical Center Institutional Review Board, IRB No. C.2016.154d). All subjects had pre-

viously undergone a liver biopsy because of the clinical suspicion of NASH. NAFLD was defined by the consumption of less than 20 and 30 g of alcohol every day for women and men, respectively, and a liver biopsy showing fatty liver disease.[16] Other common liver diseases were excluded by the self-report or previous documentation, including chronic hepatitis B or C, Wilson's disease, hemochromatosis, autoimmune hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, or previously documented NAFLD. No cirrhotic patients were identified prospectively, and a history of liver disease would have excluded any patients with any other liver disease at the baseline. Histology was assessed by a single hepatobiliary pathologist using the NASH-Clinical Research Network criteria.[17] The presence of steatohepatitis was defined by steatosis, inflammation, and cytologic ballooning. The participants were categorized based on hepatic fibrosis stage; no fibrosis (F0), mild fibrosis (F1-F2), and advanced fibrosis (F3-F4). They were also categorized by inflammatory histology grade as mild (grade 1), moderate (grade 2), or severe (grade 3), as determined by the pathologist review. The fibrosis and grade were determined at the time of the liver biopsy and were obtained from the medical records. The liver biopsies were not re-examined or confirmed as part of this retrospective study. All subjects in the repository from November 2011 to February 2016 were eligible for inclusion in the study. The primary author performed a chart review of each eligible subject to look for subjects who had a transthoracic echocardiogram performed for any indication within 1 year of the liver biopsy date.

The demographics, baseline characteristics (including past medical history), and laboratory data were obtained and cataloged in the NAFLD repository; the data was used in the analysis. Coronary artery disease and tobacco use at the time of the liver biopsy were obtained from a review of the electronic medical records. A review of the Armed Forces Longitudinal Technologies Application (AHLTA, the military outpatient record) was performed for all subjects in the NAFLD repository study to search for a transthoracic echocardiogram for any indication performed within 1 year of the liver biopsy. The echocardiograms were then reviewed independently by a level III echocardiographer. The echocardiograms were assessed for the E/e' ratio, E/A ratio, left ventricular ejection fraction (LVEF), LV mass index, left atrial (LA) volume index, tricuspid regurgitation (TR) velocity, left ventricular global longitudinal systolic strain, and estimated pulmonary arterial sys-

**Table 1.** Demographics and Baseline Characteristics of NAFL versus NASH

|  | NAFL (n=17) | NASH (n=16) | p-value |
|---|---|---|---|
| Male | 9 (53.0) | 11 (69.0) | 0.35 |
| Caucasian | 8 (47.0) | 8 (50.0) | 0.87 |
| Age (years) | 46.3±10.1 | 51.4±11.2 | 0.19 |
| T2DM | 3 (18.0) | 9 (56.0) | 0.02 |
| HLD | 13 (77.0) | 15 (94.0) | 0.15 |
| HTN | 12 (71.0) | 16 (100.0) | 0.01 |
| CAD | 0 (0.0) | 6 (38.0) | 0.00 |
| Smoking | 4 (24.0) | 2 (13.0) | 0.41 |
| BMI (kg/m$^2$) | 36.4±8.2 | 35.3±6.6 | 0.87 |
| BSA (m$^2$) | 2.2±0.3 | 2.2±0.2 | 0.71 |
| SBP (mmHg) | 136±17 | 128±11 | 0.23 |
| DBP (mmHg) | 83±13 | 78±11 | 0.22 |
| Creatinine (mg/dL) | 0.9±0.2 | 1.0±0.2 | 0.31 |
| Platelets (×10$^3$/mcL) | 264±61 | 202±44 | 0.00 |
| AST (U/L) | 30±9 | 58±32 | 0.00 |
| ALT (U/L) | 46±25 | 82±49 | 0.02 |
| LDL (mg/dL) | 106±30 | 94±43 | 0.12 |
| HDL (mg/dL) | 48±15 | 35±10 | 0.02 |
| Albumin (g/dL) | 4.4±0.4 | 4.2±0.4 | 0.02 |

