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Non-ischemic dilated cardiomyopathy and cardiac fibrosis

Bianca Olivia Cojan-Minzat[1,2] · Alexandru Zlibut[1,3] · Lucia Agoston-Coldea[1,4]

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Abstract
Cardiac fibrosis is associated with non-ischemic dilated cardiomyopathy, increasing its morbidity and mortality. Cardiac fibroblast is the keystone of fibrogenesis, being activated by numerous cellular and humoral factors. Macrophages, CD4+ and CD8+ T cells, mast cells, and endothelial cells stimulate fibrogenesis directly by activating cardiac fibroblasts and indirectly by synthetizing various profibrotic molecules. The synthesis of type 1 and type 3 collagen, fibronectin, and α-smooth muscle actin is rendered by various mechanisms like transforming growth factor-beta/small mothers against decapentaplegic pathway, renin angiotensin system, and estrogens, which in turn alter the extracellular matrix. Investigating the underlying mechanisms will allow the development of diagnostic and prognostic tools and discover novel specific therapies. Serum biomarkers aid in the diagnosis and tracking of cardiac fibrosis progression. The diagnostic gold standard is cardiac magnetic resonance with gadolinium administration that allows quantification of cardiac fibrosis either by late gadolinium enhancement assessment or by T1 mapping. Therefore, the goal is to stop and even reverse cardiac fibrosis by developing specific therapies that directly target fibrogenesis, in addition to the drugs used to treat heart failure. Cardiac resynchronization therapy had shown to revert myocardial remodeling and to reduce cardiac fibrosis. The purpose of this review is to provide an overview of currently available data.

Keywords Cardiac fibrosis · Nonischemic dilated cardiomyopathy · Cardiac magnetic resonance · Antifibrotic drugs · MicroRNAs · Cardiac resynchronization therapy

Introduction

Nonischemic dilated cardiomyopathy (NIDCM) is the most common cardiomyopathy having multiple causes and a clinical picture that includes heart failure (HF) and even sudden cardiac death (SCD) [1]. NIDCM is defined as a left ventricle (LV) enlargement and global systolic function impairment (LVEF < 45%) in the absence of coronary artery disease (CAD) or increased loading conditions (hypertension, valve disease) [2]. Due to late diagnosis and poor prognosis, the current research trend is the better understanding of its pathogenesis and the development of more efficient early diagnosis techniques [3].

Cardiac fibrosis occurs early in the progression of NIDCM, increasing cardiac rigidity, decreasing myocardial performance, and enhancing the risk of SCD and malignant arrhythmias [4, 5]. Fibrogenesis is based on myofibroblasts that come from the activation of cardiac fibroblasts (CFs) or from epithelial-to-mesenchymal trans-differentiation (EMTd). Macrophages, monocytes, T lymphocytes, mast cells, and endothelial cells are also involved in this process. Myofibroblasts enhance the synthesis of collagen fibers, fibronectin, and profibrotic mediators by various molecular pathways, disrupting the normal extracellular matrix (ECM) by increasing its myocardial percentage [6–9]. Transforming growth factor beta (TGF-β), the mothers against decapentaplegic homolog 2 and 3 (SMAD2 and SMAD3) complex, the renin angiotensin aldosterone system (RAAS), estrogens, tumoral necrosis factor alpha (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6) are the mediators that orchestrate the profibrotic process [10–14]. Serum biomarkers involved in cardiac fibrosis correlate with collagen volume fraction (CVF), and some of them could be used for prognosis and follow-up. Collagen metabolism products, galectin-3 (Gal3), soluble source of tumorigenicity 2 (sST2), and growth differentiation factor 15

✉ Lucia Agoston-Coldea
luciacoldea@yahoo.com

[1] Department of Internal Medicine, Iuliu Hatieganu University of Medicine and Pharmacy, 2-4 Clinicilor, 400006 Cluj-Napoca, Romania

[2] Department of Family Medicine, Cluj-Napoca, Romania

[3] Niculae Stancioiu Heart Institute, Cluj-Napoca, Romania

[4] 2nd Department of Internal Medicine, Emergency County Hospital, Cluj-Napoca, Romania

(GDF-15) have also a role in dynamic tracking of fibrosis. MicroRNAs (miRs) are useful in the diagnosis, assessment, and even treatment of cardiac fibrosis [15, 16]. Nowadays, cardiac magnetic resonance imaging (cMRI) with gadolinium as contrast agent represents the gold standard diagnosis and is based on T1-weighted late gadolinium enhancement (LGE) or T1 mapping (T1M) [17–19]. Major current interests are the inhibition of fibrogenesis and the induction of its reversibility. Drugs used in HF have been shown to reduce cardiac fibrosis, but new substances are emerging that target cardiac fibrosis in various pathways are emerging; some modulate collagen metabolism, while others influence some pathogenetic pathways, most commonly the TGF-β/SMADs pathway, or miRs. In addition, cardiac resynchronization therapy (CRT) is an option that seems to positively influence fibrosis, but further studies are required to establish the large-scale impact [20–24]. This review describes the latest data on the mechanisms and biomarkers of cardiac fibrosis in NIDCM subjects, advanced cMRI imaging techniques, as well as prevention and treatment opportunities in this entity.

Pathogenesis

Cardiac fibrosis is characterized by fibroblastic activation and fibrogenesis. It increases the collagen fiber synthesis that will embed into the myocardium altering its architecture and potentiating HF [11]. The keystone of the fibrogenesis is the activation of CFs. Depending on the location and ECM characteristics, cardiac fibrosis can be classified as reactive interstitial, infiltrative interstitial, and replacement fibrosis [6].

Cardiac fibrogenesis is a multistep process initiated in response to pro-inflammatory stimuli and cytokines. In myocardial injury, fibroblasts are activated and fibrogenesis is initiated leading to lack of excitation-contraction coupling with cardiac dysfunction. Besides CFs, macrophages, mast cells, lymphocytes, cardiomyocytes and vascular cells are involved in fibrogenesis [11].

Within the ECM, fibrillar collagen accumulation increases, contributing to diastolic dysfunction and activation of CFs. Nonfibrillar type VI collagen is also synthesized, yet with uncertain role in cardiac fibrogenesis. Fibrin and fibronectin are embedded in the ECM during myocardial inflammation. They facilitate CF migration, activation, and proliferation through integrins and syndecans. In addition, the matricellular protein synthesis is stimulated resulting in a newly formed ECM. These proteins are presented in Table 1 [6, 25].

Cells in cardiac fibrogenesis

CFs synthesize connective tissue adapted to the heart, which, unlike in bone or tendon, it is dense, irregular, and has a different composition. Most CFs originate from the epicardium, but there are also endocardium-derived CFs originating from interventricular septum and right ventricle (RV). It has been shown that the resident CFs are mainly responsible for cardiac fibrogenesis as a response to various stimuli [7].They recruit inflammatory cells, stimulate type I and type III collagen synthesis, determine the ECM degradation mediated by matrix-metalloproteinases (MMPs), but also its proliferation [26]. CFs are difficult to identify, but recently biomarkers with role in detecting them have been described. α-Smooth muscle actin (αSMA) plays important roles in modulating CF activity and proliferation, and also in scar contraction and ventricular remodeling [27]. Collagen 1A1 (Col1a1) and discoidin domain receptor 2 are both markers of angiotensin II-induced CF activation and collagen fiber proliferation, being involved in tissue scarring and fibrogenesis [26, 28]. Fibroblast-specific protein 1 is a nonspecific marker of activated fibroblasts being useful in quantifying fibrosis when used with other specific markers [29]. Periostin and transcription factor 21 (TCF21) occur in CFs development, subsequently modulating ECM synthesis and activating CFs [25, 26]. Platelet-derived growth factor receptor-α (PDGFR-α) activates myocardial resident CFs by stimulating receptor tyrosine kinase inhibitor pathway [30].

Myofibroblasts originate from activated CFs and circulating progenitors, endothelial cells, pericytes, and epicardial endothelial cells [6]. Up to 17% of total cardiac myofibroblasts are bone-marrow derived cells [31]. Pathological stimuli enhance TGF-β synthesis which in turn activates CFs through the exposure of type VI collagen. Cell surface integrins and syndecans determine the transduction of cellular transdifferentiation signals [6] and mechanical stress induces the synthesis of αSMA through Rho/Rho kinase pathway [32].

