OCT Imaging of Macrophages
A Bright Spot in the Study of Inflammation in Human Atherosclerosis*

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Macrophages are key inflammatory cells that are involved at every phase of atherosclerosis, from its genesis to plaque rupture and coronary thrombosis. Study of these cells in living patients is therefore critical to improving our understanding of human atherosclerosis. With this knowledge, for example, visualization of macrophage patterns within coronary fibroatheroma may provide unique signatures for diagnosing the atherosclerotic lesions that are most likely to cause a coronary event.

Given the importance of macrophages and the potential impact of their identification in vivo, the finding that intravascular optical coherence tomography (IVOCT) can be used to visualize accumulation of these cells in fibroatheromas was of great interest to many in the field (1). The key original observation was that fibroatheroma macrophages exhibit a stronger optical coherence tomography (OCT) signal intensity and variance than their surroundings. This finding has now been observed by other expert pathologists who compared OCT images versus histological findings (2). Also supporting this fact are reports using higher-resolution micro-OCT, which have proven that foamy macrophages exhibit strong OCT signals (3,4), and published positive clinical correlations between IVOCT metrics of fibroatheroma macrophage content and peripheral white blood cell count (5), high-sensitivity C-reactive protein (6), positive remodeling (7), cap rupture (8), and clinical presentation. As a result of this body of work in the field of IVOCT, there is wide consensus that this technique is capable of visualizing macrophages within fibroatheroma. As stated in the 2012 IVOCT Consensus Document in JACC (9): “Macrophages may be seen by IVOCT as signal-rich, distinct, or confluent punctate regions that exceed the intensity of background speckle noise... Macrophages should only be evaluated in the context of a fibroatheroma...”

The 2012 IVOCT Consensus Document in JACC (9) also highlights the following important features and caveats: “Macrophages may often be seen at the boundary between the bottom of the cap and the top of a necrotic core. Macrophages attenuate the IVOCT light significantly, and as a result, superficial macrophages can shadow underlying tissue, giving it the appearance of a necrotic core. Macrophage accumulations may also be confused on occasion with microcalcifications, cholesterol crystals, or internal or external elastic membrane. Noting the linear appearance of the cholesterol crystals or laminae can minimize some of these misinterpretations. It should be noted that high axial and lateral resolutions are desired for detection of macrophage accumulations. Whether or not IVOCT can identify individual macrophages is not known...”

In terms of macrophage quantification, our group developed a simple parameter that is a measure of local OCT signal intensity and local variance, termed the normalized standard deviation (NSD): $\sigma/(S_{max} - S_{min})$, in which $\sigma$ is the SD, $S_{min}$ is the minimum signal in the image, and $S_{max}$ is the maximum signal (1). Importantly, NSD was only developed and tested to determine macrophage content in plaque because it was recognized that other artery wall structures (e.g., the internal or external elastic laminae, calcification, adipocytes) would also generate a high NSD. In this initial study, NSD was found to have a good degree of correlation to the percentage area of CD68+ staining...
in cadaver fibroatheroma using linear OCT data (R = 0.84, p < 0.0001) and a moderate degree of correlation using logarithmic OCT data (R = 0.47, p < 0.05). Multiple groups have now used NSD to study macrophages seen by IVOCT (6,8,10).

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In this issue of *JACC*, Phipps et al. (11) describe research to investigate “bright spots” seen by using IVOCT and their relation to macrophages or other artery wall components. These investigators used a new method for segmenting out bright spots in the IVOCT image that employs an intensity threshold on the normalized logarithm IVOCT data which accounts for the distance between the catheter and the surface of the vessel. The authors implemented this algorithm on IVOCT images obtained from 14 cadaver coronary arteries. Bright spots were identified and compared with matching histological findings, including CD68 immunohistochemistry.

