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Original Article

Early and late response-to-injury in patients undergoing transradial coronary angiography: arterial remodeling in smokers

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Abstract: Objectives: To investigate the effect of smoking on vascular response to transradial coronary angiography (TCA). Background: Cigarette smoking is the most important modifiable cardiovascular risk factor associated with endothelial dysfunction. Methods: Radial artery flow-mediated vasodilation (RA-FMD), local stiffness (fractional diameter change), intima-media thickness (IMT), luminal and external arterial diameter were measured in 40 current smokers (CS) and former smokers (FS) at 6-14 months at the site of previous TCA and contralateral control artery. Vascular regenerative capacity was studied as chemotactic cell migration *in vitro* and *ex vivo* (n=10) and the time course of endothelial functional recovery following RA-FMD up to 72 h after TCA (n=10). Results: At 10 ± 3 months after TCA, subjects exhibited significant local stiffening and increased IMT as compared to the control arm. These late structural changes were significantly more pronounced in CS as compared to FS. IMT thickening correlated with packyears, number of daily cigarettes, and inversely with RA-FMD. Nitric oxide synthase (NOS)-dependent chemotaxis of CS' circulating angiogenic cells was impaired. *Ex vivo* incubation of endothelial cells with CS' plasma inhibited NOS-dependent endothelial wound closure and chemotaxis. *In vivo*, TCA acutely decreased RA-FMD. At 24 h, RA-FMD had recovered in FS but remained impaired at 24 h and only recovered at 48 h in CS. Conclusion: In active smokers, transradial coronary angiography is associated with delayed early recovery from transient endothelial dysfunction, decreased NOS-dependent vascular regeneration, and late arterial remodeling pointing towards potential harmful effects of transradial coronary angiography on vascular function in distinct subsets of patients.

Keywords: Smoking, intima media thickness, transradial coronary angiography, endothelial function, prevention

Introduction

Ever since the first successful diagnostic transradial coronary catheterization (TCA) by Campeau in 1989, the radial artery (RA) approach has gained increasing acceptance and has become a standard approach in most centers. TCA was shown to be safe with lower rates of access site complications, shorter hospital stay, and improved patient comfort as compared with the transfemoral access [1]. Nevertheless, radial artery occlusions might be underestimated at discharge [2] and the injury inflicted by the radial sheath may lead to later intimal hyperplasia or even occlusion [3], potentially limiting the quality of the artery for later use as a bypass graft, or dialysis shunt. Recently, we have shown that TCA leads to an acute, yet transient, impairment of endothelium-dependent vasodilation of the radial and brachial artery [4]. Furthermore, our data suggested that the regain of vasodilator function was significantly slowed in smokers as compared to the non-smokers. It was previously shown, that active smoking and passive smoke exposure not only impairs endothelial function, but also cellular processes important for endothelial regeneration and maintenance. *In vitro*, cigarette smoke causes generation of reactive oxygen species, impairs nitric oxide (NO) production, and causes apoptosis and activation of endothelial cells, all of which may contribute to the vascular toxicity of cigarette smoke [5]. We have shown that even plasma taken from

non-smokers who were briefly exposed to second hand smoke blunts eNOS-dependent chemotaxis in circulating angiogenic cells (CACs) and endothelial cells [6]. However, whether smoking in the context of a defined mechanical injury to the RA during TCA negatively affects the homeostatic repair processes necessary to inhibit long term consequences by means of structural vascular remodeling is unclear.

Therefore, the aim of the study was to investigate the effect of current smoking on the late arterial remodeling in response to injury of the RA during TCA. To investigate the impact of smoking status on early regenerative response, we performed cell migration experiments *in vitro* and *ex vivo* and followed the early recovery of endothelial function.

Materials and methods

Study population

In a first series (Series 1) we recruited patients, that had undergone first time elective TCA with a transradial access at 6-14 months prior to screening date that were current smokers (CS, n=17) or former smokers (FS, n=23) as defined by smoking abstinence of >1 year prior to TCA. In this group, late arterial remodeling was studied. In a second (Series 2(a), n=10) and third series (Series 2(b), n=10), we recruited CS and FS that were scheduled for first time elective cardiac catheterization. Blood was drawn from subjects in series 2(a) to study regenerative processes *in vitro* and *ex vivo*. The time course of endothelial functional recovery was studied in series 2(b). See **Figure 1** for study flow.

Inclusion criteria were the indication for a TCA by a cardiologist and a positive smoking history. Patients were excluded from the study, if they had undergone previous radial cannulations or had an abnormal Allen test consistent with insufficient ulnar collateral supply. Other exclusion criteria were acute inflammation (C-reactive protein >0.5 mg/dl), malignancies, heart rhythm other than sinus rhythm, and heart failure New York Heart Association functional class III to IV, and terminal renal failure. CAD patients were on standard optimal medical therapy with statin, beta-blocker, ACE-inhibitor, aspirin, or clopidogrel. The study protocol was approved by the ethics committee of the Heinrich Heine University Duesseldorf.

Study protocol

Series 1 – Late arterial remodeling: In a first series (total n=40, CS n=17, FS n=23), we investigated late arterial structural remodeling as fractional diameter change (FDC), intima-media thickness (IMT), luminal and external arterial diameter, and radial artery endothelial function by flow-mediated vasodilation (RA-FMD) in patients that had undergone first TCA at 6-14 month prior to inclusion. Vascular ultrasound exams included measurements of the cannulated radial artery in CS and FS (inter-individual control) and of the contralateral control arm without an intervention (intra-individual control).

Series 2 – (a) In vitro/ex vivo regenerative capacity: In a second group of CS (n=5) and FS (n=5), we quantitated the number of CACs in circulating blood and determined the migratory capacity after *ex vivo* culture and collected plasma. We tested the impact of the plasma on endothelial wound closure and endothelial cells migratory capacity *ex vivo*.

