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van Mil, ACCM, Pouwels, S, Wilbrink, J, Warlé, MC and Thijssen, DHJ

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1 **CAROTID ARTERY REACTIVITY PREDICTS EVENTS IN**
2 **PERIPHERAL ARTERIAL DISEASE PATIENTS**

3
4 **ANKE CCM VAN MIL, MSc.,^{1,3} SJAAK POWELS, PhD.,² JELMER WILBRINK, MSc.,¹**
5 **MICHIEL C WARLÉ, MD.,² DICK HJ THIJSSEN, PROF.^{1,3}**

6
7 Radboud Institute for Health Sciences, ¹Department of Physiology and ²Department of
8 Surgery, Radboud university medical center, Nijmegen, the Netherlands

9 ³Research institute for Sport and Exercise Sciences, Liverpool John Moores University,
10 Liverpool, United Kingdom

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18 **Author for correspondence:**

19 Prof. Dr. Dick Thijssen, Research Institute for Sport and Exercise Sciences, Liverpool John
20 Moores University, Tom Reilly Building, Byrom Street L3 3AF, Liverpool, United Kingdom

21 Email: D.Thijssen@ljmu.ac.uk, Tel: +441519046264

22
23 **Running title:** Event prediction with carotid reactivity
24
25

1 **LIST OF ABBREVIATIONS**

2 ABPI Ankle brachial pressure index

3 AUC Area under the curve

4 BMI Body mass index

5 CAR Carotid artery reactivity

6 CI Confidence interval

7 cIMT carotid intima-media thickness

8 CPT Cold pressor test

9 CV Cardiovascular

10 HR Hazard ratio

11 OR Odd's ratio

12 PAD Peripheral arterial disease

13 WHR waist-to-hip ratio

14

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16 **Clinical Trial Registration.** www.trialregister.nl/trialreg/index.asp, **NTR-4117.**

17

18 **KEYWORDS:** Endothelial function; prognosis; cardiovascular disease; atherosclerosis.

1 INTRODUCTION

2 Peripheral arterial disease (PAD) is the result of atherosclerotic arterial stenosis and
3 occlusions in the larger vessels supplying the lower extremities.¹ Patients with PAD have a
4 markedly increased risk for future cardiovascular mortality and morbidity.¹⁻⁴ Endothelial
5 dysfunction contributes to the development and progression of PAD.^{5, 6} Endothelial function,
6 often examined as the brachial artery reactivity response to ischaemia, in PAD patients is
7 impaired⁷⁻⁹ and relates to future CV events.^{10, 11} The recent AHA/ACC-guideline on the
8 management of PAD highlighted the need for an easy, simple and rapid test of endothelial
9 function to predict future adverse events in PAD.¹² Although brachial artery reactivity shows
10 predictive capacity for future CV events,^{10, 11} concerns have been raised regarding practical
11 limitations that prevents the clinical application of this technique.

12

13 Carotid artery reactivity (CAR) testing is a simple, non-invasive procedure to examine
14 endothelial function. It involves measuring the carotid artery diameter responses to the
15 sympathetic stimulation produced by the cold pressor test (CPT).^{13, 14} The carotid arteries, like
16 coronary arteries,^{13, 14} dilate in response to the CPT in healthy subjects, whereas this dilation
17 is attenuated, or reversed to vasoconstriction, in patients with cardiovascular disease.¹³⁻¹⁶
18 Interestingly, the coronary arteries' response to CPT is a strong, independent predictor of
19 cardiovascular events,^{17, 18} but it is not clear if the carotid response to CPT (i.e. CAR) also
20 predicts future cardiovascular events. This study is to our knowledge, the first to examine the
21 prognostic value of the CAR in patients with PAD. We hypothesized that CAR-induced
22 vasoconstriction would predict future CV events in patients with PAD, independent of subject
23 characteristics and clinical status.

1

2 **METHODS**

3 **Participants and study approval**

4 We recruited 172 patients with PAD scheduled for a routine visit at the vascular laboratory
5 (Department of Surgery, Radboud University Medical Center, Netherlands) for the study
6 (Figure 1). We included PAD patients with present or prior Fontaine classification 2B-3-4,
7 age ≥ 18 yr, and the ability to provide informed consent. We excluded patients with Raynaud's
8 phenomenon, chronic pain syndrome, open wounds on the upper extremities, arterial-venous
9 shunts, scleroderma, coronary, central and/or peripheral arterial disease interventions within
10 the prior < 1 week, and unstable angina pectoris, myocardial infarction, stroke or heart failure
11 within the prior 3 months. Patients provided written informed consent prior to participation.
12 The study was approved by the local Ethics Committee (NL-46109.091.13) in accordance
13 with the latest revision of the Declaration of Helsinki. This study was registered as [NTR-4117](#)
14 (Netherlands Trial Registration).

