During the study period, a total of 2,323 TTE studies were performed in 251 LVAD patients. Of them, 10 patients (4.0%) underwent a total of 12 CE studies (0.5%) (2 patients had repeat CE examinations). All patients underwent CE due to suboptimal endocardial border definition during noncontrast TTE. All but 1 patient was supported by a HeartMate II (Thoratec Corporation, Pleasanton, California) continuous-flow LVAD, and this patient was supported by a HeartMate XVE (Thoratec Corporation) pulsatile-flow LVAD. CE was performed successfully in all patients. Definity contrast was used in 9 CE examinations, and Optison was used in 3. Representative 2-dimensional echocardiography images before and after contrast administration are shown in Figure 1. The use of contrast aided image interpretation in 10 CE examinations (83%) and did not change image interpretation in 2 (17%). CE contributed to a change in LVAD patient management in 5 examinations (42%), including adjustment of pump speed in 3 patients, intensification of inotrope support in 1 patient after identification of severe right ventricular systolic dysfunction, and intensification of anticoagulation therapy in 1 patient after identification of a previously unrecognized nonobstructive left ventricular apical thrombus adjacent to the LVAD inflow cannula (Fig. 1, Online Video 1). No adverse events or known side effects of ultrasound contrast agents were reported during or after CE in any patient. Importantly, no changes in device function parameters were noted during or after CE.

To our knowledge, this is the first report to describe the use of CE in multiple LVAD patients. CE was successfully performed using a standard imaging protocol used for non-LVAD patients. Uncertainty regarding both the risk of causing adverse reactions or device function interference and the feasibility of diagnostic image acquisition during continuous blood flow into the device cannula has likely led to a significant underuse of CE in this population. Even at our institution, CE was used in only 0.5% of TTE studies performed on LVAD patients, although it is likely that many more would have benefited from its use. By comparison, CE is used in ~5% of all resting TTE studies in our laboratory. In summary, CE was feasible and safe and improved image interpretation in a small sample of LVAD patients undergoing clinically indicated echocardiography. CE should be used as needed during the echocardiographic evaluation of LVAD patients with technically difficult images.

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APPENDIX

For a supplemental video, please see the online version of this article.

Ex Vivo Cryoimaging for Plaque Characterization

Histopathology is the gold standard for the validation of arterial plaque characterization by in vivo methods. Volumetric comparison of images with histopathology, however, can be cumbersome and cost prohibitive. Cryoimaging provides real-time, ultra-high-resolution anatomical bright-field and fluorescence data by alternatively sectioning and imaging a tissue block face (1,2). Plaque morphology is minimally altered, permitting precise volumetric tissue characterization. Fibrous cap inflammation and elastin are important biomarkers that correlate with plaque stability. With accurate characterization of these constituents, cryoimaging can detect a volumetric plaque transition to a vulnerable state.

We sought to characterize fibrous cap inflammation and elastin in coronary plaques by cryoimaging. We also introduce a method for the use of cryoimaging as an adjuvant for plaque validation through a single-case volumetric comparison of cross-sections from intravascular optical coherence tomography (OCT) with cryoimaging and histopathology.

A total of 18 atherosclerotic plaques were evaluated from 10 coronary arteries of male cadavers (mean age 50.2 ± 6.2 years). Vessels were explanted and stored at 4°C no longer than 48 h. A vessel was fixed to a plastic cylindrical rig and infused with optimal cutting temperature gel. The optical coherence tomographic imaging catheter was inserted through the ostial end of the vessel, and images were captured every 200 μm by an automated 10 mm/s pull-back. Images were screened for fibroatheromatous and fibrous segments using consensus criteria (3). The start and end frames for plaque were bookmarked for analysis by cryoimaging. The vessel was sectioned into 5-cm cylindrical blocks and stored at −80°C. Cryoimaging was executed as previously described (2). Images were acquired in 5-μm intervals until reaching the plaque of interest. A 3-mm slab was sectioned and saved for histological analysis. Slabs were frozen (n = 7) or fixed in 10% formalin and embedded in paraffin (n = 11). Slices were sectioned 7 μm thick and stained. Cryoimaging was restarted until another plaque was reached or the vessel terminated.

Slides were digitally scanned and reviewed by 1 pathologist (N.P.Z.). Fibrous caps were stratified into inflamed or noninflamed groups on the basis of 2 thresholds for percent of capsule occupied by macrophage (0% and 10%). Images from cryoimaging were split into color channels, and mean intensity was measured over the fibrous cap pixel area. Univariate and multivariate logistic regression with stepwise selection were performed. Odds ratios and areas under the receiver operating characteristic curves were calculated. A p value <0.05 was considered statistically significant.

