Emerging imaging targets for infiltrative cardiomyopathy: Inflammation and fibrosis

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Molecular imaging in infiltrative cardiomyopathies is increasingly penetrating the clinical arena. Current approaches target the infiltrate directly, or its metabolic, physiologic, or functional consequences. Inflammation may not just play a role as the infiltrative mechanism itself. It is also thought to play a key role in the development and progression of heart failure in general, because it promotes the development of tissue fibrosis. The cascade leading from tissue damage to inflammation and further to fibrosis and loss of function has emerged as a therapeutic target. This review focuses (1) on novel tracers of inflammation, which are on the brink of clinical applicability and may be more specific than the gross metabolic marker F-18 deoxyglucose; and (2) on novel biologic imaging targets in fibrosis, which may be exploited for interrogation of the crosstalk between inflammation and loss of contractile function. Ultimately, the success of any novel molecular imaging assay will depend on whether it can be used for successful guidance of novel, targeted therapies aiming at tissue regeneration. (J Nucl Cardiol 2019;26:208–16.)

Key Words: Molecular imaging • infiltrative cardiomyopathy • inflammation • fibrosis

INTRODUCTION

While the treatment of ischemic, arrhythmogenic, and valvular heart disease remains dominated by mechanical interventions and broadly applicable ''blockbuster'' drug therapy, it is the field of cardiomyopathies where specific, targeted therapies are increasingly emerging. This is an opportunity for the establishment of specific molecular imaging approaches, which may—similar to emerging practice in neurology and oncology—guide those therapies by identifying suitable candidates and early therapy response.

Molecular imaging is used to identify the infiltrate itself, such as transthyretin or light chain amyloid in cardiac amyloidosis[1,2] or inflammatory granuloma in cardiac sarcoidosis.[3,4] Moreover, imaging has been used to identify the functional consequences of cardiac infiltration, e.g., by detecting microvascular dysfunction as a consequence of sarcoid and amyloid deposition,[5,6] scar/fibrosis as a consequence of burnt-out sarcoidosis,[7] or impaired contractile patterns and deformation as a consequence of restrictive cardiomyopathy.[8,9] An additional opportunity for molecular imaging is to interrogate the pathways leading from early infiltration to the final consequence of myocardial dysfunction due to irreversible tissue damage. Here, the early response to tissue injury, which triggers a repair effort by recruiting inflammatory cells, which then stimulate a molecular and cellular cascade leading to replacement fibrosis, is a major area of interest for both novel regenerative therapies and imaging assays.[10,11] Those may play a role in infiltrative cardiomyopathies, but also in other cardiovascular diseases in the future.[12] Accordingly, this review focuses on novel imaging targets related to inflammation and fibrosis and their emerging role in cardiomyopathy.

THE ROLE OF INFLAMMATION AND FIBROSIS IN HEART FAILURE PROGRESSION

Inflammation is the primary infiltrative substrate in cardiac sarcoidosis, where it forms the typical non-caseating granuloma, consisting of epitheloid cells, giant

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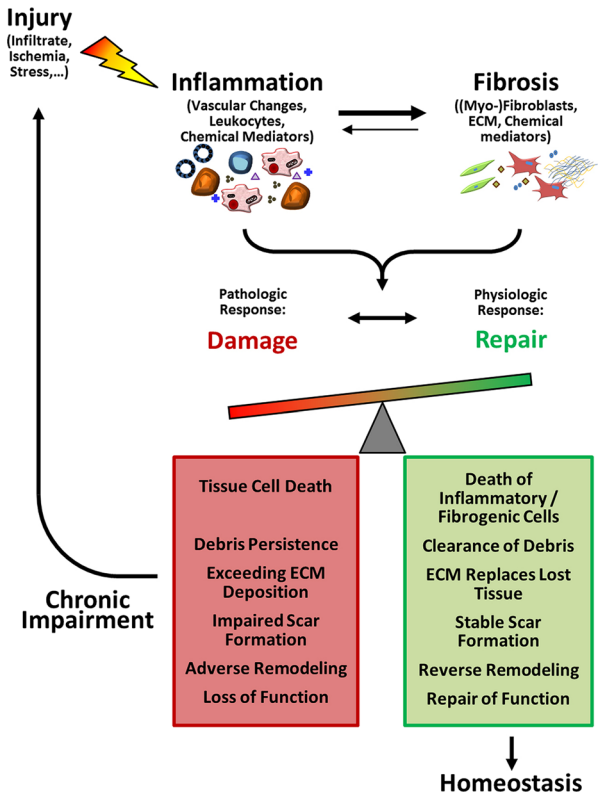

