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Vegan Diet Advice Might Benefit Liver Enzymes in Nonalcoholic Fatty Liver Disease: an Open Observational Pilot Study

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ABSTRACT

Background & Aims: The Western diet is rich in saturated fats, refined sugars and meat consistent with a high-energy load and secondary risk of increased metabolic diseases including nonalcoholic fatty liver disease (NAFLD). However, no data are available on potential benefit of vegan diets in NAFLD and/or nonalcoholic steatohepatitis (NASH). We aimed to study prospectively the effect of a vegan diet, excluding all animal products on liver chemistry in a group of consecutive NAFLD patients.
Methods: This was a prospective, pilot study run on 40 consecutive patients affected by NAFLD. Eight subjects refused to join the study for poor diet palatability, leaving 32 patients (19 males, mean age 50 years), with abnormal measures of liver function who agreed to adhere to a vegan diet for six months. The caloric intake was tailored by the dietitian to obtain a weight loss ≥5% of body weight in overweight patients [body-mass index (BMI) ≥25] and ranged from 1500 Kcal to 1800 Kcal. Patients were contacted monthly by phone to reinforce diet and lifestyle advice and were seen at the gastrointestinal clinic when doubtful about diet advice.
Results: At six-month follow-up, 6 subjects did not attend the clinic leaving only 26 patients for data analysis. Initial anthropometric values were mean weight 78 kg (range 52-95), mean body mass index (BMI) 26.8 Kg/m^2 (range 20.3-31.2). Liver function tests showed mean ALT value 99 U/L (SD±45), mean AST value 54 U/L (SD±44), mean GGT value 160 U/L (SD±122), pre-treatment. After six months mean body weight was 73 Kg (range 52-87), mean BMI was 25.2 Kg/m2 (range 20.3-29.7) ($p<0.001$ compared to baseline for both parameters). Liver enzymes improved to a mean of ALT value 36 U/L (SD±21), AST value 27 U/L (SD±10) and GGT value 55 U/L (SD±57), respectively ($p<0.001$ compared to baseline for all enzymes). Normalization of liver function tests as a whole was observed in 20/26 patients (76.9%). A loss of ≥ 5% of body weight was observed in 12 patients (46.1%), but it did not correlate with the normalization of liver function tests ($p=0.5$).
Conclusions: Our data provide preliminary evidence of improved liver enzymes in NAFLD patients with a strict vegan diet and although our study sample is limited, decreased body weight did not seem critical to the outcome.

Key words: non-alcoholic fatty liver disease – NAFLD – vegan diet – liver enzymes – transaminase – physical activity – liver disease – body weight – body mass index – NASH – lifestyle intervention.

Abbreviations: ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; BMI: body mass index; GGT: gamma-glutamyl transferase; Med-Diet: Mediterranean diet; NAFLD: nonalcoholic fatty liver disease; NASH: non-alcoholic steatohepatitis.

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is defined as the presence of excessive hepatic fat accumulation (hepatic steatosis) after the exclusion of secondary causes of fat accumulation in the liver (alcohol consumption, medications, and other medical conditions) [1]. The histologic diagnosis criteria include the presence of steatosis in >5% of hepatocytes and the imagistic diagnosis criteria include a proton density fat fraction (providing a rough estimation of the volume fraction of fatty material in the liver) >5.6% assessed by proton magnetic resonance spectroscopy or quantitative fat/water selective magnetic resonance imaging (MRI) [1].

Nonalcoholic fatty liver disease is part of the metabolic syndrome characterized by diabetes mellitus, pre-diabetes (insulin resistance), obesity, elevated blood lipids and high

blood pressure [1-3]. Not all NAFLD patients have the manifestations of the metabolic syndrome.

Although NAFLD was not recognized as a particular entity before 1980, today the disorder is affecting one in three adults and one in ten children in the United States [3-5]. The global prevalence of NAFLD is 25.24% with wide geographical variation across the world. Countries from Middle East and South America have the highest prevalence rates (around 30%) [4, 5].

The perspective of NAFLD has moved from the initial concept of limited chronic liver disease to the multidisciplinary paradigmatic pattern which involves all fields of clinical medicine.