(b) Early recovery of endothelial function: We studied in a third series of CS (n=5) and FS (n=5) the early time course of RA-FMD at -1 h (baseline) up to 72 h after TCA.

Ultrasound measurements of RA-FMD, arterial diameters, IMT, and FDC

FMD was measured as previously described [6]. Briefly, the diameter and flow velocity of the RA was measured using a 12 MHz transducer (Vivid I, GE) and automatic edge-detection software (Brachial Analyzer, Medical Imaging Applications, Iowa City, Iowa) yielding standard deviations of mean differences between repeated measurements of less than 1%. Reactive hyperemia was induced by 5 min of lower arm occlusion with a sphygmomanometric cuff inflated to 200 mmHg. After cuff deflation, 20, 40, 60, and 80 sec, the diameter was assessed and FMD calculated as maximal relative diameter gain relative to baseline. External diameters reflected the media adventitia interface. Internal/luminal arterial diameters were calculated as diastolic external diameter-(2*IMT). IMT was measured with an automatic contour detection software between the intimal and adventitial layers (Vivid I, GE) at identical sites used for RA-FMD measurements. Local

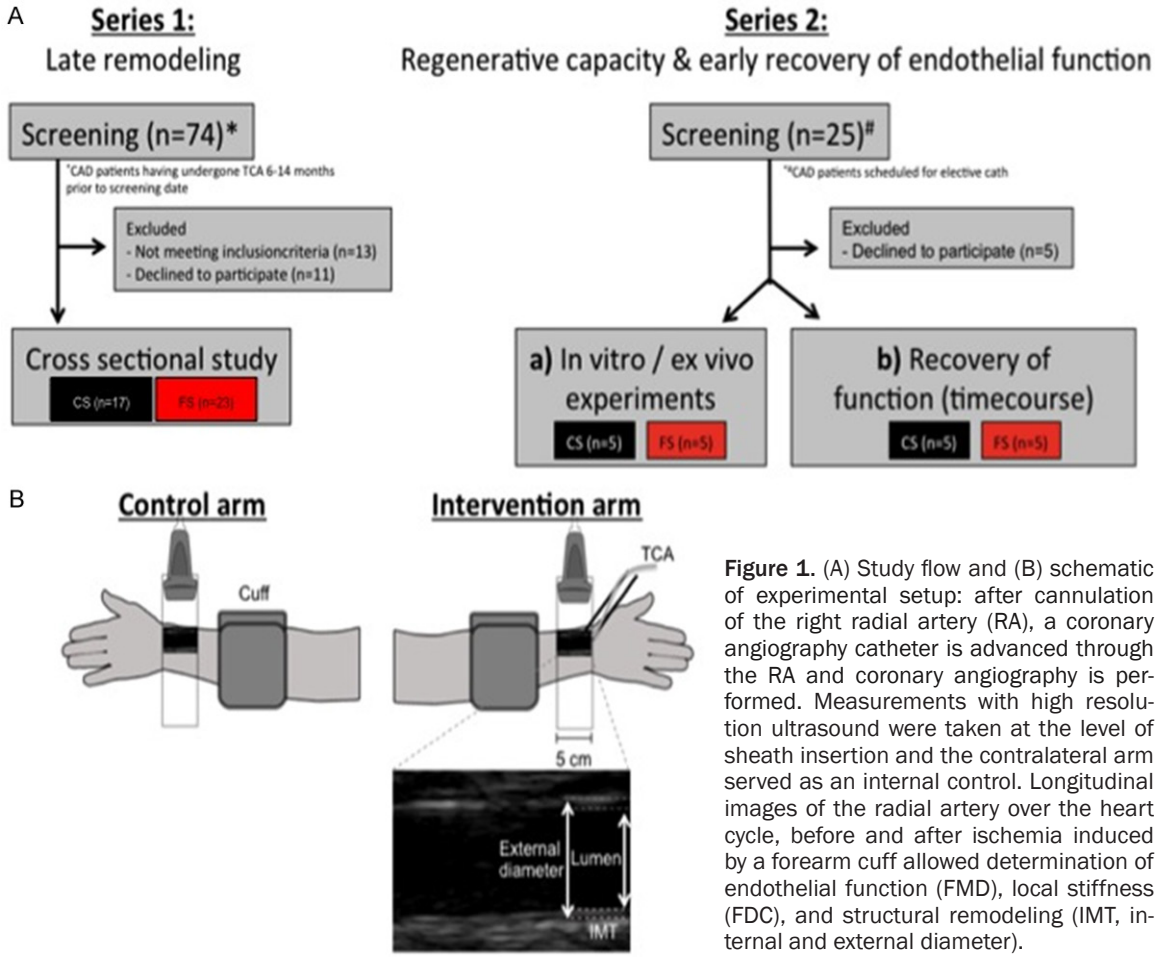

Figure 1. (A) Study flow and (B) schematic of experimental setup: after cannulation of the right radial artery (RA), a coronary angiography catheter is advanced through the RA and coronary angiography is performed. Measurements with high resolution ultrasound were taken at the level of sheath insertion and the contralateral arm served as an internal control. Longitudinal images of the radial artery over the heart cycle, before and after ischemia induced by a forearm cuff allowed determination of endothelial function (FMD), local stiffness (FDC), and structural remodeling (IMT, internal and external diameter).

arterial stiffness was determined at the site of RA-FMD measurements as a fractional diameter change (FDC) during the heart cycle and calculated as $diameter_{systolic}$-$diameter_{diastolic}$/ $diameter_{diastolic}$.

