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Association of circulating hepatocyte growth factor and risk of incident peripheral artery disease: The Multi-Ethnic Study of Atherosclerosis

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Abstract

Higher levels of hepatocyte growth factor (HGF) have been associated with the presence of peripheral arterial disease (PAD) but prospective associations are unknown. We examined the association of circulating HGF levels with incident PAD. Between 2000 and 2002, HGF was measured in 6742 Multi-Ethnic Study of Atherosclerosis (MESA) participants without PAD. Incident clinical PAD, adjudicated on the basis of a positive history for the presence of disease-related symptoms or treatment, was ascertained through 2015. Incident low ankle-brachial index (ABI), defined as an ABI <0.9 and a decline of ≥0.15, was assessed among 5736 individuals who had an ABI >0.9 at baseline and ≥1 follow-up ABI measurement 3-10 years later. There were 116 clinical PAD and 197 low ABI events that occurred over a median follow-up of 14 years and 9 years, respectively. After adjustment for demographic and clinical variables, a standard deviation increment of HGF (303 ng/l) was associated with an increased risk of clinical PAD (hazard ratio: 1.21; 95% confidence interval (CI) 1.05, 1.39) but not a low ABI (rate ratio: 1.03; 95% CI 0.85, 1.25). In conclusion, higher HGF levels were modestly associated with an increased risk of developing clinical PAD.

Keywords

peripheral arterial disease; ankle-brachial index; inflammation; risk factors

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Introduction

Lower extremity peripheral artery disease (PAD) is a significant public health problem, affecting at least 8.5 million over the age of 40 in the United States and 200 million individuals globally.[1, 2] Over 20% of men and women seen in primary care medical practices aged 70 years or older or aged 50 through 69 years with history of cigarette smoking or diabetes have a low ankle-brachial index (ABI).[3] PAD is associated with a significant increase in morbidity and mortality.[4] Continued investigation into identifying additional risk factors is important to improve our understanding of the mechanisms that cause PAD.

Inflammation is considered to be involved in the development of PAD.[5, 6] In this regard, inflammatory markers involved in the endothelial cellular adhesion pathway have been associated with an increased risk of PAD and represent a potential mechanism for how inflammation may contribute to PAD.[7–11] However, studies determining the relationship of these markers with incident PAD are limited and have focused only on soluble intracellular adhesion molecule and vascular adhesion molecule in predominantly White populations.[7, 9, 12]

Hepatocyte growth factor (HGF) is a cellular adhesion protein with pleiotropic properties including anti-fibrotic, anti-inflammatory and proangiogenic actions.[13] Despite its presumptive protective properties, high levels of circulating HGF have been associated with higher rates of cardiovascular disease, including stroke, coronary heart disease (CHD) and heart failure.[14–18] Higher levels of HGF have been cross-sectionally associated with the presence of PAD, but the association of HGF with incident disease has not been reported.[11] Therefore, the purpose of this study was to examine the association of circulating HGF levels with incident PAD in a well-characterized multi-ethnic cohort.

Patients and Methods

Cohort

The Multi-Ethnic Study of Atherosclerosis (MESA) is a National Heart, Lung, and Blood Institute funded multicenter longitudinal community-based study. The study recruited 6814 adults aged 45 to 84 years and free of clinically recognized cardiovascular disease from 6 field centers (Baltimore, Maryland; Chicago, Illinois; Forsyth County, North Carolina; Los Angeles, California; New York, New York; and St Paul, Minnesota) to undergo baseline examination between 2000 and 2002.[19] The study participants self-identified with 1 of 4 race/ethnic groups: non-Hispanic white (38%), African (28%), Hispanic (22%), and Chinese (12%) American. Follow-up visits 2, 3, 4, 5, and 6 were carried out in 2002– 2004, 2004–2005, 2005– 2007, 2010– 2012, and 2016–2018, respectively. Institutional review boards at each site approved the study, and all participants gave informed consent.

