Atherosclerosis 347 (2022) 47–54

Contents lists available at ScienceDirect

Atherosclerosis

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

The relation of RAAS activity and endothelin-1 levels to coronary atherosclerotic burden and microvascular dysfunction in chest pain patients

Ruurt A. Jukema [a], Ruben W. de Winter [a], Pepijn A. van Diemen [a], Roel S. Driessen [a], A.H. Jan Danser [d], Ingrid M. Garrelds [d], Pieter G. Raijmakers [b,a], Peter M. van de Ven [c], Paul Knaapen [a], Ibrahim Danad [a,1], Guus A. de Waard [a,*,1]

[a] Department of Cardiology, Amsterdam University Medical Centers, Location VU Medical Center, Amsterdam, the Netherlands
[b] Radiology, Nuclear Medicine & PET Research, Amsterdam University Medical Centers, Location VU Medical Center, Amsterdam, the Netherlands
[c] Epidemiology & Data Science, Amsterdam University Medical Centers, Location VU Medical Center, Amsterdam, the Netherlands
[d] Department of Pharmacology and Vascular Medicine, Department of Internal Medicine, Erasmus MC, Rotterdam, the Netherlands

ARTICLE INFO

Keywords:
Coronary artery disease
Renin-angiotensin-aldosterone-system (RAAS)
Renin
Endothelin-1

ABSTRACT

Background and aims: In this study, we investigated whether increased renin angiotensin aldosterone system (RAAS) activation and endothelin-1 levels are related to coronary artery calcium (CAC) score, total plaque volume (TPV), high risk plaque, hyperemic myocardial blood flow (MBF) and coronary microvascular dysfunction (CMD).

Methods: In a prospective, observational, cross-sectional cohort, renin as a marker for RAAS activation and endothelin-1 were measured in peripheral venous blood of 205 patients (64% men; age 58 ± 8.7 years) with suspected coronary artery disease (CAD) who underwent coronary computed tomography angiography (CCTA), [^{15}O]H_2O positron emission tomography (PET) perfusion imaging and invasive fractional flow reserve (FFR) measurements. Patients were categorized into three groups based on FFR (≤0.80) and hyperemic MBF <2.3 ml/min/g: [1] obstructive CAD (n = 92), [2] CMD (n = 26) or [3] no or non-obstructive CAD (n = 85).

Results: After correction for baseline characteristics, including RAAS inhibiting therapy, renin associated positively with CAC score and TPV, but not with hyperemic MBF ($p < 0.01$; $p = 0.02$ and $p = 0.23$). Patients with high risk plaque displayed higher levels of renin (mean logarithmic renin 1.25 ± 0.43 *vs.* 1.12 ± 0.35 pg/ml; $p = 0.04$), but not endothelin-1. Compared to no or non-obstructive CAD patients, renin was significantly elevated in obstructive CAD patients but not in CMD patients (mean logarithmic renin 1.06 ± 0.34 *vs.* 1.23 ± 0.36; $p < 0.01$ and 1.06 ± 0.34 *vs.* 1.16 ± 0.41 pg/ml; $p = 0.65$). Endothelin-1 did not differ between the three patient groups.

Conclusions: Our report provides evidence that RAAS activity measured by renin concentration is elevated in patients with coronary atherosclerosis and high risk plaque but not in patients with CMD, whereas endothelin-1 is not related to either.

1. Introduction

Stable ischemic heart disease is a multifactorial entity that consists of either epicardial atherosclerotic coronary artery disease (CAD), coronary microvascular dysfunction (CMD) or a combination thereof [1]. Both CAD and CMD are characterized by impaired myocardial perfusion leading to inducible myocardial ischemia [2]. We hypothesized that an increased coronary vasoconstrictive state might be involved in the reduced myocardial perfusion observed in patients with either CAD or CMD. To investigate this hypothesis, we assessed levels of renin as a marker of renin angiotensin aldosterone system (RAAS) activation and endothelin-1 in patients without obstructive CAD, patients with CAD and patients with CMD. We chose to assess RAAS activation since it is reported to be elevated in inflammatory diseases, most notably in atherogenesis [3]. Moreover, RAAS activation is associated with endothelial dysfunction induced CMD and RAAS inhibiting therapy has been linked to increased capillary density [4,5]. We chose to investigate the influence of endothelin-1, because it has been recognized as the most

* Corresponding author. Department of Cardiology, Amsterdam UMC, Vrije Universiteit Amsterdam, De Boelelaan 1117, 1081 HV, Amsterdam, the Netherlands.
E-mail address: g.dewaard@amsterdamumc.nl (G.A. de Waard).
[1] These authors share last authorship.

https://doi.org/10.1016/j.atherosclerosis.2022.03.017
Received 24 December 2021; Received in revised form 23 February 2022; Accepted 15 March 2022
Available online 17 March 2022

potent coronary vasoconstrictor in humans and is as such related with endothelial dependent CMD [6]. Also, endothelin-1 is related to endothelial cell injury and is reported to be elevated in patients with atherosclerosis [7]. In this study, we aim to investigate whether RAAS activation and circulating levels of endothelin-1 are associated with the extent of CAD and plaque phenotype as assessed by coronary computed tomography angiography (CCTA), the depth of myocardial ischemia by [^{15}O]H_2O positron emission tomography (PET) imaging, and CMD as defined by a normal invasive FFR measurement but abnormal [^{15}O]H_2O PET perfusion.

2. Patients and methods

2.1. Patient population

This is a sub study of the Prospective Comparison of Cardiac PET/CT, SPECT/CT Perfusion Imaging and CT Coronary Angiography With Invasive Coronary Angiography (PACIFIC trial; Clinical Trial Registration: NCT01521468) [8]. The PACIFIC trial consists of a homogenous, prospectively enrolled, population of 208 patients with stable chest pain without a history of CAD and with an intermediate pre-test probability of obstructive CAD as defined by the Diamond and Forrester criteria [9]. Patients underwent both CCTA and quantitative [^{15}O]H_2O PET myocardial blood flow. Invasive coronary angiography was performed within two weeks after the diagnostic imaging and FFR measurements were performed on all major coronary arteries. Peripheral venous blood of 205 patients was drawn on the day of invasive coronary angiography. All enrolled subjects gave written informed consent and the study was performed in accordance with the Declaration of Helsinki. The local ethics committee of the Amsterdam University Medical Center approved the study.

