Microvascular function measurement in mice: From large to small

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Received Mar 2, 2020; accepted Mar 2, 2020

doi:10.1007/s12350-020-02097-1

There is a growing body of interest in cardiac microvascular function because it plays an important role in various cardiac disease conditions. Therefore, efforts have been made to develop non-invasive techniques to measure microvascular function. In this issue of the journal, Guerraty et al developed a micro-SPECT-based approach to assess microvascular function in *in vivo* mice. They applied two independent approaches to measure microvascular function: (1) myocardial blood flow measurement using ^{99m}Tc-sestamibi as a flow tracer and (2) intramyocardial blood volume measurement using ^{99m}Tc-red blood cells. Although there are issues to be addressed, they provided an important framework for non-invasive assessment of microvascular function in mice, where a number of disease models are readily available. Thus, their approaches are encouraging for facilitating better understanding of pathophysiology underlying microvascular disease models, and thereby the development of therapeutic options in future.

Key Words: Microvascular function • mouse • SPECT • myocardial blood flow • intramyocardial blood volume

See related article, pp. 2647–2656

There is a growing body of interest in cardiac microvascular function because it plays an important role in various cardiac disease conditions, such as ischemic heart disease, cardiomyopathy, or heart failure.^{1–4} Therefore, efforts have been made to develop non-invasive techniques to measure microvascular function, including echo, MRI, CT, and nuclear imaging.⁵ Of these, nuclear imaging has advantages over other imaging modalities in view of quantification. In particular, cardiac PET imaging with flow tracers such

as ¹³N-ammonia or ¹⁵O-water is known as an established technique to measure myocardial blood flow and flow reserve,⁶ which is a marker of microvascular function in the absence of epicardial coronary arterial stenosis. However, the assessment of microvascular function is often complicated due to the coexistence of epicardial coronary stenosis in addition to microvascular dysfunction. To overcome this limitation, intramyocardial blood volume has been proposed as an alternative measure of microvascular integrity even in the presence of obstructive coronary artery disease.⁷ In the study using dogs, intramyocardial blood volume changes under resting conditions measured using fast CT paralleled changes in the microcirculation after embolization, which was true even in the presence of epicardial artery stenosis, suggesting that intramyocardial blood volume can serve as a surrogate marker of microvascular function. In a recent study by Mohy-ud-Din et al using dogs and a clinical SPECT scanner dedicated for cardiac imaging equipped with state-of-the-art cadmium zinc telluride (CZT) detectors,⁸ they demonstrated that non-invasive quantification of intramyocardial blood volume

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J Nucl Cardiol 2021;28:2657–9.

1071-3581/\$34.00

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is feasible using ^{99m}Tc -labeled red blood cells (^{99m}Tc -RBC) and SPECT as compared to the reference standard measured by *ex vivo* CT.

It is well accepted that pre-clinical animal imaging provides unique opportunities for better understanding of disease-related pathophysiology because animals of various disease models can be assessed under well-controlled conditions. Until recently, researches on microvascular function have been performed using large animals such as dogs as described above.^{7,8} The advantages of using large animals include that the methodology developed for large animals can be easily applied to clinical settings. However, the variability of disease models in such large animals is rather limited, where genetically modified models are not available. The rodents such as mice, on the other hand, are easy to handle, affordable, and more importantly a number of disease models including those with genetic modification are readily available. Therefore, there is a need for the development of strategies to assess microvascular function that can be used in small animals.

In this context, Guerraty et al developed a micro-SPECT-based approach to assess microvascular function in *in vivo* mice.⁹ In this study, they applied two independent approaches to measure microvascular function: (1) myocardial blood flow measurement using ^{99m}Tc -sestamibi as a flow tracer and (2) intramyocardial blood volume measurement using ^{99m}Tc -RBC. However, there are technical challenges to mouse imaging because mice are much smaller than large animals or human. Therefore, whether the strategies developed for large animals could be applied to mice was uncertain. To address this issue, they used U-SPECT system (MILabs, Netherlands) equipped with multi-pinhole collimators dedicated for mice imaging, resulting in a high spatial resolution of 0.35 mm in full-width at half maximum (FWHM).¹⁰ Furthermore, they introduced a unique strategy to correct for spill-over and partial volume effect using mixing matrix. To obtain myocardial blood flow from dynamic ^{99m}Tc -sestamibi imaging, they applied 1-compartment model analysis and thereby compared it with those by microsphere method as the gold standard. They observed flow related increase in K1 (flow index), although as expected, it plateaued at high flow rates. They also found that unlike large animals or human, the relationship between partial volume effect in the myocardium (R_T) and fractional blood volume (FBV), which is expressed as $R_T = 1 - \text{FBV}$, does not hold true in mice. Instead, the mixing matrix approach, which was introduced in the present study, appeared to work well. As for intramyocardial blood

volume measurement, they observed increase in it in response to hyperemia induced by isoflurane, indicating that intramyocardial blood volume can serve as a surrogate marker of microvascular function. Thus, both approaches based on myocardial blood flow and intramyocardial blood volume measurements to quantify microvascular function appears to be relevant even in small mice.