Measurement of HGF

At the baseline examination, venous blood was obtained from fasting participants and serum separation was performed within 30 min of phlebotomy with aliquots subsequently stored at −70 °C. Levels of serum HGF protein were measured with a quantitative sandwich enzyme-linked immunosorbent assay (ELISA) using the Human HGF Immunoassay kit (R&D

Systems, Minneapolis, MN) on 6769 individuals and on a sub-sample of 2403 individuals at Exam 2 as part of the Multi-scale Biology of Atherosclerosis in the Cellular Adhesion Pathway (HL98077, MESA Adhesion Ancillary Study). The lower limit of detection was 40 ng/l and the inter-assay laboratory coefficient of variations were 12.0%, 8.0%, and 7.4% at respective mean concentrations of 69, 204, and 4080 ng/l for lyophilized manufacturer's controls, and 10.4% at a mean concentration of 688 ng/l for an in-house pooled serum control.

Incident PAD – Clinical PAD and Low ABI

During follow-up, clinical PAD was identified by self-report of a PAD diagnosis by the participant at: (1) MESA clinic visits, (2) follow-up phone call, or, (3) participant notification. A PAD diagnosis was also found during review of medical records for other events. Follow-up for this analysis extended through 2015. Two physician members of the MESA mortality and morbidity review committee independently classified events. The full committee made final classifications if there were disagreements. "Definite" PAD required more than a physician diagnosis as follows.

PAD was defined as symptomatic disease including intermittent claudication, ischemic ulcers, or gangrene. The disease had to be symptomatic and have a diagnostic procedure or require therapeutic intervention. Physician adjudicators recorded the diagnosis ≥1 of: (1) lower extremity claudication, (2) atherosclerosis of arteries of the lower extremities, (3) arterial embolism and/or thrombosis of the lower extremities, or, (4) abdominal aortic aneurysm. In addition to symptoms, participants had to have ≥1 of the following: a) Ultrasonographically- or angiographically-demonstrated obstruction or ulcerated plaque (≥50% of the diameter or ≥75% of the cross-sectional area) demonstrated on ultrasound or angiogram of the iliac arteries or below, b) Absence of pulse by Doppler in any major vessel of the lower extremities, c) Exercise test positive for lower extremity claudication, d) Surgery, angioplasty, or thrombolysis for PAD, e) Amputation of one or more toes or part of the lower extremity because of ischemia or gangrene, f) Exertional leg pain relieved by rest in combination with either physician-diagnosed claudication diagnosed or an ABI≤0.8.

As for subclinical PAD, ABI was performed at the baseline examination, as well as clinic visits 3 and 5. To obtain the measurements used to calculate the ABI, participants rested supine for 5 min, and then systolic blood pressures were measured in both arms and legs with the appropriate-sized cuffs. For each leg, the systolic blood pressure in each posterior tibial and dorsalis pedis artery was measured using a continuous-wave Doppler ultrasound 5 mHz probe. The leg-specific ABI was calculated as the higher systolic blood pressure in the posterior tibial or dorsalis pedis divided by the average of the left and right brachial pressures. In the event that left and right brachial pressures differed by ≥10 mmHg, the higher of the brachial pressures was chosen, since subclavian stenosis could be present. The lower of the 2 leg-specific ABIs was used for analysis.

Individuals with a history of lower extremity revascularization and those with an ABI ≤0.90 at the baseline visit were excluded from the clinical PAD and low ABI analyses, respectively. Participants with evidence of non-compressible vessels (ABI >1.4) at baseline were also excluded from the low ABI analyses only. Incident clinical PAD required a

physician-adjudicated diagnosis of “definite” PAD as defined above. Incident low ABI was defined as a decline in ABI of at least 0.15 and to 0.90 or less in either leg. The approach for ABI decline was used to limit the impact of regression to the mean and measurement error and avoid small clinically insignificant changes being included in the incident low ABI definition.[20] If only 1 follow-up ABI was available, then that was used for the analysis. If both follow-up ABIs were available, then exam 5 was used unless the participant already met criteria for ABI decline at exam 3.

Measurement of covariates

Standardized questionnaires were used at baseline to obtain age, sex, race/ethnicity, level of education, annual household income, physical activity, alcohol consumption, smoking history, and medication usage, including statin, antihypertensive, and antidiabetic use. Physical activity was recorded as participant-reported number of intentional exercise metabolic equivalent (MET)-min/week. Alcohol consumption was categorized as current, former, or never and also by self-reported number of drinks/week. Cigarette smoking was calculated in pack-years and also defined as current, former, or never. Aspirin use was defined as a self-reported use of at least 3 days/week.