2.2. CCTA acquisition and coronary plaque analysis

CCTA was performed using a 256-slice CT scanner (Philips Brilliance iCT, Philips Healthcare, Best, the Netherlands) with a section collimation of 2 × 128 × 0.625 mm as described previously [10]. In short, prior to the scanning protocol, sublingual nitroglycerine was administered to all patients with metoprolol on indication, aiming for a heart rate <65 beats/min. Coronary artery calcium scoring (CACS) in Agatston units was obtained during a single breath-hold on a noncontrast computed tomography scan. All coronary segments with a diameter ≥2 mm were assessed by an experienced reader blinded to invasive physiological results using axial, multiplanar reformation and cross-sectional images. Coronary artery calcium (CAC) score was calculated according to the Agatston scoring system using Agatston units. Total plaque volume (TPV) (mm^3) was calculated by summing volumes of separate plaques along each coronary artery. A plaque was considered high-risk if at least 2 out of 4 major adverse plaque characteristics were present, namely: spotty calcifications (calcified plaque comprising <90° of the vessel circumference and <3 mm in length), positive remodelling (vessel area at the site of the maximal lesion to that of a proximal reference point, with an index of >1.1 representing positive remodelling), low attenuation (any voxel within plaque of <30HU) and napkin ring sign (plaque core with low attenuation surrounded by a rim-like area of higher attenuation) [11].

2.3. [^{15}O]H_2O PET imaging and assessment

The PET scans were performed using a Gemini TF64 PET/CT scanner (Philips Healthcare, Best, The Netherlands). Patients were instructed to refrain from intake of products containing caffeine or xanthine during 24 h prior to the scan. A 6-min dynamic scan protocol, commencing simultaneously with an injection of 370 MBq [^{15}O]H_2O during resting and adenosine (140 μg/kg/min), induced hyperemic conditions. The dynamic scan sequence was followed by a low-dose CT-scan for attenuation correction. In-house developed software (*CardiacVUer*, Amsterdam UMC, Vrije Universiteit Amsterdam, The Netherlands) allowed for the generation of parametric images of quantitative hyperemic myocardial blood flow (MBF) in ml/min/g [12] for each of the 17 left ventricle segments according to the standard American Heart Association model with standardized allocation of segments to the three vascular territories [13]. Coronary flow reserve (CFR) was defined as the ratio between hyperemic MBF and resting MBF. For analysis, hyperemic MBF was used because of its greater ability to identify ischemia and better prognostic value compared to CFR or resting MBF using [^{15}O]H_2O as tracer [14,15].

2.4. Invasive coronary angiography and FFR

Invasive coronary angiography was performed according to standard clinical protocols [8]. Patients were instructed to refrain from the intake of products containing caffeine during 24 h prior to the invasive angiography. All major coronary arteries and side branches >2.0 mm were interrogated with FFR. Operators refrained from FFR measurement in tight lesions (>90% diameter stenosis) for safety reasons. A 0.014-inch sensor-tipped guidewire, introduced through a 5- or 6-French guiding catheter, was used to measure FFR. To induce maximal coronary hyperemia, adenosine was administered either systemically through intravenous infusion of 140 μg/kg/min into a large vein, or intracoronary as a 150 μg bolus. FFR was calculated as the ratio of mean distal intracoronary to aortic guiding pressure.

2.5. Patient categories

By combining the FFR with hyperemic MBF, the study population was stratified into three different patient categories: [1] if FFR is abnormal in at least one coronary artery, the patient has obstructive epicardial CAD [2]. If hyperemic MBF is abnormal, but FFR is normal, CMD likely explains the hypoperfusion [3]. If both hyperemic MBF and FFR are normal, the patient was considered to have no or non-obstructive CAD [16,17]. A hyperemic MBF <2.30 ml/min/g in at least two adjacent segments and FFR ≤0.80 were considered abnormal [18,19]. Subtotal lesions and chronic total occlusions were scored as an abnormal FFR.

2.6. Endothelin-1 and renin analysis

Patients were fasting for at least 12 h, resting for at least 10 min, and sitting in an upright position before blood sampling was performed. The samples were drawn in the morning to avoid the influence of the circadian rhythm. Samples were centrifuged at 3500*g* for 15 min and stored at −80 °C. None of the samples were thawed and refrozen before analysis. Renin was quantified as a measure of RAAS activation using a radioimmunometric assay as reported by Ramsay et al. (Cisbio, Saclay, France) [20]. Endothelin-1 was measured using a Quantikine enzyme-linked immunosorbent assay (ELISA; R&D systems, Minneapolis, USA) [20].

2.7. Statistical analysis

Continuous variables are expressed as mean ± SD or median (interquartile range) where appropriate. Categorical variables are presented as frequencies with percentages. Normality of data was examined by means of QQ-plots. In the case of skewed data, data were log transformed to allow for parametric testing. The independent sample's T-test was used to compare means for continuous variables between two groups and the chi-square test for categorical variables. Means of the three patient categories were compared using one way analysis of variance (ANOVA). In case the overall F-test for the ANOVA was significant, *post hoc* pairwise comparisons between patient categories were performed with Bonferroni correction for multiple comparisons. The

relation between endothelin-1 and renin, CAC score, TPV and hyperemic MBF was assessed by Pearson's correlation coefficient. An analysis adjusted for baseline characteristics, and RAAS inhibiting therapy was additionally performed using multivariable linear regression. A *p*-value <0.05 was considered statistically significant. All analyses were performed using IBM SPSS (SPSS Statistics 26, IBM, Armonk, New York).

3. Results

3.1. Study population

[^{15}O]H_2O PET imaging and (quantitative) plaque analysis were available in 199 out of 205 patients. All 205 patients underwent coronary angiography with invasive FFR measurements. The majority of patients were male (64%), on average 58 ± 8.7 years old and 76 patients (37%) used a RAAS blocker. As expected, renin levels were higher in patients using RAAS inhibitors (mean logarithmic 1.32 ± 0.41 *vs.* 1.05 ± 0.30 pg/ml; $p = 0.01$). Baseline characteristics are given in Table 1, 2 and 3.

