Atherosclerosis 290 (2019) 214–221

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Preoperative hypertension is associated with atherosclerotic intraplaque hemorrhage in patients undergoing carotid endarterectomy

Leonie M.M. Fassaert[a], Nathalie Timmerman[a], Ian D. van Koeverden[b], Gerard Pasterkamp[c], Dominique P.V. de Kleijn[a], Gert J. de Borst[a,*]

[a] Department of Vascular Surgery, University Medical Centre Utrecht, University Utrecht, Utrecht, the Netherlands
[b] Department of Experimental Cardiology, University Medical Centre Utrecht, University Utrecht, Utrecht, the Netherlands
[c] Laboratory of Clinical Chemistry and Hematology, University Medical Centre Utrecht, University Utrecht, Utrecht, the Netherlands

HIGHLIGHTS

- Preoperative hypertension is associated with more vulnerable carotid plaques.
- These vulnerable carotid plaques contain more macrophages, lipid core and IPH.
- Similar associations are found for diastolic BP in a iliofemoral-cohort.
- Increased diastolic BP associates with more macrophages, lipid core and IPH.

ARTICLE INFO

Keywords:
Plaque
Atherosclerotic
Hypertension
Carotid endarterectomy
Systolic pressure
Diastolic pressure

ABSTRACT

Background and aims: Both hypertension and atherosclerotic plaque characteristics such as intraplaque hemorrhage (IPH) are associated with cardiovascular events (CVE). It is unknown if hypertension is associated with IPH. Therefore, we studied if hypertension is associated with unstable atherosclerotic plaque characteristics in patients undergoing carotid endarterectomy (CEA).

Methods: Prospectively collected data of CEA-patients (2002–2014) were retrospectively analyzed. Blood pressure (BP) was the mean of 3 preoperative measurements. Preoperative hypertension was defined as systolic BP ≥ 160 mmHg. Post-CEA, carotid atherosclerotic plaques were analyzed for the presence of calcifications, collagen, smooth muscle cells, macrophages, lipid core, IPH and microvessel density. Associations between BP (systolic and diastolic), patient characteristics and carotid plaque characteristics were assessed with univariate and multivariate analyses with correction for potential confounders. Results were replicated in a cohort of patients that underwent iliofemoral endarterectomy.

Results: Within CEA-patients (n = 1684), 708 (42%) had preoperative hypertension. Increased systolic BP was associated with the presence of plaque calcifications (adjusted OR1.11 [95% CI 1.01–1.22], $p = 0.03$), macrophages (adjusted OR1.12 [1.04–1.21], $p < 0.01$), lipid core > 10% of plaque area (adjusted OR1.15 [1.05–1.25], $p < 0.01$), IPH (adjusted OR1.12 [1.03–1.21], $p = 0.01$) and microvessels (adjusted beta 0.04 [0.00–0.08], $p = 0.03$). Increased diastolic BP was associated with macrophages (adjusted OR1.36 [1.17–1.58], $p < 0.01$), lipid core (adjusted OR1.29 [1.10–1.53], $p < 0.01$) and IPH (adjusted OR1.25 [1.07–1.46], $p < 0.01$) but not with microvessels nor plaque calcifications. Replication in an iliofemoral-cohort (n = 657) showed that increased diastolic BP was associated with the presence of macrophages (adjusted OR1.78 [1.13–2.91], $p = 0.01$), lipid core (adjusted OR1.45 [1.06–1.98], $p = 0.02$) and IPH (adjusted OR1.48 [1.14–1.93], $p < 0.01$).

Conclusions: Preoperative hypertension in severely atherosclerotic patients is associated with the presence of carotid plaque macrophages, lipid core and IPH. IPH, as a plaque marker for CVE, is associated with increased systolic and diastolic BP in both the CEA and iliofemoral population.

* Corresponding author. Department of Vascular Surgery, G04.129, University Medical Center Utrecht, University of Utrecht, Heidelberglaan, 100 3584 CX, Utrecht, the Netherlands.
E-mail address: G.J.deBorst-2@umcutrecht.nl (G.J. de Borst).

https://doi.org/10.1016/j.atherosclerosis.2019.09.008
Received 14 June 2019; Received in revised form 29 July 2019; Accepted 19 September 2019
Available online 22 September 2019

1. Introduction

Hypertension is one of the most common risk factors that affects 20–30% of the world population [1]. Hypertension is associated with cardiovascular events (CVE) such as myocardial infarction and stroke that have atherosclerosis as their underlying pathology.

In primary prevention, intensive blood pressure (BP) lowering in high-risk patients strongly reduces myocardial infarction and stroke [2]. Studies found a linear relation between high systolic BP levels and stroke risk, in which each 10 mmHg reduction in systolic BP was associated with a one-third decrease of stroke incidence [3,4]. The risk of cardiovascular death increased gradually with increased systolic and diastolic BP levels [5]. In secondary prevention, BP lowering is associated with a reduction in stroke [6].

CVE such as myocardial infarction and stroke mostly occur due to underlying atherosclerosis, with activation of platelets on a ruptured or an eroded plaque surface. The association between BP and CVE is well established, but the association between BP and plaque progression is much less investigated. Lowering of systolic BP is associated with attenuation of coronary plaque progression while lowering of diastolic BP is correlated with lower coronary plaque volume [7–9]. Increased BP is also associated with progression of carotid intima media thickness [10]. This shows that BP is associated with both plaque volume and progression. However, no clear reversible process has been described.

On coronary plaque characteristics, a study demonstrated in a cross-sectional study design that increased diastolic BP was correlated with higher plaque volumes, more fibrous and more calcified plaques measured by CTA [9]. No correlation between systolic BP and plaque volume or more fibrous and calcified plaques was found. These patients were well-controlled, with systolic BP ranging from 95 to 154 mmHg. Two follow-up studies revealed that increased baseline systolic BP and diastolic BP were associated with an increase in atheroma volume of the coronary plaque after 1 or 2 years, as assessed with intravascular ultrasound. [7,11] It is unknown, however, if high BP is associated with IntraPlaque Hemorrhage (IPH) in coronary plaques.

