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Total number of authors:
13

Published in:
Journal of Nuclear Cardiology

Link to article, DOI:
10.1007/s12350-023-03265-9

Publication date:
2023

Document Version
Publisher's PDF, also known as Version of record

Link back to DTU Orbit

Citation (APA):

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[\textsuperscript{68}Ga]Ga-NODAGA-E[(cRGDyK)\textsubscript{2}] angiogenesis PET following myocardial infarction in an experimental rat model predicts cardiac functional parameters and development of heart failure

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Received Jul 20, 2022; accepted Mar 11, 2023
doi:10.1007/s12350-023-03265-9

Background. Angiogenesis has increasingly been a target for imaging and treatment over the last decade. The integrin \(\alpha\textsubscript{v}\beta\textsubscript{3}\) is highly expressed in cells during angiogenesis and are therefore a promising target for imaging. In this study, we aimed to investigate the PET tracer [\textsuperscript{68}Ga]Ga-RGD as a marker of angiogenesis following MI and its ability to predict cardiac functional parameters.

Methods. First, the real-time interaction between [\textsuperscript{68}Ga]Ga-RGD and integrin \(\alpha\textsubscript{v}\beta\textsubscript{3}\) was investigated using surface plasmon resonance (SPR). Second, an animal study was performed to investigate the [\textsuperscript{68}Ga]Ga-RGD uptake in the infarcted area after one and four weeks following MI in a rat model (MI = 68, sham surgery = 36). Finally, the specificity of the [\textsuperscript{68}Ga]Ga-RGD tracer was evaluated ex vivo using histology, autoradiography, gamma counting and flow cytometry.

Results. SPR showed that [\textsuperscript{68}Ga]Ga-RGD has a high affinity for integrin \(\alpha\textsubscript{v}\beta\textsubscript{3}\), forming a strong and stable binding. PET/CT showed a significantly higher uptake of [\textsuperscript{68}Ga]Ga-RGD in the infarcted area compared to sham one week \((p < 0.001)\) and four weeks \((p < 0.001)\) after MI. The uptake of [\textsuperscript{68}Ga]Ga-RGD after one week correlated to end diastolic volume \((r = 0.74,\)
p < 0.001) and ejection fraction (r = − 0.71, p < 0.001) after four weeks.

**Conclusion.** This study demonstrates that $[^{68}\text{Ga}]\text{Ga-RGD}$ has a high affinity for integrin $\alpha_v\beta_3$, which enables the evaluation of angiogenesis and remodeling. The $[^{68}\text{Ga}]\text{Ga-RGD}$ uptake after one week indicates that $[^{68}\text{Ga}]\text{Ga-RGD}$ may be used as an early predictor of cardiac functional parameters and possible development of heart failure after MI. These encouraging data supports the clinical translation and future use in MI patients. (J Nucl Cardiol 2023;30:2073–84.)

**Key Words:** Myocardial biology • coronary artery disease • myocardial ischemia and infarction • positron emission tomography

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**INTRODUCTION**

Adverse cardiac remodeling after myocardial infarction (MI) is a process of structural and functional changes which could lead to heart failure. Remodeling of the myocardium occurs as a response to inadequate repair after an MI.1–3 The repair of the myocardium after MI consists of three phases: the acute, the proliferation and scar maturation phase. During the proliferation phase, endothelial cells proliferate and infiltrate the infarcted area, leading to the formation of a dense microvascular network, which supplies oxygen and nutrients to the infarcted area.4,5 This process is known as angiogenesis and is essential to repair after MI. The third phase is the maturation phase, in which most of the myofibroblasts transition to a phenotype that promotes scar maturation.3,7 Angiogenesis peaks seven days after MI and slowly decreases over the next 14 to 28 days.8 Since angiogenesis is vital to repair, the targeting and stimulation of angiogenesis has been of clinical interest for many years.

