Molecular imaging of ventricular remodeling

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INTRODUCTION

Cardiovascular diseases remain the number one cause of morbidity and mortality, both in the Western world and developing countries and in men and women alike.[1] In 2005, the main cause of death in the USA due to disease of the heart alone was more than of all neoplastic disease combined. It is expected that these numbers will continue to increase in the coming decades due to escalating proportions of obesity and the aging population. In addition to cardiac disease, cerebrovascular disease, diabetes, and hypertension result in substantial morbidity and mortality. Therefore, cardiovascular diseases as a whole are killer number one in the Western world, and will most likely remain to be so due to adverse lifestyle changes, including unhealthy diets and lack of exercise.

Myocardial infarction (MI) is the number one cardiac disease and often strikes the individual unexpectedly; in 50% of cases MI is the first symptom of coronary atherosclerosis. Atherosclerosis is characterized by a chronic inflammatory response resulting in the formation of multiple plaques in the lumen of the artery. This can happen gradually as a result of progressive plaque growth or suddenly as a result of plaque rupture and, subsequently, thrombosis causing acute MI (AMI).

The improvements in treatment of AMI have resulted in better survival and a decrease in the acute complications of MI, such as acute congestive heart failure (CHF), myocardial rupture, arrhythmias, and conduction system disorders. However, with more patients surviving the initial stage of AMI, the development of late complications of AMI become a more prominent health care problem.

The development of left ventricular dilatation and loss of pump function in the years following the acute myocardial injury, induced by the formation of the scar, and the impact of the local loss of function on pressure and tensile forces in the noninfarcted left ventricle, are the subject of intense research. Many trials in this area have convincingly shown that inhibition of the renin–angiotensin system, through either ACE inhibitors and/or angiotensin receptor 1 blockers, preserve cardiac function and decrease mortality post MI.[2] In addition, intervention in mineralocorticoid signaling has proven to preserve cardiac function and decrease mortality significantly.[3,4] Despite these advances, still a substantial fraction, about one-third of AMI patients, will develop pump function disorders of the left ventricle in the long run. The outcome of a relatively recent biomarker known as NT-pro Brain Natriuretic Peptide (NT pro-BNP), which is released by cardiomyocytes after the occurrence of ventricular malfunction, has proven its usefulness in diagnosing heart failure.[5] Nevertheless, it is hard to predict in which individual patient CHF will occur. Therefore, there is still a lot to be learned and to be gained from research in cardiac infarct healing and adverse left ventricular remodeling.

An upcoming diagnostic tool in analyzing the risk of cardiovascular disease in patients is cardiac imaging.[6] The capability to visualize macroscopic cardiovascular structures and the anatomical and functional consequences of cardiac diseases in patients has made a remarkable progression in the last decades. The development of coronary angiography (CAG), echocardiography, magnetic resonance imaging (MRI), and multi-detector CT (MDCT) has improved our approach in diagnosing cardiovascular disease such as atherosclerosis or left ventricular function. However, most of these imaging technologies are able to diagnose the end stage of the disease, rather than the beginning of the disease or even pre-disease states. The next frontier in imaging will be the development of the capability to image fundamental biological or molecular changes which cause cardiovascular disease and are able to predict disease outcome at an early stage. For this purpose, imaging tools other than those mentioned above have to be developed.[7]

Imaging techniques, which visualize the fundamental biological characteristics resulting in cardiovascular disease, may provide the potential to predict cardiovascular catastrophe as an early diagnostic tool.[8] With the introduction of molecular imaging, the opportunities to detect changes in biology of infarcted hearts and, therefore,

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cardiac remodeling in vivo have increased significantly in the past decade.

It can be hypothesized that the ability to visualize interstitial processes on a molecular level, which precede the geometric and functional deterioration of the left ventricle, should help to better predict the likelihood and rate of remodeling and development of HF.[9] Several key biological features provide attractive targets for molecular imaging in heart failure. First, the development of imaging techniques visualizing the biological events of angiogenesis and fibrogenesis, which are considered key processes in myocardial scar formation and left ventricular remodeling, may provide attractive targets for the identification of patients at risk to develop a failing heart. The use of agents targeted to the integrin alpha v beta 3 (avB3) may offer diagnostic means to achieve this goal.

Second, apoptosis, a form of programmed cell death (PCD), has shown to play a key role in the process of gradual loss of pump function in animal models of heart failure, caused by different triggers. Therefore, imaging apoptosis may provide a means to identify hearts that are in the process of substantial cardiomyocyte loss and subsequent loss of pump function. The use of radiolabeled Annexin A5, which shows strong affinity to apoptotic cells which have externalized phosphatidyl serine, may provide an opportunity to develop such a heart failure imaging diagnostic test.

Finally, it is well known that the activation of the renin–angiotensin axis plays a pivotal role in the development of heart failure post AMI. Therefore, imaging of the different components of renin–angiotensin neurohumoral axis may give an additional diagnostic tool for better identification of patients prone to develop heart failure. This brief review will discuss the recent progresses made in molecular cardiac imaging, focusing mainly on the achievements made in avB3 imaging, apoptosis imaging, and imaging of the activation of the renin–angiotensin system.

