

Aortic flow propagation velocity, epicardial fat thickness, and osteoprotegerin level to predict subclinical atherosclerosis in patients with nonalcoholic fatty liver disease

Didem Oğuz, Hakan Ümit Ünal¹, Hacer Eroğlu*, Öykü Gülmez, Halime Çevik**, Armağan Altun

Department of Cardiology, *Biochemistry, **Radiology, Başkent University İstanbul Hospital; İstanbul-Turkey

¹Department of Gastroenterology, Şişli Florence Nightingale Hospital; İstanbul-Turkey

ABSTRACT

Objective: Nonalcoholic fatty liver disease is the most common cause of liver dysfunction in Western countries and an independent risk factor for atherosclerotic heart disease. Appropriate noninvasive parameters are lacking for optimal risk stratification of cardiovascular disease in these patients. We evaluated several recently discovered noninvasive parameters for atherosclerosis in patients with nonalcoholic fatty liver disease: epicardial fat thickness, aortic flow propagation velocity, and osteoprotegerin level.

Methods: Forty-one patients (27 men and 14 women; mean age, 37.9±8.9 years) with nonalcoholic fatty liver disease and 37 control subjects (17 men and 20 women; mean age, 34.5±8.6 years) were enrolled in this observational case-control study. Patients with nonalcoholic fatty liver disease diagnosed at a gastroenterology outpatient clinic were included. Patients with cardiac pathology other than hypertension were excluded. Epicardial fat thickness and aortic flow propagation velocity were measured by echocardiography. The serum concentration of osteoprotegerin was measured using a commercial enzyme-linked immunosorbent assay kit.

Results: Nonalcoholic fatty liver disease patients exhibited a significantly lower aortic flow propagation velocity (155.17±30.00 vs. 179.00±18.14 cm/s, p=0.000) and significantly higher epicardial fat thickness (0.51±0.25 vs. 0.29±0.09 cm, p=0.000) than control subjects. Osteoprotegerin levels were higher, but not significant, in patients with nonalcoholic fatty liver disease (28.0±13.0 vs. 25.2±10.8 pg/mL, p=0.244). Binary logistic regression analysis showed that aortic flow propagation velocity (OR, -0.973; 95% CI, 0.947–0.999) and waist circumference (OR, -1.191; 95% CI, 1.088–1.303) were independent predictors of nonalcoholic fatty liver disease.

Conclusion: In this study, epicardial fat thickness and osteoprotegerin level were higher and aortic flow propagation velocity was lower in patients with nonalcoholic fatty liver disease. Early detection of abnormal epicardial fat thickness and aortic flow propagation velocity may warrant a search for undetected cardiovascular disease in patients with nonalcoholic fatty liver disease. (*Anatol J Cardiol* 2016; 16: 974-9)

Keywords: nonalcoholic fatty liver disease, subclinical atherosclerosis, osteoprotegerin, epicardial fat thickness, aortic flow propagation velocity

Introduction

Nonalcoholic fatty liver disease (NAFLD) is the most common cause of liver dysfunction in Western countries. Additionally, it is an independent risk factor for atherosclerotic heart disease (1). Several noninvasive markers for atherosclerotic heart disease have recently been discovered, including epicardial fat thickness (EFT), aortic flow propagation velocity (APV), and osteoprotegerin (OPG) level (2–4).

Aortic distensibility and aortic strain are elasticity indices that reflect aortic stiffness. Impaired aortic distensibility has been related to increased cardiovascular mortality in different populations (5, 6). APV, which is also used to evaluate aortic stiffness, might be an indicator of the presence of atherosclerotic

heart disease. Another echocardiographic marker for atherosclerotic heart disease is EFT. Human epicardial adipose tissue is described as a potential paracrine and/or endocrine tissue, specifically within the context of cardiovascular risk (7–9). OPG, a cytokine also known as osteoclastogenesis inhibitory factor, has been found to have a higher plasma concentration in patients with than without atherosclerotic heart disease (10–13). Several authors have concluded that OPG appears to be a promising biomarker of atherosclerosis that is independently associated with traditional risk factors of subclinical and clinical atherosclerotic disease (14–16).