To develop and optimize treatment that promotes angiogenesis, it is crucial to establish a non-invasive method for the real-time monitoring of angiogenesis. Positron emission tomography (PET) is a modality with high sensitivity and acceptable resolution that enables continuous in vivo monitoring of angiogenesis in human subjects. The integrin $\alpha_v\beta_3$ has been extensively studied and the highest expression of this integrin has been found in activated endothelial cells undergoing angiogenesis.9–11 The integrin $\alpha_v\beta_3$ is expressed at low levels in normal healthy tissue like intestinal, vascular and smooth muscle cells.12 Other cell types with expression of integrin $\alpha_v\beta_3$ is bone resorbing osteoclasts, activated macrophages, angiogenic endothelial cells and migrating smooth muscle cells.13 The tripeptide motif Arg-Gly-Asp (RGD) is a specific ligand to $\alpha_v\beta_3$ and has been the major peptide used in the molecular imaging of $\alpha_v\beta_3$, since it is recognized by the $\alpha$-subunit of the integrin14,15 on the endothelial cell. Several different RGD-based PET tracers have previously been used for imaging integrin, primarily in animal studies. The tracers differ in characteristics like linkers, chelators, radionuclides.16–18.

The aim of this study was to investigate the emerging PET radiotracer $[^{68}\text{Ga}]\text{Ga-NODAGA-E[(cRGDyK)]}_2$ ($[^{68}\text{Ga}]\text{Ga-RGD}$) angiogenesis PET following myocardial September/October 2023

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**METHODS**

**Study design**

**Binding kinetics** The assessment of real-time biomolecular interaction was performed using a Biacore X100 (Biacore, Uppsala, Sweden), with the determination of binding kinetics of $[^{68}\text{Ga}]\text{Ga-RGD}$ and integrin...
To evaluate the assay development and results, vitronectin and fibronectin was used as comparison.

**Ethical statement** The Danish Animal Experiments Inspectorate approved experimental protocols (Permit No. 2016-15-0201-00920). All animal procedures performed are in accordance with the guidelines in Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes.

**Animal experiment** 104 animals were included and underwent open chest surgery. The rats were randomly assigned to either sham or LAD ligation to induce an experimental myocardial infarction in a 1:3 ratio. One week after operation, the surviving rats (n = 88) were PET/CT scanned with 2-deoxy-2-[18F]fluoroo-D-glucose (2-[18F]FDG) and the following day [68Ga]Ga-RGD. After the [68Ga]Ga-RGD scan 52 of the rats were euthanized and the excised heart used for flow cytometry or autoradiography and histology. Four weeks after the operation, a follow-up scan of 2-[18F]FDG and [68Ga]Ga-RGD were performed (n = 36). Following [68Ga]Ga-RGD PET/CT scan the animals were euthanized, and myocardial tissue used for either

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**Figure 1.** Workflow of the study. At first, the rats underwent either sham or permanent LAD occlusion. The rats were PET/CT scanned after 1 week and follow-up scanned 4 weeks after operation. LAD, left anterior descending coronary artery; [68Ga]Ga-RGD, [68Ga]Ga-NODAGA-E[(cRGDyK)]2; PET, positron emission tomography; CT, computed tomography.
flow cytometry or histology and autoradiography (flowchart depicted in Figure 1).

See supplementary for full description of methods.

RESULTS

Integrin αvβ3, SPR experiments by single cycle kinetics

The binding between integrin αvβ3 and [68Ga]Ga-RGD showed a strong and stable interaction in the presence of Mg2+ and an even slower dissociation rate constant in the presence of Mg2+ and Mn2+ (Figure 2).

Integrin αvβ3 is the natural receptor for vitronectin, and SPR showed likewise a strong interaction with vitronectin in the presence of Mg2+ with no change in the presence of Mg2+ and Mn2+ and thereby validate the SPR analyses of integrin αvβ3 and [68Ga]Ga-RGD.

Fibronectin showed steady state interaction with integrin αvβ3. (supplementary figure S1 and table S1).

Animal experiments

Myocardial infarction extent In 1 examination out of the total 124 (0.8%), 2-[18F]FDG-PET were of poor quality and excluded from the dataset (1 week: sham = 1). In the MI group, PET showed a lack of 2-[18F]FDG uptake in the anterolateral wall of the left ventricle corresponding to the infarcted area. As expected, the extent of the defect was higher in the MI group, compared to sham after one week (p < 0.001).

