

# Investigation of the relationship between carotid intima-media thickness and angiotensin-like factor 3 levels in metabolic dysfunction-associated steatotic liver disease

Received: 14 November 2025

Accepted: 21 January 2026

Published online: 30 January 2026

Cite this article as: Kadioglu Yenyurt E., Duran E.N., Dumur S. *et al.* Investigation of the relationship between carotid intima-media thickness and angiotensin-like factor 3 levels in metabolic dysfunction-associated steatotic liver disease. *Sci Rep* (2026). <https://doi.org/10.1038/s41598-026-37389-y>

Elif Kadioglu Yenyurt, Eda Nur Duran, Seyma Dumur, Iskender Ekinici, Isa Yalcinkaya, Muge Ustuner, Huseyin Emre Carpan, Gulden Anataca, Murat Akarsu, Ummihan Topal, Hafize Uzun & Omur Tabak

We are providing an unedited version of this manuscript to give early access to its findings. Before final publication, the manuscript will undergo further editing. Please note there may be errors present which affect the content, and all legal disclaimers apply.

If this paper is publishing under a Transparent Peer Review model then Peer Review reports will publish with the final article.

**Investigation of the Relationship Between Carotid Intima-Media Thickness and Angiopoietin-Like Factor 3 Levels in Metabolic Dysfunction–Associated Steatotic Liver Disease**

Elif Kadioglu Yeniyurt<sup>1</sup>, Eda Nur Duran<sup>1</sup>, Seyma Dumur<sup>2</sup>, Iskender Ekinci<sup>3</sup>, Isa Yalcinkaya<sup>4</sup>, Muge Ustuner<sup>1</sup>, Huseyin Emre Carpan<sup>1</sup>, Gulden Anataca<sup>1</sup>, Murat Akarsu<sup>1</sup>, Ummihan Topal<sup>5</sup>, Hafize Uzun<sup>2</sup>, Omur Tabak<sup>1</sup>

<sup>1</sup>Health Sciences University, Kanuni Sultan Suleyman Training and Research Hospital, Department of Internal Medicine, Istanbul, Turkiye.

<sup>2</sup>Istanbul Atlas University, Faculty of Medicine, Department of Medical Biochemistry, Istanbul, Turkiye.

<sup>3</sup>Department of Internal Medicine, Bezmialem Vakif University, Istanbul, Turkiye.

<sup>4</sup>Health Sciences University, Basaksehir Cam and Sakura City Hospital, Department of Hematology, Istanbul, Turkiye

<sup>5</sup>Health Sciences University, Kanuni Sultan Suleyman Training and Research Hospital, Department of Radiology, Istanbul, Turkiye.

**Short Title:** ANGPTL3 and Carotid Intima-Media Thickness in MAFLD

**Corresponding Address:**

Prof. Dr. Omur Tabak

Health Sciences University, Kanuni Sultan Suleyman Training and Research Hospital, Department of Internal Medicine, Istanbul, Turkiye.

e-mail: [omurtabak@yahoo.com.tr](mailto:omurtabak@yahoo.com.tr)

**Abstract**

**Background:** Metabolic dysfunction–associated steatotic liver disease (MASLD), formerly known as non-alcoholic fatty liver disease (NAFLD), is a common condition characterized by excessive fat accumulation in the liver and is closely associated with metabolic syndrome (MetS) and insulin resistance. This study aimed to evaluate the relationship between carotid intima-media thickness (CIMT), a marker associated with cardiovascular risk, and circulating levels of angiopoietin-like protein 3 (ANGPTL3) in patients with MAFLD. **Methods:** The study included 88 patients diagnosed with MAFLD and 88 age- and sex-matched healthy controls who visited the internal medicine clinic between August and November 2022. Data on patient characteristics, laboratory values, and associated diseases were taken from the hospital database. Serum ANGPTL3 levels were measured using venous blood samples, and results with  $p < 0.05$  were considered significant. **Results:** Serum ANGPTL3 levels and CIMT were significantly higher in the MAFLD patient group compared to controls. ROC analysis showed that a hepatic steatosis index  $>33.18$  predicted MAFLD with 97.8% sensitivity and 83.33% specificity; ANGPTL3 levels  $>242$  predicted MAFLD with 51.11% sensitivity and 83.33% specificity; and CIMT  $>6$  mm (corresponding to 0.6 mm) predicted MAFLD with 83.33% sensitivity and 91.11% specificity. **Conclusion:** Our study demonstrated increased serum ANGPTL3 levels in patients with MAFLD. Dyslipidemia observed in MAFLD may be mediated through this hepatokine, suggesting that ANGPTL3 could serve as a non-invasive biomarker for MAFLD diagnosis. Furthermore, serum ANGPTL3 may have potential as a biomarker for liver fibrosis assessment in future studies.

**Keyword:**Metabolic dysfunction–associated steatotic liver disease; carotid intima-media thickness; angiopoietin-like factor; dyslipidemia

## Introduction

Non-alcoholic fatty liver disease (NAFLD) encompasses a spectrum of liver disorders defined by steatosis in more than 5% of hepatocytes in individuals with little or no alcohol consumption (1). It ranges from simple steatosis (“silent liver disease”) to non-alcoholic steatohepatitis (NASH) (2). About 10–25% of patients with simple steatosis progress to NASH, and 5–8% of these develop cirrhosis within five years (3), which can lead to liver failure or hepatocellular carcinoma (4,5). Recently, international consensus statements have introduced the term metabolic dysfunction–associated steatotic liver disease (MASLD) to replace MAFLD, emphasizing metabolic dysfunction as the central defining feature of the disease (6). However, as the present study was designed and conducted before the formal adoption of this terminology, the term MAFLD is retained throughout the manuscript.

MAFLD is closely associated with metabolic dysfunction, including obesity, insulin resistance, dyslipidemia, and metabolic syndrome (MetS), and its prevalence has increased in parallel with rising rates of these conditions worldwide (7,8).

Angiopietin-like protein 3 (ANGPTL3) is predominantly expressed in hepatocytes and plays a key role in lipid metabolism by inhibiting lipoprotein lipase activity, leading to increased circulating triglyceride-rich lipoproteins (9).

Although accumulating evidence suggests a potential association between ANGPTL3 and MAFLD, findings across existing studies remain inconsistent, and the underlying pathophysiological mechanisms have yet to be fully elucidated (10). Moreover, data evaluating ANGPTL3 in conjunction with vascular surrogate markers, such as carotid intima–media thickness (CIMT), are scarce. Considering these gaps, further investigation is warranted to clarify the clinical relevance of ANGPTL3 in MAFLD. Therefore, this study aimed to assess the relationship between serum ANGPTL3 levels and CIMT in patients with MAFLD.