Endothelial cell and CAC experiments

In a subgroup of CS and FS (Series 2(a), n=10), CAC numbers and functional activity were determined in venous blood samples taken into heparinized vacutainer tubes (R&D) from a cubital vein at hospital admission before TCA. CAC number in whole blood was measured by flow-cytometry as CD34/KDR double-positive cells in the lympho-mononuclear cell gate [6, 7]. Functional CAC characterization was performed after *ex vivo* expansion. Peripheral blood mononuclear cells (PBMCs) were isolated based on the Ficoll method (Vacutainer CPT, Becton Dickinson, Franklin Lakes, NJ) and cultured for 7 days on fibronectin-coated plates. To confirm the endothelial phenotype and survival, we performed fluorescent staining to detect lectin-binding and acLDL-uptake. Chemotaxis towards a VEGF gradient (Sigma, 50 ng/ml in EBM-2, 0.5% BSA) was quantified using a modified Boyden chamber. CACs were plated in the upper of two chambers (Corning Transwell) and number of migrated cells counted on the lower side of the dividing membrane after 6 h.

To test whether CS plasma inhibited regenerative capacity of endothelial cells, plasma was obtained from venous blood of CS and FS. Scratch assays and cell migration assays were performed with HUVECs (Lonza, cultured to maximum of passage 3) incubated with (a) basal cell medium, (b) plasma of CS, and (c) FS. Cell migration was quantified by a transwell chemotaxis assay using a modified Boyden chamber. Migration of both CACs and HUVECs was measured as follows: cells ($2*10^4$) were

Table 1. Clinical and procedural characteristics of study population; series 1: Late remodeling (mean ± SD)

A.	Subject characteristics	CS	FS	*p*
	n (male/female)	17/0	21/2	
	Age (yrs)	58 ± 11	60 ± 10	*0.545*
	CAD (1, 2, 3)	11/2/6	14/3/7	
	PAD	0	0	
	Carotid disease	0	0	
	BMI (kg/m²)	27 ± 2	27 ± 3	*0.493*
	GFR (ml/min)	79 ± 18	79 ± 15	*0.946*
	CRP (mg/dl)	0.3 ± 0.6	0.3 ± 0.4	*0.765*
	HR (bpm)	66 ± 7	68 ± 6	*0.271*
	SBP (mmHg)	132 ± 8	129 ± 12	*0.332*
	DBP (mmHg)	83 ± 9	82 ± 7	0.675
	Packyears (n)	44 ± 20	32 ± 20	*0.057*
	HbA1c	5.9 ± 0.6	5.7 ± 1.3	*0.592*
	Total cholesterol (mg/dl)	202 ± 37	197 ± 25	*0.649*
	LDL (mg/dl)	136 ± 38	136 ± 15	*0.990*
	HDL (mg/dl)	48 ± 5	49 ± 4	*0.839*
	Beta-blocker (%)	100	82	
	Statin (%)	90	82	
	ACEI/ARB (%)	90	94	
	Clopidogrel (%)	90	53	
	Aspirin (%)	100	92	
B.	Procedural characteristics	CS	FS	*p*
	Time after TCA (months)	10 ± 6	10 ± 6	*0.868*
	Elective TCA (%)	100	100	
	Stable CAD (%)	100	100	
	NSTEMI/STEMI (%)	0	0	
	5F sheath (%)	76	74	
	6F sheath (%)	24	26	
	Irradiation time (min)	11 ± 14	10 ± 10	*0.750*
	Contrast volume (ml)	53 ± 40	54 ± 34	0.940
	Dose-area product (mGy*cm²)	3,601 ± 3,569	3,485 ± 2,489	*0.916*
	Number of catheters (n)	3.7 ± 0.8	3.3 ± 0.6	*0.442*
	Heparin (U/l)	3,700 ± 1,100	3,480 ± 940	*0.880*
	PCI (%)	33	22	
	$GP_{IIaIIIb}$-inhibitor (%)	6%	4%	

plated in EBM-2 medium (without other supplements, containing 63 mg/l L-arginine) after supplementation of (a) either 0.5% BSA, (b) 10% plasma from smokers (containing approximately 5% albumin), and (c) 10% plasma from non-smokers in the upper of 2 chambers divided by a membrane with 8-µm pores (Corning Transwell). We tested the chemotactic properties of vascular endothelial growth factor (VEGF, Sigma) at 50 ng/ml added to the lower chamber; the NOS inhibitor NG-nitro-L-arginine (100 µmol/l) was added to both the upper and lower chamber: The number of migrated cells was determined on 5 random 100x optical fields per membrane after 6h incubation. To distinguish the effects on chemokinetic from chemotactic capacity, BSA or plasma were added to upper and lower chambers. Scratch assays were performed in confluent HUVECs on fibronectin coated cell culture slides (Chambertech). A pipette tip was used to induce a scratch. Closure of the endothelial wound was evaluated until completion by blinded investigators in hourly intervals using an inverted microscope.

Statistical analyses

Results are expressed as means ± standard deviation (SD). Comparisons between groups were analyzed by ANOVA (gateway test) and, if significant, consecutive post-hoc test (Bonferroni) performed. Intra-individual comparisons were analyzed using repeated measurements ANOVA. Linear relationships between continuous variables were expressed as Pearson's r. Statistical significance was assumed at $p \leq 0.05$. All statistical analyses were performed using PASW Statistics 18.

Results

Baseline characteristics

See **Table 1** for clinical and procedural characteristics of series 1. (Characteristics of series 2 subjects supplied as Supplemental Tables 1 and 2) Between FS (black) and CS (red), there were no significant differences in age, presence of 1, 2, or 3-vessel coronary-artery-disease, blood pressure, and cholesterol, CRP, glomerular filtration rate (GFR), and fasting glucose levels. Packyears were significantly lower