Patients with carotid artery stenosis have an increased risk of CVE and death. Removal of the carotid plaque by carotid endarterectomy (CEA) is effective for severe symptomatic carotid artery stenosis in reducing the risk of future stroke and stroke-related death [12,13]. A possible association between BP and carotid atherosclerotic plaque characteristics such as IPH has not been studied despite that carotid plaque IPH is associated with CVE [14]. For this, we hypothesize that pre-operative hypertension is associated with vulnerable plaque characteristics such as IPH in carotid plaques.

In this study, we investigated the association of pre-operative systolic and diastolic BP, on histological carotid plaque characteristics including IPH in patients undergoing CEA. As atherosclerosis is considered a systemic disease, we replicated the results of this CEA cohort in a separate cohort of patients with peripheral artery disease undergoing iliofemoral endarterectomy (IFE).

2. Patients and methods

This study was conducted in accordance with the declaration of Helsinki. Ethical approval (TME/C01.18) was provided by the Medical Research Ethics Committee United (MEC-U) of St. Antonius Hospital Nieuwegein, The Netherlands on April 10, 2002.

2.1. Patient population

All patients in this study were included in the Athero-Express Biobank (AE biobank). The study protocol has been published before [15]. In short, the AE biobank is a large ongoing prospective biobank study performed in two tertiary referral hospitals in The Netherlands, namely the St. Antonius Hospital in Nieuwegein and the University Medical Center in Utrecht. This biobank collects carotid atherosclerotic plaques and preoperative blood specimens of patients undergoing carotid and/or iliofemoral endarterectomy (IFE). All consecutive patients undergoing CEA or IFE were eligible for this study. Patients were recruited on ward during admission the day prior to surgery. Written and oral information concerning participation was provided, and informed consent was signed. The indication for CEA for asymptomatic patients was based on the recommendations published by the Asymptomatic Carotid Surgery Trial (ACST), and for symptomatic patients the indication was based on recommendations based on the European Carotid Surgery Trial and the North American Symptomatic Carotid Endarterectomy Trial (NASCET) [12,13,16]. Plaque removal was conducted by experienced vascular surgeons in accordance with local and international guidelines. Patients were followed for three years post-surgery for the occurrence of cardiovascular events by annual standardized questionnaires and by checking medical files. In case further information was required to define whether a cardiovascular event has occurred, the general practitioner was consulted. From the included patients, 2029 out of 2341 (87%) had available follow-up data. Reasons for loss to follow-up included no response to follow-up questionnaires, referral to another hospital, unknown contact details because patients moved away or switch to different general practitioner of any reason.

2.2. Inclusion and exclusion criteria

For the present study, patients undergoing CEA or IFE between 2002 and 2014 with available plaque histology and preoperative BP measurements were included. Clinical data were extracted from patient files and collected through standardized questionnaires. Biochemical data were obtained preoperatively as part of the standard preoperative work-up, either during the visit to the outpatient clinic or on the ward. The mean of three pre-operative BP measurements was used for analyses. Patients were excluded in case no preoperative BP or histologic plaque assessment were available. Patients operated for restenosis of the carotid or iliofemoral arteries were excluded for analysis.

2.3. Sample collection

The sample collection protocol of the Athero-Express biobank had been described earlier [17]. To summarize: preoperatively a blood sample was collected and stored at −80° C. Routine laboratory measurements of total cholesterol, triglycerides, HDL, LDL and creatinine were assessed. The atherosclerotic plaque was processed immediately after surgery and divided into segments of 5-mm thickness. The section with the largest plaque burden was defined as the culprit lesion and was subjected to immunohistochemical staining. Segments were fixated in 4% formaldehyde, decalcified for one week in ethylenediaminetetraacetic acid (to soften the calcification in the plaque for handling purposes without fully dissolving it) and embedded in paraffin. This 5 mm-segment of the culprit was cut into 5ηm slices for histological analysis.

2.4. Histological assessment

Histological slides were assessed by a previously validated protocol [15]. In short, plaque specimens were stained to examine the plaque characteristics as following: CD68 for macrophages, α-actin to identify smooth muscle cells, Picro-sirius Red (PSR) for collagen, hematoxylin eosin for general overview including calcifications, hematoxylin eosin and PSR for lipid core and CD34 for microvessels. Hematoxylin and eosin staining and fibrin by Mallory's phosphotungstic acid hematoxylin staining were used to identify the presence of luminal thrombi and intraplaque hemorrhage (IPH). Semi-quantitative scoring at 40× magnification was performed for the amount of collagen, calcification, macrophage infiltration and smooth muscle cell content and was scored as (1) no or minor staining along part of the luminal border of the plaque or (2) moderate or heavy staining along the entire luminal border or evident parts within the lesion. IPH was defined as the

Table 1
Patient characteristics of the CEA and IFE cohorts.

Patient characteristics	CEA (n = 1684)[a]	IFE (n = 657)[a]
Systolic BP, mmHg, mean [SD]	155 [26]	148 [23]
Diastolic BP, mmHg, mean [SD]	82 [13]	78 [13]
Sex, male (%)	1153 (69)	484 (74)
Age, years mean [SD]	69 [9] (35–93)	67 [9]
BMI, mean [IQR]	26 [4]	26 [4]
Current smoker, n (%)	581 (35)	267 (41)
Alcohol use > 10 units per week, n (%)	416 (25)	228 (35)
Diabetes mellitus, n (%)	392 (23)	202 (31)
Renal function, eGFR in ml/min/1.73m^2 median [IQR]	72 [27]	76 [27]
History of CAD, n (%)	519 (31)	286 (44)
Treated hypertension, n (%)	1297 (77)	553 (84)
PAOD, yes, n (%)	361 (21)	–
Clinical presentation		
Asymptomatic, n (%)	223 (13)	–
Ocular, n (%)	268 (16)	–
TIA, n (%)	722 (43)	–
Stroke, n (%)	464 (28)	–
Fontaine classification		
Fontaine IIb	–	308 (47)
Fontaine III	–	156 (24)
Fontaine IV	–	109 (17)
Stenosis ipsilateral		
50–70%, n (%)	118 (7)	51 (8)
70–99, n (%)	1528 (91)	458 (70)
Stenosis contralateral		
0–49%, n (%)	834 (50)	98 (15)
> 50%, n (%)	696 (41)	167 (25)
Year of surgery		
2002–2003, n (%)	248 (15)	39 (6)
2004–2005, n (%)	355 (21)	124 (19)
2006–2007, n (%)	285 (17)	121 (18)
2008–2009, n (%)	195 (12)	114 (17)
2010–2011, n (%)	314 (19)	164 (25)
2012–2013, n (%)	211 (13)	95 (15)
2014, n (%)	76 (4.5)	–
Triglycerides in mg/dL, median [IQR]	1.5 [1.0]	1.7 [1.2]
Total cholesterol in mg/dL, median [IQR]	4.4 [1.7]	4.4 [1.5]
HDL in mg/dL, median [IQR]	1.1 [0.4]	1.1 [0.4]
LDL in mg/dL, median [IQR]	2.4 [1.3]	2.4 [1.2]
Statin use, yes n (%)	1295 (77)	493 (75)
Antiplatelet use, n (%)	1491 (89)	548 (83)
Anti-coagulant use, n (%)	199 (12)	115 (18)
Diuretic use, n (%)	590 (35)	300 (46)
RAAS medication use, n (%)	854 (51)	417 (64)
B-blocker use, n (%)	738 (44)	301 (46)

