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High baseline fetuin-A levels are associated with lower atherosclerotic plaque progression as measured by 3D ultrasound

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ABSTRACT

Background and aims: The glycoprotein fetuin-A has anti-inflammatory effects, increases insulin resistance and plays an important role in calcium metabolism. The aim of our study was to assess the predictive value of fetuin-A on atherosclerotic plaque progression in comparison to the established cardiovascular biomarker high sensitivity C-reactive protein (hsCRP).
Methods: In this prospective, single center-, cohort study, we included 194 patients with at least one cardiovascular risk factor or established cardiovascular disease (CVD). Over a period of 4 years, each patient underwent 3D plaque volumetry of the carotid and femoral arteries on a yearly basis. To evaluate the predictive value of biomarkers in terms of plaque progression, the baseline values of fetuin-A and hsCRP were correlated with the plaque progression from baseline to the last follow up visit.
Results: 171 patients were included in the final analysis. Baseline fetuin-A levels showed a significant negative correlation with plaque progression ($r = -0.244$; $p = 0.001$). In contrast, baseline hsCRP levels showed no correlation with plaque progression ($r = 0.096$, $p = 0.20$). In the ROC-analysis, fetuin-A had a significantly better predictive value than hsCRP (fetuin-A AUC 0.67; $p = 0.001$ vs hsCRP AUC 0.49; $p = 0.88$) with an optimal cut-off value at 712 μg/ml. In patients with high fetuin A levels (>712 μg/ml), a significantly lower plaque progression was observed compared to the group with low fetuin-A levels <712 μg/ml (high fetuin-A 197 mm^3 vs. low fetuin-A 279 mm^3; $p = 0.01$)
Conclusions: Higher fetuin-A levels appear to predict lower atherosclerotic plaque progression in patients with or at risk of cardiovascular disease.

1. Introduction

Cardiovascular diseases (CVD) are the major cause of death in high-income countries. The majority of cardiovascular events like stroke and myocardial infarction occurs on the basis of atherosclerosis. In order to evaluate the risk of individual patients, the use of established risk-stratification scores e.g. the ESC-SCORE or the Framingham-Risk-Score (FRS), is recommended. However, the predictive value of current risk scoring systems is limited. Most heart attacks and strokes occur in patients who are classified as low or intermediate risk by current scoring systems [1–3]. One approach to improve the predictive value of risk stratification scores, is to include biomarkers into these models. In the last decades intensive research on numerous inflammatory biomarkers has been undertaken, consistent with the view of atherosclerosis as an inflammatory disease [4]. The best documented of these inflammatory biomarkers is high sensitivity C-reactive protein (hsCRP), which is currently the only inflammatory biomarker recommended by the ESC guidelines [2]. The ESC states that not all

Abbreviations: ABI, ankle-brachial-index; CBVD, cerebrovascular disease; CPP, carotid plaque progression; CPV, carotid plaque volume; CVD, cardiovascular diseases; CVRF, cardiovascular risk factor; FPP, femoral plaque progression; FPV, femoral plaque volume; FRS, Framingham Risk Score; hsCRP, high sensitivity C-reactive protein; IMT, intima media thickness; PAD, peripheral arterial disease; PWV, pulse wave velocity; SD, standard deviation; TPP, total plaque progression; TPV, total plaque volume; 3D, three-dimensional.

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potentially useful circulatory and urinary biomarkers have undergone state-of-the-art assessment of their added value on cardiovascular risk prediction. Systematic reviews suggest, that the vast majority of currently known cardiovascular biomarkers has limited ability to improve risk classification. Hence, further research on additional biomarkers is necessary [2,5,6].

Human fetuin-A is a 62 kDa liver-derived glycoprotein, belonging to a family of four structurally related plasma proteins, containing cystatin-like protein domains [7]. Fetuin-A is considered to represent 50% of the serum capacity to prevent calcium and phosphate precipitation [8]. Besides its effect on calcium metabolism, fetuin-A shows also effects on inflammation and insulin resistance. In experimental studies with fetuin-A knockout mice, diffuse extraosseous calcifications in vessels and cardiac valves have been detected. Further research in animal models showed, that fetuin-A is more likely to inhibit intimal than medial calcification [9–11]. The results of these animal models have been transferred to clinical studies. In a cohort study of 238 patients on peritoneal dialysis, low serum fetuin-A levels showed significant associations with atherosclerosis and valvular calcification [12]. Due to its role in calcium homeostasis and inflammation, fetuin-A is linked to the pathogenesis of atherosclerosis [13,14]. In the vascular system, fetuin-A acts as a potent inhibitor of hydroxyapatite formation [9]. In a prospective population-based study, Laughlin et al. reported that low levels of fetuin-A predicted greater risk for cardiovascular mortality in patients without diabetes [15]. Moreover, in patients who present with stable angina pectoris, fetuin-A levels are significantly lower than in non-cardiac patients [13,16]. In patients on dialysis, low fetuin-A levels were associated with stronger vascular calcification and higher mortality [17]. Dependent on the mode of stimulation, fetuin-A can act as negative acute phase protein and form an inflammatory counterpart to hs-CRP. This was shown by a study performed on the French Registry of Acute ST-Elevation and Non-ST-Elevation Myocardial Infarction (FAST-MI), where the combination of high hs-CRP and low fetuin-A was associated with the highest mortality after acute coronary syndrome of all subgroups. This trial further showed, that high levels of fetuin-A were associated with a significantly reduced 1-year cardiovascular mortality [18].

