We appreciate the interest of Dr Virmani and her colleagues in our article. The study of vulnerable plaque remains controversial and is thus open to dynamic and creative insights. We hoped that our paper would be provocative and initiate some discussion in that regard.

As Dr Virmani implies, the argument concerning the adequacy of currently available animal models of atherosclerosis will likely never be settled. As we point out in our discussion, we do not contest the biological limitations of animal models of atherosclerosis. However, if research on vulnerable plaque is to proceed, some animal models will be needed; we firmly believe that the one we have used in our study is the first step toward the development of this goal. We also recognize the different utility of various models; perhaps there will never be a single perfect model for all atherosclerosis research needs. The model remains a work in progress, and we continually strive to improve and advance the model itself as well as the techniques used to evaluate it. We agree that the model used in this study included young pigs (12 weeks old) displaying early stage fibro-proliferative lesions that are still not representative of complex human atherosclerosis. Longer-term studies in our atherosclerotic model are currently being conducted and use several noninvasive imaging techniques that will help evaluate the natural evolution of these lesions. We are learning to improve our histological techniques and are glad to clarify that by protocol, all lesions found after endovascular imaging undergo frozen section analysis and then are embedded in paraffin for further staining and morphometric analysis. Although only paraffin sections were selected for illustrations, frozen sections were also used for Oil Red O, macrophages, and neovessel staining.

Beyond the technical discussion, however, comes the broader perspective that we wanted to be the central message of our paper. Traditionally, translational research has been conducted in a stepwise fashion—from cells through in vivo animal testing and eventually to human trials. The rapid adoption of virtual histology illustrates that observance of these rigorous traditional standards is difficult. The technology is market-approved and widely available. Clinical trials are being conducted that use it to label different forms of atherosclerosis in patients. Conceivably, we will soon witness a closer look at its development reveals inevitably that its validation has not followed the traditional step-wise pattern and has glaring gaps—lack of in vivo validation being the most important one. For example, the original study used by Dr Virmani and others to support the “validation” of virtual histology used a different image processing unit to the technology clinically used today. In the same study, ex vivo postmortem pathologic specimens were used to develop various radiofrequency patterns characteristic for several “key” plaque components in artificially isolated “region of interests”. Fundamentally identical patterns are applied to provide color maps of lesions in vivo, even though we know the amount of assumption that goes with that translation—the ex vivo specimens do not undergo that same mechanical deformation that has been observed in vivo, and there is a strong possibility that ex vivo fixation and processing alter the physicochemical features of the same tissue. Yet the technology is used in human studies under the assumption that it represents accurate characterization of human atherosclerosis in vivo.

Even if we assume that the results of our study are biased by an imperfect animal model of coronary lesions, one must wonder why, in an animal model with lesions that are homogeneous and “simple” to detect and characterize, virtual histology falsely misclassified all plaque components in a very consistent and reproducible manner in most of the analyzed samples. In addition, the intra- and interobserver variability shown in the paper is likely consistent with that which occurs in the environment seen in day-to-day clinical practice. We do not believe these findings support the “misclassification of adventitia” as calcium, because most of the false readings were intraplaque.

Thus, the premise that we used an inappropriate animal model to generate erroneous conclusions on accuracy of virtual histology is one we contest vigorously. Porcine models have been universally used to validate several imaging and therapeutic devices, including intravascular ultrasound (IVUS)-based technologies.1 Rather, we wanted our paper to (1) highlight the challenges associated with validation of endovascular imaging techniques for characterization of atherosclerosis, and (2) provoke a discussion leading to more critical and cautious adoption of these novel technologies remembering that their validation today is imperfect—mainly because of the absence of a reliable in vivo test system representative of human atherosclerosis. That the paper resonated well with the reviewers and concurs with other experts in the field confirms to us that we have accomplished these goals reasonably well.2–3

The long-term objective of the vulnerable plaque field is ultimately focusing in the potential preemptive intervention of focal nonobstructive lesions in asymptomatic patients. In view of recent data,4 the only way to justify such approach is...
the development of highly accurate detection technologies that are reproducible and capable of predicting clinical events. It is imperative that such technology development occurs in a responsible manner that strictly follows rigorous validation plans. Although virtual histology seems promising and is currently at the forefront of this approach, its clinical usefulness awaits the results of ongoing clinical trials and in our opinion still remains in the field of virtual reality.

Disclosures

None.

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Animal Models and Virtual Histology
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