Therapeutic Targets in Heart Failure

Refocusing on the Myocardial Interstitium

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New therapeutic targets, agents, and strategies are needed to prevent and treat heart failure (HF) after a decade of failed research efforts to improve long-term patient outcomes, especially in patients after hospitalization for HF. Conceptually, an accurate assessment of left ventricular structure is an essential step in the development of novel therapies because heterogeneous pathophysiologies underlie chronic HF and hospitalization for HF. Improved left ventricular characterization permits the identification and targeting of the intrinsic fundamental disease-modifying pathways that culminate in HF. Interstitial heart disease is one such pathway, characterized by extracellular matrix (ECM) expansion that is associated with mechanical, electrical, and vasomotor dysfunction and adverse outcomes. Previous landmark trials that appear to treat interstitial heart disease were effective in improving outcomes. Advances in cardiovascular magnetic resonance now enable clinicians and researchers to assess the interstitium and quantify ECM expansion using extracellular volume fraction measures and other derangements in cardiovascular structure. These capabilities may provide a mechanistic platform to advance understanding of the role of the ECM, foster the development of novel therapeutics, and target specific disease-modifying pathways intrinsic to the ventricle. Refocusing on the interstitium may potentially improve care through the identification and targeted treatment of key patient subgroups. (J Am Coll Cardiol 2014;63:2188–98) © 2014 by the American College of Cardiology Foundation

The Need to Refocus on the Myocardial Interstitium in Heart Failure

Unsuccessful efforts to lower high event rates in heart failure patients. Heart failure (HF) is the most common reason for hospitalization in older patients. Mortality and rehospitalization rates 90 days after hospitalization for heart failure (HHF) remain as high as 15% and 30%, respectively (1). Accordingly, HHF incurs the greatest costs among Medicare patients (2). These high event rates after HHF require urgent attention (2,3). Yet, over the past decade, a strikingly high number of phase 3 trials attempting to improve intermediate- to long-term clinical outcomes failed to produce positive results. One probable reason why benefits observed during phase 2 trials have not translated into benefits in large phase 3 trials is a lack of in-depth basic understanding of the effects of novel drugs on cardiac structure and function (4,5).

In parallel with unsuccessful clinical trials, efforts to develop and adhere to performance measures to improve HF outcomes have not been effective (6). Reflecting our incomplete understanding (7) and the paucity of evidence, current HF performance measures place no emphasis on identifying and addressing the fundamental mechanisms of HF (8). The cascade of derangements that culminate in HF and HHF are complex and heterogeneous, with diverse underlying cardiac substrates and diverse initiating and amplifying mechanisms that are incompletely understood (7). If therapeutic advances are to be made, it is critical to identify both the modifiable and nonmodifiable conditions that impair left ventricular (LV) systolic and diastolic function (9). In this issue of the Journal, the Review by Butler and colleagues (2) provides an up-to-date and comprehensive review of HHF with a focus on the role of the interstitium. The authors detail the extensive in-depth research and clinical trials that have focused on characterizing the interstitium and its role in HHF and review the new mechanistic insights and potential novel therapeutic targets for interstitial heart disease. The authors also offer a rigorous evaluation of how to incorporate the interstitium into future studies and therapeutic efforts to improve outcomes of HHF.

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diastolic function and contribute to post-discharge morbidity and mortality (9).

**Extracellular matrix as a therapeutic target.** Considerable data suggest that both preventing and ameliorating extracellular matrix (ECM) expansion from excess collagen remain important components of therapeutic success (10). ECM expansion is one of the hallmarks of pathological LV remodeling (11–16) and may promote disease progression in many causes of HF (11–14). This concept is not new (11,17), but the importance of ECM expansion in the hierarchy of changes in HF and its value as a therapeutic target remain unclear.