The monocytes/macrophages (Mo/Mf) differentiate into myofibroblasts in response to diverse cytokines and produce various inflammatory mediators and profibrotic growth factors. Alternative activation of M2-type macrophages via IL-3 and IL-4 is associated with cardiac fibrosis. Conversely, due to their ability to phagocyte cell debris and apoptotic myofibroblasts, recent data suggest a possible antifibrotic effect of Mo/Mf [6]. Mast cells (MCs) are identified in all forms of myocardial injury, these being especially involved in fibrogenesis initiation. They can directly activate myofibroblasts and enhance fibrogenesis through tryptase, histamine, TGF-β1, and chymase stimulation [8]. Activated lymphocytes such as CD8+ and Th1, Th2, Th17, and T-reg CD4+ subsets promote cardiac fibrogenesis, myocardial remodeling, and cardiac dysfunction. Particularly, Th1 cells activate CFs by stimulating TGF-β via integrin-α4 [9, 33]. Lymphocyte T depletion has also been shown to reduce cardiac fibrosis [34]. Endothelial cells (ECs) promote cardiac fibrosis by enhancing angiotensin II (AG II) induced profibrotic factors and inflammatory cytokines, and also by EMTd. Conversely, ECs can produce antifibrotic mediators such as chemokine interferon-γ-inducible protein-10 (IP-10)

Table 1 Matricellular proteins involved in cardiac fibrosis

Class	Mechanism	Effect
Thrombospondins	TSP-1	▪ Angiostatic mediator
	▪ ↑ TGF-β signalization	▪ Profibrotic effects
	▪ ↓ MMPs	
	▪ ↑ CFs matrix deposition	
	TSP-2	▪ Inversely correlated with the occurrence of DCM
	▪ Cardiomyocyte mediator	
	TSP-4	▪ Antifibrotic effects
	▪ ↑ Heart contractility	
Tenascin-C	• Interacts with fibronectin	• Cardiac fibrosis
	• ↑ Myofibroblast recruitment	• Myocardial remodeling
	• Mechanism: not yet described	
Osteopontin	• Dual role: matricellular protein and cytokine	• Myocardial dysfunction
	• ↑ Collagen deposition	• DCM development
	• ↓ MMPs, ↑ GFs signaling, ↑ Mf	
	• ↑ Aldosterone and AG II-mediated cardiac fibrosis	
Osteonectin	• ↑ Cell-ECM interactions and collagen binding	• Cardiac fibrosis
	• ↑ TGF-β signalization	• Myocardial dysfunction
	• ↑ Cell activation and cellular trans-differentiation	
Periostin	• Localized in activated myofibroblasts	• Diabetic DCM
	• ↑ Fibroblast recruitment	• Fibrosis
	• ↑ Collagen synthesis	• Increased LV rigidity
	• Mechanism: ↑ TGF-β/SMAD pathway activate periostin	
CTGF	• ↑ TGF-β signalization	• Cardiac fibrosis

CFs cardiac fibroblasts, *CTGF* connective tissue growth factor, *DCM* dilated cardiomyopathy, *ECM, extracellular matrix*; *GFs* growth factors, *Mf* macrophage, *MMPs* matrix metalloproteinases, *SMAD* small mothers against decapentaplegic, *TGF-β* transforming growth factor-β, *TSP-1* thrombospondin-1, *TSP-2* thrombospondin-2, *TSP-4* thrombospondin-4

that inhibit CFs and hypoxia-inducible factor-1 which in turn deplete TGF-β levels [6]. In elevated myocardial pressure, a cardiomyocyte-specific TGF-β pathway is activated, generating myocardial remodeling [35] (Fig. 1).

Humoral factors and molecular pathways involved in cardiac fibrosis

TGF-β is a cytokine with major roles in cardiac fibrogenesis in NIDCM subjects. The profibrotic effect occurs in case of prolonged exposure to elevated levels of TGF-β [11]. It enhances myofibroblast activation, MC migration, and cardiac hypertrophy and also inhibits type I T helper lymphocytes [10]. Through its receptors (TGF-βR), type I and type II TGF-β receptors, TGF-β activates SMAD2/3 pathways, stimulating alternative pathogenetic pathways and regulating cell synthesis and differentiation promoting fibrogenesis. They will bind to SMAD4 forming a complex that will internalize into the cell nucleus and initiate the fibrotic response. Conversely, profibrotic conditions are able to activate the AMP-activated protein kinase α pathway which can antagonize SMAD3, counteracting its profibrotic effects [6, 10, 36, 37] [37]. TGF-β also activates protein kinase B (AKT) via the Ras homolog gene family-member A-dependent pathway which in turn activates the SMAD2 pathway while also determining EMTd; thus, it renders the production of ECM [36]. The activation of type II TGF-βR, transforming growth factor beta-activated kinase 1 (TAK1) is potentiated which in turn disrupts the normal myocardial cells and increases fibrogenesis [11]. Extracellular regulated protein kinases (ERKs) are also upregulated, playing major roles in load-induced cardiac fibrosis [6].

AG II is an important oligopeptide that renders cardiac fibrosis regardless of etiology. It potentiates cardiac fibrogenesis by interacting with its receptors, angiotensin II receptor type I and type II (AGTR1 and AGTR2) and by enhancing TGF-β production [12]. AG II stimulates myofibroblast cell adhesion and ECM production. Alternatively, by activating nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), it stimulates the synthesis of type I collagen and connective tissue growth factors (CTGF) [38]. Therefore, the blockade of RAAS could reverse cardiac fibrosis [6]. Endothelin 1 (ET-1) may induce cardiac fibrosis by stimulating the proliferation and activation of CFs,

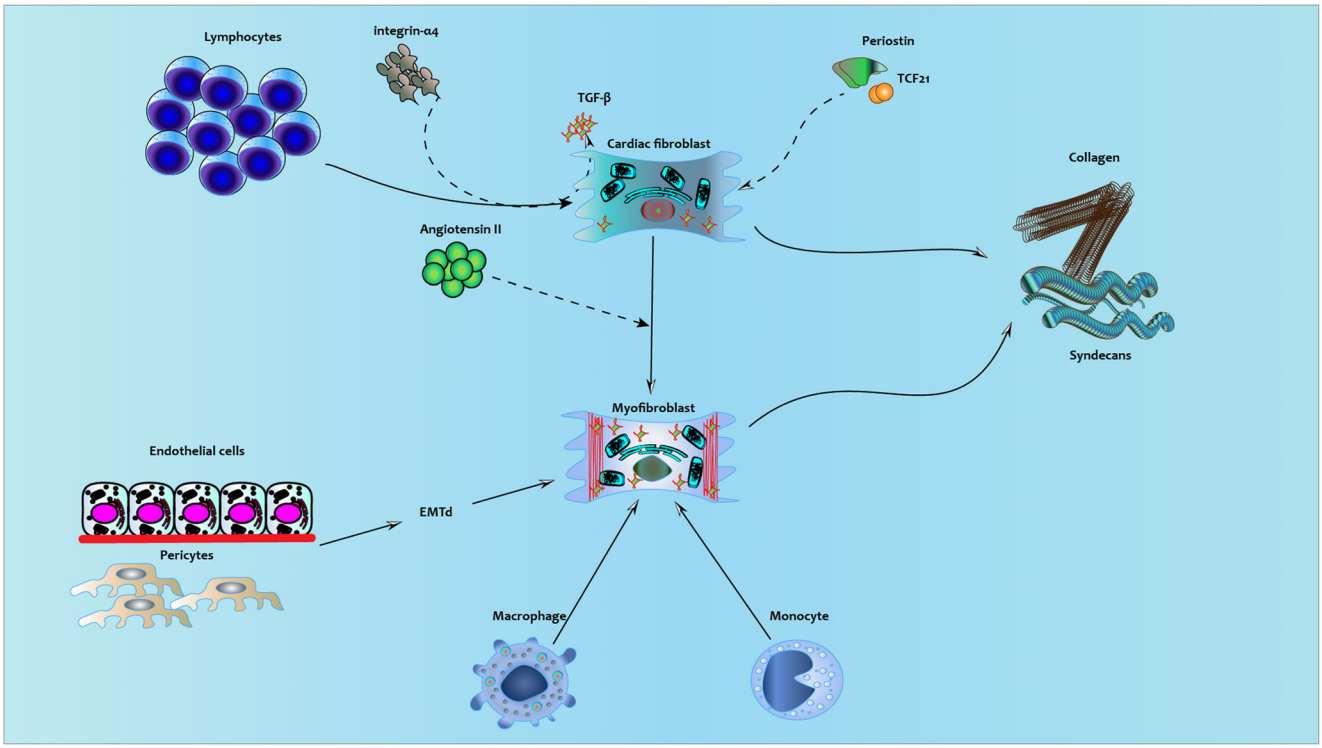

Fig. 1 Cellular involvement in cardiac fibrogenesis. Lymphocytes activate cardiac fibroblast via integrin-α4 and stimulate the production of transforming growth factor-β (TGF-β), while periostin and TCF-21 directly activate them. Endothelial cells and pericytes undergo Epithelial-mesenchymal trans-differentiation (EMTd) in myofibroblast. Macrophages and monocytes among with angiotensin II stimulate myofibroblasts. Finally, activated cardiac fibroblast and myofibroblasts produces collagen and syndecans

respectively, by enhancing collagen synthesis, but further studies are required in order to define the exact mechanisms [11].

TNF-α enhances collagen production and CF activation. TNF-α is associated with TGF-β and in murine models, with fibrogenesis and early onset of NIDCM [11, 39]. Interleukin-6 (IL-6) determines cardiac fibrosis along with AG II, but further studies are required to correctly establish the mechanisms [13]. IL-1β potentiates myocardial inflammation, apoptosis, fibrosis, and myocardial dysfunction. It decreases calcium channel functionality, reduces intracellular levels of phospholamban and sarcoplasmic ATPase, increases levels of nitrous oxide system which in turn induces mitochondrial toxicity, and stimulates TGF-β synthesis [40].

Recent studies have shown the role of the several molecular pathways involved in cardiac fibrosis. Wnt/β-catenin pathway inhibits the destruction complex formed of axin, adenomatosis polyposis coli (APC), protein phosphatase 2a (PP2a), glycogen synthase kinase 3 (GSK3), and casein kinase 1α leading to β-catenin accumulation. It also enhances myofibroblast infiltration by stimulating CF activation and epithelial to mesenchymal differentiation, hence determines myocardial dysfunction and NIDCM [41]. Moreover, in murine subjects, the CFs β-catenin knockout reduced cardiac fibrosis by downregulating Col1a1, collagen 3A1 (Col3a1), and periostin [42]. Lastly, the activating protein-1 (AP-1) stimulates the production of fibronectin, collagen, intracellular and vascular cell adhesion molecules, potentiating inflammation and cardiacfibrosis [11] (Fig. 2).