AVB3 IMAGING AS A TOOL TO IDENTIFY POST MI REMODELING

As described above, early diagnosis of the impact of the infarct on adverse left ventricular remodeling in the individual patient could prevent worsening of left ventricular pump function by adapting treatment to the individual needs and risks. Two main events that occur in the infarcted area are angiogenesis and collagen deposition. The formation of new blood vessels is crucial for infarct healing, and is induced by several factors, such as basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF).[10] Among other regulators of angiogenesis, the avB3 integrin has been identified as a critical angiogenic modulator. Angiogenic vessels display increased expression of this critical integrin.[11] This discovery led to the development of imaging tools to detect tumor angiogenesis with the use of avB3 integrin targeting agents.[12,13]

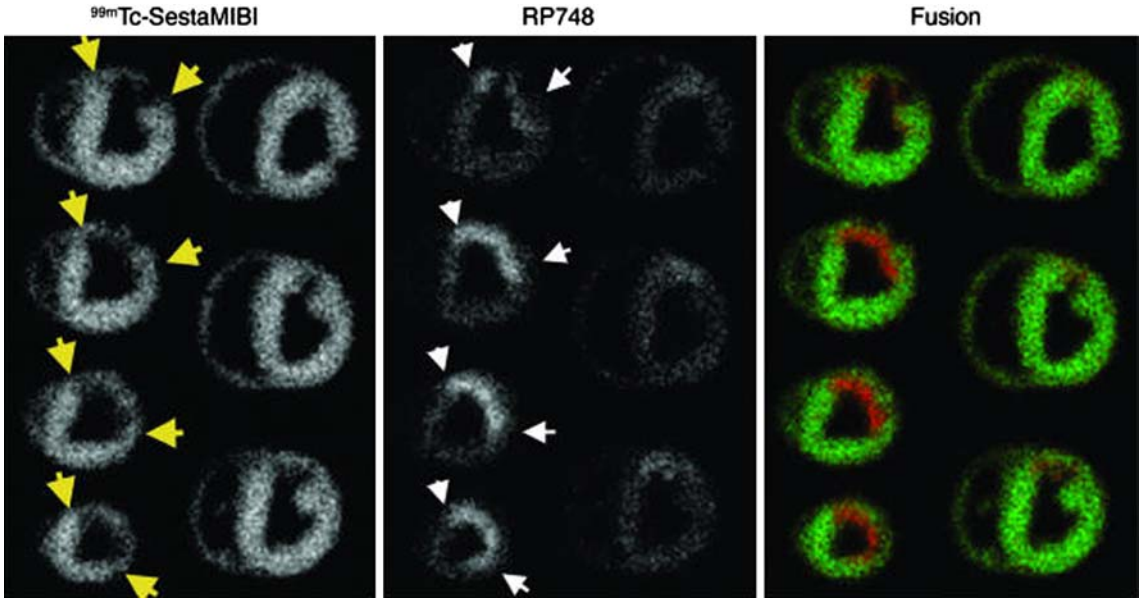

Figure 1. Short axis imaging data 3 weeks after induction of LAD infarction in a dog model. At the site of the perfusion defect (*left panel*), uptake of the indium-labeled avB3 RP 748 targeting tracer was observed (*middle panel*). *Left panel* shows the fusion data.

