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# Coronary Intimal Thickening in Newborn Babies and ≤1-Year-Old Infants

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**Abstract**

We performed a morphological characterization of intimal thickenings in coronary arteries in the very early stages of life to obtain insights into initial coronary atherogenesis. We examined specimens from 67 infants who had died of noncardiac causes within their first year of life. Serially cut sections were stained with hematoxylin-eosin, Azan, Alcian blue, acetic orceine, and immunotypified for CD68, CD34, and α-smooth muscle (SM) actin. Substantial changes were detected in about 1 of 3 participants. Alterations ranged from focal areas with mild myointimal thickening to diffuse moderate thickening. In those lesions, smooth muscle cells (SMCs) showed loss of polarity, infiltrating the subendothelium, mostly with rupture of the internal elastic lamina and without neoangiogenesis. Morphometrically, in musculoelastic intimal thickenings, neointimal thickness averaged 58.3 ± 17.8 μm, affecting 46% of the internal elastic membrane perimeter; lumen stenosis averaged 13.7% ± 5.0%. These lesions can be present very early in life and SMCs seem to play an essential role.

**Keywords**

coronary artery, early atherosclerosis, smooth muscle cells, subintimal thickening

## Introduction

In 1953, the medical community was surprised when Enos, Holmes, and Byer described the occurrence of grossly visible lesions in the coronary arteries of young U.S. soldiers killed in the Korean war,[1] although Zeek had already concluded in 1930 that arteriosclerosis may occur at any age.[2]

Traditionally, fatty streaks have been considered the initial manifestation of atherosclerosis, because they represent the earliest lipid-containing lesion.[3] Fatty streaks consist of extracellular lipids with scattered macrophages, which differ from lesions found in early xanthelasmata of familial hypercholesterolemia or in cholesterol-fed rabbits.[3] Napoli et al[4,5] have documented that fatty streak formation may begin in fetal life, being greatly favored by maternal hypercholesterolemia during pregnancy. In fact, in the Fate of Early Lesions in Children (FELIC) study, they found that maternal hypercholesterolemia induces changes in the fetal aorta that determine the long-term susceptibility of children to fatty streak formation and subsequent atherosclerosis.[5]

Ikari et al[6] evaluated the time course of formation of intimal thickening, limited to the proximal left anterior descending coronary artery, in 91 autopsies of participants aged between 17 weeks' gestation and 23 months after birth. They suggested that coronary intimal cells migrated after replication of medial smooth muscle (SM), as seen in models of carotid artery balloon injury.[6]

However, the initial sequence of events in the development of atherosclerosis in the coronary tree is still debated, as other investigators subscribe to a different view, with initial lesions of coronary arteries being characterized by proliferation of intimal smooth muscle cells (SMCs), which would cause intimal thickening prior to any evidence of visible lipid deposition.[7-14]

Intimal thickening consists primarily of proliferation of SMCs that are strongly α-actin positive, surrounded by a proteoglycan-rich matrix, while macrophages are rarely detected. These lesions are most prominent at branch points. Consistent with the hypothesis that SMC proliferation is a key event in atherosclerosis is the observation that in adult atherosclerotic plaques SMCs are characterized by specific changes in gene expression and chromosomal alterations.[15-18]

If intimal thickening is an initial event, and yet frank atherosclerotic lesion are already present at a very young age, one should expect to see intimal thickening already evident at very

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early stages of life. Indeed, Rapola and Pesonen[19] first reported occurrence of lesions resembling preatherosclerotic changes in coronary arteries of autopsied babies; they described intimal and medial changes, with SMC proliferation leading to thickening of the arterial wall.[19] Subsequently, coronary intimal proliferations were observed in 95% of an autopsy series of infants between 1 and 5 years.[20] These reports are consistent with the hypothesis that intimal thickening is an early event. Accordingly, Nakashima et al[21] reported that diffuse intimal thickening develops from an early age in human arteries before atherosclerosis evolves, and they suggested that it plays an important role in atherogenesis. Thereafter, in 2007, they demonstrated that fatty streaks develop following deposition of lipids and proteoglycans in the outer layer of preexisting diffuse intimal thickening.[22] These findings were considered pathological intimal thickenings and the key to understanding early plaque progression in atherosclerotic disease.[23]

However, description of intimal thickenings in the first few months of life and their detailed morphometric assessment is lacking.

