The Association of Alanine Aminotransferase Levels With Myocardial Perfusion Imaging and Cardiovascular Morbidity

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Abstract
Introduction: Studies suggest that non-alcoholic fatty liver disease (NAFLD) is associated with an independent risk of cardiovascular disease (CVD). We utilized a large cohort of patients undergoing myocardial perfusion imaging (MPI) with single photon emission computed tomography (SPECT) to determine the association between alanine aminotransferase (ALT) as a surrogate marker for presumed NAFLD, and the presence of myocardial ischemia and mortality. Methods: We retrospectively assessed SPECT-MPI results and medical records of individuals evaluated between 1997 and 2008. We excluded patients with known non-NAFLD liver diseases, ALT values <17 or >340 U/L and absent liver tests. Elevated ALT cases were classified as presumed NAFLD. The primary endpoint was abnormal SPECT-MPI. Secondary endpoints included cardiac death, acute myocardial infarction and all-cause mortality. Results: Of 26,034 patients who underwent SPECT-MPI, 11,324 met inclusion criteria. 1635 (14.4%) patients had elevated ALT. SPECT-MPI results did not differ significantly between subjects with elevated ALT and controls. Elevated ALT was associated with increased risk for the composite endpoint of cardiac death or acute myocardial infarction at 5-year follow-up (hazard ratio [HR] 1.3, 95% confidence interval [CI] 1.01-1.67) and in all-cause mortality (HR 1.27, CI 1.02-1.58) but only in patients with normal SPECT-MPI. Conclusions: The long-term mortality of patients with abnormal SPECT-MPI is not modulated by ALT, likely reflecting an already high risk and established CVD. However, patients with normal SPECT-MPI are at increased risk for a future cardiac event if they have an elevated ALT level, suggesting an important role for NAFLD in earlier stages of CVD.

Keywords
fatty liver, cardiovascular disease, metabolic syndrome, perfusion imaging, heart disease risk factors

Introduction
Non-alcoholic fatty liver disease (NAFLD) has become the leading etiology for chronic liver disease in western and industrialized countries.1,2 NAFLD is strongly associated with the growing epidemic of obesity and metabolic syndrome,3,4 all tightly linked through common pathophysiologic mechanisms such as insulin resistance (IR).5 Due to its wide prevalence in the western world, elevated ALT levels in the absence of an alternative liver diagnosis are a common surrogate marker for presumed NALFD (PNAFLD) in large population-based studies.6-10 A growing body of evidence suggests that NAFLD is strongly associated with evolution and progression of cardiovascular disease (CVD),11-14 which in turn has a greater impact on survival in this population than liver disease progression. Patients with NAFLD are at increased risk for the
development of cardiac ischemia and heart failure, cardiac arrhythmias such as atrial fibrillation and valvular heart disease.\textsuperscript{15} Furthermore, the degree of hepatic steatosis, as well as fibrosis stage have been shown to be negatively correlated with cardiac and autonomic dysfunction.\textsuperscript{16} Moreover, NAFLD is associated not only with overt CVD manifestations but also with early markers of subclinical atherosclerosis such as elevated coronary calcium score, increased carotid intima-media thickness (C-IMT) and left ventricular hypertrophy.\textsuperscript{17-19} However, unlike the metabolic syndrome and its leading component IR, which have long been recognized as risk factors for CVD,\textsuperscript{20} a definite link between NAFLD and CVD, that is not via the common dysmetabolic environment not been established yet.

The majority of studies exploring the association between NAFLD and CVD thus far have focused on structural components, while alterations in cardiovascular (CV) perfusion and function and its long-term effect on clinical outcomes has not been studied extensively in this population.

Myocardial perfusion imaging (MPI) with single photon emission computed tomography (SPECT) allows noninvasive quantitative assessment of myocardial ischemia in patients with suspected or known coronary heart disease during physiological or vasodilator induced stress.\textsuperscript{21-23} SPECT-MPI is also a useful modality for predicting future coronary events and may therefore be useful for assessment of CV risk in targeted populations such as patients with NAFLD.\textsuperscript{24}

In this study our aim was to determine the association between elevated ALT, a surrogate marker for PNAFLD and the presence of myocardial ischemia as assessed by SPECT-MPI, as well as the effect of the elevated marker on the subsequent incidence of CV events and all-cause mortality.