The diagnosis of NAFLD is usually first suspected in obese subjects who are found to have elevated liver enzymes during routine blood testing. However, NAFLD can be present with normal liver blood tests [6-9]. The disease can be diagnosed incidentally on standard imagistic investigations such as abdominal ultrasound or abdominal computer tomography scan (CT) showing the accumulation of fat in the liver. Apart from liver biopsy, no other reliable method of diagnosis is available for the diagnosis of NAFLD or non-alcoholic steatohepatitis (NASH) [6-9].

Obesity was demonstrated to be the most common cause of liver steatosis. The genetic background has been incriminated as a key element in the pathogenesis of NAFLD because ethnic variability is correlated with the incidence of the disease [10].

The accumulation of hepatic triacylglycerol is followed by abnormal hepatic catabolism and impaired insulin mediated suppression of hepatic glucose and very low-density lipoprotein production. Hypertriglyceridemia, hyperglycemia and hyperinsulinemia are evidenced in the next phase of the pathogenic cycle [11-13].

Furthermore, a considerable amount of carbohydrates is diverted to the liver and will be stored as fat at this level due to insulin resistance which modifies glycogen synthesis [12-14]. The excessive intracellular fatty acid accumulation triggers oxidative stress, which leads to the elevation of cytokine levels, further accelerated by mitochondrial dysfunction and insulin resistance. Several studies showed that prediction factors for the progression of the disease include age, alanine aminotransferase (ALT) values, glycemic levels, body-mass index (BMI) and waist circumference [11-13].

A strong effort on developing potential therapies for NAFLD has been pursued in recent years mostly founded on improvement in insulin resistance [14]. Lifestyle modifications, weight loss, and drug treatment may all improve insulin resistance thus becoming potential targets for trials [14, 15]. In addition, agents capable of decreasing oxidative stress and apoptosis or bearing cytoprotective properties have been evaluated mostly in uncontrolled trials [15]. More recently, the effective management of specific components of the metabolic syndrome such as diabetes mellitus, hypertension, and dyslipidemia has been suggested as adjunctive treatment options [16].

While no firm recommendations of pharmacological therapy are currently approved for the treatment of NAFLD, physical exercise and diet remain an essential part of the treatment [11, 15, 17]. Contrariwise, the majority of the studies demonstrated that weight reduction is not mandatory to restore normal liver function [13-15].

Current international guidelines recommend that the primary goal of nutritional therapy is to reduce energy intake and to achieve a 10% reduction in body weight followed by implementation of a Mediterranean style low-carb diet (Med-diet) as a basic nutritional approach [11, 15, 17]. Additional statements of the international guidelines recommend avoiding alcohol and unnecessary medications [11, 15, 17].

Future development of efficient pharmacologic agents for NAFLD treatment is an area of global research and the main strategies currently being evaluated by physicians and scientists include weight reduction (diet, exercise, bariatric surgery), lipid lowering medications, decreasing liver inflammation with pharmacological agents with an antioxidant, anti-apoptotic and anti-cytokine effect [11, 15, 17].

The Western diet is rich in saturated fats, refined sugars and meat consistent with a high-energy load and secondary risk of increased metabolic diseases including NAFLD [18, 19]. Vegan diets are low in saturated fats and refined sugars, providing a significant intake of naturally occurring antioxidant compounds [20]. A vegan diet contains only plants (such as vegetables, grains, nuts and fruits), foods made from plants and excludes all forms of food prepared from animal products (meat, eggs, milk) [20-22]. People choose to follow a vegan diet for various reasons, ranging from religion, ethics and environmental concerns, not only the desire to improve their own health. Moreover, veganism is defined as a way of living that attempts to exclude all forms of animal exploitation and cruelty, whether for food, clothing or any other purpose [21, 22].

If the vegan diet is not planned properly, with the help of a specialized dietician, the lack of essential nutrients, such as calcium, iron and vitamin B12, might lead to impaired health outcomes [22]. Nevertheless, a balanced vegan diet and understanding the process as a whole, determine all the lacking nutrients to be acquired [21, 22].

