Circulating endothelial progenitor cells correlate with erectile function in patients with coronary heart disease

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Introduction

Erectile dysfunction (ED) is defined as the inability to attain or maintain penile erection sufficient for satisfactory sexual performance.1 In the past, ED was mainly attributed to psychological disorders. Since the last two decades, somatic factors such as arterial dysfunction, venous leakage, neural or hormonal disorders, or a combination thereof were suggested to play the major roles in development of ED.2,3 Recent studies, as the ‘Massachusetts Male Aging Study’ and the ‘Cologne Male Survey’, revealed a strong association of cardiovascular risk factors and ED with a prevalence of 30–75% of ED in cardiovascular high-risk patients.4,5 Decreased production of nitric oxide by the endothelial monolayer of the penile arteries and the corpus cavernosum has been mechanistically implicated. Thus, endothelial dysfunction, as the initial step of cardiovascular diseases is related to decreased erectile function.

Bone marrow-derived endothelial progenitor cells (EPC) circulate in the peripheral blood and have been related to repair mechanisms in endothelial dysfunction and atherosclerosis due to their ability of proliferation and differentiation into endothelial cells.6–10 Moreover, cardiovascular risk factors are known to decrease levels of EPC with subsequent increase of cardiovascular events and cardiovascular deaths.11,12 Hence, circulating levels of EPC are thought to be a link between cardiovascular risk factors and endothelial dysfunction with a potential influence on erectile function. To determine the possible association and role of EPC and erectile function, patients from the EPCAD-study (Circulating Endothelial Progenitor Cells and Cardiovascular Morbidity and Mortality in Patients with Coronary Artery Disease study) were evaluated within the EROSS-Programme (Evaluation of Role of Sexual Dysfunction in the Saarland-Programme) regarding erectile and sexual function.

Methods

Study population

A total of 340 male patients with coronary artery disease from the EPCAD-study, who underwent coronary angiography were asked to participate in the study.12 Patients without signs of coronary artery disease, malignant disease, or severe acute ischaemia other than myocardial infarction were excluded. Of this, 211 patients refused participation or returned the questionnaire incomplete. Thus, 119 consecutive patients were included. Informed consent was obtained from all patients. The study protocol of the EROSS-Programme was approved by the Local Ethics Committee.

Angiography and flow cytometry

Methods of the EPCAD-study are described elsewhere.12 Briefly, patients underwent cardiac catheterization. Coronary artery

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Aims The aim of the study was to determine the influence of endothelial progenitor cells (EPC) on erectile dysfunction (ED). EPC play a major role in repair mechanisms of the endothelial monolayer, but the role of EPC in ED is unclear.

Methods and results Circulating levels of CD34+/KDR+ and CD133+ EPC were determined in 119 patients with known coronary artery disease. ED was evaluated with an ED-score generated from the KED questionnaire. Prevalence of ED was 59.7%. In univariate analysis, age, hypertension, reduced left ventricular ejection fraction (LVEF), diabetes, and circulating levels of CD133+ EPC, but not cardiovascular drug treatment were associated with ED. Body mass index (BMI) was positively (r = 0.319, P = 0.003) and high-density lipoprotein was negatively (r = −0.246, P = 0.034) correlated with ED. Adjustment for age, diabetes, hypertension, BMI, smoking, LVEF, use of statins and lower urinary tract symptoms, and prior coronary intervention revealed low levels of circulating immature CD133+ EPC as independent risk factor for ED (95% CI: 11.183 to −1.7371, P = 0.008).

Conclusion Reduced levels of circulating CD133+ EPC are an independent risk factor for ED. Thus, EPC may be a link between cardiovascular risk factors, endothelial dysfunction, and ED.
disease was scored with one- to three-vessel disease depending on the extent of stenosis (≥50%) and the number of affected coronary arteries by two independent interventional cardiologists. Cardiovascular risk factors and previous events were evaluated using medical records and personal interviews.