15

16 **Experimental design**

17 Patients abstained from strenuous exercise for 24 hours, fasted for ≥ 6 hours, and abstained
18 from caffeine and vitamin C, which are known to alter endothelial function, for ≥ 18 hours
19 prior to testing in accordance with guidelines on assessing endothelial function.²⁰

20

21 **Experimental measures**

22 General characteristics. Age and sex were obtained from the electronic patient records. A
23 physician obtained the history of smoking, hypercholesterolemia, hypertension, diabetes and
24 medication use. Height (in m), weight (in kg), and waist-hip ratio (WHR) were measured by a
25 research nurse. The same vascular surgeon assigned the patient's Fontaine clinical

1 classification. Patients with Fontaine stage ≥ 2 performed walking tests to distinguish between
2 Fontaine stages. Finally, the ankle-brachial pressure index (ABPI) was measured based on
3 clinical requirements following recent guidelines.²¹ The highest systolic pressure in the right
4 and left posterior tibial or dorsal pedis artery and in the right brachial artery was measured
5 twice, and the average of those two measurements was used to calculate the ABPI for each
6 leg. The lowest ABPI of two legs was used for analysis. For the purpose of analysis, we
7 compared those above versus below the median ABPI.

8

9 Carotid artery reactivity. Patients rested on a comfortable bed in a temperature controlled
10 room for at least 5 minutes. Participants were in the supine position with the neck extended
11 for assessment of the carotid artery. Left carotid artery diameter was recorded continuously
12 for 30-seconds before and for 90-seconds during immersion of the hand up to the wrist in ice
13 slush (4 °C). Images were obtained using a L9-3 MHz linear array probe attached to a high
14 resolution ultrasound machine. When an optimal image was found, the probe was held stable
15 and the ultrasound parameters were set to optimise the longitudinal, B-mode image of the
16 lumen-arterial wall interface. Following a 30-second baseline assessment of carotid artery
17 diameter, the hand was immersed for 90-seconds with simultaneous and continuous
18 assessment of carotid artery diameter.

19 CAR% responses were assessed for diameter. Analysis of the carotid artery diameter was
20 performed by a single blinded investigator using custom-designed edge-detection and wall-
21 tracking software, which is largely independent of investigator bias.²² Details of this
22 technique can be found elsewhere.²³ Baseline diameter was calculated as the mean of data
23 acquired across the 30 seconds preceding the CPT test. After submersion of the hand in ice
24 slush, data were calculated as the mean value for 10-second intervals, involving 8-10 full
25 cardiac cycles. Based on this data we calculated the peak diameter change (i.e. the 10-second

1 bin with the highest value, CAR%). The peak diameter change can refer to a maximum
2 constriction or dilation. The direction of this change was determined by a positive (i.e.
3 dilation) or negative (i.e. constriction) area under the curve.

4 Reproducibility (coefficient of variation, CV) of diameter responses to CPT were previously
5 assessed with a 1- and 24-hour intervals in 50 subjects. Within-day CV for baseline and peak
6 diameters were 2.2 and 2.6%, whilst day-to-day CV were 2.3% and 2.7%. Furthermore, the
7 CAR% (i.e. maximum change in diameter) showed a within-day reproducibility of 2.6% and
8 between-day reproducibility of 2.8%.¹⁴

9
10 Intima-media thickness. Carotid artery intima-media thickness (cIMT), a marker for vascular
11 structure, is related to future development of PAD.²⁴ To examine whether the CAR relates to
12 future CV events, independent of the cIMT, we examined cIMT from the same section of the
13 artery as the CAR. We obtained continuous recordings of the cIMT for 10 seconds. Analyses
14 were performed by a blinded researcher, using observer-independent edge-detection and wall-
15 tracking software.²⁵ For the purpose of analysis, we compared those above versus below the
16 median cIMT.

17

18 **Follow up and assessment of adverse events**

19 After 12 months of follow-up, adverse events were extracted from medical records and
20 verified by a blinded vascular surgeon (MW). The Dutch National Death Registration was
21 used to determine mortality. Death certificates of patients who experienced a fatal event were
22 obtained when available to categorise death into CV or non-CV related mortality. Adverse
23 events were categorised into; 1. Cardio- and cerebrovascular events (“CV events”; CV-related
24 mortality, myocardial infarction, coronary revascularisation procedures, transcranial ischemic
25 attack, cerebrovascular accident, carotid surgery, major- and minor amputations, and ischemic

1 bowel disease), 2. “Clinical progression” that is related to PAD (loss of patency (i.e., the
2 presence of restenosis in a previous endovascular reconstructed vessel), endovascular
3 reconstructive surgery using percutaneous transluminal angioplasty, and worsening in
4 Fontaine-classification), and 3. “All-cause Mortality”. We also grouped these 3 categories to
5 capture all adverse events (“Adverse events”). Only the first event was included in the
6 analyses for patients who experienced more than 1 event. All indications for PTA and
7 revascularisation surgery were discussed prior in a multidisciplinary team of vascular
8 surgeons and interventional radiologists, whilst preference of patient and interventional were
9 taken into consideration. All involved members of the multidisciplinary team were blinded to
10 the outcome of the CAR test.

