Correlation of epicardial and systemic flow-mediated vasodilation in patients with atypical angina but no evidence of atherosclerotic disease

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BACKGROUND: Atypical angina represents a diagnostic challenge and can be observed in the absence of significant coronary atherosclerosis. Endothelial dysfunction is a relevant marker of prognosis, considering cardiovascular events. The aim of the present study was to compare flow-mediated vasodilation (FMD) in systemic peripheral and epicardial coronary arteries. If noninvasive measurements of FMD in systemic arteries correlated with invasive measurements of coronary FMD, this may facilitate diagnostic approaches and determination of prognosis in patients with atypical angina in the future. Patients with atherosclerosis were excluded, because structural changes of coronary vessels may impair adequate comparison.

METHODS: Endothelial function (ENF) of epicardial and systemic arteries was examined in 61 consecutive patients with atypical angina in whom significant atherosclerosis was excluded by coronary angiography. ENF of the epicardial arteries was examined during heart catheterization, measuring diameter changes of the proximal left anterior descending coronary artery (LAD) in response to reactive hyperemia, induced by locally administered adenosine via infusion catheter to the mid-segment of the LAD (coronary FMD [FMDc]). ENF of the radial artery was examined with high-resolution ultrasound, measuring peripheral FMD (FMDp) in response to reactive hyperemia induced by distal cuff occlusion. Endothelium-independent vasoreactivity to glycerol trinitrate was assessed.

RESULTS: In patients with atypical angina in the absence of atherosclerosis, there was a significant correlation in ENF between coronary and systemic arteries ($r=0.437$; $P=0.001$). The underlying disease was myocardial inflammation (Inf) in 48 patients, in whom the mean ($\pm$ SD) ENF of epicardial (FMDc-Inf $3.40\pm5.55\%$) and systemic (FMDp-Inf $3.69\pm2.93\%$) arteries was significantly impaired ($P<0.001$), compared with 13 control (Co) patients who had normal myocardial biopsies (FMDc-Co $14.51\pm8.62\%$; FMDp-Co $7.69\pm3.42\%$). FMD of coronary ($r=-0.353$; $P=0.005$) and systemic ($r=-0.542$; $P<0.001$) arteries correlated significantly with myocardial inflammation and endothelial activation.

CONCLUSIONS: There was a significant correlation in FMD between coronary and systemic arteries in patients with atypical angina but without significant atherosclerosis. Inflammatory processes are associated with endothelial dysfunction of both vascular regions.

Key Words: *Cardiomyopathy; Coronary circulation; Endothelium-dependent vasodilation; Flow-mediated vasodilation; Inflammation; Peripheral circulation*

La corrélation de la vasodilatation épicaudique et systématique flux-dépendante chez les patients atteints d'une angine atypique, sans trace d'athérosclérose

HISTORIQUE : L'angine atypique constitue un problème diagnostique et peut s'observer en l'absence d'athérosclérose coronarienne marquée. Le dysfonctionnement endothélial est un marqueur pertinent du pronostic, compte tenu des événements cardiovasculaires. La présente étude visait à comparer la vasodilatation flux-dépendante (VFD) des artères coronaires périphériques systémiques et épicaudiques. Si des mesures non effractives de la VFD des artères systémiques étaient corrélées avec des mesures effractives de la VFD des artères coronaires, la démarche diagnostique et la détermination du pronostic des patients atteints d'une angine atypique seraient facilitées. Les patients athéroscléreux étaient exclus, car des modifications structurelles aux vaisseaux coronaires peuvent nuire à une bonne comparaison.