Importantly, ECM expansion from excess collagen and its adverse effects are modifiable. Collagen regression occurs in humans after treatment with angiotensin-converting enzyme inhibitors (18,19), angiotensin receptor blockers (20), and mineralocorticoid antagonists (21). Cardiac functional parameters and coronary flow reserve then improve (18–21). Regression of the collagen volume fraction appears modest in these studies, limited to ~20% relative change and 1% absolute change over ~10 months. Nonetheless, these agents improved outcomes in landmark large-scale trials with longer treatment duration.

Renin-angiotensin-aldosterone system (RAAS) activation occurs in the myocardium (22), and RAAS modulators improve survival regardless of blood pressure–lowering effects (23,24), even in the absence of clinical HF (24). These agents also decrease the risk of HHF (25–30). Patients with HF appear to benefit most from RAAS modulation if there is underlying myocardial fibrosis (21,31). Serelaxin, the most recent drug generating promising results in HHF patients (32), is known to reverse myocardial ECM expansion and myocardial fibrosis (33,34).

**Vulnerability Associated With Interstitial Heart Disease**

**Interstitial heart disease.** More than 20 years ago in the *Journal*, Karl Weber (17) crystallized the concept of “interstitial heart disease,” characterized by myocardial ECM expansion from excess collagen based on pioneering work by his group and others. Within this paradigm, the myocardium has both myocyte and interstitial compartments that are regulated independently (11). The activated fibroblast and the expanded ECM that it regulates assume a central role in the pathophysiology of HF and pathological LV hypertrophy (11). Maladaptive ECM changes perturb myocardial architecture and functional properties, occurring specifically as a result of fibroblast activity rather than myocyte activity (11,17).

Consequences of increased myocardial collagen content include mechanical (18,20,21), electrical (35–38), and vasomotor (19) dysfunction and diminished tolerance to ischemic insults (39–41)—all elements of cardiac vulnerability (42). Vulnerable interstitium has been reported in sudden cardiac death victims (38), and bandlike myocardial fibrosis can resemble hepatic fibrosis patterns seen in cirrhosis (43). Similar to other organs (44), fibrosis in the heart also disrupts organ architecture and culminates in organ dysfunction, with an important difference being that the heart is a highly dynamic organ where the dysfunction may be more sinister, leading to greater vulnerability to adverse outcomes. Fibrosis is associated with a number of conditions (Table 1) as well as with normal aging, in which comorbidities and heart failure are more prevalent (45–47).

**Interstitial heart disease can affect myocyte function.** ECM expansion regardless of cause can have direct deleterious effects on myocyte and cardiac function. First, specific activation of myocardial fibroblasts (sparing myocytes) causes excess collagen accumulation and myocardial fibrosis and leads to a HF phenotype (48). This finding suggests that there is potential for a primary role of the fibroblast in the development of HF.

Second, in cardiac amyloidosis, there is marked myocardial ECM expansion from noncollagenous interstitial amyloid protein accumulation (49–51). Smoldering myocyte loss occurs, leading to a chronic low-level troponin increase (52–56). This marked ECM expansion from amyloid protein also leads to severe cardiac dysfunction, manifested by a pronounced increase in B-type natriuretic peptide and an inexorable deterioration in clinical status with early mortality despite a relatively preserved ejection fraction (52). This clinical observation is important because, unlike collagen accumulation, ECM expansion due to accumulation of inert amyloid protein is a primary interstitial process and not a secondary phenomenon following myocyte injury. Therefore, ECM expansion, regardless of cause, can have
direct deleterious consequences for cardiac structure and function and lead to progressive HF.

**Uncertainty about the role of the fibroblast versus myocyte in interstitial heart disease.** The extent to which primary ECM expansion from fibroblast activation drives myocyte dysfunction or the extent to which primary myocyte disease and loss leads to ECM expansion in HF remains incompletely understood (Fig. 1). There is uncertainty about where the interstitium ranks in the hierarchy of changes associated with the development of HF. Nonetheless, one could postulate that antagonizing fibroblast activation and myocardial ECM expansion with agents that modulate the RAAS (and the adrenergic system that also can affect fibroblast activity [57]) may contribute to the success of pharmacological therapy with these drugs in large HF trials (25–30).