Figure 1. Crosstalk between inflammation and fibrosis and relationship with functional outcome. Tissue injury triggers an inflammatory response which in turn stimulates fibrosis. The interplay must be balanced to achieve repair and regeneration, or it may be imbalanced and contribute to further damage by stimulating a vicious circle of impairment. *ECM*, extracellular matrix.

cells, lymphocytes and plasma cells.[4] Immune cells also infiltrate the myocardium in response to infective (viral) myocarditis or in autoimmune myocarditis as part of the primary disease process.[13] Depending on duration and severity of the disease (and success of therapy), the disease-host interaction may develop toward spontaneous resolution of inflammation without loss of function, or toward progressive replacement fibrosis and scarring with subsequent restriction and functional impairment.

However, aside from direct infiltration by inflammatory cells or infectious pathogens as the cause of cardiomyopathy, it should be noted that inflammation is a general reaction to any kind of tissue injury, which is needed for replacement of damaged cells and adequate repair. If the inflammatory response to tissue injury is inadequate, then it may contribute to further aggravation of damage and adverse outcome.[14] After myocardial infarction, e.g., an inflammatory response can be observed (and identified by imaging) in the infarct region within hours to days after the acute event.[10,15] If this response is inadequate (exaggerated or suppressed), it is thought to contribute to ventricular remodeling and adverse outcome.[14,16] Accordingly, post-infarct inflammation has emerged as a therapeutic target, with the goal of achieving early balance in order to maintain homeostasis and successful repair with limited damage.[17]

A similar hypothesis exists for inflammation in heart failure and nonischemic cardiomyopathy, where the damage of myocytes by any injury mechanisms triggers a molecular inflammation pattern which in turn may contribute to sustained dysfunction and further damage.[18,19] Therefore, inflammation may be an attractive target for molecular imaging not just in inflammatory infiltrative cardiomyopathy, but also in other infiltrative cardiomyopathies, where it may be related to progression and outcome and become a target for drug therapy.[20]

Myocardial fibrosis is closely linked with inflammation. It also reflects a consequence of different cardiac pathologies and their associated remodeling processes. The most prominent cause is ischemia and infarction, but it also plays a key role in infiltrative cardiomyopathy and almost all other nonischemic cardiomyopathies.[21] Fibrosis is triggered by inflammation,[17] but also by other factors such as mechanical stress, and it is characterized by an excessive formation of extracellular matrix (ECM). Under physiologic conditions, the formation of ECM aims at stabilization of structure and prevention of loss of integrity. However, excessive ECM deposition leads to stiffness and impairment of myocardial function and ultimately supports heart failure progression.

In summary, the pathogenetic origin of any cardiomyopathy causes injury, which will trigger an inflammatory response to the tissue damage, which leads to tissue fibrosis with the primary goal of adequate repair. If repair cannot be achieved—due to persistence of the cause of injury, or due to inadequate repair mechanisms (which include inflammation and fibrosis pathways)—then a chronic condition will emerge, with a perpetuous vicious circle of further aggravation (Figure 1). This defines inflammation and fibrosis pathways as potentially attractive targets for specific molecular intervention aiming at improved repair—which in turn makes them attractive targets for molecular imaging (Table 1).

