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REVIEW TOPIC OF THE WEEK

Circulating Biomarkers of Myocardial Fibrosis

The Need for a Reappraisal

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ABSTRACT

Myocardial fibrosis impairs cardiac function, in addition to facilitating arrhythmias and ischemia, and thus influences the evolution and outcome of cardiac diseases. Its assessment is therefore clinically relevant. Although tissue biopsy is the gold standard for the diagnosis of myocardial fibrosis, a number of circulating biomarkers have been proposed for the noninvasive assessment of this lesion. A review of the published clinical data available on these biomarkers shows that most of them lack proof that they actually reflect the myocardial accumulation of fibrous tissue. In this "call to action" article, we propose that this absence of proof may lead to misinterpretations when considering the incremental value provided by the biomarkers with respect to traditional diagnostic tools in the clinical handling of patients. We thus argue that strategies are needed to more strictly validate whether a given circulating biomarker actually reflects histologically proven myocardial fibrosis before it is applied clinically. (J Am Coll Cardiol 2015;65:2449–56) © 2015 by the American College of Cardiology Foundation.

The search for biomarkers of structural myocardial remodeling with potential usefulness for the clinical handling of cardiac diseases has been a prolific field in the last few years. The investigation of circulating biomarkers for myocardial fibrosis, 1 key component of structural myocardial remodeling, has been accelerating at a remarkable pace. These investigations have deluged the clinical and research communities, however, with numerous candidates, few of which are likely to survive as useful clinical tools in terms of diagnosis, prognosis, and therapy monitoring (1,2). One possible explanation for this failure is that most of the proposed biomarkers lack proof that they actually reflect the quantitative and qualitative changes in collagen tissue characteristic of myocardial fibrosis. The present article focused on the necessity of accurately histologically validating each circulating molecule before it can be considered as a true biomarker of myocardial fibrosis in cardiac patients.

THE RELEVANCE OF ASSESSING MYOCARDIAL FIBROSIS IN CARDIAC PATIENTS

The predominance of the synthesis of collagen types I and III over their degradation results in the

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ABBREVIATIONS AND ACRONYMS

C_IVF = myocardial collagen type I volume fraction

C_{III}VF = myocardial collagen type III volume fraction

CVF = collagen volume fraction

DCM = dilated cardiomyopathy

HF = heart failure

HHD = hypertensive heart disease

LV = left ventricular

LVAD = left ventricular assist device

MMP = matrix metalloproteinase

PICP = carboxy-terminal propeptide of procollagen type I

PIIINP = amino-terminal propeptide of procollagen type III

TGF = transforming growth factor

accumulation within the myocardium of an excess of collagen type I and type III fibers that characterizes fibrosis (**Figure 1**). Two distinct patterns of collagen accumulation can be distinguished in myocardial fibrosis (3): focal, to replace dead cardiomyocytes and form scars (replacement fibrosis), and diffuse, which occurs in the interstitial and perivascular space without notable cell loss (reactive fibrosis). Although both patterns are observed in the heart after acute myocardial infarction, the second affects a large portion of the elderly population, and it is often a common feature of chronic cardiac diseases such as hypertensive heart disease, aortic valve stenosis, diabetic cardiomyopathy, and hypertrophic cardiomyopathy. The present review therefore focuses on reactive myocardial fibrosis.

The composition of fibrotic tissue in reactive myocardial fibrosis is characterized by an excess of collagen type I, highly cross-linked, large-diameter fibers, to the detriment of collagen type III, essentially non-cross-linked, small-diameter fibers (4) (**Figure 1**). Because of the different biophysical properties of the 2 types of collagen, small increases in the collagen type I:III content ratio have been shown to enhance myocardial stiffness. Finally, changes in collagen organization (i.e., alignment of collagen fibers relative to the cardiomyocytes), also seen in myocardial fibrosis, impair the transmission of the force generated by these cells to the ventricular chamber, thus exerting a detrimental effect on myocardial contractility.