The aim of the current study was to systematically look for possible occurrence of intimal thickening in coronary arteries in the very early stages of life and to perform a morphologic-morphometric characterization. To this goal, we studied autopsy material of infants who had died in their first year of life with no evidence of structural heart disease.

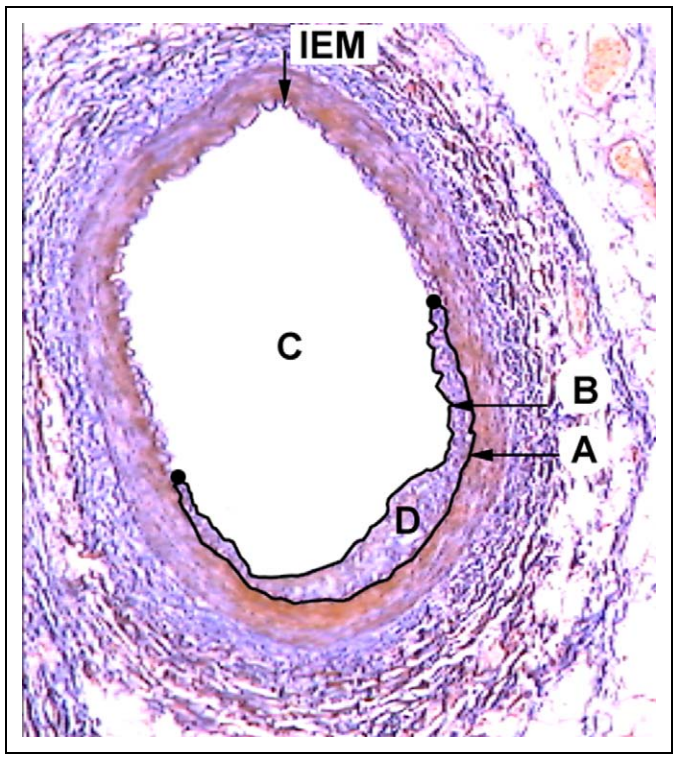


**Figure 1.** Epicardial coronary artery (left anterior descending branch) with musculoelastic thickening. A, Perimeter affected of the IEM. B, Internal perimeter of the lesion. C, Luminal area. D, Area of neointimal proliferation. Mallory trichrome ×40. IEM indicates internal elastic membrane.

## Methods

### *Population*

We examined 67 hearts obtained from infants who had died between 1 day and 12 months of age and in whom autopsy had excluded structural heart disease (see Results). Only cases where pregnancy had been without complications were evaluated. Cases in whom the mother had any known disease, or history of drug or alcohol abuse during pregnancy, were excluded. Written informed consent was obtained from parents by the Coroner's office (Buenos Aires, Argentina) before performing the autopsy.

### *Sample Handling*

Autopsies were performed within 6 to 18 hours from death. Hearts were fixed in toto by 48 hours immersion in 10% buffered formaldehyde (pH 7). The major epicardial coronary arteries were isolated along their entire length and excised transversely to their longitudinal axis, in segments approximately 3 to 4 mm long. Segments were dehydrated, embedded in paraffin blocks, and serially cut at 3-μm thickness. Sections were stained with hematoxylin-eosin and Heidenhain trichrome (Azan) for plain histological examination, Alcian blue (at pH 0.5 and 2.5) for acid mucopolysaccharides detection, acetic orceine for elastic fibers identification, or processed according to specific immunocytochemical methods (see below).

### *Morphometric Study*

Tissue histomorphometric analysis and planimetry were performed using a Nikon Eclipse E400 microscope and an image analysis program (Image Pro Plus for Windows, v3). Sections were magnified and digitized, lumen area and plaque area measured, and percentage luminal stenosis calculated. Intimal proliferation was defined[20] as musculoelastic thickening characterized by intimal proliferation of SMCs within a split and fragmented internal elastic membrane, and with evidence of further deposition of collagen and elastin (Figure 1). Neointimal thickness and total perimeter of the internal elastic lamina affected by neointimal proliferation were also measured (Figure 1). "Loose fibrous plaques" were defined as focal raised lesions consisting of an intimal SMC proliferation enmeshed in a connective tissue matrix.[20]