Stenosis ipsilateral and stenosis contralateral refer to either carotid artery or iliofemoral artery. Degree of stenosis of the carotid artery was determined by the NASCET criteria. Baseline characteristics stratified for systolic and diastolic hypertension of CEA-cohort and IFE-cohort are presented in Supplemental Data.

composite of a luminal thrombi or intraplaque hemorrhages, hematoxylin-eosin and fibrin, assessed by Mallory's phosphotungstic acid hematoxylin staining. The presence of either luminal thrombosis, intraplaque hemorrhage or both was considered as positive plaque thrombosis. IPH is scored as present or absent.

Polarized light was used to assess the area of the lipid core of the plaque, expressed as a percentage of the total plaque area. In addition, macrophages and smooth muscle cells were quantified as the percentage of plaque area with the use of computerized analyses using AnalySIS 3.2 software (Soft Imaging Systems GmbH, Münster, Germany). Microvessels were counted in 3 hotspots of the plaque and subsequently averaged per slide. All histologic slides were assessed by two independent dedicated experts, who were blinded for patient characteristics and outcomes. Good inter-observer and intra-observer similarities have been confirmed previously (K 0.6–0.9) [18].

2.5. Study endpoints

The primary endpoint of this study was to determine the relation between preoperative BP and the atherosclerotic plaque characteristics. Preoperative BP was defined as BP measured on the outpatient clinic or ward before surgery. BP measurements used in this study were the mean of three available preoperative BP measurements. Preoperative hypertension was defined as systolic BP ≥ 160 mmHg. Secondary, in order to obtain more information about the role of preoperative BP on the atherosclerotic plaque, results of the CEA-cohort were validated in an iliofemoral cohort. The secondary endpoint of this study was to determine the association of preoperative BP and secondary composite CVE during the three years after surgery. A composite endpoint of CVE included stroke, myocardial infarction, peripheral events or any cardiovascular death.

2.6. Statistical analyses

To evaluate whether an increased preoperative BP was associated with the presence of vulnerable atherosclerotic plaque characteristics, we analyzed our data for systolic and diastolic BP separately. Systolic and diastolic BP measurements were analyzed as a continuous variable, by steps of 20 mmHg Data was inspected for missing data. Baseline characteristics of patients with preoperative measured hypertension

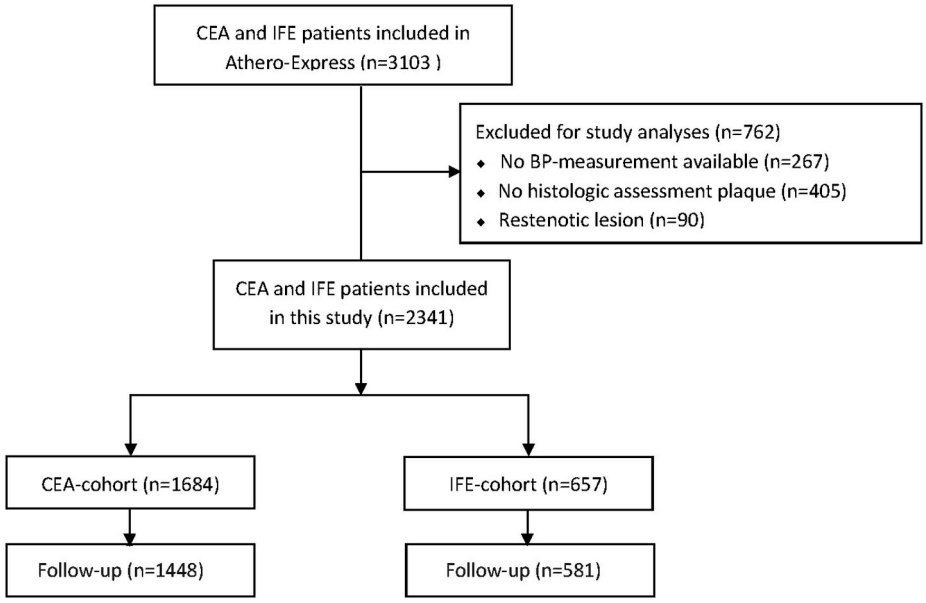

Fig. 1. Flowchart of included patients in study.
CEA: carotid endarterectomy; IFE: iliofemoral endarterectomy; BP: blood pressure.

(systolic BP ≥ 160 mmHg) were compared to those with normotensive preoperative BP measurement (systolic BP < 160 mmHg). The chi-square test was used for categorical variables and an independent T-test or Mann-Whitney *U* test for continuous variables, as appropriate. Linear regression and logistic regression analysis were performed to investigate the correlation between plaque characteristics and BP, as appropriate. Non-normally distributed quantitative histological parameters, including macrophages, smooth muscle cells (SMC) and microvessels, required logarithmic transformation before entering into linear regression models. To adjust for potential confounders, multivariate logistic regression analysis and linear regression analysis was performed. Baseline characteristics that showed an association of $p < 0.20$ with BP levels as well as with the plaque characteristic of interest were considered as potential confounders for multivariate analyses (Supplemental Tables 1 and 2). Since previous studies showed time-dependent trends in plaque characteristics and simultaneously improvement in risk factor management, year of inclusion was not included as a confounder because lowering of BP is suggested to be an underlying etiological factor (Table 1). Based on literature, symptoms status was added to the model [17,19].