IMAGING OF INFLAMMATION

Apart from sarcoidosis as a primary inflammatory disease, the detection of active immune cell infiltrates in the failing heart may generally provide insight into the progression of cardiomyopathy and the response to anti-inflammatory, but also other heart failure therapies.[22]

Table 1. Opportunities for novel tracers in cardiomyopathy

Target mechanism	Disease target	Promise
Inflammation (various cellular and molecular components, e.g., neutrophils, M1 vs M2 macrophages, T-cells, cytokines, chemokines, …)	Sarcoidosis	Identify cardiac involvement more specifically by circumventing problems of insufficient myocyte FDG uptake suppression
		Determine disease activity, predict response to anti-inflammatory therapy
		Monitor success of anti-inflammatory therapy
	Myocarditis	Detect disease and determine disease activity more specifically than FDG
	Heart failure (general)	Determine activity of proinflammatory mechanisms leading to adverse outcome (using specific tracers)
		Determine activity of reparative, anti-inflammatory mechanisms (using specific tracers)
		Select candidates for novel anti-inflammatory therapies by defining individual pro- vs anti-inflammatory condition
		Monitor effect of novel anti-inflammatory therapies
Fibrosis (myofibroblasts, activating factors, collagen)	Infiltration (Sarcoid, amyloid, myocarditis)	Determine likelihood of irreversible damage by infiltration and subsequent heart failure progression
	Heart failure (general)	Determine severity of profibrotic environment as predictor of disease progression
		Select candidates for novel antifibrotic and anti-inflammatory therapies by defining individual inflammation/fibrosis crosstalk
		Monitor effect of novel targeted therapies