## Materials and Methods

### *Study design*

This study employed a prospective, single-center, case–control design. It was conducted at the University of Health Sciences, Istanbul Kanuni Sultan Suleyman Training and Research Hospital from August to November 2022. Planned sample size was reviewed during the institutional ethics committee approval process and was considered adequate for the exploration objectives of the study.

### *Subjects*

The study enrolled 88 patients with MAFLD who were followed in the Department of Internal Medicine at the University of Health Sciences, Istanbul Kanuni Sultan Suleyman Training and Research Hospital. An additional 88 age- and sex-matched healthy volunteers served as the control group; they were recruited during routine medical check-ups and had no history or findings suggestive of chronic disease or active infection.

#### *Inclusion criteria*

Participants in the patient group were adults ( $\geq 18$  years) who did not consume alcohol, had confirmed diagnosis of MAFLD, and consented to join the study. The control group consisted of healthy volunteers aged 18 years or older, without a history of alcohol intake, MAFLD, or any chronic comorbid condition, who also agreed to participate voluntarily.

#### *Exclusion criteria*

Participants were excluded if they were younger than 18 years of age, pregnant or lactating, or had a history of alcohol use. Individuals with active infections, malignancies, chronic kidney disease requiring dialysis, severe malnutrition, or advanced (stage III–IV) heart failure were also not eligible. In addition, patients with chronic inflammatory disorders, coronary artery disease (including those with prior stent implantation or bypass surgery), or a previous cerebrovascular event were excluded from the study.

#### *Data Collection*

During enrollment, information regarding participants' age, sex, and smoking habits was obtained. Height and weight were recorded to compute body mass index (BMI), defined as weight in kilograms divided by the square of height in meters. The waist and hip circumferences of each subject were measured, and the waist-to-hip ratio was calculated to assess fat distribution. Information about additional medical conditions, such as type 2 diabetes mellitus (T2D), prediabetes, high blood pressure, dyslipidemia, and hypothyroidism, was also recorded. MetS was diagnosed according to the International Diabetes Federation (IDF) criteria, which focus on central obesity in combination with other metabolic risk factors (11).

#### *Sample collection*

After a minimum overnight fast of eight hours, venous blood samples were collected from the antecubital region of each participant between 8:00 and 9:00 a.m. The samples were immediately centrifuged at 4000 rpm for 10 minutes, and the resulting serum fractions were stored at  $-80$  °C until analysis. All samples were thawed once before testing, and measurements were conducted collectively in a single analytical session by researchers blinded to clinical information.

### *Parameters*

#### *Hepatic Steatosis Index (HSI)*

The hepatic steatosis index (HSI) is a non invasive calculation that incorporates biochemical and anthropometric measurements to estimate the likelihood of MAFLD. The index was computed using the following equation:  $HSI = 8 \times (ALT/AST) + BMI$  (+2 if diabetic, +2 if female) (12).

#### *Ultrasonographic Evaluation*

CIMT was assessed using a high-resolution B-mode ultrasound device (Esaote My Lab 9, Italy) fitted with a 4–15 MHz linear multifrequency probe. All scans were performed by a single experienced radiologist who was blinded to participants' laboratory results. Participants were examined in the supine position, with the head gently extended and rotated 30–45° away from the side under evaluation.

After a resting period of about 15 minutes in a quiet room, both common carotid arteries were visualized in longitudinal and transverse planes. Measurements were taken on the far wall of the distal segment of the common carotid artery, approximately 1 cm proximal to the carotid bulb, by determining the distance between the lumen–intima and media–adventitia interfaces.

The mean value of three successive readings was used for statistical analysis. CIMT measurements were recorded and analyzed in units of 0.1 mm.

Hepatic steatosis was diagnosed by ultrasonography based on routinely used imaging features, including increased hepatic echogenicity compared with the renal cortex and reduced visualization of intrahepatic vascular structures. Steatosis severity was classified as grade 1 (mild) or grade 2 (moderate) according to routine clinical ultrasonographic reports, reflecting the extent of these qualitative findings. All examinations were performed by a single experienced radiologist who was blinded to clinical and laboratory data (13).

#### *Assessment of serum ANGPTL3 levels*

Serum ANGPTL3 levels were measured with a sandwich ELISA kit following the instructions provided by the manufacturer (Angiopietin-Like Factor- 3, Cat No: E-EL-H0336, elabscience, USA). All samples were examined twice. Both the coefficients of intra-assay and inter-assay variations for ANGPTL3 were less than 10.

#### *Statistical analysis*

All data analyses were carried out using IBM SPSS Statistics for Windows, version 26.0 (IBM Corp., Armonk, NY, USA). Categorical variables were presented as counts and

percentages, whereas continuous variables were reported as mean  $\pm$  Standard deviation (SD) or median with minimum and maximum values, depending on their distribution.

The normality of continuous variables was assessed with the Shapiro–Wilk test. For group comparisons, the independent-samples *t*-test was used for normally distributed data, and the Mann–Whitney *U* test was used for non parametric data. Associations between categorical parameters were evaluated using the chi-square test. Correlations were analyzed through Pearson’s correlation coefficient for normally distributed variables and Spearman’s rank correlation coefficient for non-normally distributed data. Receiver operating characteristic (ROC) curve analysis was employed to identify optimal cut-off points for parameters associated with MAFLD prediction. A *p*-value of  $<0.05$  was considered indicative of statistical significance.

## Results

A total of 88 patients with MAFLD and 88 age- and sex-matched healthy individuals were included in the study. The mean age of the MAFLD group was  $42.7 \pm 7.5$  years, while that of the control group was  $44.7 \pm 9.0$  years, and the difference was not statistically significant ( $p = 0.283$ ). Each group consisted of 44.4% males ( $n = 40$ ) and 55.6% females ( $n = 50$ ). Ultrasonographic evaluation revealed grade 1 steatosis in 54.5% ( $n = 49$ ) and grade 2 steatosis in 45.5% ( $n = 41$ ) of the MAFLD patients. The frequency of diabetes mellitus (T2D) was significantly higher in the MAFLD group compared with the control group ( $p = 0.009$ ). Weight and body mass index (BMI) were significantly higher in the MAFLD group than in controls (both  $p < 0.001$ ). According to BMI classification, 2.2% of MAFLD patients were normal weight, 71.1% overweight, and 26.7% class I obese, whereas all control subjects were within the normal BMI range. Waist circumference (WC), hip circumference (HC), waist-to-hip ratio (WHR), systolic blood pressure (SBP), mean blood pressure (MBP), fasting glucose, and HOMA-IR levels were all significantly higher in the MAFLD group (all  $p < 0.001$ ). The prevalence of MetS was 58.9% in the MAFLD group and 2.2% in controls ( $p < 0.001$ ) (Table 1).