11

12 **Statistical analysis**

13 Prior to our study, we aimed to include 200 PAD patients. This group size is in line with
14 previous studies examining the prognostic value of measures of vascular health,^{10, 19} whilst
15 this group size also accounts for potential drop out (10-15%) and access to sufficient PAD
16 patients (n=400/annum, 50% inclusion rate). Data are presented as mean±SD or n (%) unless
17 stated otherwise. Statistical analysis was performed using IBM SPSS Statistics 21.0 (IBM
18 SPSS, IBM Corp., Armonk, NY, USA). Baseline characteristics were assessed for normality
19 with the Shapiro-Wilk test. We adopted unpaired Student’s t-tests (Mann-Whitney U-test for
20 non-normally distributed parameters) to compare subject characteristics, co-morbidities,
21 clinical status, and medication use between PAD patients with carotid constriction vs.
22 dilation. We used logistic regression to assess if subject characteristics, co-morbidities,
23 clinical status, or medication could predict presence of carotid constriction.

24 Analyses to examine whether presence of carotid constriction could predict future events,
25 analyses were performed separately for “CV events”, “Clinical progression”, “All-cause

1 mortality” and “Adverse events”. Cumulative event rates of carotid constriction and dilation
2 were estimated with Kaplan-Meier survival analyses, and were calculated with the log-rank
3 test. Cox proportional hazard models were used to calculate hazard ratios, including
4 correction for confounding variables (age, sex, BMI, and WHR). These variables were
5 selected based on prior evidence of an association with measures of endothelial function.^{14, 20}
6 Analyses were repeated in subgroups in whom we examined cIMT (n=169) or ABPI (n=142).
7 We examined if these clinical measures were related to increased risk for future events,²⁶ and
8 whether they altered the analyses related to carotid measurement.

9

10 **RESULTS**

11 Subject characteristics, co-morbidities, clinical status and adequate imaging was obtained in
12 all 172 PAD patients. Median change in carotid artery diameter was 0.8% (95% Confidence
13 Interval -19.3 – 11.6%). Carotid constriction occurred in n=82 patients, whereas vasodilation
14 occurred in 90 patients. Seventy patients (41%) experienced ≥ 1 event, which included loss of
15 patency (n=18), increase in Fontaine-classification (n=15), percutaneous transluminal
16 angioplasty (n=44), transient ischemic attack (n=1), myocardial infarction (n=4),
17 cerebrovascular accident (n=3), coronary revascularisation procedures (n=1), major (n=7) and
18 minor (n=1) amputation, and ischemic bowel disease (n=1). Ten PAD patients died; from CV-
19 related mortality (n=4), cancer-related mortality (n=2), and unknown cause (n=4). We found
20 no baseline differences between PAD patients with carotid constriction versus dilation in
21 subject characteristics or co-morbidities. Patients with carotid constriction reported lower
22 antiplatelet drugs usage (Table 1). Logistic regression revealed that none of the subject
23 characteristics, co-morbidities, clinical status or medication use could predict if a PAD patient
24 would demonstrate carotid constriction (backward likelihood ratio analysis, all parameters
25 $P > 0.05$). When comparing patients with and without an adverse event, those with events

1 showed higher prevalence of hypercholesterolemia. Other factors did not differ between
2 groups (Supplemental data 1).

3

4 **Prognostic value of carotid constriction for future adverse events.**

5 Kaplan-Meier survival curves demonstrated that PAD patients with carotid constriction report
6 a higher incidence of CV events ($P=0.007$), clinical progression ($P=0.005$) and adverse events
7 ($P=0.006$) compared to carotid dilation (Figure 3). There were no significant differences
8 between groups for all-cause mortality (Log-rank, $P=0.417$, Figure 3).

9 Using a multivariate Cox proportional hazard model, with the fully adjusted model correcting
10 for potential confounders (i.e. age, sex, WHR and BMI), PAD patients with carotid
11 constriction continued to demonstrate higher risk for CV events (HR 4.1, 95%CI 1.3-12.5),
12 clinical progression (HR 2.0, 95%CI 1.2-3.3) and adverse events (HR 1.8, 95%CI 1.1-3.0),
13 but not all-cause mortality (HR 1.4, 95%CI 0.4-5.1, Table 2).

14

15 Added value of clinical measures. In 30 subjects, we were unable to perform a valid ABPI
16 because of non-compressible arteries or amputation. We were unable to perform analysis of
17 cIMT in 3 participants because of technical problems. Analyses for cIMT ($n=169$) and ABPI
18 ($n=142$) showed no significant effect using the Kaplan-Meier survival analysis for adverse
19 events (Figure 4), CV events (Log-rank $P=0.674$ and 0.457 , respectively), clinical
20 progression (Log-rank $P=0.484$ and 0.153 , respectively) or all-cause mortality (Log-rank
21 $P=0.198$ and 0.795 , respectively).