The historical difficulty of measuring human ECM expansion noninvasively has obscured its role in cardiovascular disease for clinicians and researchers (58,59). Whether ECM expansion is generally a cause or effect of myocardial insults, or both, is still unclear. The phenomenon of replacement fibrosis after myocyte necrosis (11) creates a “chicken-and-the-egg” situation: which came first when ECM expansion is encountered, ECM expansion or myocyte loss, if any? Until recently, there has not been a robust noninvasive method to detect and quantify the full range of ECM expansion (14,58), rendering it difficult to track and thus masking its importance. The positive correlation between LV mass and ECM expansion (43,60–62) suggests that myocyte loss is not the sole mechanism.

**Basic Pathogenesis of Interstitial Heart Disease**

The ECM functions as a scaffold for cardiomyocytes and transmits contractile forces. In the absence of cardiac amyloidosis or myocardial edema, thick type I fibrillar collagen fibers provide tensile strength and constitute most of the ECM, even when there is ECM expansion (22). For this reason, cardiac ECM expansion is often considered synonymous with myocardial fibrosis. Type III collagen fibers, which are thinner than type I collagen fibers and provide elasticity, glycosaminoglycans (e.g., hyaluronan), glycoproteins, and proteoglycans, are also important ECM components (11). Fibroblasts are the most numerous cells in the myocardium and are central to collagen and ECM homeostasis (63). Fibroblasts secrete extracellular procollagen chains that assemble into fibrils and become cross-linked by lysyl oxidase. Collagen cross-linking is an important post-translational modification because it increases myocardial tensile strength (64) and is associated with diastolic dysfunction (18,65) and aging (63). Collagen cross-linking also resists degradation by matrix metalloproteinases and may prevent chamber dilation by increasing the integrity of the cardiac matrix (22).

Myocardial fibrosis and interstitial heart disease occur when collagen homeostasis becomes dysregulated and excess...
collagen accumulates in the interstitium secreted by activated fibroblasts, termed myofibroblasts. This transformation of fibroblasts into myofibroblasts involves the expression of alpha-smooth muscle actin, characteristic of smooth muscle cells, as well as the appearance of an extensive, synthetically active endoplasmic reticulum (11). The potential sources of cardiac myofibroblasts include not only resident cardiac fibroblasts (63) but also myofibroblasts from hematopoietic and endothelial origins (22). Documented sources of myofibroblasts in cardiac fibrosis include circulating and resident fibroblast progenitors (including fibrocytes), epicardial epithelial cells undergoing epithelial-to-mesenchymal transition, and endothelial cells undergoing endothelial-to-mesenchymal transdifferentiation (22). Cardiac fibroblasts are sensitive to a wide variety of molecules that affect their proliferative response to pathological stimuli (63,66).

**Drivers of interstitial heart disease.** Although many causes of interstitial heart disease have been identified (Table 1), data regarding the precise regulation of collagen and other components of the ECM and the wide array of molecular drivers of interstitial heart disease are incomplete (22). Key mediators include the RAAS system; transforming growth factor beta, which may affect all cell types involved in cardiac fibrosis; reactive oxygen species; tumor necrosis factor (TNF) alpha; and various cytokines that have been reviewed previously (22,44,63). Importantly, the degrees to which these factors explain individual variation in ECM expansion remain unclear. Many elements of the innate and adaptive immune response participate in the differentiation and activation of fibroblasts underlying interstitial heart disease, which were recently reviewed in detail (44). Yet, individuals appear to vary widely in their tolerance of myocardial insults, predisposition to fibroblast activation, and subsequent interstitial heart disease. These important issues deserve further study because a greater understanding may produce novel therapies.