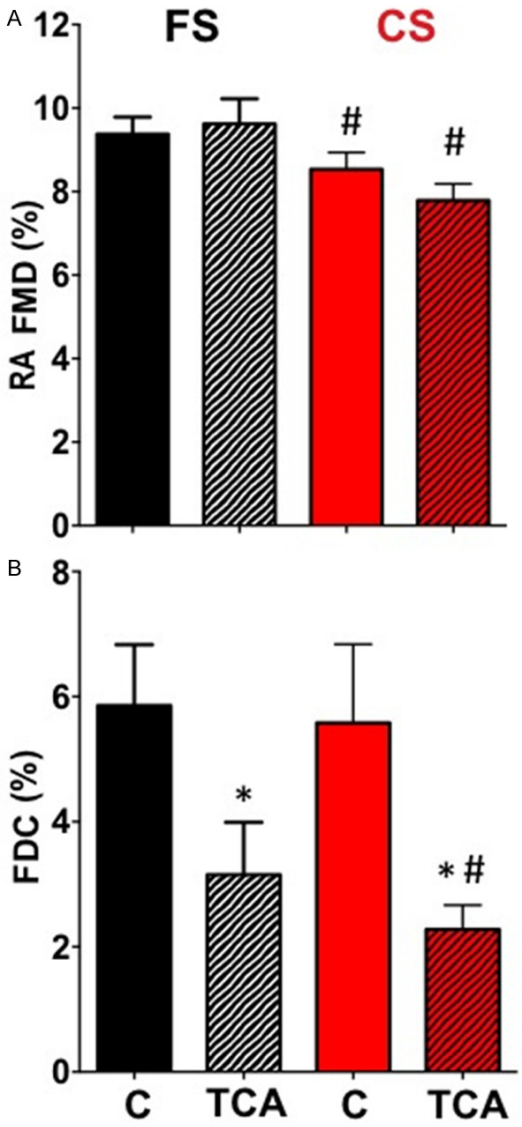

Figure 2. Endothelial function and late local arterial stiffness following TCA. Radial artery (A) flow-mediated dilation (FMD) and (B) FDC were measured 10 months after a transradial catheterization (TCA) in the interventional arm and the contralateral control arm in current smokers (CS, red) and former smokers (FS, black). *p<0.05 vs *control arm* #p<0.05 vs. *FS* (n=40). "C" denominates control arm and "TCA" the arm which was used as access site for TCA.

in the FS (34.0 ± 5.1) as compared to the CS (54.3 ± 6.4, *p=0.012*). Cotinine levels were significantly lower in FS confirming current non-smoking status. The procedural characteristics did not differ between CS and FS.

Current smoking promotes structural remodeling of the RA following TCA

We investigated the RA at the area of the sheath insertion for the TCA and contralateral control arm at 10 ± 3 months after subjects had undergone their first TCA (**Figure 1B**). RA-FMD was significantly lower in CS as compared to FS (**Figure 2**); no significant differences were found between the TCA and control arm. FDC was decreased in the TCA arm of both CS and FS as compared to control arm suggesting increased local stiffness due to TCA. However, the FDC was significantly lowered in CS as compared to FS suggesting more pronounced local arterial stiffening due to smoking status (4.0 ± 1.2% vs. 1.2 ± 0.9%, *p=0.003 vs. FS*). We observed that IMT was significantly increased in both CS and FS as compared to the respective non-cannulated contralateral control arm (**Figure 3**). IMT in the TCA arm of CS was significantly greater as compared to FS (0.44 ± 0.07 mm vs. 0.37 ± 0.05 mm, *p=0.001 vs FS*). The IMT on the control arm was not significantly different between CS and FS (0.32 ± 0.06 mm vs. 0.31 ± 0.05 mm, *p=0.726 vs FS*). Intimal thickening (Delta TCA and control arm) was approximately doubled in CS (IMT_{Delta} CS: 0.096 ± 0.088 mm, FS: 0.053 ± 0.058 mm, *p=0.04*). Furthermore, we detected a significant increase of RA external diameter in CS following TCA (2.97 ± 0.30 mm vs. 2.71 ± 0.39 mm, *p=0.027 vs. FS*). The RA luminal diameter did not differ significantly in the interventional arms (2.03 ± 0.37 mm vs. 2.01 ± 0.33 mm, *p=0.81 vs. FS*). On the contralateral control arm, no significant differences in the external (2.73 ± 0.33 mm vs. 2.72 ± 0.35 mm, *p=0.96 vs. FS*) and luminal diameter (2.10 ± 0.37 mm vs. 2.12 ± 0.35 mm, *p=0.82 vs. FS*) were detected.

Univariate correlations were found between IMT and packyears (r=0.57, p=0.002) and daily cigarette consumption (r=0.7, p=0.001, **Figure 4**). Furthermore, a negative univariate correlation existed between IMT and FMD (r=-0.44, p=0.041, n=40) in the intervention arm.

Impairment of in vitro and ex vivo regenerative capacity in CS

We observed significantly lower numbers of CD34/KDR (FS: 0.42 ± 0.19%PBMNC, CS: 0.07 ± 0.06%PBMNC, *p=0.04*) in CS as compared to FS. We tested the migratory capacity of CACs cultured from CS and FS blood (**Figure 5**). Although CACs of CS exhibited similar random cell movement (chemokinesis) as compared to FS CACs, these cells exhibited practically abol-

