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Original Article

Prognostic value of fibrosis-related markers in dilated cardiomyopathy: A link between osteopontin and cardiovascular events

Paweł Rubiś[a,*], Sylwia Wiśniowska-Śmiałek[a], Ewa Dziewięcka[a], Lucyna Rudnicka-Sosin[b], Artur Kozanecki[a], Piotr Podolec[a,c]

[a] Department of Cardiac and Vascular Diseases, John Paul II Hospital, Krakow, Poland
[b] Department of Pathology, John Paul II Hospital, 31-202 Krakow, Poland
[c] Jagiellonian University, Medical Collage, Krakow, Poland

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ABSTRACT

Introduction: Serum markers of fibrosis provide an insight into extracellular matrix (ECM) fibrosis in heart failure (HF) and dilated cardiomyopathy (DCM). However, their role as predictors of cardiovascular (CV) events in DCM is poorly understood.
Methods: This is an observational, prospective cohort study. 70 DCM patients (48 ± 12.1 years, ejection fraction – EF 24.4 ± 7.4) were recruited. Markers of collagen type I and III synthesis – procollagen type I and III carboxy- and amino-terminal peptides (PICP, PIIICP, PINP, PIIINP), fibrosis controlling factors – ostepontin (OPN), transforming growth factor (TGF1-β) and connective tissue growth factor (CTGF), and matrix metalloproteinases (MMP-2, MMP-9) and tissue inhibitor (TIMP-1), were measured in serum. All patients underwent endomyocardial biopsy. The end-point was combined with CV death and urgent HF hospitalization. Patients were divided into two groups: those who did (group 1, n = 45) and did not reach (group 2, n = 25) an end-point.
Results: Over a 12-month period of observation, 6 CV deaths and 19 HF hospitalizations occurred. Qualitative and quantitative measures of ECM fibrosis were similar in both groups. The levels of all of the markers of collagen synthesis, TGF1-β, MMP-9 and TIMP-1 were similar, however, OPN, CTGF and MMP-2 were significantly lower in group 1.
Conclusions: Invasively-determined fibrosis levels were not related with CV outcomes in DCM. Out of the 11 markers of fibrosis under study, only OPN was found to be related to CV outcomes. OPN is not only the pivotal protein controlling fibrosis, but may also serve as a biomarker associated with prognosis.

1. Introduction

Alterations in the extracellular matrix (ECM) result in cardiac fibrosis. Reactive and diffuse myocardial fibrosis is one of the main features of dilated cardiomyopathy (DCM) [1]. ECM fibrosis has numerous adverse effects on cardiac morphology and function, including an increase in collagen type I at the expense of collagen type III, which substantially increases left ventricular (LV) stiffness and alters LV filling, and produces abnormalities in collagen fiber alignment which then reduce myocardial force transmission, resulting in contractility impairment [2]. Another negative effect is the production of excess collagen tissue which may then result in either a conduction blockade or re-entrant arrhythmias [3]. Therefore, without doubt, ECM fibrosis contributes significantly to the transition from compensated and oligo-symptomatic DCM to overt heart failure (HF) [4]. Despite the well-known role of fibrosis in DCM, very few studies have studied the association between fibrosis and mortality. Moreover, in the majority of studies thus far, ECM fibrosis has been assessed non-invasively with cardiac magnetic resonance (CMR) [5,6,7] rather than via endomyocardial biopsy (EMB), the latter being the gold standard of myocardial structural assessment [8]. The most common localization of fibrosis in DCM is interstitial (pericellular). Post-mortem studies, along with paired LV and right ventricular (RV) biopsies, have shown that ECM fibrosis in DCM is diffuse. Thus, RV biopsy, which is the most commonly performed procedure, should provide data similar to that obtained from LV biopsy. The Masson's trichrome stain is particularly useful in the assessment of the main component of connective tissue e.g. collagen, which is the main component of interstitial fibrosis. In addition, myocardial fibrosis can be assessed indirectly by means of a wide array of imaging

* Corresponding author at: Department of Cardiac and Vascular Disease, John Paul II Hospital 31-202 Krakow, Pradnicka street 80, Poland.
E-mail address: pawelrub@poczta.onet.pl (P. Rubiś).