Arterial blood of 20 mL was obtained from the femoral artery before catheterization. After isolation of mononuclear cells with the Ficoll density gradient, blood was prepared for fluorescent-activated cell sorting. CD34⁺/KDR⁺- and CD133⁺-cells were detected using immunofluorescent cell staining. CD133⁺-cells were not specified regarding the co-expression of CD34. EPC were measured in absolute cell counts obtained after measuring of the Ficoll density gradient, blood was prepared for fluorescent-activated cell sorting. CD34⁺/KDR⁺- and CD133⁺-cells were detected using immunofluorescent cell staining. CD133⁺-cells were not specified regarding the co-expression of CD34. EPC were measured in absolute cell counts obtained after measuring of the Ficoll density gradient, blood was prepared for fluorescent-activated cell sorting. CD34⁺/KDR⁺- and CD133⁺-cells were detected using immunofluorescent cell staining.

**Evaluation of ED**

The KEED (Cologne questionnaire of erectile dysfunction) was used for assessment of ED. The questionnaire was validated previously in the ‘Cologne Male Survey’ and includes sociodemographic characteristics, medical history, prior surgery as well as questions dealing with sexual desire and activity and general satisfaction.⁵ The ED category consists of seven questions generating an ED-score with a range from 5 to 35 points. An increasing sum score indicates a decrease of erectile function with a cut-off for ED of more than 17 points.

Lower urinary tract symptoms (LUTS) appearing in patients with benign prostate hyperplasia or after prostate surgery are known to cause ED.⁵ Thus, the modified international prostate symptom score (IPSS) from the ‘Cologne Male Survey’ was used for evaluation of LUTS. LUTS was diagnosed by affirming at least two of seven questions.

**Statistical analysis**

All data are expressed as mean ± SD. Statistical significance was assumed at a P-level < 0.05. Due to the high prevalence of ED, there is no Gaussian distribution of the KEED-score with the Shapiro-Wilk test. Thus, non-parametric tests were applied for statistical analysis. Means between two categories were compared with the two-sided Mann-Whitney U test. Relation of variables was performed with Spearman-correlation. EPC levels were analysed as variables after log-transformation (log base 10) to normalize distribution. Multivariable regression analysis was performed to determine association of circulating EPC levels and ED. The ED-score as the dependent variable as well as number of circulating EPC (independent variable) were included as continuous variables. Confounders previously shown in univariate analysis (P < 0.05) were included in the model; hypertension, diabetes mellitus, LUTS, smoking prior ACB-surgery, or PTCA as categorical- and age, body mass index (BMI), and left ventricular ejection fraction (LVEF) as continuous variables. Levels of high-density lipoproteins were not included into multivariable regression because of the small number of patients with known plasma concentrations (n = 58). Regarding the effects of lipid-lowering drugs on EPC, current use of statins was included into multivariable regression additionally. Statistical analysis was performed with SPSS 12.0 for Windows.

**Results**

**Baseline characteristics**

Totally, 119 male patients from the EPCAD-study with angiographically diagnosed coronary artery disease completed the ED questionnaire. Average age was 64.1 ± 11.1 years (range 32–86 years). Most patients were married (76%), 12% divorced, 4% widowed, and 8% were single. Patients living in a stable partnership were 87%. Detailed patients characteristics are shown in Table 1.

**ED and cardiovascular risk factors**

Prevalence of ED was 59.7%. LUTS were present in 64 patients (53.8%) with a mean sum score of the IPSS of 3.6 ± 1.7 points. In univariate analysis, the IPSS was associated with an increased ED-score indicating a decrease of erectile function (24.3 ± 8.9 vs. 17.8 ± 7.4, P < 0.0001).

Univariate analysis revealed a significant influence of hypertension (P = 0.017) and diabetes (P = 0.012) on decreased erectile function. Prior stroke (P = 0.238) and myocardial infarction (P = 0.685), pelvic surgery (P = 0.484), NYHA classification (P = 0.931), former smoking (P = 0.200), hyperlipoproteinaemia (P = 0.734), and use of cardiovascular drugs [angiotensin-converting enzyme-inhibitors (ACE-I) (P = 0.430), angiotensin receptor blockers (ARB) (P = 0.900), calcium channel blockers (P = 0.301), beta-receptor blockers (P = 0.100), diuretics (P = 0.070), statins (P = 0.519), and acetylsalicylic acid (P = 0.850)] did not affect erectile function significantly. In contrast, smoking was associated with an increased erectile function (P < 0.032).

Age (r = 0.489, P < 0.0001) and BMI of patients (r = 0.319, P = 0.003) correlated positively with ED, whereas plasma concentrations of high-density lipoprotein showed a negative correlation (r = -0.2463, P = 0.034). Total cholesterol (P = 0.512) and low-density lipoproteins (P = 0.084) did not correlate with ED.