MÉTHODOLOGIE : Les auteurs ont examiné la fonction endothéliale (FEN) des artères épicaudiques et systémiques de 61 patients consécutifs atteints d'une angine atypique chez qui, par coronarographie, on avait exclu une athérosclérose marquée. Ils ont examiné la FEN des artères épicaudiques pendant un cathétérisme cardiaque, mesurant les fluctuations du diamètre de l'artère interventriculaire antérieure (AIA) en réponse à une hyperanémie réactive induite par de l'adénosine administrée localement par cathéter d'infusion à la section médiane de l'AIA (VFD coronaire [VFDc]). Ils ont examiné la FEN de l'artère radiale par échographie à haute résolution, mesurant la VFD périphérique (VFDp) en réponse à l'hyperanémie induite par occlusion du ballonnet distal. Ils ont évalué la vasoréactivité endothéliale indépendante au moyen de trinitrate de glycérol.

RÉSULTATS : Chez les patients atteints d'une angine atypique sans trace d'athérosclérose, on remarquait une corrélation significative de la FEN entre les artères coronaires et systémiques ($r=0.437$; $p=0.001$). La maladie sous-jacente était une inflammation myocardique (inf) chez 48 patients, chez qui la FEN moyenne ($\pm$ ÉT) des artères épicaudiques (VFDc-inf $3,40\pm5,55\%$) et systémiques (VFDp-inf $3,69\pm2,93\%$) étaient gravement endommagée ($p<0,001$) par rapport à celle de 13 sujets témoins (tém) dont les résultats de biopsie myocardique étaient normaux (VFDc-tém $14,51\pm8,62\%$; VFDp-tém $7,69\pm3,42\%$). La VFD des artères coronaires ($r=-0,353$; $p=0,005$) et systémiques ($r=-0,542$; $p<0,001$) était corrélée de manière significative avec une inflammation myocardique et une activation endothéliale.

CONCLUSIONS : On remarquait une importante corrélation de la VFD entre les artères coronaires et systémiques chez les patients atteints d'une angine atypique sans athérosclérose marquée. Les processus inflammatoires s'associent à un dysfonctionnement endothélial des deux régions vasculaires.

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Endothelial function plays a central role in the development of vascular disease and represents a relevant marker of prognosis: endothelial dysfunction in coronary artery disease is associated with an increased risk of adverse coronary events (1-3), and after transplantation, it may predict transplant failure (4,5). Endothelial function has been examined in peripheral and coronary arteries. It has been difficult, however, to establish an association between the different vascular regions (6), because different methods of testing endothelial function have been applied, and because patients with coronary artery disease have been included, in whom fixed structural changes (ie, calcified plaque) of the coronary arteries, but not in the systemic circulation, may impair adequate comparison. For these reasons, we examined patients with atypical angina in the absence of significant atherosclerosis – to exclude impairment of vasodilation by structural changes of the vessel wall – and we chose flow-mediated vasodilation (FMD) to examine endothelial function of the coronary arteries and the systemic circulation.

Atypical angina may be caused by endothelial dysfunction, which, in turn, can be caused by inflammatory processes: endothelial dysfunction has been observed in association with inflammatory immune responses and markers of systemic inflammation (C-reactive protein) (7-13). Myocardial inflammation, endothelial activation and virus persistence in myocardial biopsies have been associated with endothelial dysfunction of the peripheral arteries and coronary microcirculation (14-16). Proinflammatory cytokines, which regulate inflammatory responses in the myocardium and local cardiac vessels, may also circulate and be responsible for functional changes in peripheral vessels. On the other hand, proinflammatory cytokines may be associated with generalized vascular inflammation and systemic expression of cell adhesion molecules. We therefore aimed to not only compare endothelial function in different vascular regions, but also to examine the impact of inflammatory processes on endothelial function in these vascular regions.

METHODS

Study population

Sixty-one consecutive patients with atypical angina in whom significant atherosclerosis had been excluded by coronary angiography before measurements of epicardial artery endothelial function were taken. Left ventricular ejection fraction (EF) and pressures were documented. Right ventricular catheterization was performed to obtain endomyocardial biopsies and hemodynamic measurements. To minimize other confounding factors on endothelial dysfunction, patients with diabetes, more than one other risk factor for arteriosclerosis (17-20), systemic arteriosclerosis, severely impaired left ventricular contractility (EF less than 35%) (21-24), active systemic inflammation (7-13) or other severe disease were excluded from the present study. All cardiovascular medication with possible impacts on endothelial function (24,25) was ceased according to half-life before the study, although this may not have been necessary (26).