The male-female paradigm in cardiac fibrosis

The male-female difference in cardiovascular pathology is widely recognized. It has been observed that in women over 50 years, there is an overexpression of ECM with increased cardiac fibrosis. Corroborated data emphasizes that female subjects, both murine and human, are more protected from cardiac fibrosis due to estrogens, predominantly 17β-estradiol (E2) [14]. E2 acts through its cytosolic receptors (E2R), ERα and ERβ, either at the nuclear level (genomic pathway) or through the G protein-coupled receptor 30 (GPR30) receptors at the membrane level (nongenomic pathway) [43].

In this matter, various experimental studies have been conducted and concluded that estrogens exert antifibrotic effects on CFs through various mechanisms. In ovariectomized mice, the E2 and GPR30 G1 agonist were able to inhibit the effects of isoproterenol and the fibrosis induced by TGF-β [44, 45]. E2 was also able to prevent AG II and arterial hypertension induced fibrosis via ERβ by reducing the levels of TGF-β, collagen, and fibronectin [14]. E2 is able to block ERK signalization by reducing the synthesis of reactive oxygen species (ROS) and by inhibiting NF-κB and mitogen-activated

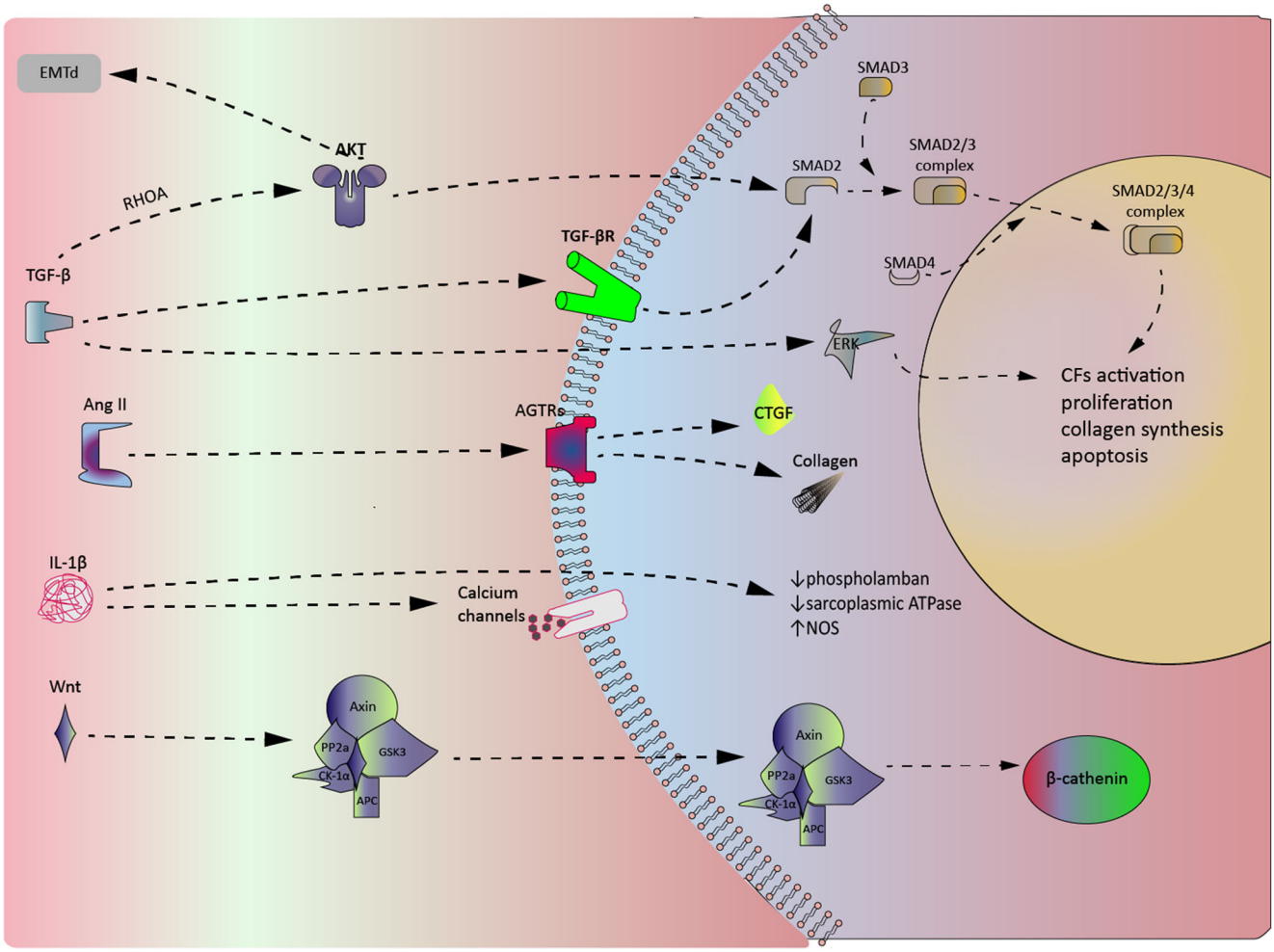

Fig. 2 Molecular mechanisms in cardiac fibroblasts. Transforming growth factor-β (TGF-β) through its specific receptors activate the mothers against decapentaplegic homolog 2 and 3 pathways (SMAD2/3) that binds with SMAD4 forming a complex that will stimulate cardiac fibroblast (CFs) activation, proliferation, collagen synthesis, and cell apoptosis. TGF-β activates ERK inducing the same effects as SMADs. TGF-β also enhances protein kinase B (AKT) via Ras homolog gene family, member A (RHOA) that stimulates epithelial-mesenchymal trans-differentiation (EMTd) and activates SMAD2. AG II through its receptors (AGTRs) enhances collagen synthesis and stimulates CTGF. Interleukin-1β disrupts calcium channels and decreases the production of phospholamban and sarcoplasmic ATP-ase and increases nitrous oxide systems (NOS). The Wnt creates a complex formed of axin, adenomatosis polyposis coli (APC), protein phosphatase 2a (PP2a), glycogen synthase kinase 3 (GSK3), and casein kinase 1α (CK-1α) that will enter the CFs and will stimulate β-cathenin synthesis

protein kinase (MAPK) lowering the expression of αSMA [14]. Moreover, by interacting with cyclic adenosine monophosphate (cAMP) and protein kinase A (PKA), ERβ is able to lower the levels of TGF-β and brake the activation of SMAD3, decreasing the expression of fibronectin, type I and type III collagen [46]. Even though the protective effects of estrogens on female CFs are clearly established, on male CFs, they are contradictory. In male CFs, studies have shown that E2 renders fibrogenesis by increasing the synthesis of ECM proteinsdue to male-specific posttranslational modifications of ERs [47]. Interestingly, the activation of GPR30 expressed in male CFs exerted antifibrotic effects by reducing the levels of αSMA, type I and type III collagens and fibronectin, but the underlying mechanisms are not fully elucidated and further studies are required in order to correctly identify them [48].

Cardiac fibrogenesis in NIDCM

In both familial and nonfamilial NIDCMs, cardiac fibrosis is a progressive process that occurs from the onset of the disease with significant consequences on prognosis and mortality. NIDCM induced by sarcomere proteins mutations involve most commonly myosin and troponin, determining cardiomyocytes' degeneration and interstitial fibrosis [4]. The truncating titin mutation, the most common cause of familial NIDCM, disturbs the mitochondrial energetic metabolism and alters cell cytoskeleton, potentiating cardiomyocyte dysfunction and inflammation, resulting in interstitial myocardial fibrosis and increased risk of arrhythmogenesis [49]. While in subjects with lamin A/C mutations there is an increased production of fibronectin, syndecans, nidogen and

decorin, with enhanced activation of TGF-β and SMAD2/3 [50], in subjects with SCN5A mutations, the sodium channel homeostasis is disturbed and calcium pumps are imbalanced, damaging the myocardium and promoting fibrogenesis [51]. In toxic NIDCM, fibrogenesis varies depending on the toxin and often is being identified in the phase IV studies. Recently, it has been shown that doxorubicin (DXO) is able to modify the structure of ECM, to inhibit the activity of topoisomerase IIβ and to stimulate the synthesis of ROS, potentiating cardiomyocyte apoptosis and fibrogenesis [52]. It is presumed that apoptosis could be also determined by the interaction of DXO with substance P and neurokinin receptor 1 (SP/NK-1R). Moreover, its profibrotic effects are due to CF activation and in chronic administration, it enhances the production of TGF-β, MMP-2, MMP-9, fibronectin, and col1α1 [52–54]. Arsenic trioxide is able to upregulate profibrotic genes, to potentiate EMTd through AKT/GSK-3/Snail pathway and to stimulate TGF-β resulting in cardiac fibrogenesis and QT prolongation [55, 56]. In patients treated with anti-CTLA4 (cytotoxic T lymphocyte-associated protein-4), the presence of myocardial fibrosis was identified, while in those treated with anti-PDL-1 (programmed death-ligand 1) agents, it was shown to stimulate pulmonary fibroblastic proliferation, but the cardiac effects have not yet been studied [57–59].

Regarding the illicit drugs, alcohol is able to enhances the synthesis of TGF-β and myostatin and determines ECM disorganization, apoptosis and cardiomyocyte necrosis, both directly and through its metabolism products, acetaldehyde adducts and lipid peroxidation [60, 61]. It is known that cocaine and methamphetamine are associated with myocardial fibrosis, but further studies are needed to elucidate the underlying mechanisms [62, 63]. Several infectious agents are able to enhance cardiac fibrogenesis in NIDCM. Through its direct cytopathic effect, coxsackievirus B3 determines fibrosis secondary to myocarditis [64]. It also increases the production of type I collagen and TGF-β, the activation of CFs and inflammatory cells, potentiating cardiac fibrosis [65, 66]. In HIV-positive subjects, cardiac fibrosis develops due to excessive accumulation of collagen, increased synthesis of TGF-β and other cytokines, activated ROS and decreased ECM degradation [67]. In addition, antiretroviral therapy enhances TGF-β/SMADs pathway potentiating cardiac fibrogenesis in HIV patients [68].

