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Associations between N-terminal procollagen type III, fibrosis and echocardiographic indices in dogs that died due to myxomatous mitral valve disease

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Abstract *Objectives:* To evaluate associations between N-terminal procollagen type III (PIIINP), a serum biomarker of collagen biosynthesis, and myocardial fibrosis in dogs with naturally-occurring myxomatous mitral valve disease (MMVD).
Animals: Twenty-two dogs with echocardiographically-confirmed MMVD were prospectively recruited from a hospital population. All died as a result of MMVD and their hearts were available for post mortem examination.
Methods: Echocardiographic measurements and serum PIIINP concentrations were obtained from all dogs prior to death or euthanasia. Serum PIIINP concentrations

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(μg/mL) were measured using a validated commercially available radioimmunoassay. Myocardial tissue samples were collected post mortem and myocardial fibrosis was scored. The average fibrosis score for all cardiac sites in the heart was designated the global fibrosis score (GFS). The average fibrosis score for all papillary muscle sites was designated the papillary fibrosis score (PFS). Univariate and multivariate linear regression analyses were used separately to evaluate associations between GFS and PFS, respectively, and PIIINP and echocardiographic variables.
Results: Left ventricular end-diastolic diameter normalized for body weight (LVEDDN) and PIIINP were weakly independently positively associated with both GFS and PFS. LVEDDN and PIIINP were weakly negatively correlated.
Conclusions: Both LVEDDN and serum PIIINP increase with increasing fibrosis score, although these relationships were not strong enough to be clinically useful. Although LVEDDN and PIIINP were positively correlated with fibrosis, PIIINP decreased with increasing LVEDDN, suggesting a complex interplay between fibrosis and remodeling in MMVD.

Abbreviations

ACE	angiotensin converting enzyme
CKCS	Cavalier King Charles spaniel
ECG	electrocardiogram
ECM	extracellular matrix
GFS	global fibrosis score
LA/Ao	left atrial to aortic root diameter ratio
LVEDD/ LVFWd	ratio of left ventricular end-diastolic diameter to left ventricular free wall thickness in diastole
LVEDDN	left ventricular end-diastolic diameter normalized for body weight
MMVD	myxomatous mitral valve disease
PFS	papillary fibrosis score
PIIINP	N-terminal procollagen type III

Introduction

Procollagen molecules consist of the characteristic triple α-helical structure of mature collagen between globular amino- and carboxy-terminal domains.[1] Amino-terminal peptide of procollagen type III (PIIINP) is released into the circulation during both synthesis and degradation of collagen type III, as cleavage of the N-terminal fragment is not essential prior to fibril formation during synthesis.[2,3] In human patients with cardiomyopathy, PIIINP is strongly associated with myocardial collagen type III fraction and is therefore considered a marker of myocardial fibrosis.[4,5] Measurement of serum markers of collagen turnover has not been reported in human patients with mitral valve prolapse.

Mild to moderate myocardial interstitial and perivascular fibrosis is a common post mortem finding in dogs with naturally-occurring myxomatous mitral valve disease (MMVD),[6–8] and survival times (from diagnosis of congestive heart failure to death or euthanasia due to cardiac disease) are negatively associated with myocardial fibrosis scores at post mortem.[8] It is possible, therefore, that a biomarker of myocardial fibrosis might be clinically valuable for staging and prognostication in canine MMVD.

A previously validated radioimmunoassay is available for measurement of serum PIIINP concentrations in dogs.[9] Canine PIIINP is a stable molecule that does not appear to require careful sample handling or to degrade significantly during storage at −80 °C.[9,e] It is not known whether serum PIIINP concentrations reflect myocardial fibrosis in dogs with MMVD, however, a previous study in dogs with naturally-occurring MMVD demonstrated a negative relationship between serum PIIINP concentrations and left ventricular size.[10] It is possible, therefore that overall there is a decrease in extracellular matrix (ECM) turnover in MMVD or that an increase in turnover of collagen type III is not an important component of the development of myocardial fibrosis in this disease. The former is supported by the observation that experimentally-induced mitral regurgitation (MR) is associated with a decrease in interstitial collagen content and collagen organization in dogs,[11–14] while the latter is supported by the observation that eccentric cardiac remodeling in response to volume overload

[e] Hezzell, MJ. Identification of clinical variables associated with ventricular remodeling and survival in canine mitral valve disease. PhD Thesis, London 2012.