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12-month patterns of serum markers of collagen synthesis, transforming growth factor and connective tissue growth factor are similar in new-onset and chronic dilated cardiomyopathy in patients both with and without cardiac fibrosis

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ABSTRACT

Background: The dynamics of the extracellular matrix (ECM) fibrosis process in dilated cardiomyopathy (DCM) may be assessed non-invasively by means of serum markers of fibrosis. *Aim:* To explore the kinetics of serum markers of fibrosis during a 12-month follow-up in DCM. *Methods:* We included 70 consecutive DCM patients (pts) (48 ± 12.1 years, EF 24.4 ± 7.4%) with new-onset (n = 35, duration < 6 months) and chronic DCM (n = 35, > 6 months). Markers of collagen type I and III synthesis – procollagens type I and III carboxy- and amino-terminal peptides (PICP, PINP, PIIICP, PIIINP), and ECM metabolism controlling factors – tumor growth factor beta-1 (TGF1-β), and connective tissue growth factor (CTGF) – were measured in serum at baseline, and at 3- and 12-month follow-up. All pts underwent endomyocardial biopsy to determine the presence and extent of ECM fibrosis. *Results:* Markers of collagen type I synthesis (PICP and PINP) were almost homogenously increased over the 3- and 12-month period, whereas PIIINP values decreased and PIIICP levels were unchanged in new-onset and chronic DCM, and in pts with and without ECM fibrosis. Both TGF-β and CTGF levels decreased over the observation period. Kinetics of serum markers of collagen synthesis and fibrosis controlling factors did not differ between DCM pts categorized according to disease duration and fibrosis status. *Conclusions:* The kinetics of collagen type I and III synthesis in DCM move in opposite directions, with production of collagen type I consistently increasing, and the synthesis of collagen type III decreasing. Levels of TGF and CTGF, which are proven fibrosis-stimulating factors, had a tendency to decrease. Regardless of disease duration or fibrosis status, the kinetics of serum markers of collagen synthesis, TGF and CTGF were similar in DCM. A better understanding of the kinetics of serum markers of fibrosis in DCM may help to develop more tailored therapeutic approaches to fibrosis.

1. Introduction

Qualitative and quantitative abnormalities of the extracellular matrix (ECM) are ubiquitous in dilated cardiomyopathy (DCM) and heart failure (HF) [1]. The main components of ECM are collagens type I and III, which make up 80% and 10% of all fibrillar proteins, respectively [2]. The physical properties of collagens significantly differ; collagen type I is thicker and highly cross-linked, thereby providing resistance, whereas collagen type III consists of small-diameter fibers, and is essentially non-cross-linked, thus providing elasticity [3]. Interstitial or reactive ECM fibrosis is frequently reported in DCM [4]. ECM fibrosis in DCM is characterized by an increase in collagen type I accumulation in the interstitium with a relative decrease in collagen type III deposition, resulting in a substantial shift in the ratio of those two collagens [5]. The net result of these changes of collagen components is a global increase in left ventricular (LV) stiffness. Endomyocardial biopsy (EMB) is still a reference method for ECM fibrosis assessment [6]. However, sampling errors, due to the patchy distribution of fibrosis, safety issues, *know-how*, and costs are major limitations on the wide-spread use of EMB [7]. Therefore, a

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number of blood molecules have been proposed as markers of ECM fibrosis. Among the many molecules tested so far, only procollagen type I, the carboxy-terminal propeptide (PICP) and procollagen type III amino-terminal propeptide (PIIINP), which are formed during extracellular conversion of procollagens into mature fibril-forming collagens and released into the bloodstream in 1:1 stoichiometric ratio, have been unequivocally confirmed to be related to ECM fibrosis [8,9]. The process of ECM fibrosis is controlled by several factors, including the renin-angiotensin-aldosterone system, and chemokines, such as transforming growth factor beta (TGFβ) as well as its downstream signal mediator – connective tissue growth factor (CTGF) [10,11]. Measurements of fibrosis-linked biomarkers may provide insight into ongoing ECM remodeling in the heart. However, it should be emphasized that in the majority of studies only single (baseline) measurements of fibrosis-related biomarkers have been performed. Thus, the kinetics of serum markers of fibrosis over a 12-month observation period has not been formally studied. Most notably, patterns of change in serum markers of fibrosis in DCM have not been directly addressed. Moreover, it is unknown whether changes in the serum markers of fibrosis over an observational period are related to either disease duration or fibrosis status in DCM.

Therefore, we aimed to investigate the time course profile of selected serum markers of fibrosis: markers of collagen synthesis, TGFβ, and CTGF after a period of 12 months, with interim measurements in the 3rd month, in cases of DCM sub-divided on the basis of the duration of the disease and fibrosis status.