There are several issues, however, that still need to be addressed by further studies as follows:

1. As they acknowledge, ^{99m}Tc -sestamibi is not an ideal tracer for flow measurement especially at high flow rates. Although they showed that low flow can accurately be assessed, it is likely that hyperemic flow such as that induced by vasodilators would be underestimated with ^{99m}Tc -sestamibi due to its limited extraction fraction at high flow rates. In this regard, ^{201}Tl may be a better tracer to track hyperemic flow, although its physical characteristics for imaging are inferior to ^{99m}Tc -labeled agents.
2. Serial measurements could be limited by high dose radiation exposure for a small mouse. For example, 4mCi of ^{99m}Tc -sestamibi or ^{99m}Tc -RBC for a 30 g mouse should be equivalent to 8000 mCi for a 60 kg man, which is not negligible particularly for multiple imaging. In this regard, micro PET imaging offers much higher sensitivity and is more suitable to reduce radiation exposure, although its limited spatial resolution (~ 0.7 mm in FWHM) as compared to the state-of-the-art micro-SPECT system (0.35 mm) may be an issue.¹¹ Alternatively, newer generation cadmium zinc telluride (CZT)-based micro-SPECT systems¹² may be an option for low dose imaging as indicated in clinical studies.^{13,14}
3. The results may be specific for U-SPECT system, where PET-like dynamic imaging is feasible owing to its stationary detector and collimator design. Therefore, whether the results presented in that study are directly applicable to more generalized micro-SPECT system is unknown.
4. The methodology presented in that study was tested only in normal mice. Therefore, more validations using pathological models, where microvascular dysfunction is most likely to exist, are necessary before it is widely used.

Despite these challenges mentioned above, Guerraty et al provided an important framework for non-invasive assessment of microvascular function in mice,⁹ where a number of disease models are readily available. Thus, their approaches are encouraging for facilitating better understanding of pathophysiology underlying

microvascular disease models and thereby the development of therapeutic options in future.

Disclosures

Dr. Matsunari has no relevant disclosures.

References

1. Schelbert HR. Coronary circulatory function abnormalities in insulin resistance: insights from positron emission tomography. *J Am Coll Cardiol* 2009;53:S3-8.
2. Miller WL, Behrenbeck TR, McCollough CH, Williamson EE, Leng S, Kline TL, et al. Coronary microcirculation changes in non-ischemic dilated cardiomyopathy identified by novel perfusion CT. *Int J Cardiovasc Imaging* 2015;31:881-8.
3. Shah SJ, Lam CSP, Svedlund S, Saraste A, Hage C, Tan RS, et al. Prevalence and correlates of coronary microvascular dysfunction in heart failure with preserved ejection fraction: PROMIS-HFpEF. *Eur Heart J* 2018;39:3439-50.
4. Camici PG, Magnoni M. How important is microcirculation in clinical practice? *Eur Heart J Suppl* 2019;21:B25-7.
5. Chung KS, Nguyen PK. Non-invasive measures of coronary microcirculation: Taking the long road to the clinic. *J Nucl Cardiol* 2018;25:2112-5.
6. Schelbert HR. Positron emission tomography measurements of myocardial blood flow: assessing coronary circulatory function and clinical implications. *Heart* 2012;98:592-600.
7. Wu XS, Ewert DL, Liu YH, Ritman EL. In vivo relation of intramyocardial blood volume to myocardial perfusion. Evidence supporting microvascular site for autoregulation. *Circulation* 1992;85:730-7.
8. Mohy-Ud-Din H, Boutagy NE, Stendahl JC, Zhuang ZW, Sinusas AJ, Liu C. Quantification of intramyocardial blood volume with (99m)Tc-RBC SPECT-CT imaging: A preclinical study. *J Nucl Cardiol* 2018;25:2096-111.
9. Guerraty M, Johnson L, Blankemeyer E, Rader D, Moore S, Metsler S. Development and feasibility of quantitative dynamic cardiac imaging for mice using μ SPECT. *J Nucl Cardiol* 2020 (in press).
10. van der Have F, Vastenhouw B, Ramakers RM, Branderhorst W, Krah JO, Ji C, et al. U-SPECT-II: An ultra-high-resolution device for molecular small-animal imaging. *J Nucl Med* 2009;50:599-605.
11. Miyazaki Y, Kobayashi M, Komatsu R, Hayashi A, Yonezawa S, Kawai K, et al. Effect of surrounding materials on iterative reconstruction-based line-source response function, and annihilations outside the source assessed by a small animal PET scanner. *Ann Nucl Med* 2014;28:512-22.
12. Matsunari I, Miyazaki Y, Kobayashi M, Nishi K, Mizutani A, Kawai K, et al. Performance evaluation of the eXplore speCZT preclinical imaging system. *Ann Nucl Med* 2014;28:484-97.
13. Nkoulou R, Fuchs T, Pazhenkottil AP, Wolfrum M, Buechel RR, Gaemperli O, et al. High efficiency gamma camera enables ultra-low fixed dose stress/rest myocardial perfusion imaging. *Eur Heart J Cardiovasc Imaging* 2019;20:218-24.
14. Yamada Y, Nakano S, Gatate Y, Sugi K, Okano N, Muramatsu T, et al. Shortened acquisition time in simultaneous 99mTc-tetrofosmin and 123I-beta-methyl-p-iodophenyl pentadecanoic acid dual-tracer imaging with cadmium-zinc-telluride detectors in patients undergoing primary coronary intervention for acute myocardial infarction. *Nucl Med Commun* 2019;40:1130-7.

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