Methods and representative images from OCT, cryoimaging, and histopathology are provided in Figure 1. A total of 3,246 optical coherence tomographic frames, 22,864 cryoimaging frames, and 126 histological slides were evaluated. OCT accurately screened 81%

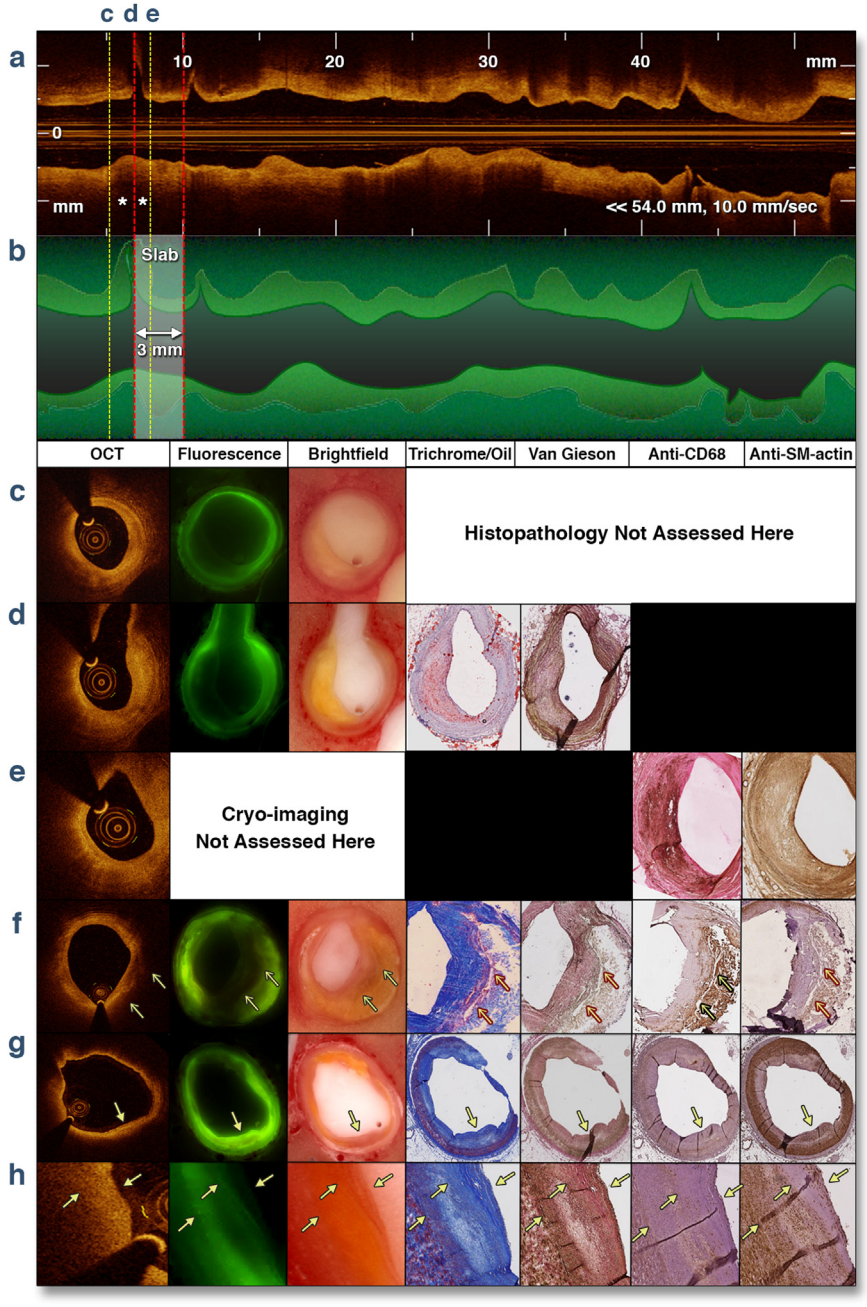

Figure 1. Illustration of Plaque Evaluation Method: Volumetric Coregistration of Images From OCT, Cryoimaging, and Histopathology

(A) Optical coherence tomography (OCT) was performed, and images were screened for plaque type. This is a longitudinal optical coherence tomographic image with a fibroatheromatous plaque of interest (4 to 15 mm). **Red dashed lines** indicate the start (7 mm) and end (10 mm) frames for subsequent histological analysis. **(B)** Schematic of cryoimaging. A bookmarked 3-mm segment was sectioned for histological analysis. Examples of coregistered images provided from **(C)** frames preceding the region of interest, **(D)** starting at the region of interest, and **(E)** within the region of interest. Images in **(D)** provide the opportunity for frame comparison among all 3 modalities. Masson's trichrome was used to assess the fibrous cap (blue), Verhoeff's Van Gieson for elastin content (yellow-black), anti-CD68 for macrophage (brown), and anti–smooth muscle actin for smooth muscle cells (dark brown). Lesions per row: **(F)** dim brown appearance of macrophage **(open arrows)** by fluorescence and **(G,H)** bright green streaks of elastin **(closed arrows)** by fluorescence. **(H)** Less inflamed fibrous tissue appears homogenously dim by fluorescence and pink to pale white by bright-field (between **closed arrows** on the right). The **asterisk** indicates target lipid plaque; slab = 3-mm plaque of interest.