Myocardial fibrosis is involved in the pathophysiology and clinical course of cardiac diseases (**Figure 1**). In fact, both quantitative and qualitative aspects of myocardial fibrosis (as evaluated on biopsy samples) have been shown to be associated with increased left ventricular (LV) stiffness and diastolic dysfunction (5), impaired LV contraction and systolic dysfunction (6), arrhythmias (7), and impaired coronary blood flow (8) in patients with heart failure (HF) of various etiologies. In addition, the presence of severe fibrosis in biopsy samples is reportedly a useful indicator for long-term mortality in patients with HF (9,10). Importantly, fibrosis can be seen in the myocardium of HF patients despite the fact that they are receiving adequate treatment as recommended by official guidelines (5–12). In addition, the extent of fibrosis may be an important factor in predicting the effectiveness of long-term HF therapy (e.g., beta-blocker therapy) (13). Therefore, the assessment of myocardial fibrosis is important in gaining a better pathophysiological understanding of the clinical picture, as well as establishing the prognosis and determining therapy in patients with HF.

VALIDATION OF A CIRCULATING MOLECULE AS A BIOMARKER OF MYOCARDIAL FIBROSIS

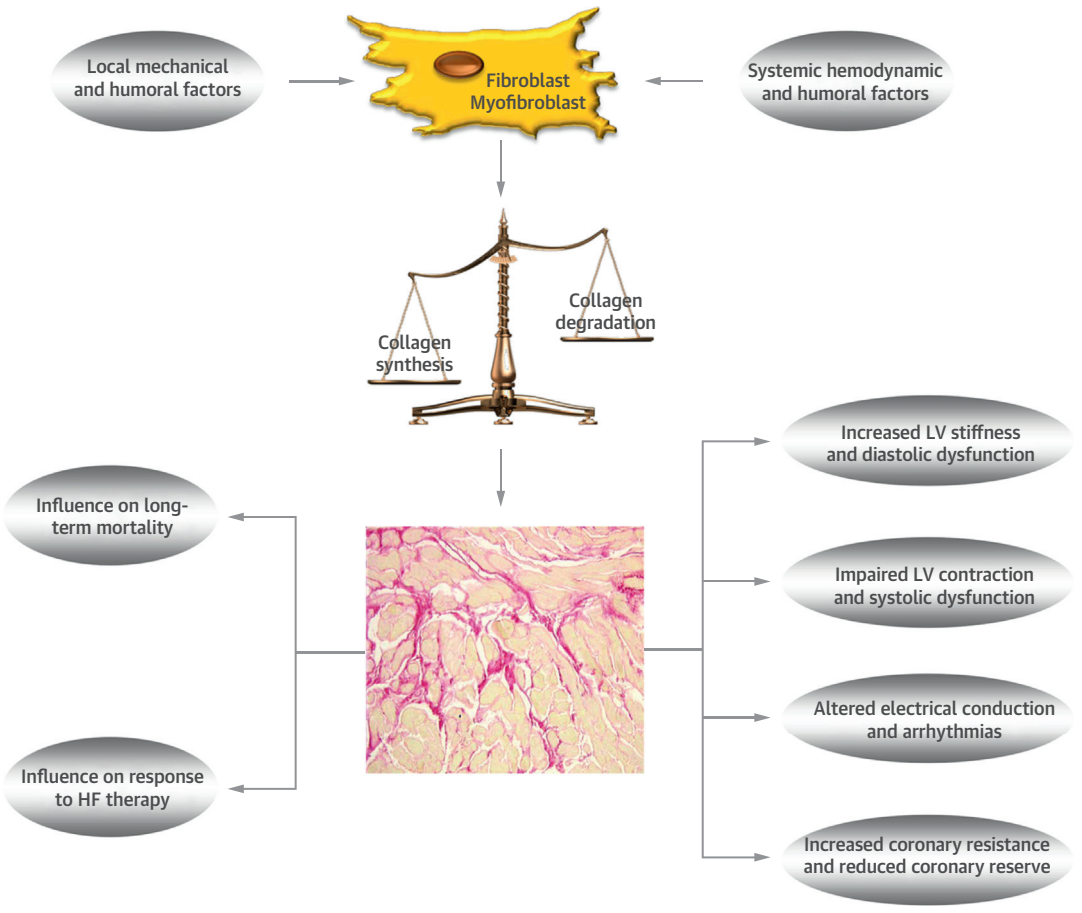
Any candidate biomarker of myocardial fibrosis must be compared with the current gold standard for diagnosis of myocardial fibrosis, which is the histopathological analysis of myocardial tissue. In addition to its clinical roles, endomyocardial biopsy may be used as a research tool to better understand the cellular and molecular pathophysiology of cardiac diseases, as well as to identify new diagnostic and therapeutic targets (as reviewed by Cooper et al. [14]). In this conceptual framework, the identification of a given circulating molecule as a true biomarker of myocardial fibrosis requires demonstration that its blood levels directly correlate with quantitative parameters used to define fibrosis in endomyocardial biopsy specimens.