Values are presented as mean±standard deviation or number (%).
NAFL, nonalcoholic fatty liver; NASH, nonalcoholic steatohepatitis; T2DM, type 2 diabetes mellitus; HLD, hyperlipidemia; HTN, hypertension; CAD, coronary artery disease; BMI, body mass index; BSA, body surface area; SBP, systolic blood pressure; DBP, diastolic blood pressure; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

**Table 2.** TTE Parameters in NAFL versus NASH Patients, Advanced-stage versus Non-advanced Stage NAFLD Patients, and High-grade versus Lower-grade NAFLD Patients

| TTE Parameters | NAFL (n=17) | NASH (n=16) | p-value | Non-advanced NAFLD[a] (n=26) | Advanced Stage NAFLD[b] (n=7) | p-value | Lower-grade NAFLD[c] (n=22) | High-grade NAFLD[d] (n=11) | p-value |
|---|---|---|---|---|---|---|---|---|---|
| E/e' | 8.8±2.0 | 11.8±3.3 | 0.004 | 9.7±3.0 | 12.4±2.7 | 0.021 | 9.3±2.7 | 12.2±2.9 | 0.00 |
| E/A | 1.2±0.5 | 1.1±0.4 | 0.533 | 1.2±0.4 | 1.2±0.5 | 0.880 | 1.1±0.4 | 1.2±0.4 | 0.36 |
| Diastolic dysfunction | 1 (6.0) | 5 (31.0) | 0.051 | 3 (12.0) | 3 (43.0) | 0.077 | 2 (9.0) | 4 (36.0) | 0.06 |
| LVEF (%) | 62.0±8.0 | 64.0±6.0 | 0.309 | 63.0±7.0 | 64.0±7.0 | 0.747 | 63.0±8.0 | 63.0±7.0 | 0.90 |
| LV Mass Index (by BSA, g/m$^2$) | 81.2±18.7 | 82.3±20.0 | 0.790 | 79.5±17.6 | 89.9±23.3 | 0.352 | 80.6±16.8 | 84.0±23.6 | 0.64 |
| LA volume index (by BSA, mL/m$^3$) | 19.8±7.4 | 23.5±7.1 | 0.168 | 21.3±7.3 | 22.8±8.0 | 0.780 | 20.9±7.7 | 23.1±6.9 | 0.40 |
| Average strain (%) | -17.2±2.6 | -18.3±3.5 | 0.345 | -17.9±2.8 | -17.3±4.3 | 0.503 | -17.6±2.5 | -18.1±4.1 | 0.87 |
| TR velocity (cm/s) | 2.2±0.6 (n=8) | 2.4±0.3 (n=8) | 0.721 | 2.2±0.5 (n=12) | 2.6±0.3 (n=4) | 0.379 | 2.2±0.3 (n=10) | 2.5±0.3 (n=6) | 0.37 |
| Estimated PASP (mmHg) | 25.6±7.8 (n=7) | 26.0±12.3 (n=8) | 0.779 | 25.7±6.2 (n=11) | 26.0±18.6 (n=4) | 0.661 | 25.4±6.9 (n=9) | 26.3±14.5 (n=6) | 0.53 |

Values are presented as mean±standard deviation or number (%).
TTE, transthoracic echocardiogram; NAFL, nonalcoholic fatty liver; NASH, nonalcoholic steatohepatitis; NAFLD, nonalcoholic fatty liver disease; LVEF, left ventricular ejection fraction; LV, left ventricular; LA, left atrial; BSA, body surface area; TR, tricuspid regurgitation; PASP, pulmonary artery systolic pressure.
[a]Non-advanced stage NAFLD=NASH Stage 1-2 + NAFL; [b]Advanced-stage NAFLD=NASH Stage 3-4; [c]Lower-grade NAFLD=NASH Grade 1 + NAFL; [d]Higher-grade NAFLD=NASH Grade 2-3.

tolic pressure (PASP) according to recommendations from the American Society of Echocardiography and the European Association of Cardiovascular Imaging.[18] The echocardiograms were also assessed for diastolic dysfunction. The presence of any abnormal parameter consistent with diastolic dysfunction would indicate the presence of diastolic dysfunction (as "yes, present" or "no, not present").