22 The cox proportional hazard models for the carotid constriction (including models 1 and 2)
23 were repeated for the subgroups with data on cIMT and ABPI. Adding cIMT or ABPI to the
24 fully adjusted model did not alter the HR of carotid constriction for future CV events, clinical
25 progression, all-cause mortality or adverse events (Supplemental material 2).

1 **DISCUSSION**

2 This is the first study, to our knowledge, to examine the relation between the carotid response
3 to the cold pressor test and future CV events in PAD patients. We found that patients who
4 demonstrated carotid constriction during the cold pressor test had a 4.1-, 2.0- and 1.8-times
5 increased risk at 1-year of developing a CV event, clinical deterioration and other adverse
6 events, respectively, compared to those with carotid dilation. Importantly, the ability of the
7 carotid vasomotor response to predict CV events was independent of subject characteristics
8 and more predictive than other common clinical measures such as ABPI and cIMT. This
9 suggests that a measure of (generalised) vascular health is more important than the extent of
10 the (localised) atherosclerotic lesion in PAD patients. Therefore, a simple and non-invasive
11 measure of carotid artery endothelial function can identify PAD patients at increased risk for
12 future adverse CV events and clinical progression.

13

14 **Dilation versus Constriction**

15 We^{14, 27} and others^{13, 15, 16} have demonstrated that the CPT produces a gradual dilator or
16 constrictor response. Similarly, normal coronary arteries show an endothelium-mediated
17 dilation that is mediated by the CPT-induced catecholamine-release, which exceeds the direct
18 constrictor effects of catecholamines on smooth muscle cells.^{15, 16} However, endothelial
19 dysfunction and/or (partial) endothelial damage impedes endothelium-mediated dilation
20 leading to vasoconstriction.¹⁵ Similar responses in the carotid artery probably explain the
21 distinct dilator or constrictor responses we observed in our study. Surprisingly, other subject
22 characteristics, co-morbidities and clinical measures did not differ between PAD patients
23 demonstrating constriction or dilator responses. This suggests that CV risk factors do not
24 contribute to the distinct vasomotion responses between PAD patients, and that the PAD

1 disease state, rather than subject characteristics or CV risk factors contributes to carotid artery
2 endothelial dysfunction.

3

4 **Relation between the carotid vascular response and subsequent events**

5 Current CV risk factors do not predict future CV events in patients with PAD.¹² This
6 highlights the potential utility of the carotid artery vasomotor response in predicting future
7 CV events. Others have demonstrated that brachial artery flow-mediated dilation predicts
8 future CV events in PAD patients,^{10, 11} but this technique is more difficult than our approach
9 of simply measuring the relatively large carotid diameter (i.e. ~7.5 mm) in response to the
10 CPT. Previous studies have also not measured clinical progression, whereas we found that
11 carotid constriction had a 2-fold increased risk for loss of patency, endovascular
12 reconstructive surgery and/or worsening in Fontaine-classification. This knowledge may
13 allow clinicians to treat more aggressively in those patients at risk for clinical deterioration.

14

15 Others have found that the coronary artery response to CPT predicts future CV events.^{17, 18}
16 Results from the present study suggest that the carotid artery response to the CPT is a
17 surrogate for coronary arteries. This is supported by our previous within-subject observation
18 that a subject's carotid and coronary response to the CPT are similar.^{14, 27} This observation
19 also supports the concept that atherosclerosis is a whole body, generalised disease of the
20 endothelium, such that an abnormal response in one vascular bed is likely also present other
21 vascular beds. Consistent with this concept is the observation that abnormal brachial artery
22 dilator responses to endothelial stimulation using increased flow⁶ or acetylcholine²⁸ are
23 associated with abnormal coronary artery responses to endothelial stimulation.

24

1 ABPI is useful in both the diagnosis of PAD²⁹ and predicting the need for revascularisation.^{2,}
2 ^{30, 31} Examining cIMT has also been useful in predicting atherosclerotic risk in some, but not
3 all, studies of the general population.^{24, 32} We found no relation between either ABPI or cIMT
4 and future CV events, clinical progression or adverse events. Furthermore, adding these
5 clinical measures to the statistical model did not alter the relationship between carotid artery
6 reactivity and future (CV) events. The finding that ABPI and cIMT are not related to future
7 events contrasts with previous work performed in the general population,^{2, 30, 31} but is largely
8 in agreement with studies performed in PAD.^{12, 33} Abnormal ABPI and cIMT indicate the
9 presence of atherosclerosis, whereas coronary responses to CPT reflect endothelial function.
10 This suggests that in individuals with known CVD, the impact of the atherosclerotic process
11 on the endothelium is more important than the atherosclerotic lesion in predicting CV events.

