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Original Article

The proposed new classification of coronary microcirculation as the predictor of the heart failure progression in idiopathic dilated cardiomyopathy

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ABSTRACT

Background: The appropriate condition of the coronary microcirculation is essential for proper cardiac muscle activity. The understanding of the pathological microcirculation changes in different stages of idiopathic dilated cardiomyopathy (IDCM) could provide a reliable background for proper therapeutic decisions and prognosis.
Methods and results: The study population consisted of 116 patients (86.2% males, mean age 50.4±13.2 years) with IDCM and heart failure. In samples from left ventricular endomyocardial biopsy, the coronary microcirculation was evaluated by staining with hematoxylin and eosin, Masson's trichrome, and anti-CD34 antibody. The microvessel density (MVD) was calculated. Also, the electron microscopic evaluation of the extracellular matrix capillaries was performed.
Samples were assigned to one of four types according to the microcirculation condition: 1, normal microvessels (MVs) (18 patients); 2, mostly normal, some MVs with slightly decreased lumen diameter and thickened wall, absent/mild intravascular fibrosis, and MVD decrease (37 patients); 3, MVs with moderately decreased lumen diameter and thickened wall, moderate intravascular fibrosis, and MVD decrease (45 patienst); and 4, MVs with significantly decreased lumen diameter and thickened wall, significant intravascular fibrosis, and MVD decrease (16 patients).
Taking all types of the proposed classification into consideration, in type 4, clinical (incidence of New York Heart Association 3 and 4, dyspnea on exertion, pulmonary congestion) and echocardiographic (left atrial and right ventricular diameter, left ventricular mass and ejection fraction, tricuspid annular plane systolic excursion, early diastolic mitral annular velocity measured at the interventricular–septal annulus [E'med], ratio of early diastolic mitral inflow velocity to E'med) parameters were worst. Only atrial fibrillation, diabetes, tricuspid annular plane systolic excursion, and the type of the microcirculation significantly correlated with the incidence of cardiovascular hospitalizations in the linear regression models.
Conclusion: The condition of the coronary microcirculation corresponds with the heart failure progression in patients with IDCM.

1. Introduction

Contemporary markers for the heart failure (HF) progression are characterized by low sensitivity and specificity [1]. The high incidence of HF, as well as the high hospitalization and mortality rates due to HF, justifies the search for novel indicators of the poor outcome. Most of the previous studies concentrated on the contractile apparatus and the extracellular matrix remodeling. In experimental models, it was proven that contractility impairment in the nonischemic HF was the result of the mismatch between angiogenesis intensity and cardiomyocyte hypertrophy [2,3]. Available data suggest that the structural and functional abnormalities of the coronary circulation occur along with the idiopathic dilated cardiomyopathy (IDCM) development [4–7].

In everyday clinical practice, microcirculation is assessed functionally. It is based on the assumption that coronary microcirculation (prearterioles, arterioles, capillaries) is the main factor determining coronary blood flow when no lesion is present in epicardial arteries [4,8,9]. Endomyocardial biopsy (EMB) is the only technique enabling its visualization in living patients [5,10].

The aim of the study was to assess the density and the structure of the coronary microcirculation in a large and representative group of patients with IDCM and to compare the findings with parameters of the HF progression.

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2. Methods

2.1. Study population

Patients hospitalized in the Department of Invasive Cardiology Central Clinical Hospital of the Ministry of the Interior were enrolled prospectively and consecutively. The inclusion criteria were age >18 years, clinical diagnosis of IDCM, left ventricular end-diastolic diameter (LVEDD) ≥56 mm, left ventricular ejection fraction (LVEF) below 45%, and hemodynamic stability. The exclusion criteria were a significant coronary artery lesion on coronarography (defined as the presence of any stenotic lesion with >50% diameter stenosis) and myocarditis confirmed by immunohistochemical staining and/or polymerase chain reaction of EMB specimens positive for cardiotropic viral genomes. The local ethics committee approved the study protocol. Informed written consent was obtained from each study participant. The study was conducted in accordance with the Declaration of Helsinki.

2.2. Endomyocardial biopsy

EMB of the left ventricle was carried out under continuous ECG monitoring. Six tissue samples were collected using a 7 F bioptome (Cordis; Johnson & Johnson Co., New Brunswick, NJ, USA).

2.3. Echocardiography

A comprehensive, standard transthoracic echocardiography examination was carried out using a commercial diagnostic ultrasound system equipped with a 3.5-Hz transducer (iE 33; Philips Medical System, Best, the Netherlands). All measurements were taken according to the guidelines of the European Association of Cardiovascular Imaging by an experienced cardiologist blinded to the patients' clinical history [11].

