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Circulating GDF-15 levels predict future secondary manifestations of cardiovascular disease explicitly in women but not men with atherosclerosis

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ABSTRACT

Background: Elevated serum levels of growth differentiation factor-15 (GDF-15), is an established risk factor for a range of cardiovascular diseases.
We aimed to evaluate the predictive value of plasma GDF-15 as a biomarker for secondary cardiovascular events (CVE) in patients with atherosclerosis undergoing carotid endarterectomy (CEA). Secondly, we determined whether plasma GDF-15 was associated with carotid plaque characteristics.
Methods: Circulating GDF-15 levels were determined by Luminex assay in a cohort of 1056 patients from the Athero-Express biobank. Composite endpoint was defined as major CVE, death and peripheral vascular interventions. Findings were validated in 473 patients from the independent Carotid Plaque Imaging Project biobank.
Results: GDF-15 levels did not associate with secondary CVE in the total cohort. However, following a significant interaction with sex, it was found to be strongly, independently predictive of secondary CVE in women but not men (quartile 4 vs. quartile 1: HR 3.04 [95% CI 1.35–6.86], $p = 0.007$ in women vs. HR 0.96 [95% CI 0.66–1.40], $p = 0.845$ in men). This was also observed in the validation cohort (women: HR 2.28 [95% CI 1.04–5.05], $p = 0.041$), albeit dependent upon renal function. In addition, GDF-15 was associated with the presence of plaque smooth muscle cells and calcification.
Conclusion: High circulating GDF-15 levels are predictive of secondary CVE in women but not in men with carotid atherosclerotic disease undergoing CEA, suggesting a potential use for GDF-15 as a biomarker for secondary prevention in women. Sex differences in the role of GDF-15 in atherosclerotic disease deserve further interest.

1. Introduction

Cardiovascular disease (CVD) remains one of the leading causes of morbidity and mortality in both men and women worldwide. Atherosclerosis is a complex chronic inflammatory process underlying cardiovascular diseases such as stroke. Patients with carotid atherosclerosis are at high risk of developing future cardiovascular atherosclerotic events (CVE). Atherosclerotic plaque composition in men undergoing carotid endarterectomy (CEA) has previously been found to be independently predictive of secondary CVE in all vascular territories [1,2].

Growth differentiation factor-15 (GDF-15), a member of the transforming growth factor (TGF-β) cytokine family, is normally weakly expressed in most parenchymal tissues [3]. During acute phase responses, stimulated by pro-inflammatory cytokines interleukin-1 (IL-1), tumour necrosis factor alpha (TNFα), and TGF-β, GDF-15 becomes highly expressed by macrophages.

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GDF-15 has been located in human carotid atherosclerotic plaques, co-localized with macrophages [4]. It has been found to be both detrimental and protective in experimental atherosclerotic mouse models: deficiency of GDF-15 attenuated early atherosclerotic lesion formation and improved the stability of plaques due to impaired macrophage migration and increased induction of collagen deposition [5,6]. Overexpression of GDF-15 on the other hand, has also shown to be protective in the atherosclerotic process, with GDF-15 reducing atherosclerotic lesion size [7].

Elevated GDF-15 levels have been established as a predictive factor for several cardiovascular diseases including in patients with known CVD presenting with acute coronary syndrome [8] and chronic heart failure [9] as well as for all-cause and cardiovascular-mortality in healthy populations free from CVD [10].

Given the increasing number of individuals who are requiring regular treatment to prevent further CVE, the identification of patients at the highest risk is important. Therefore our primary objective was to investigate circulating GDF-15 as a marker of prognosis of secondary CVE in men and women with atherosclerosis undergoing CEA. Given the previously reported behaviour of GDF-15 in atherosclerotic plaques, and the prognostic value of atherosclerotic plaque characteristics, our secondary objective was to assess the association between GDF-15 and plaque components. Finally, due to previously observed sex differences in this cohort, we tested for sex interactions of GDF-15 with secondary outcome and sex interactions in the associations between GDF-15 levels and plaque characteristics.

2. Methods

2.1. Study population

The study included patients from the Athero-Express (AE) biobank, a longitudinal study of patients undergoing CEA, as described in detail previously [11]. In short, this biobank includes all patients undergoing CEA at two Dutch Hospitals: the University Medical Centre, Utrecht and St. Antonius Hospital, Nieuwegein. Patients unable to provide consent for any reason were excluded. Indications for a CEA were reviewed by a multidisciplinary vascular team and were based on recommended criteria of the Asymptomatic Carotid Atherosclerotic Study, the North American Symptomatic Carotid Endarterectomy Trial (NASCET), and the European Carotid Surgery Trial (ECST) [11]. Patients completed a questionnaire at baseline regarding medication use, cardiovascular risk factors and medical history. The institutional review boards of the two participating hospitals approved the study.