In the last years, the development of 3D ultrasound has brought new opportunities for the quantitative assessment of subclinical atherosclerosis. This was first shown by Sillesen et al. in the BioImage-Study [19]. Previously we have reported that peripheral atherosclerotic plaque volume measured by 3D ultrasound increases with the number of CVRF and the number of vascular beds involved [20]. Moreover, the presence of chronic kidney disease and male sex is associated with higher plaque volume [21]. The biomarker neutrophil gelatinase-associated lipocalin, but not hsCRP was shown to correlate with peripheral plaque volume at baseline [22]. The purpose of our study was to evaluate the effect of cardiovascular biomarkers, in particular fetuin-A and hsCRP, on atherosclerotic plaque progression. Plaque progression was objectified by quantitative assessment via 3D-Ultrasound. To our knowledge, this is the first study to examine this relationship.

2. Methods

2.1. Study design

The study was a prospective observational single-center cohort study (Correlation of atherosclerotic Plaque Volume and Intima Media Thickness with soluble P-Selectin (ClinicalTrials.gov identifier: NCT01895725)). The baseline data and inclusion/exclusion criteria of the study have been published before [20].

Briefly, patients between 30 and 85 years of age (male and female) with established cardiovascular disease (coronary artery disease (CAD), cerebrovascular disease (CBVD) or peripheral arterial disease (PAD)) or at least one traditional cardiovascular risk factor (CVRF) were included. We defined family history of cardiovascular disease as the occurrence of a premature cardiovascular event (myocardial infarction, stroke or critical limb ischemia) in a first-degree relative (<55 years for male and <65 years for female relatives). For the diagnosis of diabetes, a fasting glucose level ≥126 mg/dl or the use of diabetes medication were considered relevant. Arterial hypertension was defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg and/or current antihypertensive therapy. For hyperlipidemia, a low-density lipoprotein value ≥ 160 mg/dL and/or triglycerides ≥150 mg/dL and/or the use of lipid-lowering drugs were required. Patient screening took place between 2013 and 2016 at the outpatient clinic of the Department of Internal Medicine III (cardiology and angiology) of the Medical University of Innsbruck. The study was carried out in accordance with the declaration of Helsinki and the study protocol was approved by the Ethics Committee of the University of Innsbruck (Project identification code-UN5048). After obtaining written and informed consent, each patient received at baseline ultrasound measurements, physical examination and routine laboratory. In addition, a detailed history of cardiovascular disease and risk factors, coexisting diseases and current medication as well as smoking status was recorded for each patient. The patients were followed up for four years with re-examination once per year. Only patients that completed all follow-up exams were included in the analysis.

2.2. Ultrasound imaging, routine laboratory and additional examination

Each patient underwent measurements of the intima-media-thickness (IMT) in the common carotid arteries of both sides, following the recommendations of the Mannheim consensus [23]. For the IMT measurements, we used a Philips iU22 system equipped with a linear L9-3 probe using a built in, automatic mean IMT-calculation software. IMT was measured ECG triggered at least 1 cm proximal to the flow divider along a segment of 10 mm free of plaques. Plaque volumetry was performed using the same machine equipped with a VL13-5 3D probe and plaque quantification software to assess plaque volume 6 cm within the bifurcation and the visible parts of the internal and common carotid arteries on both sides. As recommended by the Mannheim consensus, we defined plaque as local structure extending at least 0,5 mm into the arterial lumen or 50% of the surrounding IMT, or showing a thickness >1,5 mm from the media-adventitia interface to the intima-lumen interface. Identical measurements were performed in the common and superficial femoral artery. (femoral IMT and femoral plaque volume). Plaque progression was determined as the difference in plaque volume between the 4th follow-up visit and baseline measurement. The reliability of the method was investigated by calculating the inter-observer variability of 3 different observers, which showed a very good agreement between the raters with an intra-class correlation coefficient of 0,95 (95% CI, 0,82–0,99) [20]. Routine laboratory analyses included total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, hs-CRP, fasting glucose, HbA1C, creatinine and estimated glomerular filtration rate (eGFR). The eGFR was calculated according to the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula and chronic kidney disease (CKD) was defined as an eGFR <60 ml/min/1,73 m^2. Furthermore, one extra-EDTA whole blood sample was obtained and centrifuged to separate the cellular parts from EDTA plasma. The EDTA-plasma was used to measure the concentration of fetuin-A by means of commercially available ELISA (R&D systems human

fetuin-A quantikine ELISA kit). At each visit measurements of the blood pressure, the ankle-brachial index (ABI) and the pulse wave velocity were performed. ABI and pulse-wave velocity were measured by an automated system (AngE Pro4, SOT Medical Systems, Maria Rain, Austria). For each patient, the FRS was calculated and the patient was assigned to the corresponding risk category (low/intermediate risk group or high-risk group) [24].