Several randomized controlled studies demonstrated that a vegan diet is more efficient for weight loss than other types of diets including a vegetarian diet or a Med-diet [23-27]. Moreover, a vegan diet has been reportedly effective in improving a number of cardiovascular risk factors such as insulin resistance, glycemic control, dyslipidemia and hypertension [19, 23-27] However, no data are available on the potential benefits of vegan diets for NAFLD. We speculated a vegan diet could improve liver function in NAFLD by lowering both diet related offensive factors (eg saturated fats) and body weight. To this aim, we studied prospectively the effect of a vegan diet, excluding all animal products on liver chemistry in a group of consecutive NAFLD patients.

METHODS

This was a prospective, pilot study run on 40 consecutive consulters affected by NAFLD diagnosed according to the American Association for the Study of Liver Diseases (ASSLD) Guidelines [28] beginning on June 2015 at the Gastrointestinal Clinic of the University of Verona. The study was approved by our local Institutional Review Board (file

#2089 CESC) and all participants signed an informed consent form. Patients were referred by either general practitioners or internists for suspected NAFLD with persistent (more than 6 months) increment of liver enzymes. Alcohol abuse, viral and hereditary liver disease etiology were all excluded by history and appropriate testing, as indicated. At initial evaluation, all patients underwent a liver ultrasound scan to confirm the diagnosis [28, 29]. In addition, a transient elastography study was performed to all patients in order to identify non-invasively advanced liver fibrosis in all subjects [26]. Since all but two patients did not show features of advanced fibrosis (Stage 0-1), we decided not to proceed with imaging at follow-up. Eight subjects refused to join the study for poor diet palatability, leaving 32 patients (19 males, mean age 50 years, range 30-74 years), with abnormal liver chemistry who agreed to adhere to a vegan diet for six months. Abnormal liver chemistry was defined as any enzyme abnormality found among aspartate transaminase (AST), ALT, and gamma-glutamyl transferase (GGT). All subjects were followed up by both a dedicated dietitian and a vegan diet follower physician (A.F.). The caloric intake was tailored by the dietitian to obtain a weight loss ≥5% of body weight in overweight patients (BMI ≥25 kg/m^2) and ranged from 1500 to 1800 Kcal. Patients were also instructed to increase their level of physical activity, but no advice on specific training was provided. Patients were considered compliant to this advice when reporting any limitations of the amount of time spent being sedentary by replacing sedentary time with physical activity of any intensity. Patients were contacted monthly by phone to reinforce diet and lifestyle advice. Patients were seen at the gastrointestinal clinic when doubtful about diet advice. Cardio-metabolic risk factors were assessed and recorded in all subjects. Diabetes and impaired fasting glucose were diagnosed according to the 2013 diagnostic guidelines of the American Diabetes Association [30]. In particular, ongoing diabetic therapy, glycated hemoglobin (HbA1C) level of 6.5% (48 mmol/mol) or higher on two separate tests or a fasting blood sugar level equal or higher than 126 mg/dL (7 mmol/L) or a random blood sugar level of more than 200 mg/dL or 11.1 mmol/L after a meal, were considered indicative of diabetes. Impaired fasting glucose was defined as fasting plasma glucose levels between 100 and 125 mg/dL (between 5.6 and 6.9 mmol/L). Dyslipidemia was defined according to 2013 ESC/EAS guidelines with plasma triglycerides >1.7 mmol/L, low density lipoprotein cholesterol >190 mg/dL (4.9 mmol/L), high density lipoprotein cholesterol <1.0 mmol/L (in males) or <1.3 mmol/L (in females) or current lipid lowering therapy [31]. The high blood pressure report was based on the referring physician diagnosis with all affected patients managed by pressure-lowering pills. Potential diet influence on comorbidities was limited to ongoing drug monitoring for the study was focused on liver disease.

Categorical data was presented as absolute and relative frequencies. Normal and non-normal distributed continuous data were presented as mean along with standard deviation and median with interquartile range. Comparisons between baseline and after the diet measurements of continuous variables were done with t-test for dependent data, in case of normally distributed data, or Wilcoxon signed rank test, in case of non-normal distributed data. For all tests a 0.05 threshold for statistical significance was used, and two-tailed p-values. All analyses were performed in R environment for statistical computing and graphics (R Foundation for Statistical Computing, Vienna, Austria), version 4.0.1.

RESULTS

At six-month follow-up 6 subjects did not attend the clinic, leaving only 26 patients for data analysis. Contacted by phone, 4 subjects reported joining a different program for poor palatability of the diet, one moved far away, and one did not provide any explanation for drop out. The sample consisted of subjects with a mean age of 49 years (11.9 standard deviation), ranging from 30 to 76 years. The characteristics of patients are presented in Table I.