Among the metabolic disorders, diabetes mellitus (DM) is best correlated with cardiac fibrosis and cardiomyopathy. DM determines cardiac impairment and diabetic cardiomyopathy both by potentiating ischemia and by stimulating alternative nonischemic pathological processes. The main nonischemic mechanisms of cardiac fibrosis are due to hyperglycemia, advanced glycation end-product (AGEs), ROS and neurohumoral activation, CF proliferation with high levels of αSMA [69], myofibroblast activation, and EMTd. Neurohumoral activation and hyperglycemia are able to activate the synthesis of TGF-β, the RAAS, and ROS. AG II activates the SMAD pathway directly by increasing TGF-β synthesis, stimulates TGF-β activity through endoglin, and induces ROS gene. Recent studies have shown that in subjects with DM, there are increased levels of profibrotic cytokines such as TNF-α and IL-1β. AGEs interact with their own receptors (RAGEs) and increases collagen and laminin synthesis, activates CFs and macrophages, and enhances TGF-β and AG II activity [70]. Moreover, recent data emphasize that hyperglycemia activates calcium-sensing receptors (CaSR) increasing type I and type III collagen synthesis, stimulating CF proliferation and enhancing TGF-β/SMADs pathway, potentiating cardiac fibrosis [71]. However, due to the complex mechanisms by which DM promotes myocardial ischemia, the mechanisms cannot be completely separated, and this pathological entity must be considered as a complex of ischemic and nonischemic factors. All these are summarized in Table 2.

Assessment of cardiac fibrosis in NIDCM

Endomyocardial biopsy and pathological aspects

Although cMRI examination can accurately evaluate and quantify the degree of myocardial fibrosis, pathological examination of endomyocardial biopsy (EMB) samples is still considered the gold standard diagnostic tool. Histopathological examination (HPE) in the complete assessment of NIDCM with cardiac fibrosis includes the description of the endocardium, interstitium, and myocardium, as well as the quantification of the fibrotic load. In the heart affected by NIDCM, the endocardium can be thickened, infiltrated with inflammatory cells and CFs, as well as murine thrombi and subendocardial fibrosis can also be detected. Within the myocardium, modified cardiomyocytes, myocardial disruption and interstitial fibrosis are frequently described. Cardiomyocytes present abnormalities in terms of number, size, and shape, and also within their intracellular compounds. These cells present large variations, hypertrophic, atrophic, and normal cardiomyocytes being identified. While their cytoplasm present vacuolar degeneration, myocytosis, reduction in myofibrils, and various pigment accumulation, their nuclei have variable dimensions, irregular forms, and variable pleomorphism [72].The interstitial structure is the main site of cardiac fibrosis. Initially, acute inflammation occurs with interstitial edema and activated inflammatory cells, activating the production of collagen fibers and therefore rendering the definitive cardiac fibrosis. Intramural blood vessels are characterized by perivascular inflammatory infiltrate leading to thickened vascular wall reduced blood flow [73].

The essential histopathological parameters that are widely used in the evaluation of heart's fibrotic load of the heart tissue is CVF and the two specific forms, namely type I collagen volume fraction (C1VF) and type III collagen volume

Table 2 Main causes of fibrosis in DCM and their pathogenetic keypoints

Category	Substance/mutation	Mechanism
Genetic	SPs	▪ ↑ Cardiomyocyte degeneration ▪ ↑ Interstitial fibrosis ▪ Mechanism: not yet elucidated
	TTN	▪ ↓ Mitochondrial metabolism ▪ ↓ Cytoskeleton ▪ ↑ Cardiomyocyte dysfunction ▪ ↑ Myocardial inflammation ▪ ↑ Interstitial fibrosis ▪ ↑ Arrhythmogenic risk
	Lamin A/C	▪ ↑ TGF-β/SMADs pathway ▪ ↑ Fibronectin, syndecans, nidogen, decorin ▪ ↑ Cardiac fibrosis
	SCN5A	▪ ↓ Na^{+}channel homeostasis ▪ ↑ Ca^{2+}imbalancement
CHT drugs	DXO	▪ ↓ TI-IIβ ▪ ↑ ROS ▪ ↑ SP/NK-1R mediated apoptosis ▪ ↑ TGF-β signalization ▪ ↑ Fibronectin ▪ ↑ Col1α1 ▪ ↑ MMP-2, MMP-9 ▪ ↑ Cardiomyocyte apoptosis and cardiac fibrosis
	ATO	▪ ↑ PKB/GSK-3/Snail pathway induced EMTd ▪ ↑ TGF-β signalization ▪ ↑ cardiac fibrosis and QT prolongation
	Anti-CTLA-4/anti-PDL-1	▪ ↑ T cell dysregulation, activation, infiltration ▪ ↑ IL-6 and IFN-γ ▪ ↑ $CD8^{+}$T cells induced myocarditis
Illicit drugs	Alcohol	▪ ↑ Myostatin ▪ ↑ TGF-β signalization ▪ ↑ ECM disruption ▪ ↑ Cardiomyocyte apoptosis and necrosis ▪ ↑ Acetaldehyde adducts and lipid peroxidation
	Cocaine/METH	▪ ↑ Excitation-contraction coupling disruption ▪ ↑ CA hyperactivation
Infectious agents	CVB3	▪ ↑ Direct cytopathic effect ▪ ↑ Collagen ▪ ↑ TGF-β ▪ ↑ CFs and inflammatory cells activation ▪ ↑ Cardiac fibrosis
	HIV	▪ ↑ Collagen ▪ ↑ TGF-β ▪ ↑ Inflammatory cytokines ▪ ↑ ROS ▪ ↓ ECM degradation ▪ ART → ↑ TGF-β/SMADs pathway
Diabetes mellitus	▪ Glycemia ▪ AGEs ▪ ROS ▪ NHA	▪ ↑ TGF-β/SMADs pathway ▪ ↑ RAAS ▪ ↑ ROS ▪ ↑ TNF-α ▪ ↑ IL-1β ▪ ↑ Type I and type III collagen ▪ ↑ Laminin ▪ ↑ CFs and Mf activation ▪ ↑ CaSR activation

AGEs advanced glycation end-product, *Anti-CTLA-4* anti-cytotoxic T lymphocyte-associated protein-4, *Anti-PDL-1* anti-programmed cell death 1 ligand, *ART* antiretroviral therapy, *ATO* arsenic trioxide, *CA* catecholaminergic, *CaSR* calcium-sensing receptors, *CD8+* cluster of differentiation 8+, *CFs* cardiac fibroblasts, *CHT* chemotherapy, *Col1α1* collagen type I alpha 1, *CVB3* coxsackievirus B3, *DXO* doxorubicin, *ECM* extracellular matrix, *EMTd* epithelial-mesenchymal trans-differentiation, GSK-3 glycogen synthase kinase 3, *IFN-γ* interferon-γ, *IL-1β* interleukin-1beta, *IL-6* interleukin-6, *METH* methamphetamine, *Mf* macrophage, *MMP-2* matrix metalloproteinase-2, *MMP-9* matrix metalloproteinase-9, *NHA* neurohormonal activation, *PKB* protein kinase B, *ROS* reactive oxygen species, *SCN5A* sodium channel NaV1.5, *SMADs* small mothers against decapentaplegic, *Snail* zinc finger protein SNAI1, *SP/NK-1R* substance P and neurokinin receptor 1, *SPs* sarcomeric proteins, *TGF-β* transforming growth factor-β, *TI-IIβ* topoisomerase-IIβ, *TNF-α* tumor necrosis factor-alpha, *TTN* titin

(C3VF). This parameters are able to quantitatively determine the extension of myocardial fibrosis, but their cutoff values for fibrosis grading have not yet been established [74, 75]. In a recent clinical study conducted on 172 subjects with nonischemic HF, Aoki et al. identified that CVF, as a marker of severe myocardial fibrosis, acted as an significant predictor for all cause mortality and for cardiac events in subjects with nonischemic HF with reduced ejection fraction (HFrEF) [76]. Still, the current usage of EMB is mainly prohibited due to the chance of missing out the fibrotic areas and the increased risk of major cardiovascular complications.

Serum biomarkers of cardiac fibrosis in NIDCM

According to WHO, a biomarker represents any measurement that is able to demonstrate the interaction between a biological system and a potential hazard, being characterized by its relevance, viability and reproducibility [77]. The current research trend is to early identify cardiac fibrosis by noninvasive serum biomarkers. These substances may raise suspicion of cardiac fibrosis and some of them may be correlated with disease progression. Due to the fact that NIDCM is commonly associated with cardiac fibrosis, the identification and usage of such biomarkers are required. To evaluate the ability of a biomarker to identify cardiac fibrosis, its levels need to be compared with the gold standard diagnosis tool for cardiac fibrosis which is represented by CVF. Subsequently, the biomarker's dynamics are evaluated in relation to fibrosis progression or regression. Numerous potential biomarkers have been studied, but only a few have proved their utility, namely type 1 procollagen C-terminal pro-peptide (PICP), amino-terminal pro-peptide of type III procollagen (PIIINCP), Gal3, sST2, GDF-15, and circulating miRs [15, 16].