2.3. Statistical analysis

Normal distribution was tested by the test of Kolmogorov-Smirnov. The baseline data for continuous variables are shown as median and corresponding interquartile range (IQR).

Categorical variables are presented as absolute numbers and percentages. Total plaque volume (TPV) was calculated by adding the sum of femoral and carotid plaque volume of both sides. Total plaque progression (TPP) was defined as the difference between the TPV at the 4th follow-up visit and the TPV at baseline. For further statistical analysis the study population was divided into a high (high-TPP) and a low (low-TPP) plaque progression group, using the 75th percentile of the TPP distribution as cut-off value. The distribution of a continuous variable by a categorical variable was tested with the Mann-Whitney-U test. For the comparison of categorical variables, the chi-square-test was used. The linear bivariate correlation was assessed with Spearman correlation. To calculate the predictive value of fetuin-A and hsCRP, a ROC analysis was performed and the area under the curve (AUC) was determined. AUC values were compared using a non-parametric approach. After determining the optimal cut-off value of fetuin-A by ROC analysis, the study population was divided into a high-fetuin-A group and a low-fetuin-A group. The fetuin-A groups were then compared to each other in terms of mean plaque progression. To analyze the difference in TPV development between high-fetuin A and low-fetuin A group over the course of the follow up visits, we performed a mixed ANOVA with repeated measures. For the determination of the effect size, ηp^2 was calculated. The Greenhouse-Geisser adjustment was used to correct violations of sphericity. Multiple testing was corrected using the Bonferroni correction. Multivariate linear regression was used to analyze the relationship between CVRF, biomarkers and TPP. For the evaluation of the effect of fetuin-A on the predictive value of Framingham Risk Score, a binary logistic regression model was calculated. In the binary regression model, we combined two risk-categories (low-intermediate and high-risk) with two fetuin-A categories (low and high) resulting in four combinations available for the evaluation of the predictive value. The cut-off values of the risk categories were selected following the recommendations of the FRS [24]. For all statistical tests, a two tailed p-value of <0,05 was considered as significant. Statistical analysis was conducted with SPSS Statistics Version 27,0 (IBM Corp, Armonk, NY, USA).

3. Results

3.1. Baseline characteristics

For 194 patients who underwent 3D-ultrasound plaque volumetry each year, baseline fetuin-A levels were measured. Because of withdrawn consent and missing data, 171 patients who completed the full follow up were available for statistical analysis. The median age of the 171 study patients was 69 years, with a significant higher age in the high-TPP group. The majority of all study participants was male (55.6%); however, women were significantly more often present in the low-TPP group (51.6%) than in the high-TPP group (23.3%, $p = 0.026$). The most prevalent cardiovascular risk factor of the study population was hyperlipidaemia with 87.1%, with no significant difference between high- and low-TPP groups. 64.9% of the study population suffered from hypertension, with equal distribution in both study groups (61.7% in low-TPP vs. 74.4% in high-TPP, $p = 0.13$). Besides this, 29.2% of all patients had a positive family history for CVD, 14% suffered from diabetes and more half of the patients received lipid lowering therapy. The median LDL-cholesterol was higher in the low-TPP group, with a difference not reaching statistical significance (122 mg/dl in low-TPP vs. 106 mg/dl in high-TPP, $p > 0.05$). In the high-TPP group more patients received lipid-lowering therapy, with also this difference not being statistically significant (53.1% in low-TPP vs. 55.8% in high-TPP, $p > 0.05$). The baseline characteristics of the study population are presented in Table 1.

3.2. Distribution of baseline atherosclerotic plaque volume, intima-media-thickness, ankle brachial index and pulse wave velocity

The distribution of carotid, femoral and total plaque volume is presented in Table 2. The baseline TPV was 441 mm^3 in the high-TPP group compared to 169.5 mm^3 in the low-TPP group ($p > 0.001$). Similar to the TPV, the carotid and femoral plaque volume at baseline were significantly higher in the high-TPP group as well. Carotid IMT, ABI and PWV are considered to be predictors of subclinical atherosclerosis and cardiovascular mortality.

Although the difference in carotid IMT was small, the high-TPP group showed a statistical significantly wider carotid IMT than the low-TPP group (0.82 mm high-TPP vs. 0.71 mm low-TPP, $p = 0.001$). Likewise, the high-TPP group showed a significantly increased PWV (7.0 m/s high-TPP vs. 6.03 m/s low-TPP, $p = 0.002$). The ABI showed no significant difference between the two groups (see Fig. 1).

3.3. Correlation of biomarkers with baseline-plaque volume and plaque progression

We examined the correlation between different biomarkers and atherosclerotic plaque volume as measured by 3D-ultrasound. Fetuin-A values showed a high significant negative correlation with TPV measured on baseline visit ($r = -0.233$, $p = 0.002$), whereas hs-CRP did not correlate with the TPV ($r = 0.135$, $p = 0.07$). When we investigated the association of fetuin-A levels with plaque progression we found a highly significant negative correlation with TPP ($r = -0.244$, $p = 0.001$), with the carotid plaque progression ($r = -0.17$, $p = 0.02$) and an even stronger negative correlation with femoral plaque progression ($r = -0.20$, $p = 0.007$). On the other side, baseline levels of hs-CRP showed no significant correlation with total plaque progression ($r = 0.096$, $p = 0.20$), femoral plaque progression ($r = 0.095$, $p = 0.21$) or carotid plaque progression ($r = 0.039$, $p = 0.60$). Details for correlation between biomarkers and total plaque progression are shown in Fig. 2.