3.2. The association with atherosclerosis and plaque morphology

Renin was associated with CAC score (Pearson r = 0.22; $p < 0.01$) and TPV (Pearson r = 0.23; $p < 0.01$) (Fig. 1). Correlation plots for the relation between renin and CAC score and TPV stratified for RAAS inhibitor use are given in Fig. 2, showing correlations in the same direction but somewhat smaller ones in patients not using RAAS inhibitors. Endothelin-1 was not found to be associated with CAC score (Pearson r = 0.07; $p = 0.33$) or TPV (Pearson r = 0.04; $p = 0.55$) (Fig. 3). Fig. 4 shows higher renin levels in patients with high risk plaque compared to patients without high risk plaque (mean logarithmic renin 1.25 ± 0.43 *vs.* 1.12 ± 0.35 pg/ml; $p = 0.04$). In patients on RAAS inhibiting medication, renin was elevated in those with high risk plaque (n = 13) compared to patients without high risk plaque (n = 61; mean logarithmic renin 1.57 ± 0.55 *vs.* 1.27 ± 0.36 pg/ml; $p = 0.02$). This difference was not seen for patients not using RAAS inhibiting medication (mean logarithmic renin 1.12 ± 0.30 *vs.* 1.02 ± 0.30 pg/ml; $p = 0.10$ for high risk plaque and no high risk plaque, respectively). After correction for baseline characteristics including sex and the use of RAAS inhibitors, increased levels of renin remained independently associated with CAC score and TPV (Table 4). Endothelin-1 levels did not differ between patients with and without high risk plaque (mean logarithmic endothelin-1 0.30 ± 0.15 *vs.* 0.29 ± 0.16 pg/ml; $p = 0.83$, Fig. 5).

Table 1
Baseline characteristics; mean ± SD or N (%).

Characteristics	N = 205
Male	131 (64%)
Age, years	58.3 ± 8.7
BMI, kg/m^2	27.0 ± 3.8
Cardiovascular risk factors	
Diabetes mellitus	33 (16%)
Hypertension	96 (47%)
Hypercholesterolemia	81 (40%)
Current smoker	40 (20%)
Family history of CAD	106 (52%)
Medication	
Acetylsalicylic acid	178 (87%)
Beta-blocker	132 (64%)
Calcium channel blocker	61 (30%)
ACE-inhibitor	40 (20%)
Angiotensin-II receptor blocker	36 (18%)
Statin	158 (77%)
Symptoms	
Typical AP	71 (35%)
Atypical AP	72 (35%)
Aspecific chest pain	62 (30%)

ACE: angiotensin converting enzyme; AP: angina pectoris; ARB: angiotensin II receptor blocker BMI: body mass index; CAD: coronary artery disease.

Table 2
Baseline imaging and laboratory characteristics; mean ± SD, median (interquartile range) or N (%).

Baseline imaging and laboratory characteristics per patient	N = 205
Laboratory parameters	
Endothelin-1 (pg/ml)	2.11 (1.50–2.47)
Logarithmic endothelin-1 (pg/ml)	0.29 ± 0.15
Renin (pg/ml)	13.49 (7.97–22.88)
Logarithmic renin (pg/ml)	1.15 ± 0.37
[^{15}O] H_2O PET	
Global hMBF (ml/min/g)	2.74 ± 1.10
Ischemic PET (<2.3 ml/min/g)	106 (52.5%)
CCTA	
High risk plaque	47 (22.9%)
Napkin sign	20 (9.8%)
Low attenuation plaque	59 (28.8%)
Spotty calcifications	27 (13.2%)
Positive remodelling	52 (25.4%)
Total plaque volume (MM^3)	319.30 (72.78–707.93)
Coronary artery calcium score (HU)	170.10 (10.00–486.70)
Invasive coronary angiography	
Obstructive CAD (FFR ≤0.80 or (sub)total lesion)	98 (47.8%)

CAD: coronary artery disease; CCTA: coronary computed tomography angiography; hMBF: hyperemic myocardial blood flow; HU: hounsfield unit; PET: positron emission tomography.

3.3. The association with global hyperemic MBF

A weak to moderate negative correlation was seen for renin levels and global hyperemic MBF (Pearson r = −0.19; $p < 0.01$, Fig. 1). This correlation was seen in both patients using a RAAS inhibitor and patients not using a RAAS inhibitor (Fig. 2). After correction for baseline characteristics and RAAS inhibiting therapy, renin was not independently associated with global hyperemic MBF ($p = 0.23$, Table 4). Endothelin-1 was not related to global hyperemic MBF (Pearson r = 0.01; $p = 0.85$, Fig. 3).

3.4. Relation to coronary microvascular dysfunction and obstructive CAD

Fig. 6 shows that renin was elevated in patients with FFR based obstructive CAD (n = 92; 45.3%) when compared to no or non-obstructive CAD patients (n = 85; 41.9%, mean logarithmic renin 1.23 ± 0.36 *vs.* 1.06 ± 0.34 pg/ml; $p < 0.01$). Renin was not significantly elevated in CMD patients (n = 26; 12.8%) compared to patients with no/nonbstructive CAD (mean logarithmic renin 1.16 ± 0.41 *vs.* 1.06 ± 0.34 pg/ml; $p = 0.65$). Endothelin-1 levels did not differ between patients with obstructive CAD, CMD or with no or non-obstructive CAD (mean logarithmic endothelin-1: 0.29 ± 0.15, 0.27 ± 0.14 and 0.30 ± 0.16 pg/ml; $p = 0.64$). The same results were found if patients were categorized based on an abnormal FFR (≤0.80) and abnormal global hyperemic left ventricle perfusion <2.65 ml/min/g.

4. Discussion

This is the first study that provides an overview of the influence of RAAS activation and endothelin-1 on CAD in relation to calcified coronary atherosclerotic plaque, high risk plaque, hyperemic MBF and coronary microvascular dysfunction in stable chest pain patients. The main findings can be summarized as follows: higher levels of circulating renin indicative of RAAS activation are associated with CAD ranging from

Table 3
Baseline characteristics per patient group.