The aim of this study was to evaluate OPG, APV, and EFT for atherosclerotic heart disease in patients with NAFLD, which is an independent risk factor for atherosclerotic heart disease.

Address for correspondence: Dr. Didem Oğuz, Başkent Üniversitesi İstanbul Hastanesi, Kardiyoloji Bölümü, İstanbul-Türkiye

Phone: +90 216 554 15 00-1615 Fax: +90 216 651 98 58 E-mail: di_oguz@hotmail.com, didemo@baskent-ist.edu.tr

Accepted Date: 10.02.2016 **Available Online Date:** 28.03.2016

©Copyright 2016 by Turkish Society of Cardiology - Available online at www.anatoljcardiol.com
DOI:10.14744/AnatolJCardiol.2016.6706



Methods

Study population

In total, 41 patients with NAFLD (27 men and 14 women; mean age, 37.9±8.9 years) and 37 control subjects (17 men and 20 women; mean age, 34.5±8.6 years) were enrolled in this observational case-control study. The patients had been consecutively observed at our gastroenterology outpatient clinic over the past 12 months. Liver ultrasound (US) examinations were performed to assess the degree of steatosis. All US scans were performed by the same operator, who was blinded to the patients' clinical status. The exclusion criteria were coronary artery disease; a positive treadmill exercise test result; diabetes mellitus; a body mass index (BMI) of >35 kg/m²; bundle branch block; paced rhythm; atrial fibrillation; Q waves; left ventricular hypertrophy or strain on the electrocardiogram at rest; significant valvular heart disease; congenital heart disease; aortic aneurysm; systemic diseases that affect the aorta; severe obstructive pulmonary disease; chronic renal disease; treatment with estrogen, amiodarone, steroids, tamoxifen, diltiazem, or herbals; history of abdominal surgery; history or suspicion of viral hepatitis; and a daily alcohol intake exceeding 20 g/day. All participants included in the control group were judged to have normal results on liver function tests and were confirmed to have a normal liver by US scanning. All participants provided informed written consent. The study protocol conformed with the Declaration of Helsinki.

Anthromorphometry, blood pressure measurement, and laboratory testing

All participants underwent physical examination, anthropometric measurements, and biochemical screening. BMI was defined as weight (in kilograms) divided by the square of the height (in meters). Waist circumference (in centimeters) was recorded as the average of two measurements at the midpoint between the lowest rib and the iliac crest while the participant was standing. Blood pressure was measured using a mercury sphygmomanometer in a quiet room after at least 10 min of rest. The average of three measurements was used. Blood samples were obtained after 12 h of fasting. The levels of low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL), triglyceride, total cholesterol, glucose, creatinine, hemoglobin, aspartate aminotransferase, and alanine aminotransferase were measured by routine laboratory techniques.

The serum concentration of OPG was measured using a commercial enzyme-linked immunosorbent assay (ELISA) kit (model number: BMS201 INST) (eBioscience, Vienna, Austria). Serum samples were collected and subsequently stored at -80°C until OPG analysis. The investigator who analyzed the OPG serum concentrations was blinded to all clinical information. Both the monomeric and dimeric forms of OPG and OPG bound to RANKL were detected in this assay. A monoclonal IgG antibody was used as the capture antibody, and a biotin-conjugated polyclonal antihuman OPG antibody was used as the detection antibody. The detection limit of this assay system was 2.5 pg/mL; the intra-

assay and inter-assay coefficients of variation were typically 7% and 8%, respectively, at low and high concentrations of OPG.