3.5. Fetuin-A and the prediction of low plaque progression

The median fetuin-A concentration in the low-TPP group was significantly higher than in the high-TPP group (790.24μg/ml low-TPP vs. 694.8 μg/ml in high-TPP, $p < 0.001$). In order to determine the optimal cut-off value for the prediction of lower plaque progression, a ROC analysis was performed (Fig. 3). With an area under the curve (AUC) of 0.67 (95% CI 0.58–0.76; $p = 0.001$) and an optimal cut-off value at 712 μg/ml fetuin-A showed a sensitivity of 68.5% and a specificity of 58.1% in the prediction of lower plaque progression. Since hsCRP is considered to be a predictor of plaque progression, ROC-analysis for hs-CRP was also performed in terms of prediciton of high plaque progression. However, in our study population the AUC of hs CRP was not a significant predictor for

Table 1
Baseline characteristics of the study population. Continuous variables are presented as median and corresponding interquartile range (IQR). Categorical variables are shown as absolute numbers and percentage.

	Study population (n = 171)	Low-TPP (n = 128)	High-TPP (n = 43)	p-value
Age, years	69 (62–77)	66 (61–75.5)	73 (66–80)	**0.001**
Female, n (%)	76 (44.4)	66 (51.6)	10 (23.3)	**0.01**
Body mass index, kg/m2	25.7 (23.9–28.4)	25.8 (23.9–28.7)	25.3 (24.1–28.4)	n.s.
Hypertension, n (%)	111 (64.9)	79 (61.7)	32 (74.4)	n.s.
Family history for CVD, n (%)	50 (29.2)	42 (37.6)	8 (18.6)	n.s.
Smoking, pack years	22.5 (10–40)	20 (10–35)	40 (25–45)	**0.001**
Hyperlipidemia, n (%)	149 (87.1)	112 (87.5)	37 (86)	n.s.
Diabetes mellitus, n (%)	24 (14)	17 (13.3)	7 (16.3)	n.s.
hs-CRP, mg/dl	0.17 (0.08–0.35)	0.17 (0.08–0.34)	0.13 (0.08–0.37)	n.s.
Fetuin-A, μg/ml	757.5 (646.1–896.7)	790.2 (676.0–929.9)	694.8 (566.8–806.8)	**0.001**
Total cholesterol, mg/dl	196.5 (172–233.25)	200 (175–237)	189 (159–214)	n.s.
LDL-cholesterol, mg/dl	118 (96.75–149.25)	122 (99–153)	106 (85.0–135.25)	n.s.
HDL-cholesterol, mg/dl	59 (48–76)	60.5 (48–75)	58 (48–78)	n.s.
Triglyceride mg/dl	124 (90.5–176)	133.5 (99–153)	109.5 (90.5–143.25)	n.s.
Creatinine, mg/dl	0.91 (0.82–1.07)	0.89 (0.79–1.02)	1.00 (0.87–1.16)	**0.002**
eGFR, ml/min/1,73 m^2	74.06 (63.7–86.1)	74.82 (65.29–86.44)	71.76 (57.52–81.05)	n.s.
Antiplatelet therapy, n (%)	60 (35.1)	40 (31.3)	20 (46.5)	n.s
Lipid lowering therapy	92 (53.8)	68 (53.1)	24 (55.8)	n.s.
Antihypertensive therapy	98 (57.3)	71 (55.5)	27 (62.8)	n.s.
Any vascular disease, n (%)	48 (28.1)	31 (24.2)	17 (39.5)	n.s.
Framingham Risk Score, %	13.7 (7.9–25.3)	11.2 (7.3–21.6)	21.6 (11.2–29.4)	**0.001**
CAD, n (%)	30 (17.5)	21 (14.4)	9 (20.9)	n.s.
CBVD, n (%)	18 (10.5)	13 (10.2)	5 (11.6)	n.s.
PAD, n (%)	15 (8.8)	7 (5.5)	8 (18.6)	**0.008**

TPP – total plaque progression; CVD - cardio vascular disease; LDL - low density lipoprotein; HDL - high density lipoprotein; hs–CRP - high sensitive C-reactive protein; CAD - coronary artery disease, CBVD - cerebro vascular disease, PAD - peripheral arterial disease.

Table 2
Distribution of baseline total plaque volume, carotid intima-media thickness, ankle brachial index and pulse wave velocity. Variables are presented as median and corresponding interquartile range (IQR).