## 1. Statistical analysis

Statistical tests were performed to compare the baseline characteristics and laboratory values between NAFL and NASH (Table 1). Transthoracic echocardiogram data were compared as follows: 1). NAFL vs. NASH; 2). Non-advanced NAFLD (NAFL+NASH Stage 1-2) vs. advanced NAFLD (NASH stage 3-4); 3). Low-grade NAFLD (NAFL+NASH grade 1) vs. high-grade NAFLD (NASH grade 2-3) (Table 2). The cardiac function between NAFL and NASH was also compared between patients with and without type 2 diabetes (Table 3). The mean and standard deviations were reported for summary statistics. Owing to the small sample sizes, the p-values reported were from the Mann-Whitney *U* test. The categorical data are reported as percentages and analyzed using a Chi-Squared or Fisher Exact Test where appropriate. Furthermore, multivariable logistic regression was performed to determine if the E/e' ratio and other statistically significant factors from the baseline characteristics were independently associated with NASH. Only the E/e' ratio and HDL could be included in the model because of the lack of power. This data is presented with the ORs and their corresponding 95% confidence intervals and p-value. Statistical analysis was performed using SPSS

software v22.0 (SPSS Inc., Chicago, IL, USA).

## RESULTS

Two hundred and sixty-three subjects were eligible for chart review; 43 had transthoracic echocardiograms within 1 year of biopsy, and 33 patients had sufficient data to complete the analysis. The analysis included 17 subjects with NAFL and 16 with NASH.

Of the 17 NAFL subjects, the indications for TTE included four subjects for "screening", three for "dyspnea", three for "abnormal electrocardiogram", two for "edema", and one each for "chest pain", "arrhythmia", "syncope", "valvular disease", and "murmur". Of the 16 NASH subjects, the indications for TTE included four subjects for "dyspnea", two for "screening", two for "arrhythmia", two for "valvular disease", two for "abnormal clinical finding", and one each for "chest pain", "hypertension", "murmur", and "syncope".

Only subjects with stage F0-F3 were included in the study and analysis. Of the 16 NASH subjects, five, four, and seven had stage 1, 2, and 3 fibrosis, respectively. NASH patients with grade 1-3 inflammation were included. Five had grade 1 inflammation, nine had grade 2 inflammation, and two had grade 3 inflammation. The mean age was 48.8±10.8 years, with 61% male and 48% Caucasian. Approximately 36%, 18%, 18%, 85%, and 85% of subjects had T2DM, coronary artery disease, reported tobacco use, hypertension, and hyperlipidemia, respectively. The NASH subjects were more likely to have T2DM, hypertension, and coronary artery disease. The NASH subjects had lower platelets, higher AST,

**Table 3.** TTE Parameters in NAFL versus NASH without and with Type 2 Diabetes

| Outcome | No type 2 diabetes | | | Type 2 diabetes | | |
|---|---|---|---|---|---|---|
| | NAFL (n=14) | NASH (n=7) | p-value | NAFL (n=3) | NASH (n=9) | p-value |
| LVEF (%) | 63.3±6.2 | 65.3±6.2 | 0.50 | 56.3±14.0 | 63.6±6.8 | 0.64 |
| LV mass index (by BSA, g/m$^2$) | 77.4±17.4 | 79.1±18.8 | 0.50 | 98.8±16.4 | 84.7±21.6 | 0.23 |
| E/A ratio | 1.3±0.5 | 1.2±0.9 | 1.00 | 1.1±0.4 | 1.0±0.3 | 0.93 |
| E/e' ratio | 8.6±2.0 | 11.8±3.1 | 0.01 | 9.7±2.6 | 11.8±3.5 | 0.31 |
| LA volume index (by BSA, mL/m$^3$) | 18.1±5.5 | 21.0±5.2 | 0.35 | 28.0±10.9 | 25.4±8.0 | 0.52 |
| TR Velocity (cm/s) | 2.1±0.6 (n=7) | 2.3±0.3 (n=5) | 0.87 | 2.7 (n=1) | 2.6±0.3 (n=3) | 0.65 |
| Estimated PASP (mmHg) | 25.6±7.9 (n=7) | 21.0±11.4 (n=5) | 0.63 | N/A (n=0) | 34.3±10.1 (n=3) | N/A |
| Average Strain (%) | -17.1±2.5 | -19.4±3.8 | 0.20 | -18.0±3.5 | -17.5±3.3 | 0.78 |