12

13 A potential limitation of our study is that we did not include other biomarkers, such as high-
14 sensitive C-reactive protein, which are demonstrated to have potential prognostic value in
15 PAD for future CV events.^{37, 38} Unfortunately, we did not assess these biomarkers to examine
16 the potential added value of combining these markers with the CAR, which may be relevant
17 since these biomarkers may provide additional information to our in vivo measure of
18 endothelial function.

19

20 Clinical relevance. The carotid artery reactivity to CPT procedure is easy to perform, low-
21 cost, non-invasive, and requires minimal time and equipment. The simplicity of the test is
22 supported by an excellent reproducibility.¹⁴ Moreover, in contrast to the majority of subject
23 and/or disease characteristics, the carotid artery vasomotor response to CPT identified
24 subjects with an increased risk for future events. The clinical relevance of the CAR may relate
25 the identification of PAD patients who are more vulnerable to non-adherence and/or

1 complications during surgery. For example, although symptomatic PAD should all be on drug
2 therapy, compliance to therapy is relatively poor.³⁴⁻³⁶ The CAR-test may help to identify
3 individuals in whom it is of special importance to maintain compliance to drug therapy.
4 Additionally, cardiovascular co-morbidity in PAD patients is associated with increased
5 perioperative cardiovascular risk. Future studies are needed to assess the potential added
6 value of the CAR to estimate perioperative risk in PAD patients.

7
8 In conclusion, our study provides the first evidence that carotid artery reactivity, independent
9 of subject characteristics and clinical measures such as cIMT and ABPI, predicts future
10 adverse (CV) events and clinical progression in PAD patients. The presence of carotid artery
11 constriction during the CPT is associated with a 4-fold increased risk for future CV events
12 and 2-fold increased risk for clinical progression. These observations suggest that the carotid
13 artery reactivity should be further evaluated for its ability to predict future risk in patients
14 with PAD.

15

16 **AUTHOR CONTRIBUTIONS**

17 DHJT and MW designed the study. DHJT ensured funding of the project. ACCMM, MW, SP
18 and JW were involved in data collection. ACCMM and DHJT performed the statistical
19 analyses. All authors contributed to the interpretation of the data, writing of the manuscript
20 and provided approval of the final version.

21

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4

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7

8 **DISCLOSURES**

9 No conflicts of interest, financial or otherwise, are declared by the author(s).

10

11 **AFFILIATIONS**

12 From the Radboud Institute for Health Sciences, ^A Department of Physiology (A.v.M., D.T.,
13 J.W.) and ^B Department of Surgery (S.P., M.W), Radboud university medical center,
14 Nijmegen, the Netherlands; Research institute for Sport and Exercise Sciences, Liverpool
15 John Moores University, Liverpool, United Kingdom (A.v.M., D.T.)

16

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- 6

1 **FIGURES.**

2 **FIGURE 1.** CONSORT diagram of the study.

3 **FIGURE 2.** Set-up of the practical performance of the test (A). Screen-shot of the
4 assessment of carotid artery diameter, with the yellow box indicating the region of interest
5 within the automated software performed analysis of the diameter (i.e. yellow lines on the
6 artery wall) (B). Data from a representative subjects demonstrating carotid artery diameter
7 dilatation (C) and constriction (D) during the cold pressor test. Both panels represent the
8 carotid artery diameter (in cm) across the 30-s baseline (up to the vertical dashed line; the
9 start of the cold pressor test) and 90-s during the cold pressor test. Data were analysed in 10-
10 second bins to identify presence of dilatation or constriction. More detailed findings of this
11 procedure is presented in the methods section.

12 **FIGURE 3.** Kaplan-Meier survival curves for adverse events (A), CV events (B), clinical
13 progression (C) and all-cause mortality (D) in PAD patients (n=172) across a 1-year follow-
14 up. We have dichotomised PAD patients in those who demonstrate coronary constriction
15 (CAR constriction, dotted line) or dilation during the CPT (CAR dilation, solid line). P-values
16 relates to a Log-rank test.

17 **FIGURE 4.** Kaplan-Meier survival curves for cIMT (A, 169 PAD patients), and ABPI (B,
18 142 PAD patients) related to occurrence of adverse events across a 1-year follow-up. The
19 solid line represents the cIMT and ABPI above the median, the dotted line refers to the cIMT
20 and ABPI below the median. P-values relates to a Log-rank test.