**Methods**

**Study Population and Patient Data**

The study was approved by the Soroka University Medical Center ethics committee. We performed a retrospective analysis of all individuals who underwent SPECT-MPI at Soroka University Medical Center (SUMC) between 1997 and 2008. SUMC is a 1200 bed academic center which serves as the only tertiary care hospital in the Negev region of Israel. All subjects are identified by a national ID number and were members of Clalit Health Services, the largest health care provider in Israel. Clalit maintains a computerized database, with complete records of patients’ medical history, laboratory and imaging test results, past and current medications and mortality data.

The following data were extracted: demographics, body mass index (BMI), medical history including existing ICD diagnoses of traditional cardiac risk factors and use of alcohol, liver enzymes, serologic tests for viral hepatitis, immunologic tests for anti-nuclear antibodies (ANA) and anti-smooth muscle antibodies (ASMA), detailed SPECT-MPI study data, cardiac events and mortality data. The cause of death was verified by individual chart review of all mortality cases.

We excluded subjects with absence of liver enzyme tests within a year of the SPECT-MPI, known diagnosis of hepatitis B (HBV) or hepatitis C (HCV), serological evidence of HBV and HCV, positive ANA or ASMA, alcohol consumption (>20 g/d) and pregnant women from 3 months pre-term to nine months post parturition. Subjects with ALT < 17 U/L were excluded due to known association with frailty.\textsuperscript{25,26} Subjects with ALT >340 U/L, 10 times the upper limit of the local laboratory normal value were excluded due to a presumed liver injury not associated with NAFLD.\textsuperscript{27}

Subjects were defined as having systemic arterial hypertension (HTN), diabetes mellitus (DM) or dyslipidemia if they had a documented diagnosis or by the use of blood pressure lowering medications, insulin or oral hypoglycemic agents or lipid-lowering medications, respectively. Obesity was defined as a BMI > 30 kg/m\textsuperscript{2}. Metabolic syndrome was defined as the presence of at least 3 of the following: HTN, DM, dyslipidemia and obesity. As waist circumference, HDL and triglyceride levels were not available, obesity and dyslipidemia, respectively, were used as proximal terms for metabolic syndrome definition. Family history of premature coronary artery disease (CAD) was defined as a CAD event occurring in a first degree relative (men ≤ 55 years or women ≤ 65 years).

**SPECT-MPI Protocol and Analysis**

Standard protocols were used to trigger ischemia with either treadmill (Bruce protocol) or pharmacologic stress testing using intravenous administration of dipyridamole or dobutamine prior to the administration of the radiotracer (technetium sestamibi or thallium). Image acquisition was performed in accordance with the ASNC guidelines.\textsuperscript{28} Semi-quantitative analysis of the stress and rest images was performed. Perfusion defects (reversible and fixed) were expressed in percentage myocardium unit, calculated from summed scores (summed stress, summed rest and summed difference scores) as previously described.\textsuperscript{29} The degree of perfusion defect reversibility (ischemia) was classified as: 0\% (normal), 1\%-5\% (mild), and 6\% or greater (moderate–severe).\textsuperscript{30} Abnormal myocardial perfusion was defined as a summed stress score (SSS) of greater than 1.

During the study period, all scans were performed using the same Siemens E-Cam dual head gamma camera. Reconstruction and analysis of the studies were performed using the same dedicated workstation (Philips).

**Definition of the Study Groups and Measurement of Liver Enzymes Groups**

Serum ALT activity was measured using standard lab technique within 1 year of SPECT-MPI study and subjects were divided into deciles according to ALT levels. Because of relatively flat outcomes below ALT of 35 U/L and above 17 U/L (Supplementary Figure 1), analysis was dichotomized to normal and elevated ALT, using a cutoff of 45 U/L in men and 34 U/L in women. These values are at the upper limit of normal
for ALT values in our institute’s laboratory. Subjects with elevated ALT and no other causes were defined as having PNAFLD.

