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FAPI-PET in Cardiovascular Disease

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PET probes targeting fibroblasts are frequently used for varying applications in oncology. In recent years, the clinical spectrum has been expanded towards cardiovascular medicine, e.g., after myocardial infarction, in aortic stenosis or as a non-invasive read-out of atherosclerosis. We herein provide a brief overview of the current status of this PET radiotracer in the context of cardiovascular disease, including translational and clinical evidence. In addition, we will also briefly discuss future applications, e.g., the use of fibroblast-targeting PET to investigate bilateral organ function along the cardiorenal axis.

Introduction

While the concept of disturbed coronary artery flow in patients with atherosclerosis and after acute myocardial infarction (AMI) is well understood, myocardial ischemia is accompanied by a complex interaction of (sub) cellular processes.[1] Recent efforts turned towards inflammatory pathways in an acute setting and major clinical trials testing anti-inflammatory medication have led to varying effects on structural parameters and clinical outcome[1-3] or even increased rates of fatal infection.[3] Such inconsistent benefits highlight the urgent need to determine the optimal time-point to initiate anti-inflammatory therapies. In this regard, positron emission tomography (PET) probes deciphering the current inflammatory status in the myocardium may serve as a surrogate marker to determine patients at risk even to identify the optimal time window for therapy on-set (Fig. 1).[4,5] After acute inflammation, however, cardiac repair is characterized by activated fibroblasts, along with aggregation of structural proteins shaping the extracellular matrix (ECM).[6] While this phenomenon can be cardioprotective, overzealous activation triggers an excessive accumulation of those proteins, ultimately leading to fibrosis, stiffness and finally, heart failure.[6,7] Of note, such a remodeling of the ECM matrix is a shared feature not only in individuals suffering from AMI, but also in other cardiovascular diseases, including, but not limited to aortic stenosis (AS), arrhythmogenesis or atherosclerosis.[8-10] Currently emerging fibroblast activation protein (FAPI) molecular imaging probes (Fig. 2), however, can monitor respective fibrotic reprogramming in the injured myocardium,[11] thereby allowing to image late stages of fibrosis.[12] In the present review, a brief overview on the current status of such profibrotic activity-targeting PET radiotracers in cardiovascular disease is provided. We will focus on three selected scenarios, including MI, AS following transcatheter aortic valve replacement (TAVR) and atherosclerosis. In addition, we will discuss the potential of fibroblast-targeting PET biomarkers for image-piloted reparative strategies after primary cardiac damage or even improved bilateral heart-kidney outcome.

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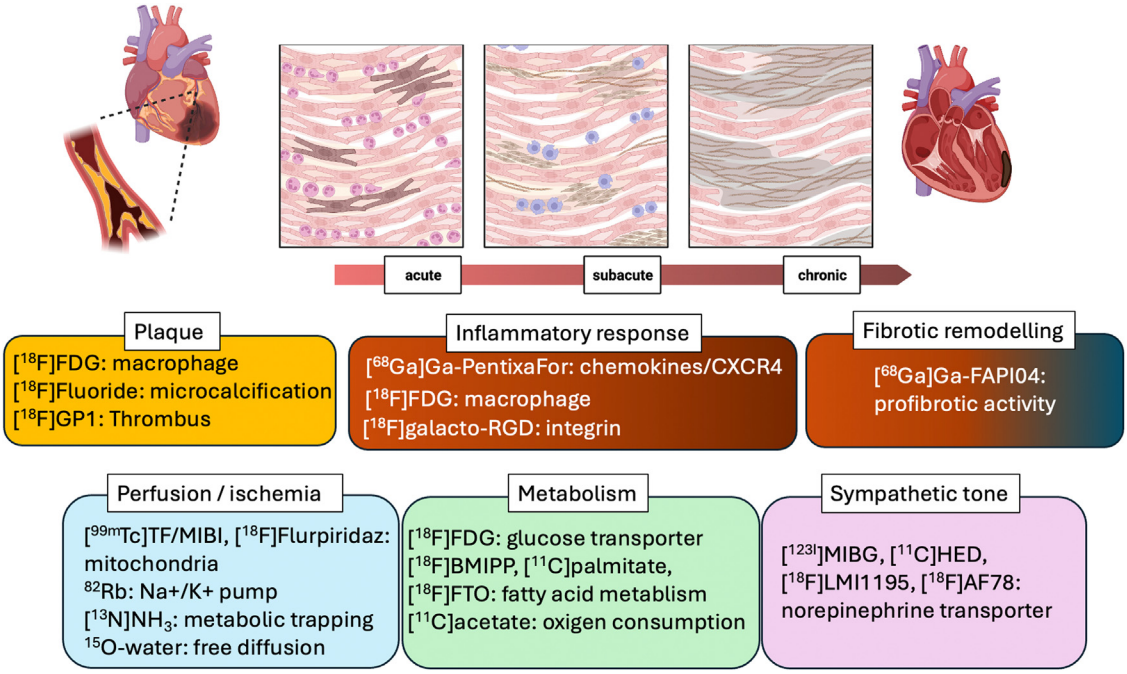