Body mass index (BMI) was calculated as weight in kg divided by height in m squared. Three separate systolic and diastolic resting blood pressure measurements were taken in seated participants, with the last 2 measurements being averaged for analysis. Hypertension was defined as a self-report of physician diagnosis and use of an anti-hypertensive medication, or systolic blood pressure ≥140, or diastolic blood pressure ≥90 mmHg.

Total and high-density lipoprotein (HDL) cholesterol, triglycerides, and glucose were measured from fasting blood samples. Low-density lipoprotein (LDL) cholesterol was calculated by the Friedewald equation in those with triglycerides <400 mg/dl. Diabetes was defined as a fasting glucose ≥126 mg/dl or use of anti-diabetic medications. High-sensitivity C-reactive protein (hsCRP) and serum cystatin C were determined with a BNII nephelometer (N Latex Cystatin C & N High Sensitivity CRP; Dade Behring Inc., Deerfield, IL). The estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration equations.[21]

Statistical methods

Descriptive characteristics were computed overall and by quartiles of baseline HGF. Incidence rate of clinical PAD was computed with Poisson regression per 100 person-years of follow-up. Kaplan-Meier plots were drawn to present the survival curves by quartiles of baseline HGF. Log rank tests were used to compare the survival distributions.

Cox hazard models were used to estimate the hazard ratio (HR) of incident clinical PAD associated with HGF levels. For the analysis of low ABI, we used Poisson regression with offset to accommodate differential time to exposure, to estimate the rate ratios (RR) of low ABI associated with HGF levels. Several nested models were used for both analyses: Model 1 adjusting for age, gender, and race/ethnicity; Model 2 further adjusting for diabetes, hypertension, smoking (current/former/never and pack-years), alcohol use, lipid lowering therapy, physical activity, BMI, eGFR, hsCRP, ABI, HDL-cholesterol, and total cholesterol.

Analyses were additionally adjusted for log-transformed coronary artery calcium if any significant associations were observed in Model 2. We used generalized additive models (GAMs) with splines for continuous exposures to address the functional form for both analyses of clinical PAD and low ABI. We found non-linearity in the upper tail of baseline HGF when modeling clinical PAD. We thus performed a subgroup analysis excluding participants in the upper 1% of baseline HGF that is the 68 individuals with baseline HGF higher than 1863 ng/l. In this subset the generalized linear models showed no further departure from linearity. We found no meaningful departures from linearity in the full cohort when modeling low ABI.

The association of change in HGF, between exam 1 and exam 2, with PAD was assessed using Cox proportional hazards and Poisson regression for clinical PAD and low ABI, respectively. HFG exam 2 values were additionally adjusted for and the exam 1 covariates were used to avoid an effect of the HGF change on covariates for these analyses. Participant who developed interim events clinical PAD or low ABI were excluded from the respective analyses. With only 37 events of clinical PAD in this subset we had low power to see an association.

P-values and confidence intervals are not adjusted for multiple testing. Analyses were conducted using R environment for statistical computing.[22]

Results

A total of 6742 participants were included in the incident clinical PAD analysis (mean age=62 years; 53% female; 39% white, 27% African-American 22% Hispanic, and 12% Chinese). Table 1 shows that participants with higher baseline HGF levels were older, less likely to be physically active, and more likely to be female, be Hispanic, have a smoking history, have diabetes, have lower total cholesterol, and report using both anti-hypertensive and lipid lowering medications. These participants also had a higher BMI, higher SBP, higher hsCRP, lower eGFR, and lower HDL-cholesterol.

The Kaplan Meier clinical PAD-free survival curves according to baseline HGF values are shown in Figure 1. The log rank test p values were <0.01. The clinical PAD incidence rates per 100-person years of follow-up were 0.058 (95% confidence interval (CI) 0.020, 0.168), 0.065 (95% CI 0.025, 0.172), 0.141 (95% CI 0.072, 0.278), and 0.318 (95% CI 0.182, 0.557) for quartiles 1 to 4, respectively.