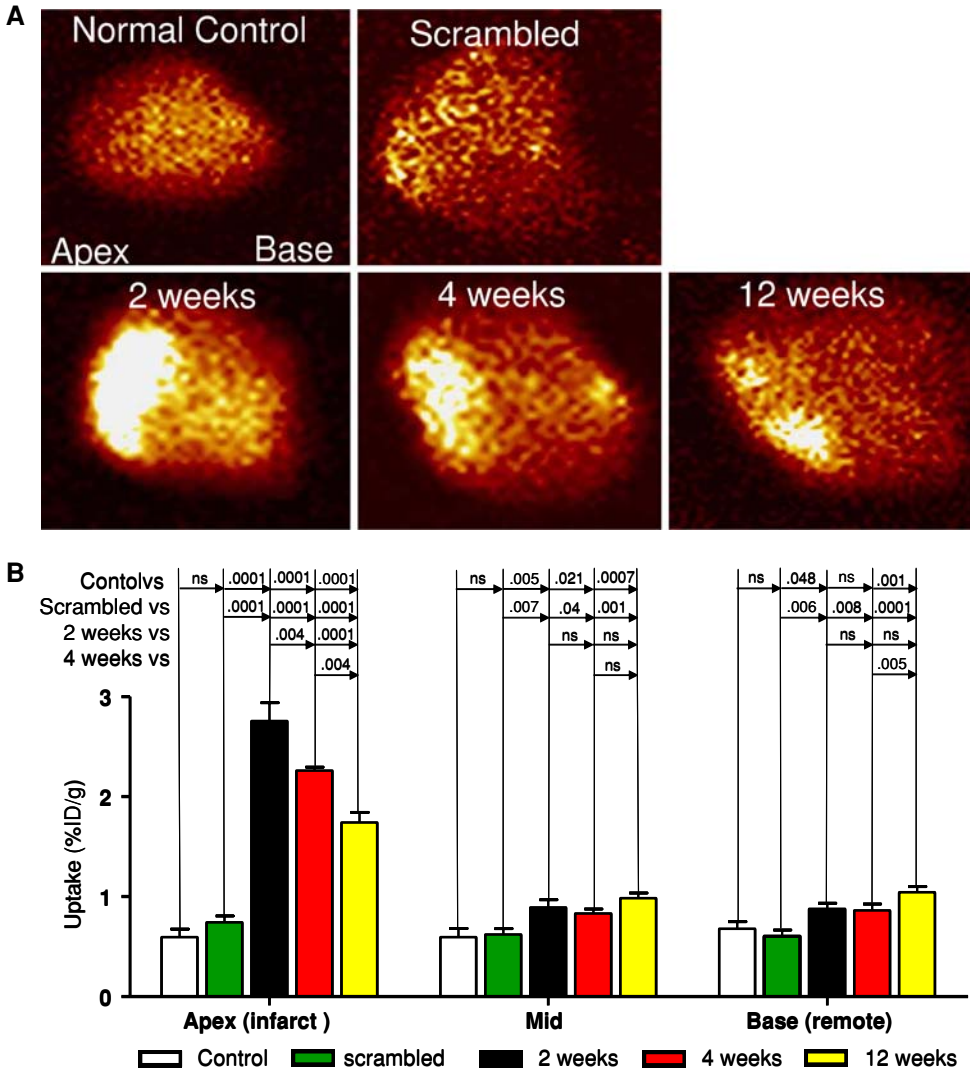

Figure 2. **A**, Nuclear imaging showed that no uptake was seen of the avB3 targeted tracer was seen in normal controls and in mice with infarcted hearts injected with a scrambled variant of the targeting peptide. Uptake of the tracer peaked at 2 weeks post MI, and decreased gradually at 4 weeks and 12 weeks, respectively. **B**, The in vivo imaging data are confirmed by quantitative measurement (% injected dose per gram) in the infarct zone (*left panel*). In the mid sections and base sections no significant uptake of the tracer was observed.

In addition, imaging of avB3 integrin has been used to noninvasively visualize angiogenesis in infarcted hearts. Meoli et al used indium-111-RP748 (^{111}In-RP748), a radiolabeled avB3 targeted compound, to image angiogenesis in infarcted canine hearts with SPECT imaging.[14] ^{111}In-RP748 uptake peaked at 1 week after reperfusion and was associated with avB3 integrin expression on endothelial and vascular smooth muscle cells. In addition, they used the same targeting agent in infarcted dog hearts which showed uptake of ^{111}In-RP748 at the side of the perfusion defect using SPECT imaging (Figure 1).

Recently, ^{18}F-Galacto-RGD, a PET tracer targeting specifically avB3 integrin expression, has been introduced.[15] Higuchi et al used this tracer to monitor avB3 integrin expression in rat hearts after ischemia/reperfusion, and reported results comparable with ^{111}In-RP748 imaging.[16] Clinical use of the ^{18}F-Galacto-RGD PET tracer in a patient with MI was reported shortly.[17]

Apart from its crucial involvement in angiogenesis, avB3 integrin also has been linked to the development of fibrosis,[18,19] which led our group to evaluate whether avB3 integrin imaging could be used to visualize