Results of multivariate logistic regression analyses of CEA-cohort will be compared to results of an iliofemoral cohort. Sub-analysis was performed to assess the relation between BP and secondary events within three years post-procedural. Cox-regression analysis was used. Values with a $p < 0.05$ were considered statistically significant. SPSS version 24.0 (SPSS Inc, Chicago, Illinois) was used for all statistical analyses.

3. Results

3.1. Patient population

A total of 2383 patients who underwent carotid endarterectomy (CEA) and 720 patients who underwent femoral iliac endarterectomy (IFE) were included. After exclusion of patients with missing preoperative BP measurements or missing plaque histology, and restenotic lesions, 1684 CEA-patients and 657 IFE-patients were included in the analysis. 26 CEA-patients (1.5%) were also included in IFE-cohort (see flowchart; Fig. 1).

3.2. CEA-cohort

Of the CEA-cohort, the majority of patients were male (68%) with a median age of 70 years (62–76, interquartile range, IQR). At the moment of inclusion, diabetes mellitus was reported in 23% of the patients, smoking in 35% and preoperative hypertension in 42%. In 87% of the patients, the carotid artery stenosis was symptomatic; these symptoms were mostly transient ischemic attacks (43%). The median timing between index event and surgery was 30 days [IQR61]. Patients with preoperative systolic hypertension were significantly older, were less often diabetic or had a history of coronary artery disease, had decreased kidney function and a more severe stenosis degree. Moreover, these patients had higher total cholesterol levels, higher LDL levels and less often used statins. Patients with preoperative systolic hypertension were operated more often in the earlier years (2002–2005) than in the later years (2010–2015). Remarkably, 80% of the patients with preoperative systolic hypertension used antihypertensive medications, for preoperative diastolic hypertension this was 75% (Table 1).

Univariate logistic regression analyses showed a positive association between systolic BP (per 20 mmHg) and the presence of calcification, macrophage content, lipid core, IPH and the number of microvessels in the atherosclerotic plaque.

After adjustment for potential confounders, the association between increased systolic BP and macrophages (OR 1.12, 95% CI 1.04–1.21, $p < 0.01$), the presence of lipid core ≥ 10% and ≥40% (OR 1.15, 95%CI 1.05–1.25, $p < 0.01$ and OR 1.13, 95% CI 1.03–1.23, $p = 0.01$, for 10% and 40%, respectively), calcification (OR 1.11, 95% CI 1.01–1.22, $p = 0.03$), IPH (OR 1.18, 95% CI 1.03–1.21, $p = 0.01$) and number of microvessels (OR 0.04, 95% CI 0.00–0.08, $p = 0.03$) remained statistically significant (Table 2).

In addition, for diastolic BP (per 20 mmHg) univariate analysis showed that the presence of more macrophages (OR 1.36, 95%CI 1.17–1.58, $p < 0.01$), a lipid core ≥10% and ≥40% (OR1.29, 95% CI 1.10–1.53, $p < 0.01$ and OR1.25, 95% CI 1.05–1.49, $p = 0.01$ for 10% and 40%, respectively) and IPH (OR1.25, 95% CI 1.07–1.45, $p < 0.01$) were associated. After adjustment for potential confounders, these vulnerable plaque characteristics retained a strong association with high diastolic BP (Table 2 and Fig. 2).

Table 2
CEA plaque characteristics and systolic and diastolic BP.

Systolic BP (per 20 mmHg)				
Semi-quantitative plaque characteristics	**Odds ratio unadjusted [95% CI]**	***p*-value univariate**	**Odds ratio adjusted [95% CI]**	***p*-value multivariate**
Moderate/heavy calcification	1.10 [1.02–1.19]	0.01	1.11 [1.01–1.22]	0.03[a]
Moderate/heavy collagen	1.00 [0.91–1.10]	0.97	1.01 [0.92–1.11]	0.83[b]
Moderate/heavy SMC	1.00 [0.92–1.08]	0.90	0.95 [0.86–1.04]	0.26[c]
Moderate/heavy macrophages	1.10 [1.02–1.19]	0.01	1.12 [1.04–1.21]	< 0.01[d]
Presence of lipid core 10%	1.14 [1.05–1.25]	< 0.01	1.15 [1.05–1.25]	< 0.01[e]
Presence of lipid core 40%	1.12 [1.03–1.21]	0.01	1.13 [1.03–1.23]	0.01[f]
Presence of IPH	1.10 [1.02–1.19]	0.02	1.12 [1.03–1.21]	0.01[g]
Continuous plaque characteristics	**Beta unadjusted [95% CI]**	***p*-value univariate**	**Beta adjusted [95% CI]**	***p*-value adjusted [95% CI]**
Mean number of micro vessels per hotspot	0.04 [0.01–0.07]	0.01	0.04 [0.00–0.08]	0.03[h]
Diastolic BP (per 20 mmHg)				
Semi-quantitative plaque characteristics	**Odds ratio unadjusted [95% CI]**	***p*-value univariate**	**Odds ratio adjusted [95% CI]**	***p*-value multivariate**
Moderate/heavy calcification	1.05 [0.91–1.21]	0.54	1.08 [0.90–1.28]	0.41[i]
Moderate/heavy collagen	0.88 [0.74–1.05]	0.14	0.91 [0.76–1.09]	0.32[j]
Moderate/heavy SMC	0.97 [0.83–1.13]	0.69	0.84 [0.69–1.01]	0.06[k]
Moderate/heavy macrophages	1.32 [1.14–1.52]	< 0.01	1.36 [1.17–1.58]	< 0.01[l]
Presence of lipid core 10%	1.30 [1.11–1.53]	< 0.01	1.29 [1.10–1.53]	< 0.01[m]
Presence of lipid core 40%	1.22 [1.04–1.44]	0.01	1.25 [1.05–1.49]	0.01[n]
Presence of IPH	1.22 [1.05–1.41]	0.01	1.25 [1.07–1.46]	< 0.01[o]
Continuous plaque characteristics	**Beta unadjusted [95% CI]**	***p*-value univariate**	**Beta adjusted [95% CI]**	***p*-value adjusted [95% CI]**
Mean number of micro vessels per hotspot	0.04 [-0.02-0.11]	0.15	0.022 [-0.51-0.10]	0.55[p]