After full adjustment, higher baseline HGF levels were associated with a significantly increased risk of developing clinical PAD (HR per SD increment 1.21, 95% CI 1.05, 1.39) (Table 2 & Figure 2). This association was unchanged after additional adjustment for coronary artery calcium (HR per SD increment 1.21, 95% CI 1.04, 1.41). In the analysis limited to individuals with baseline HGF <1863 ng/l, we observed 114 events among the 6674 participants. After full adjustment, higher baseline HGF levels were similarly associated with an increased risk of developing clinical PAD (HR per SD increment 1.35, 95% CI 1.06, 1.73) (Figure 2).

Higher baseline HGF levels were also associated with an increased risk of incident low ABI, but only in minimally adjusted analyses (RR 1.09, 95% CI 1.00, 1.19) (Table 2). The association was significantly attenuated after adjustment for established risk factors (RR 1.03, 95% CI 0.85, 1.25). Changes in HGF between exam 1 and exam 2 was not associated with risk of either incident clinical PAD or low ABI (Table 3).

Discussion

Higher HGF levels were associated with a 21% higher risk of incident clinical PAD. In contrast, change in HGF was not associated with a higher risk of developing either clinical PAD or a low ABI.

Literature evaluating the relationship between HGF and PAD is based entirely on cross-sectional analyses. In over 100 consecutive patients undergoing coronary angiogram, plasma HGF level was an independent predictor for the presence of aorto-iliac disease.[23] Similarly, plasma HGF levels were significantly higher among individuals with angiographically proven clinical PAD compared with those without evidence of PAD.[24] Finally, in a study of over 2000 MESA participants, each SD increment of HGF was associated with a 20% greater odds of lower ABI category, defined as ≤1.0 after adjustment for traditional risk factors.[11]

While our findings are the first to demonstrate a prospective relationship between HGF and incident clinical PAD, a similar association has been reported between HGF and development of clinical disease in other vascular beds. For example, among post-menopausal women participating in the Women's Health Initiative, those in the highest quartile of baseline HGF values had an almost 40% higher risk of developing a stroke when compared with those in lowest quartile of baseline HGF values.[14] Prior analyses involving this MESA cohort found that each SD increment of HGF was associated with a 17% and 20% increased risk of incident stroke and coronary heart disease, respectively.[25, 26] The magnitude of these relative risks are similar to what was reported here for incident clinical PAD.

We did not find a significant association for HGF levels and risk of incident low ABI, which is in contrast to what has been reported for changes in subclinical disease found in other vascular territories for this cohort. That is, each SD increment of HGF was cross-sectionally associated with an increase in coronary artery calcium and carotid intima-media thickness of 37 Agatston units and 0.04 mm, respectively.[26] Similarly, change in HGF has been shown to be an independent predictor of incident CHD.[27] While the reasons for these discrepancies are uncertain, some important differences were present. Change in ABI was reported as a prospective measure and according to a clinically important threshold value instead of as a continuous change. The power to detect whether changes in HGF were associated with clinical PAD development was limited, 37 clinical PAD events compared with 183 CHD events. Despite this limited power change, HGF was nearly associated with an increased clinical PAD risk (p=0.057), and it is possible that a significant relationship would have been found with more events.

HGF has been proposed as a clinical biomarker for predicting cardiovascular disease. HGF is an endothelium-specific growth factor present in the vascular endothelium produced in response to endothelial damage and higher levels are associated with hypertension, diabetes mellitus, increased age, and BMI.[28–33] Circulating HGF level is also an early surrogate marker of acute arterial thrombus and is elevated in acute myocardial infarction and in occlusive PAD with evidence of collateral development.[34–36] The increase in HGF production can occur at sites remote from the site of disease.[37, 38] Reversal of HGF upregulation of these factors has been reported following revascularization.[35] Rather than being a risk factor for atherosclerotic disease, HGF may actually have a therapeutic role. HGF has been implicated in tissue repair, activation of anti-apoptotic pathways, tissue regeneration, wound healing, and angiogenesis, and was reported to augment collateral vessel development.[39–42] Intramuscular gene transfer of naked plasmid DNA containing the sequence encoding HGF in patients with critical limb ischemia resulted in a significant increase in ABI and improved clinical symptoms, including ischemic ulcer, decrease amputation rate, and mortality out to 2 years.[43]