Overall, the algorithm of Phipps et al. (11) performed reasonably well compared with matching CD68+ histology, demonstrating a sensitivity of 80% and a specificity of 49%. The overall accuracy was similar to that of an expert IVOCT reader’s delineation of macrophages, which had a sensitivity of 46% and a specificity of 76%; total agreement between the algorithm and expert was only 53%, however. This low overlap in a sense illustrates the value of a combined approach: the quantitative algorithm was more sensitive to subtle bright spots, whereas the human eye was more capable of disregarding nonmacrophage results. As mentioned in the authors’ discussion, a suitable approach may be to use their algorithm to identify candidate bright spots. Subsequently, another algorithm/expert reader can determine whether these bright spots are within plaque or fibroatheroma.

When IVOCT bright spots determined by using the algorithm of Phipps et al. (11) were compared with corresponding CD68+ staining and microscopic morphology, the findings support the field’s current understanding about the capability of IVOCT to image macrophage accumulations. In particular:

1. The majority (57%) of bright spot regions identified anywhere in the artery walls were associated with macrophages. This high percentage is remarkable because these bright spots were detected in all anatomical/architectural regions within the artery wall, including the adventitia, media, and intima. The analysis also evaluated IVOCT data within calcified plates of fibrocalcific plaques that clearly do not contain intact macrophages. Again, specificity would increase significantly if this analysis was restricted to noncalcified fibroatheromatous plaque, as is the standard in the field.

2. Bright spots in the context of thin-cap fibroatheroma were caused by macrophages in 94% of cases. This result also supports the concept that the specificity of IVOCT is high for macrophages in fibroatheroma. This finding is significant because thin-cap fibroatheroma is the predominant plaque presumed to be at risk for cap rupture, and inflammation within the cap is thought to be an important predisposing risk factor for this event.

3. Not all bright spots are caused by macrophages; some are caused by other artery wall or plaque components, such as cholesterol crystals, elastin/collagen, and microcalcifications. These results should help with the understanding of the interpretation of bright regions within IVOCT images. This information may be useful when training IVOCT readers to avoid diagnostic pitfalls and consider other sources of high IVOCT signal regions. It clearly is important to look at the morphological context in which these regions are identified to evaluate whether these signal-rich spots or regions are macrophages.

In addition to confirming our current understanding of macrophage diagnosis in IVOCT, the investigators (1) uncovered interesting new findings about bright spots and their relation to macrophages. For example, they discovered that some areas with CD68 positivity did not exhibit bright spots according to IVOCT. One plausible hypothesis for this result, suggested by the authors, is that there may be different types of macrophages (e.g., M1 vs. M2 macrophages) that differ in their backscattering properties. Shadowing by macrophages, a well-known phenomenon in IVOCT images, was further expanded upon here by the discovery that macrophages which elicited shadowing were also frequently associated with other particulate tissues, such as microcalcifications and cholesterol crystals. This finding suggests that phagocytosis of these components may cause higher IVOCT light scattering and attenuation.

Although the work of Phipps et al. (11) has nicely added to the literature, confirming our understanding of IVOCT imaging of macrophage accumulations and further identifying potential pitfalls in diagnosis, it is important to remember that even equipped with accurate macrophage-finding methods, we must still consider the context of these bright spots before ascribing clinical or scientific relevance. Indeed, it may be better to assess which regions are inflamed and which regions are not and what are the structural
and functional implications of this inflammation. For example, an isolated few bright spots are probably not clinically significant, whereas a large accumulation of many bright spots within a thin fibrous cap may be of more concern.

Details about coronary wall patterns of macrophage infiltration in patients have previously been difficult to obtain. The recognition that IVOCT can visualize macrophage accumulations and its confirmation in the paper by Phipps et al. (11) are “bright spots” in our quest to study human coronary atherosclerosis and diagnose vulnerable plaque in patients. Continued pursuit of such research will further build on IVOCT’s diagnostic capabilities in the coronary wall so that we may advance our understanding of inflammation in this disease and ultimately provide better care to our patients.

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