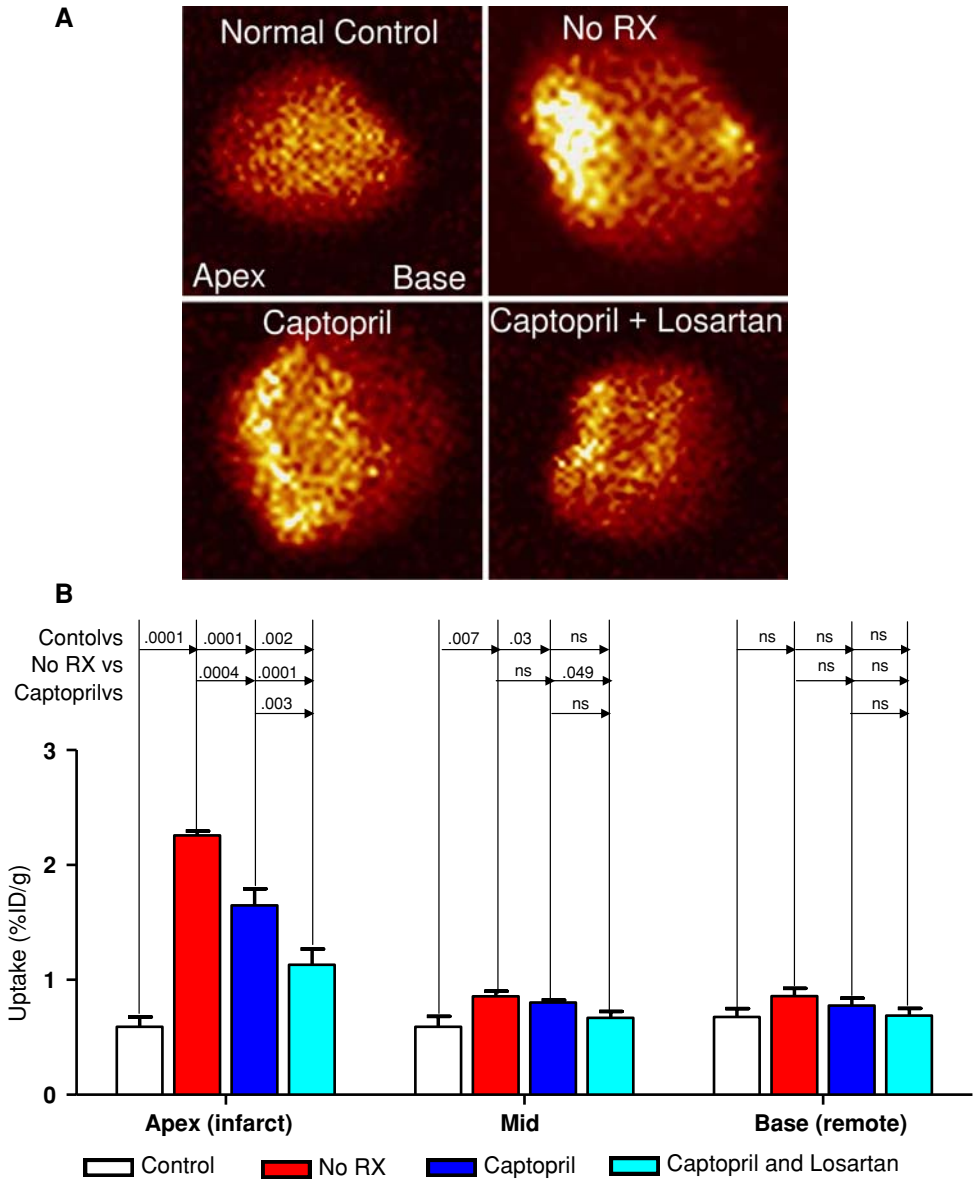

Figure 3. **A**, The effect of treatment with captopril and/or losartan post MI. In the treated groups, less uptake of the avB3 targeting tracer was observed using captopril and an even more extensive reduction when the treatment of captopril was combined with losartan. **B**, The imaging data were confirmed by quantitative measurement (% injected dose per gram).

fibrogenesis in the infarcted heart.[20] MI was induced in mice, which received a ^{99m}Tc-labeled Cy5.5-RGD imaging peptide (CRIP) intravenously for microSPECT imaging of integrin at 2, 4, and 12 weeks after MI (Figure 2).

The uptake of CRIP was most pronounced in the infarcted area, and peaked at 2 weeks after MI. Immunological analysis linked CRIP uptake to myofibroblasts, and CRIP uptake paralleled with the production of newly formed collagen, with a yellow/green birefringence. In addition, several mice were treated with antagonists of the renin–angiotensin system, which is known to be involved in pathological myocardial remodeling. Interestingly, mice treated with captopril or with captopril and losartan displayed significantly reduced uptake of the CRIP (Figure 3). Thus, apart from the potential value of radiolabeled CRIP in visualizing collagen formation, it may also fulfill the need for a surrogate endpoint marker for therapeutic interventions.

In conclusion, imaging of avB3 integrin has the potential to identify HF-prone patients early after MI,