Characteristics	Obstructive CAD n = 92	CMD n = 26	Non-obstructive CAD n = 85	*p* value
Male	79 (86%)	18 (69%)	33 (39%)	<0.01
Age, years	58.9 ± 7.7	58.5 ± 8.6	57.9 ± 9.6	0.73
BMI, kg/m^2	27.2 ± 3.6	26.9 ± 3.9	26.8 ± 3.9	0.77
Cardiovascular risk factors				
Diabetes mellitus	18 (20%)	6 (23%)	8 (9%)	0.10
Hypertension	42 (46%)	16 (62%)	38 (45%)	0.30
Hypercholesterolemia	45 (50%)	12 (46%)	23 (27%)	<0.01
Current smoker	15 (16%)	8 (31%)	17 (20%)	0.26
Family history of CAD	47 (51%)	12 (46%)	45 (53%)	0.83
Medication				
Acetylsalicylic acid	84 (92%)	21 (81%)	72 (85%)	0.16
Beta-blocker	65 (71%)	16 (62%)	51 (60%)	0.26
Calcium channel blocker	29 (32%)	8 (31%)	24 (28%)	0.87
ACE-i/ARB	35 (39%)	10 (39%)	31 (37%)	0.96
Statin	78 (86%)	18 (69%)	60 (71%)	0.03
Symptoms				
Typical AP	38 (41%)	10 (39%)	23 (27%)	0.13
Atypical AP	28 (30%)	10 (39%)	33 (39%)	0.47
Aspecific chest pain	26 (28%)	6 (23%)	29 (34%)	0.49

BMI: body mass index; ACE-i: angiotensin converting enzyme inhibitor; AP: angina pectoris; ARB: angiotensin II receptor blocker; AP: angina pectoris; CAD: coronary artery disease; CMD: coronary microvascular dysfunction.

calcified plaque burden to adverse plaque characteristics but not with CMD, whereas endothelin-1 was not found to be associated with any of these parameters of atherosclerosis or CMD.

Studies have shown that the beneficial effect of RAAS inhibition is greater than expected by their ability to lower blood pressure alone [21]. Indeed, the renin-angiotensin II pathway has been considered a potent modulator of oxidative stress, coronary inflammation and endothelial dysfunction. Injured arteries recruit inflammatory cells by angiotensin II and these inflammatory cells produce angiotensin II, resulting in a positive feedback loop that accelerates atherosclerosis [3]. In fact, it is not completely unraveled to which extent locally produced renin-angiotensin contributes to the development of atherosclerosis compared to its uptake from the systemic circulation. Interestingly, we found that renin levels were significantly elevated in patients with high risk plaque, a finding in keeping with a previous experimental study conducted with *ApoE* knockout mice, which showed that angiotensin II induced atherosclerotic plaque vulnerability [22]. Our study adds to the body of existing literature that increased systemic renin levels were associated with total atherosclerotic burden as reflected by total CAC score, TPV, and more frequent high risk plaque features on CCTA.

Multiple studies have shown that RAAS activation is related to hyperemic MBF and endothelial independent CMD. A placebo controlled study using [^{15}O]H_2O PET derived myocardial perfusion demonstrated a significant improvement of myocardial blood flow in ischemic regions of symptomatic chest pain patients (n = 18) after ACE inhibition quinaprilat was administered intravenously [23]. More recently, a small (n = 14) NH_3 PET study showed a significant improvement of hyperemic myocardial perfusion in hypertrophic cardiomyopathy patients with CMD after 6 months of treatment with perindopril [24]. Indeed, we found a relation between renin and coronary atherosclerosis (i.e. more coronary calcium and/or plaque) and one would expect a lower perfusion in patients with atherosclerosis. In univariable analysis, we found a weak to moderate relation between renin and global hyperemic MBF, however, this relation was not significant after correction for covariables. Although we did not find a convincing relation between renin and myocardial perfusion, we cannot exclude this relationship either based on the results of the univariable analysis. Nevertheless, a large overlap is seen between renin levels of patients with and without a large atherosclerotic burden, high risk plaque, myocardial ischemia and CMD, which may in part be attributed to the complex interaction of renin-angiotensin II with cardioprotective cytokines such as adiponectin [25].

Endothelin-1 is the most potent endogenous vasoconstrictor hitherto discovered, and several studies have linked it to development of atherosclerosis and CMD. Elevated levels of endothelin-1 were seen in limb ischemia, coronary graft failure and at plaque sites with high macrophage content. Interestingly, its vasoconstrictor effects are more potent in the coronary vascular bed owing to the unique adaptive characteristics of the coronary microvasculature to epicardial flow in comparison to other arterial sites. A study by Lerman and colleagues demonstrated a significant correlation between plasma endothelin-1 and the number of sites of atherosclerotic disease involvement (r = 0.89; $p < 0.001$) in symptomatic atherosclerotic patients [7]. In addition, in a study of 510 patients, serum endothelin-1 levels were independently associated with coronary calcifications in symptomatic chest pain patients, and Reriani et al. showed, in a double blind placebo controlled trial, that endothelial function improved after endothelin-A

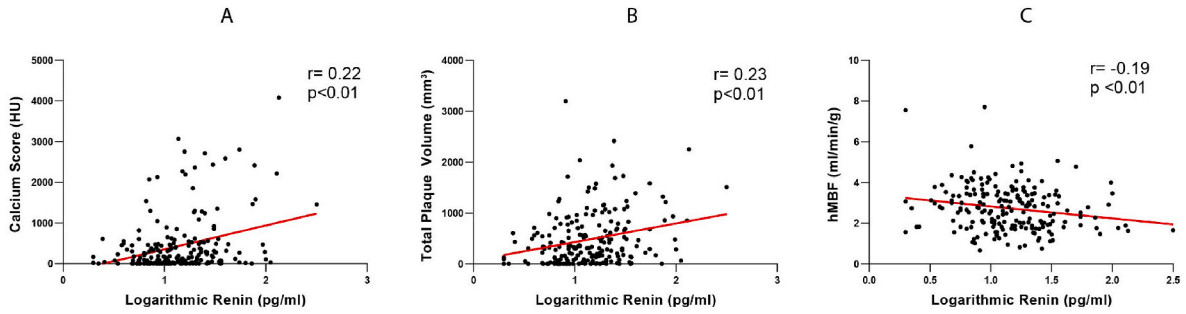

Fig. 1. Renin and its correlation with the coronary artery calcium score (A), total plaque volume (B) and global hyperemic MBF (C) on a per patient basis. MBF: myocardial blood flow; HU: hounsfield units.