2.4. Histopathology and immunohistochemistry

Two to three (in case of discrepancies in the assessment) tissue samples from each patient were evaluated histologically with hematoxylin and eosin (H&E) and Masson's trichrome (TM) methods. The immunohistochemical staining with anti-CD34 antibodies was used to estimate microvessel density (MVD) (IS 632; DAKO, Glostrup, Denmark).

2.5. Electron microscopy

One tissue sample was used for the electron microscopic (EM) examination. Those samples were fixed in 2% paraformaldehyde and 2.5% glutaraldehyde in cacodylate buffer and postfixed in 2% OsO_4. After dehydration in a series of increasing alcohol (EtOH) concentrations (30%–99.9%) and propylene oxide, tissue samples were embedded in Epon blocks. Then material was processed for EM and analyzed (Jeol JEM 1011, Japan).

2.6. Morphometry

The microvessel lumen diameter, wall thickness, and structure as well as peri-/intravascular fibrosis were assessed in tissue sections stained with H&E and TM. MVD was assessed in tissue sections stained with anti-CD34 antibodies with the use of morphometric image analysis software (ImageJ, USA). MVD was calculated as a mean density from four surface areas 300 μm × 300 μm. MVD was expressed per unit area (0.09 mm^2). Two independent pathologists evaluated two sections prepared from each biopsy sample. Image analysis was performed using the same lighting conditions and optical settings for each sample. A morphometric EM examination was conducted in order to assess the ultrastructure of the extracellular matrix capillaries.

2.7. Statistics

The continuous variables were expressed as mean ± standard deviation; the intergroup differences were tested using Student's t test, whereas analysis of variance was used for multiple group comparisons. The categorical variables were expressed as numbers and percentages; the intergroup differences were tested using the χ^2 test. The relation between variables was assessed using linear regression. Survival was analyzed using univariate and multivariate Cox proportional hazard regression. All tests were two sided with a significance level of $P<.05$. A commercial statistical package (SPSS 19.0; IBM, Armonk, NY, USA) was used for all statistical analyses.

3. Results

3.1. Study population

The study population consisted of 116 Caucasian patients (86.2% males, mean age 50.4±13.2) with IDCM hospitalized between January 2000 and December 2012. The characteristics of the entire study population are presented in Table 1. The average follow-up lasted 41.2± 26.2 months (minimum of 9.2 months, maximum of 139.5 months).

3.2. Ultrastructural changes

The endothelial luminal surface was smooth, smooth with present microvilli (filopodia), or high and hypertrophic. If the basement membrane was observed, it was well defined, but more often the basement membrane-like material was recognized. The nucleus of the endothelial cell either had a normal morphological structure or invaginations of the membrane existed. In many samples, erythrocytes adherent to endothelial surface were present. Mitochondria of the endothelial cells were few and oval, whereas in other samples, the increase in their number and size, the change of a shape (to polymorphic or round), and often the decrease in the number of cristae were noticed. Pinocytic vesicles were either few in number and small or abundant and large. The lumen of capillaries was either normal or deformed and narrowed. In some samples, necrosis of capillaries with the high and hypertrophic endothelium was observed.

3.3. Classification of myocardial microcirculation in patients with IDCM

The analysis of the microvessel lumen diameter, wall thickness and structure, peri-/intravascular fibrosis, and MVD in light microscopy enabled to distinguish four morphological types (Table 2, Figs. 1–2). In the EM evaluation, features of the extracellular matrix capillaries (including the presence of hypertrophy, erythrocyte adherence, microvilli, pinocytic vesicles, equivalents of vascular loops in light microscopy, and features of young and forming vessels) were taken into account (Table 3, Fig. 3). Based on those parameters, study population was assigned to one of four types of myocardial microcirculation: type 1, 18 patients; type 2, 37 patients; type 3, 45 patients; and type 4, 16 patients.

Because the analyzed lesions may be heterogeneous in IDCM, problems with the assignment to a specific type were probable. In such cases, in order to avoid the incorrect assignment, an additional biopsy sample was evaluated.

3.4. Relation between MVD and left ventricular mass

In the linear regression, MVD declined along with the left ventricular mass increase. The correlation was negative, significant, but weak (beta=−0.27, $R^2=0.07$, $P=.04$).

3.5. Clinical parameters and microcirculation classification

There were no statistically significant differences in the basic characteristics of types 1–4 (Table 1).