2. Methods

2.1. Study population

The study population consisted of 70 consecutive DCM patients, recruited over a period of 15 months, who provided written informed consent, and which was approved by the relevant institutional committees and the Ethical Committee. DCM was diagnosed in line with the current European Society of Cardiology 2007 guidelines [12]. In order to be recruited, the patients had to remain in an uncompromised condition, graded in line with the New York Heart Association (NYHA) class I-III, for at least two preceding weeks. Furthermore, we made sure to include the same number of patients (n = 35 per each group), with new-onset defined as HF symptoms lasting ≤6 months (group 1), and chronic DCM (group 2) when the duration of symptoms was longer than 6 months. The duration of HF symptoms was measured based on the time which had elapsed from the beginning of typical HF symptoms (such as dyspnea on exertion or at rest, paroxysmal nocturnal dyspnea, orthopnea, palpitations, and/or edemas) to the index hospitalization or ambulatory visit to cardiology clinics; this measurement was then utilized to qualify patients for inclusion into either the new-onset or chronic group. Additionally, patients with conditions known to be implicated in collagen turnover, including bone and joint diseases, chronic liver insufficiency, peripheral atherosclerosis, and neoplasms, were excluded from the study. Assessment of the patients' status, echocardiographic examinations, and blood sampling were repeated at the 3- and 12-month stages, following their inclusion. A study flow diagram is presented in Fig. 1.

2.2. Echocardiography

Echocardiographic examinations complied with the recommendations of the European Associations of Cardiovascular Imaging [13]. All echocardiographic investigations were made on commercially available equipment (Vivid 7 GE Medical System, Horten, Norway) with a phased-array of 1.5–4 MHz transducer. The conventional M-mode, 2-dimensional and Doppler parameters were calculated. All measurements were obtained from the mean of 3 beats for patients with sinus rhythm, and 5 beats for those with atrial fibrillation. Chamber diameters, areas, and volumes were normalized for body surface area (BSA).

2.3. Endomyocardial biopsy (EMB)

EMB procedures were performed via a femoral or jugular vein approach [7]. Long (104 cm), flexible, disposable biopsy forceps, 7 French size, with small jaws (Cordis®, Johnson & Johnson Co., Miami Lakes, FL, USA) were used. Simultaneous fluoroscopic guidance and bioptom curvature ensured the precise biopsy of the right ventricular interventricular septum. Up to five myocardial samples were obtained, and stored in formalin for light microscopic examinations. The presence and levels of fibrosis were determined qualitatively by an experienced pathologist who had been blinded to the clinical data. Specimens for fibrosis assessment were stained with Masson's trichrome, where fibrotic areas stained blue and normal muscle fibers stained red. We defined ECM fibrosis as the disproportionate accumulation of fibrillar collagen between intermuscular spaces previously devoid of collagen, which may also compress surrounding cardiomyocytes. Patients were diagnosed as either fibrosis-positive or negative.

2.4. Laboratory measurements

Venous blood for biomarker assessment was drawn at baseline, and after 3 and 12 months, following an overnight fast, typically between 8 and 9 a.m. After being centrifuged, the supernatant was stored at −20 °C until time of assay. The levels of all markers and fibrosis controlling factors were determined in plasma with available ELISA tests, such as Procollagen I N-Terminal Propeptide (PINP), Procollagen III N-Terminal Propeptide (PIIINP), Procollagen I C-Terminal Propeptide (PICP), Procollagen III C-Terminal Propeptide (PIIICP), Connective Tissue Growth Factor (CTGF) (all from Cloud Clone Corp. Houston, TX, USA); and TGF-β1 (Diaclone SAS, Besancon Cedex, France). All laboratory work was conducted by technicians unaware of the clinical and sample status. Normal serum ranges for PICP, PINP, PIIICP, PIIINP, TGF-β1, and CTGF were provided by the manufacturers. These ranges were 0.064–0.186 ng/ml for PICP; 30.2–55.1 pg/ml for PINP; 5.2–35.5 ng/ml for PIIICP; 26.9–63.6 ng/ml for PIIINP; 4.6–14.7 ng/ml for TGF-β1, and 2.3–42.5 ng/ml for CTGF. Intra-assay and inter-assay coefficients of variation were found to be < 7%.

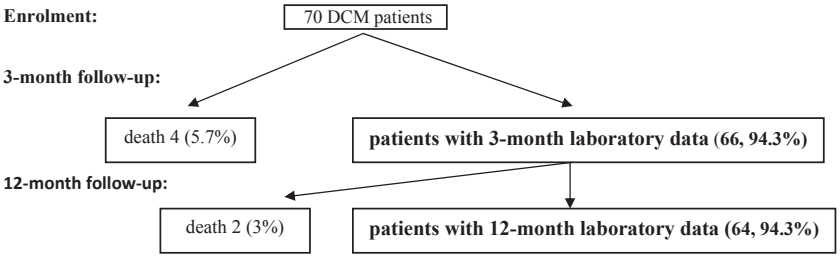

Fig. 1. Study flow chart.