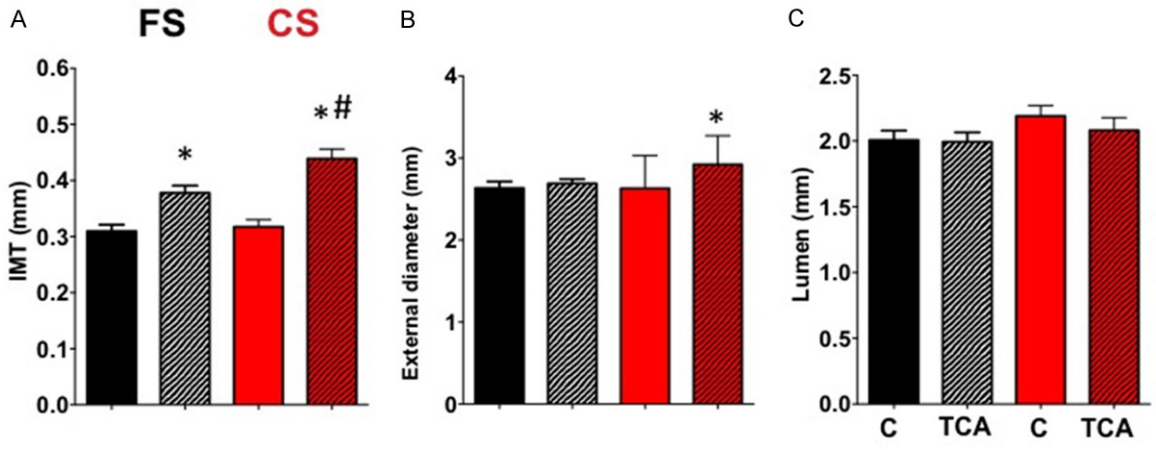

Figure 3. Late arterial remodeling of radial artery following TCA. (A) IMT, (B) external diameter, and (C) lumen were measured 6-14 month after a transradial catheterization (TCA) at the interventional arm and at the contralateral control arm in current smokers (CS, red) and former smokers (FS, black). CS exhibited significantly greater external diameter due to greater intima media thickness (IMT) as compared to FS and control arm (C). *p<0.05 vs. *control arm* #p<0.05 vs. *FS*. "C" denominates control arm and "TCA" the arm which was used as access site for TCA.

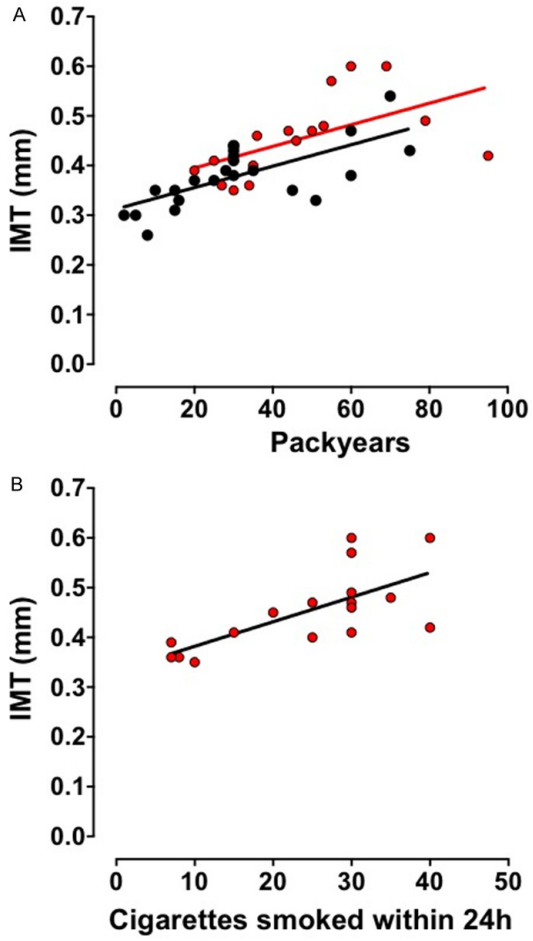

Figure 4. Correlation of IMT and smoking: (A) Packyears and IMT in CS (r=0.57, p<0.002, n=17) and in FS (r=0.63, p<0.001, n=23) and (B) IMT and the number of cigarettes consumed daily in CS (r=0.7, p<0.001, n=17).

ished chemotaxis i.e. CACs did not migrate towards a VEGF cytokine gradient. FS CAC chemotaxis was inhibited by L-NMMA confirming that NOS activity was required to allow chemotaxis [6, 8]. L-NMMA did not affect chemotaxis in CS suggesting that CS CACs had lost NOS activity. Similar results with impaired NOS dependent chemotaxis were seen in the second series of experiments in which endothelial cells (HUVEC) were incubated with CS and FS plasma. Furthermore, plasma isolated from CS impaired endothelial wound healing. Incubation with CS plasma almost doubled wound closure time similar to prolonged closure times that we observed in the presence of NOS inhibitor L-NMMA.

Current smoking delays early recovery of vasodilator dysfunction

CS exhibited significantly lower RA-FMD at baseline (7.3 ± 1.0% vs. 9.5 ± 0.7%, *p=0.004,* **Figure 6**). At 6 h after TCA, RA-FMD values decreased significantly in both groups (4.1 ± 0.7% vs. 4.7 ± 1.2%, *p=0.912 between groups*). Whereas in FS RA-FMD had returned to baseline at 24 h (9.1 ± 1.3%, *p=0.465 vs. baseline*), CS remained impaired at 24 h (5.3 ± 0.7%, *p=0.043 vs. baseline*) and recovered at 48 h (7.0 ± 0.7%, *p=0.712 vs. baseline*). To determine the contribution of endothelium-dependent vasomotor dysfunction to the impairment of FMD after catheterization, we measured the endothelium-independent smooth muscle response to oral GTN after FMD measurements.