2.2. Tissue collection and histological examination

As per a standardized protocol, atherosclerotic plaques, collected during CEA, were immediately processed and divided into 5 mm segments along the longitudinal axis. The culprit lesion, identified as the segment with the largest plaque burden, was fixed in formalin (4%), embedded in paraffin and then histologically examined. Plaque characteristics were scored previously by two independent observers blinded to clinical outcome with good intraobserver and interobserver reproducibility [11].

2.3. Measurement of biomarkers

Blood was drawn from patients immediately prior to surgery from the radial arterial sheath. Presenting symptoms formed part of the inclusion criteria for indication for surgery ranging from asymptomatic patients to patients presenting with a stroke. A custom-built Luminex Screening assay (R&D Systems) was used in combination with the "Bio-Plex Multiplex system (Bio-Rad)" to perform the analysis of plasma GDF-15. Patients with GDF-15 levels that were above detection limit of assay ($n = 1$), or analyzed but were outside the range of the calibration curve ($n = 1$) due to possible technical error, were excluded from the current study.

2.4. Follow-up and clinical outcome

Patients were followed up from inclusion date for three years using annual questionnaires. In addition, the electronic hospital medical files were reviewed regarding CVE. In the case of non-responses, or if a response suggested a CVE, the general practitioner or specialist was contacted for further information. Cardiovascular outcome was defined as a composite end-point of vascular death (due to myocardial infarction, stroke, ruptured abdominal aortic aneurysm, heart failure, as well as sudden death of unspecified cause), non-fatal MI, non-fatal stroke in addition to secondary vascular interventions. The latter included both coronary and peripheral interventions and amputations that had not already been planned at the time of primary carotid endarterectomy.

2.5. Validation cohort

Four hundred and seventy-three patients who underwent CEA (due to stroke, amaurosis fugax, transient ischemic attack related to carotid stenosis >70% or asymptomatic with stenosis >80%) between 2005 and 2012 were included from the Carotid Plaque Imaging Project (CPIP) biobank cohort. The Swedish Cause of Death and National inpatient Health Registers were used to identify post-operative CVE occurring up to seven years after surgery. GDF-15 in plasma was measured by PEA Proseek Multiplex CVD^{96x96} reagents kit (Olink Bioscience, Uppsala, Sweden). A more extensive description of the cohort can be found in the supplementary methods.

2.6. Statistical analysis

Cardiovascular risk factors were compared across quartiles of GDF-15 using the χ^2 test for categorical variables due to a skewed distribution of levels. One-way analysis of covariance (ANOVA) (parametric) and the Kruskall-Wallis (non-parametric) test were used for continuous variables where appropriate.

Given the number of missing values, cardiovascular risk factor variables were imputed using the single imputation method with the "MICE" package in R studio for the subsequent analyses [12]. These variables were: high density lipoprotein (HDL) n missing 319 (30.2%), low density lipoprotein (LDL) n missing 325 (30.8), triglycerides n missing 321 (30.4%), total cholesterol n missing 306 (29.0%), body mass index (BMI) n missing 40 (3.7%), hypertension (HTN) n missing 22 (2.1%), peripheral intervention n missing 2 (0.2%), history of coronary artery disease (CAD) n missing 1 (0.1%), antiplatelet therapy n missing 4 (0.4%), statin therapy n missing 2 (0.2%), presenting symptoms n missing 8 (0.8%), estimated glomerular filtration rate (eGFR) n missing 89 (8.4%), and contralateral stenosis n missing 118 (11.2%). Univariable ordinal regression analysis was performed to determine inflammatory markers (IL-6, TGF-β, TNF-α, IL-1, VEGFA, IL-10 and high sensitivity c-reactive protein (hsCRP)) associated with GDF-15 quartiles. For sex-specific associations, GDF-15 was split into sex-specific quartiles in order to be analyzed as a categorical variable.

Regression modelling was also performed in order to analyze the relationships between GDF-15 and the plaque characteristics: fat content, collagen (no/minor vs. moderate/heavy), percentages of macrophages, smooth muscle cells (SMC) (no/minor vs. moderate/heavy), calcium (no/minor vs. moderate/heavy), presence of plaque hemorrhage (PH) (no vs. yes) and microvessel density.