Figure 1 PET probes that can be applied after myocardial infarction. Those radiotracers provide a read-out of the entire pathophysiological cascade after the acute event, including early inflammation, fibrotic healing or remodeling. Created with biorender.com.

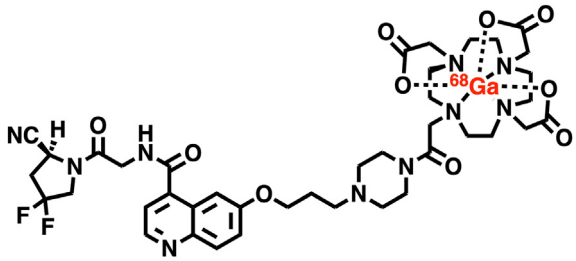

Figure 2 Chemical structure of the PET agent (^{68}Ga)-labeled fibroblast activation protein inhibitor 4 (FAPI-04).

Myocardial Infarction - Translational Evaluation

Varasteh and coworkers were among the first to investigate the ^{68}Gallium-labeled FAP inhibitor 04 ([^{68}Ga]Ga-FAPI04) in rats after MI using a dedicated micro PET/CT. In a longitudinal scan setting, the FAPI-based PET signal peaked in the injured area at day 6 after the acute event (Fig. 3). To confirm specificity, the authors also reported on successful blocking of the signal by co-injecting non-labeled FAPI-04. In an ex-vivo analysis using immunofluorescence staining, Varasteh et al also demonstrated presence of FAP-avid myofibroblasts in the infarcted myocardium. A drawback of this preclinical investigation included open thoracotomy to conduct left coronary artery ligation, which also caused increased radiotracer accumulation due to wound healing.[11] Diekmann et al then also investigated [^{68}Ga]Ga-FAPI04 6 to 11 days after coronary intervention in MI patients and reported on an exceeding fibrotic PET signal relative to the reference perfusion radiotracer. On concomitant cardiac magnetic resonance imaging (cMRI), 50% of segments without late gadolinium enhancement also had increased [^{68}Ga]Ga-FAPI04 uptake, indicated that activation of myofibroblasts also occur in non-infarcted myocardium.[13] This is in line with a (non-PET) pig study, which also reported on interstitial fibrosis in adjacent myocardium after MI.[14] The observed increase on [^{68}Ga]Ga-FAPI04 uptake on later stages in rats (day 6) and humans (day 6 to 11)[11,13] is in line with previous reports on inflammatory-targeted molecular imaging investigating the C-X-C motif chemokine receptor 4 (CXCR4)-directed PET agent [^{68}Ga]Ga-PentixaFor after AMI.[4,15] For the latter imaging probe, Thackeray et al had already demonstrated a peak in the infarcted area 3 days after coronary ligation,[15] while in humans, such an increased CXCR4 PET signal was demonstrated after a median of 4 days following reperfusion therapy.[4] As such, those reports provide translational evidence that dedicated PET probes may allow for a non-invasive read-out of the inflammation-fibrosis cascade post-MI in a longitudinal setting. Of note, both CXCR4- and FAPI-targeted imaging probes at baseline have also provided predictive value for later functional decline or major cardiovascular events.[4,16] Diekmann and coworkers applied [^{68}Ga]Ga-FAPI04 11 days post-MI and determined the value of imaging activated fibroblasts in the injured myocardium for later functional outcome by using cMRI at baseline and during follow-up. While [^{68}Ga]Ga-FAPI04 did not match with cMRI-derived myocardial tissue-specific parameters, it showed a negative correlation with later obtained left ventricular ejection fraction (Fig. 4). The authors concluded that profibrotic activity-targeting PET has an incremental value in addition to morphological information obtained by cMRI, in particular on ventricular remodeling after MI.[16]

