Unique Protocol ID: DCM-AHEAD

Brief Title: Diabetes and Lipid Accumulationand Heart Transplant (DCM-AHEAD)

Official Title: Lipid Accumulation in Heart Transplant From Non-diabetic Donors to Diabetic Recipients

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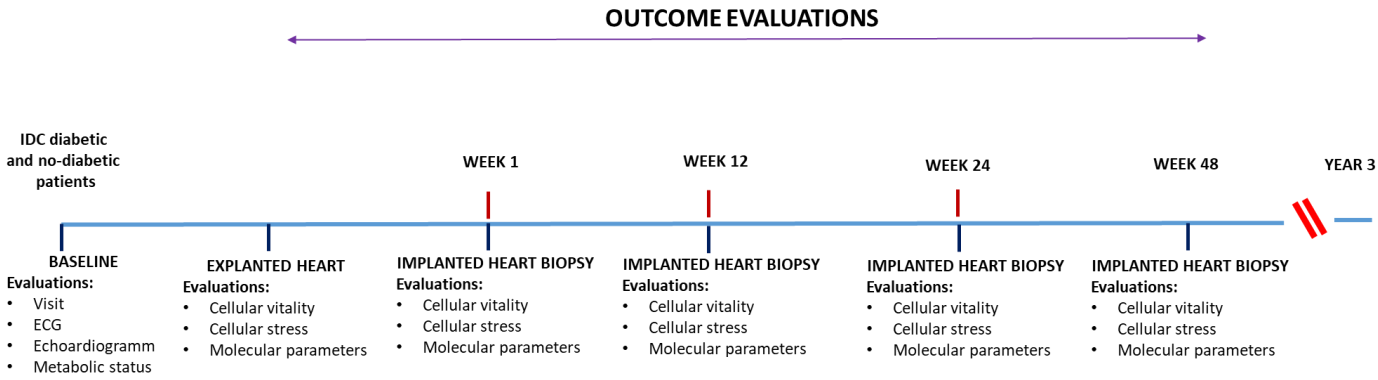
BACKGROUND

Idiopathic dilated cardiomyopathy (IDC) is defined by the presence of left ventricular systolic dysfunction in the absence of an abnormal loading condition or significant coronary heart disease (1). IDC is the leading cause of end-stage heart failure (HF) and is responsible for half of all heart transplants (HTx) (1). Endocrine disorders, including diabetes, are known to be associated with IDCs. Diabetes mellitus (DM), which is present in 75% of patients with idiopathic IDC, is an independent risk factor for the development of heart failure and death in IDC (2-4). Therefore, DM may exacerbate the need for HTx; moreover, diabetic patients are less suitable for HTx and DM remains an independent risk factor for death even after HTx (5). Recent studies have revealed the presence of diabetic cardiomyopathy, a condition of myocardial dysfunction without coronary heart disease (6, 7). This term was first introduced by Rubler et al. (8) in 1972, which highlighted patients with diabetes and congestive heart failure with normal coronary arteries. The pathophysiological mechanisms by which diabetes affects the development and progression of diabetic heart disease are not known. Therefore, the aim of our study will be to evaluate, in the diabetic heart removed, the presence of any cellular alterations related to diabetic disease. In addition, the progression of such lesions in the transplanted heart in diabetic patients will be evaluated.

MATERIALS AND METHODS

Study protocol

A prospective observational study will be conducted with a follow-up duration ranging from a minimum of 12 months to a maximum of 36 months (Figure).



Patients

A cohort of consecutive patients (diabetics vs. non-diabetics) with IDCM and NYHA class III/IV heart failure refractory to maximum medical therapy and treated with cardiac transplantation at the Division of Cardiac Surgery of the University of Campania "Luigi Vanvitelli" will be evaluated prospectively. At the Department of Medical Sciences will be conducted the clinical follow-up for cardiometabolic evaluation (eg routine, glycemic compensation). The molecular and cellular study will be conducted at the department of biochemistry. The follow-up will be 12 months. Patients will be recruited aged > 18, <75 years, with indication to receive a heart transplant (survival score for accepted heart failure (HFSS) at high risk, peak VO2 <10 ml / kg / min after reaching the anaerobic threshold; recurrent symptomatic ventricular arrhythmias refractory to medical treatment, ICD and surgical), affected by IDCM with heart failure in class III / IV NYHA refractory to maximal medical therapy; diabetic and non-diabetic patients. Patients with non idiopathic dilated heart disease (valvular disease, ischemic-infarct heart disease, etc.) will be excluded. All patients will be included in the study after signing the informed consent to participate in the study. The study will be performed according to the Helsinki Declaration.

Explanted heart anlysis

Myocardial tissues from hearts removed from diabetics vs. non-diabetics will be analyzed from a histological, microscopic and metabolic functional point of view. From the residual portion of the heart removed, after routine histological analyses conducted according to the "Heart Transplant Clinical Guidelines 2017". (9), a portion of muscle tissue (quantity 5-10 mm3) will be removed, from which 3 portions will be obtained: one portion will be incorporated into the OCT compound for immunohistochemical analysis, a second portion will be immediately frozen in liquid nitrogen and stored at -70°C for WB evaluation, and a third portion will be used for quantitative evaluations performed by ELISA.