Our study has limitations. Although the diagnosis of clinical PAD involved a comprehensive adjudication process, the number of overall events was low (<2%). This hindered our ability to detect any differences in associations of HGF and incident PAD stratified by race/ethnicity, as has been previously reported for HGF and incident CHD.[26] Our ability to determine whether changes in HGF impacted PAD risk was hindered because of both limited power as follow-up HGF measurement was only repeated in a subset of participants (only 37 events occurred) and a short duration of time. Coefficient of variation for HGF measurement were not insignificant and some misclassification may have occurred. Incident low ABI may have been inadequately assessed, particularly in diabetics, due to exclusion of participants with an ABI >1.4 at baseline. ABI measurements did not include a post-exercise value and, therefore, may not have detected PAD in some individuals. Lastly, causality cannot be inferred on the basis of this observational study and we cannot exclude the possibility that unmeasured or inadequately measured confounders may account for the observed associations.

In conclusion, higher HGF levels are associated with a higher risk of developing clinical PAD independent of traditional risk factors in a diverse, population-based US cohort of men and women. Further studies are needed before findings can be potentially translated into something clinically actionable. Corroborating results in other prospective cohorts, discerning a possible mechanistic role of HGF in the development of PAD, and determining whether HGF has actual value as a prognostic risk marker of PAD will be important to better understand the relevance of the current findings.

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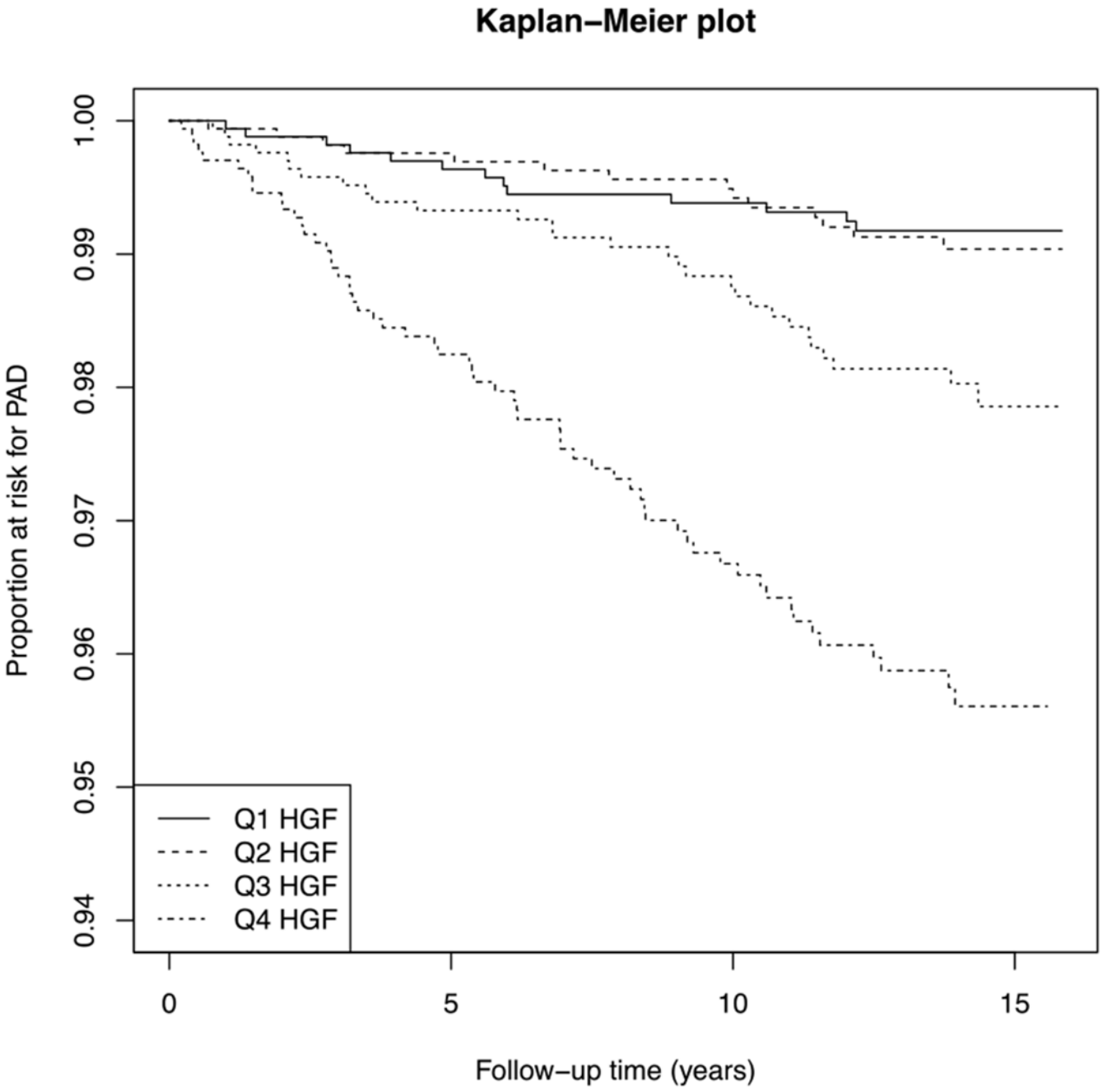