After four weeks, the extent of the defect in the MI group was still larger than sham (p < 0.001). (Table 1).
in vivo $^{68}$Ga-Ga-RGD PET-uptake measurements of $^{68}$Ga-Ga-RGD in %ID/g are shown in Table 1. The intraclass correlation coefficient for %ID/g of the anterior wall (0.84; 95% CI 0.75–0.9) and posterior wall (0.86; CI 95% 0.79–0.91) measurements were good.

One week after total chronic occlusion of the LAD, the MI group showed a significantly higher uptake of $^{68}$Ga-Ga-RGD by in vivo PET, compared to the sham group in the anterior wall of the left ventricle (LV) (Supplementary figure S4). After four weeks, the MI group still showed a significantly higher uptake of the $^{68}$Ga-Ga-RGD in the anterior wall, compared to sham animals. In the non-infarcted posterior LV wall, there was no difference in uptake of $^{68}$Ga-Ga-RGD after one and four weeks, when comparing the MI group with the sham group (Figure 3).

EDV and EF measured from gated 2-$^{18}$F-FDG-PET and correlation to $^{68}$Ga-Ga-RGD ECG-gated 2-$^{18}$F-FDG-PET/CT was used to analyze EDV, ESV and EF. The MI groups had a significantly higher EDV after one week compared to the sham group ($p = 0.015$). After four weeks the EDV in the MI group had increased and was significantly higher than the sham, indicating a progression towards a heart failure phenotype in the MI group (Table 1). The MI group had a significantly lower EF, compared to sham after one week. After four weeks, the difference in EF was still evident. There was no difference in EF from one week to four-week scan between the groups (Table 1). There was a positive correlation between mean $^{68}$Ga-Ga-RGD uptake (%ID/g) in the anterior LV wall after one week and the four weeks end diastolic volume ($r = 0.74, p < 0.001$). A negative correlation was observed between max $^{68}$Ga-Ga-RGD uptake (%ID/g) at one week and EF at four weeks ($r = -0.71, p < 0.001$) (Figure 4).

In multiple regression analysis with EDV after four weeks, only %ID/g is significant in predicting EDV after four weeks (%ID/g $p = 0.005$, LVEF acute $p = 0.305$, FDG extent acute $p = 0.127$).

Table 1. Baseline and follow-up characteristic in PET/CT

<table>
<thead>
<tr>
<th></th>
<th>Infarct</th>
<th>Sham</th>
<th>P</th>
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<tbody>
<tr>
<td>1 week</td>
<td></td>
<td></td>
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<tr>
<td>Infarct N = 55</td>
<td>14.5 ± 1.2</td>
<td>54 ± 1.4</td>
<td>0.15 ± 0.005</td>
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<tr>
<td>Sham N = 33</td>
<td>476 ± 10</td>
<td>148 ± 5</td>
<td>0.09 ± 0.003</td>
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<tr>
<td>[68Ga-Ga-RGD PET]</td>
<td>Left ventricular end-diastolic volume, (ul)</td>
<td>0.09 ± 0.003</td>
<td></td>
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<tr>
<td>[%ID/g anterior wall]</td>
<td>0.09 ± 0.003</td>
<td></td>
<td></td>
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<tr>
<td>[%ID/g posterior wall]</td>
<td>0.09 ± 0.005</td>
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<tr>
<td>[%ID/g blood pool]</td>
<td>0.09 ± 0.005</td>
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<tr>
<td>Target-to-background ratio</td>
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<th>Sham N = 12</th>
<th>P</th>
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<td>Infarct N = 5</td>
<td>18 ± 2</td>
<td>568 ± 13</td>
<td>0.11 ± 0.0005</td>
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<td>Sham N = 3</td>
<td>266 ± 13</td>
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<tr>
<td>[%ID/g]</td>
<td>0.07 ± 0.002</td>
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<tr>
<td>[%ID/g]</td>
<td>0.1 ± 0.003</td>
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<tr>
<td>[%ID/g]</td>
<td>0.2 ± 0.004</td>
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<tr>
<td>Target-to-background ratio</td>
<td>1.5 ± 0.05</td>
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</tbody>
</table>

In vivo $^{68}$Ga-Ga-RGD PET-uptake PET-uptake measurements of $^{68}$Ga-Ga-RGD in %ID/g are shown in Table 1. The intraclass correlation coefficient for %ID/g of the anterior wall (0.84; 95% CI 0.75–0.9) and posterior wall (0.86; CI 95% 0.79–0.91) measurements were good.