Informed consent was obtained from all patients before angiography; inclusion ensued after atherosclerosis was excluded by angiography. The study protocol was approved by the local ethics committee.

Endothelial function

Endothelial function of epicardial arteries: Endothelial function of the left anterior descending coronary artery (LAD) was assessed. By means of coronary angiography, diameter changes in response to reactive hyperemia (coronary FMD [FMDc]),

Figure 1 Endothelial function testing in coronary arteries: coronary angiogram with Doppler wire and infusion catheter (cath) placed in the mid-segment of the left anterior descending artery, to measure flow-mediated vasodilation in the proximal segment of the left anterior descending artery in response to locally administered adenosine (distal)

compared with glycerol trinitrate MDc (GTN-MDc), were detected, referring to the standard protocols (27,28) (Figure 1).

Diagnostic left heart catheterization and coronary angiography were performed by a standard percutaneous femoral approach. After completion of the diagnostic catheterization, an additional 5000 U of heparin and 500 mg of acetylsalicylate were given intravenously, and an 8-F guiding catheter was introduced into the LAD. A Doppler-tipped guidewire (FloWire, floppy 0.014 in, 15 MHz, pulse repetition frequency 15 kHz to 120 kHz, [Cardiometrics, USA]) was inserted into the mid-segment of the LAD and carefully positioned to obtain a stable flow velocity signal. A monorail infusion catheter (multifunctional probing catheter, 0.018+0.014 in; Boston Scientific, USA) was placed in the mid-segment of the LAD, just proximal to the tip of the Doppler wire, for local application of adenosine (Figure 2). After stable baseline conditions were obtained, adenosine (0.12 mg/mL) was infused at a rate of 2 mL/min for 3 min via the infusion catheter to the mid-segment of the LAD. Shear stress-mediated, endothelium-dependent vasodilation was measured in the proximal segment of the LAD, which was exposed to increased flow, but not directly to the endothelium-independent vasodilator adenosine (Figure 2). Reactive hyperemia (increased flow) in the proximal segment of the LAD was induced by direct adenosine-mediated vasodilation in the microcirculation, distal to the infusion catheter. After a return to the basal state (3 min), GTN (0.2 mg) was injected into the ostium of the left main stem via a guiding catheter to assess the maximal endothelium-independent dilator capacity of the entire LAD, including the proximal segment. Throughout the study, phasic and mean intracoronary blood flow velocity (via FloWire), heart rate and aortic pressure (via guiding catheter) were continuously measured. Serial manual injections of nonionic contrast medium were performed at baseline, at the end of the adenosine infusion and after injection of GTN. Blood flow velocity was measured with FloMap equipment (Volcano Therapeutics, The Netherlands). Diameter changes in the LAD were measured by quantitative coronary angiography (Philips Inturis CardioView, QCA V3.3; Pie Medical

Figure 2) Endothelium-dependent, flow-mediated vasodilation in systemic peripheral arteries (FMDp) in relation to endothelium-dependent, flow-mediated vasodilation in coronary arteries (FMDc). There was a significant correlation ($r=0.536$; $P<0.001$)

Imaging, The Netherlands). Coronary angiography was performed with a simultaneous biplane, multidirectional and isocentric x-ray system, and videodigitized end-diastolic frames were analyzed. Diameter and flow changes in response to infusion of vasoactive medication were expressed as per cent change to baseline.

Endothelial function of systemic arteries: Endothelial function of the radial artery was assessed as previously described (13,14). By means of high-resolution ultrasound, diameter changes in response to reactive hyperemia (FMD), compared with GTN-MD, were detected, referring to standard protocols (29,30). Accuracy and reproducibility have been documented (30); a low coefficient of variation for measurements of arterial diameter and a high correlation between consecutive control measurements have been demonstrated (29). FMD, in response to reactive hyperemia, represents endothelium-dependent vasoreactivity, whereas GTN-MD indicates smooth muscle cell function and is independent of endothelial function. Reactive hyperemia, induced by distal cuff occlusion and release, leads to a release of endothelium-dependent vasodilator substances mediated by shear stress rather than ischemic metabolites (29,30).