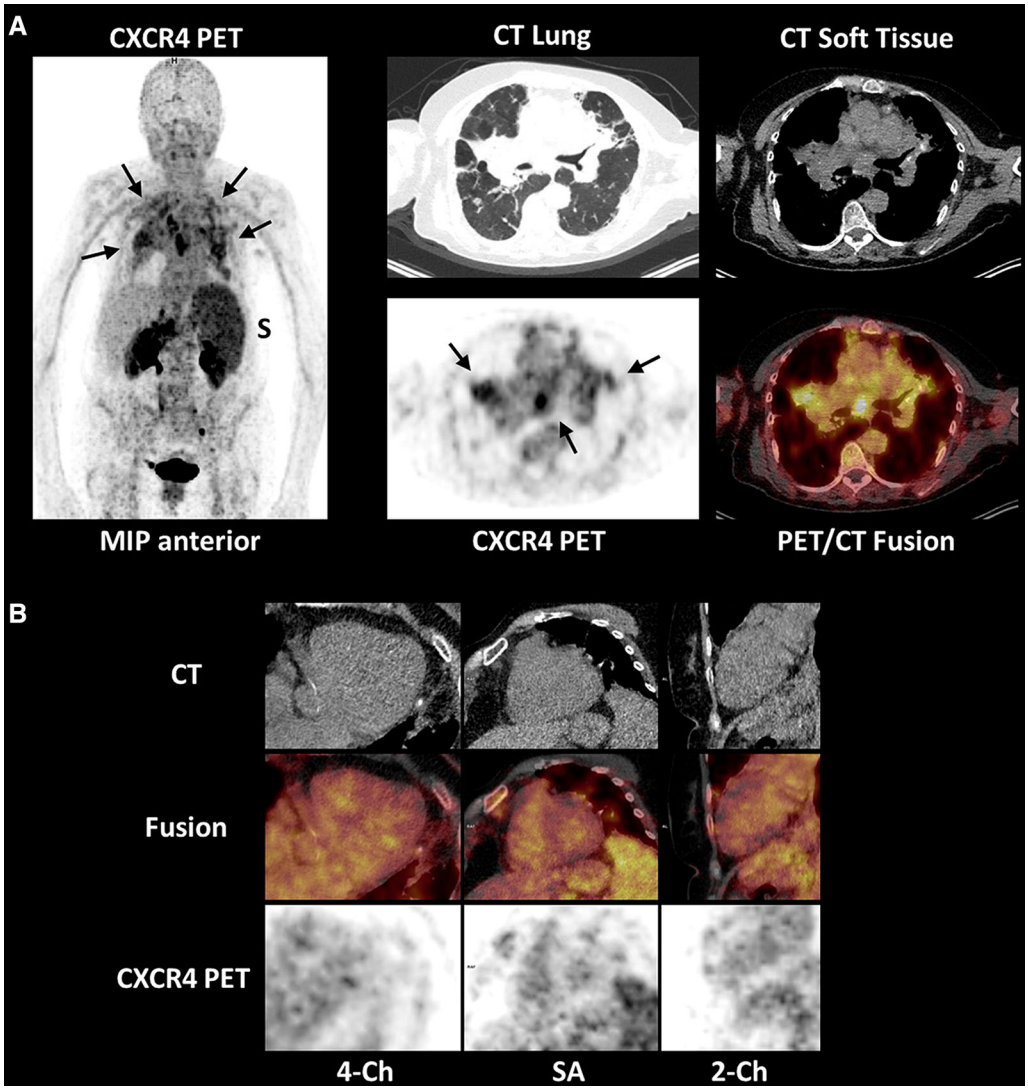

Figure 2. Clinical case study imaged with a new inflammation-specific tracer. Shown are whole-body and cardiac PET/CT images using the chemokine receptor CXCR4-targeted tracer, ^{68}Ga-pentixafor, in a patient with newly diagnosed sarcoidosis, confirmed by hilar lymph node biopsy. **A** Whole-body images show clear uptake in multiple lung lesions and mediastinal lymph nodes (arrows), along with enlarged and active spleen (S), consistent with the presence of CXCR4-positive leukocytes and thus with active systemic inflammation. **B** Cardiac images show lack of background activity and definite absence of focal lesions, ruling out the presence of cardiac involvement. *CT*, computed tomography; *PET*, positron emission tomography; *CXCR4*, CX-motive chemokine receptor type 4; *MIP*, maximum intensity projection; *4-Ch*, four-chamber view; *SA*, short axis view; *2-Ch*, dual-chamber view.

This is best investigated in the setting of early post-infarct myocardial inflammation, where experimental and clinical works have shown that the severity of inflammation in the infarct region can be determined by imaging and is predictive of subsequent remodeling and adverse outcome.[15,16] The situation from an imaging point of view is more challenging in nonischemic cardiomyopathies because the myocardial inflammation signal may be less intense and more diffuse.

So far, immune cell imaging has mostly relied on the enhanced glucose metabolism exhibited by activated inflammatory cells. Neutrophils and proinflammatory M1-like macrophages display high glucose metabolism and accumulate ^{18}F-FDG, which allows PET-based detection of an inflammatory infiltrate in sarcoidosis, and of inflammation within the infarct territory after myocardial infarction—if cardiomyocyte uptake is effectively suppressed.[10,15,16] Especially in a failing