To examine the risk of future secondary CVE in relation to plasma GDF-15 levels, multivariable cox proportional hazard models were used adjusting for covariates, selected in the following way: univariable cox proportional hazard models assessing outcome and plasma GDF-15 along with each baseline cardiovascular risk factor were analyzed. Variables with a *p*-value of <0.05 in the models were selected as covariates

for the final multivariable model. These were: age, gender, HDL, triglycerides, CAD, history of peripheral intervention, presenting symptoms and contralateral stenosis. eGFR was also forced in the final model due to previously observed literature regarding their associations with circulating GDF-15 [13]. A second full, multivariable model was analyzed with the simultaneous addition of hsCRP and N-terminal pro b-type natriuretic peptide (NTproBNP) as additional covariates. A multiplicative interaction term between sex and GDF-15 was also included in the full model along with the aforementioned covariates. As this showed a significant sex interaction ($p < 0.10$), analyses were performed in a sex-stratified manner with the same covariates as above excluding gender. As there were differences in risk factors between men and women at baseline, these were added to the sex-stratified models as additional covariates in separate analyses.

The incremental prognostic value of GDF-15 was assessed by comparing the areas under the curve (AUCs) of receiver operating characteristics (ROC) curves with and without GDF-15. In addition, the integrated discrimination improvement index (IDI) was calculated using the "survIDINRI" package for R, to assess the improvement of risk prediction [14–16].

The level of significance for all analyses was set at α <0.05. R software for statistical computing, version 3.2 [17] was used for all analyses.

3. Results

3.1. Baseline characteristics

The study included 1056 patients with a mean age of 68.7 (standard deviation (sd) 9.3) years. The median GDF-15 level was 1251.68 ng/l (Interquartile range (IQR) 829.15, 2028.69). Increasing GDF-15 quartiles were directly associated with age ($p < 0.001$), history of diabetes ($p < 0.001$), history of CAD ($p < 0.001$), hsCRP levels ($p < 0.001$) and

high NTproBNP levels ($p < 0.001$) (Table 1). Increasing quartiles of GDF-15 inversely associated with eGFR ($p < 0.001$).

The study consisted of 724 men and 332 women. Women were more likely to have a history of HTN ($p = 0.007$) but there was no difference in antihypertensive use between men and women ($p = 0.511$). Women were also more likely to be current smokers ($p = 0.043$), however men were more likely to have a history of CAD ($p = 0.004$) (Table 2). Women also had a lower eGFR ($p = 0.007$). When stratified into sex-specific quartiles (Supplementary Table 1a and b), increasing GDF-15 quartiles directly associated with age, a history of diabetes, history of CAD, increasing hsCRP levels, increasing NTproBNP levels and inversely correlated with eGFR in both men and women (all *p*-values <0.001).

3.2. Associations of inflammatory markers with GDF-15 in the derivation cohort

HsCRP was significantly associated with increasing levels of GDF-15, for 1 increase in hsCRP levels, the odds of quartile four vs. the other three quartiles combined is 5.02 (95% CI 2.14–13.67). No significant associations were found between circulating GDF-15 and the circulating inflammatory markers IL-6, TGF-β, TNF-α, or IL-1. No significant associations were found between circulating GDF-15 and the circulating pro-tumorigenic factors VEGFA and IL-10.

3.3. Secondary outcome

The median follow up time was 2.98 years (IQR 2.00–3.08). The total number of events was 273 (205 in men and 68 in women). We did not find an association of plasma GDF-15 with risk of secondary outcome in terms of composite CVE (quartile 4 vs. quartile 1: HR 1.42 [95% CI 0.97–2.07], $p = 0.073$) in a multivariable cox proportional hazard model adjusting for age, gender, HDL, triglycerides, history of CAD, history of peripheral intervention, presenting symptoms, contralateral

Table 1
Baseline characteristics of the derivation cohort stratified by Plasma GDF-15 quartile.