Transthoracic echocardiographic examination

Echocardiographic examination was performed at rest in the left lateral decubitus position. Measurements were obtained using an Acuson Sequoia 512 system (Siemens, Munich, Germany) with a 4-MHz transducer according to the American Society of Echocardiography measurement guidelines. Echocardiographic evaluation was performed by the same experienced cardiologist who had no clinical information about the patients. The epicardial fat was defined as the echo-free space between the outer wall of the myocardium and the visceral layer of the pericardium. EFT was measured perpendicularly on the free wall of the right ventricle at end-diastole for three cardiac cycles. The aortic annulus was used as the anatomical landmark with which to standardize the measurement point. The average measurement of three cardiac cycles was used for the statistical analysis. Color M-mode Doppler recordings were obtained from the suprasternal window in the supine position. The cursor was located parallel to the main flow direction in the descending aorta. The Nyquist limit was adapted to 30–50 cm/s, switching to the M-mode with the recorder sweep rate of 200 mm/s. The M-mode spatiotemporal velocity map in the shape of a flame was displayed. If the slope of the flame was unclear, baseline shifting was used to change the aliasing velocity until a clear delineation of the velocity slope was obtained. APV was then calculated by dividing the distance between the points corresponding to the beginning and end of the propagation slope by the duration between the corresponding time points. Thus, APV corresponds to the velocity at which the flow is propagating down the artery. The mean of at least three measurements was recorded as APV.

Exercise electrocardiography

A symptom-limited treadmill exercise electrocardiographic test was performed with the modified Bruce protocol to distinguish lower risk of CVD patients more precisely. The test result was defined as positive if the horizontal or down-sloping ST-segment depression measured ≥1 mm at 80 ms after the J point in two or more contiguous leads, and typical angina increased in severity with exercise.

Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software, version 18 (SPSS Inc., Chicago, Illinois, USA). Statistical significance was defined as $p < 0.05$. Results are given as mean±standard error (SE) and median [interquartile range (IQR)]. Variable distributions were assessed with the Kolmogorov–Smirnov normality test. Statistical comparison between groups was assessed by student's t-test, the Mann–Whitney U test, or the Pearson chi-square test. Correlation analyses were performed using Spearman or Pearson's coefficients to assess relationships of mea-

Table 1. Clinical and biochemical characteristics of patients with nonalcoholic fatty liver disease and controls

	Controls (n=37)	Patients with NAFLD (n=41)	P
Gender, male/female, n	17/20	27/14	NS
Age, years	34.5±8.6	37.9±8.9	NS
BMI, kg/m ²	24.20±3.26	29.50±3.97	<0.001
SBP, mm Hg	118.0±13.8	129.3±14.8	0.001
DBP, mm Hg	75.0±11.5	79.6±12.5	NS
Heart rate, beats/min	78.9±13.9	76.6±13.4	NS
Pulse pressure, mm Hg	43.0±10.6	49.2±12.3	0.020
Waist circumference, cm	89.5±9.7	106.0±11.9	<0.001
Smoking	13	15	NS
HT	3	9	NS
Anti-HT treatment	3	7	NS
Family history of CAD	7	5	NS
Hemoglobin, g/dL	13.77±1.40	15.0±1.20	<0.001
Creatinine, mg/dL	0.77±0.12	0.86±0.14	0.002
FBG, mg/dL	91.2±7.0	98.6±9.9	<0.001
LDL, mg/dL	118.0±33.0	140.7±40.3	0.009
HDL, mg/dL	49.0±9.9	39.7±8.9	<0.001
Total cholesterol, mg/dL	183.5±36.1	212.3±42.8	0.002
Triglycerides, mg/dL	87(75–101)	158(126–198)	<0.001

BMI - body mass index; CAD - coronary artery disease; DBP - diastolic blood pressure; FBG - fasting blood glucose; HDL - high-density lipoprotein cholesterol; HT - hypertension; LDL - low-density lipoprotein cholesterol; NAFLD - nonalcoholic fatty liver disease; NS - nonsignificant; SBP - systolic blood pressure. Student's t-test or the Mann-Whitney U test was used for comparison of groups. Categorical variables were compared using the Pearson chi-square test

ures. Binary logistic regression analysis was used to determine the independent predictors of NAFLD.

Results

Forty-one patients with NAFLD (mean age, 37.9±8.9 years) and 37 controls (mean age, 34.5±8.6 years) participated in this study. The mean values for the main clinical and laboratory characteristics in the NAFLD and control groups are reported in Table 1. The age, sex distribution, diastolic blood pressure, heart rate, history of hypertension, medications, family history of coronary artery disease, and smoking ratio were similar between the NAFLD and control groups. Patients with NAFLD had significantly higher BMI, waist circumference, lipid profile, systolic blood pressure, fasting blood glucose, hemoglobin, and creatinine values than the controls (Table 1). The median value of EFT was 0.40 cm (0.36–0.55 cm) in patients with NAFLD and 0.29 cm (0.23–0.33 cm) in controls, with a statistically significant difference (p<0.001) (Table 2). APV was 155.17±30.00 cm/s in patients with NAFLD and 179.40±18.14 cm/s in controls, with a statistically significant difference (p<0.001) (Table 2). The OPG level