PICP and PIIICNP Being part of the heterotrimeric structure of type I collagen, PICP is a peptide released in the blood when type 1 collagen is polymerized in extracellular fibrils. It is considered to be a marker of type 1 collagen synthesis, fibroblastic activity, bone formation, and various pathological processes [78]. Recent clinical studies have confirmed the use of PICP in the evaluation of cardiac fibrosis. Serum levels of PICP have been significantly correlated with CVF in hypertensive subjects both with and without HF [75] and, besides, Lopez et al. have shown that in those with HF, PICP is alsocorrelated with C1VF [79]. In addition, these serum values decreased simultaneously with CVF values in subjects treated with antifibrotic drugs such as losartan and torasemide [75]. It has also been shown that spironolactone was able to reduce the myocardial fibrotic load confirmed both by CVF and serum PICP, in patients with NIDCM [80]. Regarding PIIINCP, it is synthesized when procollagen type III converts into collagen type III [74]. Plasma PIIINCP levels are also associated with CVF and C3VF in patients with NIDCM [75]. Moreover, PIIINCP has been positively correlated with ventricular dilation and inversely associated with the improvement of systolic function in NIDCM patients [81].

Gal3 Gal3 is a β-galactoside-binding protein which is found in various organs and tissues having important roles in cell adhesion, macrophage activation, angiogenesis, chronic inflammation, apoptosis, and fibrosis [82].

Experimental studies Nowadays, more and more studies confirm the primary role of Gal3 in cardiac fibrogenesis. Calvier et al. have shown that Gal3 is associated with cardiac fibrosis, while its inhibition diminishes the progression of it [83]. In a murine study, MacKinnon et al. identified the profibrotic effects of Gal3, being able to activate myofibroblasts, to control the immune-inflammatory modulation and to enhance the TGF-β signaling. Conversely, they have shown that the inhibition of Gal3 was correlated with decreased fibrosis due to TGF-β drop and lack of fibroblastic activation [84]. Furthermore, Wu et al. have found that in animal model, Gal3 was correlated with E/e' as a marker of diastolic dysfunction induced by aortic banding and they also identified 32% higher values of Gal3 in stretched cardiomyocytes compared to normal ones [85]. Besides, in a recent experimental study conducted on mouse cardiomyocyte cell culture, it has been identified that mechanical stress, protein kinase C and AG II render the activation of Gal3 and its profibrotic effects. They have also shown that in animal models with HF, Gal3 levels were increased along with the αSMA, actin, and type I collagen [86]. Strong evidence emphasize that the administration of Gal3 in the pericardium renders cardiac fibrogenesis by interacting with syndecans and activating local macrophages [87]. Similarly, Liu et al. identified that the pericardial administration of Gal3 in rats has important profibrotic effects is by enhancing the TGF-beta expression and SMAD3 phosphorylation [88]. An interesting study conducted by Martinez et al. have shown that in human CF cell cultures, cardiothropin-1 (CT-1) upregulated Gal3 along with αSMA, vimentin, CTGF, TGF-β, osteopontin, collagen type I, as well as the proinflammatory markers IL-6, monocyte chemotactic protein 1 (CCL-2), and IL-1β. Conversely, in Gal3-knocked down cells, CT-1 did not render the expression of vimentin, CTGF, osteopontin, collagen type I, nor IL-6 and CCL-2. Furthermore, in the same published study, the authors showed that in animal models, CT-1 enhanced the expression of Gal3 which in turn was correlated with perivascular fibrosis [89].

Clinical studies The role of Gal3 in cardiac fibrogenesis, pulmonary hypertension, and HF in human subjects have recently confirmed in clinical studies [90]. In a study conducted on 3353 patients from Framingham Offspring Cohort, Ho et al. identified that Gal3 was associated with increased LV mass and enhanced risk of HF and also with all-cause mortality in

this population [91]. Moreover, de Boer et al. have shown that in patients with HF, Gal3 is an independent marker for rehospitalization for HF and all-cause mortality, especially in those with HFpEF, and it is also associated with inflammatory biomarkers [92]. In a study conducted on 150 patients with NIDCM, Vergaro et al. identified a significant correlation between serum Gal3 and myocardial replacement fibrosis determined by cMRI-LGE. In their research, Gal3 expressed a cutoff value of 14.6 ng/mL with a moderate sensibility and specificity for predicting the presence of LGE in cMRI [93]. Furthermore, Martinez et al. confirmed the role of CT-1 in potentiating Gal3 levels and its profibrotic effects even in human subjects with HF [89].

sST2

ST2 is part of the interleukin 1 receptor family having two isoforms, one transmembrane (ST2L) and another soluble circulating (sST2), and are synthetized mainly by CFs and cardiomyocytes. It is now recognized that ST2 specifically interacts with IL-33 providing a dual effect. By the interaction of IL-33 with ST2L, it provides cardioprotective effects, while its interaction with sST2 competes with ST2L acting as a receptor decoy and antagonizing its beneficial cardiac effects [94].

Recent data emphasize the diagnostic and prognostic value of sST2 in patients with acute and advanced chronic HF [95]. It has been shown that sST2 is correlated with increased LV end-diastolic filling pressure (E/e' ratio > 15), NYHA classification and NT-proBNP levels [16]. In a study conducted on 876 patients with chronic HF, Bayes-Genis et al. confirmed that sST2 was superior to Gal3 in terms of risk stratification [96]. Furthermore, Santhanakrishnan et al. identified that patients with HF, sST2 serum levels were significantly higher in subjects with HFpEF compared to normal subjects, but after adjustment for age, sex and other comorbidities the correlation did not persist [97]. When compared between subjects with HFpEF, the sST2 serum levels are lower in the group with normal LV diastolic filling pressure (E/e' ratio < 8) as opposed to those with undeterminable (E/e' ratio = 8–15) or increased LV diastolic filling pressure (E/e' ratio > 15) with a cut off value higher than 13.5 ng/mL [98]. Furthermore, in a study, it is identified that the levels of sST2 were associated with elevated secondary pulmonary hypertension in patients with HF and chronic obstructive pulmonary disease, but further studies are required in order to establish the correct association between sST2 and HF [99].

Regarding the prognostics, more and more data confirm the role of sST2 in prognostic evaluation in patients with HF and NIDCM. A study indicates a cut off value for sST2 of 17.53 ng/mL for mortality prediction [100]. sST2's mortality prediction capacity is confirmed in another study conducted in pediatric patients with NIDCM and moreover, it confirms its usefulness in monitoring the outcome [101].

GDF-15

GDF-15 is a cytokine part of the TGF-β superfamily with important implications in inflammation, metabolism, and cancer. More and more evidence emphasized the primary roles of GDF-15 in the development and progression of cardiovascular disease and underline its diagnostic and prognostic importance, but studies are still few and incipient. It has been shown that in some clinical studies, GDF-15 was able to increase the prognostic value of other biomarkers used for predicting cardiac mortality in patients with HF and also in identifying asymptomatic HF [102]. A recent study conducted by Wang et al. emphasized a similar diagnostic ability of GDF-15 with NT-proBNP in subjects with HFpEF, while serum levels of GDF-15 were superior to NT-proNBP in identifying the degree of HF even after adjusting for confounders [103]. In a small but interesting study conducted on 32 patients with NIDCM, Nair et al. have shown that GDF-15 was significantly correlated with MMPs and sST2 levels as markers of myocardial dysfunction and fibrosis. They have also shown that GDF-15 was positively associated with the NYHA class, NT-proBNP levels, LVEF and LV diastolic function [104]. Furthermore, GDF-15 was significantly associated with markers of fibrogenesis such as type I collagen carboxy-terminal peptide and PIIINCP. It is also correlated with E/e' ratio, left atrial pressure, and inversely with LVEF. Thus, GDF-15 appears to play roles in cardiac fibrosis, myocardial remodeling, and cardiac dysfunction, but further studies are needed to elucidate underlying mechanisms [105].

Circulating miRs

miRs are fragments of noncoding RNAs that play a primary role in controlling gene expression through gene silencing, either by blocking translation or inducing degradation of messenger RNAs. Their malfunction may induce aberrant gene expression with important pathogenetic consequences [106]. miRs are stable molecules that can be determined in all the body fluids and can be used in diagnostic and therapeutic purposes. Recent data identified an important number of miRs with implications in the whole spectrum of cardiovascular pathology, but the research is in the beginning [107]. Many miRs are described in cardiac fibrogenesis, but the most studied are miR-21, miR-29, and miR-133. miR-21 is associated with myocardial remodeling and fibrosis.

In an experimental study conducted on rat CFs, Cao et al. have shown that miR-21 was able to activate CF proliferation by enhancing signal transducer and activator of transcription 3 (STAT3) pathway and in turn decreasing the cell adhesion molecule 1 (CADM1), thus promoting cardiac fibrogenesis

[108]. Furthermore, in the study of Yuan et al., TGF-β rendered the overexpression of miR-21 in a dose-dependent manner, while miR-21 blockage inhibited the CF proliferation. In addition, they have also demonstrated that by directly blocking the inhibitory SMAD7 pathway, miR-21 was able to activate TGF-β and SMAD2/3 pathway enhancing the synthesis of αSMA and collagen fibers, thus promoting cardiac fibrogenesis [109]. Nevertheless, Brønnum et al. have shown in an experimental research that miR-21 was capable to inhibit the protein sprouty homolog 1 (SPRY1) pathway, an inhibitor of ERK/MAPK pathway and in turn to render EMTd and fibroblastic phenotype expression [110]. The role of miR-21 in both in ischemic and NIDCM have been recently confirmed by Li et al. [111], but further studies are required in order to correctly establish the underlying mechanisms and utility.