Biochemical parameters are summarized in Table 2. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), low-density lipoprotein cholesterol (LDL-C), triglyceride (TG), total cholesterol, C-reactive protein (CRP), HbA1c, and ferritin levels were significantly elevated in the MAFLD group compared with controls (all  $p < 0.05$ ), whereas high-density lipoprotein cholesterol (HDL-C) and vitamin D levels were significantly lower (both  $p < 0.01$ ). No significant differences were observed between the two groups regarding creatinine, thyroid-stimulating hormone (TSH), total protein, albumin, or platelet count ( $p >$

0.05). The mean HSI, serum ANGPTL3 concentration, and CIMT were all significantly higher in patients with MAFLD compared to healthy controls ( $p < 0.001$  for all). Detailed comparisons are presented in Table 3.

When the MAFLD group was analyzed by sex, diabetes status, obesity class, and presence of metabolic syndrome, serum ANGPTL3 levels did not differ significantly among subgroups ( $p > 0.05$  for all).

In the MAFLD group, ANGPTL3 levels showed a weak but statistically significant positive correlation with systolic blood pressure (SBP;  $r_s = 0.216$ ,  $p = 0.041$ ) and body mass index (BMI;  $r_s = 0.240$ ,  $p = 0.023$ ). No significant association was found with diastolic blood pressure (DBP;  $r_s = -0.072$ ,  $p = 0.498$ ). No significant correlations were observed with the other variables.

A strong positive correlation was observed between ANGPTL3 and CIMT ( $r = 0.687$ ,  $p < 0.001$ ) (**Figure1**).

Receiver operating characteristic (ROC) curve analysis revealed that an HSI cut off value of  $>33.18$  predicted the presence of MAFLD with 97.78% sensitivity and 83.33% specificity. An ANGPTL3 level of  $>242$  ng/mL predicted MAFLD with 51.11% sensitivity and 83.33% specificity, while a CIMT value of  $>6$  mm (corresponding to 0.6 mm) predicted MAFLD with 83.33% sensitivity and 91.11% specificity. The area under the ROC curve (AUC) was 0.958 for HSI, 0.749 for ANGPTL3, and 0.932 for CIMT (all  $p < 0.001$ ) (**Figure2**).

## Discussion

The evidence linking serum ANGPTL3 to MAFLD is still relatively limited. Although a few studies have indicated that ANGPTL3 levels may be higher in patients with NASH than in healthy individuals, the current body of literature is small and heterogeneous. Notably, there is a lack of large, prospective studies that evaluate ANGPTL3 across the full clinical spectrum of MAFLD or in clearly defined subgroups. For this reason, existing findings should be interpreted as preliminary. Expanding research into whether ANGPTL3 can serve as a marker of MAFLD severity or progression—particularly in populations that have not yet been investigated could significantly advance understanding in this area.

In the present study, patients with MAFLD showed markedly higher HSI values, serum ANGPTL3 concentrations, and CIMT measurements compared with healthy controls. In the MAFLD group, serum ANGPTL3 levels showed weak but significant positive correlations with SBP and BMI, while no association was observed with DBP. ANGPTL3 also demonstrated a strong positive correlation with CIMT. ROC analyses further revealed

that HSI values above 33.18 and CIMT measurements greater than 6 mm (corresponding to 0.6 mm) predicted MAFLD with high sensitivity and specificity. Several non-invasive indices are available for the assessment of hepatic steatosis. In the present study, HSI was preferred due to its simplicity and widespread clinical use, with previously demonstrated diagnostic performance in identifying hepatic steatosis. Although an ANGPTL3 level above 242 ng/mL showed moderate sensitivity, its specificity remained strong. Taken together, these observations indicate a possible link between ANGPTL3, hepatic fat accumulation, and early vascular alterations in patients with MAFLD.

BMI remains the most commonly used measure for evaluating overweight and obesity. However, other anthropometric indicators—including waist circumference, hip circumference, and the waist-to-hip ratio—are increasingly preferred, as they better reflect central adiposity. These metrics have been consistently associated with several chronic diseases such as diabetes, cardiovascular disorders, and various malignancies (14). In our study, higher BMI values were generally associated with higher steatosis grades, which aligns with previous research linking increased BMI to more advanced hepatic fat accumulation (15). Patients with higher body weight and BMI also tended to show higher steatosis grades. Patients with MAFLD exhibited significantly larger waist circumferences than controls, and body weight, BMI, and waist circumference were positively correlated within the MAFLD group. Height did not differ between groups. Armstrong et al. (16) evaluated 1,118 individuals with elevated liver enzymes and found that 26% (n = 295) had abnormalities attributable to MAFLD. Among these patients, 40.7% had elevations in two or more liver enzymes; GGT increased in 70%, ALT in 51%, and AST in 26% of cases. In agreement with these data, the present study showed significantly higher AST and ALT levels in the MAFLD group. Collectively, these findings highlight the strong association between adiposity and the severity of hepatic steatosis. They also emphasize the value of anthropometric measurements in assessing MAFLD risk. Chronic inflammation and immune dysregulation driven by T2D and insulin resistance are also thought to contribute to MAFLD development (17). This relationship has been clearly demonstrated in numerous studies linking T2D and insulin resistance with MAFLD. Consistent with the literature (18), this study found that patients with MAFLD had significantly higher glucose, HbA1c, and HOMA-IR levels compared to healthy controls, and the proportion of diabetic patients was also higher. These results reinforce the critical role of insulin resistance and glycemic dysregulation in the pathogenesis of MAFLD.

Numerous studies in literature have reported significant elevations in total cholesterol, triglycerides, and LDL-C levels, alongside a marked decrease in HDL-C(19,20). Far fewer studies have examined the relationship between metabolic risk factors and advanced liver disease, and definitions of relevant prognostic factors vary. However, recommendations from the largest and highest-quality studies indicate that both lipid abnormalities (low HDL-C and high triglycerides) and hypertension are independently associated with severe liver disease (21). Consistent with the literature, the results of this study showed that patients with MAFLD had significantly higher total cholesterol, LDL-C, and triglyceride levels, while HDL-C levels were significantly lower compared to healthy controls. These findings emphasize the strong association between dyslipidemia and MAFLD, highlighting the importance of lipid management in reducing disease progression risk.