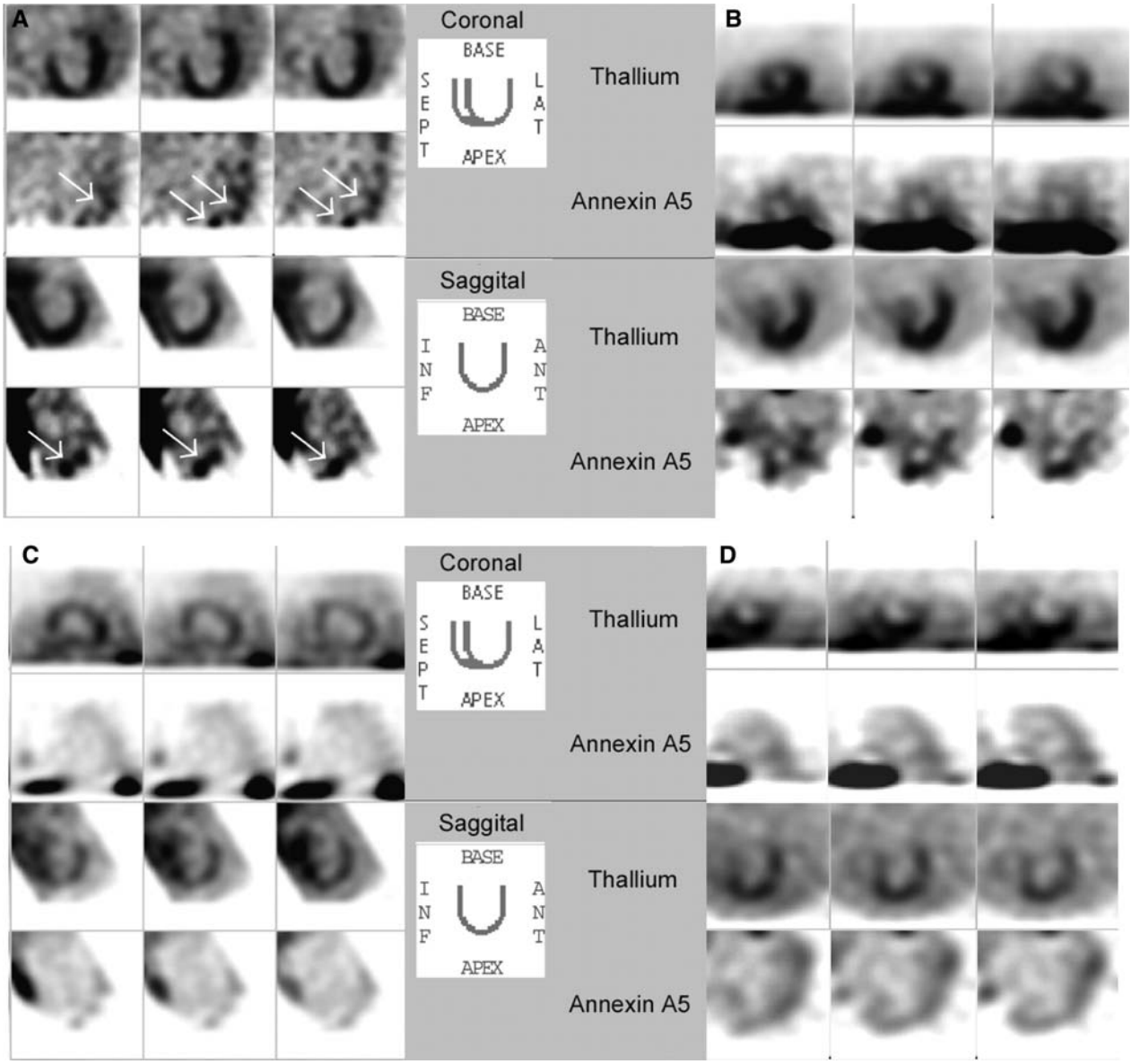

Figure 4. Uptake of ^{99m}Tc-labeled Annexin A5 in advanced cardiomyopathy. **A**, Patient with dilated cardiomyopathy (DCMP) and reduced left ventricular function showing focal uptake in the anterolateral region of the heart. **B**, Patient with DCMP in acute heart failure showing global uptake. **C**, DCMP in a patient with no symptoms of heart failure, notice no uptake is present. In **D**, relative of DCMP patient, absence of uptake of ^{99m}Tc-labeled Annexin A5 (adapted from Kietselaer et al[30]).

and thereby will help to optimize medical treatment. However, prospective clinical trials are needed to investigate whether the potential of integrin imaging translate into clinically useful diagnostic tests.

APOPTOSIS AS A TARGET FOR MOLECULAR IMAGING IN CHF

Apoptosis plays a key role in the process of degradation of cardiomyocytes resulting in ventricular dysfunction.[21-24] Research in animal models of CHF has demonstrated that interference in apoptosis pathways delays the ongoing process of pump function disorders resulting in heart failure.[25]

One of the main biochemical characteristics of apoptosis is caspase 3 activation. The process of activation of caspase 3, an apoptosis-related cysteine protease, begins with the release of cytochrome C into the cytoplasmic area, mainly caused by oxidative stress and cytokinemia. Caspases have numerous substrates, including contractile proteins, such as troponin-T. In addition, in most cells, caspase activation results in

activation of DNA fragmentation enzymes. However, it is thought that the activation of DNA fragmentation enzymes in heart muscle cells is compensated for by different antiapoptotic pathways. The consequence of apoptosis activation in cardiomyocytes is that these cells become dysfunctional, but still survive due to the preservation of DNA. This means that restoration of a healthy environment could potentially restore individual cardiomyocyte function. Another consequence of caspase 3 activation is that it results in alterations in phospholipid distribution in the sarcolemmal lipid bilayer, causing revelation of phosphatidyl serine (PS) to the surface of the cell membrane.[26,27] Theoretically, the extent of PS externalization reveals an indication of the degree of apoptosis in the heart. Accordingly, it is plausible that PS can be a used as a target to detect activated cell death in heart failure. The detection of PS exposure has been proven extensively by radionuclide imaging using ^{99m}Tc-labeled Annexin A5.[28,29]

Kietselaer et al recently demonstrated the feasibility of Annexin A5 imaging to reveal PCD in a small group of patients with recently diagnosed advanced heart failure. Imaging of ^{99m}Tc-labeled Annexin A5 was performed using a SPECT in nine CHF patients with advanced nonischemic cardiomyopathy (hypertrophic, $N = 1$, dilated, $N = 8$). To include a similar genetic background as the hypertrophic cardiomyopathy patient, the same imaging procedure was performed on two relatives who did not suffer from CHF. Left ventricular uptake of Annexin 5 was demonstrated focal, multifocal, or diffuse in a total of five patients.[30] All patients in whom ^{99m}Tc-labeled Annexin A5 uptake was present in the left ventricle were diagnosed with a significant decrease of left ventricular function as demonstrated by follow-up echocardiography 1 year later (Figure 4).