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modalities. Although widely available, echocardiography and nuclear imaging have low specificity for ECM fibrosis detection, whereas positron emission tomography (PET) has high specificity but its availability is limited. Therefore, CMR is currently the method of choice for cardiac fibrosis assessment. CMR with delayed imaging following administration of gadolinium contrast allows the visualization of regional myocardial fibrosis via areas of late gadolinium enhancement (LGE) [5,6]. Moreover, assessment of diffuse fibrosis can be achieved with post-contrast enhanced T1 and T2 mapping. However, there are few studies with conflicting results on biopsy-determined fibrosis and outcomes in DCM and HF.

Increased collagen synthesis and degradation are indicated in the pathology of ECM fibrosis in DCM [9]. Measurement of collagen byproducts in blood is relatively straightforward and provides data which have led to insights into the dynamics of ECM fibrosis. These studies have shown that synthesis of collagen type I prevails over collagen type III during the ECM fibrosis process in DCM [10]. It is also known that transforming growth factor (TGF), acting via connective tissue growth factor (CTGF), and ostepontin (OPN) are both major cytokines implicated in the initiation and progression of the fibrosis process [11,12]. In addition, during ECM fibrosis, there has been found to be an overexpression of matrix metalloproteinases (MMPs) that escape the normal controls of tissue inhibitors (TIMPs) [13]. The various links between serum markers of fibrosis and invasively-determined ECM fibrosis have been extensively studied. However, to date, clear associations have only been found for two collagen synthesis by-products: C-terminal propeptide of procollagen type I (PICP) and the N-terminal propeptide of procollagen type III (PIIINP). The important role of various molecules, such as TGF, CTGF, cardiotrophin-1, OPN, MMPs and TIMPs in the fibrotic process have been described in animal models. Still, the significance of serum levels of these molecules and ECM fibrosis in humans is less clearly understood. Bearing in mind the crucial roles of these molecules in the pathology of fibrosis, it is of little wonder that they were thought to be associated with survival in patients with cardiac diseases. However, in the majority of studies so far, unselected HF populations have been investigated, and thus these findings may not necessarily apply to specific diseases, such as DCM.

In the present study, we examined the associations between biopsy-detected ECM fibrosis and a wide array of serum markers of fibrosis, including markers of collagen synthesis, fibrosis controlling factors, and the MMP/TIMP system with regards to cardiovascular (CV) outcomes in a contemporary DCM cohort.

2. Material and methods

2.1. Study population

This is a single-center, observational, prospective cohort study. Over a period of 14 months, 70 consecutive patients with DCM were recruited for this study. The diagnosis of DCM was confirmed according to the European Society of Cardiology (ESC) 2007 guidelines having excluded significant coronary artery disease, primary heart valve disease, congenital heart disease, and arterial hypertension [14]. All patients had significantly dilated LV (>117% of predicted LV end-diastolic diameter) and depressed systolic function with ejection fraction (EF) below 35%. For the purposes of inclusion, the patients had to have stable HF symptoms, in line with the New York Heart Association (NYHA) class I–III, for at least the preceding two weeks. Study participants were optimally treated according the current ESC guidelines and had their HF-medications appropriately up-titrated [15]. Furthermore, the presence of concomitant non-cardiac diseases, such as bone and joint diseases, chronic liver insufficiency, peripheral atherosclerosis, and neoplasms affecting collagen metabolism and the circulating levels of procollagens also served as exclusion criteria [16]. Each patient was observed for 12 months from the time of inclusion, with one interim visit after 3 months, and no patient was lost to follow-up. At the 12-month point of follow-up, CV death had occurred in 6 (8.6%) patients, and urgent HF hospitalization in 19 (27.1%) patients. Based on this data, patients were divided into two groups: those who did not have a combined end-point (group 1, n = 45), and those in whom a combined end-point occurred (group 2, n = 25). All patients were willing to participate, and signed informed consent forms which were approved, along with the study protocol, by the relevant institutional committees and the Ethical Committee.

Table 1
Baseline characteristics of the study population.