Previous studies commonly report elevations in inflammatory mediators such as IL-1 $\alpha$ , IL-1 $\beta$ , IL-6 and TNF- $\alpha$  in individuals with hepatic steatosis (22). C-reactive protein (CRP) is a commonly used, readily available, dependable, and affordable acute-phase reactant for evaluating inflammation, applied in the diagnosis and surveillance of numerous disorders (23). Several publications have proposed that CRP may also be useful in identifying MAFLD and estimating its severity (24,25). In our study, CRP values were clearly higher in the MAFLD group compared with healthy individuals, a finding that is compatible with the existing literature. This supports the notion that systemic inflammation contributes to the pathogenesis of MAFLD and suggests that CRP could serve as a practical marker for assessing both the presence and extent of the disease.

CIMT is widely used as a surrogate marker of cardiovascular risk and vascular or endothelial inflammation (26). Previous clinical studies have shown that CIMT increases with the severity of hepatic steatosis in patients with MAFLD, supporting an association between liver fat accumulation and early atherosclerotic changes (27). However, not all clinical studies have reported a direct relationship between the severity of hepatic steatosis and carotid intima-media thickness, suggesting that CIMT may be more strongly influenced by accompanying MetS features rather than liver fat burden alone (28). Therefore, the association between increased CIMT and MAFLD observed in this study should be interpreted as an indirect relationship, primarily mediated by shared metabolic risk factors such as insulin resistance, dyslipidemia, and obesity, which have been shown to be associated with increased CIMT in patients with MAFLD(29,30).

In a large meta-analysis conducted by Simon et al.(26), which included 37,197 individuals, the authors reported that every 0.1 mm increase in CIMT was associated with a

10–15% higher risk of myocardial infarction and a 13–15% higher risk of cerebrovascular events after adjustments for age and sex. Importantly, they emphasized that CIMT remained an independent predictor of cardiovascular and cerebrovascular risk even when demographic characteristics and conventional risk factors were taken into account (26). Consistent with these findings, a number of studies have demonstrated a notable association between MAFLD and increased CIMT(29,31). Several clinical studies have reported higher CIMT values in patients with hepatic steatosis compared with those without steatosis. Likewise, Mladenova et al. (30)observed a higher frequency of pathological CIMT among patients with MAFLD. Collectively, these results suggest that MAFLD is closely linked to early atherosclerotic alterations and underscore the value of assessing CIMT when evaluating the cardiovascular risk profile of these patients. Overall, the evidence from both cohort studies and meta-analyses indicates that increased CIMT in individuals with hepatic steatosis may reflect early vascular changes that contribute to an elevated risk of future cardiovascular and cerebrovascular events. This reinforces the relevance of incorporating CIMT monitoring into the routine cardiovascular risk assessment of patients with MAFLD.

Several members of the angiotensin-like protein family have been shown to influence inflammatory signaling pathways, including the regulation of cytokine and chemokine responses (32). Experimental studies have suggested that ANGPTL3 may contribute to atherosclerotic processes by promoting macrophage-related inflammatory responses within vascular plaques. Although derived from experimental models, these findings provide biological context for the association between circulating ANGPTL3 levels and increased CIMT observed in the present study (33).

In their recent work, Wu et al.(34), examined how serum lipid parameters, inflammatory markers, and CIMT interact in patients with MAFLD. When we carried out a similar analysis in our own group of 125 individuals, using Pearson correlation coefficients, we observed a pattern that was broadly consistent with their findings. In our analysis, the biochemical parameters (LDL-C, HDL-C, hs-CRP, ALT and AST) showed varying correlation strengths, while CIMT values were generally higher in patients with MAFLD. This observation, although not unexpected, adds to the growing impression that vascular alterations begin relatively early in the course of MAFLD. From a clinical standpoint, disturbances in lipid metabolism together with low-grade inflammation seem to contribute, at least in part, to these CIMT elevations.

Wu et al. (34) also reported findings from an experimental MAFLD model, showing that increases in aortic wall thickness and CIMT occurred before overt atherosclerotic plaque

formation. These experimental observations provide mechanistic insight into early vascular alterations associated with hepatic steatosis but cannot be directly extrapolated to clinical disease.

In our study, patients with MAFLD exhibited significantly higher CIMT values compared with healthy controls. Taken together, these findings support the concept that MAFLD is associated with early vascular changes beyond the liver, while acknowledging that experimental and clinical evidence should be interpreted within their respective methodological contexts.

ANGPTL3 is a key regulator of lipid metabolism that increases plasma triglyceride, total cholesterol, and LDL-C levels. The plasma levels of ANGPTL3 play a critical role in triglyceride-rich lipoprotein metabolism (35,36). ANGPTL3 is mostly expressed in the liver. Tikka et al. (37) reported that in individuals with ANGPTL3 deficiency, a liver-specific mechanism is associated with an insulin-sensitive phenotype. Altun et al. (38), in a cohort study, showed that ANGPTL4 regulates plasma lipoprotein levels by inhibiting LPL activity and found a relationship between serum ANGPTL4 levels, obesity, and hepatic steatosis in adolescents. Zhang et al. (39), in a study on mice, reported that insulin may affect ANGPTL8 expression in hepatocytes and adipocytes. Another study indicated that blocking ANGPTL3 represents a new therapeutic approach for MAFLD, demonstrating both efficacy and safety (40). In this present study, ANGPTL3 levels were found to be higher in MAFLD patients compared to healthy individuals.

In clinical studies, findings regarding ANGPTL3 and liver-related outcomes have been inconsistent. While genetic studies have reported no clear association between triglyceride-lowering ANGPTL3 variants and adverse liver outcomes (41), some pharmacological approaches targeting ANGPTL3 have been associated with changes in hepatic fat content, whereas others have not (42,43). These discrepancies highlight the need for further well-designed clinical studies to clarify the role of ANGPTL3 in MAFLD.

In the present study, serum ANGPTL3 levels were significantly higher in patients with MAFLD compared with healthy controls. Together with the observed associations with CIMT and HSI, these findings suggest that ANGPTL3 may be involved in the metabolic and vascular alterations accompanying MAFLD, although causal relationships cannot be inferred.

Consistent with previous MAFLD studies, ROC analysis demonstrated that CIMT and HSI showed discriminatory ability for identifying MAFLD. Serum ANGPTL3 levels also showed moderate diagnostic performance in this context.

Taken together, higher ANGPTL3 concentrations, along with increased CIMT and HSI values, may reflect early metabolic and vascular alterations associated with MAFLD. These findings suggest the presence of overlapping metabolic and cardiovascular pathways in MAFLD, rather than implying a direct causal relationship.