Table I. Patients' characteristics

Characteristic	NAFLD patient (n=26))
Age (years), media (SD)	49.92 (11.91)
Gender (Male), n (%)	18/26 (69.23)
Hypertension (%)	11/26 (42.31)
Diabetes or impaired fasting glucose n (%)	11/26 (42.31)
Statin treatment n (%)	9/25 (36)
Dyslipidemia n (%)	15/26 (57.69)
Diet calorie content n (%)	22/26 (84.62)
Subjective report of incremented physical activity n (%)	12/26 (46.15)
Follow-up (days), median (IQR)	268 (158 - 324)

SD: standard deviation; IQR: interquartile range.

Initial anthropometric values were mean weight 78 kg (range 52-95 Kg), mean BMI 26.8 (20.3-31.2). Liver chemistry showed mean ALT value 99 U/L (SD±45), mean AST value 54 U/L (SD±44), mean GGT value 160 U/L (SD±122), pre-treatment. After six months, the mean body weight was 73 Kg (range 52-87 Kg), mean BMI was 25.2 (range 20.3-29.7). Liver chemistry improved to a mean of ALT value 36 U/L (SD±21), AST value 27 U/L (SD±10) and GGT value 55 U/L (SD±57), respectively.

Normalization of liver chemistry as a whole was observed in 20/26 patients (76.9%). A loss of ≥5% of body weight was observed in 12 patients (46.1%), but it did not correlate with normalization of liver chemistry (p=0.5).

Following the vegan diet the subjects weight, body mass index, as well as ALT, AST and GGT decreased statistically significantly (Table II). However, the differences in time for albumin and platelet count were not statistically significant. Drug prescription for cardio-metabolic diseases was left unaltered by the vegan diet in all but four patients (3 diabetics, 1 high blood pressure patient), where drug schedule was decreased by the referring physician.

DISCUSSION

Nonalcoholic liver fatty liver disease has emerged as a major etiology of chronic liver disease with a population prevalence

Table II. The evolution of subjects' characteristics, before and after the vegan diet

Characteristic	Baseline	Folow-up	Difference (95% CI)	p
Weight (kg), median (IQR)	79 (73 - 87)	74 (69 - 81)	-5 (-7.2 - -4)	< 0.001=
BMI (kg/m2), mean (SD)	27.01 (2.85)	25.35 (2.45)	-1.66 (-2.22 - -1.1)	< 0.001
Alanine transaminase (U/L), median (IQR)	68 (49 - 126)	32 (25 - 43)	-36 (-72.5 - -25)	< 0.001=
Alanine transaminase (U/L), median (IQR)	68 (49 - 126)	32 (25 - 43)	-36 (-72.5 - -25)	< 0.001=
Gamma-glutamyl transpeptidase (U/L), median (IQR)	95 (76 - 163)	39 (31 - 57)	-56 (-164.5 - -39)	< 0.001=
Albumin (g/L), mean (SD)	43.02 (3.83)	43.66 (3.9)	0.64 (-0.21 - 1.49)	0.134
Platelets *100/mL, mean (SD)	232.12 (65.31)	231.38 (64.52)	-0.73 (-19.3 - 17.83)	0.936

SD: standard deviation; IQR: interquartile range; CI: confidence interval; BMI: body mass index

