

# Is Pathologic Intimal Thickening the Key to Understanding Early Plaque Progression in Human Atherosclerotic Disease?

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The term “Pathologic Intimal Thickening” (PIT) was recently introduced to define an early stage of atherosclerosis described in human coronary lesions found at autopsies of sudden death victims.<sup>1</sup> This descriptive identifier is based on the AHA type III (intermediate) lesion and, as originally presented by Stary and colleagues, it’s believed to be the morphological and chemical bridge to more advanced plaques.<sup>2</sup> The precise histological features and clinical relevance of PIT remains unsettled, and use of the term is still far from widespread. In short, PIT identifies a lesion with an extracellular lipid pool with intimal smooth muscle cell loss typically adjacent to the medial wall in addition to varying degrees of macrophage infiltration near the lumen. These morphological features indicate a progressive lesion in the earlier stages of atherosclerosis, although there is yet the presence of a necrotic core. As recently studied by Nakashima and colleagues in this issue of *Arteriosclerosis, Thrombosis, and Vascular Biology*, it may provide a key in settling the chicken-versus-egg debate of atherosclerotic plaque progression: does lipid come first, or do macrophages?<sup>3</sup> Is PIT the precursor lesion of fibroatheroma? The study in this issue uses 3-dimensional histology to attempt to address some of these issues, and, in doing so, may raise as many questions as it answers.

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Although there are many detailed autopsy studies describing various lesion morphologies, little is known how human atherosclerosis progresses from early to more advanced plaques, marked by the formation of a necrotic core. This important question remains, in part, from a lack of direct experimental testing in prospective models of human disease. Moreover, potentially relevant finding in animals are difficult to associate with humans because the pathologic change of atherosclerosis in man cannot be definitely equated with animals. Although the categorization of human lesions has provided valuable information concerning descriptive morphological events, in particular those of fatal plaques,<sup>1</sup> static two-dimensional images afforded by conventional histology offer limited insight into spatial relationship or sequences of events critical to lesion formation. Therefore, one of the

major obstacles to understanding how atherosclerosis develops and progresses arises from the inability to achieve biological visualization of arterial wall.

The importance of the early neointima as a fertile soil for the development of plaque cannot be overemphasized.<sup>4</sup> The presence of a thickened intimal layer, observed in 30% of newborns, is a fundamental structural difference that separates human arteries from animals, which the intima is primarily defined as a single layer of endothelial cells separated from underlying media by a relatively thin basement membrane and rare smooth muscle cells. This earliest intimal layer is exposed to various blood borne components and positive hemodynamic forces, which strongly influence atherosclerosis development in the arterial wall. The earliest pathologic events are not restricted to cellular elements alone as the extracellular matrix proteins also contribute significantly. The influence of the extracellular matrix proteins in early lesion formation was first put forth in a seminal paper by Tabas and colleagues, describing their selective ability to accommodate enzymes that specialize in lipid retention.<sup>5</sup> This initial process is thought critical to formation of the necrotic core, although the precise sequence of events directing its development remain unresolved. The pervasive question of how necrotic cores form is pivotal because lesions with lipid-rich cores eventually retain the potential to become unstable and rupture, which is the underlying cause in approximately 65% of all sudden coronary deaths.<sup>1,6</sup> Indeed, revisiting the early plaque to resolve the question of how lipid pools convert to more advanced fibroatheromatous plaques (lesions with necrotic cores) presents one of the most challenging issues.

## Advanced Coronary Lesions Occur at Specific Anatomic Sites

Complex atheromatous lesions consisting of plaques with defined necrotic cores include fibroatheromas, rupture prone (thin-cap fibroatheromas), acute and healing ruptures, and lesions complicated by intraplaque hemorrhage. These plaques are predominantly found in the proximal portions of the left anterior descending, left circumflex, and the mid-portion of the right artery.<sup>7</sup> This anatomic relationship is no coincidence because many lesions occur near flow dividers as there is ample evidence to suggest that atherosclerotic lesion size and vulnerability can be manipulated by changing patterns of blood flow.<sup>8</sup> Essential hemodynamic elements underlying the pathology of atherosclerosis involve shear stress, oscillatory (bidirectional) flow, and local eddies and/or boundary layer separation. The influence of hemodynamic

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forces along with changes in proteoglycan composition is one of the primary contributors to early lesion development because mechanical strain is thought to influence a variety of proatherogenic genes,<sup>9</sup> in particular those involved in the production and/or processing of extracellular matrix proteins.