### *Immunocytochemical Study*

Immunophenotyping of cells present in vessels was performed with monoclonal antibodies to identify lymphocyte subsets (T cells: CD45RO, Biogenex, San Ramon, California; B cells: CD20, Dako Co; cytotoxic/suppressor: CD8, Dako Co (Dako Corporation, Santa Barbara, CA, USA); helper/inducer: OPD4, Biogenex); macrophages (CD68, Dako Co); endothelial cells (CD31, CD34, Biogenex; factor VIII, Ylem-Milano Milan,

**Table 1.** Causes of Death

| n | Male | Female | Cause of death |
|---|---|---|---|
| 2 | 2 | 0 | Anencephalia |
| 6 | 4 | 2 | Anoxic encephalopathy |
| 3 | 2 | 1 | Traumatic brain injury |
| 2 | 1 | 1 | Meningitis |
| 2 | 2 | 0 | Pneumonia |
| 52 | 27 | 25 | Sudden infant death syndrome |

Italy); and SMCs (α-SM-actin; antimouse α-actin monoclonal antibody concentrated MU128-UC clon 1A4, Biogenex). Detection systems were (1) streptavidin-biotin-peroxidase and/or alkaline phosphatase (Biogenex) and (2) Dako enhanced polymer one-step staining (EPOS) (Dakopatts, Carpinteria, California). When double immunostaining was required, EPOS System, a single step with peroxidase-conjugated primary antibodies was performed, followed by a second detection with streptavidin-biotin-alkaline phosphatase. Development of peroxidase reaction was carried out with 3,3-diaminobenzidine and Fast Red, to visualize alkaline phosphatase reactions; the first antigen stained brown and the second bright red. Negative controls were run simultaneously.

### *Statistical Analysis*

Data are expressed as mean ± SD or median and interquartile range (IQR), as appropriate. Histologic results were compared by 2-tailed unpaired *t* test. Nonparametric statistics were performed using the Mann-Whitney test. A $P < .05$ was considered significant. Analyses were performed using Statistics version 7.

## Results

### *Population*

Sixty-seven cases were studied (29 females and 38 males), 90 (60-210) days of age (median, IQR). A specific cause of death was established in 15 cases (22.4%); the remaining infants were assumed to have died because of sudden infant death syndrome (SIDS; Table 1).

### *Morphometric Study*

Morphometric data are detailed in Table 2. One or more alterations of coronary arteries were observed in 24 cases (35.3%). Median age was 105 days (IQR 83-210) in cases with positive coronary artery specimens and 90 days (IQR 30-240) in cases with morphologically normal coronary arteries. This difference was not significant. Of note, 12.5% of specimens from ≤30 days old babies already showed positive for lesions.

Alterations ranged from mild to moderate myointimal thickening. No specific distribution pattern was apparent, although when intimal thickening was present, the left anterior descending coronary artery was always involved to some extent.

In most cases, alterations were represented by rather diffuse musculoelastic intimal thickening (Figure 2). In some cases, thickening was localized, appearing like a cushion (Figure 3A). The subendothelial connective tissue was infiltrated to a variable extent by SMCs, monocytes, and amorphous deposits; lymphocytes were rare. Smooth muscle cells showed loss of polarity and tended to form columns perpendicular to the axis of the media and to infiltrate the subendothelial connective tissue, mostly with rupture of the internal elastic membrane (Figure 3B). Increased amounts of mucoid ground substance were also frequently observed. The endothelium was morphologically intact, and its surface smooth and devoid of thrombi. In 1 case, a loose fibrous plaque was found to be obstructing the lumen by 50%.

In some cases (Figure 4), fragmentation of the internal elastic membrane and of the elastic fibers of the media was evident; areas of focal thinning were also present in the media, particularly evident at sites of greater SMC proliferation. Smooth muscle cells lost their polarity, with their long axis perpendicular to the media and migrating into the subendothelial tissue. The endothelium was morphologically preserved and no thrombi could be observed.

As intermingled lesions with components of both categories were frequently observed, musculoelastic intimal thickening and nonstenotic plaques were considered together for the morphometric assessment (Table 2). In these cases, lumen stenosis averaged 13.7% +/− 5.0%, and 45.6% of the internal elastic membrane's perimeter was involved.