up to 33%, depending on the sample studied [4, 5]. If not treated, it may progress to NASH, fibrosis and cirrhosis with a potential chance of hepatocellular carcinoma [1]. NAFLD etiology is multifactorial and it is strongly associated with the components of metabolic syndrome, mainly obesity, dyslipidemia and diabetes mellitus [9]. Lifestyle modifications and incremental physical activity remain the cornerstone of NAFLD management. Recently, vegetarian diets have become increasingly popular and reportedly beneficial for diseases such as diabetes, heart disease, stroke and even cancer [32]. In this regard, the Academy of Nutrition and Dietetics has released the statement that "appropriately planned vegetarian, including vegan, diets are healthful, nutritionally adequate, and may provide health benefits in the prevention and treatment of certain diseases" [32]. A vegan diet could be the "magic bullet" capable of addressing both the NAFLD and cardiovascular comorbidities. However, no study has addressed the potential benefit of a low-fat diet withdrawing all animal nutrients on liver function in NAFLD. Therefore, we are the first to report on the beneficial effect of a vegan diet on a limited sample of NAFLD patients. Our data are consistent with the effective management of altered liver function in NAFLD by a combination of vegan diet advise and general suggestion of increased physical activity. Almost half of our patients did lose weight by limited calorie deprivation. This is in agreement with the general assumption that vegan diet followers are thinner for the diverse diet composition when compared to meat lovers [32]. We could not exclude the observed weight loss was relevant to the treatment outcome. However, it seems unlikely because the weight loss did not correlate with improved liver function thus orienting towards the beneficial effect of meat abstinence. Cardiovascular comorbidities in our patients were not adequately addressed for both the small study sample and the focus on liver enzymes. However, a few of our patients reported a decreased drug schedule to control diabetes, which is encouraging. Vegan diet advice mechanism/s of action is/are unclear, but a complex interplay between avoiding offending meat byproducts and increasing vegetable antioxidants compounds is speculated [33, 34]. Alternatively, beneficial modulation of the gut microbiota due to the increased assumption of fermentable fibers might be as relevant on improving liver enzymes [35, 36]. Compliance to such a restrictive diet might be a prescription limitation as well as long term efficacy of vegan diet advice on liver function and histology. Vegetarian diet motivation does not rely exclusively on health improvement, but compassion toward animals and a desire to better protect the environment are relevant as well [32]. However, additional controlled studies to compare the effect of a vegan diet to diverse diet advice on NAFLD and/or progression to NASH seem warranted. Moreover, a diet approach with limited meat proteins has been reported effective as well for NAFLD management in both western and eastern study populations, thou not universally. A Med-Diet is a diet pattern characterized by the main consumption of plant-based foods and fish with reduced consumption of meat and dairy products [37]. A study performed by Gelli et al. [37] analyzed the effect of a counseling-supported treatment with the Med-Diet and increased physical activity on the severity of NAFLD. A total of 46 patients were included in the study and effect of lifestyle modifications on NAFLD severity was evaluated at baseline with liver ultrasonography scan (US), liver enzymes and metabolic parameters. In addition, biochemistry was assessed at mid- and end-evaluations (3rd and 6th month) and US was also performed at end-intervention. The results showed that all liver enzymes tested significantly decreased during the treatment with particular evidence for the ALT values (altered values reduced from 67% down to 11%). Moreover, the percentage of patients with steatosis grade equal to or higher than 2 was reduced from 93% to 48% and steatosis regressed in 20% [37]. At the end of the Med-Diet the majority of the analyzed parameters (BMI, waist circumference, waist-to-hip ratio, AST, ALT, GGT, serum glucose, dyslipidemia, and NAFLD liver fat score) improved between baseline and end-treatment, a result resembling our data. However, all of the patients met both the goal of moderate-intense physical activity for 3 hours per week and attended focused lifestyle counseling support which might have been key to the outcome. In addition, patients with metabolic comorbidities were excluded from the study. On the contrary, a study performed by Baratta et al. [38] aimed to investigate the relationship between NAFLD and adherence to a Med-Diet in a large cohort of patients with cardio metabolic risk factors screened for the presence of liver steatosis. Ultrasound was the method used for the evaluation of liver steatosis and a nine-item dietary questionnaire was used to study diet adherence. A total of 584 consecutive outpatients were divided into low, intermediate, and high diet adherence. Liver steatosis was present in 82.7% of patients, decreasing from the low to high adherence group (and using logistic regression

analysis, hypertriglyceridemia, Med-Diet adherence, and high waist circumference) were all significantly associated with NAFLD [38]. The results highlighted the existence of an inverse relationship between Med-Diet and NAFLD, suggesting that a nutritional approach low in animal byproducts may be beneficial for NAFLD management [38].