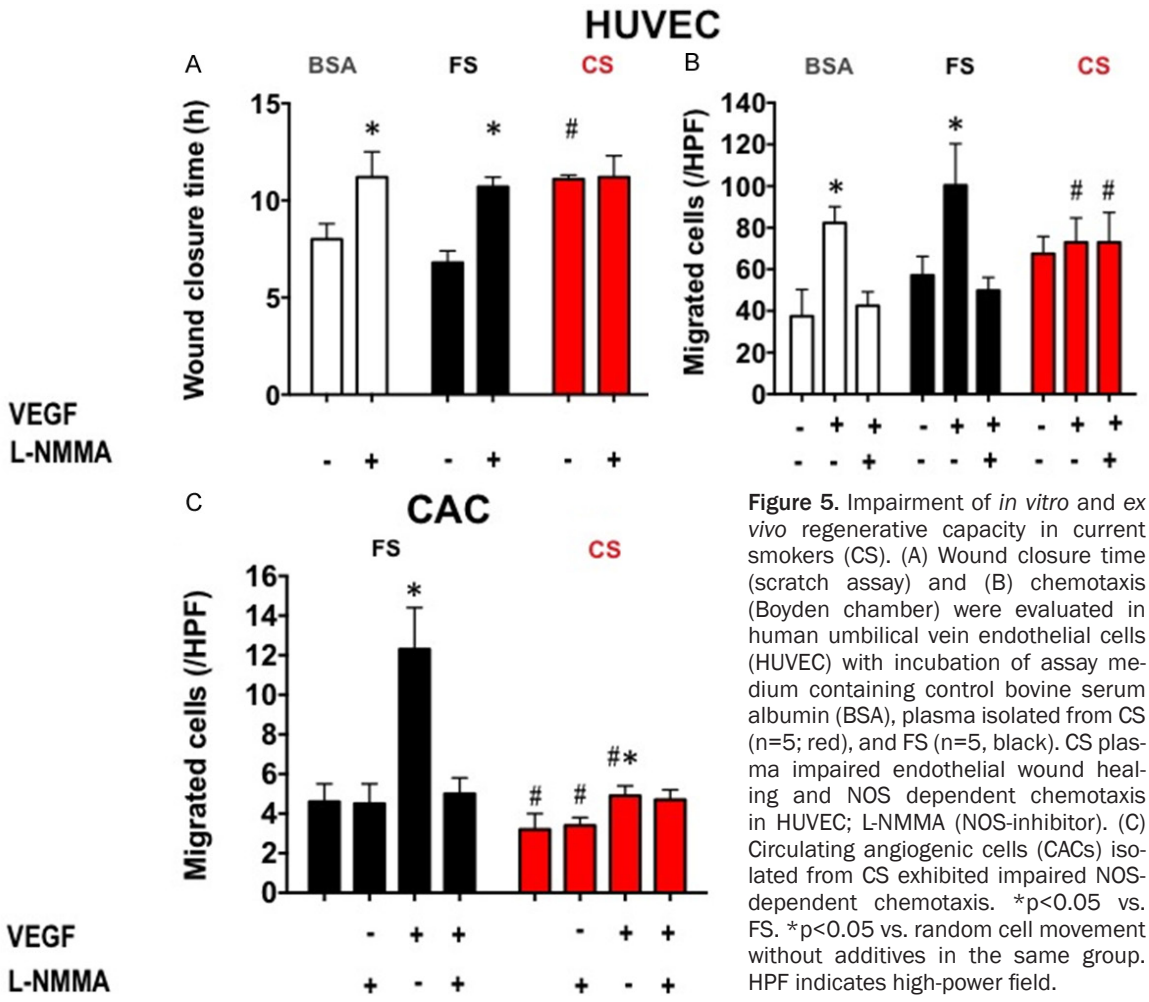

Figure 5. Impairment of *in vitro* and *ex vivo* regenerative capacity in current smokers (CS). (A) Wound closure time (scratch assay) and (B) chemotaxis (Boyden chamber) were evaluated in human umbilical vein endothelial cells (HUVEC) with incubation of assay medium containing control bovine serum albumin (BSA), plasma isolated from CS (n=5; red), and FS (n=5, black). CS plasma impaired endothelial wound healing and NOS dependent chemotaxis in HUVEC; L-NMMA (NOS-inhibitor). (C) Circulating angiogenic cells (CACs) isolated from CS exhibited impaired NOS-dependent chemotaxis. *p<0.05 vs. FS. *p<0.05 vs. random cell movement without additives in the same group. HPF indicates high-power field.

Our results show that the GTN response was not significantly different between CS and FS at baseline (13.4 ± 0.5% vs. 14.1 ± 0.7%, *p=0.116 between groups*) and all consecutive time points suggesting that smoking status did not influence smooth muscle function and that the degree of mechanical injury was comparable between groups. GTN significantly decreased in both groups at 6 h (7.4 ± 2.0% vs. 7.1 ± 0.6%, *p=0.787 between groups)*, remained decreased at 24 h (10.1 ± 0.8% vs. 10.7 ± 1.7%, *p=0.541 between groups)*, and returned to baseline values at 48 h (13.2 ± 1.1% vs. 14.4 ± 1.4%, *p=0.154 between groups)*. Measurements at the control arm remained unaffected.

Discussion

Smoking leads to endothelial dysfunction promoting structural remodeling

We [4] and others [9] have previously shown that active cigarette smoking and even exposure to secondhand smoke [6] leads to acute impairment of endothelial function and might also have a longer-lasting effects by negatively impacting vascular repair mechanisms, including the migratory function of endothelial cells. Endothelial dysfunction is a major mechanism by which cigarette smoking promotes atherosclerosis [10, 11]. In the context of the present study, we showed that in current smokers the recovery of endothelial function was slower and the increase of IMT secondary to mechanical vascular irritation was more severe as compared to former smokers. In animal models, denudation of arteries leads to intima hyperplasia that is enhanced by exposure to cardiovascular risk factors [12]. Several studies demonstrated correlations with cardiovascular risk factors, including aging, systolic blood pressure [13], hypercholesterolemia [14], glucose level [15], and smoking status with impaired endothelial function. But also structural changes as measured by IMT are strongly associated with