Uptake of $^{68}$Ga-Ga-RGD following myocardial infarction correlates with $\alpha_v \beta_3$ expression on endothelial cells. Cellular distribution of $\alpha_v \beta_3$ was evaluated in whole rat hearts by flow cytometry one and four weeks after MI. One week after MI there was an increase in the amount of immune cells and endothelial cells compared to the sham group (p = 0.0006 and 0.018, respectively) which four weeks after MI returned to the cellular composition to the levels of sham treated animals (p > 0.05 for all cell types; Figure 5A). The overall amount of cells positive for $\alpha_v \beta_3$ across cell types increased drastically 1 week after MI (p < 0.0001) and returned to sham levels...
4 weeks after MI ($p > 0.05$; Figure 5B). Besides the changes in cellular composition of the heart and the overall increase in $\alpha_v$ and $\beta_3$, one week after MI, myocytes increased their expression of $\alpha_v$ and $\beta_3$ ($p = 0.0016$ and $< 0.0001$, respectively) while only $\beta_3$ was increased on endothelial cells and immune cells ($p < 0.0001$ and $= 0.0026$, respectively). The increased expression of $\beta_3$ returned to sham levels in all cell types four weeks after MI ($p \geq 0.05$) while $\alpha_v$ had a statistically insignificant trend towards being slightly increased across cell types (Figure 5C).

Correlating the investigated cellular findings to the mean $\%$ID/g $[^{68}\text{Ga}]\text{Ga-RGD}$ uptake revealed the best correlation for $\alpha_v$ expression on endothelial cells ($r = 0.52$, $P = 0.002$) which was less apparent on immune cells and myocytes (Figure 5D) as well as other possible correlations (Supplementary Figure S2).

**Histological analysis of the heart** The presence of an infarcted area in the myocardium was confirmed by histological evaluation of the heart. The infarcted area on HE stains showed regions with a lack of myocytes and many cells with atypical nuclei (Figure 6). This was more profound and transmural at four weeks compared to after one week. MT stains showed collagen accumulation (fibrosis) in the infarcted area, represented as blue staining. This was more delineated at four weeks compared to after one week. When measuring fibrosis density (percentage of total area) on MT-stained cross-sections of the heart (Figure 7 A,B), there was a significantly higher degree of fibrosis in the MI group, compared to sham after one week and after four weeks (sham: 1.1 ± 1.3; one week: 11.7 ± 10; four weeks: 9.1 ± 7.0, $p = 0.047$, Figure 7 D).

Immunohistochemically staining for the integrin $\beta_3$ chain showed a stronger positivity for $\beta_3$ in infarcted tissue compared to healthy tissue (Figure 7 C, red
arrow), with the $\beta_3$ being primarily present in the wall of the vessel.

**$[^{68}\text{Ga]}\text{Ga-RGD uptake after ischemia measured by autoradiography and gamma counting of the heart ex vivo**** For autoradiography analysis, the sham group, showed no focal $[^{68}\text{Ga]}\text{Ga-RGD accumulation in the myocardium, only homogeneous background activity in the myocardium. In the MI group, an increased focal $[^{68}\text{Ga]}\text{Ga-RGD uptake was observed, corresponding to the infarcted area. This was evident both one week and four weeks after MI. Gamma counting showed a significantly higher %ID/g of $[^{68}\text{Ga]}\text{Ga-RGD in the MI group, compared to the sham group. After one week, the mean %ID/g were 0.12 vs 0.068 (p < 0.001) and after four weeks 0.11 vs 0.063 %ID/g (p < 0.001). There was a significant correlation between gamma counting and autoradiography ($r = 0.62, p < 0.001$).