Study Endpoints
The primary hypothesis for the study was that association exists between PNAFLD and the presence of myocardial ischemia as assessed by SPECT-MPI. The primary imaging endpoint was an abnormal myocardial perfusion result on SPECT-MPI. Secondary clinical endpoints were a composite of cardiac death and acute myocardial infarction (MI) during 5 years of follow-up following the SPECT-MPI, all-cause mortality, cardiac death and MI during this time period. Cardiac death was defined as a death occurring during hospitalization for a primary diagnosis of acute coronary syndrome or heart failure or within 30 days of discharge, or by sudden death at home in patients with a preexisting diagnosis of ischemic heart disease (IHD) and no malignancy, chronic lung disease, or dementia. A myocardial infarction event was defined by a hospitalization with a primary diagnosis of MI.

Statistical Analysis
Patient characteristics and outcomes were compared using chi-square or Fisher’s exact tests for categorical variables and ANOVA test for continuous variables. Non-normally distributed variables are presented as medians with interquartile range (IQR) and compared with Kruskal-Wallis test. Survival estimates were calculated by the Kaplan-Meier method and compared using the log-rank test. We used Cox proportional hazards regression models for multivariable analysis of survival to the endpoints. Covariates were selected based on the clinical significance and results of univariate analyses (P < .05 for inclusion). The final covariates selected were age, gender, obesity, HTN, dyslipidemia, DM, congestive heart failure, family history of cardiac disease and smoking.

Results

Patient Population
During the study period a total of 26,034 patients underwent SPECT-MPI. We excluded 78 patients due to HCV or HBV infection, 200 patients for alcohol consumption, 8745 patients for missing liver enzyme results within 1 year from SPECT-MPI study, 7 patients with ALT >340 U/L and 5680 patients with ALT < 17 U/L. There were no exclusions due to a positive ANA or ASMA serology result. The final study population consisted of 11,324 subjects (Supplementary Figure 2).

The most common indications for the SPECT-MPI exam were the evaluation of chest pain at 41.2% of exams (4673) followed by cardiac risk stratifications at 8.3% of exams (940).

SPECT-MPI Test Results
Abnormal myocardial perfusion was detected in 4576 (40.4%) subjects overall and was found less frequently in subjects with elevated ALT in comparison to the normal ALT group (P < .001, Table 2). Moderate reversible ischemia was present in 2310 (20.4%) subjects. The percentage of subjects with moderate reversible ischemia and the percentage of myocardium exhibiting overall stress and reversible perfusion defects were lower in the elevated ALT group (P = .02), but the difference was small in magnitude (0.3% of myocardial unit) and not clinically meaningful (Table 2). There was no significant difference between the groups in the percentage of myocardium exhibiting a fixed perfusion defect (P = .37). Thus, elevated ALT was not associated with an increased risk for abnormal SPECT-MPI results. Due to differences in the clinical characteristics found between the 2 groups we performed an adjusted multivariable logistic regression analysis in order to estimate the OR for abnormal perfusion and moderate reversible ischemia following an elevated ALT result (Table 3). The analysis revealed a significantly increased risk (OR 1.21, P = .04) for an abnormal perfusion test following an elevated ALT. However, no significant risk was found for moderate reversible ischemia.
In order to assess the adjusted risk for myocardial perfusion defects following an elevated ALT we performed a multivariable linear regression analysis (Table 4). For all 3 types of perfusion defects (stress, fixed and reversible) no significantly increased risk was found for the presence of an elevated ALT (P < .001).

**Clinical Outcomes**

Subjects with elevated ALT levels showed a near-significant trend for increased risk to achieve the composite cardiac outcome on a multivariable analysis of survival (HR 1.23, CI 0.99-1.49, P = .05; Figure 1). As expected, for patients with an elevated ALT and an abnormal myocardial perfusion test, the risk of achieving the composite cardiac outcome increased significantly (HR 1.25, CI 1.13-1.38, P < .001). For patients with an elevated ALT and a normal perfusion test no increased risk was found (HR 1.23, CI 0.99-1.49, P = .05).

All-cause mortality during a five-year follow-up was not significantly associated with an elevated ALT (HR 1.16, CI 0.97-1.39, P = .1), but as expected, was increased in subjects with reversible perfusion defects (HR 1.15, CI 1.05-1.26, P < .001). We performed an adjusted multivariable sensitivity analysis, assessing the effect of elevated ALT on clinical outcomes according to the baseline SPECT-MPI findings. Interestingly, ALT was associated with a significantly increased risk for composite cardiac endpoints (HR 1.3, CI 1.01-1.67, P = .04) and for all-cause mortality (HR 1.27, CI 1.02-1.58, P = .03) in subjects without a significant reversible perfusion defect (<1% myocardium; Table 5). In contrast, in subjects with mildly abnormal or moderate perfusion defect at baseline, abnormal ALT did not increase the risk for clinical outcomes.