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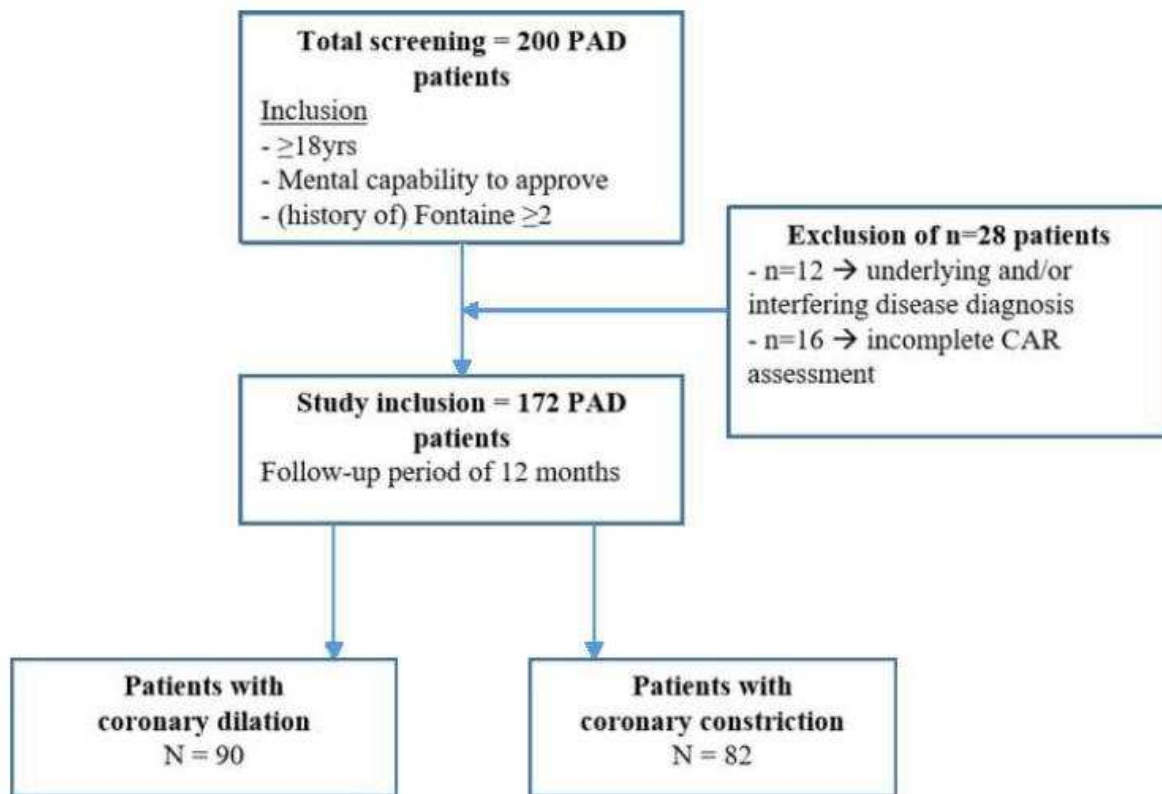
1 **Table 1.** Baseline characteristics of patients with PAD with carotid artery constriction (CAR
 2 constriction) or dilation (CAR dilation) during the CPT. *Indicate Mann-Whitney U test,
 3 presented as median [minimum – maximum]. P-value indicates difference between
 4 vasoconstriction *versus* vasodilation.

Subject characteristics	Total group n=172	CAR constriction n=82	CAR dilation n=90	P-value
Age*, y	68±10	71 [43-85]	67 [46-90]	0.223
Sex, males (%)	115 (67)	58 (71)	57 (63)	0.303
Height (m)	1.73±0.09	1.73±0.10	1.72±0.10	0.464
Weight (kg)	79.8±14.6	80.1±14.3	79.6±14.9	0.804
Body-mass index (kg/m ²)	27±4	27±4	27±4	0.900
Waist-to-hip ratio	1.01±0.10	1.02±0.10	1.00±0.10	0.210
Smoking, yes n (%)	55 (32)	28 (34)	27 (30)	
History n (%)	97 (56)	45 (55)	52 (58)	0.839
Comorbidities				
Hypertension, n (%)	138 (80)	66 (80)	72 (80)	0.936
Hypercholesterolemia, n (%)	133 (76)	61 (74)	72 (80)	0.380
Diabetes Mellitus, n (%)	46 (27)	22 (27)	24 (27)	0.873
Medication use				
Antiplatelet drugs, n (%)	135 (78)	58 (71)	77 (86)	0.018
Acetylsalicylic acid (ASA)	125 (73)	55 (67)	70 (78)	
Plavix (clopidogrel)	4 (2)	1 (1)	3 (3)	0.667
Dual therapy (combined)	6 (3)	2 (2)	4 (4)	
Statins, n (%)	141 (82)	71 (87)	70 (78)	0.133
Beta-blockers, n (%)	89 (52)	40 (49)	49 (54)	0.458
ACE inhibitors, n (%)	59 (34)	26 (32)	33 (37)	0.494
Proton pump inhibitors, n (%)	87 (51)	40 (49)	47 (52)	0.652
Clinical status				
Mild ischaemia (Fontaine 1-2A), n (%)	62 (36)	25 (30)	37 (41)	0.143
Moderate-severe ischaemia (Fontaine 2B-3-4), n (%)	110 (64)	57 (70)	53 (59)	
ABPI (n=142)	0.65±0.22	0.65±0.22	0.66±0.21	0.679
ABI left rest (n=135)	0.72±0.22	0.73±0.21	0.76±0.25	0.402
ABI right rest (n=132)	0.73±0.22	0.74±0.25	0.74±0.21	0.978
Carotid IMT* (mm)	0.79 [0.15-2.78]	0.80 [0.15-2.78]	0.78 [0.35-2.06]	0.860
Baseline carotid diameter* (mm)	7.5±1.1	7.7 [4.4-10.5]	7.3 [5.1-10.9]	0.358
CAR%	0.8 [-19.3 - 11.6]	1.3 [-9.5 - 11.6]	-1.2 [-19.3 - 9.6]	0.068*