### Noninvasive Metrics of ECM Expansion: the Extracellular Volume Fraction

**A novel measure.** It is challenging to understand pathophysiological processes when they are difficult to measure. Previously, ECM expansion had been difficult to image and quantify with any technique (14,58,59,67,68), but technological advances in cardiovascular magnetic resonance (CMR) now permit quantification of prognostically relevant ECM expansion (15,16) and its apparent treatment response (15). The extracellular volume fraction (ECV) technique quantifies ECM expansion by measuring the volume fraction of the interstitial space (Fig. 2). ECM expansion in the absence of amyloid or edema typically reflects excess type I collagen (12). The change in T1 before and after contrast allows computation of gadolinium (Gd) contrast concentration. The proportional uptake of Gd contrast in the myocardium relative to plasma (i.e., 1 − hematocrit) measures the volume fraction of the myocardial extracellular space after equilibrium occurs (67,69–71). Isolated postcontrast myocardial T1 measures are also related to the collagen volume fraction (72,73), but are confounded by a variety of clinically relevant factors (74). ECV does not have similar limitations and exhibits higher correlation with the collagen volume fraction (75–77). Other non-CMR techniques to estimate ECM expansion have been reviewed previously and are not discussed here (58,59,68).

ECM expansion measurement introduces a useful intermediate phenotype to demonstrate the efficacy of ECM regression for novel treatments without resorting to large-scale trials with prohibitively high costs. Demonstrating the efficacy of any new therapy is challenging because it must be incremental to established treatments. Rather than enrolling thousands of patients to be followed for many years to demonstrate improved outcomes at an astronomical cost exceeding available resources, a more realistic initial approach might be to track the ECV response before embarking on large-scale ventures.

Quantifying the myocardial ECM has the potential to assess the risk of death or HHF (15,16) and optimize the clinician’s choice of diagnostic and therapeutic strategies. For therapeutic development, ECM expansion measures can elucidate mechanisms of the response (e.g., ECM regression), which may then be important for phase 3 trial design (4). There is considerable heterogeneity in the degree of ECM expansion in the human myocardium (15,16,49,78) that may vary in both the timing and extent of a response to a treatment (21,31). This variability is relevant for trial design; response to new interventions and risk/benefit ratios may vary according to the degree of myocardial health characterized by the degree of myocardial ECM expansion.

**Robustness of ECV measures.** Myocardial ECV is a CMR measure of ECM expansion and correlates highly with the collagen volume fraction in human myocardium (R² = 0.7 to 0.9) (75–77,79), regardless of whether it is apparent in late gadolinium enhancement (LGE) images. ECM expansion can vary spatially, from focal deposits to diffuse deposition distributed throughout the entire myocardium (50,80). Gd tracks thin collagen strands with high fidelity at the cellular level (81). ECV measurement is reproducible between CMR scans performed on different days (67,77,82), translating into fewer subjects required for clinical trials (83). ECV detects subclinical changes (50,71), but ECV measurement algorithms are not yet standardized and remain vendor and center dependent (74). ECV also remains limited by partial volume effects constrained by limited spatial resolution whereby larger pixels straddle tissue borders; thus, ECV is at present not well suited for right ventricular or left atrial sampling given their thinness (74,84). The same concepts for ECV measurement can be extended to computed tomography using iodinated contrast (85,86).

ECV is a promising tool for monitoring the ECM response to therapy. Still, more work regarding optimization, automation, and standardization is required (74). Notably, these limitations also apply to the ejection fraction,
which is measured in different ways across and within modalities but remains an important clinical metric.

**Initial ECV results.** Initial results with ECV measures are encouraging. ECV can detect disease not readily apparent with conventional LGE (49,50,67,71,75,78,87,88). ECM expansion measured by CMR in humans correlates positively with LV mass (15,16,78), reflecting previous pathological observations (38,43,60–62). Regarding this issue, further work is needed to understand complex relationships between the myocyte and interstitial compartments and how ECM expansion from myocardial fibrosis or even amyloid may affect myocyte energetics (13,89). Interestingly, CMR detects lower ECM in patients receiving RAAS antagonists, although the magnitude of the reduction appears modest (15). ECV may be suitable for evaluating serial changes in ECM (67,82,83).