The percentage of total myocardial tissue occupied by collagen fibers or myocardial collagen volume fraction (CVF) can be determined with automated image analysis systems in myocardial samples with collagen-specific staining. Similarly, the use of monoclonal antibodies against collagen type I and collagen type III allows the determination of the myocardial CVF occupied by either collagen type I (C_IVF) or collagen type III (C_{III}VF) fibers, respectively. Thus, myocardial fibrosis is characterized by abnormally high values of CVF, C_IVF, and C_{III}VF and/or of the ratio of C_IVF:C_{III}VF (15).

Because of the patchy distribution of myocardial fibrosis, the greatest potential limitation to endomyocardial biopsy evaluation is sampling error. Therefore, the analysis of several tissue fragments is important for diagnostic accuracy and interpretation. A biopsy of the left ventricle may seem diagnostically more contributive than a biopsy of the right ventricle in some cardiomyopathies. However, Pearlman et al. (16), using postmortem tissue from cardiac patients without HF, found that myocardial fibrosis (assessed as the CVF) is a generalized process similarly affecting the 2 cardiac chambers. Furthermore, we reported that fibrosis (assessed as the CVF) present in biopsy specimens of the right side of the interventricular septum is similar to fibrosis present in the free wall of the left ventricle in HF patients (17).

Importantly, the endomyocardial biopsy is a safe procedure. It has a rate of transient complications of <0.5% and a risk of cardiac perforation with tamponade of <0.05% (18).

FIGURE 1 Pathophysiology of Myocardial Fibrosis



Schematic view of the general mechanisms and consequences of myocardial fibrosis. HF = heart failure; LV = left ventricular.

VALIDATION OF THE CANDIDATE BIOMARKERS: AVAILABLE CLINICAL EVIDENCE

A number of molecules, detectable in either the serum or plasma in humans by using immunoassay methods, have recently been proposed as biomarkers of myocardial fibrosis (Table 1). However, in most cases, demonstration of an association between the biomarker and histologically assessed myocardial fibrosis (i.e., the blood level of the biomarker directly correlates with either CVF or C_IVF or C_{III}VF, and after a therapeutic intervention, it changes in parallel with the changes in some of these 3 histological parameters) is lacking or remains inconclusive. Some examples may serve to illustrate the importance of this initial validation of the candidate molecules.

BIOMARKERS WITH PROVEN EVIDENCE OF THEIR ASSOCIATION WITH MYOCARDIAL FIBROSIS. As presented in Table 1, among the many circulating molecules proposed as biomarkers of myocardial fibrosis in humans, only 2 collagen-derived serum peptides have been shown to be associated with myocardial fibrosis: the carboxy-terminal propeptide of procollagen type I (PICP), formed during the extracellular conversion of procollagen type I into mature fibril-forming collagen type I by the enzyme procollagen type I carboxy-terminal proteinase, and the amino-terminal propeptide of procollagen type III (PIIINP), formed during the extracellular conversion of procollagen type III into mature fibril-forming collagen type III by the enzyme procollagen type III amino-terminal proteinase (Figure 2). This topic has been reviewed previously by Prockop and Kivirikko (19).