	Study population (n = 171)	Low-TPP (n = 128)	High-TPP (n = 43)	p-value
Total PV, mm^3	222.0 (70–455)	169.5 (51.2–374.5)	441.0 (205.0–621.0)	**<0.001**
Carotid PV, mm^3	61.0 (3.0–184.0)	35.5 (0–160)	127.0 (66.0–368.0)	**<0.001**
Femoral PV, mm^3	144.0 (33.0–250.0)	109.0 (0–221.25)	218.0 (98.0–366.0)	**<0.001**
Carotid IMT, mm	0.72 (0,63–0,83)	0.71 (0.61–0.80)	0.82 (0.68–0.91)	**<0.001**
ABI	0.92 (0.86–0.99)	0.94 (0.87–0.99)	0.9 (0.84–0.97)	n.s.
PWV, m/s	6.2 (5.49–7.67)	6.11 (5.3–7.42)	7.0 (6.05–8.21)	**0.02**

PV - plaque volume, TPP - total plaque progression, IMT - intima-media-thickness, ABI- ankle brachial index, PWV - pulse wave velocity.

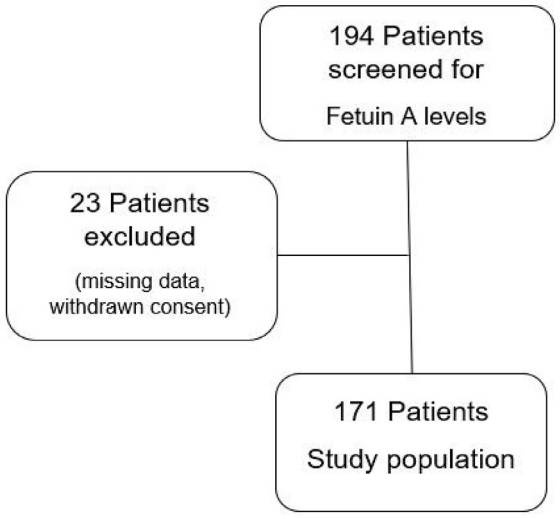

Fig. 1. Flow chart of study participants.

high-plaque progression (AUC 0.49, 95% CI 0.38–0.59, p = 0.88). The patient group with fetuin-A levels above the cut-off value showed significant lower TPP than patients with lower fetuin-A levels (p = 0.01) as shown in Fig. 4. For the analysis of plaque volume over the course of the follow up visits, mixed analysis of variance (ANOVA) was performed. In the mixed ANOVA there was a significant main effect for follow-up visits on plaque development ($F(2.19,368.83) = 75.26$, $p < 0.001$, $p\eta^2 = 0.3$). Besides this, the fetuin-A group also had a significant main effect on plaque development, meaning that the fetuin-A groups differed significantly over the course of time. ($F(1,168) = 4.72$, $p = 0.03$, $p\eta^2 = 0.02$). Due to missing homogenity of covariances, the interaction between the subject factors was not interpreted (see Fig. 5).

3.6. Multivariate regression analysis

In our study, patients with high-fetuin-A had significantly more diabetic patients (20.6% high-fetuin-A vs. 4.4% low-fetuin-A, p = 0.003), significantly more female patients (52.9% high-fetuin-A vs. 30.9% low-fetuin-A, p = 0.005) and significantly lower creatinine levels (0.88 mg/dl high-fetuin-A vs 0.95 mg/dl low-fetuin-A) compared to those with low fetuin_A. There was no significant difference in other risk factors or laboratory parameters, including the eGFR (data not shown). For the evaluation of fetuin-A as independent predictor for TPP, a multivariate regression model was calculated. The multivariate regression model for the prediction of TPP included traditional CVRF (age, sex, arterial hypertension,