Technical settings: The radial artery was examined by two-dimensional ultrasound images, with a 10 MHz linear array transducer and a standard 128XP-10C ultrasound system (Acuson, USA). The transducer was positioned distal to the elbow to achieve a longitudinal picture of the radial artery. Transmit zone, depth and gain were set to optimize images of the lumen-arterial wall interface, and images were magnified by resolution box function; machine operating parameters were not changed during the study. Diameters were measured by means of a computerized edge-detection program (Information Integrity, USA); the images were electrocardiogram (ECG)-triggered at end-diastole throughout the study. Arterial flow velocity was measured by pulsed Doppler signal at a 70° angle to the vessel throughout the study.

Study protocol: The subject lay at rest for at least 10 min before beginning the scan for endothelial function. A resting scan was recorded for 1 min. A pneumatic tourniquet, placed at the subject's wrist, was then inflated to a pressure of 300 mmHg for 3 min. The release would immediately induce increased blood flow in the subject's forearm for a few seconds, which represented

the stimulus for endothelium-dependent vasodilation. Vasodilation was generally maximal after 60 s, when flow had already normalized. The vessel was continuously scanned during the procedure, from baseline to 5 min after release of the cuff. A break of 10 min, with the patient continuously supine, was required before the scan for endothelium-independent vasodilation was started. After a resting scan, 400 µg of GTN was administered sublingually, and the scan continued for 5 min after application. Maximal vasodilation generally occurs 4 min to 5 min after GTN administration. All scans were performed by one experienced person. The computer-assisted calculation of vessel diameters was conducted in a blinded fashion. The ECG was monitored continuously, and blood pressure was controlled throughout the study. All cardiovascular medication was ceased 12 h to 48 h before the study, depending on half-life.

Calculations: FMD represents the percentage of diameter increase caused by shear stress compared with baseline:

$$\text{FMD (\%)} = \frac{\text{maximum diameter} - \text{base diameter}}{\text{base diameter}}$$

GTN-MD represents the percentage of diameter increase induced by GTN compared with baseline. Reactive hyperemia was considered to be adequate if maximum Doppler flow velocity recorded within the first 15 s after cuff deflation or if maximum flow recorded during adenosine infusion exceeded baseline Doppler flow velocity by at least a factor of two.

Myocardial biopsies

Endomyocardial biopsies from the right ventricular septum were obtained using a standard percutaneous transvenous femoral approach with a standard biptome.

For immunohistological evaluation, the samples were prepared and evaluated as described previously (26,31,32). Immunohistologically stained leukocytes (CD2+, CD3+, CD4+, CD8+ and activated CD45-RO+ lymphocytes, and macrophages) were counted per high-power field (400-fold magnification, equivalent to 0.28 mm²) by a Leica MDRD microscope (Leica, Germany) in all available fields (more than 10 fields per antibody). Endothelial expression of human leukocyte antigen (HLA)-1, HLA-DR and intercellular adhesion molecule (ICAM)-1 was semiquantitatively scaled 1 (normal), 2 (intense) or 3 (abundant), according to the intensity of immunoperoxidase staining of endothelial cells. Endothelial activation was graded according to the sum of endothelial expression of HLA-1, HLA-DR and ICAM-1 – 3 to 4 (normal), 5 to 7 (moderate) and 8 to 9 (abundant). Myocardial inflammation was confirmed in myocardial biopsies, if more than seven CD3+ lymphocytes per square millimetre of tissue were identified and/or if endothelial expression of cell adhesion molecules was enhanced. Myocardial biopsies were examined and analyzed by two independent, blinded observers.