### Three-Dimensional Assessment of Early Atherosclerosis

The study by Nakashima and colleagues in this issue of *Arteriosclerosis, Thrombosis, and Vascular Biology* redefines the methodology by which we examine atherosclerotic plaques using 3D reconstruction of histological sections to gain better understanding of early lesion development in human coronary arteries.<sup>3</sup> Serial and step sections were obtained from the right coronary together with the acute marginal branch collected from autopsy subjects (mean age,  $32 \pm 11$  years) who died of non-cardiac causes. The lesions were graded semi-quantitatively based on the ratio of lipid volume to arterial wall volume analyzed in 3D images.

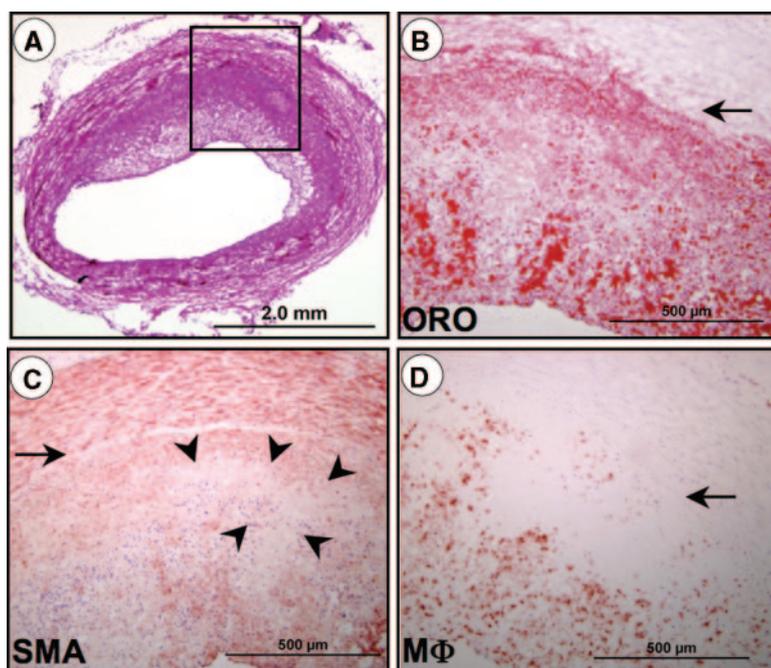
The authors report the earliest change in the arterial wall (defined as grade 0) as diffuse intimal thickening, consisting of smooth muscle cells and extracellular matrix with little or no accumulated lipid, but a few macrophages in the superficial layer. All cases of grade 1 and most with grade 2 demonstrated what the authors define as PIT characterized by extracellular lipid deposits in the outer intimal layer with lesional macrophages being more numerous as the lipid grades increased. The majority of grade 3 lesions were considered fatty streaks where there was greater Sudan IV staining along with biglycan and decorin localized within the outer areas of the intima while macrophage/foam cells were localized near the inner intima toward the lumen.