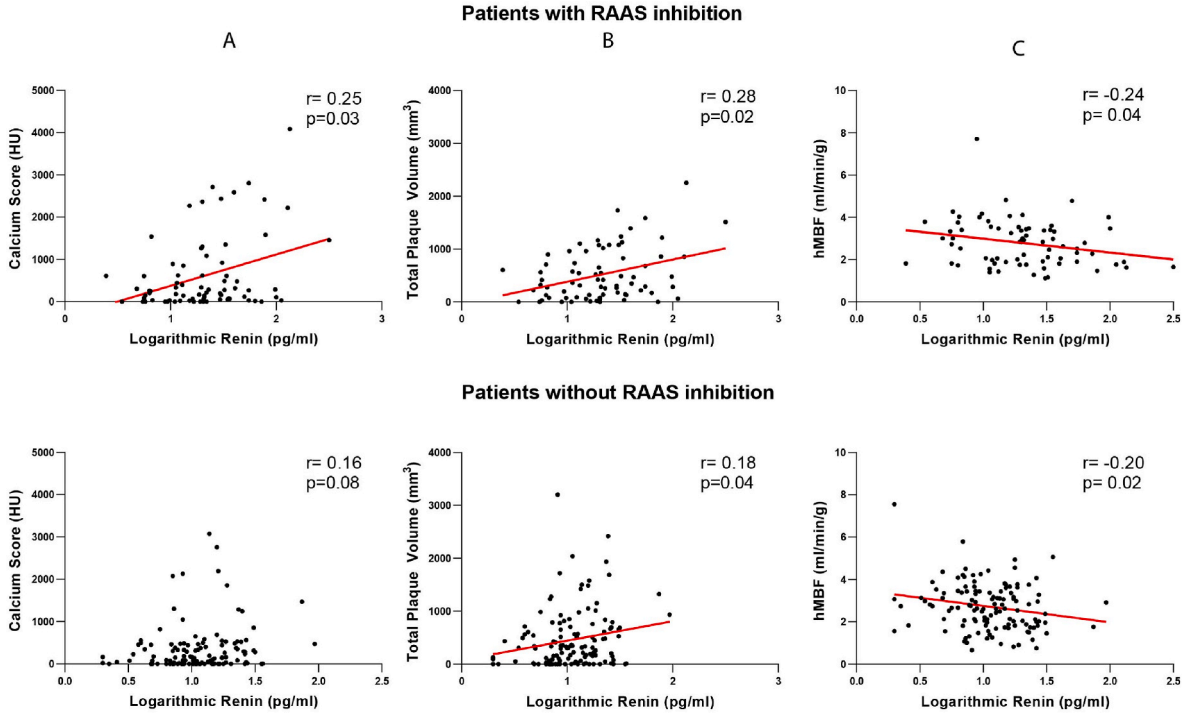

Fig. 2. Renin and its correlation with the coronary artery calcium score (A), total plaque volume (B) and global hMBF (C) on a per patient basis, split for patients using RAAS inhibitors.
hMBF: hyperemic myocardial blood flow; HU: hounsfield units; RAAS: renin angiotensin aldosterone system.

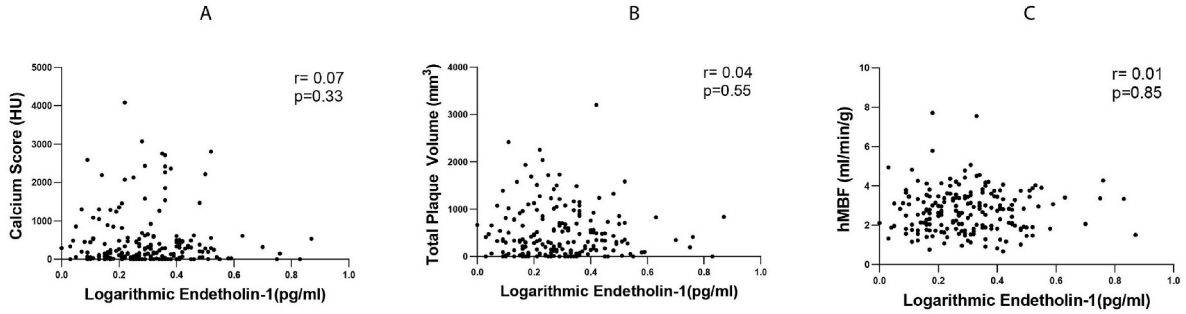

Fig. 3. Endothelin-1 and its correlation with the coronary artery calcium score (A), total plaque volume (B) and global hMBF (C). hMBF: hyperemic myocardial blood flow; HU: hounsfield units.

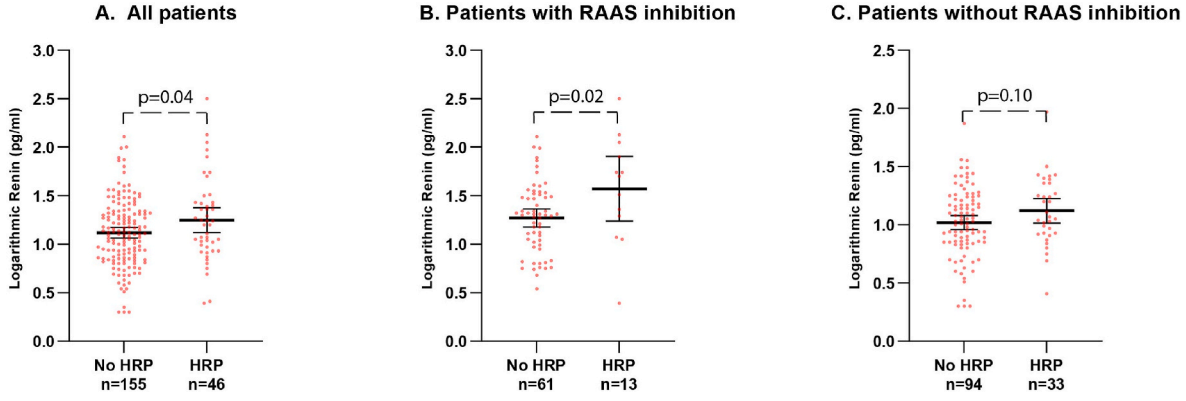

Fig. 4. Renin levels depicted (mean with 95% confidence interval) for patients with high risk plaques *versus* patients without high risk plaques for all patients (A) and split for RAAS inhibition (B and C).
HRP: high risk plaque; RAAS: renin angiotensin aldosterone system.

Table 4
The association between patient variables and coronary calcium score, total plaque volume and global hyperemic MBF.