The roles of miR-29 in modulating cardiac fibrogenesis have recently been demonstrated, yet some data emphasize its dual role in this process. It has been hypothesized that miR-29 could promote cardiomyocyte apoptosis by decreasing the expression of several antiapoptotic genes and, conversely, to hinder the synthesis of collagen fibers preventing cardiac fibrogenesis [112]. In a recent murine study, Sassi et al. have demonstrated that the lack of miR-29 expression was able to prevent myocardial remodeling and, even more than that, exogenous miR-29 blockade was able to impede the synthesis of Col1a1, Collagen 1 A2 (Col1a2), Col3a1 and lower the degree of cardiac hypertrophy and fibrosis. They have also identified that miR-29 stimulated glycogen synthase kinase 3 beta (GSK3B) and other proteins that were involved in the Wnt pathway, thus promoting cardiac fibrogenesis [113].On the contrary, in their studies, Yamada et al. and Drummond et al. highlighted the antifibrotic potential effects of miR-29, both on cardiac and extracardiac fibrosis [114, 115]. In addition to these findings, Liang et al. have found that miR-29b3p and miR-29c3p were able to block TGF-β and MMP2, and in turn TGF-β via the SMAD3 pathway was able to inactivate miR-29 molecules [116]. The adverse outcomes may be explained by the possible different roles of the miR-29 subtypes, but further studies are needed to accurately identify them.

miR-30 is significantly associated with myocardial hypertrophy, and it is inversely correlated with CTGF, collagen, and fibrosis [117]; thus, it could become a potential biomarker for cardiac fibrosis. Recently, in a murine study that evaluated the effects of miR-30 after myocardial infarction, Chen et al. demonstrated that miR-30 may be able to reduce the deposition of type I and type III collagen by directly blocking CTFC, exerting beneficial effects on cardiac fibrosis [118]. Yet, further studies must be conducted to evaluate its potential role in fibrosis in patients with NIDCM. Another miR that share similar effects of blocking CTGF is miR-133, which is also associated with cardiac fibrosis. Angelini et al. identified that miR-133 is able to reduce the expression of CTGF through the serum response factor (SRF)/miR-133/CTGF axis. Moreover, the hyperstimulation of SRF blocks miR-133 and potentiates CTGF, while normal levels of SRF do not exert any effect on miR-133 molecules [119]. Recently, it has been shown that miR-133a could be capable to hinder cardiac fibrosis by blocking the AKT pathway and by regulating β-adrenergic receptor transduction signalization [120]. The latter is achieved by downregulating β-arrestin and (cAMP)-dependent protein kinase A [121]. In the murine study of Chen et al. conducted on mice with NIDCM, it was identified that miR-133a inversely correlates with several markers of cardiac fibrogenesis namely with fibronectin, FGF1, TGF-β, AG II, and ET-1. Also, it has been shown that the overexpression of miR-133a inhibited TGF-β/SMAD2 pathway preventing diabetes induced fibrosis [122].

A recent study emphasized that the dysregulations in miRs expression in NIDCM models were able to differentiate between the compensated and the decompensated NIDCM. miR-454, miR-500, and miR-1423p were downregulated, while miR-1246 was increased and all three miRs significantly correlated with diastolic dysfunction. Moreover, miR-1246 was associated with the levels of NT-proBNP levels and with E/e' as a marker of diastolic dysfunction [123]. The use of miRs patterns has also been shown to increase diagnostic accuracy. For instance, the pattern miR-125a5p, miR-190a, miR-550a5p, and miR-638 was able to differentiate between HFpEF and HFrEF, while the pattern miR-1833p, miR-190a, miR-1933p, miR-1935p, miR-5455p differentiated HFpEF subjects from a healthy group [124]. Other two miRs capable to distinguish between HFrEF and HFpEF are miR-375 and miR-328. Furthermore, miR-375 proved increased predictive power when it was associated with NT-proBNP [125]. Combining these miRs patterns with HF markers can enhance diagnostic accuracy in subjects with NIDCM.

cMRI

cMRI is the noninvasive gold standard investigation that uses advanced imaging techniques and contrast agents to accurately assess myocardial fibrosis [17]. Using cine-imaging, chambers volumes and cardiac function are determined, while for the assessment of myocardial fibrosis, specific cMRI protocols have been developed [18]. LGE increased signal intensity in the inversion-recovery sequence can detect irreversible replacement fibrosis [126] (Fig. 3), while post-contrast T1-weighted mapping identifies diffuse interstitial fibrosis [127]. Myocardial tissue stores gadolinium depending on its pathogenetic status, and in turn, it accelerates the longitudinal relaxation time T1, changing its intensity. Local infusion, extracellular distribution, internal water exchanges, and gadolinium wash-in/wash-out are parameters that influence the final aspect of the images [19]. Gadolinium persists longer in the fibrotic area because the extracellular distribution is increased

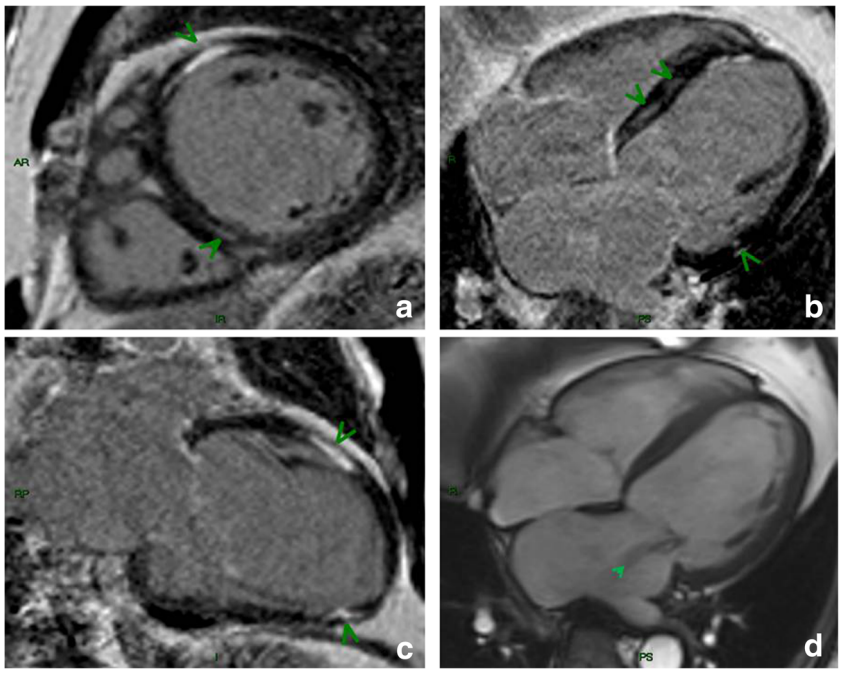

Fig. 3 Cardiac magnetic resonance imaging in a patient with clinical nonischemic dilated cardiomyopathy. Midwall late gadolinium enhancement in present in the anterior wall (arrows) in **a** short axis view. Focal midwall late gadolinium enhancement is visible on the infero-lateral wall (arrows) in **a** short axis views, **b** 4-chamber views, and **c** 2-chamber CMR views. Cine-imaging presenting mitral regurgitation (arrow) in **d** 4-chamber views

and the wash-out time is prolonged. Image acquisition is based on T1-weighted gradient echo that can be achieved within a single heartbeat by the fast and highly reproductible modified look-locker inversion-recovery (MOLLI) sequence a single heartbeat technique [128]. There is also the double-heartbeat technique that gives an impeccable contrast being preferred in NIDCM's characterization, but the long apnea and acquisition periods makes it sometimes prohibitive. These acquisitions evaluate the extent, magnitude and pattern of fibrosis with a global and segmental characterization. Specific LGE patterns can differentiate between various cardiomyopathies, inflammatory or storage myocardial diseases. LGE-identified fibrosis correlates with histological changes, fibrosis biomarkers and can assess myocardial viability [18].

In NIDCM, three LGE patterns have been described, namely midwall, subendocardial, or transmural, which suggest a prior myocardial infarction, and the absence of LGE [129]. Linear or bandlike midwall LGE occur in 28–35% cases of NIDCM [129, 130] and corresponds to irreversible replacement fibrosis and fibro-fatty degeneration. In up to 59% of patients, histological fibrosis exists yet no LGE is being detected. This corresponds to diffuse interstitial fibrosis and can be unraveled when the null point method is used in LGE image acquisition. In previous reports, the presence of LGE was associated with major cardiac events and arrhythmias, but not with the cardiac function [131–133]. Particularly, myocardial LGE in NIDCM is associated with increased risk of hospitalization and mortality and low response rate to best medical therapy [126, 134]. Moreover, in LV midwall fibrosis (LVMWF), the global circumferential strain, strain rate, and torsion are reduced, and there is an increased risk of HFrEF [135]. In order to characterize diffuse cardiac fibrosis (DCF), the T1M technique was developed. DCF is caused by interstitial fibrosis which seizes the extracellular space, and the LGE technique is no longer able to identify it. T1M assesses the intrinsic T1 relaxation time of the myocardium, and combined with inversion pulses that suppress the tissue, it highlights the presence of gadolinium within the fibrotic areas by assessing the extracellular volume fraction (ECV) [136, 137]. In fact, T1M creates a myocardial map that is built from the intensity of the signals of each cardiac voxel. The MOLLI technique is the most used in T1M, but there are other procedural methods such as the short MOLLI (ShMOLLI) specifically designed to reduce the long apnea and acquisition time in patients with severe cardiac or respiratory disease [137]. T1M can accurately assess the degree of diffuse myocardial fibrosis, and in the future, typical fibrosis patterns can be created for various diseases. Studies have shown that the average precontrast T1 relaxation time is longer in the pathological myocardium. Also, in the presence of chronic scarring, it becomes significantly longer. Despite the extraordinary usefulness of the technique, it needs further studies to validate its clinical utility [126, 137]. There are other cMRI methods that evaluate myocardial fibrosis; some have not been validated for clinical usage, while others are under development. Equilibrium contrast cMRI is based on gadolinium bolus followed by continuous intravenous infusion in order to achieve humoral equilibrium, blood volume distribution determination, and T1-weighted measurements before and after equilibrium. This method was created for accurate assessment of diffuse myocardial fibrosis, but due to a complicated and complex acquisition protocol, it is not routinely used [138].