The five patients who demonstrated no uptake of Annexin A5 remained in a stable clinical state or even showed improvement of left ventricular function (Figure 5). In line with the data in animal models of CHF, these data suggest that Annexin A5 uptake is related to dysfunction of the left ventricle. Annexin A5 may provide the potential to become a useful imaging tool to identify patients with active apoptosis in the myocardium, which most likely indicates a transition to overt heart failure. The question remains what Annexin A5 binding into the myocardium indicates, since it was suggested that cardiomyocytes activate caspases during apoptosis, but that DNAses are not activated, resulting in the concept of apoptosis interruptus.

Figure 6 shows a possible explanation for uptake of ^{99m}Tc-labeled Annexin A5 after apoptosis is stimulated. The revelation of PS is a response of caspase 3 activation. As mentioned above, different antiapoptotic

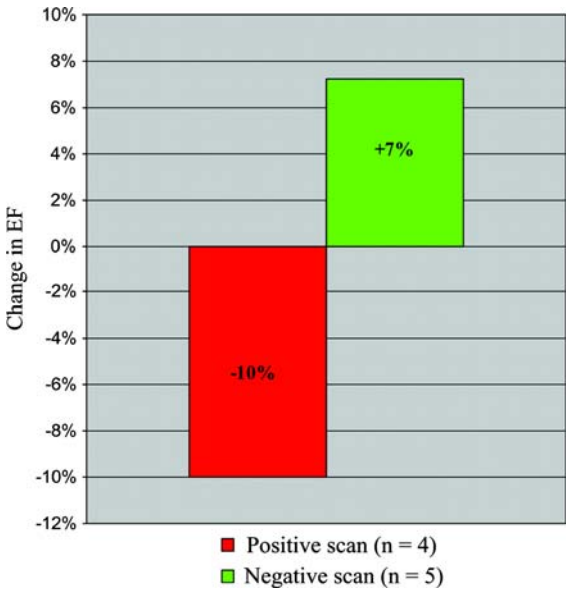

Figure 5. Differences in left ventricular ejection fraction after 1 year follow-up in CDMP patients with uptake of Annexin A5 and no uptake of Annexin A5 on SPECT (adapted from Kietselaer et al[30].

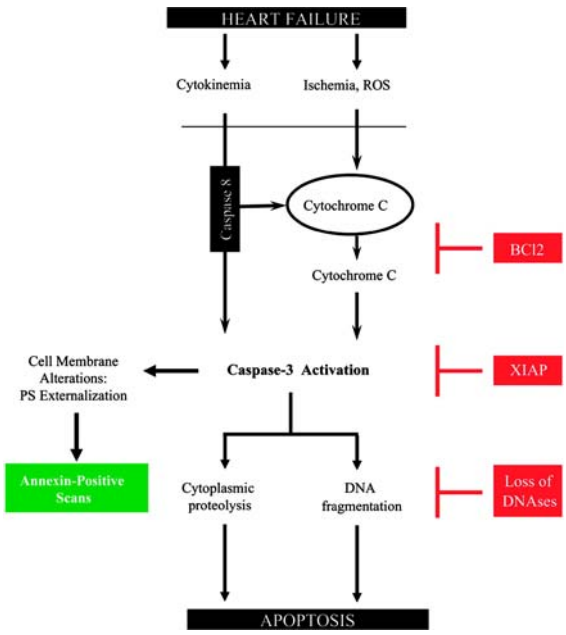

Figure 6. The concept of PS externalization in heart failure. Cytokine and oxidative stress (reactive oxygen species) inducing release of cytochrome c out of the mitochondria lead to caspase 3 activation. This results in cytoplasmic proteolysis and DNA fragmentation, ultimately leading to apoptosis. The increase of BC12- and XIAP proteins and the loss of DNAses prevent the so-called apoptosis interruptus and activate caspase 3.

pathways, preventing the degradation of DNA, compensate for the activation of DNA fragmentation enzymes in heart muscle cells. It is assumed that the quantity of PS, which manifests externally, is an indication of the amount of activated of caspase 3. Therefore, it is believed that Annexin 5 uptake not necessarily indicates loss of cardiomyocytes, but is a reflection of the activation level of caspase-3.

THE AT-1 RECEPTOR AS A TARGET FOR MOLECULAR IMAGING IN CHF

The essential role of the renin–angiotensin system in ventricular remodeling following AMI has been established in many experimental and clinical studies. Rather than circulating renin–angiotensin levels, it believed that myocardial upregulation of angiotensin converting enzyme, angiotensin II, and its receptors determine the likelihood of ventricular remodeling.[31,32] In an experimental study in mice, it was shown that transgenic mice with deficient angiotensin II type 1 receptor expression revealed negligible remodeling post MI. Moreover, a lower expression of fibrosis and transforming growth factor (TGF-B1) was demonstrated.[31] These insights from experimental models have been translated to clinical studies showing that patients with CHF patients using angiotensin receptor blockers[33,34] and/or ACE-inhibitors[35-37] have substantially improved survival. It has been suggested that maximization of antiangiotensin therapy, including increase in ACE-inhibitor dose or addition of ARB over ACE-inhibitor therapy, could further reduce morbidity[33,38] and mortality[39] in HF. It is, therefore, imaginable that accurate assessment of myocardial angiotensin receptor expression could potentially guide optimization of antiangiotensin therapy. Nowadays, diagnostic imaging of HF is focused on geometric and structural cardiac imaging.[40,41]