Table 2. Comparison of epicardial fat thickness, aortic flow propagation velocity, and osteoprotegerin level between nonalcoholic fatty liver disease and control groups

	Controls (n=37)	Patients with NAFLD (n=41)	P
EFT, cm	0.29 (0.23–0.33)	0.40 (0.36–0.55)	<0.001
APV, cm/s	179.40±18.14	155.17±30.00	<0.001
OPG, pg/mL	22.26 (19.71–25.04)	25.1 (21.06–28.6)	0.244

APV - aortic flow propagation velocity; EFT - epicardial fat thickness; NAFLD - nonalcoholic fatty liver disease; OPG - osteoprotegerin. Binary logistic regression analysis was performed to assess the correlations between the studied parameters

Table 3. Correlation of epicardial fat thickness with clinical variables

	Correlation coefficient	P
Age	0.44	<0.001
Waist circumference	0.42	<0.001
Body mass index	0.38	0.001
Fasting glucose	0.48	<0.001
Total cholesterol	0.27	0.017
LDL cholesterol	0.33	0.004
HDL cholesterol	-0.19	0.930
Triglycerides	0.28	0.013
Osteoprotegerin	0.21	0.063
Hemoglobin	0.14	0.240

Correlation analyses were performed using Spearman or Pearson's coefficients according to the distribution of the variables. HDL - high-density lipoprotein; LDL - low-density lipoprotein

was higher in the NAFLD group than in the control group, but was statistically insignificant [25.1 pg/mL (21.06–28.6 pg/mL) and 22.26 pg/mL (19.71–25.04 pg/mL) respectively; p=0.244] (Table 2).

Among all patients, EFT was correlated with age (r=0.44, p<0.001), waist circumference (r=0.42, p<0.001), BMI (r=0.38, p=0.001), fasting blood glucose (r=0.48, p<0.001), total cholesterol (r=0.27, p=0.017), LDL (r=0.33, p=0.004), and triglycerides (r=0.28, p=0.013) (Table 3). However, there was no correlation among EFT, OPG, and HDL.

APV was correlated with waist circumference (r=-0.27, p=0.02), BMI (r=-0.28, p=0.012), and fasting blood glucose (r=-0.24, p=0.04) (Table 4).

We found a statistically significant correlation between EPF and APV (r=-0.313, p=0.006). Binary logistic regression analysis showed that APV (OR, -0.973; 95% CI, 0.947–0.999) and waist circumference (OR, -1.191; 95% CI, 1.088–1.303) were independent predictors of NAFLD.

Discussion

This study mainly evaluated the relationship between APV, EFT, and OPG levels as atherosclerotic parameters and NAFLD. We demonstrated increased EFT and OPG levels and decreased

Table 4. Correlation of aortic flow propagation velocity with clinical variables

	Correlation coefficient	P
Age	-0.280	0.140
Waist circumference	-0.270	0.020
Body mass index	-0.280	0.012
Fasting glucose	-0.240	0.040
Total cholesterol	-0.020	0.880
LDL cholesterol	0.007	0.950
HDL cholesterol	0.220	0.059
Triglycerides	-0.190	0.087
Osteoprotegerin	-0.140	0.210
Hemoglobin	0.030	0.810

Correlation analyses were performed using Spearman or Pearson's coefficients according to the distribution of the variables. HDL - high-density lipoprotein; LDL - low-density lipoprotein

APV levels in patients with NAFLD. Moreover, we showed that APV was correlated with waist circumference, BMI, and fasting glucose; EFT was correlated with APV.