Statistical analysis

Statistical analysis was performed using SPSS version 10.0 (SPSS Inc, USA). Descriptive data are expressed as mean ± SD. Quantitative data were correlated by use of the Spearman's rho rank-order analysis, calculating the coefficient of correlation r . Quantitative data were compared with qualitative data by the Kruskal-Wallis test on rank sums for independent samples, followed by a post hoc multiple comparison procedure, if appropriate. To compare quantitative data of two groups, the Mann-Whitney U test was applied. Statistical significance was inferred at $P<0.01$.

TABLE 1
Patient characteristics and endothelial function

Characteristic	Total population (n=61)	Patients without inflammation (n=13)	Patients with inflammation (n=48)	P
Age (years)	43±12	44±10	43±12	0.790
Flow-mediated vasodilation in systemic arteries (%)	4.54±3.54	7.69±3.42	3.69±2.93	<0.001*
Flow-mediated vasodilation in coronary arteries (%)	5.69±7.66	14.51±8.62	3.40±5.55	<0.001*
Endothelium-independent vasodilation in response to glycerol trinitrate in systemic arteries (%)	25.55±9.14	30.60±8.37	24.18±8.93	0.010
Endothelium-independent vasodilation in response to glycerol trinitrate in coronary arteries (%)	18.40±9.81	22.67±12.10	17.24±8.89	0.087
Baseline diameter, left anterior descending coronary artery (mm)	3.48±0.81	3.37±0.99	3.51±0.77	0.480
Diameter in response to reactive hyperemia, left anterior descending coronary artery (mm)	3.66±0.83	3.81±1.06	3.62±0.76	0.597
Diameter in response to glycerol trinitrate, left anterior descending coronary artery (mm)	4.08±0.83	4.07±1.06	4.08±0.77	0.867
Baseline diameter, radial artery (mm)	2.52±0.55	2.37±0.55	2.57±0.55	0.294
Diameter in response to reactive hyperemia, radial artery (mm)	2.63±0.55	2.54±0.56	2.66±0.54	0.560
Diameter in response to glycerol trinitrate, radial artery (mm)	3.13±0.53	3.10±0.56	3.13±0.53	0.991
Ejection fraction (%)	61±15	57±19	62±14	0.620
Left ventricular end-diastolic pressure (mmHg)	8±3	9±5	8±3	0.290
Pulmonary capillary wedge pressure (mmHg)	7±3	7±2	7±3	0.860
Cardiac output (L/min)	6±1	6±2	5±1	0.380

Data are expressed as mean ± SD. P indicates statistical differences between patients with myocardial inflammation and patients without an inflammatory immune response. *Statistically significant difference (P<0.001)

RESULTS

Patient characteristics

General characteristics: The mean (± SD) age of the 61 patients was 43.67±12.50 years; 31 were male and 30 were female. The mean left ventricular EF was 61.18±15.64%. All patients were normotensive (12 patients were treated for hypertension) and had normal lipid levels (low-density lipoprotein levels lower than 3.1 mmol/L; 11 patients were treated with statins) at the time of investigation. Eight patients were smokers. The patients were taking standard cardiovascular medication – 48 were taking angiotensin-converting enzyme inhibitors or angiotensin II type 1 antagonists, 36 were taking beta-blockers, eight were taking calcium antagonists and 13 were taking digitalis glucosides. Hemodynamic measurements are depicted in Table 1. There were no significant differences between inflammatory and noninflammatory patient groups considering these parameters.

Clinical presentation and history: Patients presented with chest pain (typical or atypical angina); 14 patients reported palpitations and 49 patients reported fatigue or exercise intolerance. At the time of inclusion into the study, all patients were in New York Heart Association class I or II (no significant differences).

Noninvasive examinations: ST segment changes were documented in the ECGs of nine patients, and arrhythmias were documented in eight patients. Bicycle ergometric ECG stress testing resulted mainly in unspecific ST-T segment changes; formal criteria for ischemia were not observed. In all patients, regional wall motion abnormalities (more than two segments) or impaired global systolic left ventricular function was demonstrated by echocardiography (Table 1). In the patients with regional hypokinesia, global EF frequently remained normal due to compensation by the remaining wall segments. Pericardial effusion was not observed.