As mentioned above, it is well known that inhibition of the renin–angiotensin system, through either ACE inhibitors and/or angiotensin receptor 1 blockers, preserve cardiac function and decrease mortality post MI.[2] Verjans et al investigated the potential of angiotensin receptor II type 1 imaging in an animal model of post MI left ventricular remodeling.[42] A fluorescent label was marked to an angiotensin peptide analogue (APA), which traced angiotensin type 1 and 2 receptors for imaging purposes. The data from the study showed distinct uptake of the fluorescent tracer in the infarct and border zone of the mouse hearts between 1 and 6 weeks post MI (Figure 7).

At time point of 12 weeks, the uptake was markedly reduced. Immunohistochemical analysis and 2-photon microscopy showed co-localization of the tracer with both myofibroblasts and collagen. No uptake of the fluorescent tracer was observed in cardiomyocytes. Upregulation of the AT1 receptor on myofibroblasts allows for growth factor (such as angiotensin-II)-induced proliferation and collagen production, which is believed to contribute to healing and the remodeling process following MI.[43,44]

The same research group created a radiolabeled imaging tracer of a well-known angiotensin II antagonist named Losartan. With the use of this analogue, SPECT-CT imaging was performed after left ventricular function was evaluated by echocardiography. These imaging studies showed that uptake of the radiolabeled Losartan product co-localized to the infarct area on SPECT-CT imaging, and that the uptake of the radiolabeled product in the infarct area was 2.4-fold higher as compared to control hearts (Figure 8). Together, these data demonstrate the feasibility of in vivo imaging through targeting of angiotensin receptors in an experimental HF model.

Furthermore, the data showed that upregulation of angiotensin receptor preceded the development of left ventricular remodeling, as detected by echocardiography. Currently, significant emphasis is being placed on the recognition of stage A and B HF patients as a strategy of prevention of more advanced HF.[45] Accordingly, development of a technology that predicts occurrence of cardiac remodeling, such as AT-1 receptor imaging, is of crucial importance, especially since the clinical practice nowadays allows diagnosis of HF only after the left ventricle has undergone adverse remodeling.

This concept is further emphasized by the recent demonstration of another strategy for imaging renin–angiotensin axis with the use of radiolabeled benzoyl lisinopril. Dilsizian et al incubated short-axis myocardial slices explanted from patients undergoing cardiac transplantation for end-stage ischemic cardiomyopathy with F-18 fluoro-benzoyl lisinopril.[46] There was specific binding of radiotracer to ACE; mean binding was 6.6 ± 5.2 compared with 3.4 ± 2.5 luminescence/mm^2 in segments pre-incubated with cold lisinopril ($P < 0.0001$). Furthermore, mean radiotracer binding was 6.3 ± 4.5 in infarcted, 7.6 ± 4.7 in peri-infarcted, and 5.0 ± 1.0 luminescence/mm^2 remote noninfarcted ($P < 0.02$) segments. Together, these imaging studies demonstrate that activation of the renin–angiotensin system can be visualized using molecular imaging technology. Both studies also showed that the components of the tissue renin–angiotensin cascade are upregulated only about 2- to 3-fold. It remains unclear whether such relatively small difference between remodeling cardiac tissue and control hearts could provide a clinically robust diagnostic strategy for imaging targeted to ATR and/or ACE.