TABLE 1 Histological Validation of Circulating Molecules Proposed as Biomarkers of Myocardial Fibrosis in Clinical Studies

	Is the Circulating Level of the Molecule Associated With Histologically Proven Myocardial Fibrosis?
Molecules related to collagen metabolism	
C-terminal propeptide of procollagen type I	Yes (6,11,20–23,29)
N-terminal propeptide of procollagen type I	Unknown
N-terminal propeptide of procollagen type III	Yes (23,24)
C-terminal telopeptide of collagen type I	Inconclusive evidence (23,24)
Matrix metalloproteinase-1	No (17)
Matrix metalloproteinase-2	Unknown
Matrix metalloproteinase-3	Unknown
Matrix metalloproteinase-8	Unknown
Matrix metalloproteinase-9	Unknown
Tissue inhibitor of metalloproteinases-1	No (17)
Tissue inhibitor of metalloproteinases-4	Unknown
Molecules related to the regulation of collagen turnover	
microRNA-21	Inconclusive evidence (26)
microRNA-29b	Unknown
microRNA-122	Unknown
microRNA-133a	Unknown
microRNA-499-5p	Unknown
Transforming growth factor-β1	Inconclusive evidence (30,34)
Growth differentiation factor-15	Inconclusive evidence (36)
Connective tissue growth factor	Inconclusive evidence (30)
Osteopontin	No (30,39)
Osteoglycin	Unknown
Syndecan-1	Unknown
Syndecan-4	Unknown
Molecules integrating cardiac stress injury, inflammation, and fibrosis	
Galectin-3	Inconclusive evidence (29,30)
Cardiotrophin-1	No (42)
Soluble ST2	Unknown
Midregional pro-atrial natriuretic peptide	Unknown
Myostatin	Unknown

The supportive references are presented in parentheses.

Serum PICP levels have been found to be highly correlated with total CVF in patients with hypertensive heart disease (HHD) without (20) and with (6,11) HF. In addition, it has been shown that there is a robust correlation between serum PICP levels and C_IVF in patients with HHD and HF (21). Of interest, serum PICP levels and CVF reportedly changed in parallel in response to losartan therapy in patients with HHD without HF (22) and in response to torasemide therapy in patients with HHD and HF (11). These levels also varied similarly in HF patients with idiopathic dilated cardiomyopathy (DCM) treated with spironolactone (23).

Serum PIIINP has been found to be highly correlated with C_{III}VF in HF patients with ischemic heart disease or idiopathic DCM (24). In addition, the reduction in the extent of CVF observed in HF patients with idiopathic DCM treated with spironolactone was accompanied by a significant reduction in serum PIIINP (23).