GDF15 quartile	1 ($n = 264$)	2 ($n = 264$)	3 ($n = 264$)	4 ($n = 264$)	*p*-value
Plasma GDF15, pg/ml (median [IQR])	643.82 [531.69, 741.50]	1028.04 [936.88, 1130.18]	1555.05 [1380.71, 1751.47]	3056.06 [2412.53, 5282.26]	<0.001
Risk factors					
Age (mean (sd))	62.84 (8.30)	68.17 (8.76)	71.04 (8.15)	72.85 (8.67)	<0.001
BMI, kg/m² (mean (sd))	26.74 (3.76)	26.07 (3.63)	26.17 (4.08)	26.26 (4.15)	0.225
Current smoker, n (%)	88 (33.6)	92 (35.1)	101 (38.8)	86 (33.0)	0.495
eGFR, CG, ml/min (median [IQR])	87.16 [71.57, 100.47]	74.44 [60.62, 89.69]	64.78 [53.70, 77.65]	57.33 [43.33, 73.71]	<0.001
Lipid parameters, mmol/l					
HDL (median [IQR])	1.10 [0.90, 1.38]	1.14 [0.95, 1.42]	1.10 [0.89, 1.36]	1.04 [0.85, 1.25]	0.007
LDL (median [IQR])	2.81 [2.28, 3.61]	2.80 [2.09, 3.45]	2.37 [1.82, 3.29]	2.50 [1.97, 3.10]	<0.001
Total cholesterol (median [IQR])	4.80 [4.06, 5.60]	4.74 [3.97, 5.59]	4.28 [3.58, 5.24]	4.35 [3.67, 5.01]	<0.001
Triglycerides (median [IQR])	1.43 [1.00, 2.00]	1.42 [1.00, 1.96]	1.30 [1.00, 1.84]	1.30 [1.00, 1.96]	0.451
Medical history					
Diabetes mellitus, n (%)	23 (8.7)	44 (16.7)	64 (24.2)	100 (37.9)	<0.001
HTN, n (%)	187 (71.9)	183 (69.6)	185 (72.8)	191 (74.3)	0.675
CAD, n (%)	55 (20.8)	78 (29.5)	89 (33.8)	100 (37.9)	<0.001
Stroke, n (%)	69 (26.1)	80 (30.3)	89 (33.7)	89 (33.7)	0.187
Peripheral intervention, n (%)	45 (17.0)	54 (20.5)	62 (23.6)	62 (23.6)	0.204
Medications					
Statin therapy, n (%)	205 (77.7)	206 (78.0)	207 (78.7)	193 (73.4)	0.459
Antiplatelet therapy, n (%)	246 (93.2)	240 (90.9)	234 (89.3)	227 (86.6)	0.084
On presentation					
Symptoms, n (%)					0.383
Asymptomatic	36 (13.7)	48 (18.3)	44 (16.8)	34 (13.0)	
TIA	130 (49.6)	108 (41.2)	107 (40.8)	110 (42.0)	
Stroke	57 (21.8)	63 (24.0)	71 (27.1)	75 (28.6)	
Ocular	39 (14.9)	43 (16.4)	40 (15.3)	43 (16.4)	
Stenosis >50%, n (%)	83 (35.0)	104 (43.5)	107 (48.0)	114 (47.7)	0.015
hsCRP, µg/ml (median [IQR])	1.55 [0.69, 3.29]	1.63 [0.74, 3.30]	2.10 [1.03, 4.54]	2.56 [1.15, 7.25]	<0.001
NTproBNP, pmol/l (median [IQR])	40.17 [30.43, 54.31]	44.81 [34.03, 62.88]	49.11 [39.12, 125.56]	78.56 [41.95, 194.24]	<0.001

Normally-distributed continuous variables are presented as means with standard deviation in parenthesis. Non-normally distributed continuous variables are expressed as medians with interquartile range (IQR) in parenthesis. Categorical variables are numbers of total with percentage in parenthesis. The *p*-value indicates the difference across all four groups. BMI: Body mass index, eGFR: estimated glomerular filtration rate, CG: Cockroft-Gault, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, CAD: coronary artery disease, HTN: hypertension, TIA: transient ischemic attack, hsCRP: high sensitive c-reactive protein, NTproBNP: Natriuretic pro B-type protein.

Table 2
Baseline characteristics of the derivation cohort stratified by sex.

	Men (n = 724)	Women (n = 332)	p-value
Age (mean (sd))	68.95 (8.78)	68.25 (10.26)	0.263
BMI, kg/m^2 (mean (sd))	26.18 (3.36)	26.61 (4.92)	0.100
Current smoker, n (%)	238 (33.1)	129 (39.7)	0.044
eGFR, CG, ml/min (median [IQR])	71.83 [57.12, 90.46]	68.19 [54.01, 85.60]	0.007
Lipid parameters, mmol/l			
HDL (median [IQR])	1.04 [0.86, 1.28]	1.23 [1.00, 1.52]	<0.001
LDL (median [IQR])	2.55 [2.00, 3.21]	2.75 [2.19, 3.61]	0.008
Total cholesterol (median [IQR])	4.40 [3.68, 5.21]	4.91 [3.92, 5.74]	<0.001
Triglycerides (median [IQR])	1.40 [1.00, 2.00]	1.38 [0.99, 1.90]	0.339
Medical history			
Diabetes mellitus, n (%)	162 (22.4)	69 (20.8)	0.616
HTN, n (%)	492 (69.5)	254 (77.9)	0.006
CAD, n (%)	241 (33.3)	81 (24.5)	0.005
Stroke, n (%)	229 (31.6)	98 (29.5)	0.537
Peripheral intervention, n (%)	144 (19.9)	79 (23.9)	0.169
Medication			
Statin therapy, n (%)	551 (76.1)	260 (78.8)	0.379
Antiplatelet therapy, n (%)	647 (89.6)	300 (90.9)	0.589
Antihypertensive therapy, n (%)	565 (77.5)	249 (75.5)	0.511
On presentation			
Symptoms, n (%)			0.955
Asymptomatic	110 (15.3)	52 (15.9)	
TIA	316 (43.9)	139 (42.4)	
Stroke	183 (25.4)	83 (25.3)	
Occular	111 (15.4)	54 (16.5)	
Stenosis >50%, n (%)	293 (45.1)	115 (39.9)	0.163
Biomarkers			
GDF-15 pg/ml (median [IQR])	1279.30 [871.67, 2056.59]	1209.51 [790.11, 1887.03]	0.035
hsCRP, μg/ml (median [IQR])	1.67 [0.81, 3.96]	2.21 [1.04, 4.69]	0.005
NTproBNP, pmol/ml (median [IQR])	48.03 [36.176100.50]	47.66 [34.63, 93.88]	0.600
Plaque characteristics			
Fat (>40%), n (%)	209 (32.0)	37 (12.7)	<0.001
Collagen (moderate/heavy), n (%)	507 (77.6)	225 (77.1)	0.908
Macrophages (moderate/heavy), n (%)	353 (54.3)	125 (43.3)	0.002
SMC (moderate/heavy), n (%)	420 (64.5)	218 (74.4)	0.003
Calcification (moderate/heavy), n (%)	289 (44.4)	137 (46.9)	0.516
PH (present), n (%)	409 (62.8)	144 (49.1)	<0.001
Microvessel density, n (%)	6.70 [3.67,11.00]	6.69 [3.30, 11.69]	0.676