*The limitations of this study*

i) MAFLD was diagnosed solely using ultrasonography, and no liver biopsies were performed. Because non-invasive fibrosis scoring methods were also not applied, information regarding fibrosis severity or histological inflammation is unavailable. As a result, it was not possible to directly examine how serum ANGPTL3 levels relate to tissue-level pathology. Future studies incorporating biopsy-proven MAFLD cases could help clarify this relationship.

ii) The study sample was relatively small and drawn from a single center, which may restrict the broader applicability of the findings.

iii) Although information regarding comorbid conditions and ongoing medications, including antidiabetic, antihypertensive, and lipid-lowering therapies, was recorded, these variables were not included in multivariate analyses due to the limited sample size and heterogeneity of treatment regimens. Therefore, the potential influence of concomitant medications on ANGPTL3 levels, lipid parameters, and CIMT cannot be fully excluded.

iv) The statistical analyses were primarily based on univariate comparisons; therefore, the observed associations may reflect shared metabolic risk factors rather than independent relationships. Multivariate regression models were not performed due to the limited sample size, and this should be considered when interpreting the findings.

While the term metabolic dysfunction-associated steatotic liver disease (MASLD) has recently been proposed to better reflect the metabolic nature of the disease, the results of the present study remain applicable, as the included patients showed metabolic characteristics that align with current MASLD definitions.

v) Although sex-specific differences in hepatic fat distribution, lipid metabolism, ANGPTL3 regulation, and cardiovascular risk have been reported, sex-stratified analyses were not performed in the present study due to the limited sample size. Therefore, potential sex-related differences in ANGPTL3 levels, carotid intima-media thickness, and metabolic parameters could not be evaluated and should be addressed in future studies.

Despite these limitations, the study showed that serum ANGPTL3 levels were higher in individuals with MAFLD than in healthy controls. This observation suggests that ANGPTL3 may reflect metabolic alterations observed in patients with MAFLD. The findings contribute to the ongoing evaluation of potential non-invasive markers associated with

MAFLD, without implying causality or definitive diagnostic utility. These findings should be interpreted as associative rather than causative, given the observational design of the study, and further prospective studies incorporating histological assessment are required to clarify the clinical relevance of ANGPTL3 in MAFLD.

### **Author contributions**

EKY, END, IE, SD, IY, MU, HEC, GA, MA, UT, HU, and OT conceptualized and designed this study. EKY, END, IE, MU, HEC, GA, and MA performed data acquisition. END and SD performed statistical analyses. All authors contributed to drafting, reviewing, and finalizing the manuscript. All authors have read and agreed to the published version of the manuscript.

### **Institutional Review Board Statement**

The study protocol was approved by the Health Sciences University Istanbul Kanuni Sultan Suleyman Training and Research Hospital Ethics Committee (number and date of approval: No. 2022.07.183 / 28.07.2022). All subjects gave their informed consent for inclusion before they participated in the study.

### **Funding**

No funding was received for conducting this study.

### **Conflicts of interest**

The authors declare no conflicts of interest.

### **Data availability statement**

The datasets used and analyzed in this study are available from the corresponding authors upon reasonable request.

### **References**

1. Sanyal AJ, Friedman SL, McCullough AJ, Dimick-Santos L, American Association for the Study of Liver Diseases, United States Food and Drug Administration. Challenges and opportunities in drug and biomarker development for nonalcoholic steatohepatitis: findings and recommendations from an American Association for the Study of Liver Diseases-U.S. Food and Drug Administration Joint Workshop. *Hepatology*. 2015 Apr;61(4):1392–405.
2. Kuchay MS, Misra A. From non-alcoholic fatty liver disease (NAFLD) to metabolic-associated fatty liver disease (MAFLD): A journey over 40 years. *Diabetes Metab Syndr*. 2020;14(4):695–6.
3. Powell EE, Wong VWS, Rinella M. Non-alcoholic fatty liver disease. *Lancet*. 2021 June 5;397(10290):2212–24.

4. Canivet CM, Bonnafous S, Rousseau D, Leclere PS, Lacas-Gervais S, Patouraux S, et al. Hepatic FNDC5 is a potential local protective factor against Non-Alcoholic Fatty Liver. *BiochimBiophys Acta Mol Basis Dis.* 2020 May 1;1866(5):165705.
5. Tilg H, Moschen AR, Roden M. NAFLD and diabetes mellitus. *Nat Rev Gastroenterol Hepatol.* 2017 Jan;14(1):32–42.
6. Eslam M, Newsome PN, Sarin SK, Anstee QM, Targher G, Romero-Gomez M, et al. A new definition for metabolic dysfunction-associated fatty liver disease: An international expert consensus statement. *J Hepatol.* 2020 July;73(1):202–9.
7. 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 July;64(1):73–84.
8. Cusi K, Abdelmalek MF, Apovian CM, Balapattabi K, Bannuru RR, Barb D, et al. Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD) in People With Diabetes: The Need for Screening and Early Intervention. A Consensus Report of the American Diabetes Association. *Diabetes Care.* 2025 July 1;48(7):1057–82.
9. Balasubramaniam D, Schroeder O, Russell AM, Fitchett JR, Austin AK, Beyer TP, et al. An anti-ANGPTL3/8 antibody decreases circulating triglycerides by binding to a LPL-inhibitory leucine zipper-like motif. *J Lipid Res.* 2022 May;63(5):100198.
10. Ke Y, Liu S, Zhang Z, Hu J. Circulating angiopoietin-like proteins in metabolic-associated fatty liver disease: a systematic review and meta-analysis. *Lipids Health Dis.* 2021 May 25;20(1):55.
11. Alberti KGMM, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, et al. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation.* 2009 Oct 20;120(16):1640–5.
12. Chung J, Park HS, Kim YJ, Yu MH, Park S, Jung SI. Association of Hepatic Steatosis Index with Nonalcoholic Fatty Liver Disease Diagnosed by Non-Enhanced CT in a Screening Population. *Diagnostics (Basel).* 2021 Nov 23;11(12):2168.
13. Gerstenmaier JF, Gibson RN. Ultrasound in chronic liver disease. *Insights Imaging.* 2014 Aug;5(4):441–55.
14. Asghari G, Nikparast A, Mahdavi M, Dehghan P, Valizadeh M, Hosseinpanah F, et al. Diagnostic performance of different anthropometric indices among Iranian adolescents for intima media thickness in early adulthood: A prospective study and literature review. *Front Nutr.* 2023;10:1098010.
15. Yuan S, Chen J, Li X, Fan R, Arsenault B, Gill D, et al. Lifestyle and metabolic factors for nonalcoholic fatty liver disease: Mendelian randomization study. *Eur J Epidemiol.* 2022 July;37(7):723–33.