Values are presented as mean±standard deviation.
TTE, transthoracic echocardiogram; NAFL, nonalcoholic fatty liver; NASH, nonalcoholic steatohepatitis; LVEF, left ventricular ejection fraction; LV, left ventricular; BSA, body surface area; LA, left atrial; TR, tricuspid regurgitation; PASP, pulmonary artery systolic pressure.

higher ALT, lower HDL, and lower albumin than the NAFL subjects (Table 1).

The transthoracic echocardiogram parameters were compared for three different groups: NAFL versus NASH, NAFLD without advanced fibrosis versus advanced stage NASH, and low-grade NAFLD and high-grade NASH. The E/e' ratio, a marker of left ventricular diastolic dysfunction, was significantly higher in NASH than NAFL (11.8±3.3 vs. 8.8±2.0, p=0.00), advanced stage NASH compared to non-advanced stage NAFLD (12.4±2.7 vs. 9.7±3.0, p=0.02), and high-grade NASH compared to lower-grade NAFLD (12.2±2.9 vs. 9.3±2.7, p=0.00) (Fig. 1). NASH showed a marginally significant association with a diastolic dysfunction compared to NAFL (31% vs. 6%, p=0.05). This trend was maintained with advanced stage NASH compared to NAFLD without advanced fibrosis (43% vs. 12%, p=0.08) and with high-grade NASH compared to low-grade NAFLD (36% vs. 9%, p=0.06). There were no significant differences nor trends towards significance were observed between these three groups in regards to other markers of cardiac dysfunction: E/A ratio, LVEF, LV mass index, LA volume index, left ventricular global longitudinal systolic strain, TR velocity, and estimated PASP.

The measures of diastolic dysfunction were also compared in patients with NAFL versus NASH in those who did and did not have T2DM. The E/e' ratio was significantly higher in NASH than NAFL in those who did not have T2DM (11.8±3.1 vs. 8.6±2.0, p=0.01), but it was not significantly higher than in those with T2DM (11.8±3.5 vs. 9.7±2.6, p=0.31). The remaining markers of cardiac dysfunction were similar in the

NASH and NAFL patients with and without T2DM.

Logistic regression was performed to determine if the E/e' ratio and other statistically significant baseline characteristics were independently associated with NASH. The baseline characteristics that were possible confounders were assessed, including T2DM, hypertension, coronary artery disease, and HDL. The full model, including T2DM, hypertension, and coronary artery disease, did not converge due to singularity. Therefore, these factors were removed, making the final model contain only the E/e' ratio and HDL. The ORs for E/e' and HDL were 1.87 (95% CI 1.13-3.12, p=0.00) and 0.89 (95% CI 0.80-0.98, p=0.00), respectively.