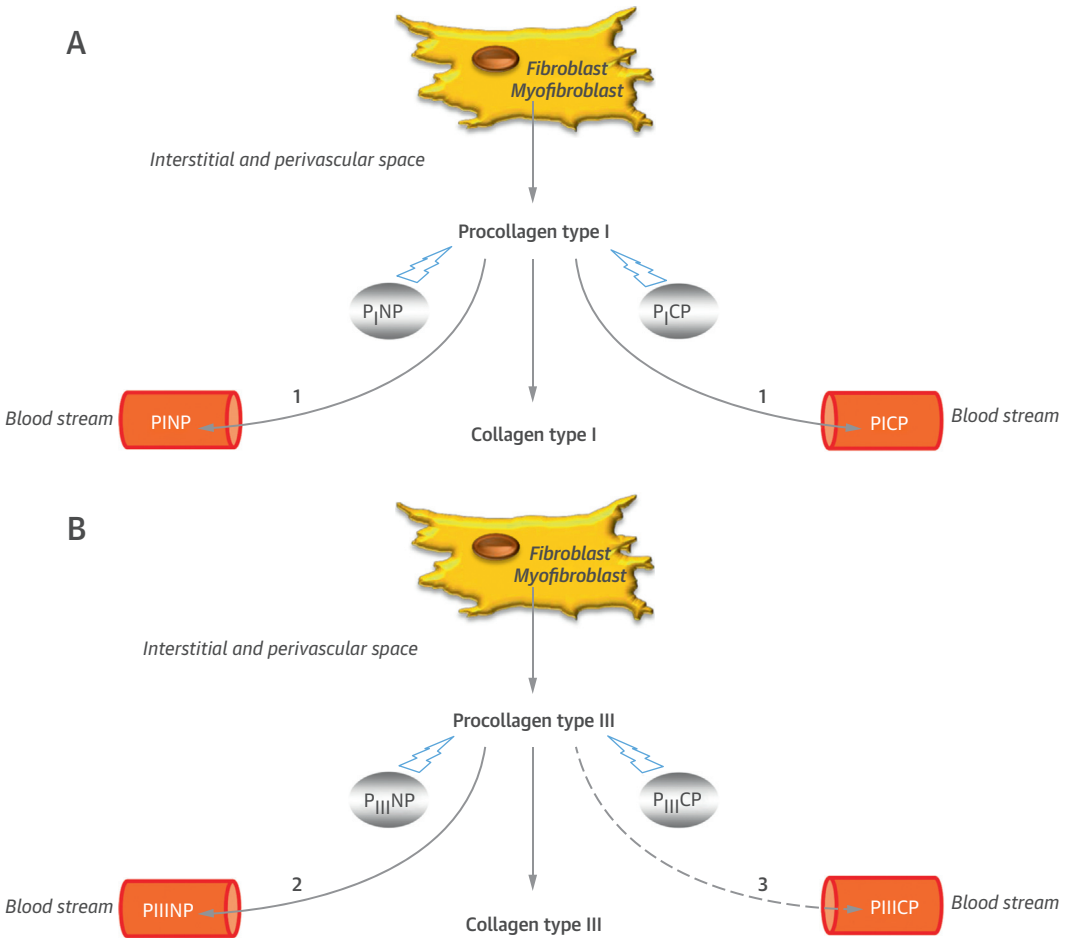
BIOMARKERS WITH INCONCLUSIVE EVIDENCE OF THEIR ASSOCIATION WITH MYOCARDIAL FIBROSIS. Although the small carboxy-terminal telopeptide released by the action of matrix metallo-proteinase (MMP)-1 on collagen type I fibers (CITP) may reflect collagen degradation (20), the information regarding its relation to myocardial fibrosis is controversial. A direct correlation of CITP with C_IVF was reported in HF patients with ischemic heart disease or idiopathic DCM (24); however, Izawa et al. (23) found that serum CITP levels were lower in HF patients with idiopathic DCM and severe myocardial fibrosis than in patients with mild to moderate fibrosis. Furthermore, the reduction in fibrosis with spironolactone in patients with severe fibrosis was accompanied by an increase in serum CITP (23).

It has been shown experimentally that microRNA-21 regulates the activity of cardiac fibroblasts and participates in the development of myocardial fibrosis (25). Interestingly, the circulating levels of microRNA-21 were directly correlated with myocardial expression of collagen type I messenger ribonucleic acid in patients with isolated severe aortic stenosis undergoing aortic valve replacement surgery (26). However, no data were provided in this study regarding CVF and C_IVF or their potential association with microRNA-21.