Patient variables	Calcium score			TPV			Global hyperemic MBF		
	Stand Beta	Unstand beta (95% CI)	*p* value	Stand beta	Unstand beta (95% CI)	*p* value	Stand beta	Unstand beta (95% CI)	*p* value
Demographics									
Male	0.22	324.62 (141.70–507.54)	<0.01	0.33	359.56 (217.02–502.09)	<0.01	−0.53	−1.22 (−1.49 to −0.95)	<0.01
BMI (per kg/m^2 increase)	0.14	27.17 (2.75–51.59)	0.03	0.11	15.20 (−3.82 to 34.21)	0.12	0.07	0.02 (-0.02 to 0.06)	0.26
Age (per year increase)	0.32	25.61 (15.64–35.58)	<0.01	0.29	18.03 (10.20–25.86)	<0.01	−0.19	−0.02 (−0.40 to 0.01)	<0.01
RAAS inhibition	0.03	40.52 (−192.12 to 272.16)	0.73	−0.11	−116.25 (−298.06 to 65.57)	0.21	0.17	0.39 (0.04–0.74)	0.03
Cardiovascular risk factors									
Diabetes mellitus	0.18	337.68 (86.52–588.83)	<0.01	0.08	117.59 (−76.86 to 312.04)	0.23	−0.08	−0.25 (−0.63 to 0.13)	0.19
Hypertension	−0.03	−41.84 (−257.86 to 174.18)	0.70	0.05	48.49 (−119.53 to 216.52)	0.57	−0,19	−0.41 (−0.74 to −0.09)	0.01
Hypercholesterolemia	0.08	101.96 (−78.23 to 282.16)	0.27	0.13	138.03 (−3.18 to 279.25)	0.06	−0.15	−0.34 (−0.61 to −0.06)	0.02
Current smoker	−0.07	−123.57 (-343.48 to 96.35)	0.27	0.02	24.98 (−145.01 to 194.97)	0.77	−0.06	−0.17 (−0.50 to 0.16)	0.31
Vasoactive substances									
Log renin (per pg/ml increase)	0.21	392.04 (137.67–646.41)	<0.01	0.18	249.86 (51.58–448.13)	0.02	−0.08	−0.23 (−0.62 to 0.15)	0.23

Regression coefficients from multivariable linear regression models with calcium score, TPV and hyperemic MBF as dependent variables. BMI: body mass index; MBF: myocardial blood flow; RAAS: renin angiotensin aldosterone system; Stand beta: standardized beta; TPV: total plaque volume; Unstand beta: unstandardized beta.

receptor antagonist therapy [26,27]. As such, endothelin-1 has been considered an important peptide in CMD and the coronary atherosclerotic process [28]. However, the present findings are in contrast to these prior observations as we did not find a relationship between endothelin-1, CAC score, TPV, hyperemic MBF or CMD. Of note, locally synthesized endothelin-1, namely in the endothelium, is 100-fold higher compared to circulating plasma levels. This suggests a strong paracrine mode of action of endothelin-1 enabling it to react swiftly to alternating local hemodynamics. Our study measured systemic endothelin-1 levels while endothelin-1 produced in the coronary vascular bed possibly plays a decisive role in coronary atherosclerosis by stimulating the formation of reactive oxygen species and hence coronary inflammation [28]. Despite the strong paracrine action of endothelin-1, most studies measure systemic endothelin-1 levels because therapeutic options are focused on systemic inhibition of endothelin-1. Furthermore, endothelin-1 stimulates cell proliferation and as such acceleration of the atherosclerotic process [28]. Importantly, the interaction of endothelin-1 with intracoronary produced prostaglandins and nitric oxide has not been investigated and may in part explain the lack of association of plasma endothelin-1 and measures of coronary atherosclerosis and CMD [29]. The upcoming randomized controlled trial Precision Medicine With Zibotentan in Microvascular Angina (NCT04097314) should further clarify whether or not blocking the endothelin receptor type A by administration of zibotentan improves exercise capacity [30]. This trial will increase our understanding of the potential role of endothelin-1 in the pathogenesis and therapeutic options pertaining to CMD.

4.1. Limitations

Our study has several limitations. Firstly, this is a cross-sectional study with a limited sample size. By design, cross-sectional studies are useful studies to assess associations, however, these studies cannot be used to determine causality. Secondly, we have measured renin and endothelin-1 at a single point in time. Caution must be taken to interpret multifactorial biomarkers like renin and endothelin-1 in the context of chronic diseases like atherosclerosis or CMD. Third, adenosine was used as the hyperemic agent for PET imaging and FFR measurement. Because adenosine is an endothelial independent vasodilator, patients with vasospastic angina based on endothelial dysfunction could have been classified into the no or non-obstructive CAD group. We did not perform intracoronary acetylcholine testing to account for this. Furthermore, we have analysed circulating, thus exocrine, renin and endothelin-1. Based on our measurements, we cannot elaborate on the paracrine activity of endothelin-1 and renin. Considering these inherent limitations to our study, all results should be considered exploratory, and replication studies are needed.

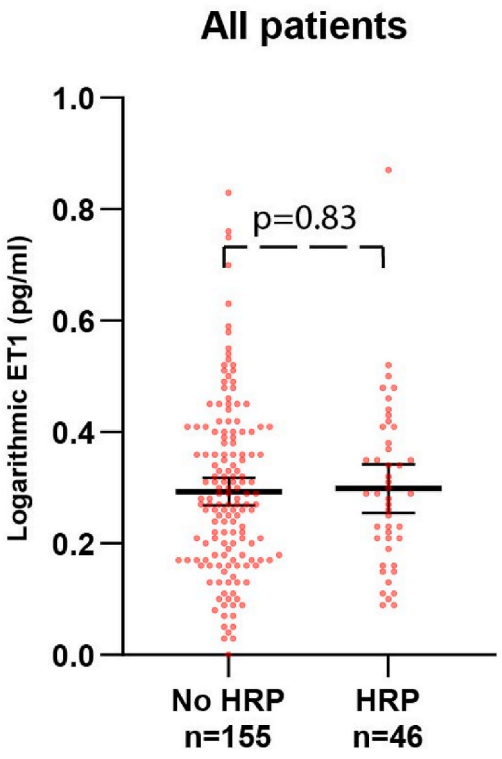

Fig. 5. Levels of endothelin-1 depicted (mean with 95% confidence interval) for patients with high risk plaques versus patients without high risk plaques. ET1: endothelin-1 HRP: high risk plaque; ET1: endothelin-1.