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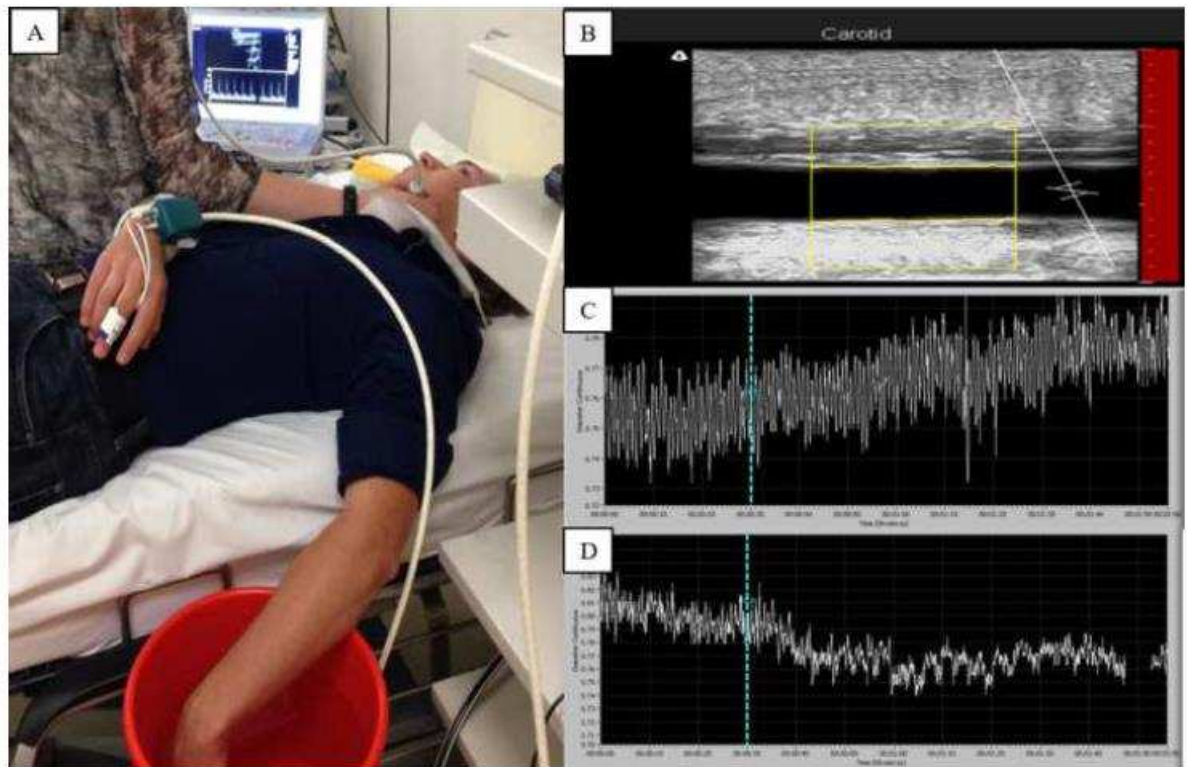
1 Figure 1