E ndo-myocardial biopsies and Follow up

Transplanted patients will be followed through an echocardiographic evaluation (enrolment and 3-6-12 months follow-up), to evaluate cardiac functional parameters, metabolic parameters and survival outcomes (death for all causes, cardiac death, re-hospitalisation for heart failure). In all transplanted patients endo-myocardial biopsies (EMB) are collected according to normal clinical practice by the "ASHI" guideline (9): weekly for the first month; every 2 weeks for the following month; 1 for the following 4 weeks; 1 for the following 6 weeks; every 3 months for the following 2 years; and finally every 6 months for the following 3 years. 2-6 (usually 4, 1 to 3 mm3) fragments of myocardial tissue will be collected from the apical segment of the right side of the interventricular septum (IVS). The collected myocardial tissue will be immediately analyzed for histopathological evaluation of rejection. From the remaining portion of tissue 3 portions will be obtained: one portion will be incorporated into the OCT compound for immunohistochemical analysis, a second portion will be frozen immediately in liquid nitrogen and stored at -70°C for PCR evaluation, and a third portion will be used for quantitative evaluations performed by ELISA.
The conservation and morpho-functional evaluation of biopsy samples will be carried out at the Bio-Bank of the University of Campania "Luigi Vanvitelli".

Biopsies analysis

PCR.

Total RNA will be isolated from human heart sample homogenates using the RNeasy Mini kit (74106, Qiagen), according to the manufacturer's protocols "Stabilization of RNA in collected animal tissues" and "Purification of total RNA from animal tissues". RNA concentration and purity will be determined using the NanoDrop 2000c (Thermo Fisher Scientific) spectrophotometer. Genomic DNA (gDNA) contamination will be removed from RNA samples prior to the Reverse Trascription (RT) phase, performed on the Gene AMP PCR System 9700 (Applied Biosystems)

using the QuantiTect Reverse Transcription Kit (205311, Qiagen), according to the "Reverse Transcription with Elimination of Genomic DNA for Quantitative, Real-Time PCR" protocol. The final step for real-time PCR analysis (qPCR) will be performed on the CFX96 Real-time System C1000 Touch Thermal Cycler (BIORAD). The analysis will be performed according to the "Two-Step RT-PCR (Standard Protocol)" protocol, using the QuantiTect SYBR Green PCR Kit (204143, Qiagen) and specific QuantiTect Primer Assays (249900, Qiagen) for each gene tested. Relative quantization of gene expression will be performed using the 2^-ΔΔΔΔCt method, using GAPDH as the cleaning control gene. P values will be calculated on the basis of a student t-test or one-way variance analysis (ANOVA), followed by Dunnett's post hoc test; $P < 0.05$ will be considered significant.

Immunofluorescence. Confocal laser scanning microscopy.

The analysis of immunofluorescence in sections of the myocardium will be performed according to the manufacturing protocol using the laser scanning confocal microscope (LSM 700; Zeiss). In particular, the complete removal of the paraffin will be carried out before proceeding to the staining of the section. For this purpose, the slides will be deparaffinated by passing them through xylenes, alcohols and water. An antigen recovery buffer (10 mM sodium citrate, 0.05% Tween 20, pH 6.0) will be added to the dewaxed and rehydrated sections and boiled in the microwave for 20 minutes. After 20 minutes, immunofluorescence staining will be performed using specific antibodies against PPAR-alpha (1µg/ml) (polyclonal rabbit, NB600-636, Novusbio, Abingdon, UK), PPAR-gamma (1µg/ml) (mouse monoclonal, orb69095, Biorbyt, Cambridge, UK) or against SREBP1 (1µg/ml) (polyclonal rabbit, ab28481, Abcam, Cambridge, UK). Secondary antibodies will be conjugated with Alexa Fluor 633 or Alexa Fluor 488 (Life Technologies Italia, Monza, Italy). Nuclear contrast staining will be performed using the VECTASHIELD® mounting medium with DAPI (H-1200, Vector Laboratories, Burlingame, USA).

Immunohistochemistry.

Oil Red O (Solvent Red 27, Sudan Red 5B, C.I. 26125, C26H24N4O) will be used for the assessment of the accumulation of intramyocytic lipids.

Statistical analysis

Study population groups (diabetics vs. non-diabetics) will be compared using the Pearson test for categorical variables and the Kruskal-Wallis test for continuous variables. Candidates for admission to the multivariate model will be identified by focusing on factors that will differ significantly (P value < 0.05) in the univariate analysis among diabetic vs non-diabetic patients. Cox regression will be used to construct the predictive model of mortality. The risk ratio for mortality will be adjusted by age, BMI, cholesterol, LDL, triglycerides and aspirin, ticlopidine, anti-aggregants, beta-blockers, ACE inhibitors or tailors, antidiabetic drugs, statins, etc. present at the time of admission to hospital for cardiac transplantation. The analysis of survival after cardiac transplantation will be performed using the Kaplan-Meier curve and the Cox regression method. Mortality curves will be obtained separately for diabetic and non-diabetic patients, and then compared using the log-rank test. All tests will be considered significant if they have a value of $p<0.05$. All tests will be conducted in 2 populations: diabetic vs non-diabetic patients after cardiac transplantation. The SPSS program (version 21, IBM SPSS) will be used for all analyses.

References

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