## DISCUSSION

Cardiac disease is the leading cause of death of patients with NAFLD, and there are numerous studies suggesting a connection between NAFLD and subclinical cardiac dysfunction. In the present study, a histologic diagnosis of NASH was associated with an increased echocardiographic marker of diastolic dysfunction compared to the less aggressive NAFL. These findings are consistent with previous research showing the relationship between the worsening severity of NAFLD and subclinical cardiac remodeling. Jung et al.[19] reported that mild and moderate-to-severe NAFLD had a higher OR of abnormal LV relaxation than the normal group (1.29 and 1.95 respectively) and increased relative wall thickness (1.26 and 1.46 respectively). Petta et al.[14] showed that more severe liver fibrosis (F3-F4 fibrosis) was associated with both morphologic and functional abnormalities compared to less severe fibrosis (F0-F2), including an increased posterior wall thickness, increased LV mass/height, increased relative wall thickness, increased left atrial volume, ejection fraction, lower lateral e', and lower E/A ratio. Furthermore, a meta-analysis by Wijarnpreecha et al.[20] compiled 12 studies and 280,645 patients and reported a significant association between patients with NAFLD and diastolic cardiac dysfunction compared to patients without NAFLD (OR 2.02; 95% CI 1.47-2.79). Interestingly, a recent study using biopsy-proven NAFLD showed no increase in the E/e' ratio at rest according to the fibrosis stage. On the other hand, there was a significant increase in stress E/e' ratio with increasing fibrosis stage. The peak VO2, ventilatory anaerobic threshold, and exercise time were inversely correlated with the fibrosis stage,
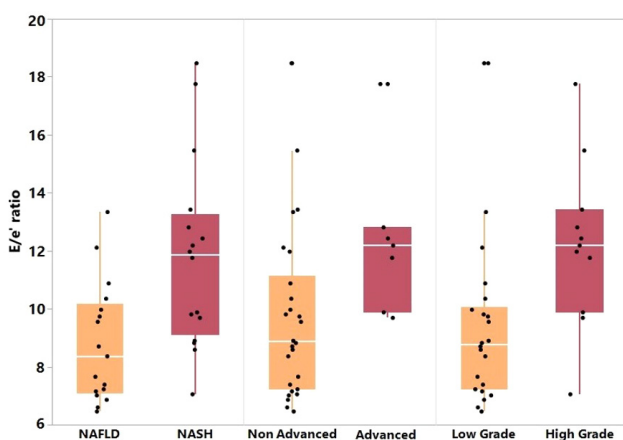


**Fig. 1.** E/e' ratio for NAFL versus NASH, non-advanced versus advanced NASH, and low grade versus high-grade NASH. NAFL, nonalcoholic fatty liver; NASH, nonalcoholic steatohepatitis.

and they all decreased with increasing fibrosis stage.[21] Although there was no difference in the rest E/e' ratio, as in the present study, this does add support to an increasing diastolic dysfunction with increasing fibrosis stage similar to the present findings.

These findings show that both an advanced stage of fibrosis and a higher grade of inflammation in NAFLD is associated with an increased marker of diastolic dysfunction. This is the first study to show a positive correlation specifically with the histologic grade of inflammation and stage of fibrosis with early cardiac dysfunction. A systemic inflammation and insulin resistance from the liver fat content likely plays a key role because of the unfavorable metabolism of fatty acids, glucose, and lipoproteins. Patients with NAFLD have increased lipolysis, lipid profiles showing increased LDL and decreased HDL, hyperglycemia with insulin resistance, and secretion of inflammatory markers, including IL-6, TNF-alpha, CRP, and others. These metabolic abnormalities and inflammatory markers have been linked with accelerated atherosclerosis and coronary artery disease.[22,23] Insulin resistance can also adversely affect the cardiomyocyte function because insulin signaling has been shown to play a role in myosin gene expression and affect the utilization of fatty acids and glucose in the cardiomyocytes.[24]

Several studies have investigated the possible association between NAFLD and the risk of CVD, but the independent prognostic role of NAFLD on CVD remains a topic of intense debate. It is unclear if the relationship between NAFLD and incidence of CVD is simply a byproduct of the shared CVD risk factors or if NAFLD actively contributes to the development of CVD. This is a relevant question because the answer could change how patients with NAFLD are screened for cardiac disease and how the treatments for NAFLD may reduce these cardiac events. The focus on identifying early cardiac disease is essential because NAFLD has been associated with several other abnormal cardiac findings, such as increased carotid-artery intima-media thickness, increased arterial stiffness, increased coronary artery calcification, and high-risk coronary plaques.[25-27] Furthermore, an increasing fibrosis stage has been correlated with increased cardiac events and increased all-cause and cardiac mortality.[28-30] Early changes in TTE could indicate an increased risk of cardiac events in the future.