Normally-distributed continuous variables are presented as means with standard deviation in parenthesis. Non-normally distributed continuous variables are expressed as medians with interquartile range (IQR) in parenthesis. Categorical variables are numbers of total with percentage in parenthesis. BMI: Body mass index, eGFR: estimated glomerular filtration rate, CG: Cockroft-Gault, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, CAD: coronary artery disease, HTN: hypertension, TIA: transient ischemic attack, hsCRP: high sensitive c-reactive protein, NTproBNP: Natriuretic pro B-type protein, SMC: smooth muscle cells, PH: plaque hemorrhage.

stenosis and eGFR. This was also the case in a full model correcting additionally for NTproBNP and hsCRP (quartile 4 vs. quartile 1: HR 1.40 [95% CI 0.96–2.05], p = 0.081) (Fig. 1a). However when stratified by sex (p-value for sex interaction in quartile 4 vs. quartile 1 0.075), plasma GDF-15 was found to be independently associated with secondary CVE in women but not in men (quartile 4 vs. quartile 1: HR 3.30 [95% CI 1.47–7.40], p = 0.005 in women vs. HR 1.14 [95% CI 0.73–1.80], p = 0.558 in men). This association in women remained significant in the full model following the addition of plasma hsCRP and plasma NTproBNP (quartile 4 vs. quartile 1: HR 3.04 [95% CI 1.35–6.86], p = 0.007) (Fig. 1b and c). Following the addition of risk factors to the sex-specific models that differed at baseline between men and women (smoking status, HTN, LDL and total cholesterol), the significant association in women remained intact (quartile 4 vs. quartile 1: HR 3.18 [95% CI 1.40–7.24], p = 0.006) and there was again no significant association in men (quartile 1 vs. quartile 4: HR 1.14 [95% CI 0.72–1.80], p = 0.568).

3.4. Associations of atherosclerotic plaque characteristics with plasma GDF-15

Univariably, plasma GDF-15 was significantly associated with moderate/heavy SMC (OR 1.27 [95% CI 1.07–1.56], p = 0.014, per sd increase in plasma GDF-15). Plasma GDF-15 was also associated with the presence of moderate/heavy calcification in the plaque (OR 1.19 [95% CI 1.05–1.37], p = 0.009 per sd increase in plasma GDF-15). These associations remained significant when adjusting for the same covariates used

previously; SMC: OR 1.32, [95% CI 1.10–1.65], p = 0.007), calcification: OR 1.19 [95% CI 1.049–1.38], p = 0.100. There were no significant sex interactions for neither plaque characteristic therefore analyses were not sex-stratified. When SMC (quartile 4 vs. quartile 1: HR 1.53 [95% CI 1.02–2.29], p = 0.038) and calcification (quartile 4 vs. quartile 1: HR 1.56 [95% CI 1.04–2.33], p = 0.032) were added to the cox regression models for the total cohort, the association between GDF-15 levels and secondary outcome became significant. In women the association attenuated with the addition of both SMC (quartile 4 vs. quartile 1: HR 2.50 [95% CI 1.06–5.87], p = 0.036) and calcification (quartile 4 vs. quartile 1. HR 2.62 [95% CI 1.14–6.06], p = 0.024) but remained significant. In men the associations between GDF-15 and outcome remained the same with the addition of SMC and calcification into the models.