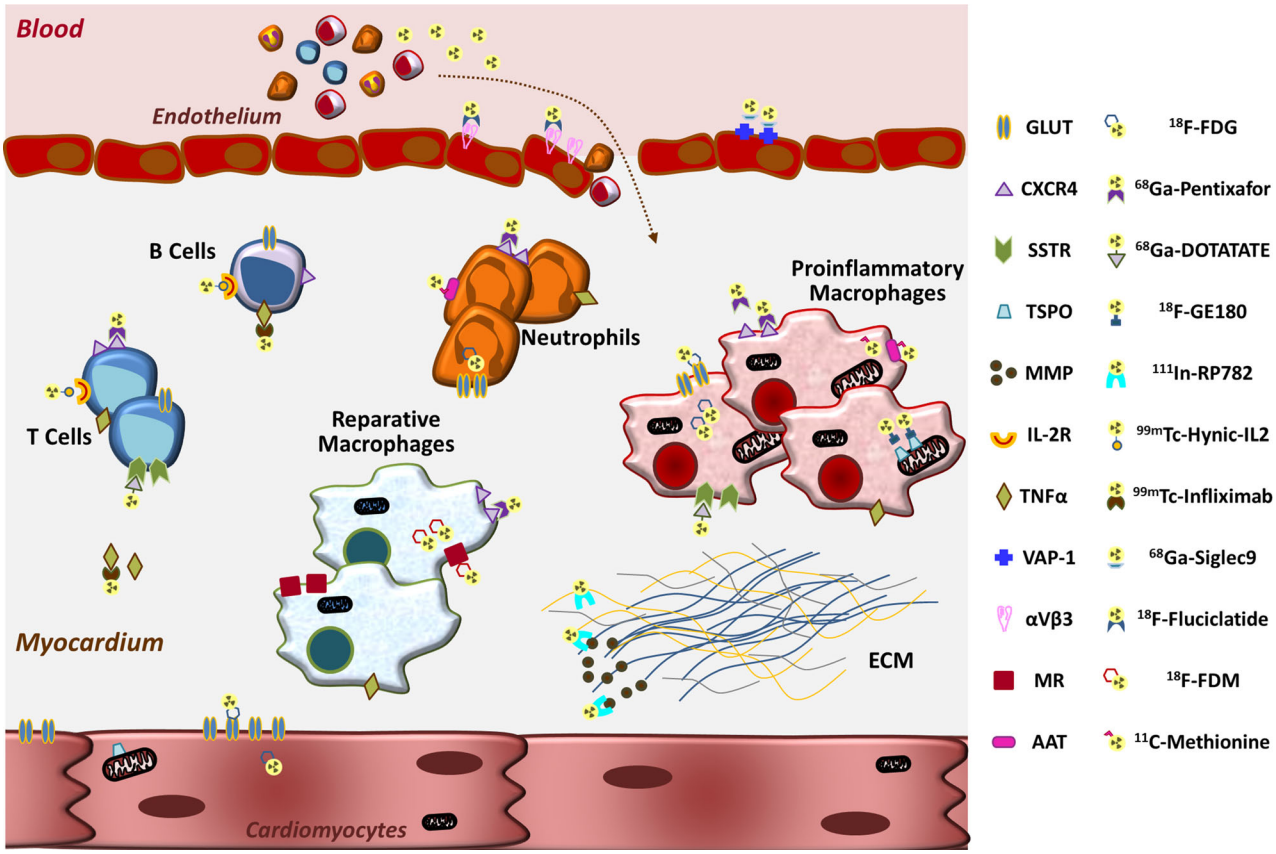

Figure 3. Cardiovascular inflammation imaging targets. Leukocyte subpopulations enter inflamed region and express various target molecules, which can be detected by specific tracers. *GLUT*, glucose transporter; *FDG*, fluorodeoxyglucose; *CXCR4*, CX-motive chemokine receptor type 4; *SSTR*, somatostatin receptor; *TSPO*, translocator protein; *MMP*, matrix metalloproteinase; *IL-2R*, interleukin 2-receptor; *IL2*, interleukin-2; *TNFα*, tumor necrosis factor alpha; *VAP-1*, vascular adhesion protein 1; *αVβ3*, alphaVbeta3 integrin; *MR*, mannose receptor; *FDM*, fluorodeoxymannose; *AAT*, amino acid transporter (all other abbreviations are drug names).

heart, however, interpretation of FDG images for inflammation may be complicated by heart failure-associated alterations in myocardial substrate metabolism which may lead to poorly suppressible myocytes, contributing to the total tracer signal.[23,24]

Hence, to circumvent the nonspecific components of the signal, radiotracers with higher leukocyte specificity and limited or no cardiomyocyte background signal are desirable.[22] A broad leukocyte signal may be achieved by direct labeling of exogenous white blood cells, as used in infection imaging with ^{111}In-oxine or ^{99m}Tc-HMPAO.[25] However, this approach requires rapid homing of the reinjected leukocytes to the target myocardial region. It does not identify tissue-resident inflammatory cells or cells activated or attracted from other sources within the body. As opposed to focal infection, the diffuse signal associated with general myocardial tissue inflammation in heart failure may thus be too weak for reliable identification by radiolabeled leukocytes, necessitating other tracer-based approaches.