Hemodynamic measurements: Left ventricular end-diastolic diameter (by echocardiography), EF (by angiography), left ventricular end-diastolic pressure, right atrial pressure, right ventricular pressure (systolic/end-diastolic), pulmonary artery pressure (systolic/diastolic/mean), pulmonary capillary wedge pressure (PCWP), cardiac output, stroke volume index and cardiac index were measured in the study population; data for EF, cardiac output, left ventricular end-diastolic pressure and PCWP are depicted in Table 1. There were no significant differences among patient groups.

TABLE 2
Immunohistology of myocardial biopsies

	Total population (n=61)	Patients without inflammation (n=13)	Patients with inflammation (n=48)	P
Endothelial activation	5.90±2.20	3.08±0.28	6.67±1.83	<0.001*
CD2+	1.60±3.00	0.46±0.31	1.89±3.30	0.002*
CD3+	1.69±3.22	0.43±0.26	2.01±3.54	0.001*
CD4+	0.70±1.34	0.18±0.19	0.83±1.47	0.012
CD8+	0.60±1.17	0.12±0.12	0.72±1.29	0.008*
CD45-RO+	1.54±2.86	0.48±0.41	1.80±3.15	0.016
Macrophages	1.70±3.29	0.80±0.42	1.93±3.66	0.071

Data are expressed as mean ± SD. P indicates statistical differences between patients with myocardial inflammation and patients without an inflammatory immune response. Leukocyte counts (CD2+, CD3+, CD4+ and CD45-RO+ lymphocytes, and macrophages) are presented per high-power field (0.28 mm²). *Statistically significant difference (P<0.01)

Markers of systemic inflammation: C-reactive protein levels were below 6 mg/L and white cell counts were normal in the study population.

Myocardial biopsies

Myocardial inflammation (leukocyte infiltrates and/or enhanced endothelial expression of adhesion molecules) was confirmed by immunohistology in 48 subjects, applying the criteria described above. Thirteen subjects had normal myocardial biopsies.

Leukocyte counts (CD2+, CD3+, CD4+ and CD45RO+ lymphocytes, and macrophages) per high-power field (0.28 mm²) are depicted in Table 2. Table 2 also includes the measurements for endothelial expression of HLA-1, HLA-DR and ICAM-1, expressed as a sum score for endothelial activation as defined above.

By definition, lymphocyte counts and endothelial activation are significantly enhanced in patients with myocardial inflammation compared with patients without an inflammatory immune response.

Figure 3) Endothelium-dependent, flow-mediated vasodilation in systemic peripheral arteries (FMDp) (**top**) and flow-mediated vasodilation in epicardial coronary arteries (FMDc) (**bottom**) in patients with myocardial inflammation compared with patients without an inflammatory immune response. The differences were statistically significant ($P < 0.001$)

Endothelial function (general characteristics)

Peripheral measurements: Heart rate and blood pressure did not change significantly during measurements. Adequate reactive hyperemia, as a stimulus for endothelium-dependent vasodilation, was achieved in all subjects. The mean ($\pm$ SD) resting diameter of the radial artery was 2.52 ± 0.55 mm; after reactive hyperemia, the mean ($\pm$ SD) diameter increased to 2.63 ± 0.55 mm, and after glycerol trinitrate, it increased to 3.13 ± 0.53 mm (Table 1).

Coronary measurements: Heart rate and blood pressure did not change significantly during adenosine infusion or GTN injection. Adequate reactive hyperemia, as a stimulus for endothelium-dependent vasodilation, was achieved in all subjects, as measured by FloWire. The mean ($\pm$ SD) resting diameter of the LAD was 3.48 ± 0.81 mm; after reactive hyperemia by adenosine, the mean ($\pm$ SD) diameter increased to 3.66 ± 0.83 mm, and after GTN, it increased to 4.08 ± 0.83 mm (Table 1).