BP, blood pressure; SMC, smooth muscle cells; IPH, intraplaque hemorrhage.
Bold values were considered statistically significant. $p < 0.05$.
[a] Corrected for age, eGFR, CAD, ipsilateral stenosis, clinical presentation, total cholesterol, gender, HDL.
[b] Corrected for ipsilateral stenosis, statins, clinical presentation.
[c] Corrected for age, clinical presentation, total cholesterol, LDL, gender, eGFR.
[d] Corrected for clinical presentation, statins, gender.
[e] Corrected for age, diabetes, CAD, statins, gender and clinical presentation.
[f] Corrected for age, ipsilateral stenosis, statins, gender, clinical presentation.
[g] Corrected for CAD, statins, gender, clinical presentation.
[h] Corrected for age, diabetes, ipsilateral stenosis, clinical presentation and triglycerides.
[i] Corrected for age, CAD, ipsilateral stenosis, clinical presentation, total cholesterol, PAOD.
[j] Corrected for ipsilateral stenosis, statins, clinical presentation, PAOD.
[k] Corrected for age, clinical presentation, total cholesterol, LDL.
[l] Corrected for clinical presentation, statins, anti-coagulant use.
[m] Corrected for age, diabetes, CAD, statins, clinical presentation.
[n] Corrected for age, ipsilateral stenosis, statins, PAOD, BMI, clinical presentation.
[o] Corrected for CAD, statins, BMI, clinical presentation.
[p] Corrected for age, diabetes, ipsilateral stenosis, clinical presentation, triglycerides, BMI (Supplemental Table 1).

3.3. Iliofemoral cohort

Replication of results in 657 iliofemoral patients showed similar trends in baseline characteristics and BP levels (Supplemental Tables 5 and 6). Univariate and multivariate analyses revealed no significant associations between systolic BP levels and vulnerable plaque characteristics. Regarding diastolic BP, increased diastolic BP showed a strong association with the presence of IPH (OR1.37, 95% CI 1.07–1.76, $p = 0.01$) (Table 3). After adjustment for potential confounders, diastolic BP remained associated with IPH (OR1.48, 95% CI 1.14–1.93, $p < 0.01$). Also, increased diastolic BP levels were positively correlated with an increased number of macrophages and the presence of a lipid core > 10% (OR 1.78, 95% CI 1.13–2.91, $p = 0.01$ and OR 1.45, 95% CI 1.06–1.98, $p = 0.02$, respectively) (Table 3 and Fig. 2).

3.4. Secondary events

Three-year follow-up data of secondary composite CVE were available in 2029 patients of the total cohort of CEA (n = 1448) and IFE (n = 581). Secondary CVE within three years' post-procedural occurred in 669 patients (370 CVE in CEA cohort and 299 CVE in IFE cohort). Secondary CVE analyses corrected for cardiovascular risk factors showed a gradually increased risk for the composite endpoint with systolic BP (adjusted HR per 20 mmHg increase 1.06, 95% CI 1.00–1.13), $p = 0.04$, but not for diastolic BP (adjusted HR per 20 mmHg 1.05, 95% CI 0.93–1.17), $p = 0.43$.

4. Discussion

The current study investigated the association between BP and carotid atherosclerotic plaque characteristics. Our results show that both increased systolic and diastolic BP levels were gradually associated with a more vulnerable plaque phenotype in patients with severe carotid artery stenosis undergoing CEA. Increased systolic BP and diastolic BP levels correlated with more macrophages, IPH and a larger lipid core. Similar trends were seen in the iliofemoral replication cohort as increased diastolic BP was associated with the presence of macrophages, IPH and a large lipid core.

Up to now, the relation between BP and carotid plaque characteristics has been unknown. Previous studies have mainly focused on

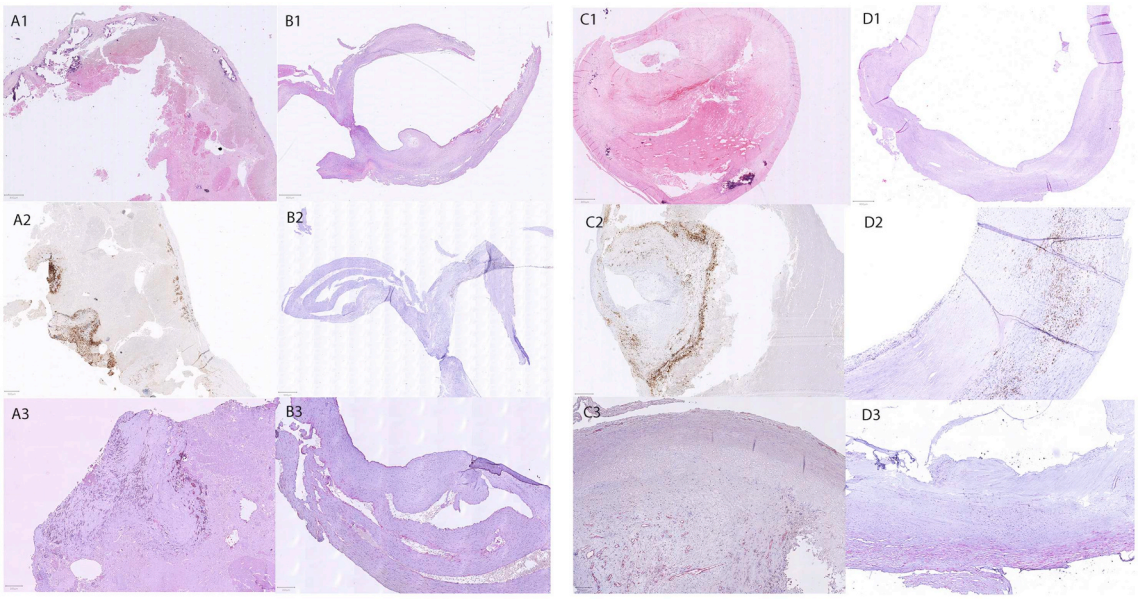

Fig. 2. Histologic panel of symptomatic and asymptomatic patients stratified for blood pressure.
(A) Symptomatic patient with high blood pressure. (A1) HE staining. (A2) CD68 staining. (A3) CD34 staining. (B) Symptomatic patient with normal blood pressure. (B1) HE staining. (B2) CD68 staining. (B3) CD34 staining. (C) Asymptomatic patients with high blood pressure. (C1) HE staining. (C2) CD68 staining. (C3) CD34 staining. (D) Asymptomatic patient with normal blood pressure. (D1) HE staining. (D2) CD68 staining. (D3) CD34 staining.