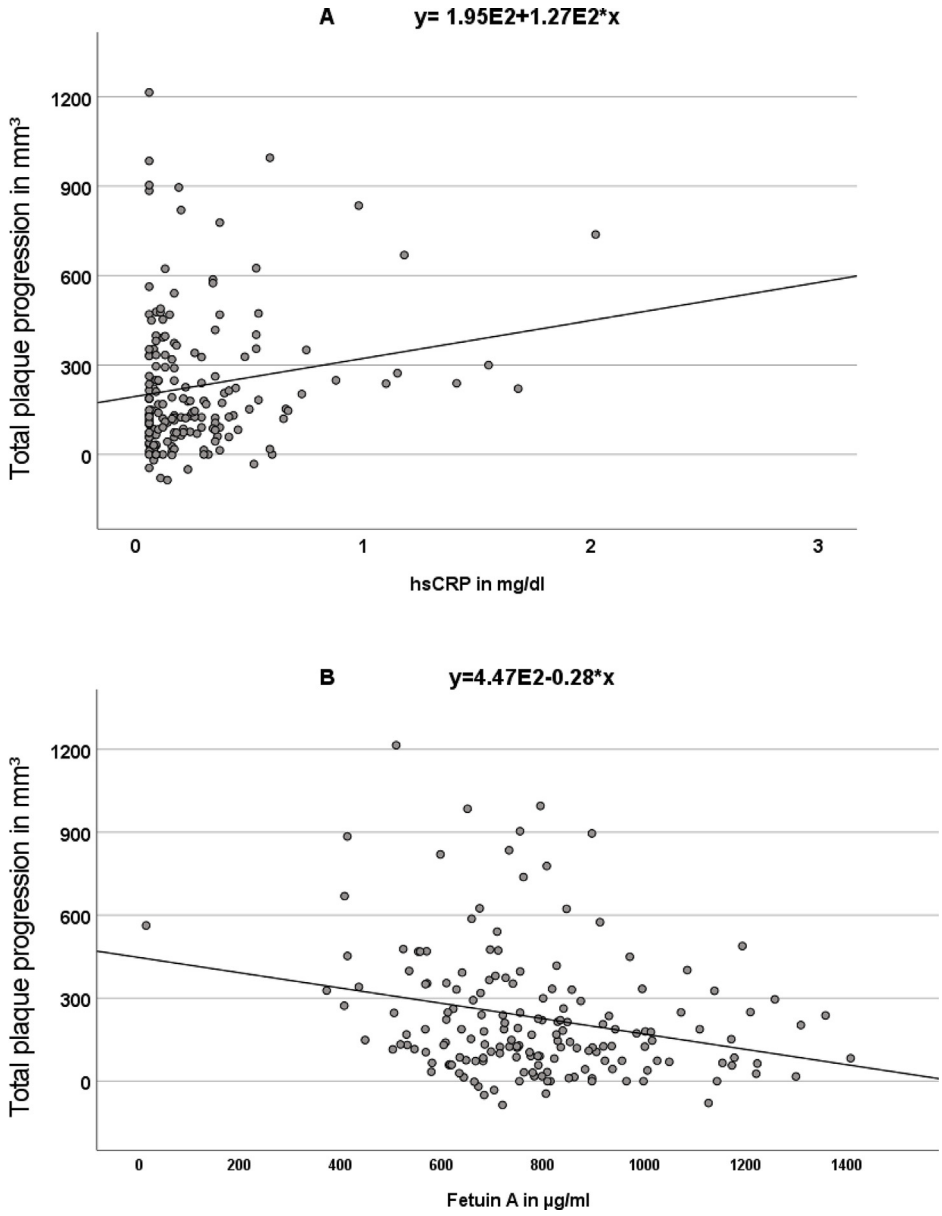

Fig. 2. Scatterplots with corresponding regression lines showing the correlation of hs-CRP (A) and fetuin-A (B) with the total plaque progression (TPP).

smoking, hyperlipidaemia, established CVD), medical therapy of CVRF (antiplatelet therapy, lipid lowering therapy, antihypertensive therapy), as well as laboratory parameters creatinine, eGFR, total cholesterol, LDL-cholesterol and the biomarkers hsCRP and fetuin-A. In the multivariate regression model, a significant association was found for age, arterial hypertension, antihypertensive therapy, antiplatelet therapy and fetuin-A ($p < 0.05$). No significant association was found for sex, smoking, established CVD, diabetes, hsCRP, creatinine, eGFR, total cholesterol, LDL-cholesterol, hyperlipidaemia and lipid lowering therapy ($p > 0.05$). As shown in Table 3 the strongest negative association with TPP was observed for antihypertensive therapy ($b = -0.27$, $p = 0.01$) and fetuin-A ($b = -0.19$, $p = 0.01$). On the other side, the strongest predictors for total plaque progression were age ($b = 0.31$, $p = 0.01$) and arterial hypertension ($b = 0.32$, $p = 0.004$).

3.7. Binary logistic regression

The binary logistic regression model was statistically significant ($p < 0.05$). The goodness-of-fit was assessed using the Hosmer-Lemeshow test, showing a good fit of the model ($p > 0.05$).

The combination of low-fetuin-A-group/intermediate-low-FRS had a significant positive association with high-TPP (OR 3.59; 95% CI 1.65–13.89; $p = 0{,}016$). The strongest association for high-TPP was found for patients with low fetuin-A levels and high-risk category (OR 7.61, 95% CI 2.77–20.87, $p < 0.001$). The results are summarized in Table 4.

4. Discussion

In our cohort of individuals with at least one CVRF or established CVD, we found that patients with high plasma fetuin-A levels had a lower atherosclerotic plaque progression compared to those with

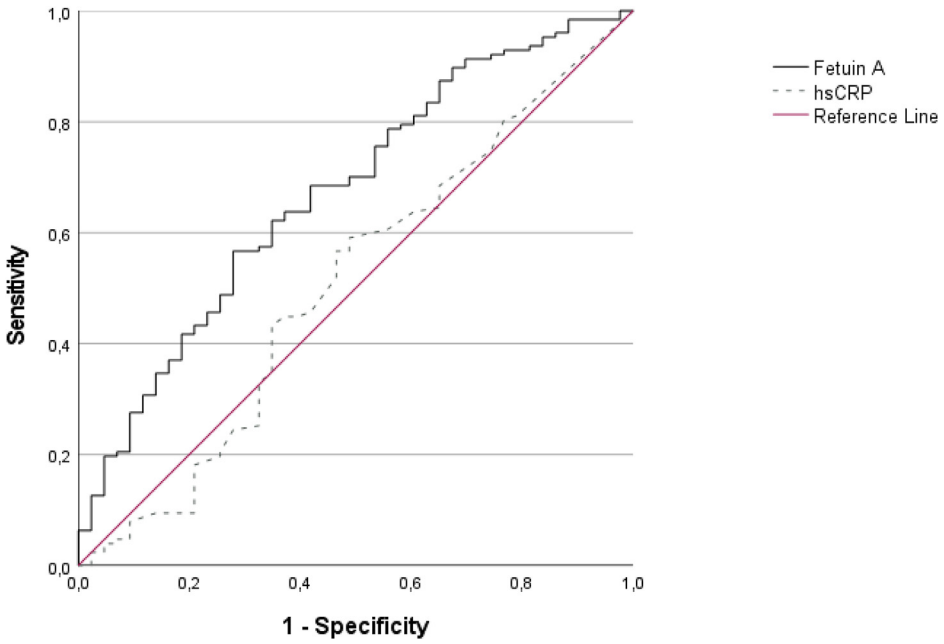

Fig. 3. Receiver operating curve (ROC) for the prediction of plaque progression by fetuin-A and high sensitivity C-reactive protein (hsCRP).