Although the available evidence suggests that the small lectin-like protein galectin-3 mediates fibrosis in different experimental models of HF (27,28), no associations were found between plasma galectin-3 levels and CVF, C_IVF, and C_{III}VF in patients with HHD and HF (29). Conversely, even though CVF increased significantly in HF patients with idiopathic DCM 6 months after left ventricular assist device (LVAD) support, plasma galectin-3 decreased significantly in the same patients (30). Although it has been shown that the matricellular protein connective tissue growth factor is up-regulated in different models of myocardial fibrosis (31,32), its plasma level did not correlate with CVF in HF patients with nonischemic DCM, and it remained stable 6 months after LVAD support in these patients, despite the fact that CVF increased significantly (30).

Abundant experimental evidence supports the notion that transforming growth factor (TGF)-β plays an important role in the pathogenesis of myocardial fibrosis (reviewed by Dobaczewski et al. [33]), and plasma TGF-β levels were found to be directly correlated with the LV myocardial expression of collagen type I messenger ribonucleic acid in patients with

FIGURE 2 Extracellular Generation and Release Into the Bloodstream of Collagen-Derived Serum Peptides



(A) The amino-terminal propeptide of procollagen type I (PINP) and the carboxy-terminal propeptide of procollagen type I (PICP) originate during the conversion of procollagen type I in collagen type I, and **(B)** the amino-terminal propeptide of procollagen type III (PIIINP) and the carboxy-terminal propeptide of procollagen type III (PIIICP) originate during the conversion of procollagen type III in collagen type III. **(1)** Since PICP is formed in a 1:1 stoichiometric ratio to the collagen type I molecule formed, the propeptide concentrations in tissue fluids and blood are direct indicators of ongoing collagen type I synthesis. **(2)** The cleavage at the amino-terminus proceeds at a relatively slow rate and, thus, partially processed procollagen molecules (known as pN-collagen type III molecules) are found on the surface of collagen type III fibers. Therefore, the PIIINP concentrations in tissue fluids and blood are not direct indicators of ongoing collagen type III synthesis. **(3)** Although PIIICP is set free, it is unknown whether it reaches the bloodstream. P_ICP = procollagen type I carboxy-terminal proteinase; P_INP = procollagen type I amino-terminal proteinase; $P_{III}CP$ = procollagen type III carboxy-terminal proteinase; $P_{III}NP$ = procollagen type III amino-terminal proteinase.

aortic stenosis undergoing valve replacement (34). However, no correlation of plasma TGF-β with either CVF or C_IVF was provided in this study. In addition, it has been reported that plasma TGF-β levels were higher in control subjects than in HF patients with idiopathic DCM that had abnormally high CVF values (30). Furthermore, although CVF increased significantly in these patients 6 months after LVAD support, plasma TGF-β did not change.

Growth differentiation factor-15 is a member of the TGF-β cytokine superfamily that has been shown to be associated with myocardial fibrosis in a genetic model of HF (35). Although plasma levels of growth differentiation factor-15 reportedly correlate directly with CVF in patients with idiopathic DCM before LVAD support, the reduction in these levels observed 1 month after LVAD support was accompanied by an increase in CVF (36).

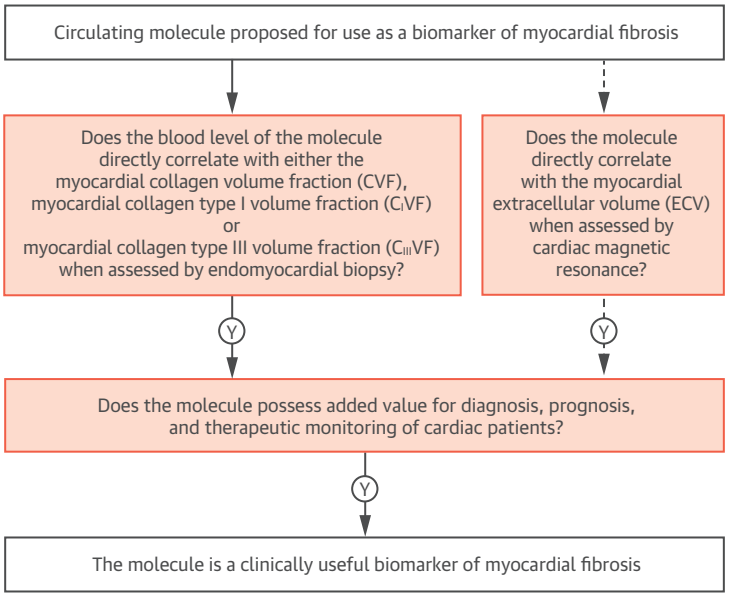
BIOMARKERS WITH LACK OF EVIDENCE OF THEIR ASSOCIATION WITH MYOCARDIAL FIBROSIS. No correlations between CVF and serum MMP-1, serum tissue inhibitor of matrix metalloproteinases-1, and