carotid intima media thickness as determined by ultrasound. The only previous study that reported on histological carotid plaque characteristics in relation with BP was performed in a small asymptomatic hypertensive CEA cohort with specific patients that had a morning BP surge (defined as an increase of SBP ≥ 50 mmHg and/or DBP ≥ 22 mmHg in the early morning) versus those without. Patients with a morning BP surge showed more unstable plaques with more inflammation compared with those without a morning BP surge [20]. In contradiction to this previous study, our two cohorts are large with mainly symptomatic patients that are both hypertensive and non-hypertensive.

Although performed in a different study population and usage of different methods for plaque characterization, our results are in line with previous studies on the association of hypertension and coronary plaque characteristics. Increase in baseline BP was associated with coronary plaque atheroma (determined by intravascular ultrasound) which matches our association of high BP and a large lipid core in the carotid plaque. Intravascular ultrasound, however, does not allow detection of macrophages and IPH.

We replicated our findings in a cohort of patients undergoing IFE. Carotid and femoral plaques have been described to have different morphology [21]. While carotid plaques show more atheromatous plaques with larger lipid cores and more macrophages and T-cells and with more metalloproteinase MMP9 than the femoral plaques [21–23], femoral plaques have more stable fibrocalcified lesions with a lower concentration of cholesterol and a higher concentration of calcium [21,22]. Next to the vascular bed, this is probably due to the timing of surgery. In symptomatic patients undergoing carotid revascularization, treatment is performed preferably within the first two weeks after index event. In contrast, in patients with intermittent claudication complaints surgery is often preceded by exercise training resulting in delayed iliofemoral surgery [24]. Despite these intrinsic differences between carotid and femoral plaque morphology, we found that systolic and diastolic BP were in both cohorts associated with IPH, underlining the systemic importance of BP on IPH.

IPH in coronary plaques has been described to contain glycophorin A and iron from erythrocytes [25]. High levels of glycophorin A and iron have been associated with a large lipid core and high influx of macrophages suggesting that IPH represent a potent atherogenic stimulus [25]. In our carotid plaques, IPH is increased with high BP together with an increased lipid core and macrophage accumulation that might point to IPH increase as a result of high BP. In accordance, IPH in carotid plaques was associated with accelerated plaque progression and increased lipid core [26].

Leakage of plaque microvessel endothelium has been held responsible for IPH in advanced human coronary atherosclerosis [27]. We only found an association of systolic BP and plaque microvessels in the CEA cohort and not in the iliofemoral cohort. One could hypothesize that increased BP could accelerate erythrocyte leakage from microvessels or cause rupture of these immature microvessels due to direct mechanic forces, both resulting in plaque instability with subsequent CVE.

Next to the association of high BP with vulnerable plaque characteristics, we found that patients despite treatment have residual hypertension, with respectively 86% and 82% of CEA and IFE patients having a systolic BP ≥ 160 mmHg while treated with antihypertensive medication. This could be either due to non-compliance of medications or unclose monitoring of the BP lowering effect by physicians. Surprisingly, diabetes and coronary artery disease were more frequently reported in patients with systolic BP ≤ 160 mmHg. This is probably induced by selection bias since patients with a history of diabetes or coronary artery disease will be subjected to more stringent secondary preventive strategies and therefore more often screened for hypertension and subsequently treated with antihypertensive medications.

Residual hypertension in our cohorts together with the association of high BP with more macrophages, lipid core and IPH as markers of the rupture-prone plaque and IPH as plaque marker associated with CVE strongly indicates that intensive BP monitoring and intensive anti-hypertensive therapy is needed for these severely atherosclerotic patients [14,28].

Table 3
Patient characteristics iliofemoral-cohort and systolic BP.

Systolic BP (per 20 mmHg)				
Semi-quantitative plaque characteristics	**Odds ratio unadjusted [95% CI]**	***p*-value univariate**	**Odds ratio adjusted [95% CI]**	***p*-value multivariate***
Moderate/heavy calcification	1.01 [0.88–1.15]	0.92	1.00 [0.88–1.15]	0.95[a]
Moderate/heavy collagen	0.97 [0.81–1.16]	0.72	1.03 [0.86–1.24]	0.73[b]
Moderate/heavy SMC	0.95 [0.83–1.10]	0.52	1.00 [0.86–1.16]	0.99[c]
Moderate/heavy macrophages	0.97 [0.81–1.16]	0.75	1.03 [0.81–1.32]	0.79[d]
Presence of lipid core, 10%	1.13 [0.97–1.33]	0.12	1.14 [0.97–1.34]	0.11[e]
Presence of lipid core, 40%	1.13 [0.74–1.71]	0.57	1.10 [0.74–1.36]	0.63[f]
Presence of IPH	1.05 [0.92–1.19]	0.48	1.21 [1.00–1.47]	0.05[g]
Continuous plaque characteristics	**Beta unadjusted [95% CI]**	***p*-value univariate**	**Beta adjusted [95% CI]**	***p*-value adjusted [95% CI]**
Mean number of micro vessels per hotspot	−0.03 [-0.12-0.07]	0.61	0.39 [-0.23-0.09]	−0.07[h]
Diastolic BP (per 20 mmHg)				
Semi-quantitative plaque characteristics	**Odds ratio unadjusted [95% CI]**	***p*-value univariate**	**Odds ratio adjusted [95% CI]**	***p*-value multivariate***
Moderate/heavy calcification	0.94 [0.73–1.20]	0.61	1.01 [0.78–1.30]	0.96[i]
Moderate/heavy collagen	0.94 [0.67–1.32]	0.72	0.90 [0.63–1.29]	0.57[j]
Moderate/heavy SMC	0.88 [0.67–1.17]	0.38	0.79 [0.59–1.05]	0.11[k]
Moderate/heavy macrophages	1.38 [0.98–1.93]	0.06	1.78 [1.13–2.91]	**0.01**[l]
Presence of lipid core > 10%	1.30 [0.96–1.76]	0.09	1.45 [1.06–1.98]	**0.02**[m]
Presence of lipid core > 40%	1.43 [0.65–3.13]	0.38	1.55 [0.71–3.42]	0.27[n]
Presence of IPH	1.37 [1.07–1.76]	**0.01**	1.48 [1.14–1.93]	< **0.01**[o]
Continuous plaque characteristics	**Beta unadjusted [95% CI]**	***p*-value univariate**	**Beta adjusted [95% CI]**	***p*-value adjusted [95% CI]**
Mean number of micro vessels per hotspot	0.02 [-0.16-0.20]	0.82	0.08 [-0.23-0.39]	0.60[p]