Figure 1.

Kaplan-Meier plot of clinical PAD-free survival according to $HGF_{exam\ 1}$ quartiles

HGF=hepatocyte growth factor; PAD=peripheral artery disease

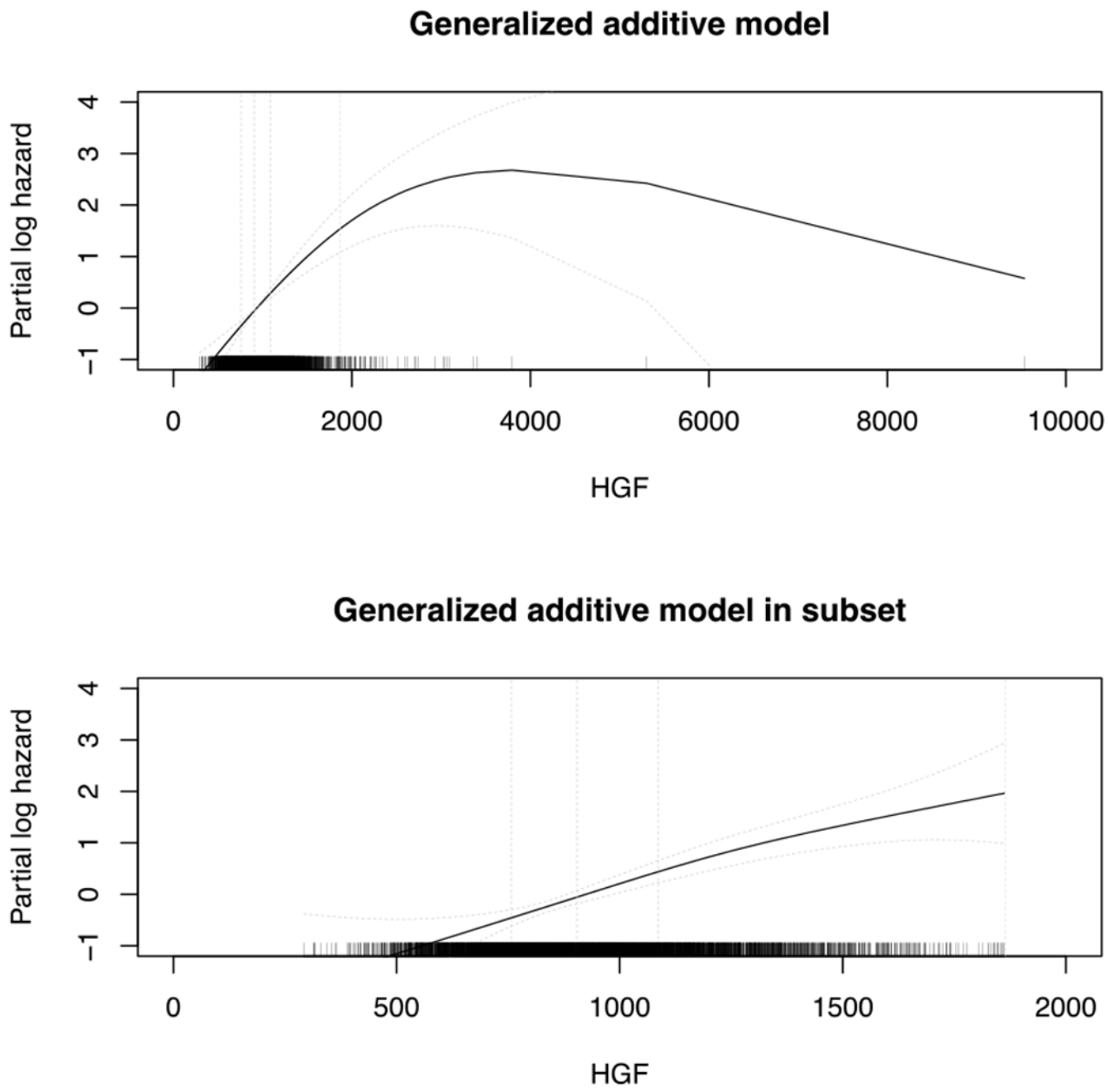

Figure 2.

GAM plot for risk of clinical PAD per standard deviation increase in HGF_{exam1} level Penalized spline with 4 degrees of freedom for HGF_{Exam1} in Model 2. Dotted curves show 95% confidence interval. Blue lines show quartile cut-offs. Top panel shows entire cohort. Bottom panel excludes those participants in the 99th percentile baseline HGF values. GAM=general additive model; HGF=hepatocyte growth factor; PAD=peripheral artery disease

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Table 1.

Baseline characteristics of MESA participants according baseline HGF quartiles (n=6742)[*].

Characteristic	Overall	Quartile 1 (<757.0 ng/l)	Quartile 2 (757.0-905.6 ng/l)	Quartile 3 (904.6-1085.9 ng/l)	Quartile 4 (>1085.9 ng/l)	p[†]
Age, years	62.1 (10.2)	58.3 (9.3)	61.3 (9.8)	63.4 (10.1)	65.6 (10.3)	<0.01
Male, n (%)	3184 (47%)	878 (52%)	787 (47%)	769 (46%)	750 (45%)	<0.01
Race, n (%)						<0.01
White	2602 (39%)	752 (45%)	631 (37%)	628 (37%)	591 (35%)	
Chinese	800 (12%)	307 (18%)	244 (14%)	156 (9%)	93 (5%)	
Black	1856 (27%)	431 (26%)	511 (30%)	476 (28%)	438 (26%)	
Hispanic	1484 (22%)	196 (12%)	299 (18%)	425 (25%)	564 (33%)	