NAFLD is a chronic liver disease that comprises a wide spectrum of hepatic damage ranging from simple steatosis and steatohepatitis to advanced fibrosis and cirrhosis (17). Although the true prevalence of NAFLD is unknown, the estimated prevalence in the general population ranges from 3% to 24% (18). In the present study, patients with NAFLD had a higher systolic blood pressure, waist circumference, fasting blood glucose, and triglyceride level and a lower HDL level. According to previous studies, NAFLD appears to be strongly associated with risk factors for atherosclerosis, such as hypertension, obesity, diabetes, high triglycerides, low HDL, insulin resistance, and metabolic syndrome; NAFLD also appears to increase with age (18). Patients with NAFLD have an increased risk of cardiovascular disease (CVD) (19–21), reportedly independent of underlying cardiovascular risk factors (22). Efe et al. (23) found that the prevalence of coronary artery disease in patients with NAFLD was significantly higher than that in patients with normal liver tissue. The association between NAFLD and the risk of CVD has been attributed to increased oxidative stress and subclinical inflammation. Fat deposition in patients with NAFLD is considered to increase the levels of free fatty acids that lead to CVD by causing inflammation (24, 25). Indeed, it is hypothesized that NAFLD is not only a risk marker of CVD but may also be involved in its pathogenesis (26). Genetic predisposition, reduced levels of adiponectin, and coagulation imbalance are the other physio-pathogenic mechanisms of CVD in NAFLD patients (27). Endothelial dysfunction is the first stage in the development of atherosclerosis and CVD. Atherosclerosis leads to increased arterial resistance through thickening and stiffening of the arterial wall. A loss of arterial elastic properties results in pathophysiological changes in circulation, including increased pulse pressure and vessel endothelial dysfunction (28). Increased arterial resistance may be reflected by a decrease in

APV. Güneş et al. (29) recently showed that the M-mode-derived propagation velocity of the descending thoracic aorta (AVP), which is an easy, practical, noninvasive measurement of arterial resistance, was associated with coronary atherosclerosis. In the present study, AVP was lower in patients with NAFLD than in controls. Additionally, AVP was correlated with waist circumference, BMI, and fasting glucose. Increased aortic stiffness has been shown to be more closely related to visceral obesity, as assessed by waist circumference, than to an increased BMI (30). However, further studies are required to determine whether the measurement of AVP may be used to improve cardiovascular risk estimation in patients with NAFLD. EFT is clinically related to cardiac and visceral adiposity, and it has been suggested as a new cardiometabolic risk factor (31). The epicardial fat tissue is in direct contact with the myocardium and major coronary arteries and their branches and is metabolically active (32, 33). Epicardial adipocytes can secrete a large number of cytokines and vasoactive peptides, all of which can independently increase cardiovascular risk (34). Echocardiographic assessment of EFT is simple, rapid, safe, and easily reproducible and may have a potential to be a simple and reliable marker of increased cardiovascular risk. In the study of Eroğlu et al. (35), EFT was found to be a marker of the presence and severity of coronary artery disease. Iacobellis et al. (36) found that EFT better predicts ultrasound-measured fatty liver than BMI or waist circumference in obese patients. In our study, we exclude morbid obese patients (BMI >35 kg/m²). Another study showed that a higher EFT is associated with the severity of liver fibrosis. They also found some alterations of cardiac functions and morphology linked to liver fibrosis (37). However, in the study of Psychari et al. (38), EFT was not found to be significantly correlated with NAFLD. In contrast to these studies, in our study, we did not include high cardiovascular risk patients and showed that EFT is correlated with age, fasting glucose, total cholesterol, LDL, triglycerides, waist circumference, and BMI, in accordance with some previous findings (39, 40).

Moreover, our data showed that EFT was significantly higher in the NAFLD than in the control group. Furthermore, EFT was correlated with APV, which is a previously described marker of increased arterial resistance. In the study of Sünbül et al. (41), patients with NAFLD have increased arterial resistance. In addition, they found that the severity of liver fibrosis and EFT are independent predictors of measurements of arterial resistance.