### *Immunocytochemical Study*

Macrophages (CD68) were at times detected at the intimal border of the lesions, penetrating the endothelium; however, no macrophages were seen within the lesions. Monocytes and/or foam cells were present in very low numbers (ie, ≤2 per ×400 magnification field); B lymphocytes were also rare (ie, ≤2 per field). Neoangiogenesis (CD31/CD34) was not detected.

## Discussion

This study shows that vessel alterations consisting of musculoelastic thickening of the intima of coronary arteries are already detectable in babies and infants ≤1 year old.

It has been previously reported that infants in their first 6 months of life may often show macrophages filled with lipid droplets accumulating in segments of coronary arteries[24]; these lesions can be found in about 2 of 3 adolescents.[7] Those observations indicate that lipid accumulation is an early phenomenon. However, aside from this specific change—which takes place mostly in the media—the intima of coronary arteries may also undergo early changes, which may become manifest even before the appearance of foam cell accumulations.

Proliferation of SMCs and its attendant subintimal thickening has long been assumed to be a benign, "adaptive" change of vessel wall accompanying growth. The fact that they are

**Table 2.** Morphometric Data of Coronary Arteries Affected by Preatherosclerotic Lesions

| Neointimal thickness (μm) | % perimeter affected IEM | Area of neointimal proliferation ($mm^2$) | % stenosis | |
|---|---|---|---|---|
| 58.3 ± 17.8 | 45.6 ± 12.0 | 0.029 ± 0.01 | 13.7 ± 5.0% | Musculoelastic intimal thickening and nonstenotic fibrous plaques (n = 22) |
| 151.4 ± 17.3 | 43.8 ± 11.0 | 0.076 ± 0.03 | 37.4% | Fibrous plaque (n = 2) |

NOTE: IEM = internal elastic membrane.

almost universally present in human arteries and show a well-organized structure has led the American Heart Association to consider intimal thickening at the site of vascular bifurcations as a normal finding.[22] Nevertheless, it is still debated whether these findings might represent an intermediate condition between normality and lipid/foam cell accumulation, being in other words, the initial step in the progression of atherosclerosis. In this respect, it should be noted that branch bifurcations are also the sites of preferential development of atherosclerotic plaques.[24] In addition, lipid deposition has been described at sites of underlying subintimal thickening and provides evidence that there is a progression toward atherosclerotic lesions.[7] At these stages, these findings are no longer considered normal and the term pathological intimal thickening would be used.[23] Finally, SMCs' monoclonality found in atherosclerotic lesions speaks in favor of its early origin.[6,16,17,25]

Little information is available about the possible course of development of human coronary artery atherosclerosis. Most data have been obtained in human aortas or in experimental models. In the peculiar condition of coronary disease development in transplanted hearts, Davies and al-Tikriti[26] defined a first stage characterized by intimal hyperplasia and disruption of the internal elastic lamina; the second stage is the migration of SMCs from the media into this thickened intima; the third stage is represented by the deposition of lipids, eventually resulting in atheroma. In this view, thickening may be a prerequisite for retention and accumulation of lipids and thus for plaque formation,[24] although if lipoprotein concentration is particularly high, macrophage/foam cells and lipids accumulate and plaques may also develop at sites without previous thickening.

## Previous Observations

Virmani et al[27] and Nakashima et al[22] had already attracted attention to the fact that coronary intimal thickening may be an early pathological change, preceding lipid/macrophage deposition. In a large series of 241 sudden death victims, Virmani et al observed that whereas coronary lesions in participants older than 50 years were richer of lipids and foam cells, occurrence of intimal thickening tended to precede lipid/macrophage deposition in "relatively young" participants (ie, <50 years old).[27] A younger cohort has more recently been investigated by Nakashima et al.[22] In 38 participants studied at autopsy (ranging between 7 and 49 years old), they reported