Targeting of amino acid metabolism, on the other hand, may be a potential strategy: The radiolabeled amino acid ^{11}C-methionine, which is clinically used for tumor imaging, has been shown to be taken up by neutrophils and proinflammatory macrophages, with only limited uptake in normal and dysfunctional cardiomyocytes.[26,27] It identifies post-infarct myocardial inflammation and may also show uptake in sarcoidosis lesions.[28] However, the availability of ^{11}C-labeled tracers is limited and inhibits broader clinical application.

More recent studies have investigated other specific targets which are found on leukocyte populations, and introduced novel tracers for clinical application. Imaging of the CX-motive chemokine receptor type 4 (CXCR4) using ^{68}Ga-pentixafor can identify a range

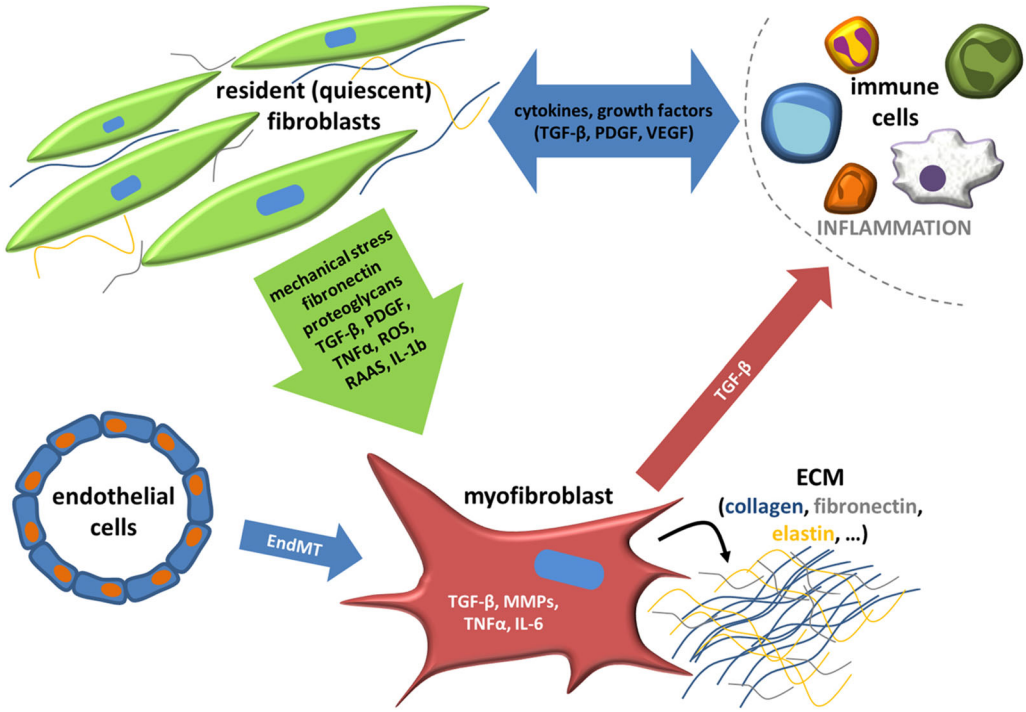

Figure 4. Biochemical and cellular processes involved in cardiac fibrosis. Resident fibroblasts differentiate into myofibroblasts, which secrete extra cellular matrix (ECM, collagen, fibronectin etc.). Immune cells, endothelial cells, and excessive ECM deposit further sustain the fibrogenetic process. *TGF-β*, transforming growths factor beta; *TNFα*, tumor necrosis factor alpha; *ROS*, reactive oxygen species; *RAAS*, renin-angiotensin-aldosterone system; *PDGF*, platelet-derived growth factor; *VEGF*, vascular endothelial growth factor; *IL*, interleukin; *MMP*, matrix metalloproteinase; *EndMT*, endothelial to mesenchymal transition.

of bone marrow-derived cells, with a signal that has been validated versus ex vivo leukocyte counts in heart tissue.[29] CXCR4 is an attractive target for imaging because it can also be blocked by small molecule inhibitors which may be introduced as novel therapy.[30] So far, ^{68}Ga-pentixafor has been successfully used for clinical imaging of post-infarct myocardial inflammation [29,31] and atherosclerosis.[32] Owing to the absence of myocardial background signal, it may also be useful in sarcoidosis or in other cardiomyopathies (Figure 2), although feasibility studies are still missing.