BP, blood pressure; SMC, smooth muscle cells; IPH, intraplaque hemorrhage.
Bold values are considered statistically significant; $p < 0.05$.
[a] Corrected for age, eGFR, smoking, CAD.
[b] Corrected for age, anti-coagulants use, CAD.
[c] Corrected for age, antiplatelet use, anti-coagulant use, smoking.
[d] Corrected for HDL, smoking, CAD.
[e] Corrected for age, smoking, antiplatelet.
[f] Corrected for age, smoking, statins.
[g] Corrected for anti-coagulant use, amputation, CAD, triglycerides.
[h] Corrected for HDL, statins, smoking, triglycerides.
[i] Corrected for age, CAD.
[j] Corrected for age, anti-coagulant use, CAD.
[k] Corrected for age, anti-coagulant use.
[l] Corrected for HDL, LDL, CAD.
[m] Corrected for eGFR, age.
[n] Corrected for age, statins.
[o] Corrected for anti-coagulant use, amputation, CAD, eGFR.
[p] Corrected for HDL, LDL, eGFR, total cholesterol, statins (Supplemental Table 2).

4.1. Limitations

Some limitations should be addressed. First, BP measurements used in the current study are in-hospital preoperative measurements that were assessed on the nursing ward conform preoperative work-up. Stress-induced factors or white coat hypertension can influence these in-hospital BP measurements. To diminish the effect of these factors, the used BP measurements are the mean of three preoperative BP measurements measured on the ward on separate moments in time. These blood pressure measurements are conform BP measurements used in large clinical trials for management of arterial hypertension and most feasible in clinical practice [29,30]. Second, although we found in both carotid and iliofemoral cohort a correlation between vulnerable plaque characteristics and increased BP, no causal relation can be proven due to the cross-sectional study design. Future prospective trials should be addressed to investigate the causality of intensive BP treatment on atherosclerotic plaque characteristics. Third, as patients undergoing carotid artery stenting (CAS) were not included in these analyses, results cannot be extrapolated for CAS-patients. Finally, one out of five patients undergoing CEA have systolic BP inter-arm difference of > 15 mmHg [31]. Nurses and healthcare takers in our hospitals are instructed to measure BP of patients undergoing CEA bilaterally, in which the side of the highest BP will be used for future BP measurements. However, there is no data available whether the mean of BP is solely based on single BP measurements of the highest arm. This might have influenced our results [31,32].

4.2. Conclusion

In conclusion, increased systolic and diastolic BP levels are associated with more carotid plaque macrophages, lipid core and IPH in patients undergoing CEA. Replication in a separate iliofemoral cohort confirmed these associations.

Conflicts of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

Financial support

Funding was from the EU 755320 Taxinomisis grant to GP, DdK and GJdB.

Author contributions

Study design: LF, IvK, NT.
Data collection: LF, NT, IvK.
Histological slides analysis: GP.
Data analysis: LF, NT.
Writing and figures: LF, NT, DK.
Revision and final approval: LF, NT, DK, GJdB, IvK, GP.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.atherosclerosis.2019.09.008.