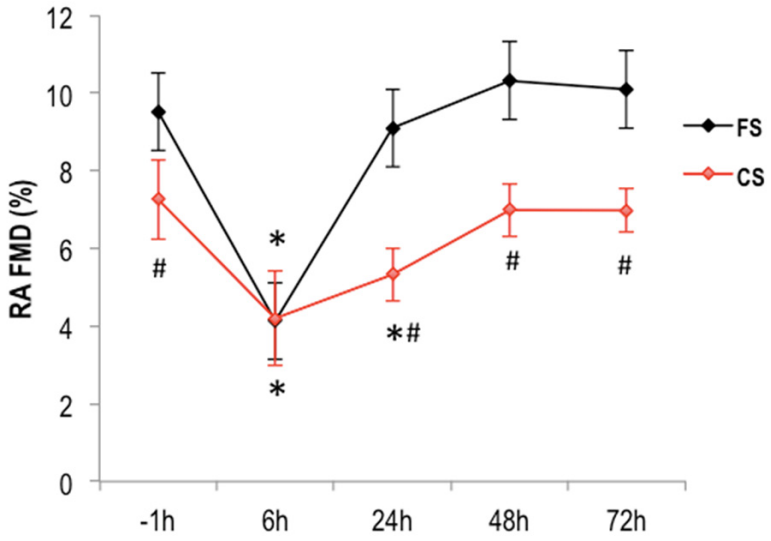

Figure 6. Early recovery of transient endothelial dysfunction after TCA (n=10). Endothelial function measured as FMD at baseline before (-1 h), after 6, 24, 48, and 72 h in current smokers (CS) and former smokers (FS) undergoing TCA. Data are presented for the radial artery FMD on the interventional arm. *p<0.05 vs. *baseline (-1 h),* #p<0.05 vs. *FS*.

these cardiovascular risk factors. IMT is associated with the degree of impaired endothelial vasomotor function, is preceded by endothelial dysfunction, affected by treatments that improve endothelial function, and, therefore, believed to represent a long term sequel of endothelial dysfunction in a broader sense [16-18].

In 1987 Glagov et al. [19] described that during atherogenesis there is an initial intimal proliferative adaptive response, which is associated with an increased wall stress, triggering intimal hyperplasia. During this process, intimal hyperplasia temporarily stabilizes wall stress leading to gradual artery enlargement, which can be interpreted as a compensatory enlargement, i.e. positive remodeling. At some point, external arterial enlargement can no longer compensate for intimal hyperplasia and the lumen diameter starts to decrease, resulting in lumen loss/stenosis and flow restriction, i.e. negative remodeling. However, these findings are based on coronary arteries that are known to be prone to atherosclerosis. Nevertheless, we propose that a similar process can takes place in vessels, which are typically not affected to a similar extent of atherosclerosis i.e. the radial artery. Interestingly, our data show enhanced IMT thickening and compensatory external arterial diameter enlargement in current smokers, while maintaining luminal diameter reminiscent of positive remodeling as described above. A potential mechanism by which smoking might fire this process is a stimulation of smooth muscle cell-proliferation and intimal hyperplasia [20]. Several studies suggest that nicotine induced vascular smooth muscle cell proliferation, promotes atherosclerosis [21], and exaggerates post-injury neointima hyperplasia in animal models [22].

Vascular injury and long-term consequences

Our data support that arterial injury induced by arterial cannulation and sheath placement leading to endothelial denudation can be a trigger for adverse structural remodeling of the radial artery. Depending on the size of the sheath in relation to the arterial luminal diameter acute vascular dysfunction is followed by intimal hyperplasia and lumen loss. Patients with repeated transradial catheterization, showed a stronger intimal thickening and luminal loss of the RA [23]. Abe and colleagues [24] showed that placement of a 6F system during transradial interventions resulted in a decreased RA diameter at 3 month follow-up. Furthermore, Uhlemann et al. demonstrated that the use of 5F sheaths for transradial access significantly decreased the rate of radial arterial occlusion by 55%, compared with 6F sheaths [2]. These data are supported by intravascular ultrasound results of Wakeyama et al. who showed a reduced lumen diameter due to intima-media-thickening after transradial intervention with 6F sheaths and that repeated access enhanced this [23]. This structural remodeling response in particular with small sheath size might have been overlooked in other studies, as many studies investigated remodeling with angiographic techniques were only lumen diameters are investigated. Using ultrasound, we were able to detect not only the luminal diameter, but also the external diameter of the RA. Importantly, we detected that intimal hyperplasia occurred in all patients with the degree of IMT thickening and adaptive arterial enlarge-

ment inversely associated with endothelial function and the degree of cigarette smoke exposure, a known endothelial toxin. Our data suggest that patients with endothelial dysfunction are at increased risk for adverse remodeling in response to (iatrogenic) arterial injury. However, longitudinal studies in larger patient groups over longer timeframes are necessary to identify determinants of adverse remodeling and vascular complications and evaluate whether the observed changes are potentially reversible.

Smoking impairs vascular regeneration

Our current data suggest that a prolonged period of endothelial dysfunction following TCA may contribute to a more pronounced IMT thickening. Endothelial dysfunction is considered as a key factor in the pathogenesis of atherosclerosis [25]. After endothelial injury, surrounding endothelial cells migrate into the denuded area, circulating angiogenic cells home to denuded areas and can help inhibiting intimal hyperplasia. eNOS plays an integral regulatory role in vascular biology, regeneration of endothelium, and CAC function and there is ample evidence that smoking leads to dysfunction of NOS [5, 11, 26]. We here show that smokers CACs exhibited similar random cell movement (chemokinesis) as compared to non smokers' CACs. Mechanistically, we show *in vitro* that CACs chemotaxis in FS was inhibited by L-NMMA confirming that NOS activity is required to allow chemotaxis [6, 8]. L-NMMA did not affect chemotaxis in CS suggesting that CACs from CS had lost NOS activity. Incubation of human umbilical vein endothelial cells with cigarette smokers plasma led to a blockade of the endothelial nitric oxide synthase (eNOS)/NO pathway and impaired VEGF- induced migration. This suggests that the mechanisms involved in smoke toxicity are similar in endothelial cells. Several studies suggested that an impairment of vascular NO availability might contribute to an accelerated intimal hyperplasia. Yoko et al. demonstrated in eNOS-KO mice a significantly enhanced neointimal formation after balloon injury [27]. Another study demonstrated that after mechanical expansion of the femoral artery resulted in rapid onset of apoptosis of medial smooth muscle cells and enlargement of the artery [28]. Also in humans, several studies have reported remodeling processes under different clinical conditions as a dynamic phenomenon that takes place alongside atherosclerotic plaque development [23, 29, 30]. This is important because a number of risk factors, in particular smoking, promote atherosclerosis, impairs regeneration [8] and moreover current smoking inhibits the NO production [31]. The NO signaling is coupled with the ability of CACs to migrate towards VEGF [8, 32]. Our current data support, that this key NOS-dependent endothelial repair mechanism is counteracted by current smoking leading to an impaired migration in smokers and potentially facilitates late intima-hyperplasia following arterial injury in active smokers. This is supported by our correlations between IMT and parameters of current smoking. In addition, pathophysiological studies addressing smoking and wound healing suggest a prolonged effect on inflammatory and reparative cell functions leading to delayed healing and complications [33]. Our data support these findings. Incubation of cells with smokers' plasma almost doubled wound closure time similar to NOS inhibitor L-NMMA, which underlines the impaired endothelial wound healing in smokers and a NOS dependent effect. Smoking cessation restores endothelium-dependent relaxations by increased release or bioavailability of NO from endothelial cells and might support wound healing effects. It is tempting to hypothesize that peri-interventional smoking cessation can help to curb vascular remodeling after TCA and thereby prevent long-term complications of this procedure and preserve the option to use the radial artery as a bypass graft or dialysis shunt. This however needs to be shown in future studies.