3.5. Incremental predictive utility of GDF-15

As our results suggest that GDF-15 predicts secondary CVE in women in our cohort but not in men, we tested to see if there was an additional value of GDF-15 as a predictor of composite events on top of the traditional biomarkers hsCRP and NTproBNP. Improvements in the AUC were seen in women, upon the addition of plasma GDF-15 to a clinical model including hsCRP and NTproBNP, although this was not significant (Fig. 2). No significant improvement was seen in men (Fig. 2). However, a more sensitive measure for prognostic value, the IDI, was significant in women with the addition of GDF-15 to the clinical model including

a) Total cohort

b) Men
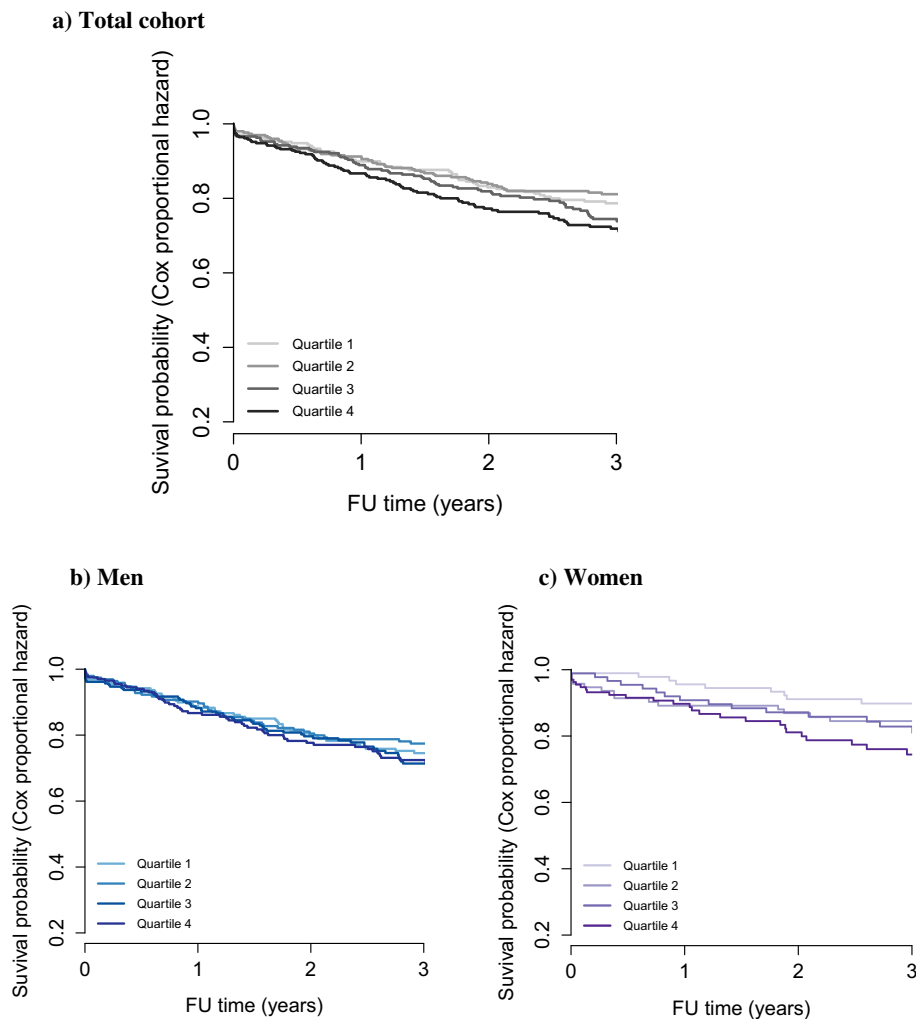
c) Women

Fig. 1. Multivariable Cox regression plots showing the predictive value of GDF-15 and composite events by quartiles in the total cohort (a), men (b), and women (c).

hsCRP and NTproBNP (IDI 0.04, [95% CI 0.01–0.10], $p = 0.007$). This was not the case in men (IDI 0.001, [95% CI 0.00–0.01], $p = 0.199$).

3.6. Validation

Our findings were validated in the CPIP biobank consisting of 311 men and 162 women undergoing endarterectomy between the years 2005–2012 at Skåne University Hospital, Sweden. The baseline characteristics of the validation cohort were largely similar to the AE discovery cohort (Supplementary Table 2). Also in line with the findings from the AE cohort, age, eGFR and presence of diabetes showed the strongest associations to quartiles of GDF-15 (Supplementary Table 3). Kaplan-Meier survival analysis showed a significant association of GDF-15 in quartiles to composite CVE (myocardial infarction, stroke, transient ischemic attack, amaurosis fugax or CV death) in women (Log rank p-value = 0.033), but not in men (Supplementary Fig. 1). In Cox regression models the highest GDF-15 quartile was significantly associated with composite CVE compared to all other quartiles during follow up, independently of age and diabetes (HR 2.28 [95% CI 1.04–5.05], $p = 0.041$) (Table 3). This association was lost with the addition of eGFR (Table 3).