Notably, lipid grades correlated positively with advancing age, but not with risk factors of total cholesterol, triglyceride levels, or smoking status. Although a history of smoking was associated with enhanced macrophage infiltration, it did not

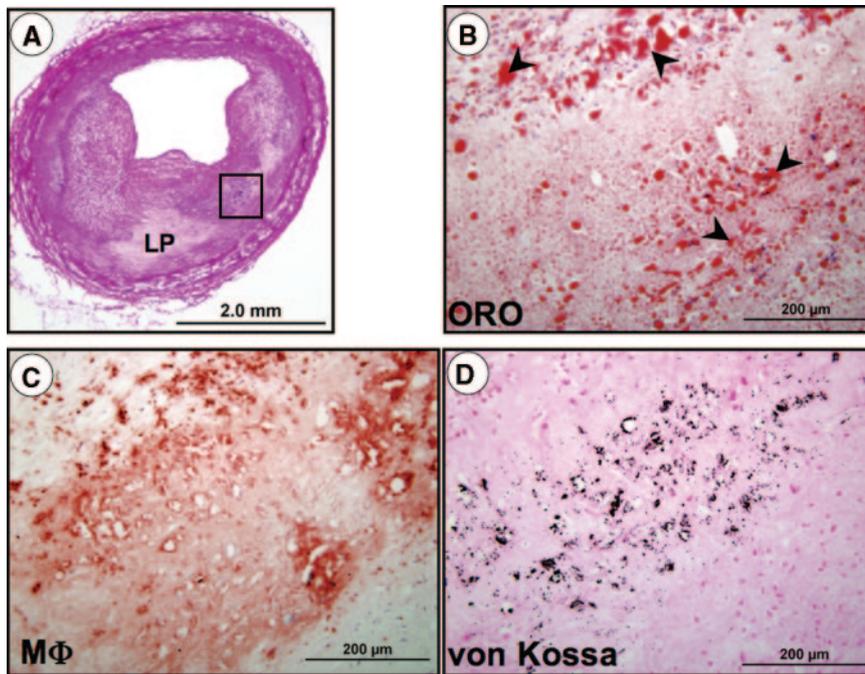
correlate with accumulated lipid. Comparison of similar aged diabetics also showed no differences in the amount of arterial wall lipid. In the majority of cases, lipid staining was distributed eccentrically and more strongly positive in the proximal and branching portions of the artery than distal regions. In lower lesion grades, the vertical distribution of lipid was more concentrated in the outer intimal surface with the proportion of lipid in this region increasing with advancing age. In contrast, the vertical distribution of macrophages was most prominent in the inner intima where there was modified LDL and monocyte chemoattractant protein-1 (MCP-1). The precise stimulus for lipid modifications or MCP-1 expression is unclear from the present data because these elements could not be attributed to traditional risk factors for cardiovascular disease.

It is clear, however, that lipid accumulation in the deep intimal layer represents the earliest stage of lesion growth marked by the expression of proteoglycans, biglycan and decorin. It is well known that transforming growth factor (TGF)- $\beta$  plays an important role in the production of proteoglycans and hence its importance in early lesion development. On the contrary, foam-cell rich fatty streaks are the less dominant early plaque types, which are unlikely to progress where in fact they are shown to regress in some regions of the thoracic and abdominal aorta later in life.<sup>10</sup> Similarly, the conversion of fatty streaks into more advanced atherosclerotic plaques in the coronary vasculature appears to be an unrelated pathological process.<sup>11</sup>

In the study by Nakashima, early lipid accumulation was mostly localized to the extracellular space between smooth muscle cells or near elastin fibers.<sup>3</sup> The distribution of lipid correlated with the expression of select proteoglycans where biglycan, decorin, and versican, as shown previously, accumulate in topographical distinct patterns within atherosclerotic lesions.<sup>12</sup> In grade 0 lesions, biglycan was distributed concentrically toward the outer side of the intima, and decorin



**Figure 1.** Human coronary lesion consistent with pathologic intimal thickening (A to D). A, Low power image shows an eccentric plaque with clinically nonsignificant stenosis. There are superficial foam cells overlying a lipid pool at the base of the plaque characterized by a significant loss of cells (hematoxylin and eosin stain). B, Oil-red-O stain shows a diffuse lipid pool deep within the plaque with more concentrated lipid-rich foam cells (evidenced by denser lipid droplets) toward the luminal surface. C,  $\alpha$ -smooth muscle actin stain highlights a discrete area of smooth muscle cell loss (arrowheads) in the outer (deeper) intima. D, CD68-positive macrophages (M $\Phi$ ) are seen bordering the lipid pool near the luminal surface with a notable absence of these cells within the deeper plaque. Panels B to D are areas represented by the black box in panel A. The arrows correspond to the internal elastic lamina.