Another target which is thought to be upregulated in activated inflammatory cells is the somatostatin receptor type 2 (SSTR2). Expression profiles suggest that SSTR2 may identify proinflammatory leukocyte subsets,[33] and PET tracers such as ^{68}Ga-Dotatate or ^{68}Ga-Dotatoc are readily available and clinically established for imaging of neuroendocrine tumors. Studies in atherosclerosis have shown superiority over FDG for imaging of active lesions[33] and suggested that the signal can be modified by SSTR2-directed therapy.[34] Also, feasibility for imaging of myocardial inflammation has been suggested in cardiac sarcoidosis,[35] after myocardial infarction and in myocarditis,[36] although the robustness of the signal in those early studies remains debatable. Of note, other studies did not observe practically meaningful imaging signals using SSTR2-targeted tracers in cardiovascular inflammatory conditions.[23,37] Hence, more work is required to determine the practical feasibility of SSTR2-targeted inflammation imaging.

Another interesting and clinically feasible target for inflammation imaging is the 18 kD mitochondrial translocator protein TSPO, which can be detected in humans using various PET tracers that have mostly been applied for neuroinflammation imaging.[38] More recent studies have suggested that TSPO is also a feasible target for imaging inflammation outside of the brain, including arthritis [39] but also atherosclerosis or vasculitis.[40,41] In heart failure, however, myocardial uptake of this mitochondrial tracer may be upregulated without signs of inflammation, probably owing to mitochondrial dysfunction.[42] This by itself may be an interesting imaging signal to identify disease activity, but it is not specific for inflammatory infiltrates anymore.

There are various additional tracers of mechanisms involved in inflammation, such as matrix metalloproteinases, interleukin-2 receptor, tumor necrosis factor alpha, vascular adhesion protein-1, or mannose receptor, which are in different stages of preclinical and early clinical development. Yet, they all have in common that they have either not yet reached human application or that they have not yet been used for cardiovascular inflammation imaging. Figure 3 provides an overview over available inflammation imaging targets and respective tracers.

What does it take to get those new tracers of inflammation to the clinics? In addition to the typical challenges for a molecular imaging agent related to tracer stability and target specificity, as well as strength and robustness of the imaging signal, there is also a need for a more precise characterization of the quality of the biologic signal. The process of inflammation includes a broad spectrum of leukocyte subpopulations and signaling molecules. Some of those components are proinflammatory and, if excessively upregulated, may contribute to adverse outcome. Yet others may be reparative and anti-inflammatory and thus contribute to a good outcome.[12] The specificity and selectivity of a tracer for those components of 'good' and 'bad' inflammation needs to be understood in order to define their practical value. The need for a more differentiated view of inflammation, where not all inflammation is the same, is further emphasized by the failure of early trials of anti-inflammatory drug therapies in heart failure.[18,20] The resultant requirement of a more detailed analysis of the individual inflammatory condition gives support for molecular imaging with well characterized inflammation tracers, which may aid in optimizing therapy development and application in the future by personalizing the individual treatment strategy.

IMAGING OF FIBROSIS

An overview of the biochemical mechanisms leading to tissue fibrosis is shown in Figure 4. Initially, resident (quiescent) cardiac fibroblasts are activated by a trigger such as an injury, (mechanical) stress, growth factors, or profibrotic cytokines.[21] Activated fibroblasts themselves express profibrotic and -inflammatory factors, and differentiate into ECM-producing activated myofibroblasts. This differentiation is supported by TGF-β (transforming growth factor beta). Additionally, TGF-β stimulates activated myofibroblasts to further produce excessive collagen and fibronectin. Importantly, resident cardiac fibroblasts are not the only source of ECM-producing myofibroblasts. Other resident and infiltrating cells may transform into activated myofibroblasts, e.g., endothelial cells, by endothelial to mesenchymal transition.[43]

It is important to note that fibrosis itself is not an irreversible process, and several approaches of antifibrotic therapies are under investigation.[44,45] For monitoring, identification, and measuring the biologic activity of fibrosis, circulating biomarkers have been investigated, of which many are informative and potentially useful.[46,47] However, circulating biomarkers reliably reflecting the exact status of cardiac fibrosis have not yet been found. Molecular imaging of the different biochemical processes involved in cardiac fibrosis has the potential to assess the fibrogenesis as well as the ECM burden regionally and organ-specific on the myocardial tissue level.