References

[1] J. Mackay, G.A. Mensah, The Atlas of Heart Disease and Stroke, World Heal Organ, 2004.
[2] X. Xie, E. Atkins, J. Lv, A. Bennett, B. Neal, et al., Effects of intensive blood pressure lowering on cardiovascular and renal outcomes : updated systematic review and meta-analysis, Lancet 387 (2016) 435–443.
[3] C.M.M. Lawes, D.A. Bennett, V.L. Feigin, A. Rodgers, Blood Pressure and Stroke. An overview of published reviews, Stroke 35 (2004) 776–785.
[4] A. Attar, M. Sayadi, M. Jannati, Effect of intensive blood pressure lowering on cardiovascular outcomes based on cardiovascular risk : a secondary analysis of the SPRINT trial, Eur. J. Prev. Cardiol. 26 (2019) 238–245, https://doi.org/10.1177/2047487318800741.
[5] Prospective Studies Collaboration, Age-specific relevance of usual blood pressure to vascular mortality : a meta-analysis of individual data for one million adults in 61 prospective studies, Lancet 360 (2002) 1903–1913.
[6] H. Zhang, L. Thijs, J.A. Staessen, Blood pressure lowering for primary and secondary prevention of stroke, Hypertension 48 (2006) 187–195.
[7] A.K. Chhatriwalla, S.J. Nicholls, T.H. Wang, K. Wolski, I. Sipahi, et al., Low levels of low-density lipoprotein cholesterol and blood pressure and progression of coronary atherosclerosis, J. Am. Coll. Cardiol. 53 (2009) 1110–1115.
[8] I. Sipahi, E.M. Tuzcu, P. Schoenhagen, K.E. Wolski, S.J. Nicholls, et al., Effects of normal, pre-hypertensive, and hypertensive blood pressure levels on progression of coronary atherosclerosis, J. Am. Coll. Cardiol. 48 (4) (2006) 4–9.
[9] M. Saleh, A. Alfaddagh, T.K. Elajami, H. Ashfaque, H. Haj-ibrahim, et al., Diastolic blood pressure predicts coronary plaque volume in patients with coronary artery disease, Atherosclerosis 277 (2018) 34–41.
[10] K. Chien, Y. Tu, H. Hsu, T. Su, H. Lin, et al., Differential effects of the changes of LDL cholesterol and systolic blood pressure on the risk of carotid artery atherosclerosis, BMC Cardiovasc. Disord. 12 (2012) 1–7.
[11] M.H. Garciá-Garciá, V. Klauss, N. Gonzalo, S. Garg, Y. Onuma, et al., Relationship between cardiovascular risk factors and biomarkers with necrotic core and atheroma size : a serial intravascular ultrasound radiofrequency data analysis, Int. J. Cardiovasc. Imaging 28 (2012) 695–703.
[12] North American Symptomatic Carotid Endarterectomy Trial Collaborators, Beneficial effect of carotid endarterectomy in symptomatic patients with high-grade carotid stenosis, N. Engl. J. Med. 325 (7) (1991) 445–453.
[13] Writing Group, A.R. Naylor, J.B. Ricco, G.J. de Borst, S. Debus, et al., Management of atherosclerotic carotid and vertebral artery disease: 2017 clinical practice guidelines of the european society for vascular surgery (ESVS), Eur. J. Vasc. Endovasc. Surg. 55 (1) (2017) 1–79.
[14] W.E. Hellings, W. Peeters, F.L. Moll, S.R.D. Piers, J. van Setten, et al., Composition of carotid atherosclerotic plaque is associated, Circulation 121 (2010) 1914–1950.
[15] B.A.N. Verhoeven, E. Velema, A.H. Schoneveld, J.P.P.M. de Vries, P. de Bruin, et al., Athero-express : differential atherosclerotic plaque expression of mRNA and protein in relation to cardiovascular events and patient characteristics . Rationale and design, Eur. J. Epidemiol. 19 (2004) 1127–1133.
[16] European Carotid Surgery Trialists' Collaborative Group, Randomised trial of endarterectomy for recently symptomatic carotid stenosis : final results of the MRC European Carotid Surgery Trial (ECST), Lancet 351 (1998) 1379–1387.
[17] B. Verhoeven, W.E. Hellings, F.L. Moll, J.P. de Vries, D.P.V. de Kleijn, et al., Carotid atherosclerotic plaques in patients with transient ischemic attacks and stroke have unstable characteristics compared with plaques in asymptomatic and amaurosis fugax patients, J. Vasc. Surg. 42 (2005) 1077–1081.
[18] W.E. Hellings, G. Pasterkamp, A. Vollebregt, C.A. Seldenrijk, J.P.M. de Vries, et al., Intraobserver and interobserver variability and spatial differences in histologic examination of carotid endarterectomy specimens, J. Vasc. Surg. 46 (2007) 1147–1154.
[19] D.P.J. Howard, G.W. vanLammeren, J.N. Redgrave, F.L. Moll, J.P.M. de Vries, et al., Histological features of carotid plaque in patients with ocular ischemia versus cerebral events, Stroke 44 (2013) 734–739.
[20] R. Marfella, M. Siniscalchi, M. Portoghese, C. Di Filippo, F. Ferraraccio, et al., Morning blood pressure surge as a destabilizing factor of atherosclerotic plaque: role of ubiquitin-proteasome activity, Hypertension 49 (4) (2007) 784–791.
[21] F. Herisson, M.-F. Heymann, M. Chétiveaux, C. Charrier, S. Battaglia, et al., Carotid and femoral atherosclerotic plaques show different morphology, Atherosclerosis 216 (2011) 348–354.
[22] S. Shaikh, J. Brittenden, R. Lahiri, P.A.J. Brown, F. Thies, et al., Macrophage subtypes in symptomatic carotid artery and femoral artery plaques, Eur. J. Vasc. Endovasc. Surg. 44 (2012) 491–497.
[23] J. Orbe, L. Fernandez, J.A. Rodr, G. Rábago, M. Belzunce, et al., Different expression of MMPs/TIMP-1 in human atherosclerotic lesions . Relation to plaque features and vascular bed, Atherosclerosis 170 (2003) 269–276.
[24] V. Aboyans, J.-B. Ricco, M.E.L. Bartelink, M. Björck, M. Brodmann, et al., ESC guidelines on the diagnosis and treatment of peripheral arterial diseases , in collaboration with the european society for vascular surgery (ESVS), Eur. Heart J. 39 (2017) 763–821 2018.
[25] F.D. Kolodgie, H.K. Gold, A.P. Burke, D.R. Fowler, H.S. Kruth, et al., Intraplaque hemorrhage and progression of coronary atheroma, N. Engl. J. Med. 349 (2003) 2316–2325.
[26] N. Takaya, C. Yuan, B. Chu, T. Saam, N.L. Polissar, et al., Presence of intraplaque hemorrhage stimulates progression of carotid atherosclerotic plaques, Circulation 111 (2005) 2768–2775.
[27] J.C. Sluimer, F.D. Kolodgie, A.P.J.J. Bijnens, K. Maxfield, E. Pacheco, et al., Thin-walled microvessels in human coronary atherosclerotic plaques show incomplete endothelial junctions, J. Am. Coll. Cardiol. 53 (17) (2009) 1517–1527.
[28] J.A. Schaar, J.E. Muller, E. Falk, R. Virmani, V. Fuster, et al., Terminology for high-risk and vulnerable coronary artery plaques, Eur. Heart J. 25 (2004) 1077–1082.
[29] ADVANCE Collaborative Group, Effects of a fixed combination of perindopril and indapamide on macrovascular and microvascular outcomes in patients with type 2 diabetes mellitus (the ADVANCE trial): a randomised controlled trial, Lancet 370 (2007) 829–840.
[30] M. Böhm, H. Schumacher, K.K. Teo, E.M. Lonn, F. Mahfoud, et al., Achieved blood pressure and cardiovascular outcomes in high-risk patients: results from ONTARGET and TRANSCEND trials, Lancet 389 (2017) 2226–2237.
[31] A. Huibers, J. Hendrikse, M.M. Brown, S.A. Pegge, M. Arnold, et al., Upper extremity blood pressure difference in patients undergoing carotid revascularisation, Eur. J. Vasc. Endovasc. Surg. 53 (2) (2017) 153–157.
[32] G. Kranenburg, W. Spiering, P.A. de Jong, L.J. Kappelle, G.J. de Borst, et al., Inter-arm systolic blood pressure differences , relations with future vascular events and mortality in patients with and without manifest vascular disease, Int. J. Cardiol. 244 (2017) 271–276.