their ratio (which may serve as an indirect index of circulating unbound active MMP-1) have been found in patients with HHD and HF (17). However, it is noteworthy that those patients with higher values of serum MMP-1 and serum MMP-1:tissue inhibitor of matrix metalloproteinases-1 ratio exhibited reduced levels of perimysial and endomysial collagen. This finding suggests that these molecules may be related more to the loss of the physiological mysial collagen scaffold than to the accumulation of pathologic non-mysial collagen.

Experimental findings support the notion that the matricellular protein osteopontin seems to mediate fibrogenic actions in HF models (37,38). However, plasma concentrations of osteopontin are reportedly not associated with CVF or C_IVF in patients with HHD and HF (39). Furthermore, in HF patients with non-ischemic DCM, plasma osteopontin levels tended to decrease whereas CVF increased significantly during LVAD support (30).

Cardiotrophin-1 is a member of the interleukin-6 superfamily that behaves in vitro as a profibrotic cytokine in cultured animal and human cardiac fibroblasts (40), and in vivo it induces cardiac fibrosis in rats (41). Recently, an association between the myocardial expression of cardiotrophin-1 and collagen types I and III has been reported in the fibrotic myocardium of patients with HF of hypertensive origin (42). However, the plasma levels of cardiotrophin-1 were not correlated with CVF, C_IVF, or C_{III}VF in the same patients.

CENTRAL ILLUSTRATION Process for the Clinical Validation of a Circulating Biomarker of Myocardial Fibrosis



López, B. et al. J Am Coll Cardiol. 2015; 65(22):2449–56.

Proposed process to be completed for validating a circulating molecule candidate as a biomarker of myocardial fibrosis. The **dashed line** indicates that before extracellular volume (ECV) measured by cardiac magnetic resonance imaging can be assumed as a representative parameter of CVF, more investigation in the field is required. CVF = myocardial total collagen volume fraction; C_IVF = myocardial collagen type I volume fraction; C_{III}VF = myocardial collagen type III volume fraction; Y = yes.

LESSONS TO LEARN FROM THE AVAILABLE EVIDENCE

It is necessary to be cautious when translating experimental data into the clinical setting. In fact, although the experimental findings may support a causative role for a given molecule in the development of myocardial fibrosis, this does not mean that in cardiac patients, the molecule's circulating levels will mirror the extension and severity of the myocardial deposition of collagen fibers. However, it is important to note that due to the nature of the clinical investigations reviewed here, the information provided in most of the studies is more of a snapshot of the evolutionary state of cardiomyopathy rather than a view on the progression of the pathologic process.

Although the endomyocardial biopsy procedure is safe, the difficulty in performing it in clinical practice for research purposes must be recognized. The use of noninvasive methods as an alternative should thus be considered. In this regard, the ability to non-invasively examine the myocardium by using cardiac magnetic resonance imaging could also be useful for the initial validation of circulating biomarkers of myocardial fibrosis. Despite restricted accessibility, cardiac magnetic resonance imaging is emerging as a modality that enables noninvasive evaluation of the myocardial interstitial space through the measurement of myocardial extracellular volume with T1 mapping techniques. Interestingly, it has been shown in patients with various cardiac diseases that cardiac magnetic resonance-measured myocardial extracellular volume correlates with CVF (as reviewed by Treibel et al. [43]). However, T1 mapping is evolving rapidly and still requires methodological standardization. Therefore, although accumulation of fibrotic tissue leading to expansion of the interstitial space may be partly responsible for the increase in the extracellular volume, whether this measurement can serve as a surrogate parameter of myocardial fibrosis for validation purposes of a candidate circulating biomarker requires further investigation.