16. Armstrong MJ, Gaunt P, Aithal GP, Barton D, Hull D, Parker R, et al. Liraglutide safety and efficacy in patients with non-alcoholic steatohepatitis (LEAN): a multicentre, double-blind, randomised, placebo-controlled phase 2 study. *Lancet*. 2016 Feb 13;387(10019):679–90.
17. Zemlianitsyna O, Polozova L, Karachentsev I, Sinaiko V, Kravchun N. Features Of Excretion Of Melatonin In Urine in Patients with Type 2 Diabetes Mellitus and Non-Alcoholic Fatty Liver Disease with Manifestations Of Fibrosis and Its Relationship with Certain Metabolic and Immunological Indicators. *Georgian Med News*. 2018 Jan;(274):103–7.
18. Hossain IA, Rahman Shah MM, Rahman MK, Ali L. Gamma glutamyl transferase is an independent determinant for the association of insulin resistance with nonalcoholic fatty liver disease in Bangladeshi adults: Association of GGT and HOMA-IR with NAFLD. *Diabetes Metab Syndr*. 2016;10(1 Suppl 1):S25-29.
19. Zou Y, Zhong L, Hu C, Zhong M, Peng N, Sheng G. LDL/HDL cholesterol ratio is associated with new-onset NAFLD in Chinese non-obese people with normal lipids: a 5-year longitudinal cohort study. *Lipids Health Dis*. 2021 Mar 25;20(1):28.
20. Zhu Z, Yang N, Fu H, Yuan G, Chen Y, Du T, et al. Associations of lipid parameters with non-alcoholic fatty liver disease in type 2 diabetic patients according to obesity status and metabolic goal achievement. *Front Endocrinol (Lausanne)*. 2022;13:1002099.
21. Jarvis H, Craig D, Barker R, Spiers G, Stow D, Anstee QM, et al. Metabolic risk factors and incident advanced liver disease in non-alcoholic fatty liver disease (NAFLD): A systematic review and meta-analysis of population-based observational studies. *PLoS Med*. 2020 Apr;17(4):e1003100.
22. Ajmal MR, Yaccha M, Malik MA, Rabbani MU, Ahmad I, Isalm N, et al. Prevalence of nonalcoholic fatty liver disease (NAFLD) in patients of cardiovascular diseases and its association with hs-CRP and TNF- $\alpha$ . *Indian Heart J*. 2014;66(6):574–9.
23. Yeniova AO, Küçükazman M, Ata N, Dal K, Kefeli A, Başığit S, et al. High-sensitivity C-reactive protein is a strong predictor of non-alcoholic fatty liver disease. *Hepatogastroenterology*. 2014;61(130):422–5.
24. Duan Y, Pan X, Luo J, Xiao X, Li J, Bestman PL, et al. Association of Inflammatory Cytokines With Non-Alcoholic Fatty Liver Disease. *Front Immunol*. 2022;13:880298.
25. Jamialahmadi T, Bo S, Abbasifard M, Sathyapalan T, Jangjoo A, Moallem SA, et al. Association of C-reactive protein with histological, elastographic, and sonographic indices of non-alcoholic fatty liver disease in individuals with severe obesity. *J Health Popul Nutr*. 2023 Apr 7;42(1):30.
26. Simon A, Megnien JL, Chironi G. The value of carotid intima-media thickness for predicting cardiovascular risk. *ArteriosclerThrombVasc Biol*. 2010 Feb;30(2):182–5.
27. Tarantino G, Costantini S, Finelli C, Capone F, Guerriero E, La Sala N, et al. Carotid intima-media thickness is predicted by combined eotaxin levels and severity of hepatic steatosis at ultrasonography in obese patients with Nonalcoholic Fatty Liver Disease. *PLoS One*. 2014;9(9):e105610.

28. Tarantino G, Finelli C, Colao A, Capone D, Tarantino M, Grimaldi E, et al. Are hepatic steatosis and carotid intima media thickness associated in obese patients with normal or slightly elevated gamma-glutamyl-transferase? *J Transl Med.* 2012 Mar 16;10:50.
29. Vu H, Khanh Tuong TT, Hoang Lan N, et al. Association between nonalcoholic fatty liver disease and carotid intima-media thickness. *Clin Ter.* 2023;174(1):42-47.
30. Mladenova IL, Tan EF, Ng JY, Sharma P. Non-alcoholic fatty liver disease (NAFLD) and its association to cardiovascular disease: A comprehensive meta-analysis. *JRSM Cardiovasc Dis.* 2025;14:20480040251325929.
31. Corey KE, Misdraji J, Gelrud L, Zheng H, Chung RT, Krauss RM. Nonalcoholic steatohepatitis is associated with an atherogenic lipoprotein subfraction profile. *Lipids Health Dis.* 2014 June 21;13:100.
32. Carbone C, Piro G, Merz V, Simionato F, Santoro R, Zecchetto C, et al. Angiopoietin-Like Proteins in Angiogenesis, Inflammation and Cancer. *Int J Mol Sci.* 2018 Feb 1;19(2):431.
33. Zhang Y, Yan C, Dong Y, Zhao J, Yang X, Deng Y, et al. ANGPTL3 accelerates atherosclerotic progression via direct regulation of M1 macrophage activation in plaque. *J Adv Res.* 2025 Apr;70:125–38.
34. Wu J, Zhang H, Zheng H, Jiang Y. Hepatic inflammation scores correlate with common carotid intima-media thickness in rats with NAFLD induced by a high-fat diet. *BMC Vet Res.* 2014 July 16;10:162.
35. Balasubramaniam D, Schroeder O, Russell AM, Fitchett JR, Austin AK, Beyer TP, et al. An anti-ANGPTL3/8 antibody decreases circulating triglycerides by binding to a LPL-inhibitory leucine zipper-like motif. *J Lipid Res.* 2022 May;63(5):100198.
36. Xu YX, Redon V, Yu H, Querbes W, Pirruccello J, Liebow A, et al. Role of angiopoietin-like 3 (ANGPTL3) in regulating plasma level of low-density lipoprotein cholesterol. *Atherosclerosis.* 2018 Jan;268:196–206.
37. Tikka A, Soronen J, Laurila PP, Metso J, Ehnholm C, Jauhiainen M. Silencing of ANGPTL 3 (angiopoietin-like protein 3) in human hepatocytes results in decreased expression of gluconeogenic genes and reduced triacylglycerol-rich VLDL secretion upon insulin stimulation. *Biosci Rep.* 2014 Dec 12;34(6):e00160.
38. Altun Ö, Dikker O, Arman Y, Ugurlukisi B, Kutlu O, Ozgun Cil E, et al. Serum Angiopoietin-like peptide 4 levels in patients with hepatic steatosis. *Cytokine.* 2018 Nov;111:496–9.
39. Zhang L, Shannon CE, Bakewell TM, Abdul-Ghani MA, Fourcaudot M, Norton L. Regulation of ANGPTL8 in liver and adipose tissue by nutritional and hormonal signals and its effect on glucose homeostasis in mice. *Am J Physiol Endocrinol Metab.* 2020 May 1;318(5):E613–24.