Dietary pattern analysis is an alternative approach to examine the association between diet and NAFLD which was adopted by Chan et al. [39] in a large sample of 797 apparently healthy Chinese adults. The study compared the effect on NAFLD prevalence of two diet-quality scores, namely Diet Quality Index-International (DQI-I) and Med-Diet Score. NAFLD was defined as intrahepatic triglyceride content at ≥5% by proton-magnetic resonance spectroscopy and diagnosed in 220 subjects (27.6%). A low DQI-I score, a tool assessing variety, adequacy, moderation and overall balance, but not Med-Diet score was associated with the prevalence of NAFLD [39]. Multivariate regression analyses showed an inverse correlation of vegetables, legumes, fruits, and dried fruits intake with NAFLD prevalence [39] thus supporting the assumption that a diverse kind of low-fat diets are associated with a reduced risk of NAFLD. Moreover, Ryan et al. [40] analyzed the association between NAFLD and Med-Diet using localized magnetic resonance and concentrations of glucose, insulin, triglycerides, ALT, and GGT. Liver biopsy was used for the diagnosis of NAFLD. All patients followed both the Med-Diet and a control diet (low fat-high carbohydrate diet), in random order with a 6-week wash-out period in- between [40]. This randomized, cross-over study confirmed that even without weight loss, Med-Diet is effective in reducing liver steatosis which is in agreement with our findings of the minor relevance of weight loss on an intervention outcome.

The histological characteristics of patients with NAFLD adherent to a Med-diet were evaluated in a cross-sectional study of 82 patients, performed by Aller et al. [41] in order to evaluate the level of adherence to the diet, a 14-Item Med-Diet Assessment Tool was applied. The results showed that 35 patients (42.7%) had a low grade of steatosis (grade 1) and 47 patients (57.3%) had a high grade of steatosis (grade 2 and 3), 56 patients (68.3%) had liver steatohepatitis and 42 patients (51.2%) had liver fibrosis [41]. Using logistic regression analysis, 1 unit of the Med-Diet Assessment Tool was associated with a lower risk of steatohepatitis and steatosis [41] providing additional evidence that greater adherence to the Med-Diet was associated with a lower risk of steatosis and steatohepatitis. However, Kontogianni et al. [42] could not confirm that adherence to a Med-Diet was associated with a lower likelihood of having NAFLD in a prospective study on 73 patients with recent NAFLD diagnosis with liver biopsy performed in almost half of them. Nonetheless, Med-Diet seemed to be associated with a lesser degree of insulin resistance as measured by the insulin resistance index [42].

A study performed by Trovato et al. [43] analyzed the relationship between Med-Diet, BMI changes, physical exercise and NAFLD in an effort to sort out the most effective treatment component. Adherence to Med-Diet Score, Bright Liver Score and liver ultrasound were used for the evaluation of 90 non-alcoholic non-diabetic patients. Although, after the first and third month of evaluation, the hepatic changes were still not significant, a significant decrease of Bright Liver Score was observed after 6 months of intervention [43]. Adherence to Med-Diet was a significant predictor of changes in the fat content in NAFLD and was independent of other lifestyle changes, resembling our data.

Our pilot study has a number of limitations. The absence of the control group raises the problem of the true effect of the intervention upon the subjects. The small sample size impeded adjustment for confounders, that might explain the evolution, although the results were statistically significant. The lack of liver histology to accurate differentiate NAFLD from NASH represent another limit. A possible regression to the mean effect might have influenced the results of the study. Additional limitation comes from the study short follow up because longer follow up might modify the compliance. Finally, patients were contacted by phone and compliance could have been limited by diet palatability. Nevertheless, its promising results should be further validated in a controlled prospective design on an adequately sized sample.

CONCLUSIONS

Our data provide preliminary evidence of improved measures of liver function associated with a strict vegan diet and although our study sample is limited, decreased body weight did not seem critical to outcome. Compliance to such a restrictive diet is an issue as well as long term efficacy of vegan diet advice on liver function and histology. However, additional controlled studies to compare the effect of a vegan diet to diverse diet advice on NAFLD and/or progression to NASH seem warranted

Conflicts of interest: L.B. and A.F. are vegan diet followers. The other authors declare no conflict of interest.

Authors' contributions: G.C. designed the study and wrote the draft. A.F. conceived and planned the study. S.L.P. revised the draft. A.D. was involved in planning and supervised the study. C.S. analyzed the data. D.C.L. processed data and verified the analytical methods. L.B. recruited patients and collected data. All of the authors provided critical feedback and approved the final version of the manuscript.

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