**ED and cardiovascular disease**

Patients with prior coronary intervention procedure or coronary artery bypass grafting had significantly higher ED-scores (23.0 ± 8.6 vs. 19.8 ± 8.8, P = 0.005), indicating a decreased erectile function. In addition, patients with
ED and EPC

EPC counts of CD133⁺ cells were transformed logarithmically with a corresponding range from 1.73 to 3.11. Patients with ED had significantly lower levels of circulating CD133⁺ cells than patients with a normal erectile function (2.47 ± 0.31 vs. 2.59 ± 0.21, \( P = 0.025 \)). In contrast, CD34⁺/KDR⁺ cells were distributed similarly in both groups (1.85 ± 0.25 vs. 1.83 ± 0.25, \( P = 0.805 \)).

Circulating CD133⁺ cells showed a negative correlation with erectile dysfunction with a decreasing ED in patients with increasing numbers of EPC (\( r = -0.274, P = 0.004 \), Figure 2). Multivariable regression analysis adjusted to age, diabetes mellitus, hypertension, smoking, LUTS, BMI, LVEF, prior ACB-surgery, or PTCA and current use of statins identified lower levels of circulating CD133⁺ cells as an independent risk factor for ED (95% CI −11.183 to −1.7371, \( P = 0.008 \), Table 2). Age, BMI, LUTS, ejection fraction, and prior ACB-surgery or PTCA were also independent predictors of ED (Table 2). In contrast, after logarithmic transformation, CD34⁺/KDR⁺ cells showed no significant effect regarding erectile function (\( P = 0.287 \)).

Discussion

ED is a frequent symptom of generalized atherosclerosis in the elderly, especially in patients with cardiovascular risk factors and diseases. Comparing with recent studies, prevalence of ED was 60% in patients with coronary heart disease.¹³ Prior studies indicated cardiovascular risk factors as predictors of decreased erectile function.³,⁴ Our results confirm these data with hypertension, diabetes mellitus, and low levels of high-density lipoprotein turned out to be an univariate, and age and BMI as independent risk factors for ED. Smoking was shown to be an established risk factor for endothelial and ED.⁴ However, in our study, current use of nicotine was not associated with ED, probably due to the small prevalence of smoking in our population (n = 29, 24%). Consequently, there was also no significant influence on ED in multivariable analysis. Moreover, prior myocardial infarction or stroke showed no association with onset or increase in ED. As ED is suggested to be an early symptom of generalized atherosclerosis and endothelial dysfunction, present coronary artery disease in all of our patients is supposed to bias and exceed the negative influence of cardiovascular risk factors and prior myocardial infarction or stroke on ED. Thus, cardiovascular risk factors or single events as a myocardial infarction seem to influence erectile function scarcely in patients with present atherosclerosis. This suggestion is supported by the finding, that prior ACB-surgery or coronary intervention is an independent predictor of ED in these patients, atherosclerosis is pronounced with an enormous impact on endothelial function in the penis with consecutive ED.

Some studies indicated that treatment of hypertension might contribute to the development of ED.⁴,¹³ Diuretics have been previously shown to impair sexual function in the THOM study. In particular, chlorthalidone was the specific drug showing an increased incidence of ED.¹⁶ After 24 months of treatment this effect was not detected anymore. For calcium-antagonists there is one study on nifedipine showing a greater deterioration in ejaculation and
Endothelial dysfunction is likely to be a decisive component of ED. \[^{30}\] Consistently with these data, our study could not demonstrate any association of cardiovascular drugs with ED in univariate analysis. Only diuretics tended to impair erectile function, which is in line with the results from the TOMH study.

In addition, statins were known to improve endothelial function and increase concentrations of circulating EPC. \[^{23,24}\] Moreover, in diabetic mice, rosuvastatin was shown to improve erectile function. \[^{45}\] In the present study, no association of erectile function and the current use of statins could be detected in univariate analysis. However, multivariable analysis revealed a trend towards an increasing erectile function in patients with statin treatment. Missing the significant level might be due to the low usage of statins in our population (60%). Furthermore, confirmation of positive effects of statins on endothelial function in the corpus cavernosum might be attenuated by present atherosclerosis in our patients.