**Figure 2.** Human coronary fibrous cap atheroma with early necrosis (A to D). A, Low power image showing an eccentric plaque with approximately 60% cross-sectional luminal narrowing. A prominent acellular lipid pool (LP) is seen at the base of the plaque. The small area within the black box represents a region of early necrotic core formation marked by macrophage infiltration, lipids, and necrosis. B, Oil-red-O staining showing the accumulation of neutral lipids; the larger dense lipid droplets (arrowheads) are localized to clusters of foam cells, as indicated in serial sections. C, Early necrotic core formation evidenced by the infiltration of numerous CD68-positive macrophages (M $\Phi$ ). The infiltration of the lipid pool by macrophages with subsequent cell death represents one of the initial steps in necrotic core formation. D, Early calcification (stippling, black reaction product) associated with the local cell death of macrophages and/or smooth muscle cells. Panels B to D are areas represented by the black box in panel A.

to a lesser extent. In all histological sections showing PIT with severe and/or diffuse lipid deposits, the distribution of apolipoproteins coincided with regions positive for Sudan IV and biglycan, however, the correlation with decorin was less consistent.

### Understanding Transitional Plaques by 3D Histology

Although it is widely accepted that the death of foamy macrophages contributes to extracellular lipid, the study by Nakashima et al strengthens the concept that earliest deposition of lipid occurs independent of foam cells.<sup>3</sup> We would like to address the concern, however, that the early lesions described in the present study do not meet our previously published criteria of PIT.<sup>1</sup> In our studies, oil red-O positive lipid pools and proteoglycans in the deep plaque near the intimal/medial border together with the loss of smooth muscle cells characterize PIT (Figure 1A to 1C). Moreover, varying degrees of inflammatory cells consisting of mostly macrophages are typically confined to the inner intima, bordering outside lipid-pool (Figure 1D). In further disagreement with the authors, it is our belief that PIT represents a transitional lesion to the more advanced fibroatheroma rather than the fatty streak. In our view, the lipid-rich lesions referred to as PIT in the current study correspond to perhaps precursor plaques, which more closely resemble our definition of diffuse intimal thickening, because there is no evidence of smooth muscle cell loss.<sup>1</sup>

Based on our observations, macrophages infiltrating into lipid pools become trapped and eventually undergo apoptosis liberating potent inflammatory cytokines and growth factors, matrix metalloproteinases (MMPs), and lipids. Extracellular matrix degradation together with macrophage cell death and early necrosis marks the conversion of PIT into an early fibroatheroma (Figure 2). The tool of

3D histology as described in the present study would help us gain a better understanding of this relationship at the spatial and molecular level. Similarly, this transition may represent the point of “angiogenic switch” where neovascularization plays a more dominant role in plaque progression.

Understanding the spatial involvement of relevant biologic markers in reference to a timeline offers, for the moment, the best view of atherosclerotic disease progression in humans. In particular, 3D analysis of plaques may help explain the most imperative question as to why certain lipid pool lesions convert to a more proinflammatory state with ensuing necrosis whereas others appear relatively quiescent for decades. Similarly this technique would allow further delineation of relevant markers of plaque progression involving focal attachment, proteolysis, cell signaling, recruitment of inflammatory cells, and cell death. Moreover, the impact of physical characteristics such as low- and high-shear stress points relative to arterial branching, necrotic core formation, and fibrous cap thinning (lesion instability) could be further defined in relation to plaque rupture. Finally, 3D spatial resolution at the molecular level would help further characterize the role of angiogenesis in morphological subtypes as a prelude to intraplaque hemorrhage and expansion of the necrotic core.<sup>13</sup>

### Disclosures

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