4. Discussion

Patients with carotid atherosclerosis are at risk of developing future CVE. GDF-15 has previously been found to be associated with risk of secondary CVE and mortality in patients with known heart failure and acute coronary syndromes. We now show that high circulating levels of GDF-15 are independently predictive of secondary composite CVE in women but not in men with atherosclerosis. This was also the case in the validation cohort, however the predictive ability of plasma GDF-15 appears to be dependent upon renal function in this cohort. It is known that eGFR is associated with plasma GDF-15 with raised levels seen in patients with chronic kidney disease [13,18]. Renal function is a major prognostic determinant for both cardiovascular and non-cardiovascular death in men and women [19]. It is not known however, if GDF-15 is causally related to cardiovascular and renal disease or whether it is a marker of disease state in general. Baseline GDF-15 levels were not significantly different between men and women in the validation cohort as in the derivation cohort with higher levels seen in men despite men having a better renal function than women. eGFR levels were similar in men and women between both the two cohorts.

In addition to sex differences in renal function in the derivation cohort, men and women also showed differences in other baseline clinical characteristics such as differences in lipid profile and history of CAD. It is important to note that these factors are also prognostic determinants; therefore the differences in predictive value of GDF-15 between men and women may be explained by these differences in risk profiles. However as we corrected for these factors in our analyses we can thus state the prognostic value of GDF-15 in women is independent of these risk factors.
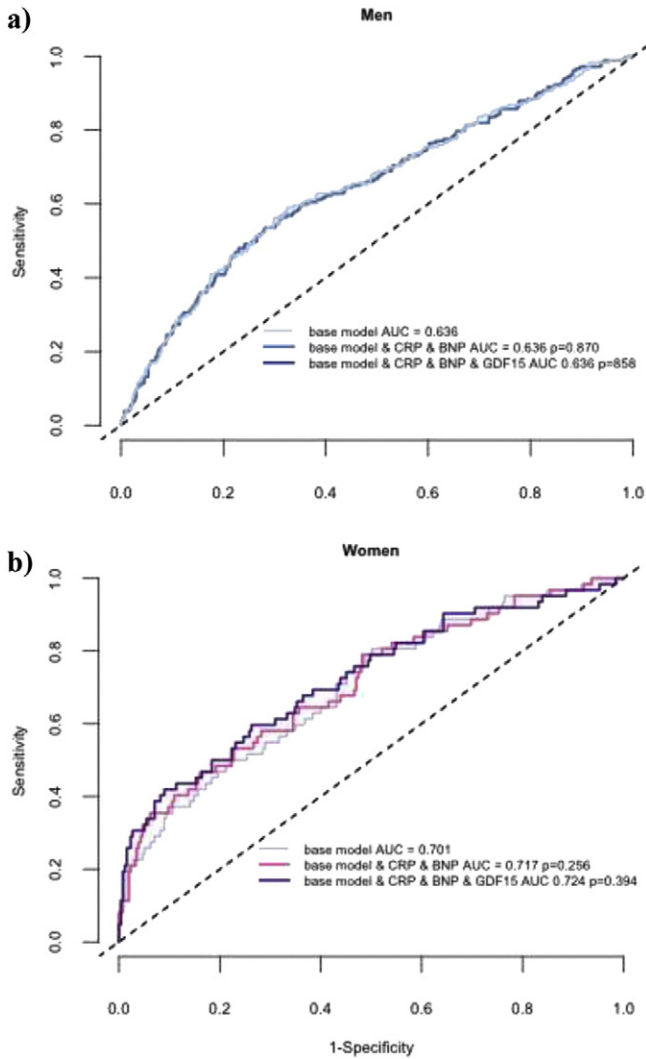
Fig. 2. Comparison of areas under the receiver operating characteristics (ROC) curves for prediction of composite outcome for GDF-15 in combination with hsCRP NTproBNP and in men (a) and women (b).

Evidence is accumulating that the underlying complex chronic disease process of atherosclerosis significantly differs between men and women. This is evident from variations found in the composition of the atherosclerotic carotid plaques obtained from men and women undergoing CEA [2,20]. In our study we found that the presence of SMC and calcification in the carotid plaque are associated with levels of GDF-15 in the total derivation cohort. SMC play an important role in the progression of atherosclerosis, forming extracellular matrix resulting in fibrous caps. Inflammatory cells and macrophages are involved in the apoptosis of SMC, explaining why on rupture of the fibrous cap during an acute event such as a stroke, macrophages are in