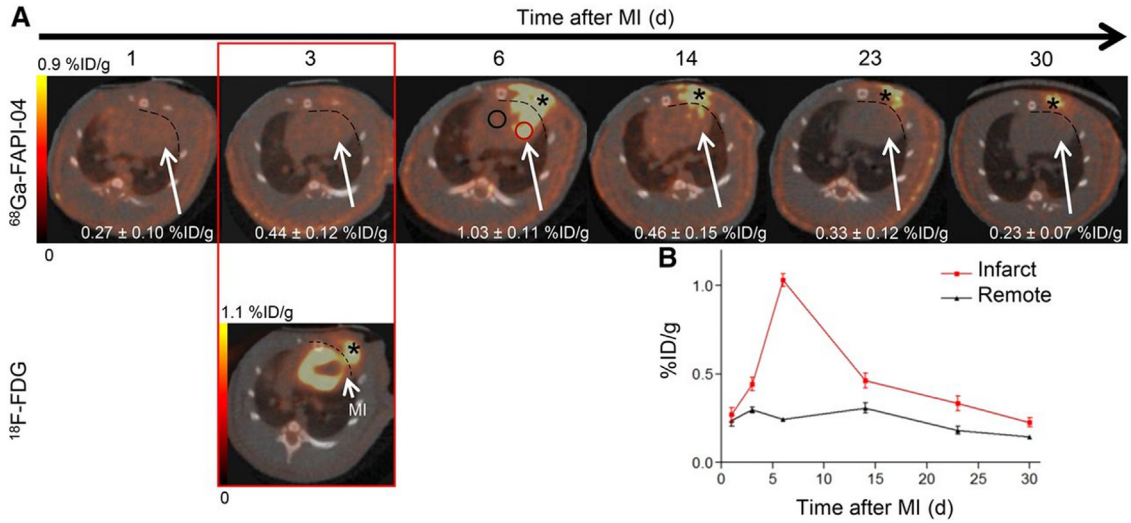

Figure 3 [^{68}Ga]Ga-FAPI-04 uptake in a longitudinal setting in rats after myocardial infarction (MI) using a micro-PET/CT scanner. (A) Transaxial [^{68}Ga]Ga-FAPI-04 PET/CT (upper rows) revealed an increased radiotracer signal 6 days after the acute event. [^{18}F]FDG (3 d after MI) is also displayed as reference (lower row). Dashed lines are used to differentiate between radiotracer accumulation in the infarcted myocardium vs. wounds initiated due to thoracotomy (to initiate coronary ligation; asterisk). At day 6 post-MI, regions of interest (circles) are placed over the infarct border zone and remote myocardium. (B) Time–activity curve for infarcted myocardium (red) also shows an increased uptake 6 days post-MI relative to other dates of imaging. This research was originally published in *JNM*. Modified from[11], copyright by the Society of Nuclear Medicine and Molecular Imaging, Inc.