40. Hu X, Fan J, Ma Q, Han L, Cao Z, Xu C, et al. A novel nanobody-heavy chain antibody against Angiotensin-like protein 3 reduces plasma lipids and relieves nonalcoholic fatty liver disease. *J Nanobiotechnology*. 2022 May 19;20(1):237.
41. Gobeil É, Bourgault J, Mitchell PL, Houessou U, Gagnon E, Girard A, et al. Genetic inhibition of angiotensin-like protein-3, lipids, and cardiometabolic risk. *Eur Heart J*. 2024 Mar 1;45(9):707–21.
42. Bergmark BA, Marston NA, Bramson CR, Curto M, Ramos V, Jevne A, et al. Effect of Vupanorsen on Non-High-Density Lipoprotein Cholesterol Levels in Statin-Treated Patients With Elevated Cholesterol: TRANSLATE-TIMI 70. *Circulation*. 2022 May 3;145(18):1377–86.
43. Gaudet D, Karwatowska-Prokopczuk E, Baum SJ, Hurh E, Kingsbury J, Bartlett VJ, et al. Vupanorsen, an N-acetyl galactosamine-conjugated antisense drug to ANGPTL3 mRNA, lowers triglycerides and atherogenic lipoproteins in patients with diabetes, hepatic steatosis, and hypertriglyceridaemia. *Eur Heart J*. 2020 Oct 21;41(40):3936–45.

**Table 1.** Analysis of demographic characteristics

Parameter		MAFLD Group	Control Group	p value
Age		42.7±7.5 (44)	44.7±9.0 (45)	0.283**
Sex	Male	40 (44.4%)	40 (44.4%)	1.000 <sup>#</sup>
	Female	50 (55.6%)	50 (55.6%)	
Steatosis Grade	1	49 (54.4%)	-	-
	2	41 (45.6%)	-	-
T2D. n (%)		9 (10.0%)	1 (1.1%)	0.009 <sup>#</sup>
Height (cm)		166.6±8.7 (166)	166.9±7.9 (164)	0.767**
Weight (kg)		79.4±9.8 (79)	64.8±7.8 (62)	<0.001**
BMI (kg/m <sup>2</sup> )		28.5±2.2 (28.4)	23.1±0.9 (23.05)	<0.001**
Obesity classification. n (%)	Normal weight	2 (2.2%)	90 (100%)	<0.001 <sup>#</sup>
	Overweight	64 (71.1%)	0 (0.0%)	
	Class I obesity	24 (26.7%)	0 (0.0%)	
Waist circumference (cm)		98.7±6.6 (98)	77.3±7.6 (76)	<0.001**
Hip circumference (cm)		97.6±4.9 (102)	92.8±5.2 (85)	<0.001**
Waist-to-hip ratio		1.01±0.05 (1.02)	0.83±0.06 (0.85)	<0.001**
Systolic BP (mmHg)		124.5±13.3 (120)	115.3±9.4 (120)	<0.001**
Diastolic BP (mmHg)		59.6±1.4 (60)	59.5±3.4 (60)	0.337**
Mean BP (mmHg)		81.2±4.5 (80)	78.1±4.1 (76.7)	<0.001**
Glucose (mg/dL)		99.8±24.7 (93)	93.4±27.3 (90)	0.009**
HOMA-IR		4.43±4.80 (3.15)	2.47±2.38 (1.9)	<0.001**
Metabolic syndrome. n (%)		53 (58.9%)	2 (2.2%)	<0.001 <sup>#</sup>
<b>Abbreviations:</b> T2D, type 2 diabetes mellitus; BMI, body mass index; BP, blood pressure; HOMA-IR, homeostatic model assessment of insulin resistance.				

<sup>#</sup>Chi-square test; \*\*Mann–Whitney U test

Data are presented as mean ± standard deviation (SD), minimum–maximum (min–max), and median values as appropriate for continuous variables; categorical variables are presented as n (%).  $p < 0.05$  was considered statistically significant.

**Table 2.** Analysis of biochemical parameters

	<b>MAFLD Group</b>	<b>Control Group</b>	
<b>Parameter</b>	<b>Mean <math>\pm</math> SD (Median)</b>	<b>Mean <math>\pm</math> SD (Median)</b>	<b><i>p</i> value**</b>
<b>Creatinine (mg/dL)</b>	0.77 $\pm$ 0.17 (0.7)	0.72 $\pm$ 0.15 (0.7)	0.064
<b>Glucose (mg/dL)</b>	99.8 $\pm$ 24.7 (93)	93.4 $\pm$ 27.3 (90)	0.009**
<b>ALT (U/L)</b>	25.2 $\pm$ 28.3 (18)	17.3 $\pm$ 4.6 (17)	0.001
<b>AST (U/L)</b>	31.5 $\pm$ 36.3 (21)	16.4 $\pm$ 9.6 (15)	<0.001
<b>LDL cholesterol (mg/dL)</b>	122.1 $\pm$ 30.4 (122)	106.1 $\pm$ 28.6 (107.5)	0.001*
<b>HDL cholesterol (mg/dL)</b>	43.0 $\pm$ 10.2 (42.7)	51.4 $\pm$ 14.1 (48.3)	<0.001
<b>Triglycerides (mg/dL)</b>	170.7 $\pm$ 92.5 (150.5)	111.2 $\pm$ 61.9 (90.5)	<0.001
<b>Total cholesterol (mg/dL)</b>	199.3 $\pm$ 33.8 (200.5)	179.8 $\pm$ 33.3 (175.5)	<0.001*
<b>CRP (mg/dL)</b>	3.35 $\pm$ 3.31 (2)	2.46 $\pm$ 5.49 (1.45)	0.001
<b>TSH (mg/dL)</b>	1.82 $\pm$ 1.76 (1.5)	1.70 $\pm$ 1.15 (1.4)	0.702
<b>Vitamin D (mg/dL)</b>	16.9 $\pm$ 9.0 (16)	20.96 $\pm$ 11.26 (19)	0.006
<b>Total protein (mg/dL)</b>	7.21 $\pm$ 0.39 (7.2)	7.08 $\pm$ 0.43 (7.1)	0.050
<b>Albumin (mg/dL)</b>	4.59 $\pm$ 0.28 (4.6)	4.62 $\pm$ 0.35 (4.6)	0.771
<b>HbA1c (mmol/mol)</b>	5.89 $\pm$ 0.91 (5.7)	5.57 $\pm$ 0.44 (5.6)	0.005
<b>Ferritin (mg/dL)</b>	109.4 $\pm$ 102.5 (86.5)	94.58 $\pm$ 236.01 (52)	0.005
<b>Platelet count (10<sup>3</sup>/<math>\mu</math>L)</b>	272.3 $\pm$ 61.4 (256)	267.51 $\pm$ 61.54 (263)	0.873
<b>Abbreviations:</b> ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRP, C-reactive protein; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TSH, thyroid-stimulating hormone.			