ECM expansion may rank highly in the hierarchy of myocardial changes that occur in HF pathophysiology.
Collagen biomarker panels (i.e., procollagen type III amino terminal peptides) appear to predict events (102,110,111) and are more widely available than ECV. Yet, consensus has not yet emerged about which biomarkers are the most robust diagnostically and prognostically (112). ECV has the advantage of being specific to the myocardium. Compared with histological measures of the myocardium, ECV (75,76) provides higher correlation values than collagen biomarkers (113). The association with outcomes may be stronger for ECV. If confirmed, the use of ECV to quantify myocardial fibrosis would require fewer study participants to identify associations in future studies (67,83). A limitation of ECV measures is that they cannot assess the precise composition and structural modifications of the ECM, such as extent of cross-linking, and the accumulation of advanced glycation end products.

**Future Directions**

**Novel therapeutics.** Because apparent amelioration of ECM expansion culminates in improved outcomes, the myocardial interstitium is an attractive target for drug development. Newer agents (Table 2) such as potent and selective nonsteroidal mineralocorticoid antagonists (114), serelaxin (32,115), and microribonucleic acids (miRs) (48,116) offer novel ways to treat ECM expansion caused by myocardial fibrosis. Some medications with demonstrable efficacy in trials have not extended to the community and have not shown similar effectiveness in routine practice (117). Newer and more potent agents to treat ECM expansion present new opportunities to bridge this gap and potentially improve outcomes in HF.

Still, some agents intended to specifically target fibrosis in HF and other disease states have failed. Previous therapeutic agents potentially targeting the interstitium and other derangements (e.g., omapatrilat [118], nesiritide [5]) have not always been successful compared with standards of care, which illustrates the challenge of developing efficacious

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**Table 2** Treatments Targeting Myocardial Fibrosis in Development

<table>
<thead>
<tr>
<th>Category</th>
<th>Name</th>
<th>Target/ Mechanism</th>
<th>Class</th>
<th>Target Disease</th>
<th>Manufacturer</th>
<th>Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth factors</td>
<td>Pirfenidone</td>
<td>TGF-β activity, inflammation</td>
<td>Small molecule</td>
<td>IPF, hepatic and renal fibrosis, hypertrophic cardiomyopathy</td>
<td>InterMune, Brisbane, California</td>
<td>Clinic (approved for IPF in EU and Japan)</td>
</tr>
<tr>
<td>ECM</td>
<td>BAY 94-8862</td>
<td>Nonsteroidal mineralocorticoid receptor antagonist</td>
<td>Small molecule</td>
<td>HHF,cardiac fibrosis</td>
<td>Bayer HealthCare, Berlin, Germany</td>
<td>1/2</td>
</tr>
<tr>
<td>ECM</td>
<td>GS-6624</td>
<td>Lysyl oxidase–like 2</td>
<td>Monoclonal antibody, small molecule</td>
<td>Cardiac fibrosis, IPF</td>
<td>Gilead Sciences, Foster City, California</td>
<td>1/2</td>
</tr>
<tr>
<td>ECM</td>
<td>Relaxin/serelaxin</td>
<td>Fibroblast, myofibroblast, collagen, MMP-9, MMP-2</td>
<td>Hormone</td>
<td>HHF, hypertensive heart disease, vasodilatation</td>
<td>Novartis/Corteva, Basel, Switzerland</td>
<td>1/2/3</td>
</tr>
</tbody>
</table>

Adapted from Wynn and Ramalingam (44).
therapies. One agent, etanercept, an anti-TNF agent believed to prevent TNF-mediated cardiac fibrosis, was paradoxically linked to increased TNF bioavailability and augmentation of TNF cytotoxicity (119,120).