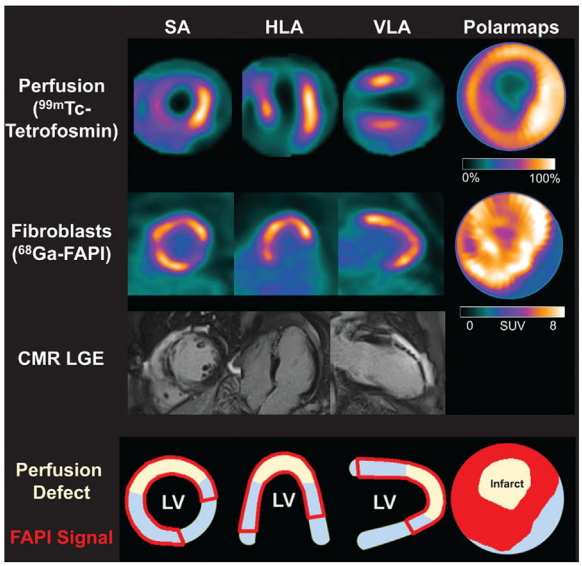

Figure 4 Perfusion SPECT (upper rows) shows a perfusion defect and increased late gadolinium enhancement (LGE) on magnetic resonance imaging in a patient after myocardial infarction. Profibrotic activity-targeting [^{68}Ga]Ga-FAPI-04 exceeds the infarcted area and the LGE signal. This research was originally published in *JNM*. Modified from[16], copyright by the Society of Nuclear Medicine and Molecular Imaging, Inc.

Aortic Stenosis

Beyond MI, [^{68}Ga]Ga-FAPI04 has been applied to other myocardial diseases, including AS. As an underlying pathophysiological rationale, cardiac fibrosis has been advocated to play an eminent significance in AS patients scheduled for TAVR. For instance, based on biopsy specimen, increased fibrotic load was tightly linked to less favorable cardiac functional outcome and higher rates of heart failure.[8] Increased fibrotic burden in the heart was also an independent predictor of cardiovascular death even during long-term follow-up,[17] thereby rendering myocardial fibroblasts as a target of interest in AS. As such, a recent multimodal imaging study applied [^{68}Ga]Ga-FAPI04 PET along with cMRI and echocardiography in 23 AS patients prior to valve replacement. The PET-based FAP-avid volume prior to intervention provided large variation, indicating that the imaging signal reflects different stages of fibroblast activation. Of note, relative to other imaging parameters, profibrotic activity derived from PET exhibited significant correlation with improved left ventricular ejection fraction after TAVR, suggesting that this image biomarker may also identify high-risks prone to later cardiac functional decline.[18]

Atherosclerosis

Fibrosis is also involved in atherosclerosis, mainly by balancing the inflammatory response (triggering plaque rupture) and profibrotic activity in a chronic setting (mainly mediating stability).[19,20] Relative to other imaging modalities, PET provides a whole-body functional read-out, which allows to decipher plaque activity in virtually every arterial segment in the body.[12,21] As such, recent efforts turned towards leveraging the advantages of hybrid imaging, with PET providing information on fibroblast activity in the vessels, while the CT component can assist in identifying plaque burden. For instance, a recent study investigated correlations between