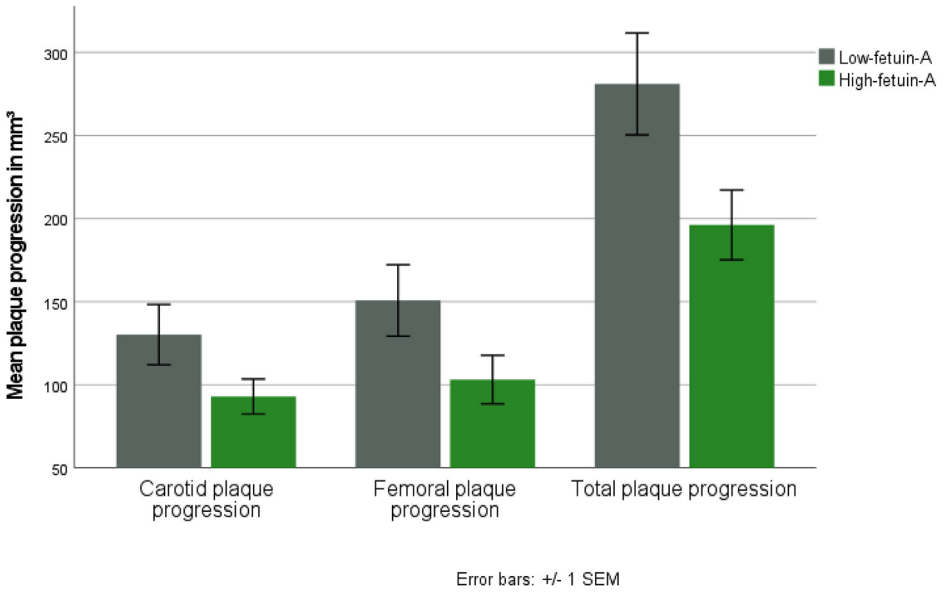

Fig. 4. Bar diagram comparing the difference in total plaque progression for low-fetuin-A (<712μg/ml) and high-Fetuin A (>712μg/ml). Patients with high fetuin-A levels show significantly lower plaque progression ($p = 0.01$).

low fetuin-A levels. To our knowledge this is the first study that demonstrates the association of fetuin-A and peripheral atherosclerosis measured by 3D plaque volumetry. The physiologic and pathophysiologic effects of fetuin-A can be attributed to three major effects: inhibition of ectopic calcification, anti-inflammatory effects and worsening of insulin resistance. Each of these effects plays an important role in the pathogenesis of atherosclerosis and in the risk for cardiovascular events. Of the positive vascular effects of fetuin-A, the inhibition of ectopic calcification is best documented [25,26]. Similar to our study Guarneri et al. showed a negative correlation between fetuin-A levels and carotid IMT in hypertensive patients [17,27].

The Cardiovascular Health Study (CHS) as well as the Carotid atherosclerosis progression study (CAPS) proved that carotid intima media thickness (CIMT) is a strong surrogate parameter of subclinical atherosclerosis [28]. In the study of Guarneri et al. the correlation was independent of the lipid profile and oxidative stress, suggesting that the relationship of fetuin-A and atherosclerosis in this case was not primarily linked to inflammatory processes [27,29]. The data of our study support these results and show a strong negative correlation between fetuin-A levels and peripheral atherosclerosis measured by 3D ultrasound.

Additionally to its calcification inhibiting effects, fetuin-A has anti-inflammatory effects by inhibiting the production of pro-inflammatory cytocine TNF and TGFβ [25]. Fetuin-A is known to be a negative-acute phase protein and to act as a counterpart of