Myofibroblasts are the major source of ECM, and thus they constitute a primary target for cardiac fibrosis imaging. Van den Borne and colleagues introduced a bimodal arginine-glycine-aspartate (RGD)-based peptide targeting integrin αVβ3 on the surface of myofibroblasts.[48] In a mouse model of MI, they were able to detect αVβ3-expressing myofibroblasts noninvasively using near-infrared optical imaging and SPECT. However, at later time points (>12 M) post MI, the integrin αVβ3 was no longer detectable, while fibrosis with large amounts of collagen (ECM) was still present. In addition, target specificity of αVβ3 integrin may be problematic, because it is overexpressed not only on myofibroblasts but also on activated endothelial cells where ti may be a marker of angiogenesis.

Another valuable target in fibrosis is collagen, the major component of ECM. Several collagen-targeting peptides have been discovered and radiolabeled for their use as imaging agents for fibrosis via PET and SPECT.[49–53] Of note, most of the investigated collagen-binding derivatives are collagelin analogs, but like collagelin itself, they all exhibit low affinities for the target, which may compromise feasibility for imaging.[51–53] However, it has been shown experimentally for some candidates that they specifically bind to collagen and detect collagen in fibrotic tissue in vivo. The ^{68}Ga-labeled [^{68}Ga]Ga-NO2A-collagelin, e.g., showed very low nonspecific background in crucial organs such as liver, heart and lungs, and ideal pharmacokinetics,[52,53] but it has not proven yet its ability to visualize collagen in fibrotic tissue in vivo.

Further promising targets for fibrosis imaging include matrix metaloproteinases (MMPs) and other integrins. Apart from their role in inflammation, several MMPs and their inhibitors are actively involved in fibrogenesis, and furthermore, MMPs are responsible for ECM degradation, the reverse mechanism. A specific probe for the detection of increased MMP-2 and -14 activities has, e.g., been developed, and it has shown

potential to selectively detect cardiac fibrosis.[54] Also, activated cardiac fibroblasts express various integrins, e.g., the integrin αVβ1 which is involved in the central process of TGF-β activation.[55] Similarly, the αVβ6 integrin is highly expressed on activated fibroblasts, and small integrin αVβ6 targeting molecules have been introduced for fibrosis imaging,[56] but have been tested outside of cardiac imaging only so far.

Thus, while first steps toward molecular imaging of cardiac fibrosis have been initiated, this field is wide and open, and still awaits further exploration. Discovering suitable targets for molecular imaging in cardiac fibrosis, holds promise because—unlike the relatively nonspecific fibrosis signal derived from CT- or CMR contrast agent enhancement—it may help to guide targeted antifibrotic therapy at an early stage where the fibrotic process is biologically active and reversible.

SUMMARY

The increasing penetration of targeted therapies in infiltrative cardiomyopathy provides a major opportunity for growth and clinical acceptance of targeted myocardial molecular imaging approaches. Realization of the future vision of image-guided regenerative therapy will require careful matching of imaging assays with therapeutic interventions, at best through a common molecular target. This will require expansion of the current armamentarium of molecular imaging agents, which may include, but should not remain limited to tracers that have their origin in other areas of molecular medicine, such as neurology and oncology. Inflammation and fibrosis are specifically attractive target areas for both imaging as well as therapy, with broad implications in cardiomyopathy but also in other cardiovascular diseases. Accordingly, future work in these areas holds strong potential to support growth of biologically targeted cardiovascular medicine.

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Disclosure

Frank M. Bengel and Tobias L. Ross do not have any conflicts of interest to disclose.

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