Determinants of ECM expansion. The factors leading to ECM expansion and especially the identification of modifiable and nonmodifiable risk factors for myocardial fibrosis remain incompletely understood. At the molecular level, angiogenic factors, various growth factors, proteolytic enzymes, and fibrogenic cytokines are among the elements involved in fibroblast regulation that lead to ECM expansion (121,122). Transforming growth factor beta, endothelin-1, angiotensin II, platelet-derived growth factor, and connective tissue growth factor appear to be some of the key proteins governing fibroblast activation/differentiation into alpha-smooth muscle actin–expressing myofibroblasts.

Figure 3  ECV Maps Display Severe Diffuse ECM Expansion Not Detectable With Conventional LGE Imaging

(A) ECV maps for an older patient with heart failure with corresponding T1 maps (T1 data are used to create ECV maps). The ECV maps reveal severe diffuse ECM expansion, readily apparent in 2 ECV display settings (black arrows); the midmyocardial ECV is 37% in this patient, which is >95th percentile in the Pittsburgh cohort. In contrast, LGE images show only a small focal abnormality in the inferior right ventricular insertion point (red arrow). (B) Semiautomated quantitative LGE thresholding techniques using 2 common methods fail to identify the severe diffuse ECM expansion present in nullified myocardium. The bright pixels highlighted in pink (red arrows) are minimal. (C) For comparison, ECV maps from a younger patient without evident cardiomyopathy and a midmyocardial ECV of 25% (which is within the normal ECV range) are shown. Midmyocardial ECV is measured to avoid contamination from partial volume effects from limited spatial resolution and/or misregistration errors depicted by the green pixels along the blood pool and myocardium interface. Note that in A and C, the ECV map window and level settings for the 2 individuals are identical. ECM = extracellular matrix; ECV = extracellular volume fraction; LGE = late gadolinium enhancement.
that can persist in an activated state (123). These molecules represent potential therapeutic targets. Connective tissue growth factor in turn is regulated by miR-30 and -133 (124); miR-21 is also a key mediator of fibroblast activation (121). miR therapies targeting the fibroblast and ECM are under active development (116).

In the setting of coronary artery disease, the mechanisms of fibrosis in noninfarcted myocardium are unclear (e.g., repetitive stunning, increased wall stress, and neurohormonal activation) as are the optimal prevention and treatment strategies. The degree to which identifiable clinical or genetic conditions explain myocardial fibrosis (accounting for measurement error) is unknown. The natural history of myocardial fibrosis once it is present and whether it is progressive and inexorable (i.e., whether “fibrosis begets fibrosis”) also remain poorly defined. The comparative efficacy of drug treatment and timing of any response has not been adequately investigated. The time and degree to which exercise can modulate myocardial fibrosis is also unknown.

Conclusions

There is an urgent need to improve outcomes in the large and vulnerable population of patients with HF, particularly those with HHF. New therapies are needed that focus on critical disease-modifying pathways intrinsic to the ventricle without adversely disrupting collagen metabolism in other organs. Myocardial ECM expansion is an important pathological abnormality, likely a major driver of disease progression in many cases leading to HF and HHF, and it remains an important therapeutic target. The terms interstitial heart disease (17) and vulnerable myocardial interstitium (38) have been coined to highlight the deleterious effects of ECM expansion. ECM expansion due to myocardial fibrosis in the ventricle is associated with mechanical, electrical, and vasomotor dysfunction, with an adverse prognosis, but it is treatable. Until recently, ECM expansion has been difficult to image and quantify. Using CMR to quantify ECM expansion, myocardial fibrosis, and other cardiac derangements in cardiovascular structure may foster drug development and provide a mechanistic foundation to improve care through targeted treatment of specific disease-modifying pathways intrinsic to the ventricle. More data are needed to explore these hypotheses, which merit further investigation.

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