Numerous randomized controlled studies have found that OPG, a soluble decoy receptor of osteoclast activator, acts as a regulatory molecule in patients with vascular calcification. In the Women on the Move through Activity and Nutrition (WOMAN) study (42), a higher OPG was associated with a higher coronary artery calcium score, whereas the Framingham Study reported a nonsignificant relationship between OPG and coronary calcification (12). The serum OPG level is associated with arterial stiffness, as measured by pulse wave velocity (43). Moreover, a high serum OPG level was reported to be an independent predictor of cardiovascular events in patients undergoing hemodi-

alysis (43). In addition, a 17-year prospective follow-up study of patients with type II diabetes showed a strong predictive value of the OPG level for all-cause and cardiovascular mortality (44). However, the present study showed that the OPG level was not significantly increased in patients with NAFLD. Moreover, there was no association between the OPG level and APV. This discrepancy may be due to the small sample size of this study as well as the patients' characteristics, which included younger ages and a more stable clinical status with a lower risk profile of CVD than in previous studies.

Study limitations

Our study has several limitations. First, because the sample size was relatively small, only a few patients in the NAFLD group had upper-limit alanine aminotransferase levels, and the degree of steatosis was not assessed. Second, NAFLD was not confirmed by liver biopsy, and for medical and ethical reasons, we excluded alcoholic liver disease and autoimmune hepatitis based on answers to a simple questionnaire.

Conclusions

In this study, we demonstrated APV is independently associated with NAFLD, even those classified as being at low risk for cardiovascular events according to traditional risk scores. In addition, the present study showed that EFT was higher in patients with than without NAFLD. Early detection of abnormal EFT and APV levels may warrant a search for undetected CVD in patients with NAFLD.

Conflict of interest: None declared.

Peer-review: Externally peer-reviewed.

Authorship contributions: Concept – D.O., Ö.G., H.Ü.Ü.; Design – D.O., H.Ç., Ö.G.; Supervision – D.O., A.A.; Fundings – Başkent Üniversitesi; Materials – D.O., H.Ü.Ü., H.Ç., H.E.; Data collection &/or processing – D.O., H.E.; Analysis &/or interpretation – D.O., H.E., H.Ç.; Literature search – D.O., A.A.; Writing – D.O., Ö.G., H.Ü.Ü., A.A.; Critical review – D.O., A.A.