FMD

In patients with atypical angina, in the absence of significant atherosclerosis, there was a significant correlation between endothelial function of the coronary arteries and endothelial function of systemic arteries ($r = 0.536$; $P < 0.001$) (Figure 2), as measured by FMD in the coronary arteries (the LAD) and the systemic peripheral arteries (the radial artery).

Figure 4) Endothelium-dependent, flow-mediated vasodilation in systemic peripheral arteries (FMDp) (**top**) and flow-mediated vasodilation in epicardial coronary arteries (FMDc) (**bottom**) in relation to endothelial activation (sum expression of human leukocyte antigen-1, human leukocyte antigen-DR and intercellular adhesion molecule-1) as determined by myocardial biopsies. There were significant correlations for FMDp ($r = -0.353$; $P = 0.005$) and FMDc ($r = -0.542$; $P < 0.001$)

Peripheral measurements: Endothelial function, as determined by peripheral FMD (FMDp) of the radial artery, was impaired in patients with myocardial inflammation (FMDp $3.69 \pm 2.93\%$) compared with control (Co) patients without myocardial inflammation (FMDp-Co $7.69 \pm 3.42\%$; $P < 0.001$) (Figure 3). Endothelial function of systemic arteries correlated significantly with endothelial activation, measured by the endothelial expression of HLA-1, HLA-DR and ICAM-1 in myocardial biopsies ($r = -0.353$; $P = 0.005$) (Figure 4).

Coronary measurements: Endothelial function, as determined by FMD of the LAD, was impaired in patients with myocardial inflammation (FMDc $3.40 \pm 5.55\%$) compared with patients without an inflammatory immune response (FMDc-Co $14.51 \pm 8.62\%$; $P < 0.001$) (Figure 3). Endothelial function of the coronary arteries (FMDc) correlated significantly with endothelial activation, measured by the endothelial expression of HLA-1, HLA-DR and ICAM-1 in myocardial biopsies ($r = -0.542$; $P < 0.001$) (Figure 4).

GTN-MD

Endothelium-independent vasodilation in response to GTN was determined in the coronary arteries (the LAD) and the systemic

arteries (the radial artery). However, because the results were within, or close to, the normal range in all subjects of the study population (GTN-MDp $25.55 \pm 9.14\%$; GTN-MDc $18.40 \pm 9.81\%$), there was no significant correlation between independent vasodilation in the coronary arteries and endothelium-independent vasodilation in the systemic arteries ($r=0.123$; $P=0.345$).

Peripheral measurements: Endothelium-independent vasodilation of the radial artery, as determined by GTN, was impaired in patients with myocardial inflammation (GTN-MDp $24.18 \pm 8.93\%$) compared with patients without an inflammatory immune response (GTN-MDp-Co $30.60 \pm 8.37\%$; $P=0.010$) (Table 1). Endothelium-independent vasodilation of systemic arteries (GTN-MDp) did not correlate with endothelial activation ($r=-0.248$; $P=0.063$).

Coronary measurements: Endothelium-independent vasodilation of the LAD, as determined by GTN, had a tendency toward impairment, but not significantly, in patients with myocardial inflammation ($17.24 \pm 8.89\%$) compared with patients without an inflammatory immune response ($22.67 \pm 12.10\%$; $P=0.087$) (Table 1). Endothelium-independent vasodilation of the coronary arteries (GTN-MDc) did not correlate with endothelial activation ($r=-0.119$; $P=0.362$).

Impact of other factors

All subjects in the study population were of middle age, with only small variations. EF and other hemodynamic measurements did not vary extensively among the study population, because patients with severely impaired left ventricular function were excluded. Therefore, in the present study, age, left ventricular EF, left ventricular end-diastolic diameter, left ventricular end-diastolic pressure, PCWP, cardiac output, cardiac index and stroke volume index had no impact on endothelium-dependent or endothelium-independent vasodilation of coronary or systemic arteries, or on endothelial activation or myocardial leukocyte infiltrates.