There were exactly 1000 cases of all-cause mortality during follow-up. The leading cause of death was congestive heart failure (280 patients, 2.5%, Table 6) followed by malignancy (258 patients, 2.3%) and overwhelming sepsis (198 patients, 1.7%). We were unable to elucidate the cause of death for 30 patients (0.3%). There were no differences in the leading causes of death between patients with or without elevated ALT levels.
This is the first study to evaluate the association between elevated ALT levels as a surrogate marker for PNAFLD and myocardial perfusion in a large-scale population-based cohort, and the first to study the association of PNAFLD with long-term mortality risk based on baseline CV risk stratification. Our main finding is that the presence of elevated ALT levels, representing PNAFLD, increases the risk of CV outcomes and all-cause mortality in subjects who had a normal SPECT-MPI. In contrast, PNAFLD did not modulate the long-term mortality or CV risk among subjects with abnormal SPECT-MPI. Interestingly, at baseline, PNAFLD was not associated with an abnormal SPECT-MPI. An increased risk for an abnormal baseline SPECT-MPI result in patients with an elevated ALT was only found in a multivariable adjusted logistic analysis. However, a similar analysis was unable to show increased risk for moderate reversible ischemia and various perfusion defects, severely reducing the clinical significance of this finding.

Several studies have demonstrated a link between NAFLD and objective evidence of CAD. Puchner et al. found that high-risk coronary plaques on coronary CT angiography were more frequent among patients with CT-defined NAFLD compared to patients without NAFLD, and similar results were reported in other studies. In another CT-defined NAFLD study, DeFilippis and colleagues demonstrated an association between NAFLD and an atherogenic dyslipidemia phenotype. Arslan et al. demonstrated that the presence of ultrasound defined NAFLD is independently associated with the presence and extent of CAD as seen during invasive coronary angiography. Fotbolcu et al. have shown that left ventricular systolic and diastolic function, presumably reflecting established CAD, is impaired in patients with NAFLD, even in the absence of metabolic syndrome. In a meta-analysis by Lu and colleagues, which included over 7000 participants from cross-sectional studies and prospective cohort studies, a model adjusted for established CV risk factors found NAFLD to be a predictor of CVD. In contrast, Ruhl et al. did not find increased CV mortality in a prospective study of patients with elevated ALT levels. Stepanova et al. were able to demonstrate an increased risk for CVD in ultrasound defined NAFLD patients. However, in their study NAFLD did not significantly increase the risk for CV mortality during long-term follow-up. Also, Alexander et al. recently performed a population based retrospective matched cohort study to estimate the incidence of acute MI in patients with NAFLD compared to the general population after adjustment for traditional risk factors and did not demonstrate increased risk in the NAFLD group.

### Table 5. Adjusted HR for the Effect of ALT on Clinical Outcomes by Baseline MPI Result (95% Confidence Intervals) for Development of Clinical Outcomes in 5 Years for Patients With Abnormal Versus Normal ALT According to Their Initial SPECT-MPI Result.

| Reversible perfusion defect | N = 8198 | N = 2309 | N = 817 |
|----------------------------|----------|----------|---------|
| Cardiac death & MI risk (95%CI) | 1.3 (1.01-1.67) | 1.14 (0.77-1.68) | 1.21 (0.65-2.24) |
| P = .04 | P = .51 | P = .54 |
| All-cause mortality (95%CI) | 1.27 (1.02-1.58) | 0.95 (0.65-1.4) | 1.09 (0.57-2.05) |
| P = .03 | P = .81 | P = .79 |
| Cardiac death (95%CI) | 1.32 (0.9-1.93) | 0.93 (0.52-1.63) | 1.96 (0.92-4.1) |
| P = .15 | P = .79 | P = .08 |
| MI (95%CI) | 1.12 (0.81-1.54) | 1.27 (0.77-2.09) | 1.26 (0.56-2.83) |
| P = .49 | P = .34 | P = .58 |

*COX model method adjusted for gender, age, diabetes mellitus, congestive heart failure, hypertension, family history of premature coronary heart disease, current smoker, obesity and dyslipidemia.