References

1. Sookoian S, Pirola CJ. Non-alcoholic fatty liver disease is strongly associated with carotid atherosclerosis: a systematic review. *J Hepatol* 2008; 49: 600-7.
2. Hirai T, Sasayama S, Kawasaki T, Yagi S. Stiffness of systemic arteries in patients with myocardial infarction. A noninvasive method to predict severity of coronary atherosclerosis. *Circulation* 1989; 80: 78-86.
3. Gatzka CD, Cameron JD, Kingwell BA, Dart AM. Relation between coronary artery disease, aortic stiffness, and left ventricular structure in a population sample. *Hypertension* 1998; 32: 575-8.
4. Blacher J, Pannier B, Guerin AP, Marchais SJ, Safar ME, London GM. Carotid arterial stiffness as a predictor of cardiovascular and all-cause mortality in end-stage renal disease. *Hypertension* 1998; 32: 570-4.
5. Laurent S, Boutouyrie P, Asmar R, Gautier I, Laloux B, Guize L, et al. Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients. *Hypertension* 2001; 37: 1236-41.
6. Stefanadis C, Dernellis J, Tsiamis E, Stratos C, Diamantopoulos L, Michaelides A, et al. Aortic stiffness as a risk factor for recurrent acute coronary events in patients with ischaemic heart disease. *Eur Heart J* 2000; 21: 390-6.
7. Mazurek T, Zhang L, Zalewski A, Mannion JD, Diehl JT, Arafat H, et al. Human epicardial adipose tissue is a source of inflammatory mediators. *Circulation* 2003; 108: 2460-6.
8. Sacks HS, Fain JN. Human epicardial fat: what is new and what is missing? *Clin Exp Pharmacol Physiol* 2011; 38: 879-87.
9. Bos D, Shahzad R, van Walsum T, van Vliet LJ, Franco OH, Hofman A, et al. Epicardial fat volume is related to atherosclerotic calcification in multiple vessel beds. *Eur Heart J Cardiovasc Imaging* 2015; 16: 1264-9.
10. Aksu F, Özçelik F, Kunduracılar H, Barutçu A, Yel M, Ümit EG, et al. The relation between the levels of osteoprotegerin and the degree of coronary artery disease in patients with acute coronary syndrome and stable angina pectoris. *Kardiol Pol* 2014; 72: 34-41.
11. Breland UM, Hollan I, Saatvedt K, Almdahl SM, Damas JK, Yndestad A, et al. Inflammatory markers in patients with coronary artery disease with and without inflammatory rheumatic disease. *Rheumatology* 2010; 49: 1118-27.
12. Lieb W, Gona P, Larson MG, Massaro JM, Lipinska I, Keaney JF Jr, et al. Biomarkers of the osteoprotegerin pathway: clinical correlates, subclinical disease, incident cardiovascular disease and mortality. *Arterioscler Thromb Vasc Biol* 2010; 30: 1849-54.
13. Abedin M, Omland T, Ueland T, Khera A, Aukrust P, Murphy SA, et al. Relation of osteoprotegerin to coronary calcium and aortic plaque (from the Dallas Heart Study). *Am J Cardiol* 2007; 99: 513-8.
14. Altun A, Uğur-Altun B, Tatlı E. Decreased serum osteoprotegerin levels in patients with cardiac syndrome X. *J Endocrinol Invest* 2004; 27: 839-43.
15. Uğur-Altun B, Altun A, Gerenli M, Tuğrul A. The relationship between insulin resistance assessed by HOMA-IR and serum osteoprotegerin levels in obesity. *Diabetes Res Clin Pract* 2005; 68: 217-22.
16. Mogelvang R, Pedersen SH, Flyvbjerg A, Bjerre M, Iversen AZ, Galatius S, et al. Comparison of osteoprotegerin to traditional atherosclerotic risk factors and high-sensitivity C-reactive protein for diagnosis of atherosclerosis. *Am J Cardiol* 2012; 109: 515-20.
17. Clark JM. Weight loss as a treatment for nonalcoholic fatty liver disease. *J Clin Gastroenterol* 2006; 40 Suppl 1: S39-43.
18. Clark JM. The epidemiology of nonalcoholic fatty liver disease in adults. *J Clin Gastroenterol* 2006; 40 Suppl 1: S5-10.
19. Targher G, Day CP, Bonora E. Risk of cardiovascular disease in patients with nonalcoholic fatty liver disease. *N Engl J Med* 2010; 363: 1341-50.
20. Targher G, Marra F, Marchesini G. Increased risk of cardiovascular disease in non-alcoholic fatty liver disease: causal effect or epiphenomenon? *Diabetologia* 2008; 51: 1947-53.
21. VanWagner LB, Ning H, Lewis CE, Shay CM, Wilkins J, Carr JJ, et al. Associations between nonalcoholic fatty liver disease and subclinical atherosclerosis in middle-aged adults: the Coronary Artery Risk Development in Young Adults Study. *Atherosclerosis* 2014; 235: 599-605.
22. Targher G, Arcaro G. Non-alcoholic fatty liver disease and increased risk of cardiovascular disease. *Atherosclerosis* 2007; 191:

- 235-40.
23. Efe D, Aygün F. Assessment of the relationship between non-alcoholic fatty liver disease and CAD using MSCT. *Arq Bras Cardiol* 2014; 102: 10-8.
 24. Perseghin G. The role of non-alcoholic fatty liver disease in cardiovascular disease. *Dig Dis* 2010; 28: 210-3.
 25. Targher G, Bertolini L, Scala L, Zoppini G, Zenari L, Falezza G. Non-alcoholic hepatic steatosis and its relation to increased plasma biomarkers of inflammation and endothelial dysfunction in non-diabetic men. Role of visceral adipose tissue. *Diabet Med* 2005; 22: 1354-8.
 26. Oni ET, Agatston AS, Blaha MJ, Fialkow J, Cury R, Sposito A, et al. A systematic review: burden and severity of subclinical cardiovascular disease among those with nonalcoholic fatty liver; should we care? *Atherosclerosis* 2013; 230: 258-67.
 27. 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.
 28. Fargion S, Porzio M, Fracanzani AL. Nonalcoholic fatty liver disease and vascular disease:state-of-the-art. *World J Gastroenterol* 2014; 20: 13306-24.
 29. Güneş Y, Tuncer M, Güntekin U, Ceylan Y, Şimşek H, Şahin M, et al. The relation between the color M-mode propagation velocity of the descending aorta and coronary and carotid atherosclerosis and flow-mediated dilatation. *Echocardiography* 2010;27:300-5.
 30. Rosito GA, Massaro JM, Hoffmann U, Ruberg FL, Mahabadi AA, Vasan RS, et al. Pericardial fat, visceral abdominal fat, cardiovascular disease risk factors, and vascular calcification in a community-based sample. The Framingham Heart Study. *Circulation* 2008; 117: 605-13.
 31. Natale F, Tedesco MA, Mocerino R, de Simone V, Di Marco GM, Aronne L, et al. Visceral adiposity and arterial stiffness: echocardiographic epicardial fat thickness reflects, better than waist circumference, carotid arterial stiffness in a large population of hypertensives. *Eur J Echocardiogr* 2009; 10: 549-55.
 32. Talman AH, Psaltis PJ, Cameron JD, Meredith IT, Seneviratne SK, Wong DT. Epicardial adipose tissue: far more than a fat depot. *Cardiovasc Diagn Ther* 2014; 4: 416-29.
 33. Fitzgibbons TP, Czech MP. Epicardial and perivascular adipose tissue and their influence on cardiovascular disease: basic mechanisms and clinical associations. *J Am Heart Assoc* 2014; 3: e000582.
 34. Iacobellis G, Barbaro G. The double role of epicardial adipose tissue as pro- and anti-inflammatory organ. *Horm Metab Res* 2008; 40: 442-5.
 35. Eroğlu S, Sade LE, Yıldırım A, Bal U, Özbiçer S, Özgül AS, et al. Epicardial adipose tissue thickness by echocardiography is a marker for the presence and severity of coronary artery disease. *Nutr Metab Cardiovasc Dis* 2009; 19: 211-7.
 36. Iacobellis G, Barbarini G, Letizia C, Barbaro G. Epicardial fat thickness and nonalcoholic fatty liver disease in obese subjects. *Obesity* 2014; 22: 332-6.
 37. Petta S, Argano C, Colomba D, Camma C, Di Marco V, Cabibi D, et al. Epicardial fat, cardiac geometry and cardiac function in patients with nonalcoholic fatty liver disease: association with severity of liver disease. *J Hepatol* 2015; 62: 928-33.
 38. Psychari SN, Rekleiti N, Papaioannou N, Varhalama E, Drakoulis C, Apostolou TS, et al. Epicardial fat in nonalcoholic fatty liver disease: properties and relationships with metabolic factors, cardiac structure, and cardiac function. *Angiology* 2016; 67: 41-8.
 39. Şengül C, Çevik C, Özveren O, Oduncu V, Sünbül A, Akgün T, et al. Echocardiographic epicardial fat thickness is associated with carotid intima-media thickness in patients with metabolic syndrome. *Echocardiography* 2011; 28: 853-8.
 40. Kim SJ, Kim HS, Jung JW, Kim NS, Noh CI, Hong YM. Correlation between epicardial fat thickness by echocardiography and other parameters in obese adolescents. *Korean Circ J* 2012; 42: 471-8.
 41. Sünbül M, Ağırbaşı M, Durmuş E, Kıvrak T, Akın H, Aydın Y, et al. Arterial stiffness in patients with non-alcoholic fatty liver disease is related to fibrosis stage and epicardial adipose tissue thickness. *Atherosclerosis* 2014; 237: 490-3.
 42. Kuller LH, Pettee Gabriel KK, Kinzel LS, Underwood DA, Conroy MB, Chang Y, et al. The Women on the Move Through Activity and Nutrition (WOMAN) study: final 48-month results. *Obesity* 2012; 20: 636-43.
 43. Lee JE, Kim HJ, Moon SJ, Nam JS, Kim JK, Kim SK, et al. Serum osteoprotegerin is associated with vascular stiffness and the onset of new cardiovascular events in hemodialysis patients. *Korean J Intern Med* 2013; 28: 668-77.
 44. Reinhard H, Lajer M, Gall MA, Tarnow L, Parving HH, Rasmussen LM, et al. Osteoprotegerin and mortality in type 2 diabetic patients. *Diabetes Care* 2010; 33: 2561-6.