Body mass index, kg/m^2, mean (SD)	28.3 (5.5)	26.6 (4.7)	28.1 (5.2)	28.7 (5.5)	30.0 (5.9)	<0.01
Smoking status, n (%)						<0.01
Never	3388 (50%)	908 (54%)	903 (54%)	823 (49%)	754 (45%)	
Former	1599 (24%)	349 (21%)	370 (22%)	417 (25%)	463 (28%)	
Current	877 (13%)	154 (9%)	175 (10%)	239 (14%)	309 (18%)	
Pack-years smoking, mean (SD)	11.3 (20.9)	9.2 (19.3)	9.2 (17.1)	12.5 (22.9)	14.2 (23.2)	<0.01
Alcohol use, n (%)						<0.01
Never	1374 (20%)	318 (19%)	351 (21%)	349 (21%)	356 (21%)	
Former	1599 (24%)	349 (21%)	370 (22%)	417 (25%)	463 (28%)	
Current	3721 (56%)	1001 (60%)	953 (57%)	917 (54%)	850 (51%)	
Diabetes, n (%)	840 (12%)	94 (6%)	169 (10%)	222 (13%)	355 (21%)	<0.01
SBP, mmHg, mean (SD)	126.7 (21.5)	121.0 (19.5)	125.7 (20.5)	128.4 (22.1)	131.2 (22.4)	<0.01
DBP, mmHg, mean (SD)	71.9 (10.3)	72.1 (10.3)	72.2 (10.0)	71.9 (10.2)	71.5 (10.5)	0.05
Total cholesterol, mg/dL, mean (SD)	194 (36)	194 (34)	197 (36)	195 (36)	191 (37)	0.03
LDL cholesterol, mg/dL, mean (SD)	117 (31)	117 (31)	120 (31)	118 (31)	114 (32)	<0.01
HDL cholesterol, mg/dL, mean (SD)	51 (15)	53 (16)	51 (14)	50 (14)	49 (14)	<0.01
Lipid lowering therapy, n (%)	1087 (16%)	209 (12%)	275 (16%)	277 (16%)	326 (19%)	<0.01
Antihypertensive use, n (%)	2503 (37%)	450 (27%)	566 (34%)	659 (39%)	828 (49%)	<0.01
Physical activity, MET-min/wk, mean (SD)	5760 (5904)	6253 (5960)	5971 (6089)	5723 (6312)	5097 (5133)	<0.01