**DISCUSSION**

The major finding of this study is that $[^{68}\text{Ga]}\text{Ga-RGD PET-uptake in the infarcted area correlates with cardiac functional parameters at a later time point. More specifically, we found that the uptake of $[^{68}\text{Ga]}\text{Ga-RGD in the infarcted area after one week correlated to the EDV and EF measured after four weeks. The $[^{68}\text{Ga]}\text{Ga-RGD tracer used was recently developed by our group,19–22 and demonstrated in the present study, a stronger binding to $\alpha_v\beta_3$ than that of its natural ligand vitronectin. We also demonstrated that after a MI, endothelial cells express a higher level of $\alpha_v\beta_3$ after one week, declining after four weeks. The focal uptake of $[^{68}\text{Ga]}\text{Ga-RGD on autoradiography was present in the infarcted area and not in healthy myocardium, correlating to measured $[^{68}\text{Ga]}\text{Ga-RGD counts in the respective slices of the heart. In vivo imaging of angiogenesis using $[^{68}\text{Ga]}\text{Ga-RGD with PET/CT showed a higher uptake of
[68Ga]Ga-RGD in the infarcted area compared to sham after both one and four weeks.

Our study is not the first to image angiogenesis based on integrin $\alpha_v\beta_3$ targeted PET tracer in a rat model of acute MI. However, in this study, we present in vivo and ex vivo data that support that binding between integrin $\alpha_v\beta_3$ and [68Ga]Ga-RGD is better than previously for other RGD targeting PET tracers, e.g., $^{64}$Cu-NOTA-PEG$_4$-cRGD, a cyclic RGD-peptide radiolabelled with $^{64}$Cu for PET imaging. This radiolabelled version has a different chelator (NOTA-PEG$_4$), a different isotope ($^{64}$Cu) and used a cyclic RGD-peptide rather than the dimeric RGD-peptide radiolabelled version used in our study (chelator: NODAGA, isotope: $^{68}$Ga). We previously showed that the dimeric $[^{68}\text{Ga}]\text{Ga-NODAGA-E[(cRGDyK)$_2$]}$ has a higher affinity towards integrin $\alpha_v\beta_3$ than the monomer.$^{23}$ SPR analysis of $^{64}$Cu-NOTA-PEG$_4$-cRGD and integrin $\alpha_v\beta_3$ showed the same KD as we present,$^{16}$ but the dissociation rate is slower between our [68Ga]Ga-RGD and integrin $\alpha_v\beta_3$ than $^{64}$Cu-NOTA-PEG$_4$-cRGD and integrin $\alpha_v\beta_3$. This could indicate a more stable complex between $[^{68}\text{Ga}]\text{Ga-RGD}$ and integrin $\alpha_v\beta_3$ than $^{64}$Cu-NOTA-PEG$_4$-cRGD and integrin $\alpha_v\beta_3$.

SPR has previously been used to assess the binding between integrin $\alpha_v\beta_3$ and RGD.$^{24}$ This study found that the binding between integrin $\alpha_v\beta_3$ and vitronectin was improved with the presence of Mn$^{2+}$. Our SPR analysis...
of vitronectin and integrin $\alpha_\beta_3$ showed the same level of affinity, which confirms the accuracy of our setup. We found that binding of $[^{68}\text{Ga}]\text{Ga-RGD}$ to integrin $\alpha_\beta_3$ was 1000-fold stronger than vitronectin in the presence of Mn$^{2+}$, suggesting that $[^{68}\text{Ga}]\text{Ga-RGD}$ binds specifically and with a stable complex to integrin $\alpha_\beta_3$.