This study has some limitations, such as the small sample size and the retrospective, single-center design. The small sample size and retrospective design made it difficult to control for possible confounders and show statistical significance between populations. For example, the significance of the E/e' ratio disappeared when breaking down patients with NAFL and NASH into those with and without T2DM because there were only three patients with NAFL and T2DM. Subjects with NASH had a significant prior medical history and laboratory abnormalities at the baseline, which could be expected based on the understanding of the disease. On the other hand, it was difficult to elucidate if these were confounding. This study also relied on transthoracic echocardiograms being performed for any indication, which may have biased the results in many ways. There were more screening TTEs in the NAFL group (four compared to two), but most patients in the NAFL and NASH groups had TTE performed for symptoms consistent with possible cardiac disease and dysfunction. This may have made the underlying cardiac dysfunction more likely and more difficult to differentiate between the groups than a larger, asymptomatic population of NAFL and NASH. Finally, the small sample size made statistical analysis difficult because the multivariable logistical regression could not be run successfully with all the significant variables, and only E/e' and HDL were assessed.

In conclusion, histologically proven NASH with advanced fibrosis or higher grade inflammation is associated with an increased E/e' ratio, a marker of diastolic dysfunction, and trended toward significance in the diastolic dysfunction overall. Overall this study adds to the growing body of evidence that demonstrates an independent correlation between the histological severity of NAFLD and subclinical cardiac dysfunction. More studies with a histological diagnosis of NASH will be needed to clarify these findings.

## REFERENCES

1. Rinella ME. Nonalcoholic fatty liver disease: a systematic review. JAMA 2015;313:2263-2273.
2. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology 2016;64:73-84.
3. Younossi Z, Tacke F, Arrese M, et al. Global perspectives on non-alcoholic fatty liver disease and nonalcoholic steatohepatitis. Hepatology 2019;69:2672-2682.
4. Lonardo A, Nascimbeni F, Mantovani A, Targher G. Hypertension,

diabetes, atherosclerosis and NASH: cause or consequence?. J Hepatol 2018;68:335-352.

5. Lonardo A, Lugari S, Ballestri S, Nascimbeni F, Baldelli E, Maurantonio M. A round trip from nonalcoholic fatty liver disease to diabetes: molecular targets to the rescue?. Acta Diabetol 2019;56:385-396.

6. Loria P, Marchesini G, Nascimbeni F, et al. Cardiovascular risk, lipidemic phenotype and steatosis. A comparative analysis of cirrhotic and non-cirrhotic liver disease due to varying etiology. Atherosclerosis 2014;232:99-109.

7. Williams CD, Stengel J, Asike MI, et al. Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: a prospective study. Gastroenterology 2011;140:124-131.

8. Hallsworth K, Hollingsworth KG, Thoma C, et al. Cardiac structure and function are altered in adults with non-alcoholic fatty liver disease. J Hepatol 2013;58:757-762.

9. Singh S, Allen AM, Wang Z, Prokop LJ, Murad MH, Loomba R. Fibrosis progression in nonalcoholic fatty liver vs nonalcoholic steatohepatitis: a systematic review and meta-analysis of paired-biopsy studies. Clin Gastroenterol Hepatol 2015;13:643-654.e1-9; quiz e39-40.

10. VanWagner LB, Wilcox JE, Colangelo LA, et al. Association of non-alcoholic fatty liver disease with subclinical myocardial remodeling and dysfunction: a population-based study. Hepatology 2015; 62:773-783.

11. Bonci E, Chiesa C, Versacci P, Anania C, Silvestri L, Pacifico L. Association of nonalcoholic fatty liver disease with subclinical cardiovascular changes: a systematic review and meta-analysis. Biomed Res Int 2015;2015:213737.

12. Bhatia LS, Curzen NP, Calder PC, Byrne CD. Non-alcoholic fatty liver disease: a new and important cardiovascular risk factor?. Eur Heart J 2012;33:1190-1200.

