Cardiovascular Pathology 24 (2015) 65–70

Contents lists available at ScienceDirect

Cardiovascular Pathology

Review Article

Novel prognostic tissue markers in congestive heart failure

James R. Stone *

Center for Systems Biology, Massachusetts General Hospital and Department of Pathology, Massachusetts General Hospital and Harvard Medical School, Boston, MA, 02114, USA

ARTICLE INFO

Article history:
Received 12 June 2014
Received in revised form 3 July 2014
Accepted 5 July 2014

Keywords:
Apoptosis
Proliferation
Ubiquitin
Cyclophilin A
Gremlin-1
Heterogeneous nuclear ribonucleoprotein C

ABSTRACT

Heart failure is a relatively common disorder associated with high morbidity, mortality, and economic burden. Better tools to predict outcomes for patients with heart failure could allow for better decision making concerning patient treatment and management and better utilization of health care resources. Endomyocardial biopsy offers a mechanism to pathologically diagnose specific diseases in patients with heart failure, but such biopsies can often be negative, with no specific diagnostic information. Novel tissue markers in endomyocardial biopsies have been identified that may be useful in assessing prognosis in heart failure patients. Such tissue markers include ubiquitin, Gremlin-1, cyclophilin A, and heterogeneous nuclear ribonucleoprotein C. In some cases, tissue markers have been found to be independent of and even superior to clinical indices and serum markers in predicting prognosis for heart failure patients. In some cases, these novel tissue markers appear to offer prognostic information even in the setting of an otherwise negative endomyocardial biopsy.

Contents

1. Introduction

Heart failure is a common clinical problem affecting 1%–2% of individuals in developed countries, with one in five people developing heart failure during their lifetime [1]. Recent advancements in treatment have improved survival, but morbidity, mortality, and treatment related costs have remained relatively high. The economic burden of heart failure in North America alone is expected to exceed $70 billion by 2030 [1]. There are multiple distinct causes of heart failure including atherosclerotic coronary artery disease and nonischemic causes of heart failure such as myocarditis, storage diseases, amyloidosis, and inherited and idiopathic cardiomyopathies. Risk stratification or assessment of prognosis is becoming increasingly important in managing patients with heart failure, with such prognostication affecting choice of therapies, decisions to implant mechanical assist devices and defibrillators, and decision making concerning admission, discharge, and follow-up [2–4]. The hope is that, by defining risk more accurately in patients with heart failure, we will be better able to identify the patients who would benefit from more aggressive therapy and follow-up without incurring the unnecessary expenses of delivering the more aggressive management to low-risk patients not requiring such treatment. For patients with nonischemic dilated cardiomyopathy, currently, risk stratification is most often based on clinical parameters such as New York Heart Association (NYHA) functional class, echocardiographic indices such as left ventricular ejection fraction (LVEF), and more recently serum markers such as the level of B-type natriuretic peptide (BNP) [2–4]. However, the shortcomings of these clinical indices to adequately identify all high-risk patients have been noted [5,6].

Endomyocardial biopsy can be very helpful to diagnose specific diseases in patients with nonischemic heart failure including myocarditis, amyloidosis, and storage diseases [7–9]. Identification of such specific disorders can substantially impact both patient management and prognosis [10–13]. However, for many patients with unexplained heart failure, endomyocardial biopsy does not yield specific diagnostic

Abbreviations: BNP, B-type natriuretic peptide; CyPA, cyclophilin A; DCM, dilated cardiomyopathy; hnRNP-C, heterogeneous nuclear ribonucleoprotein C; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association; TUNEL, terminal deoxynucleotidyl transferase–mediated deoxyuridine triphosphate nick end labeling.

Funding: This work was funded by Massachusetts General Hospital.

* Massachusetts General Hospital, Simches Research Building Room 8236, 185 Cambridge Street CPZN, Boston, MA 02114. Tel.: +1 617 726 8303; fax: +1 617 643 3566.
E-mail address: jrstone@partners.org.

http://dx.doi.org/10.1016/j.carpath.2014.07.010
1054-8807/

information. Such biopsies are often referred to as "negative" biopsies. However, such negative biopsies still contain morphologic abnormalities, which, at least in some settings, have been correlated with bad outcomes. Such morphologic abnormalities include increased cardiac myocyte size, increased cardiac myocyte nuclear area, decreased myofibril volume fraction, increased interstitial volume density, increased extracellular matrix, increased fibrosis, and, in younger patients, a low degree of lipofuscin deposition [14–21]. However, it has been difficult to implement standardized prognostication in nonischemic heart failure using these morphologic features. For some of these features, assessment is difficult and time consuming, often requiring computer-assisted morphometrics. In some instances, the association with outcome is relatively weak, yielding overall poor test characteristics, and for some features, there has been a lack of reproducibility of the association with outcome across different institutions [22–25]. In addition, while time consuming to perform, morphometric analyses are often poorly reimbursed by insurance companies and other third-party payers. However, the fact that morphologic changes in the tissue in negative cardiac specimens can be correlated with outcome in some settings suggests that there are molecular alterations in these specimens that might serve as sensitive and specific markers of outcome in this patient population. This review will summarize the current literature on utilizing tissue markers in endomyocardial biopsies and surgical myocardial resections to predict outcome in patients with nonischemic heart failure. In general, the markers discussed here can be assessed using routine formalin-fixed, paraffin-embedded tissue.