### Table 6. Causes of Death During Follow-Up.

| Elevated ALT at baseline (%) | Percent | Frequency | Diagnosis          |
|-----------------------------|---------|-----------|--------------------|
| 14.3                        | 2.5     | 280       | CHF                |
| 14                          | 2.3     | 258       | Malignancy         |
| 11.1                        | 1.7     | 198       | Sepsis             |
| 20                          | 0.8     | 85        | MI                 |
| 10.7                        | 0.5     | 56        | CVA                |
| 13.9                        | 0.3     | 36        | COPD               |
| 20                          | 0.3     | 30        | Unknown            |
| 13.3                        | 0.1     | 15        | Trauma             |
| 15.4                        | 0.1     | 13        | PVD                |
| 28.6                        | 0.1     | 7         | ESKD               |
| 16.7                        | 0.1     | 6         | PE                 |
| 20                          | 0.0     | 5         | ILD                |
| 20                          | 0.0     | 5         | SBO                |
| 33.3                        | 0.0     | 3         | NASH cirrhosis     |
| 0                           | 0.0     | 1         | Pancreatitis       |
| 0                           | 0.0     | 1         | LBO                |
| 0                           | 0.0     | 1         | Drug overdose      |
| 14.5                        | 91.2    | 10,324    | Survived at follow-up |

Abbreviations: CHF, congestive heart failure; MI, myocardial infarction; CVA, cerebrovascular accident; COPD, chronic obstructive pulmonary disease; PVD, peripheral vascular disease; ESKD, end-stage kidney disease; PE, pulmonary embolism; ILD, interstitial lung disease; SBO, small bowl obstruction; NASH, nonalcoholic steatohepatitis; LBO, large bowl obstruction.

### Discussion

This is the first study to evaluate the association between elevated ALT levels as a surrogate marker for PNAFLD and myocardial perfusion in a large-scale population-based cohort, and the first to study the association of PNAFLD with long-term mortality risk based on baseline CV risk stratification. Our main finding is that the presence of elevated ALT levels, representing PNAFLD, increases the risk of CV outcomes and all-cause mortality in subjects who had a normal SPECT-MPI. In contrast, PNAFLD did not modulate the long-term mortality or CV risk among subjects with abnormal SPECT-MPI. Puchner et al. found that high-risk coronary plaques on coronary CT angiography were more frequent among patients with CT-defined NAFLD compared to patients without NAFLD, and similar results were reported in other studies. In another CT-defined NAFLD study, DeFilippis and colleagues demonstrated an association between NAFLD and an atherogenic dyslipidemia phenotype. Arslan et al. demonstrated that the presence of ultrasound defined NAFLD is independently associated with the presence and extent of CAD as seen during invasive coronary angiography. Fotbolcu et al. have shown that left ventricular systolic and diastolic function, presumably reflecting established CAD, is impaired in patients with NAFLD, even in the absence of metabolic syndrome. In a meta-analysis by Lu and colleagues, which included over 7000 participants from cross-sectional studies and prospective cohort studies, a model adjusted for established CV risk factors found NAFLD to be a predictor of CVD. In contrast, Ruhl et al. did not find increased CV mortality in a prospective study of patients with elevated ALT levels. Stepanova et al. were able to demonstrate an increased risk for CVD in ultrasound defined NAFLD patients. However, in their study NAFLD did not significantly increase the risk for CV mortality during long-term follow-up. Also, Alexander et al. recently performed a population based retrospective matched cohort study to estimate the incidence of acute MI in patients with NAFLD compared to the general population after adjustment for traditional risk factors and did not demonstrate increased risk in the NAFLD group.
Our study cannot be compared directly to previously-reported population-based studies due to several reasons. First, all of the patients in our study had an intermediate pre-test probability of CAD, as they were all referred to a tertiary center for SPECT-MPI, a test that is indicated for cardiac risk stratification. Given the known association of NAFLD with diabetes and the metabolic syndrome, it is likely that many subjects with NAFLD were considered to have a higher risk of CAD and were referred directly to a more invasive evaluation and treatment. This would be predicted to result in under-representation of NAFLD in our study population and indeed, the 14.4% incidence in our study is markedly lower than the 30% rate reported in a population-based study from Israel during similar years. Furthermore, patients with NAFLD who were referred directly to a more invasive evaluation and treatment. This would be predicted to result in under-representation of NAFLD in our study population and indeed, the 14.4% rate reported in a population-based study from Israel during similar years. Additionally, patients with NAFLD who were referred to SPECT-MPI and are included in our study are likely to have less prominent CV risk factors than their counterparts who underwent more invasive testing. This population stratification likely explains why the PNAFLD patients in our cohort were not associated with increased rates of obesity or dyslipidemia and did not exhibit a greater risk of abnormal SPECT-MPI.