Conclusion

Our data suggest that current smoking status strongly impacts on the early recovery of endothelial function and structural maintenance of arteries following arterial injury during transradial coronary angiography interfering with NOS-dependent vascular repair mechanisms.

These data raise the question, whether or not active smokers should be subjected to coronary angiography via the transradial access route. Longitudinal longer term follow up studies in larger cohorts of active smokers are mandatory. Furthermore, the use, quality, and outcome of a radial artery bypass graft for CABG procedures, which might be accompanied also

by mechanical stress during harvesting of the artery, deserves further studies, at least in active smokers. The widespread use of transradial coronary angiography might be questionable in specific subsets of patients.

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Disclosure of conflict of interest

None.

Abbreviations

BA, brachial artery; CAC, circulating angiogentic cells; CAD, coronary artery disease; CS, current smokers; FDC, fractional diameter change; FS, former smokers; IMT, intima-media thickness; RA, radial artery; RA-FMD, radial artery flow-mediated vasodilation; TCA, transradial coronary angiography.

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Supplemental Table 1. Characteristics of study subject; Series 2(a): Regenerative capacity

Subject characteristics	CS	FS	p
n (male/female)	5/0	5/0	
Age (yrs)	57 ± 4	59 ± 6	0.595
CAD (1, 2, 3)	3/2/0	3/2/0	
PAD	0	0	
BMI (kg/m^2)	26 ± 4	27 ± 3	0.614
GFR (ml/min)	68 ± 10	68 ± 11	0.954
CRP (mg/dl)	0.3 ± 0.1	0.3 ± 0.2	0.765
HR (bpm)	61 ± 7	67 ± 16	0.502
SBP (mmHg)	125 ± 16	130 ± 11	0.596
DBP (mmHg)	76 ± 11	78 ± 5	0.637
Packyears (n)	36 ± 8	39 ± 6	0.615
Fasting glucose (mg/dl)	92 ± 11	102 ± 18	0.352
Total cholesterol (mg/dl)	169 ± 22	164 ± 26	0.742
LDL (mg/dl)	103 ± 32	102 ± 22	0.956
HDL (mg/dl)	51 ± 17	46 ± 12	0.645
Beta-blocker (%)	100	80	
Statin (%)	100	100	
ACEI/ARB (%)	80	100	
Clopidogrel (%)	100	100	
Aspirin (%)	100	100	

In vitro and *ex vivo* experiments were performed with CACs and plasma obtained from these subjects.

Supplemental Table 2. (A) Subject and (B) procedural characteristics; Series 2(b): Early recovery of endothelial function

A.	Subject characteristics	CS	FS	p
	n (male/female)	5/0	5/0	
	Age (yrs)	64 ± 11	62 ± 7	0.819
	CAD (1, 2, 3)	2/2/1	3/2/0	
	PAD	0	0	
	BMI (kg/m^2)	27 ± 2	28 ± 1	0.493
	GFR (ml/min)	79 ± 18	79 ± 15	0.946
	CRP (mg/dl)	0.3 ± 0.2	0.3 ± 0.2	1.000
	HR (bpm)	64 ± 7	66 ± 7	0.797
	SBP (mmHg)	134 ± 6	128 ± 6	0.180
	DBP (mmHg)	84 ± 6	83 ± 5	0.778
	Packyears (n)	35 ± 9	48 ± 12	0.090
	Fasting glucose (mg/dl)	86 ± 10	81 ± 10	0.504
	Total cholesterol (mg/dl)	179 ± 11	176 ± 22	0.807
	LDL (mg/dl)	129 ± 13	121 ± 24	0.534
	HDL (mg/dl)	46 ± 5	50 ± 8	0.365
	Beta-blocker (%)	80	80	
	Statin (%)	100	100	
	ACEI/ARB (%)	80	100	
	Clopidogrel (%)	100	100	
	Aspirin (%)	100	100	
B.	Procedural characteristics	CS	FS	p
	Elective TCA (%)	100	100	
	Stable CAD (%)	100	100	
	NSTEMI/STEMI (%)	0	0	
	5 F sheath (%)	100	100	
	Irradiation time (min)	10 ± 12	9 ± 12	n.s.
	Contrast volume (ml)	58 ± 36	60 ± 41	n.s.
	Number of catheters (n)	3.0 ± 0.7	3.2 ± 0.8	0.694
	Heparin (U/l)	1600 ± 1140	1400 ± 1140	0.789
	PCI (%)	0	0	