There are several commercially available microbubbles throughout the world. Although their primary indications are for either improving left ventricular opacification or enhancing liver imaging, these agents have also been used clinically for off-label applications, such as myocardial and tumor perfusion imaging.\(^1,2\) The reason such practices are permissible is that these applications provide critical additional clinically useful information with the same doses of microbubbles required for the approved indication, and the imaging techniques required to perform these off-label applications are available on almost all commercially available scanners. To get Food and Drug Administration approval for any new indication or modification of the microbubble in the United States, additional expensive clinical trials and safety data (in the case of microbubble shell modifications) would be required. This becomes especially problematic for targeted molecular imaging applications with microbubbles, where specific ligands must be attached to microbubbles in order for them to be retained at sites where receptor upregulation occurs in various disease states like reperfusion injury,\(^3\) localized myocardial inflammation after ischemia,\(^4\) and myocarditis, as is shown by Steinl et al\(^5\) in this issue of Circulation: Cardiovascular Imaging.

**See Article by Steinl et al**

In the setting of myocarditis, clinical studies and expert review papers have recommended magnetic resonance imaging as the modality of choice.\(^6,7\) However, the diagnostic accuracies of most magnetic resonance imaging protocols that rely on a combination of T2-weighted edema detection with black blood imaging, hyperemic perfusion with T1-weighted imaging, and delayed enhancement imaging to detect myocarditis are <80%. Steinl et al created a murine model of varying severities of autoimmune myocarditis and, at 21 days, intravenously injected microbubbles that were targeted to either CD4 on lymphocytes or endothelial P-selectin.\(^5\) Subsequent molecular imaging of retained targeted microbubble contrast was effective at detecting the presence of myocarditis. Traditional echocardiographic measurements like ejection fraction and left ventricular mass were not effective in distinguishing normal myocardium from active myocarditis. Contrast-enhanced molecular imaging detected both moderate and severe forms of myocarditis with targeted microbubbles, whereas other advanced forms of ultrasound imaging (longitudinal strain) were only able to identify severe forms of myocarditis.\(^3\)

Although the CD4 and P-selectin targeted microbubbles clearly could improve diagnostic accuracy, it would require conjugation of anti-P-selectin or anti-CD4 antibodies to the microbubble surface. For such a targeted microbubble to be used in clinical practice, extensive safety testing would be required first. However, the authors of this study used a third targeted microbubble that consisted of simply adding a negatively charged phospholipid to the fluorocarbon microbubble. Specifically, this was done by sonication of decafluorobutane gas with an aqueous dispersion of 1 mg/mL polyethylene glycol stearate, 2 mg/mL distearoyl phosphatidylcholine, and 0.3 mg/mL of the negatively charged phospholipid distearoyl phosphatidylserine. Although the CD4 targeted microbubbles were useful in identifying the pathogenesis of myocarditis in this setting, the incorporation of a negatively charged phospholipid like phosphatidyl serine into the bubble membrane was just as effective at myocarditis detection with molecular imaging as were the ligand-targeted microbubbles. Phosphatidyl serine within the microbubble shell seems to enhance complement-mediated opsonization and subsequent attachment to both leukocytes and inflamed microvascular endothelium.\(^4\) This same complement-mediated inflammation is also seen within the myocardial risk area after even brief periods of ischemia, and, therefore, phosphatidyl serine microbubbles have been used with molecular imaging approaches to detect ischemia reperfusion injury\(^4\) and, more recently, brief periods of remote ischemia\(^6\) (Table). These findings have significant clinical implications because phosphatidyl serine containing microbubbles are already commercially available in certain countries for liver imaging in Japan and South Korea.\(^5\)

Therefore, similar to perfusion imaging, molecular imaging may be possible with currently approved microbubbles. These off-label uses would provide critical information in areas where clinicians still lack accurate bedside imaging techniques that could aid in rapid decision making, such as detection of remote ischemia in patients with a recent episode of chest pain, rapidly defining risk area in acute coronary syndromes, and as was shown in the current study, detection of myocarditis in patients presenting with acute chest pain.
and normal coronary arteries. Because phosphatidyl serine–
coated microbubbles have already been shown to be safe in
clinical trials,10 additional studies should now be performed
to determine its efficacy in detecting these common clinical
problems with molecular echo imaging techniques.

Acknowledgments
The author thanks Carol Gould for her administrative assistance with
the preparation of the article.

Sources of Funding
This project was supported in part by the Theodore F. Hubbard
Foundation, Omaha, Nebraska.

Disclosures
Dr Porter has the following disclosures: research support, Lantheus
Medical Imaging and Astellas Pharma Inc; and Instrumentation and
Research Support, Philips Research North America and General
Electric Global Research.

References
1. Porter TR, Xie F. Myocardial perfusion imaging with contrast ultra-
jcim.2009.09.024.

RP, DeCarri JM, Weinert L, Krausz T, Lang RM. Differential diag-
nosis of cardiac masses using contrast echocardiographic perfusion

Noninvasive ultrasound imaging of inflammation using microbubbles tar-

JR. Noninvasive imaging of myocardial reperfusion injury using

5. Steinl DC, Xu L, Khanicheh E, Ellertsdottir E, Ochoa-Espinosa A,
004720.

Cooper JT, White JA, Abdel-Aty H, Gutterlet M, Prasad S, Aletas A,
Laissy JP, Paterson I, Filipchuk NG, Kumar A, Pauschinger M, Liu P;
International Consensus Group on Cardiovascular Magnetic Resonance in
Myocarditis. Cardiovascular magnetic resonance in myocarditis: a JACC
jacc.2009.02.007.

7. Lurz P, Etel I, Adam J, Steiner J, Grothoff M, Desch S, Fuerneau G,
Gutberlet M, Thiele H. Diagnostic performance of CMR imaging com-
pared with EM in patients with suspected myocarditis. JACC Cardiovas Imaging.

8. Mott B, Fackwood W, Xie A, Belcik JT, Taylor RP, Zhao Y, Davidson BP,
Lindner JR. Echocardiographic ischemic memory imaging through com-
plement-mediated vascular adhesion of phosphatidylserine-containing mi-

9. Sonturn C. Physicochemical characteristics of Sonazoid™, A
new contrast agent for ultrasound imaging. Ultrasound Med Biol
2008;34:824–833.

10. Appis AW, Tracy MJ, Feinstein MA. Update on the safety and efficacy of
commercial ultrasound contrast agents in cardiac applications. Echo Res

Key Words: [Editorsials ■ echocardiography ■ inflammation ■ microbubbles ■ molecular imaging ■ myocarditis]
Detection of Myocarditis With Molecular Echo Imaging: Another Potential Application for the Phosphatidyl Serine Microbubble

Thomas R. Porter

Circ Cardiovasc Imaging. 2016;9:
doi: 10.1161/CIRCIMAGING.116.005249
Circulation: Cardiovascular Imaging is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2016 American Heart Association, Inc. All rights reserved.
Print ISSN: 1941-9651. Online ISSN: 1942-0080

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circimaging.ahajournals.org/content/9/8/e005249

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation: Cardiovascular Imaging can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation: Cardiovascular Imaging is online at:
http://circimaging.ahajournals.org//subscriptions/