2. Cell death and proliferation markers as prognostic markers in congestive heart failure

The first group of markers to be assessed for predictive power in the setting of congestive heart failure involved proteins involved in cell death and proliferation. The general rationale for this approach was that heart failure patients with poor outcome might have decreased rates of cardiac myocyte proliferation and/or increased rates of cardiac myocyte cell death compared with heart failure patients with a better outcome. In an early study, heart tissue from 31 patients with idiopathic dilated cardiomyopathy (IDCM) who underwent partial left ventriculectomy was examined [26]. The surgical ventriculectomy specimens were subjected to terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeling (TUNEL) staining to assess for apoptosis and Ki-67 immunohistochemistry to assess for proliferation. Cardiac myocyte staining and interstitial cell staining were each quantified. The degree of staining was compared with overall survival at 6 months and 5 years after surgery. The degree of Ki-67 staining did not correlate with survival at either time point. Likewise, there was no difference in the degree of apoptosis when comparing survivors with nonsurvivors at the shorter 6-month follow-up interval. However, those patients who were alive 5 years after the surgery had significantly fewer apoptotic cardiac myocytes as well as significantly fewer apoptotic interstitial cells than those patients who died within 5 years of the surgery. Thus, a high degree of apoptosis in the myocardial tissue indicated an increased risk for death on long-term follow-up.

In a similar but larger study, intraoperative myocardial biopsies from 56 patients with IDCM undergoing mitral and tricuspid valve annuloplasty were examined [27]. The tissue was assessed for the degree of cardiac myocyte apoptosis by both TUNEL staining and immunohistochemistry for the apoptosis inhibitor Bcl-2. In addition, the tissue was assessed for the expression of the proliferation markers Ki-67 and proliferating cell nuclear antigen by cardiac myocytes. The patients were divided into two groups based on survival: those who died within 3 years of surgery and those who were alive at 7 years following surgery. For all four markers, there were statically different levels of staining between the two patient groups. Compared with the survivors, the nonsurvivors had more cardiac myocytes with apoptosis and fewer cardiac myocytes expressing the apoptosis inhibitor Bcl-2 and proliferation markers. The proliferation marker expression by cardiac myocytes may in large part be an indication of nuclear division rather than actual cell division.

Along with the previous study [26], this study confirmed that cardiac myocyte apoptosis identified by TUNEL staining is a negative predictor, with increased staining indicating a bad outcome [27]. This notion is supported by the observation that there was less expression of the apoptosis inhibitor Bcl-2 by the nonsurvivors compared with the survivors. However, Bcl-2 expression was observed in 14.6%±3.2% of the cardiac myocytes of the nonsurvivors and 21.3%±2.1% of the cardiac myocytes of the survivors, yielding an intergroup ratio of 1.46. Such a low intergroup ratio suggests that Bcl-2 staining may have poor test characteristics for prospectively predicting prognosis. This study also indicated that proliferation markers such as Ki-67 may be positive predictor markers, with positive staining indicating a good outcome [27]. However, the study highlighted a problem with Ki-67 and TUNEL staining. For both of these markers, less than 0.1% of the cardiac myocytes stain. This means that while proliferation and apoptosis markers may be useful in surgical resections and intraoperative biopsies, they are unlikely to be useful in small routine endomyocardial biopsies.

A third more recent study has examined the predictive power of multiple distinct cell death markers using endomyocardial biopsies [28]. The authors examined endomyocardial biopsies from 100 patients with IDCM. Cases with histologic features of myocarditis were excluded. The biopsies were evaluated for myocyte diameter and the degree of fibrosis. In addition, apoptosis was assessed by TUNEL staining, oncosis/necrosis was assessed by staining for complement factor C9, and autophagy was evaluated by assessing for large ubiquitin-positive vacuoles in the cardiac myocytes (Fig. 1). The study endpoint was mortality at 3 years. Only 4 TUNEL-positive apoptotic nuclei were observed in a total of 3 of the 100 biopsies, confirming that positive TUNEL staining is too infrequent to be useful in small routine endomyocardial biopsies. In univariate analyses, cardiac myocyte diameter and positive ubiquitin staining, but not C9 staining, correlated with mortality at 3-year follow-up. Ubiquitin staining had a sensitivity of 52% and a specificity of 81% for predicting bad outcome (death at 3 years), with a negative predictive value of 82% and a positive predictive value of 50%. In multivariate analysis, the simultaneous presence of both ubiquitin staining and C9 staining in the same biopsy was an independent predictor of mortality, even after inclusion of seven clinical predictors of mortality (age, gender, left ventricular end diastolic diameter, pulmonary artery wedge pressure, systolic blood pressure, presence of mitral regurgitation and presence of ventricular tachycardia). The simultaneous presence of both ubiquitin staining and C9 staining in the same biopsy had a positive predictive value of 58% and negative predictive value of 80% for mortality, with clear divergence of the survival curves (Fig. 1). Interestingly, for this patient population, the presence of staining for both ubiquitin and C9 in the same biopsy indicates the patient is more likely than not going to die within 3 years, and the absence of the dual staining indicates an 80% chance the patient will be alive in 3 years.

3. Germlin-1 as a novel prognostic marker in congestive heart failure

Gremlin-1 is a bone morphogenetic protein antagonist that plays roles in development and angiogenesis and is also up-regulated in chronic fibrosing disorders [29–34]. In a recent large outcome-based study, high expression of Gremlin-1 on endomyocardial biopsy was found to predict poor outcome in patients with heart failure [35]. In this study, endomyocardial biopsies from 214 patients with nonischemic congestive heart failure were assessed for Gremlin-1 expression (Fig. 2), routine inflammatory markers, the presence of viral genomes (parvovirus B19; human herpesviruses 6, 7, and 8; enterovirus; Epstein–Barr virus; influenza A/B; adenoviruses; herpes simplex virus; cytomegalovirus; and varicella zoster virus), and the degree of fibrosis. This group of 214 patients included patients with multiple distinct heart diseases, such as myocarditis, amyloidosis, and storage