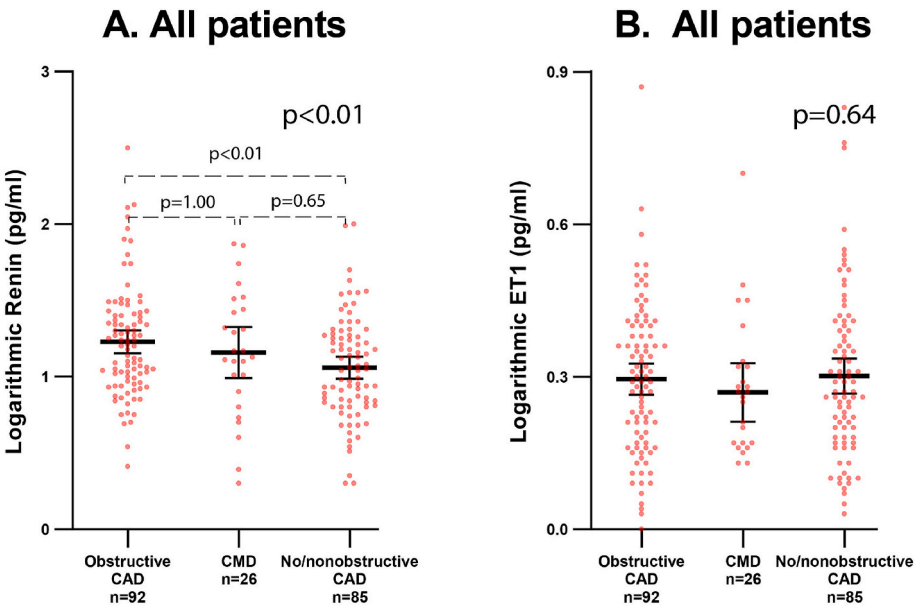

Fig. 6. Levels of renin and endothelin-1 (meant with 95% confidence interval) stratified by the three patient categories (mean with 95% confidence interval). An FFR≤0.80 and hyperemic MBF <2.30 ml/min/g in at least two adjacent segments ml/min/g were considered abnormal. CAD: coronary artery disease; CMD: coronary microvascular dysfunction; ET1: endothelin-1; FFR: fractional flow reserve; hMBF: hyperemic myocardial blood flow.

4.2. Conclusions

Our report provides evidence demonstrating that in stable chest pain patients, increased systemic RAAS activation is associated with the extent of coronary artery calcification, total plaque volume and high risk plaque features but not endothelial independent coronary microvascular dysfunction. In addition, our exploratory findings indicate that circulating endothelin-1 is not associated with any of these atherosclerotic parameters nor coronary microvascular dysfunction.

CRediT authors contribution statement

Ruurt A. Jukema: Conceptualization, Writing – Original Draft-, Writing – Review & Editing; Ruben W. de Winter: Conceptualization, Writing – Review & Editing; Pepijn A. van Diemen: Conceptualization, Writing – Review & Editing; Roel S. Driessen: Conceptualization, Writing – Review & Editing; A.H. Jan Danser: Resources, Writing – Review & Editing; Ingrid M. Garrelds: Resources, Writing – Review & Editing; Pieter G. Raijmakers: Conceptualization, Writing – Review & Editing; MDa; Peter M. van de Ven: Formal analysis, Writing – Review & Editing; Paul Knaapen: Conceptualization, Writing – Review & Editing; Ibrahim Danad: Conceptualization, Writing – Review & Editing; Guus A. de Waard: Conceptualization, Writing – Review & Editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] M. Echavarria-Pinto, J. Escaned, E. Macias, M. Medina, N. Gonzalo, R. Petraco, et al., Disturbed coronary hemodynamics in vessels with intermediate stenoses evaluated with fractional flow reserve: a combined analysis of epicardial and microcirculatory involvement in ischemic heart disease, Circulation 128 (24) (2013) 2557–2566.

[2] G.A. de Waard, C.M. Cook, N. van Royen, J.E. Davies, Coronary autoregulation and assessment of stenosis severity without pharmacological vasodilation, Eur. Heart J. 39 (46) (2018) 4062–4071.

[3] C.M. Ferrario, W.B. Strawn, Role of the renin-angiotensin-aldosterone system and proinflammatory mediators in cardiovascular disease, Am. J. Cardiol. 98 (1) (2006) 121–128.

[4] B. Hornig, U. Landmesser, C. Kohler, D. Ahlersmann, S. Spiekermann, A. Christoph, et al., Comparative effect of ace inhibition and angiotensin II type 1 receptor antagonism on bioavailability of nitric oxide in patients with coronary artery disease: role of superoxide dismutase, Circulation 103 (6) (2001) 799–805.

[5] K. Rakusan, N. Cicutti, A. Maurin, D. Guez, P. Schiavi, The effect of treatment with low dose ACE inhibitor and/or diuretic on coronary microvasculature in stroke-prone spontaneously hypertensive rats, Microvasc. Res. 59 (2) (2000) 243–254.

[6] A. Lerman, D.R. Holmes Jr., M.R. Bell, K.N. Garratt, R.A. Nishimura, J. C. Burnett Jr., Endothelin in coronary endothelial dysfunction and early atherosclerosis in humans, Circulation 92 (9) (1995) 2426–2431.

[7] A. Lerman, B.S. Edwards, J.W. Hallett, D.M. Heublein, S.M. Sandberg, J. C. Burnett Jr., Circulating and tissue endothelin immunoreactivity in advanced atherosclerosis, N. Engl. J. Med. 325 (14) (1991) 997–1001.

[8] I. Danad, P.G. Raijmakers, R.S. Driessen, J. Leipsic, R. Raju, C. Naoum, et al., Comparison of coronary CT angiography, SPECT, PET, and hybrid imaging for diagnosis of ischemic heart disease determined by fractional flow reserve, JAMA Cardiol. 2 (10) (2017) 1100–1107.

[9] G.A. Diamond, J.S. Forrester, Analysis of probability as an aid in the clinical diagnosis of coronary-artery disease, N. Engl. J. Med. 300 (24) (1979) 1350–1358.

[10] R.S. Driessen, W.J. Stuijfzand, P.G. Raijmakers, I. Danad, J.K. Min, J.A. Leipsic, et al., Effect of plaque burden and morphology on myocardial blood flow and fractional flow reserve, J. Am. Coll. Cardiol. 71 (5) (2018) 499–509.