13. Karabay CY, Kocabay G, Kalayci A, et al. Impaired left ventricular mechanics in nonalcoholic fatty liver disease: a speckle-tracking echocardiography study. Eur J Gastroenterol Hepatol 2014;26: 325-331.

14. Petta S, Argano C, Colomba D, et al. Epicardial fat, cardiac geometry and cardiac function in patients with non-alcoholic fatty liver disease: association with the severity of liver disease. J Hepatol 2015;62:928-933.

15. Simon TG, Bamira DG, Chung RT, Weiner RB, Corey KE. Nonalcoholic steatohepatitis is associated with cardiac remodeling and dysfunction. Obesity (Silver Spring) 2017;25:1313-1316.

16. Chalasani N, Younossi Z, Lavine JE, et al. The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. Hepatology 2012; 55:2005-2023.

17. Kleiner DE, Brunt EM, Van Natta M, et al. Nonalcoholic steatohepatitis clinical research network. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 2005;41:1313-1321.

18. Nagueh SF, Smiseth OA, Appleton CP, et al. Recommendations for the evaluation of left ventricular diastolic function by echocardiography: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. J Am Soc Echocardiogr 2016;29:277-314.

19. Jung JY, Park SK, Ryoo JH, et al. Effect of non-alcoholic fatty liver disease on left ventricular diastolic function and geometry in the Korean general population. Hepatol Res 2017;47:522-532.

20. Wijarnpreecha K, Lou S, Panjawatanan P, et al. Association between diastolic cardiac dysfunction and nonalcoholic fatty liver disease: a systematic review and meta-analysis. Dig Liver Dis 2018;50:1166-1175.

21. Canada JM, Abbate A, Collen R, et al. Relation of hepatic fibrosis in nonalcoholic fatty liver disease to left ventricular diastolic function and exercise tolerance. Am J Cardiol 2019;123:466-473.

22. Gaggini M, Morelli M, Buzzigoli E, DeFronzo RA, Bugianesi E, Gastaldelli A. Non-alcoholic fatty liver disease (NAFLD) and its connection with insulin resistance, dyslipidemia, atherosclerosis and coronary heart disease. Nutrients 2013;5:1544-1560.

23. Francque SM, van der Graaff D, Kwanten WJ. Non-alcoholic fatty liver disease and cardiovascular risk: pathophysiological mechanisms and implications. J Hepatol 2016;65:425-443.

24. Belke DD, Betuing S, Tuttle MJ, et al. Insulin signaling coordinately regulates cardiac size, metabolism, and contractile protein isoform expression. J Clin Invest 2002;109:629-639.

25. Lee YJ, Shim JY, Moon BS, et al. The relationship between arterial stiffness and nonalcoholic fatty liver disease. Dig Dis Sci 2012; 57:196-203.

26. Zhou YY, Zhou XD, Wu SJ, et al. Nonalcoholic fatty liver disease contributes to subclinical atherosclerosis: a systematic review and meta-analysis. Hepatol Commun 2018;2:376-392.

27. Puchner SB, Lu MT, Mayrhofer T, et al. High-risk coronary plaque at coronary CT angiography is associated with nonalcoholic fatty liver disease, independent of coronary plaque and stenosis burden: results from the ROMICAT II trial. Radiology 2015;274: 693-701.

28. Targher G, Byrne CD, Lonardo A, Zoppini G, Barbui C. Non-alcoholic fatty liver disease and risk of incident cardiovascular disease: a meta-analysis. J Hepatol 2016;65:589-600.

29. Dulai PS, Singh S, Patel J, et al. Increased risk of mortality by fibrosis stage in nonalcoholic fatty liver disease: systematic review and meta-analysis. Hepatology 2017;65:1557-1565.

30. Henson JB, Simon TG, Kaplan A, Osganian S, Masia R, Corey KE. Advanced fibrosis is associated with incident cardiovascular disease in patients with non-alcoholic fatty liver disease. Aliment Pharmacol Ther 2020;51:728-736.