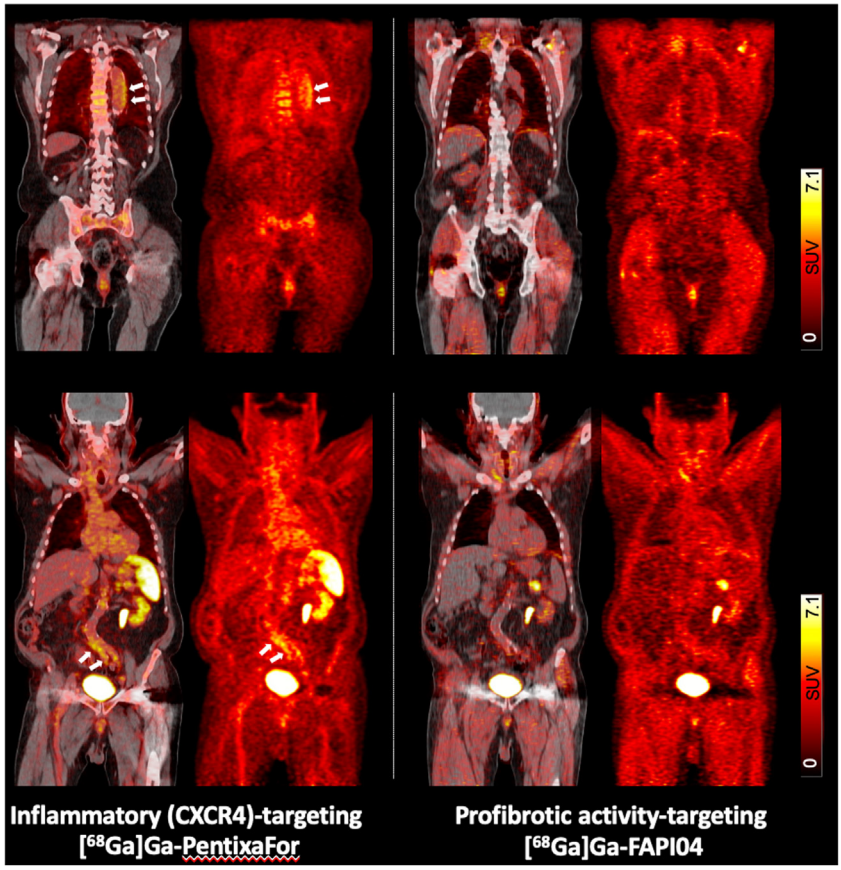

Figure 5 Different coronal maximum intensity projections for PET and PET/CT of the same cancer patient, which was imaged with C-X-C motif chemokine receptor 4 (CXCR4)-directed [^{68}Ga]Ga-PentixaFor (left) and profibrotic activity-directed [^{68}Ga]Ga-FAPI-04 (right) for oncological purposes. Focal arterial wall uptake in seen on CXCR4-directed PET as indicated by the arrows (upper rows, thoracic aorta segments; lower rows, abdominal aorta segments). On fibroblast activation PET, however, there was no relevant uptake in those arterial wall portions, suggesting that such a dual-tracer approach targeting inflammation and fibrosis may provide information on functional heterogeneity in the arterial tree.

arterial FAP uptake on PET with cardiovascular risk factors and calcified plaque burden (derived from CT). For the latter assessment, the number of plaques, thickness and circumference of calcification was investigated, thereby providing an elaborate assessment of the actual plaque load. Focal uptake in arterial segments was observed in 800 sites, while only 377 also had concomitant calcification on CT. Visual and quantitative evaluation of FAPI-avid vessel sites provided significant associations with all parameters of plaque load, but on univariate analysis, only body-mass index as a cardiovascular risk factor was associated with the number of FAPI-avid sites in the vasculature. The authors concluded that [^{68}Ga]Ga-FAPI04 can identify focal vessel wall lesions and is also linked to calcification, but not rigorously linked to cardiac risk.[11] Gallium labeling may explain this phenomenon, as fluorine radiochemistry has improved physiochemical properties leading to improved sensitivity,[22] in particular in small focal lesions such as in the vessels. Nonetheless, similar to MI,[4,15] PET radiotracers targeting CXCR4 (as a read-out of acute inflammation) and profibrotic activity (in a chronic setting) may provide further insights into the functional heterogeneity of plaque burden in patients beyond morphological (CT-based) information (Fig. 5).

Future Perspectives – Cardiorenal Crosstalk and Image-Guided Prevention

Beyond cardiovascular diseases, a recent report showed that [^{68}Ga]Ga-FAPI04 can also be applied to nephrology. For instance, in chronic kidney disease (CKD), renal fibrosis has been evaluated by this radiotracer in a translational setting. In a preclinical environment using a micro-PET, the molecular imaging signal in the kidneys was elevated in CKD animals relative to a control group and was also associated with fibrotic renal remodeling.[23] Those findings have been recently confirmed in 13 patients with suspicion for renal fibrosis. With biopsy serving as reference, [^{68}Ga]Ga-FAPI04 displayed a large variety of the PET signal (maximum standardized uptake values), which increased with worsening of renal fibrosis. Of note, PET/CT assessing profibrotic activity can then provide information on both kidneys,[24] thereby addressing potential sampling biases after renal puncture. Of note, CXCR4-targeting [^{68}Ga]Ga-PentixaFor has also been applied to determine inflammation in the kidneys, including in mice and patients after MI to investigate cardiorenal crosstalk. In patients, the renal imaging signal was linked to

worsening kidney outcome[25] and thus, inflammatory-directed and fibrosis-targeting molecular imaging may also be applicable to decipher pathophysiological interactions along the cardiorenal or renocardiac axis (depending on the *primum movens*).[26] For instance, the myocardium post-MI can initiate grave consequences for the kidneys, including acute overshooting inflammation or elevated pro-fibrotic signaling.[26] As PET probes are applied systemically, heart-kidney crosstalk can then be investigated by whole-body PET using the afore-mentioned target-specific radiotracers. This may also open avenues to implement PET-guided molecular preventive strategies to improve outcome,[5] e.g., to initiate *on-peak* anti-fibrotic treatment at the maximum of cardiac or renal target expression derived from imaging.[27,28]