Current research trend aims to perfect the usage of cMRI-LGE in diagnosing and evaluating of cardiac fibrosis. The aforementioned LGE patterns have both diagnostic and prognostic value in patients with NIDCM. Besides the fact that LVMWF is able to exclude the ischemic etiology of DCM, in patients with NIDCM, it is associated with a 9-fold increased risk of SCD, ventricular tachycardia (VT), LV-assisted device requirement, cardiac transplantation, and 12-month mortality [5, 130, 134, 139]. Furthermore, a recent published study showed that subjects with LVEF > 30% and LV LGE > 5% are at similar risk as those with LVEF < 30%, and vice versa [140]. Recent data emphasize that cMRI-LGE could become useful in guiding CRT by identifying eligible patients more accurately [134]. A recent study has emphasized the ability of LGE-cMRI in assessing heterogeneous distributed fibrosis with important roles in predicting SCD and malignant arrhythmias in subjects with NIDCM, but further studies are needed in order to refine the technique [141]. Furthermore, a recently published meta-analysis that evaluated the prognostic value of cMRI-LGE on patients with NIDCM have shown that the presence of LGE seemed to be a strong predictor for cardiovascular events, particularly for malignant ventricular arrhythmias and HFrEF [17]. Current available data regarding cMRI-LGE and cardiac fibrosis in NIDCM are presented in Table 3 [139, 140, 142–149].

Fig. 3 cMRI in a patient with clinical NIDCM

Therapeutic approaches for cardiac fibrosis in NIDCM

Until recently, cardiovascular therapy in NIDCM targeted heart failure, arrhythmias, and support for systolic function. Current trend aims at reducing cardiac fibrosis, improving overall cardiac performance and cardiac resynchronization therapy, decreasing mortality, and increasing life quality.

Drugs

Pharmacological treatment represents the first line of therapy in NIDCM. ACCF/AHA and ESC recommend the use of angiotensin-converting enzyme inhibitors (ACEIs) or angiotensin receptor blockers (ARBs), in association with β-blockers (βB) and aldosterone antagonists for improving LV dimension and function, reducing morbidity-mortality, but also reducing congestion symptoms [150, 151]. Furthermore, regarding cardiac fibrosis treatment, there are some studies that target some specific pathways and the results are promising, but further research is needed.

Recent studies have shown that ACEI and ARB could be able to inhibit AG II and TGF-β/SMADs pathway and to render the synthesis of N-acetyl seryl-aspartyl-lysyl-proline, a potent antifibrotic tetrapeptide, preventing myofibroblast activation and fibrogenesis [74, 152]. Moreover, βB, statins, and eplerenone have proven to exert antifibrotic effects [20]. Regarding specific antifibrotic therapy, direct inhibitors of TGF-β receptors such as GW 788388 and anti-TGF-β antibodies could block the activation of CFs; however, further studies are needed to strengthen such therapies [153]. TNF-α synthesis can also be blocked by p38 MAPK inhibitor [21]. Furthermore, being involved in CF adhesion and its inhibitors are able to mitigate renal fibrosis, periostin could become a potential cardiac antifibrotic target. Similarly, caveolin-1 could be a promising target, but studies are needed to evaluate this finding [154].

Modulating collagen synthesis is another way to influence cardiac fibrosis. Serelaxin, via relaxin-2 receptors, reduces inflammatory cells activation, apoptosis, ROS, and collagen synthesis, inhibiting fibrogenesis. It reduced the degree of dyspnea and mortality in those with acute HF [155]. In a murine study, Wu et al. identified that serelaxin could be able to inhibit TGF-β/SMADS pathway blocking the activation of CFs and to stimulate MMP2/TIMP2 ration enhancing ECM degradation [156]. Recently, it has been shown that polyphenols could block CTGF, TGF-β/SMADs pathway, ECM settlement, and NF-κB signaling; decrease TNF-α and ROS/ERK/TGF-β/periostin pathway; reduce ROS synthesis; and decrease Mo/Mf and MC infiltration. However, potency studies are needed to validate their clinical effects [157]. MMP inhibitors that stimulate collagen degradation could become promising therapeutic options, but the lack of their plasma stability and the insufficient data limit their use [74].

Certain miRs can be influenced either by miR mimicry and anti-miRs. The blockage of miR-21 could be able to reduce pressure-induced cardiac dysfunction and interstitial fibrosis, while antagonizing miR-208a could increase survival. The miR-29 mimicry could be capable ofTGF-β/SMADs pathway inhibition, reducing cardiac fibrogenesis [22, 74, 116]. Moreover, corroborated data have shown that decreased levels of miR-133 are correlated with increased levels of beclin-1, cardiac hypertrophy, HF severity, and inflammation, while miR-133 enhancement has beneficial effects. Interestingly, it has been shown that carvedilol could be able to upregulate miR-133, which could attenuate cardiomyocyte apoptosis [158]. In addition, low salt diet, adiponectin, and hydrogen sulfide could act as an exogenous and endogenous stimulator of miR-133. Targeting miRs is expanding, but significant studies are needed for the clinical validation of these therapies [120].

Other drugs have demonstrated their ability to reduce cardiac fibrosis. Rosiglitazone and pioglitazone reduce cardiac fibrosis through peroxisome proliferator-activated receptor-γ (PPARγ) and NF-κB, lowering CTGF levels. It also decreases myocardial remodeling by inhibiting RAAS [159]. Moreover, the combination of rosiglitazone and losartan attenuates interstitial fibrosis and collagen deposition by reducing TGF-β,

Table 3 cMRI assessed cardiac fibrosis in NIDCM

Authors	Year	Subject no.	Purpose	Conclusions	Ref
Kono et al.	2010	32 with NIDCM	Evaluate the role of cMRI-LGE in predicting NSVT and ICD implantation	The presence of cMRI-LGE was associated with higher incidence of NSVT and ICD implantation	[139]
Leyva et al.	2011	559 with NIDCM	Evaluate the usefulness of cMRI in guiding CRT and LV lead deployment	cMRI-LGE was able to accurately guide the LV lead deployment away from the myocardial fibrotic areas, therefore ensuring a correct CRT procedure	[140]
Leong et al.	2011	82 with newly diagnosed HF and NIDCM	Assess if the extent of LGE are associated with response to BMT in patients with newly diagnosed NIDCM	cMRI- LGE is a predictor for therapy response in subjects with NIDCM ($p < 0.001$). After multivariate analysis, the correlation persists ($p < 0.001$)	[141]
Klem et al.	2012	132 with NIDCM	Evaluate if cMRI detected myocardial fibrosis in risk stratification in subjects with NIDCM proposed for ICD	LGE is an independent predictor for adverse outcome in subjects for ICD and HFrEF. Significant LGE fibrosis identifies high-risk and severe outcome	[142]
Levya et al.	2012	258 with IDCM and NIDCM	Evaluate if LVMWF is a morbi-mortality predictor in subjects with DCM undergoing CRT	LVMWF was a predictor for cardiovascular mortality and hospitalization for MACE ($p < 0.0001$). The correlation persists after adjustment for confounders ($p = 0.0004$)	[137]
Gulati et al.	2013	472 with NIDCM	Evaluate if myocardial fibrosis is a predictor for mortality and SCD in subjects with NIDCM	The presence and extent of LVMWF is a predictor for all-cause mortality, cardiovascular mortality and cardiac transplantation ($p < 0.001$). The correlation persists after adjustment for confounders ($p < 0.001$)	[143]
Puntmann et al.	2016	637 with NIDCM	Evaluate the association of cardiac fibrosis assessed with LGE and T1M with all-cause mortality as primary endpoint in NIDCM subjects	Septal T1M, ECV, LGE - is predictive for all-cause mortality ($p < 0.001$). After adjustment for confounders, correlation persists. Also, septal T1M and LGE are independent predictors for outcome	[144]
Halliday et al.	2017	399 with NIDCM	Assess the association between LVMWF, risk of SCD and possible ICD implantation in NIDCM	cMRI-LGE was predictive for primary endpoint (SCD and aborted SCD) In HFpEF and NIDCM, it could identify a subgroup liable for SCD	[136]
Pi et al.	2018	172 with NIDCM	Evaluate the prognostic value of cMRI-LGE in patients with NIDCM and HFrEF	The presence of cMRI-LGE was associated with the primary outcome composite of all-cause death and heart transplantation ($p = 0.01$). The correlation persistent after adjustment for confounders ($p = 0.03$).	[145]
Oh et al.	2019	142 with NIDCM	Evaluate if newly cMRI parameters for cardiac fibrosis are associated with LV reverse remodeling after BMT	Decreased values for T1M and ECV are significantly associated with LV reverse remodeling ($p < 0.001$, $p < 0.001$)	[146]

BMT best medical therapy, *cMRI* cardiac magnetic resonance imaging, *ECV* extracellular volume, *HFpEF* heart failure with preserved ejection fraction, *HFrEF* heart failure with reduced ejection fraction, *ICD* implantable cardioverter defibrillator, *LGE* late gadolinium enhancement, *LV* left ventricle, *LVMWF* left ventricle mid-wall fibrosis, *NIDCM* nonischemic dilated cardiomyopathy, *T1M* T1 mapping

TNFα, IL-1β, and IL-6 [160]. Imatinib, a tyrosine kinase inhibitor, blocks phosphorylation and activation of PDGFRs and thus inhibits profibrotic gene expression, type I and type III collagen deposition, decreases α-SMA expression, and reduces myocardial hypertrophy [161].These drug effects are summarized in Table 4.