Reduction of L VEF in patients with chronic heart failure is correlated with an impairment of endothelial function due to neurohumoral alterations and increased levels of cytokines with a possible influence on erectile function. \[^{26,27}\] We could demonstrate a significant correlation of L VEF and erectile function with decreased ejection fraction as an independent risk factor for ED, which reflects the profound impact of cardiac function on endothelial performance per se, independently of concomitant risk factors or diseases.

Both, ED and endothelial dysfunction are related to cardiovascular risk factors. \[^{4,5,28}\] Diminished activity of the endothelial nitric oxide synthase with subsequent decreased levels of nitric oxide appears to be the main molecular mechanism of endothelial dysfunction. Cellular mechanisms of endothelial dysfunction are apoptosis and decreased regeneration of the endothelial monolayer. \[^{29}\] Regarding the amount of endothelial monolayer in erectile tissue, endothelial dysfunction is likely to be a decisive component of ED. \[^{30}\]

EPC may play a major role in repair mechanisms of the endothelial monolayer leading to improvement of endothelial function due to regeneration of the endothelial monolayer as well as neoangiogenesis. \[^{7}\] Moreover, absence of sufficient EPC in patients with endothelial cell injury may affect progression of cardiovascular disease with EPC as an independent predictor of cardiovascular outcome. \[^{11,12}\] Recent studies further demonstrated a reduction of EPC in patients with cardiovascular risk factors or chronic heart failure, but also in patients with endothelial dysfunction. \[^{11,31}\] Thus, circulating levels of EPC could be a pathophysiological link between cardiovascular risk factors, LV dysfunction, and endothelial dysfunction suggesting a significant role of EPC in the progression of atherosclerosis and cardiovascular disease. \[^{32}\] Regarding the association of cardiovascular risk factors and LVEF with ED, beneficial effects of EPC on erectile function are likely.

Circulating levels of EPC were recently shown to be decreased in patients with ED, but without discrimination between CD133\(^{\text{+}}\)- or CD34\(^{\text{+}}\)/KDR\(^{\text{+}}\)-cells and with differences in ED between patients with or without cardiovascular risk factors. \[^{33}\] These data were confirmed in the present study. In addition, circulating levels of CD133\(^{\text{+}}\)-EPC were significantly reduced in cardiovascular high-risk patients with coronary artery disease and ED. In contrast, levels of CD34\(^{\text{+}}\)/KDR\(^{\text{+}}\)-EPC did not differ in patients with or without ED. Furthermore, in multivariable analysis, we could indicate lower levels of circulating CD133\(^{\text{+}}\)-EPC as an independent risk factor for a decreased erectile function in patients with coronary artery disease. Thus, EPC may also be important for endothelial function within the erectile tissue. The group of EPC shows a wide heterogeneity with functionally important subpopulations. Especially, CD133\(^{\text{+}}\)-EPC without expression of CD34 were suggested to be immature and a precursor of CD34\(^{\text{+}}\)-EPC with a higher potency regarding vascular repair mechanisms. \[^{34}\] Regarding our results, the functional endothelial monolayer of the corpus cavernosum as the main target of endothelial dysfunction in patients with ED possibly differs from the vascular endothelium. Thus, particularly immature EPC could be able to regenerate the endothelial monolayer with subsequent amelioration of erectile function, but this remains speculative regarding the unknown CD34-expression in the CD133\(^{\text{+}}\)-EPC analysed in our study.

In conclusion, we demonstrated that decreased numbers of circulating EPC are an independent risk factor for ED, suggesting EPC as a possible link between cardiovascular risk factors and endothelial dysfunction in the penile arteries and the corpus cavernosum. Considering the putatively positive influence of EPC in patients with ED, different treatment options could be possible. A prior study indicated the beneficial effect of intracoronary infusion of autologous EPC in patients with acute myocardial infarction. \[^{35}\] Regarding technical requirements, this kind of treatment in patients with ED seems to be inconvenient. Endogenous activation of circulating levels of EPC appears to be more appropriate. Treatment with statins or the PDE-V-inhibitor vardenafil were recently shown to increase circulating levels of EPC. \[^{24,36}\] Moreover, level of physical activity was independently associated with erectile function, possibly because of increased levels of EPC after recurrent exercise. \[^{37,38}\] Therefore, besides the gain of pathophysiological understanding, our findings identify EPC as a possible novel treatment target in patients with ED.

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