(9 of 11) and 86% (6 of 7) of fibroatheromatous and fibrous plaques compared with histopathology. Two cases were classified as fibrous by OCT but fibroatheromatous by histopathology, and 1 case was classified as fibroatheromatous by OCT but fibrocalcific by histopathology.

Macrophages appeared brown with low intensity by fluorescence and pink to pale white by bright-field (Fig. 1f). Elastin appeared as bright green streaks by fluorescence and pink to pale white by bright-field (Fig. 1g). Cryoimaging green fluorescence was the best individual marker of inflammation at 0% (odds ratio: 0.93; $p < 0.05$) and 10% (odds ratio: 0.95; $p < 0.05$) macrophage, with areas under the curve of 0.958 (95% confidence interval: 0.869 to 1.000; $p < 0.001$) and 0.790 (95% confidence interval: 0.516 to 1.000; $p < 0.05$), respectively. Using green fluorescence intensity <60 as positive for inflammation (i.e., >0% macrophage), sensitivity was 91.7% ($p = 0.003$), specificity 100% ($p = 0.016$), positive predictive value 100% ($p < 0.001$), negative predictive value 85.7% ($p = 0.06$), and accuracy 94.4% ($p < 0.001$).

We demonstrate that less cryoimaging fluorescence correlates with greater density of macrophage and less elastin. These properties are consistent with studies using different excitation and emission wavelengths (4). Although this trial was small, a volumetric approach for comparing optical coherence tomographic images with cryoimaging and histopathology was successfully introduced. Early data yielded sensitivities of OCT within range reported in previous research. Errors in screening are typically related to misidentification of lipid versus fibrous and calcified plaques (5). Cryoimaging is a promising adjuvant for plaque characterization on the basis of high sensitivity and specificity for these constituents.

Clinical applications of volumetric tissue characterization by cryoimaging are numerous. Volume-rendering techniques are being developed to improve 3-dimensional coregistration between in vivo modalities and cryoimaging. Tissue volumes may be segmented and compared quantitatively to obtain more accurate results than by histopathology alone. Segmentation of elastin and inflammation during volume renderings may be analyzed via finite element analysis to understand effects of plaque deformation on genuine vessel architecture. Coupling to in vivo imaging modalities provides potential for understanding the real-time effects of therapeutic intervention (i.e., stent deployment) on fibrous cap stress. Fluorescence tagging before imaging allows volumetric quantification of desired cells or proteins.

In conclusion, this pilot study demonstrates that cryoimaging is capable of detecting fibrous cap inflammation with high sensitivity and specificity. Potential for macrophage quantification was demonstrated at 2 thresholds. Cryoimaging is a promising adjunct to histopathology for studies aiming to validate OCT for plaque characterization.

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In the Footsteps of Virchow

We read with interest the report on "Fat Deposition in Dilated Cardiomyopathy Assessed by CMR" by Lu et al. (1), because it describes a phenomenon already observed by Rudolph Virchow in 1858. In his book on cellular pathology, he devotes 2 full chapters to fatty atrophy of the heart (2). In lecture 16, he describes the fatty degeneration of the muscular substance of the heart in its different stages. Here we quote the text verbatim:

> You will observe that, even with the naked eye, certain changes can be recognized in the heart, namely a discoloration of its whole substance (which no longer presents the red hue of muscle, but wears a pale yellow tint), and besides peculiar spots on the papillary muscles. If you examine these more closely, you will perceive, in the direction of the primitive fasciculi, short, yellowish streaks which communicate so as almost to present a plexiform arrangement, and pervade the substance of the papillary muscles, whilst they offer a striking contrast to the reddish colour of proper muscular substance. This is the perfect form of genuine fatty metamorphosis of the real muscular substance of the heart, which differs most essentially from obesity of the heart, in which this organ becomes extremely fat and adipose tissue here and there so infiltrates its walls, that scarcely any muscle is to be perceived.

Unlike Virchow, the investigators of the present study have not been able to distinguish between intramyocellular and extramyocellular fat. The investigators also elected to omit the metabolic profiles of their patients. This might be of importance because we identified a subgroup of heart failure patients with severe metabolic