Flow cytometry analysis of the heart showed that the level of $\alpha_\beta_3$ positive cells were significantly higher after one week in the MI group compared to the sham in the myocytes/myofibroblasts, immune cells, and endothelial cells. The literature describes $\alpha_\beta_3$ to be involved with different actions of the different cell types, all which relates to myocardial wound healing, which is activation and migration of endothelial cells, infiltration and anti-inflammatory actions for immune cells, resistance to apoptosis for myocytes, and matrix remodeling for myofibroblasts. The flow cytometry analysis supports the many different actions of $\alpha_\beta_3$, since the levels of positive $\alpha_\beta_3$ cells were present in myocytes, immune cells and endothelial cells. However, the best correlation was between RGD uptake and endothelial cells, which indicates that even though the integrin is present in other cell types relating to myocardial wound healing, the signal detected by RGD PET is mostly derived from angiogenesis.

The percentages of $\alpha_\beta_3$ positive immune cells and endothelial cells both declined from one to four weeks after MI, indicating that the immunomodulation and formation of new blood vessels had declined. The decline in $\alpha_\beta_3$ positive cells by flow cytometry was in our study paralleled by a decline in in vivo uptake of $[^{68}\text{Ga}]\text{Ga-RGD}$.

In vivo PET imaging showed a significant increase in $[^{68}\text{Ga}]\text{Ga-RGD}$ uptake in the infarcted myocardium, compared to non-infarcted myocardium. This was evident both at one and four weeks after MI which correlated best with $\alpha_\beta_3$ expression on endothelial cells, indicating that $[^{68}\text{Ga}]\text{Ga-RGD}$ is a promising PET tracer in detecting angiogenesis and remodeling. The first scan was performed after one week, to ensure that the initial inflammatory response following MI did not affect the $[^{68}\text{Ga}]\text{Ga-RGD}$ uptake. The follow-up scan was done after four weeks, to investigate the potential shift from angiogenesis to remodeling. Several other studies have

![Figure 6. Histological verification of infarction after one and four weeks. The staining for Masson’s trichrome shows a fibrotic area corresponding to the accumulation of $[^{68}\text{Ga}]\text{Ga-RGD}$ pictured with autoradiography. MT: Masson’s Trichrome; HE: Hematoxylin & Eosin; AR: autoradiography.](image)

![Figure 7. Masson’s trichrome staining shows a clear area of fibrosis compared to healthy tissue (A, B). The immunohistochemically staining for integrin $\beta_3$ shows an accumulation in the vessel wall (C). The quantification of fibrotic tissue on MT shows a significant difference between sham and the MI groups (D). The extent of fibrosis is unchanged between week 1 and 4 in the MI group, indicating a stable and consistent ligation of the LAD.](image)
shown angiogenesis imaged with other RGD PET tracers\textsuperscript{17,18,28–30} and three clinical trial has been performed.\textsuperscript{31–33} However, there are some inconsistency towards the conclusions of these studies. Sherif et al. performed a preclinical trial with permanent ligation of the LAD artery and F-galacto-RGD 1 week after MI. In this study a low uptake of RGD was associated with lower EF and higher EDV. Jenkins et al. showed that in 21 patient with ST-elevation myocardial infarction, high uptake of F-fluciclatide RGD was predicting regions of recovery.

It is established that left ventricular dilation is correlated with subsequent heart failure independently of risk factors and EF in humans.\textsuperscript{34} Our findings of a correlation between EDV at four weeks follow-up with \textsuperscript{68}Ga-Ga-RGD uptake one week after MI, indicate that \textsuperscript{68}Ga-Ga-RGD could be used as a non-invasive method to early identify patients at risk of developing dilated cardiomyopathy following a MI. The clinical implications of such a tool may be paramount. While it seems documented in our study that \textsuperscript{68}Ga-Ga-RGD is increased in the presence of MI with high sensitivity and specificity, further studies are needed to confirm the correlation between early uptake of \textsuperscript{68}Ga-Ga-RGD and subsequent adverse remodeling.

One of the great advantages of the \textsuperscript{68}Ga-Ga-RGD tracer used in the present study is the use of a generator produced radionuclide, which circumvents the need of an on-site cyclotron. The production of \textsuperscript{68}Ga-Ga-NODAGA-EI([cRGDyK])\textsubscript{2} is fast, simple, with a high yield, and the PET tracer is stable. Other RGD PET tracers, such as those labeled with \textsuperscript{18}F, need a cyclotron close by.