Lastly, biomaterials can be used to obtain myocardial regeneration and ventricular stability, especially after acute myocardial injury. However, these methods could also be extrapolated to NIDCM. Thus, special polymers of myocardial support, implantation of special tissues containing active molecules are being tested. In a murine study conducted on canine models with ischemic HFrEF, Chaudhry et al. have shown that cardiac support device (CSD) developed by Acorn Cardiovascular Inc. that are wrapped around both LV and right ventricle (RV) could be able to normalize end-diastolic volume, reduce cardiac hypertrophy and cardiac fibrosis [162]. Even though the effects could be extrapolated to NIDCM, the results of clinical trials on CSD are questionable and yet the benefits are not fully proven. Hydrogels or cell-containing scaffolds may also be used in order to provide ventricular stability and render recovery [20].

Regarding the surgical treatment of NIDCM, several procedures such as Batista's concept of reduction in LV wall tension by partial ventriculectomy have been tested, but with questionable results. In a clinical study conducted on five patients with NIDCM that underwent modified Dor's procedure of endoventricular path plasty, Doenst et al. have shown

Table 4 Potential antifibrotic therapies in NIDCM

Category	Drugs	Details
Cardiovascular drugs	▪ ACEI	▪ ↓ AG II
	▪ ARB2	▪ ↓ TGF-β
		▪ ↑ N-acetyl seryl-aspartyl-lysyl-proline
		▪ ↓ CF activation
		▪ ↓ Fibrogenesis
		▪ ↓ Myocardial remodeling
	▪ Beta-blockers	▪ ↓ CA activation
		▪ ↑ Antifibrotic effects
Molecular targets	▪ Anti-TGF-β	▪ Direct TGF-β inhibitor - GW 788388
		▪ Anti-TGF-β antibodies
	▪ Anti-TNF-α	▪ p38 MAPK inhibitor
	▪ Future targets	▪ Periostin
		▪ Caveolin-1
Collagen synthesis	▪ Serelaxin	▪ ↓ Apoptosis
		▪ ↓ ROS
		▪ ↓ Collagen
		▪ ↓ Inflammatory cell activation
		▪ ↓ TGF-β/SMADs pathway
		▪ ↓ CF activation
		▪ ↑ MMP-2/TIMP-2 ratio
		▪ ↑ ECM degradation
Polyphenols	▪ Flavonols	▪ ↓ CTGF
	▪ Flavones	▪ ↓ TGF-β/SMADs pathway
	▪ Isoflavones	▪ ↓ TNF-α and
	▪ Anthocyanins	▪ ↓ ROS/ERK /TGF-β/periostin pathway
	▪ Flavanones	▪ ↓ ROS
	▪ Resveratrol	▪ ↓ Mo/Mf and MCs infiltration
	▪ Curcumin	▪ ↓ Dyslipidemia
	▪ Piperine	▪ ↓ Collagen synthesis
	▪ Evodiamine	
miRs	▪ miR-29 mimick	▪ ↓ TGF-β/SMADs pathway
		▪ ↓ Cardiac fibrosis
	▪ miR-133 enhancement	▪ miR-133 stimulating agents:
		– Carvedilol
		– Adiponectin
		– Hydrogen sulfide
		▪ ↓ Beclin-1
		▪ ↓ Hypertrophy
		▪ ↓ HF severity
		▪ ↓ Inflammation
		▪ ↓ Cardiomyocyte apoptosis
Antidiabetic drugs	▪ Rosiglitazone	▪ ↓ PPARγ
	▪ Pioglitazone	▪ ↓ NF-κB
		▪ ↓ RAAS
		▪ ↓ TGF-β
		▪ ↓ TNF-α
		▪ ↓ IL-1β
		▪ ↓ IL-6
		▪ ↓ Collagen deposition
		▪ ↓ Cardiac fibrosis
Tyrosine kinase inhibitor	▪ Imatinib	▪ ↓ PDGFRs activation
		▪ ↓ Profibrotic genes expression
		▪ ↓ Type I and type III collagen deposition
		▪ ↓ α-SMA
		▪ ↓ Myocardial hypertrophy

α-SMA α smooth muscle actin, *ACEI* angiotensin-converting enzyme inhibitors, *AG II* angiotensin II, *ARBs* angiotensin II receptor antagonists, *CA* catecholaminergic, *CFs* cardiac fibroblasts, *CTGF* connective tissue growth factor, *DCM* dilated cardiomyopathy, *ECM* extracellular matrix, *ERK* extracellular signal-regulated kinases, *HF* heart failure, *IL-1β* interleukin-1β, *IL-6* interleukin-6, *MAPK* mitogen-activated protein kinase, *MCs* mast cells, *Mf* macrophage, *miR-133* microRNA-133, *miR-29* microRNA-29, *MMP-2* matrix metalloproteinase-2, *Mo* monocyte, *NF-κB* nuclear factor kappa-light-chain-enhancer of activated B cells, *PDGFRs* platelet-derived growth factor receptors, *PPAR-γ* peroxisome proliferator-activated receptors-γ, *RAAS* renin angiotensin aldosterone system, *ROS* reactive oxygen species, *SMADs* small mothers against decapentaplegic, *TGF-β* transforming growth factor-β, *TIMP-2* tissue inhibitor of metalloproteinases 2, *TNF-α* tumor necrosis factor alpha

that even though this procedure could be performed safely and could immediately improve cardiac function, the long-term results are questionable. Moreover, it cannot replace heart transplantation, but it could become an alternative to it [163]. In the study of Calafiore et al. conducted on 66 patients with ischemic DCM, NIDCM and post-valvular DCM, it has been shown that surgery performed in subjects with normal or moderately impaired RV function, pulmonary artery systolic pressure (PAPs) < 45 mmHg and elective surgery have better outcome than in those with severely impaired RV function, PAPs ≥ 45 mmHg, necessity of intra-aortic balloon pump and/or inotropes [164]. Furthermore, in a study conducted on 56 subjects with NIDCM, Isomura et al. have demonstrated increased survival rate and better outcome due to intraoperative echocardiography in selecting operative procedure [165]. Recently, Suma et al. have found that in patients with NIDCM and HFrEF, NYHA class III/IV, septal akinesia and enlarged LV diastolic diameter, septal anterior ventricular exclusion with mitral reconstruction was associated with increased LVEF, decreased LV diastolic diameter, LV end-diastolic and end-systolic volumes, decrease in NTproBNP levels and in NYHA class [166]. Surgical treatment in NIDCM may be useful in certain situations, but it is grappling with important limitations due to the increased rate of periprocedural complications, high-risk interventions, reduced experience, and due to studies in small groups of patients.

CRT

CRT or biventricular pacing is a recently developed method of approaching patients with HFrEF and significant electrical and mechanical dyssynchrony [23]. NIDCM with HFrEF represent 43% of the patients with CRT [24]. This procedure is performed by placing two leads, one in RV at the apex and the other in LV through the coronary sinus at the lateral or posterolateral wall. Afterwards, the leads will synchronously stimulate both ventricles; thus, the electrical and mechanical dyssynchrony is solved and myocardial reverse remodeling is initiated. CRT is performed in patients with LVEF < 35%, electrical dyssynchrony with QRS complex > 120 ms with left bundle branch block and mechanical intraventricular, interventricular, and atrioventricular dyssynchrony [23].

Perspectives

The impact of CRT on cardiac fibrosis is being studied, but things are just at the beginning. In a recent murine study that evaluated the impact of CRT on myocardial fibrosis, Wang et al. have shown that CRT normalized QRS duration, reduced intraventricular dyssynchronism, and improved EF. Furthermore, they have shown that CRT was correlated with the reduction of myocardial fibrosis from LV quantified histologically by CVF, but insignificantly in RV, and was also associated with the reduction of TGF-β and SMAD2/3 pathway expression [167]. Thus, the early results are promising, but future studies are needed to accurately evaluate CRT on cardiac fibrosis in humans.

NIDCM is a severe form of primary myocardial disease characterized by progressive HF, with a poor prognosis, but still better than in ischemic HF. In patients with HFrEF, the 5-year-mortality rate exceeds 50%. Interestingly, in the study of Broch et al., patients with newly diagnosed NIDCM had better outcome when treated accordingly with the latest guidelines [168]. Furthermore, it is known that survival improvement in NIDCM patients are due to early diagnosis, heart transplantation, and HF management strategies [169]. Cardiac fibrosis is an important issue that should be evaluated promptly in each patient with NIDCM because it has a major impact on morbidity and mortality. Recent data certify that pathogenetic mechanisms in NIDCM stimulate various fibrogenesis pathways. However, some pathogenetic pathways are well explained, others require further studies to accurately identify them. Diagnosis by serum biomarkers begins to take shape and the use of miRs is a promising option for the future, yet validation studies are needed. Lately, EMB was replaced by cMRI with gadolinium administration having a high diagnostic accuracy. Given the consequences of cardiac fibrosis, the proper identification of therapeutic agents is another challenging aspect of the near future. There are drugs that exert antifibrotic effects and promising therapies targeting fibrosis are being developed, but things are still in the early beginning.

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Compliance with ethical standards

Competing interests The authors declare that they have no competing interests.

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