Parameter	group 1 (n = 45)	group 2 (n = 25)	p-value
Age [years]	46.6 ± 12.6	50.6 ± 10.9	0.19
Sex [male/female]	40 (89%)/5 (11%)	23 (92%)/2 (8%)	0.68
BMI [kg/m^2]	27.3 ± 5.2	27.1 ± 5.6	0.85
NYHA class	2.47 ± 0.76	2.76 ± 0.66	0.11
Duration [months]	**17.8 ± 28.2**	**36 ± 44.3**	**0.04**
LBBB [n, %]	16 (35.6%)	11 (44%)	0.49
LVESd/BSA [mm/m^2]	**28.2 ± 5.8**	**33.4 ± 8.2**	**0.003**
LVEDd/BSA [mm/m^2]	**33.8 ± 5.4**	**38.8 ± 8.4**	**0.004**
LVESvol/BSA [ml/m^2]	89.8 ± 41.7	107.5 ± 59.2	0.16
LVEDvol/BSA [ml/m^2]	118.1 ± 49.1	142.3 ± 73.8	0.13
EF [%]	25.1 ± 7.2	23.1 ± 7.6	0.29
E/E'(average sep + lat)	20.2 ± 12.5	20.8 ± 9.9	0.83
ECM fibrosis [n,%]	16 (35.6%)	8 (32%)	0.76
CVF [%]	6.7 ± 7	4.4 ± 3.6	0.4
CO [l/min]	**4.3 ± 1.3**	**3.4 ± 0.7**	**0.001**
PA mean [mmHg]	**19.7 ± 8.5**	**29.3 ± 12.1**	**0.001**
PCWP mean [mmHg]	**13 ± 7.5**	**19.7 ± 8**	**0.002**
PH [n, %]	**11 (24.4%)**	**16 (66.7%)**	**0.001**
VO_2peak [ml/kg/min]	**18.3 ± 6.1**	**14.5 ± 5.5**	**0.04**
Hb [g/dl]	14.7 ± 1.5	14 ± 1.7	0.11
creatinine [umol/l]	87.1 ± 20.2	110.4 ± 87.6	0.09
hs-troponin T [ng/ml]	0.02 ± 0.014	0.026 ± 0.023	0.21
hs-CRP [mg/dl]	10.7 ± 26.2	7.4 ± 18.4	0.59
NT-proBNP [pg/ml]	**2426.8 ± 4247**	**5069.7 ± 6852**	**0.05**
Beta-blocker [n, %]	44 (98%)	25 (100%)	0.45
ACE-I [n, %]	43 (95.6%)	23 (92%)	0.54
ARB [n, %]	2 (4.4%)	0	0.28
MRA [n, %]	42 (93.3%)	24 (96%)	0.64
Furosemide [n, %]	**23 (51.1%)**	**19 (76%)**	**0.05**
CRT/ICD [n, %]	14 (31.1%)	13 (52%)	0.08

Data are presented as mean ± SD or n (%).
BMI – body mass index, NYHA – New York Heart Association class, LBBB – left bundle branch block, LVESd/BSA – indexed to body surface area left ventricular end-systolic diameter, LVEDd/BSA – indexed LV end-diastolic diameter, LVESvol/BSA – indexed LV end-systolic volume, LVEDvol/BSA – indexed LV end-diastolic volume, EF – ejection fraction, E/E' (average sep + lat) – ratio of early mitral inflow E-wave and early myocardial E' velocity (E' – is an average of septal and lateral myocardial velocity), CVF – collagen volume fraction, CO – cardiac output; PA mean – mean pulmonary artery pressure, PH – pulmonary hypertension, VO_2peak – peak oxygen uptake, Hb – hemoglobin, CK-MB – myocardial fraction of creatine kinase, hs-troponin T – high sensitivity troponin T, hs-CRP – high sensitivity C-reactive protein, NT-proBNP – amino-terminal pro B-type natriuretic peptide, ACE-I – angiotensin converting enzyme inhibitor, ARB – angiotensin receptor type 1 blocker, MRA – mineralocorticoid receptor antagonist, CRT – cardiac resynchronization therapy, ICD – implantable cardioverter-defibrillator.
Bold values indicate that these particular parameters were statistically significant (p-value < 0.05).

2.2. Endomyocardial biopsy (EMB)

EMB procedures were performed by experienced operators via a femoral or jugular vein [17]. Long (104 cm), flexible, disposable biopsy forceps 7 French size with small jaws (Cordis®, Johnson & Johnson Co, Miami Lakes, FL, USA) were used for the procedure.