Characteristic	Overall	Quartile 1 (<757.0 ng/l)	Quartile 2 (757.0-905.6 ng/l)	Quartile 3 (904.6-1085.9 ng/l)	Quartile 4 (>1085.9 ng/l)	p†
C-reactive protein, mg/L, mean (SD)	3.8 (5.8)	2.5 (4.8)	3.2 (4.8)	4.0 (5.4)	5.4 (7.5)	<0.01
eGFR, mL/min/1.73m^2, mean (SD)	81.2 (18.5)	82.8 (15.5)	81.7 (19.8)	80.8 (17.3)	79.5 (20.7)	<0.01

HGF=hepatocyte growth factor, SBP=systolic blood pressure, DBP=diastolic blood pressure, LDL=low-density lipoprotein, HDL=high-density lipoprotein, eGFR=estimated glomerular filtration rate, MET= metabolic equivalent

*Continuous variables are expressed as mean (SD). Categorical variables are n (percent).

†Comparisons were made across quartiles using linear trend test across quartiles.

Table 2.

Associations of baseline HGF with incident PAD*.

	Clinical PAD (n=6742)					Low ABI (n=5736)				
	Events/# at risk	**Model 1† HR (95% CI)**	**p**	**Model 2‡ HR (95% CI)**	**p**	**Events/# at risk**	**Model 1 RR (95% CI)**	**p**	**Model 2 RR (95% CI)**	**p**
Continuous										
Per SD unit (303 ng/l)	116/6742	1.17 (1.11, 1.23)	<0.001	1.21(1.05, 1.39)	0.007	197/5736	1.09 (1.00, 1.19)	0.027	1.03 (0.85, 1.25)	0.754

HGF=hepatocyte growth factor, PAD=peripheral arterial disease, ABI=ankle-brachial index

*Results of multivariable Cox Proportional Hazards Models (clinical PAD) and Poisson regression models (low ABI)

†Model 1 adjusted for age, sex, and race/ethnicity

‡Model 2 adjusted for Model 1 + diabetes, hypertension, smoking (current/former/never and pack-years), alcohol use, lipid lowering therapy, physical activity, body mass index, estimated glomerular filtration rate, C-reactive protein, ABI, high-density lipoprotein cholesterol, and total cholesterol

Table 3:

Association of change in HGF from exam 1 to exam 2 and development of PAD*.

	Clinical PAD (n=2413)					Low ABI (n=2223)				
	Events/# at risk	Model 1† HR (95% CI)	p	Model 2‡ HR (95% CI)	p	Events/# at risk	Model 1 RR (95% CI)	p	Model 2 RR (95% CI)	p
Per SD unit increase										
$HGF_{exam\ 2}$ (302 ng/l)		1.15 (1.03, 1.29)	0.012	1.21 (0.99, 1.47)	0.057		1.13 (0.96, 1.33)	0.137	1.20 (0.98, 1.47)	0.083
$HGF_{exam2\ -\ exam\ 1}$ (202 ng/l)	37/2413	0.91 (0.68, 1.23)	0.54	0.86 (0.65, 1.14)	0.31	59/2223	1.09 (0.76, 1.57)	0.624	1.07 (0.77, 1.49)	0.667

HGF=hepatocyte growth factor, PAD=peripheral arterial disease, ABI=ankle-brachial index

*Results of multivariable Cox Proportional Hazards Models (clinical PAD) and logistic regression models (low ABI)

†Model 1 adjusted for age, sex, and race/ethnicity

‡Model 2 adjusted for Model 1 + diabetes, hypertension, smoking (current/former/never and pack-years), alcohol use, lipid lowering therapy, physical activity, body mass index, estimated glomerular filtration rate, C-reactive protein, ABI, high-density lipoprotein cholesterol and total cholesterol.

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