[11] N. Nerlekar, F.J. Ha, C. Cheshire, H. Rashid, J.D. Cameron, D.T. Wong, et al., Computed tomographic coronary angiography-derived plaque characteristics predict major adverse cardiovascular events: a systematic Review and meta-analysis, Circ. Cardiovasc. Imag. 11 (1) (2018), e006973.

[12] H.J. Harms, P. Knaapen, S. de Haan, R. Halbmeijer, A.A. Lammertsma, M. Lubberink, Automatic generation of absolute myocardial blood flow images using [15O]H2O and a clinical PET/CT scanner, Eur. J. Nucl. Med. Mol. Imag. 38 (5) (2011) 930–939.

[13] M.D. Cerqueira, N.J. Weissman, V. Dilsizian, A.K. Jacobs, S. Kaul, W.K. Laskey, et al., Standardized myocardial segmentation and nomenclature for tomographic imaging of the heart - a statement for healthcare professionals from the Cardiac Imaging Committee of the Council on Clinical Cardiology of the American Heart Association, Circulation 105 (4) (2002) 539–542.

[14] I. Danad, V. Uusitalo, T. Kero, A. Saraste, P.G. Raijmakers, A.A. Lammertsma, et al., Quantitative assessment of myocardial perfusion in the detection of significant coronary artery disease: cutoff values and diagnostic accuracy of quantitative [(15) O]H2O PET imaging, J. Am. Coll. Cardiol. 64 (14) (2014) 1464–1475.

[15] M.J. Bom, P.A. van Diemen, R.S. Driessen, H. Everaars, S.P. Schumacher, J. T. Wijmenga, et al., Prognostic value of [15O]H2O positron emission tomography-derived global and regional myocardial perfusion, Eur. Heart J. Cardivasc. Imag. 21 (7) (2020) 777–786, https://doi.org/10.1093/ehjci/jez258.

[16] N.P. Johnson, R.L. Kirkeeide, K.L. Gould, Is discordance of coronary flow reserve and fractional flow reserve due to methodology or clinically relevant coronary pathophysiology? JACC Cardiovasc. Imag. 5 (2) (2012) 193–202.

[17] M. Echavarria-Pinto, J. Escaned, E. Macías, M. Medina, N. Gonzalo, R. Petraco, et al., Disturbed coronary hemodynamics in vessels with intermediate stenoses evaluated with fractional flow reserve: a combined analysis of epicardial and

microcirculatory involvement in ischemic heart disease, Circulation 128 (24) (2013) 2557–2566.
[18] P.A. Tonino, B. De Bruyne, N.H. Pijls, U. Siebert, F. Ikeno, M. van' t Veer, et al., Fractional flow reserve versus angiography for guiding percutaneous coronary intervention, N. Engl. J. Med. 360 (3) (2009) 213–224.
[19] I. Danad, V. Uusitalo, T. Kero, A. Saraste, P.G. Raijmakers, A.A. Lammertsma, et al., Quantitative assessment of myocardial perfusion in the detection of significant coronary artery disease: cutoff values and diagnostic accuracy of quantitative [(15) O]H2O PET imaging, J. Am. Coll. Cardiol. 64 (14) (2014) 1464–1475.
[20] S.G. Ramsay, K.D. Dagg, I.C. McKay, B.J. Lipworth, C. McSharry, N.C. Thomson, Investigations on the renin-angiotensin system in acute severe asthma, Eur. Respir. J. 10 (12) (1997) 2766–2771.
[21] P. Verdecchia, G. Reboldi, F. Angeli, R. Gattobigio, M. Bentivoglio, L. Thijs, et al., Angiotensin-converting enzyme inhibitors and calcium channel blockers for coronary heart disease and stroke prevention, Hypertension (Dallas, Tex : 1979) 46 (2) (2005) 386–392.
[22] L. Mazzolai, M.A. Duchosal, M. Korber, K. Bouzourene, J.F. Aubert, H. Hao, et al., Endogenous angiotensin II induces atherosclerotic plaque vulnerability and elicits a Th1 response in ApoE-/- mice, Hypertension (Dallas, Tex : 1979) 44 (3) (2004) 277–282.
[23] C.A. Schneider, E. Voth, D. Moka, F.M. Baer, J. Melin, A. Bol, et al., Improvement of myocardial blood flow to ischemic regions by angiotensin-converting enzyme inhibition with quinaprilat IV: a study using [15O] water dobutamine stress positron emission tomography, J. Am. Coll. Cardiol. 34 (4) (1999) 1005–1011.
[24] M.G. De Gregorio, C. Fumagalli, A. Tomberli, K. Baldini, G. Puccini, G. d'Amati, et al., Myocardial blood flow in patients with hypertrophic cardiomyopathy receiving perindopril (CARAPaCE): a pilot study, J. Cardiovasc. Med. 22 (6) (2021) 511–513.
[25] A.Y. Jang, P.E. Scherer, J.Y. Kim, S. Lim, K.K. Koh, Adiponectin and cardiometabolic trait and mortality: where do we go? Cardiovasc. Res. (2021) 1–11.
[26] P. Qing, X.L. Li, Y. Zhang, Y.L. Li, R.X. Xu, Y.L. Guo, et al., Association of big endothelin-1 with coronary artery calcification, PLoS One 10 (11) (2015), e0142458.
[27] M. Reriani, E. Raichlin, A. Prasad, V. Mathew, G.M. Pumper, R.E. Nelson, et al., Long-term administration of endothelin receptor antagonist improves coronary endothelial function in patients with early atherosclerosis, Circulation 122 (10) (2010) 958–966.
[28] T.M. Kolettis, M. Barton, D. Langleben, Y. Matsumura, Endothelin in coronary artery disease and myocardial infarction, Cardiol. Rev. 21 (5) (2013) 249–256.
[29] J.H. Traverse, D. Judd, R.J. Bache, Dose-dependent effect of endothelin-1 on blood flow to normal and collateral-dependent myocardium, Circulation 93 (3) (1996) 558–566.
[30] A.J. Morrow, T.J. Ford, K. Mangion, T. Kotecha, R. Rakhit, G. Galasko, et al., Rationale and design of the medical research council's precision medicine with zibotentan in microvascular angina (PRIZE) trial, Am. Heart J. 229 (2020) 70–80.