The interpretation of the serum levels of the 2 biomarkers with proven evidence of its association

with myocardial fibrosis (i.e., PICP, PIIINP) is a complex issue that deserves some consideration. First, their tissue and organ origin are still unclear. For instance, a net release of PICP (6), but not of PIIINP (44), from the heart has been reported in patients with HF. In addition, whereas some authors have found a net release of PIIINP from the heart in patients with aortic stenosis (45), others failed to find such a release in patients with the same cardiac valve disease (44), patients with hypertrophic cardiomyopathy (46), and HF patients with ischemic heart disease or idiopathic DCM (47). Second, the diagnostic usefulness of these biomarkers may vary among cardiac diseases. In fact, whereas the association between serum PICP and C_IVF was observed in HF patients with HHD (6,21) but not in HF patients with ischemic heart disease or idiopathic DCM (24), the opposite was true for serum PIIINP and C_{III}VF (6,21,24). Third, changes in serum concentrations of these biomarkers in patients with cardiovascular diseases may represent integrated abnormalities of the cardiovascular collagen (e.g., an excess of collagen types I and III is present in the arterial wall of hypertensive patients with arterial stiffness). Fourth, the presence of concomitant noncardiac diseases affecting collagen metabolism may also affect the circulating levels of these molecules (48). This finding may be highly relevant in conditions involving chronic liver insufficiency (because PICP and PIIINP are cleared via uptake by endothelial cells in the liver) and in the setting of metabolic bone diseases with high collagen type I turnover.

Finally, as demonstrated experimentally (49) and clinically (21,50), the effects of an increase in collagen quantity on LV hemodynamics are modified not only by the relative abundance of collagen type I over collagen type III but also by the degree of collagen cross-linking among the fibrils forming the fiber. Therefore, to have a more global idea of how myocardial fibrosis influences cardiac function, biomarkers assessing the quality of the myocardial network are also necessary.

CLINICAL IMPLICATIONS

Cardiac diseases evolving with HF in which myocardial fibrosis is a determinant of outcome represent a major disease burden and, in many cases, their prevalence is increasing. Therefore, the investigation of circulating biomarkers to track myocardial fibrosis needs to be a major focus in the coming years. In this conceptual framework, the candidate biomarker must be initially tested to see whether it robustly reflects myocardial fibrosis (**Central Illustration**). If this is the case, then it must be tested to see whether it possesses added value in terms of diagnosis, prognosis, and therapeutic monitoring when compared to the available biomarkers (**Central Illustration**). For the first step, the application of specific protocols aimed to assess associations of biomarker levels with histological (or cardiac magnetic resonance) parameters of myocardial fibrosis is mandatory. Additionally, the use of panels combining imaging and circulating biomarkers may integrate different levels of information, overcome methodological limitations, and contribute to a better profiling of each individual patient with a view to personalize the therapy.

To develop strategies aimed to validate biomarkers of myocardial fibrosis useful in the clinical handling of cardiac patients, collaboration between academia, industry, and government agencies is required. This joint effort should result in the creation of consortia that would allow the identification of a comprehensive panel of circulating (and imaging) biomarkers of myocardial fibrosis to be concomitantly analyzed in a pooled sample of randomized, hypothesis-driven clinical trials. The success of these initiatives will depend on circulating biomarkers for myocardial fibrosis being successfully incorporated into day-to-day clinical practice, thus contributing to achievements in personalized medicine in the cardiologic domain.

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