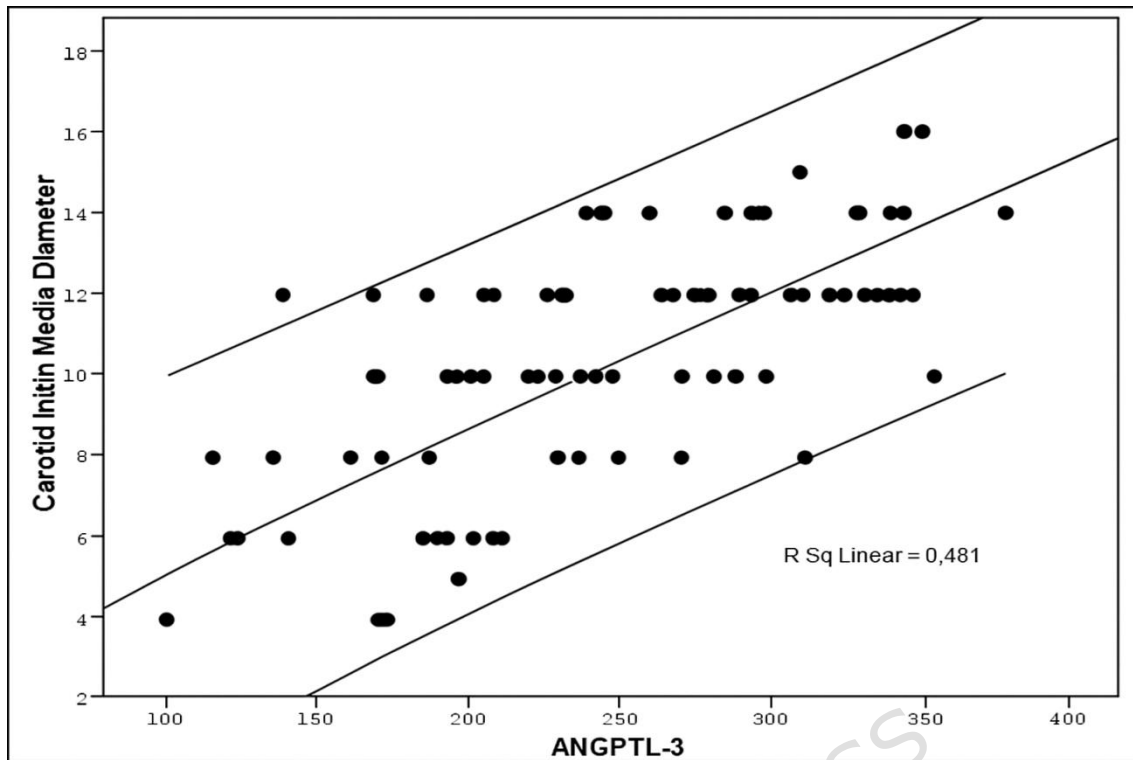
Data are presented as mean  $\pm$  standard deviation (SD) and median (min–max) as appropriate.

\*Student's t-test and \*\*Mann–Whitney U test were used as applicable.  $p < 0.05$  was considered statistically significant.

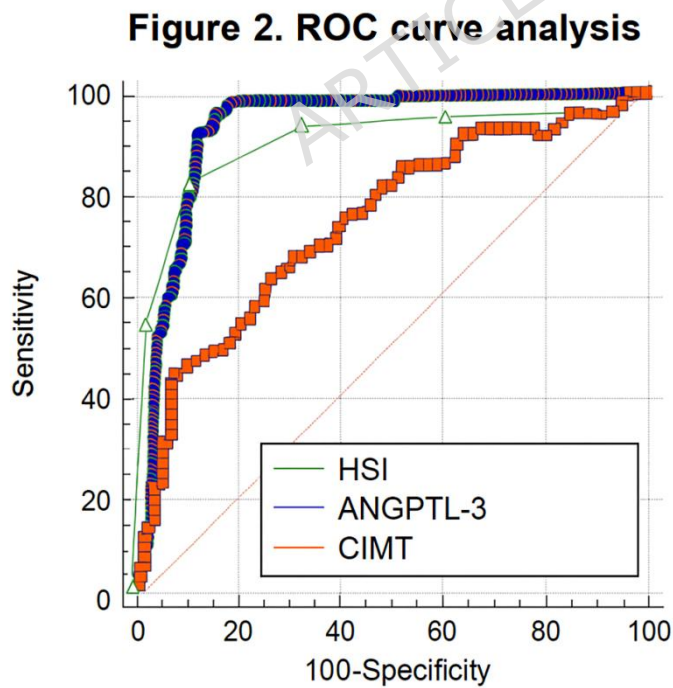
**Table 3.** Comparison of hepatic steatosis index, serum ANGPTL-3, and carotid intima–media thickness (CIMT) between MAFLD and control groups

Parameter	MAFLD group (Mean $\pm$ SD / Median)	Control group (Mean $\pm$ SD / Median)	p value
Hepatic steatosis index (HSI)	39.6 $\pm$ 4.5 (38.97)	31.6 $\pm$ 2.4 (31.56)	<0.001
Serum ANGPTL-3 (ng/mL)	247.8 $\pm$ 66.3 (244.5)	189.7 $\pm$ 54.4 (177)	<0.001
Carotid intima–media thickness (CIMT) (mm)	10.47 $\pm$ 3.03 (11)	5.07 $\pm$ 1.23 (5)	<0.001

CIMT values are expressed in units of 0.1 mm.



**Figure 1.** Correlation between serum ANGPTL3 levels (ng/mL) and carotid intima–media thickness (CIMT) in patients with MAFLD. *CIMT values are expressed in units of 0.1 mm.*



**Figure 2.** Receiver operating characteristic (ROC) curves for hepatic steatosis index (HSI), serum ANGPTL3 levels (ng/mL), and carotid intima–media thickness (CIMT) in patients with MAFLD. *CIMT values are expressed in units of 0.1 mm.*

ARTICLE IN PRESS