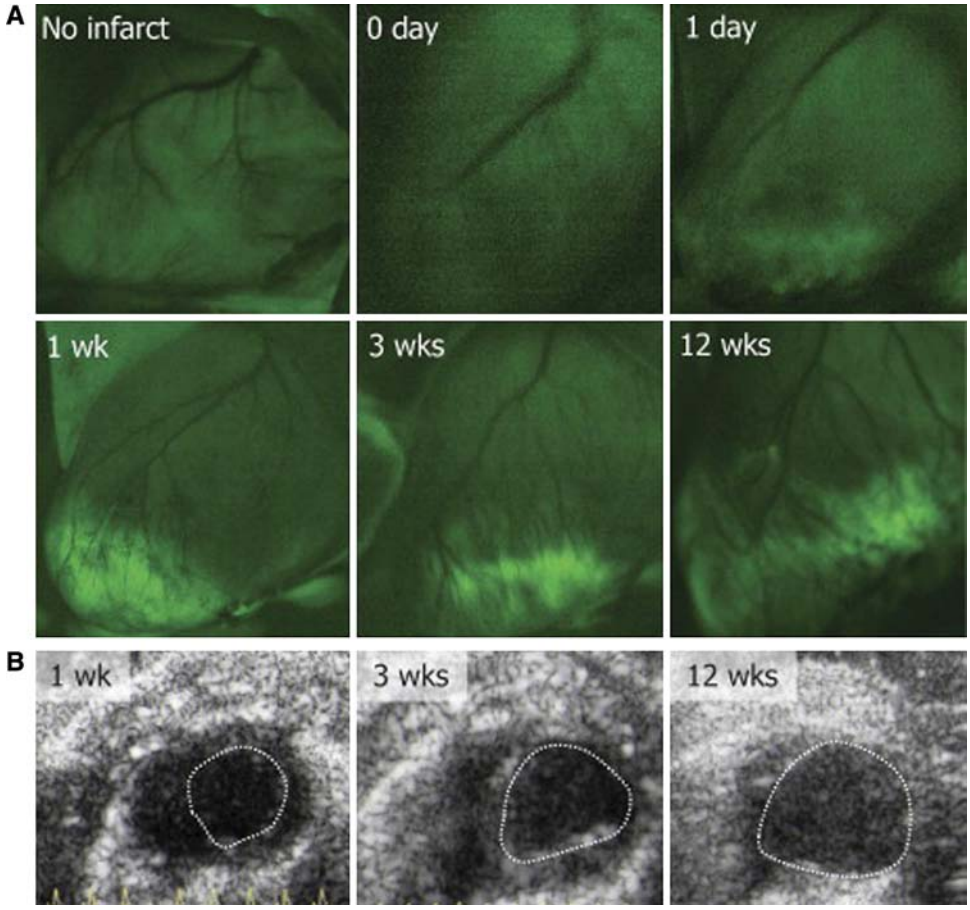

Figure 7. A, Imaging data show the uptake of the fluorescent AT1 receptor-targeted tracer at different time points after MI. The uptake peaks at 1 week post AMI, and then gradually decreases over time. In **B**, the concomitant echocardiographic data are shown, indicating a clear increase in LV end diastolic diameter.

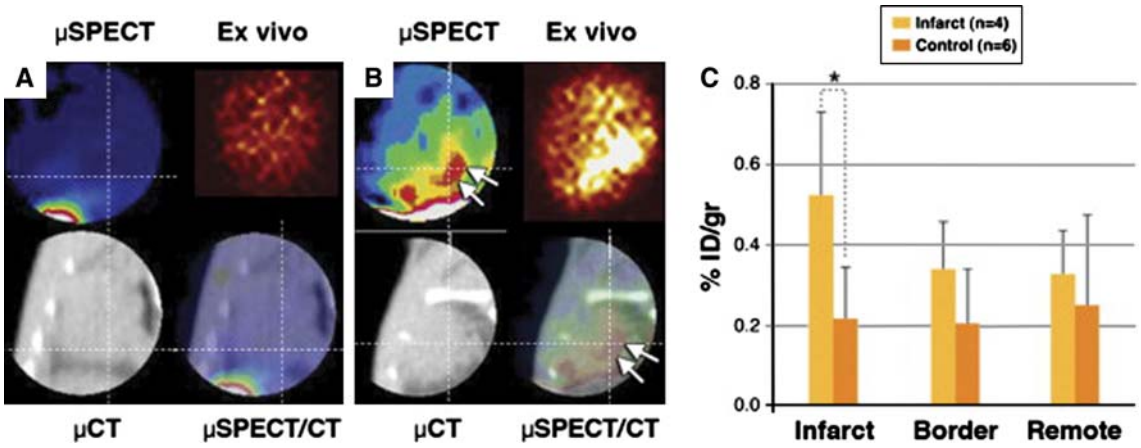

Figure 8. In **A**, the control mouse does not show uptake of the technetium-labeled losartan product on SPECT-CT imaging. In **B**, in a mouse 3 week post MI clear uptake of the tracer can be seen on SPECT-CT imaging. In **C**, quantitative analysis (% injected dose per gram) shows a 2.4-fold uptake of the tracer in the infarct area. In the border zone and remote zone no significant differences were seen.

OUTLOOK

Based on the preclinical and clinical data obtained in molecular imaging of adverse left ventricular remodeling, the current outlook for the development of such an imaging technology is promising. For both the imaging of avB3 integrin and imaging of phosphatidyl serine expression as a reflection of caspase 3 activation, the preliminary data provide a sufficient basis for the design and execution of novel studies. For imaging of the components of the renin–angiotensin system, the extent of uptake may be insufficient to form a basis for clinical applications. However, before one of these technologies could be adopted as diagnostic tests for routine clinical use, large clinical studies need to be conducted to address key questions. For instance, it is still unknown whether the uptake of avB3 targeting tracers is robust enough to uncover patients that are at the brink of developing adverse remodeling post MI. In addition, it remains to be seen whether the extent of the uptake of the tracer can be modulated by treatment with different regimes of CHF treating compounds. Studies focused on addressing these questions are under way.

For imaging phosphatidyl serine exposure in the heart using Annexin A5, as a reflection of caspase-3 activation, the outlook depends largely on the availability of clinically graded Annexin A5 imaging diagnostic kits. With the availability of clinical Annexin A5 imaging kits, studies could be designed to further explore the predictive value of Annexin A5 in patients with failing hearts and to study the effect of therapeutic interventions.

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