Second, a direct comparison between our patient cohort and previously reported large population-based studies of NAFLD is problematic, as the gold standard for the diagnosis of NAFLD is an invasive liver biopsy, an approach usually not feasible in large studies. Thus, all reported large population-based studies utilized different surrogate markers for NAFLD, making a direct comparison problematic. In our study, the effect of PNAFLD on cardiac outcomes and overall mortality was limited to subjects with a normal SPECT-MPI at baseline. Acute myocardial infarction is the result of atherosclerotic plaque rupture, which is not necessarily preceded by symptoms of angina and a clinically significant atherosclerotic coronary stenosis that would be diagnosed by SPECT-MPI. NAFLD with its underlying pro-inflammatory state and associated metabolic risk factors may lead to the development of coronary microvascular dysfunction in the absence of epicardial CAD. Therefore, a normal SPECT-MPI seems insufficient to exclude future occurrence of acute coronary events in this specific population. In contrast, subjects with an abnormal SPECT-MPI, reflecting an already compromised coronary blood flow, are at higher risk (probably not further modulated by the presence of NAFLD) for a future cardiac event. An alternative explanation for this finding is that subjects with an abnormal SPECT-MPI were subsequently offered more aggressive therapy for their CAD and associated comorbidities, which modified their risk profile for adverse CV outcome irrespective of the presence of NAFLD. It is also possible that in these high-risk patients, the presence of NAFLD may not portend an even higher risk.

The strength of our study is the use of a large database of more than 10,000 patients with 5 years of follow-up. Given the infrastructure of the Israeli healthcare system, we were able to capture near complete data on all participants. Our study’s main limitation is in the assumption that ALT elevation (in the absence of other identifiable causes) reflects the presence of NAFLD, thus fitting the definition of PNAFLD. Although the gold standard for diagnosing NAFLD is a liver biopsy, this is not practical in large-scale epidemiological studies such as ours, and neither is imaging, especially in a retrospective analysis. However, the surrogate use of elevated aminotransferases, in the absence of alcohol abuse or viral hepatitis, to define PNAFLD has been well-validated. In fact, in relying on elevated enzymes with our cut-off values, we are likely underestimating the true prevalence of NAFLD in our population, as even in subjects with normal liver enzymes, NAFLD and even NASH are common. It is possible that our failure to show abnormal SPECT-MPI tests among the presumed NAFLD patients as compared to our control patients is due to the inclusion of subjects with NAFLD and normal or mildly elevated ALT in the control group. It is also possible that our study was not powered enough to show significant differences which would have been apparent in a larger study. For example, patients with an elevated ALT were found to have an increased HR for all-cause mortality during a five-year follow-up, however the finding was not statistically significant. Furthermore, ALT was found to increase the risk for an abnormal SPECT-MPI result but only in a multivariable analysis.

In conclusion, our study evaluates for the first time the association of PNAFLD with SPECT-MPI results and mortality. Our results show that the interaction of PNAFLD with CV outcomes is complex and is highly dependent on the selected study population. Further research is indicated to identify the underlying mechanisms by which NAFLD may contribute to the development and progression of CVD.

Author Contributions
DY: Conceptualization, Data curation, Writing—Original draft, Review and editing; RT: Formal analysis, Resources, Writing—Review and editing; VN: Supervision, Resources, Writing—Review and editing; AS: Resources, Writing—Review and editing; AW: Resources, Writing—Review and editing; YR: Formal analysis, Writing—Review and editing; OE: Conceptualization, Supervision, Writing—Review and editing.

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