Conclusions

Profibrotic activity-directed PET probes can monitor fibrotic reprogramming at later stages after cardiac injury. After acute MI, the [^{68}Ga]Ga-FAPI04 PET signal exceeded the underperfused area in the myocardium and was also linked to cardiac functional deterioration during follow-up. In AS patients scheduled for TAVR, a broad variation of the baseline FAP-avid volume indicates that the PET signal reflects different stages of fibroblast activation. In atherosclerosis, a tight link between focal arterial wall uptake and calcified plaque load was recorded and the fibrotic status provided by [^{68}Ga]Ga-FAPI04 may be of relevance to identify calcified plaques prone to rupture. As radiotracers are administered systemically, future studies may also focus on applications beyond cardiovascular disease to investigate bilateral organ function, e.g., fibrotic changes along the cardiorenal axis in patients after MI.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Rudolf A. Werner reports a relationship with German Research Foundation that includes: funding grants. Rudolf A. Werner reports a relationship with Novartis that includes: consulting or advisory. Rudolf A. Werner reports a relationship with PentixaPharm that includes: consulting or advisory and travel reimbursement. Takahiro Higuchi reports a relationship with German Research Foundation that includes: funding grants. Takahiro Higuchi reports a relationship with Okayama University that includes: funding grants. Takahiro Higuchi reports a relationship with Japan Society for the Promotion of Science that includes: funding grants. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Takahiro Higuchi: Writing – review & editing, Writing – original draft, Visualization, Supervision, Investigation. **Sebastian E. Serfling:** Supervision, Resources, Methodology, Data curation. **David M. Leistner:** Writing – review & editing. **Thimoteus Speer:** Writing – review & editing, Validation, Supervision. **Rudolf A. Werner:** Writing – original draft, Supervision, Investigation.

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References

1. Matter MA, Paneni F, Libby P, et al: Inflammation in acute myocardial infarction: The good, the bad and the ugly. Eur Heart J 45(2):89-103, 2024
2. Nidorf SM, Fiolet ATL, Mosterd A, et al: Colchicine in patients with chronic coronary disease. N Engl J Med 383(19):1838-1847, 2020
3. Ridker PM, Everett BM, Thuren T, et al: Antiinflammatory therapy with canakinumab for atherosclerotic disease. N Engl J Med 377(12):1119-1131, 2017
4. Werner RA, Koenig T, Diekmann J, et al: CXCR4-targeted imaging of post-infarct myocardial tissue inflammation: prognostic value after reperfused myocardial infarction. JACC Cardiovasc Imaging 15(2):372-374, 2022
5. Hess A, Derlin T, Koenig T, et al: Molecular imaging-guided repair after acute myocardial infarction by targeting the chemokine receptor CXCR4. Eur Heart J 41(37):3564-3575, 2020
6. Fan D, Takawale A, Lee J, et al: Cardiac fibroblasts, fibrosis and extracellular matrix remodeling in heart disease. Fibrogenesis Tissue Repair 5 (1):15, 2012
7. Tillmanns J, Hoffmann D, Habbaba Y, et al: Fibroblast activation protein alpha expression identifies activated fibroblasts after myocardial infarction. J Mol Cell Cardiol 87:194-203, 2015
8. Puls M, Beuthner BE, Topci R, et al: Impact of myocardial fibrosis on left ventricular remodelling, recovery, and outcome after transcatheter aortic valve implantation in different haemodynamic subtypes of severe aortic stenosis. Eur Heart J 41(20):1903-1914, 2020
9. Vasquez C, Mohandas P, Louie KL, et al: Enhanced fibroblast-myocyte interactions in response to cardiac injury. Circ Res 107(8):1011-1020, 2010
10. Ding N, Yang C, Ballew SH, et al: Fibrosis and inflammatory markers and long-term risk of peripheral artery disease: The ARIC study. Arterioscler Thromb Vasc Biol 40(9):2322-2331, 2020
11. Varasteh Z, Mohanta S, Robu S, et al: Molecular imaging of fibroblast activity after myocardial infarction using a (68)Ga-labeled fibroblast activation protein inhibitor, FAPI-04. J Nucl Med 60(12):1743-1749, 2019