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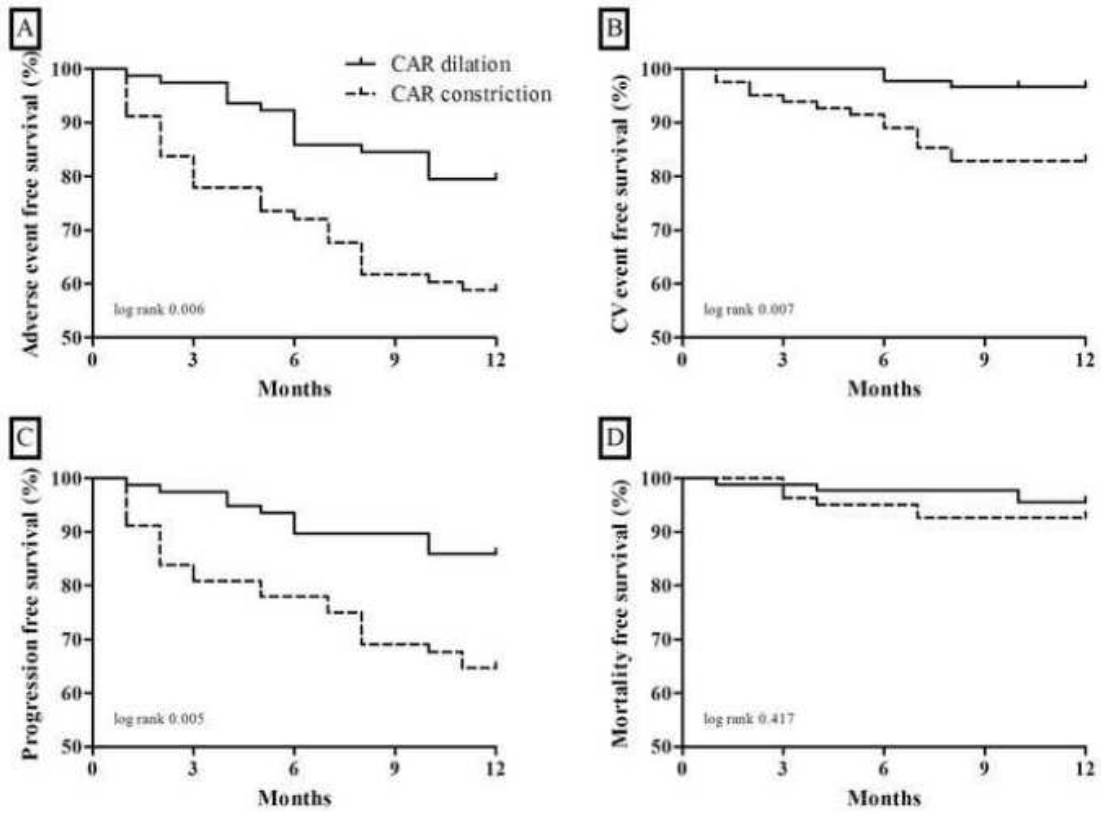
1 Figure 2



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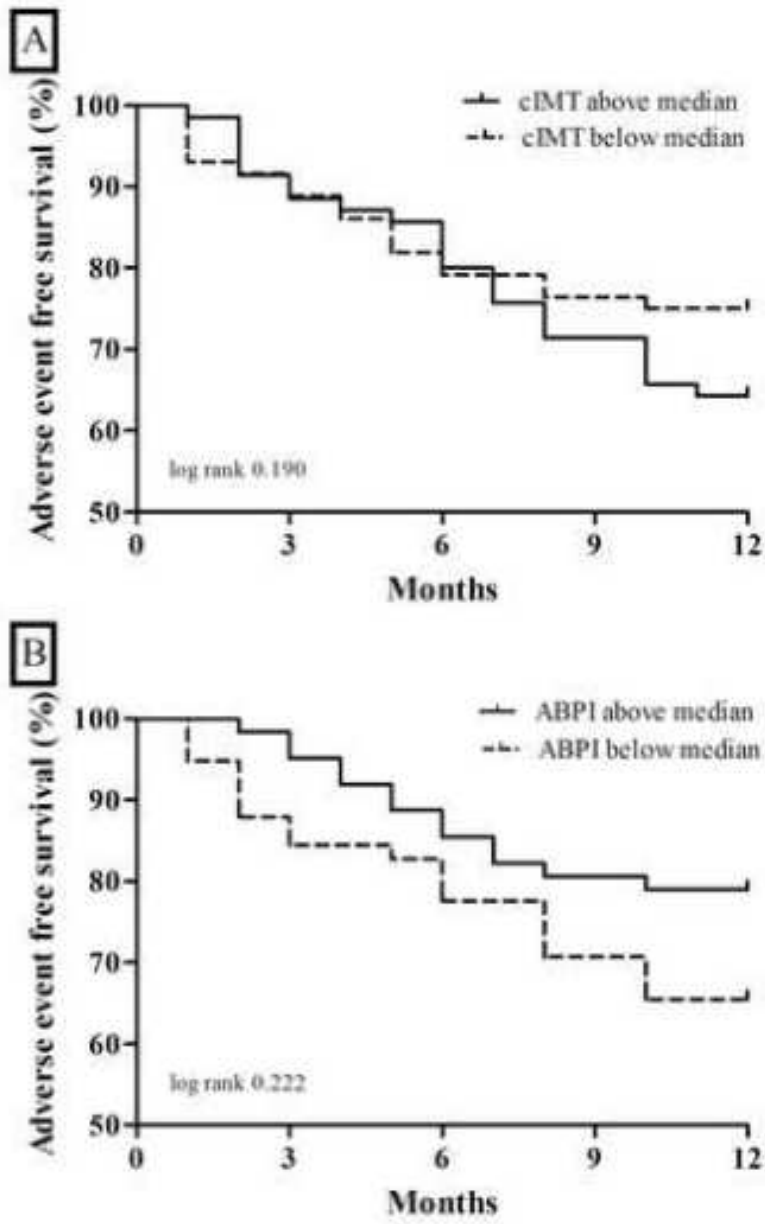
1 Figure 3



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1 Figure 4



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