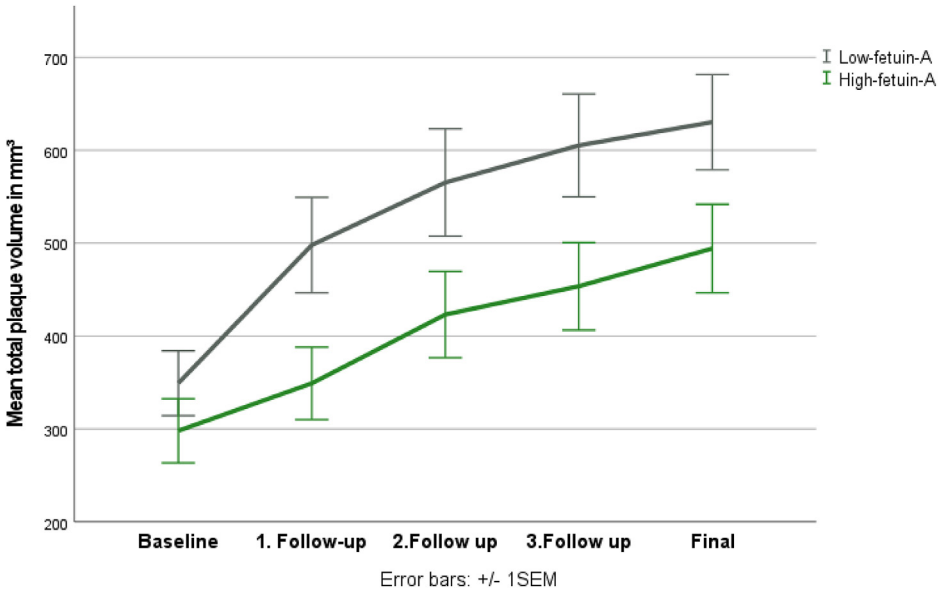

Fig. 5. Mean total plaque volume by study-visit and fetuin-A level. Low fetuin A (<712.43μg/ml), high-fetuin A (>712.43μg/ml). Follow-up visits were carried out once per year. Error bars show ± 1 standard error of the mean (SEM).

Table 3
Multivariate regression model of total plaque progression.

	b	p
Total Plaque Progression (TPP)		
Age	**0.311**	**0.01**
Arterial hypertension	**0.323**	**0.004**
Antiplatelet therapy	**0.187**	**0.02**
Antihypertensive therapy	**−0.269**	**0.01**
Fetuin A	**−0.189**	**0.01**

Coefficient of determination (model quality): Adjusted $R^2 = 0.271$, $p < 0.001$. Contributions of the parameters are presented via the standardized regression coefficient b.

Table 4
Binary logistic regression. Prediction of high total plaque progression by FRS and fetuin-A.

	OR	95%CI	p
Low-fetuin-A/low-intermediate risk	**3.59**	**1.27–10.**	**0.016**
High-fetuin-A/high-risk	**4.79**	**1.65–13.89**	**0.004**
Low-fetuin-A/high-risk	**7.61**	**2.77–20.87**	**0.01**

Predictive value of different FRS risk categories in terms of plaque progression after combination with fetuin-A group. The Odds ratio for the prediction of high total plaque progression is shown compared to hig3h-fetuin-A/low-intermediate risk group.

hsCRP.

In the Nurses' Health-Study higher fetuin A levels were associated with a lower CAD risk, only when the corresponding hsCRP levels were also high [30]. These antagonistic effects were confirmed by the studies conducted by Lim et al. and Chen et al. [18,26] In our study population however there was no significant difference in serum hsCRP levels between high-TPP and low-TPP groups. Hs-CRP is currently the only inflammatory biomarker that is recommended in current ESC guidelines [2]. In our study, hs-CRP showed no significant correlation with baseline plaque volume. This has also been shown in a previous publication [22].

In contrast to the mechanisms discussed so far, fetuin-A exerts also pro atherogenic effects by increasing insulin resistance [25,26]. Due to these opposing pathways, the data on fetuin-A are controversial and sometimes ambiguous. The influence of fetuin-A seems to differ depending on the studied population [27]. For patients with end stage renal disease, dialysis patients and the general population, lower fetuin-A levels are associated with an increased cardiovascular risk [15,26,31,32]. In diabetic patients however, high fetuin-A levels lead towards an exacerbation of insulin resistance and thus to higher cardiovascular mortality [33]. Likewise patients with non-alcoholic fatty liver disease show higher mortality with higher fetuin-A levels [34,35]. In a meta analysis from 2019, Xie et al. conclude that high fetuin-A levels are associated with lower all-cause mortality in patients with established CAD [36].The participants of our study represent mainly patients with low-to moderate cardiovascular risk and a relatively small proportion of diabetics (14%). Hence, our study results are consistent with the predicted effects. In our data there was no significant difference in plaque progression between diabetic and non diabetic subjects. However, the possibilities for subgroup analysis of diabetic patients were limited due to the low number of diabetic patients in the study population. This is the first study presenting the association of fetuin-A and subclinical atherosclerosis measured by 3D ultrasound. With our data, we want to contribute to a deeper understanding of the role of fetuin-A in the development of atherosclerosis.

The strength of our study is the prospective design with yearly examinations documenting the progression of plaque volume by a reliable method (3D ultrasound). The major limitation is the small sample size, which makes further subgroup analysis e.g. in diabetic patients impossible. Although the sample size is similar to the sample size of comparable trials, research with more patients is necessary. Furthermore, the trial was only conducted at one center.

Conclusion

In conclusion, we can say that fetuin-A appears to be a useful biomarker in the risk evaluation of patients with CVRF or established CVD with the potential to further improve risk stratification by the FRS. Additional work will be necessary to confirm these data in a larger cohort.

Ethics approval and consent to participate

The study protocol has been accepted by the Ethics Committee of the Medical University of Innsbruck and complies with the Declaration of Helsinki. A written informed consent was provided from all participants before inclusion into the study.

Availability of data and materials

All data generated or analysed during this study are included in this published article-within the manuscript and its supplementary information files.

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Declaration of competing interest

We do not have to report any conflicts of interest.

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