**STUDY LIMITATIONS**

This study investigated permanent ligation of the LAD as a MI model. To assess the broader applicability of \textsuperscript{68}Ga-Ga-RGD studies in reperfusion models with transient occlusion of the LAD should be conducted in the future. A reperfusion model would be a more clinically relevant and translate better to investigate acute myocardial infarction in humans.

This study was not designed to investigate heart failure following MI. To establish if \textsuperscript{68}Ga-Ga-RGD uptake correlates with heart failure, the animals needed to be observed for a longer period of time.

In this study the functional cardiac parameters were EDV, ESV and EF. To assess the cardiac function in greater detail, magnetic resonance imaging or a perfusion tracer such as \textsuperscript{13}N-NH\textsubscript{3} could be used to evaluate myocardial blood flow and myocardial flow reserve. However, \textsuperscript{13}N-NH\textsubscript{3} requires an on-site cyclotron, making it difficult to examine in a rodent model.\textsuperscript{35}

**CONCLUSION**

This study demonstrates that \textsuperscript{68}Ga-Ga-RGD has a high affinity for integrin $\alpha_v\beta_3$ which enables the evaluation of angiogenesis following an MI, using PET/CT. The in vivo RGD uptake after one week correlated to ejection fraction and end diastolic volume after four weeks, indicating that \textsuperscript{68}Ga-Ga-RGD may be used as an early predictor of cardiac functional parameters and possible development of heart failure after an MI. These encouraging data support a clinical translation.

**NEW KNOWLEDGE GAINED:**

The PET tracer \textsuperscript{68}Ga-Ga-NODAGA-EI([cRGDyK])\textsubscript{2} forms a stable and strong binding to the integrin $\alpha_v\beta_3$, which enable the detection of angiogenesis. The angio-genic response after one week correlated to predictors of early onset of heart failure phenotype.

**Author contributions**

SB participated in conception of the study as well as collection of the data, analysis, drafting the manuscript and final approval of the manuscript. JKJ, CEG, BF, JSM and AC contributed with data collection, analysis and revision of the manuscript. LRP, EC and CC contributed to data analysis and revision of the manuscript. TB, PH, RSR and AK contributed with conception and design of the study as well critical revision of the manuscript and final approval.

**Funding**

Open access funding provided by Royal Danish Library. The authors received funding from the European Union’s Horizon 2020 research and innovation program under grant agreements no. 670261 (ERC Advanced Grant) and 668532 (Click-It), the Lundbeck Foundation, the Novo Nordisk Foundation, the Innovation Fund Denmark, the Danish Cancer Society, Arvid Nilsdon Foundation, the Neye Foundation, the Research Foundation of Rigshospitalet, the Danish National Research Foundation (grant 126), the Research Council of the Capital Region of Denmark, the Danish Health Authority, the John and Birthe Meyer Foundation and Research Council for Independent Research.

**Disclosure**

A.K. is an inventor on patents covering the PET tracer used (EP3706809A1 and US16/762,873). No other potential conflicts of interest relevant to this article exist.
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References


6. van der Laan AM, Piek JJ, van Royen N. Targeting angiogenesis directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.


68Ga-PRGD2 PET/CT for \( \alpha_v \beta_3 \)-integrin imaging of myocardial 
infarction and stroke. Theranostics 2014;4:778-86.
33. Jenkins WSA, Vesey AT, Stirrat C, Connell M, Lucatelli C, Neale 
A, et al. Cardiac \( \alpha_v \beta_3 \) integrin expression following acute 
34. Yeboah J, Bluemke DA, Hundley WG, Rodriguez CJ, Lima JAC, 
Herrington DM. Left ventricular dilation and incident congestive 
heart failure in asymptomatic adults without cardiovascular 
disease: Multi-Ethnic Study of Atherosclerosis (MESA). J Card 
Fail 2014;20:905-11.
35. Ghotbi AA, Clemmensen A, Kyhl K, Follin B, Hasbak P, 
Engstrøm T, et al. Rubidium-82 PET imaging is feasible in a rat 

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