12. Werner RA, Thackeray JT, Diekmann J, et al: The changing face of nuclear cardiology: Guiding cardiovascular care toward molecular medicine. J Nucl Med 61(7):951-961, 2020
13. Diekmann J, Koenig T, Zwadlo C, et al: Molecular imaging identifies fibroblast activation beyond the infarct region after acute myocardial infarction. J Am Coll Cardiol 77(14):1835-1837, 2021
14. Nagaraju CK, Dries E, Popovic N, et al: Global fibroblast activation throughout the left ventricle but localized fibrosis after myocardial infarction. Sci Rep 7(1):10801, 2017
15. Thackeray JT, Derlin T, Haghikia A, et al: Molecular imaging of the chemokine receptor CXCR4 after acute myocardial infarction. JACC Cardiovasc Imaging 8(12):1417-1426, 2015
16. Diekmann J, Koenig T, Thackeray JT, et al: Cardiac fibroblast activation in patients early after acute myocardial infarction: Integration with MR tissue characterization and subsequent functional outcome. J Nucl Med 63(9):1415-1423, 2022
17. Herrmann S, Fries B, Salinger T, et al: Myocardial fibrosis predicts 10-year survival in patients undergoing aortic valve replacement. Circ Cardiovasc Imaging 11(8):e007131, 2018
18. Diekmann J, Neuser J, Rohrich M, et al: Molecular imaging of myocardial fibroblast activation in patients with advanced aortic stenosis before transcatheter aortic valve replacement: A pilot study. J Nucl Med 64 (8):1279-1286, 2023
19. Monslow J, Todd L, Chojnowski JE, et al: Pure E. fibroblast activation protein regulates lesion burden and the fibroinflammatory response in Apoe-deficient mice in a sexually dimorphic manner. Am J Pathol 190 (5):1118-1136, 2020
20. Basatemur GL, Jorgensen HF, Clarke MCH, et al: Vascular smooth muscle cells in atherosclerosis. Nat Rev Cardiol 16(12):727-744, 2019
21. Slart R, Writing g, Reviewer g, Members of EC, Members of EI, Inflammation, et al: FDG-PET/CT(A) imaging in large vessel vasculitis and polymyalgia rheumatica: joint procedural recommendation of the EANM, SNMMI, and the PET Interest Group (PIG), and endorsed by the ASNC. Eur J Nucl Med Mol Imaging 45(7):1250-1269, 2018
22. Sanchez-Crespo A: Comparison of Gallium-68 and Fluorine-18 imaging characteristics in positron emission tomography. Appl Radiat Isot 76:55-62, 2013
23. Mao H, Chen L, Wu W, et al: Noninvasive assessment of renal fibrosis of chronic kidney disease in rats by [(68)Ga]Ga-FAPI-04 small animal PET/CT and biomarkers. Mol Pharm 20(5):2714-2725, 2023
24. Zhou Y, Yang X, Liu H, et al: Value of [(68)Ga]Ga-FAPI-04 imaging in the diagnosis of renal fibrosis. Eur J Nucl Med Mol Imaging 48 (11):3493-3501, 2021
25. Werner RA, Hess A, Koenig T, et al: Molecular imaging of inflammation crosstalk along the cardio-renal axis following acute myocardial infarction. Theranostics 11(16):7984-7994, 2021
26. Rangaswami J, Bhalla V, Blair JEA, et al: Cardiorenal syndrome: Classification, pathophysiology, diagnosis, and treatment strategies: A scientific statement from the American Heart Association. Circulation 139(16): e840-e878, 2019
27. Werner RA, Pomper MG, Buck AK, et al: SPECT and PET radiotracers in renal imaging. Semin Nucl Med 52(4):406-418, 2022
28. Toyama Y, Werner RA, Ruiz-Bedoya CA, et al: Current and future perspectives on functional molecular imaging in nephro-urology: Theranostics on the horizon. Theranostics 11(12):6105-6119, 2021