abundance and there are only a few SMC [21]. We show that women have a higher number of SMC than men, which is indicative of a more stable plaque, with women being shown previously in this cohort to have a more stable plaque phenotype than men [20]. However, the prognostic value of plasma GDF-15 in women is independent to the presence of SMC. Calcification is believed to enhance the migration of SMC and also plays a role in the proliferation of SMC during the process of atherosclerosis [22]. We show that plasma GDF-15 is also predictive of secondary outcome in the total cohort independently to the presence of SMC and calcification. Therefore, the predictive value of plasma GDF-15 in this cohort of carotid atherosclerotic patients cannot be explained by carotid plaque characteristics. In addition to plaque characteristic, atherosclerotic plaque morphology also differs between men and women with men more likely to have a ruptured plaque as the substrate for thrombotic events whereas women are more likely to suffer from plaque erosions as the substrate for events [23]. The mechanisms underlying plaque erosions point to endothelial dysfunction [24]. GDF-15 has been found to negatively impact endothelial function [25] and not only has smoking been recently found to induce GDF-15 [26], but it also has direct effects upon the endothelium itself and is also associated with plaque erosion [23]. As women in our cohort were more likely to be smokers, the explanation as to why we only show an association between GDF-15 and CVE in women may involve the mechanism of endothelial dysfunction, i.e. microvascular inflammation which is usually more likely to be observed in women than in men.

In the normal physiological state, GDF-15 is only weakly expressed and is not expressed in the adult myocardium. Stimulated by pro-inflammatory cytokines IL-1, TNFα, and TGF-β, GDF-15 becomes highly expressed during acute phase responses. A study by de Jager et al. found that GDF-15 expression in human plaques was higher in unstable versus advanced stable lesions. This study also found that leukocyte GDF-15 deficiency profoundly inhibited early lesion formation and resulted in increased atherosclerotic plaque stability due to impaired macrophage migration and increased induction of collagen deposition [5]. Macrophages are major contributors in the process of atherosclerosis and play a similar role in other chronic inflammatory diseases such as rheumatoid arthritis. GDF-15 has been postulated to be a by-product of macrophage activation and has also been shown to play a role in rheumatoid arthritis. These autoimmune diseases are more prevalent in women than in men [27] and result in increased vascular reactivity. This culminates in microvascular spasm and microvascular dysfunction, conditions which are also more commonly seen in women with non-obstructive CAD. The reasoning behind the stark difference in prevalence of autoimmune diseases between men and women remains unclear but does highlight the likely differences in the underlying mechanisms of atherosclerosis between men and women. We showed a significant association between increasing levels of circulating GDF-15 and circulating hsCRP but we were unable to find any correlation between circulating GDF-15 and other markers of inflammation such as IL-6, TGF, TNF, or IL-1. Previously GDF-15 has been found to positively correlate with CRP in patients with acute coronary syndrome [28], in patients undergoing cardiopulmonary bypass grafting [29], and in a population free from overt CVD [13]. This could indicate that GDF-15 is upregulated

Table 3
Cox proportional hazards regression models of secondary cardiovascular events in men and women with high and low GDF-15 levels in plasma in the validation CPIP Cohort.

GDF-15 quartile	Men			Women		
	1 + 2 + 3 (n = 233)	4 (n = 78)	p-Value	1 + 2 + 3 (n = 122)	4 (n = 40)	p-value
Plasma GDF-15 (a.u.)	<1630	>1630		<1640	>1640	
Composite CVD events, n (%)	47 (20%)	18 (23%)	0.585	20 (16%)	13 (33%)	0.028
HR (95% CI), unadjusted	1	1.37 (0.80–2.37)	0.254	1	2.58 (1.28–5.21)	0.008
HR (95% CI), adjusted for age	1	1.19 (0.66–2.17)	0.563	1	2.61 (1.25–5.42)	0.010
HR (95% CI), adjusted for age and diabetes	1	1.23 (0.70–2.18)	0.697	1	2.28 (1.04–5.05)	0.041
HR (95% CI), adjusted for age, diabetes and eGFR	1	1.04 (0.55–1.95)	0.904	1	2.01 (0.89–4.56)	0.095

eGFR: estimated glomerular filtration rate, n.s: non-significant.

in chronic CVD specific diseases compared to traditional inflammatory biomarkers.

4.1. Limitations

One limitation is that the patients included in this study may undergo a change in their cardiovascular risk factors during the follow-up time. This information is not recorded and therefore these variables could not be used as possible covariates in our multivariable analyses.

5. Conclusion

The explanation behind sex differences in the pathophysiology of atherosclerosis and in the mechanism of GDF-15 within atherosclerosis remains unsolved but clearly merits further investigation. This is of utmost importance as there is future potential for serum GDF-15 to be implemented into clinical practice to be used as a biomarker for prediction of secondary events in women with atherosclerosis.

High circulating GDF-15 predicts the risk of secondary cardiovascular outcome in women with severe carotid atherosclerosis undergoing CEA but not men.

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Conflicts of interest

There are no conflicts of interest to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ijcard.2017.03.101.

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