DISCUSSION

Atypical angina represents a diagnostic challenge, with diagnoses ranging from severe coronary stenosis to functional disorders. Endothelial dysfunction may explain symptoms in patients with angina but no evidence of significant coronary atherosclerosis. Furthermore, endothelial dysfunction is a relevant marker of prognosis, considering cardiovascular events (1-5). In the absence of manifest atherosclerosis, endothelial function is impaired in patients with risk factors for atherosclerosis (17-20,25) and heart failure (21-24), as well as in those in inflammatory states (7-16). In the present study, atypical angina was associated with endothelial dysfunction and inflammatory processes, especially in the myocardium. We cannot exclude that patients with subclinical atherosclerosis were included in our study, because intravascular ultrasound was not part of the study protocol. However, this would not have altered our conclusions. We excluded patients with more than one risk factor for atherosclerosis or diabetes, but slight impact of a single risk factor (with a potential increase in oxidative stress and inflammatory responses) remains a potential cause or confounder of endothelial dysfunction in patients with atypical angina.

In the present study, we demonstrated a correlation between FMD in peripheral systemic arteries and FMD in coronary arteries in patients with angina symptoms but without significant atherosclerosis. This finding is clinically relevant, because it may facilitate diagnostic approaches and determination of prognosis in patients with atypical angina in the future. In these patients, noninvasive measurement of FMD in systemic arteries is sufficient

and invasive measurements of FMD in coronary arteries may not be required, because both measurements of FMD correlate. The results of the present study cannot be extended to patients with manifest atherosclerosis or coronary artery disease, who were excluded. This is because fixed structural changes of coronary vessels, such as calcified plaque, can impair endothelium-dependent and -independent vasodilation in the affected arteries, and therefore impair adequate comparison of endothelial function in different vascular regions.

In the present study, we compared endothelial function in coronary and epicardial arteries, testing the same mechanism of endothelial function in both vascular regions: FMD induced by reactive hyperemia or shear stress, which appears to be the most physiological stimulus for endothelium-dependent vasodilation in coronary and systemic arteries. We found a significant correlation between the endothelial function of epicardial arteries and the endothelial function of systemic arteries. The reason for the difficulties in establishing associations between coronary and systemic endothelial function in previous studies may be that different methods of testing endothelial function were applied (6).

To put the present study into context with previous studies, we also evaluated endothelial function with respect to the results of myocardial biopsies. Besides confirming the results for systemic arteries published previously (14,15), we found that the endothelial function of epicardial arteries was significantly impaired in patients with myocardial inflammation and endothelial activation compared with patients without an inflammatory immune response, and that the severity of the endothelial dysfunction of epicardial arteries correlated significantly with the extent of endothelial activation.

Potential mechanisms for the link between epicardial and systemic endothelial dysfunction in patients with atypical angina but without significant atherosclerosis may be circulating cytokines or generalized vascular disease associated with inflammatory processes. Myocardial inflammation, detected in myocardial biopsies, may reflect a systemic inflammatory immune response rather than a localized disease of the heart. Exercise intolerance may reflect peripheral endothelial dysfunction and impairment of coronary flow.

Endothelium-independent vasodilation was mainly within the normal range; therefore, a correlation between epicardial and systemic GTN-MD was not observed. In patients with myocardial inflammation and endothelial activation, but not in patients with endothelial activation alone, GTN-MD was significantly lower than in patients without an inflammatory immune response; however, it was still within the normal range.

The correlation in FMD between epicardial and systemic arteries in patients with atypical angina but without significant atherosclerosis is of special importance for future investigations. Endothelial function of peripheral arteries, which can be measured noninvasively, may indicate coronary endothelial function, which has to be measured invasively. Clinically, this finding is especially important, because endothelial dysfunction, on one hand, may explain symptoms such as atypical angina or exercise intolerance, and on the other hand, it represents a marker of prognostic relevance (1-5) and may influence therapeutic decisions.

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