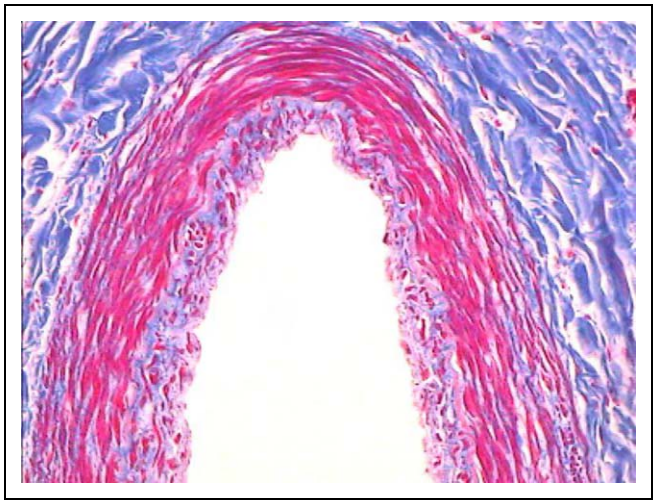

**Figure 2.** Intima-medial thickness with subendothelial proliferation of smooth muscle cells in an epicardial coronary artery of a patient, who died at 2 months of age due to sudden infant death syndrome (SIDS). Azan trichrome ×40.

the earliest lesion in the arterial wall (defined as grade 0; see Figures 4A and C of their paper[22]) to be intimal thickening, consisting of SMCs and extracellular matrix with little or no accumulated lipids and a few macrophages in the superficial layer. This picture is remarkably similar to Figures 2 and 3A of the current article. Grades 1 and 2, instead, were characterized by extracellular lipid deposition in the outer intimal layer, with macrophages being more numerous in the lesion as the lipid deposition score increased.[22] Of note, in the sections showing severe lipid deposits, the distribution of apolipoproteins coincided with areas also positive for intimal proteoglycans.[22] Our findings obtained in an autopsy series of noncardiac deaths that occurred in the first months of life confirm and extend the observations of Virmani et al[27] and Nakashima et al.[22] Furthermore, having been carried out in newborn babies and infants up to 12 months, they set earlier in life the hypothesis put forward that "... intimal thickening may be the key to understanding early plaque progression in human atherosclerotic disease."[23]

Usually, fetal and infant arteries adjust to normal asymmetries in hemodynamic forces to maintain optimal flow/wall stress at all points along the artery course.[28,29] Indeed, Virmani et al[30] postulated that adaptive intimal thickening rapidly occurs in most arteries once flow is established, in utero or soon after birth. Therefore, it is not surprising that these arteries

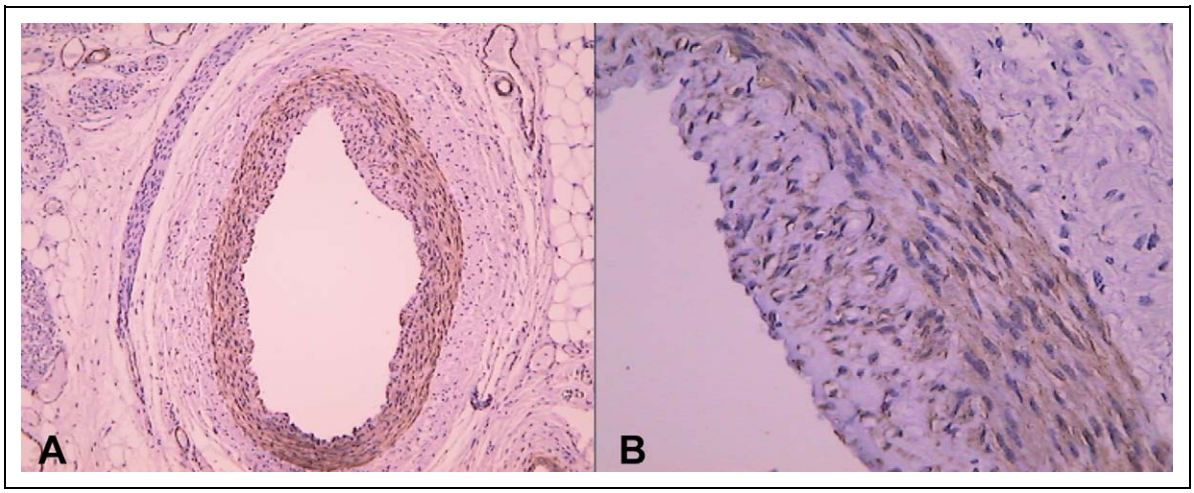


**Figure 3.** A, Epicardial coronary artery showing a loose fibrous plaque with a loose subendothelial connective tissue infiltrated with proliferating smooth muscle cells. Immunohistochemistry; anti-α-SMA ×40. B, Same section as in A. Intima-medial thickness showing positivity for α-actin. Coronary artery; anti-α-SMA ×100. SMA indicates smooth muscle actin.

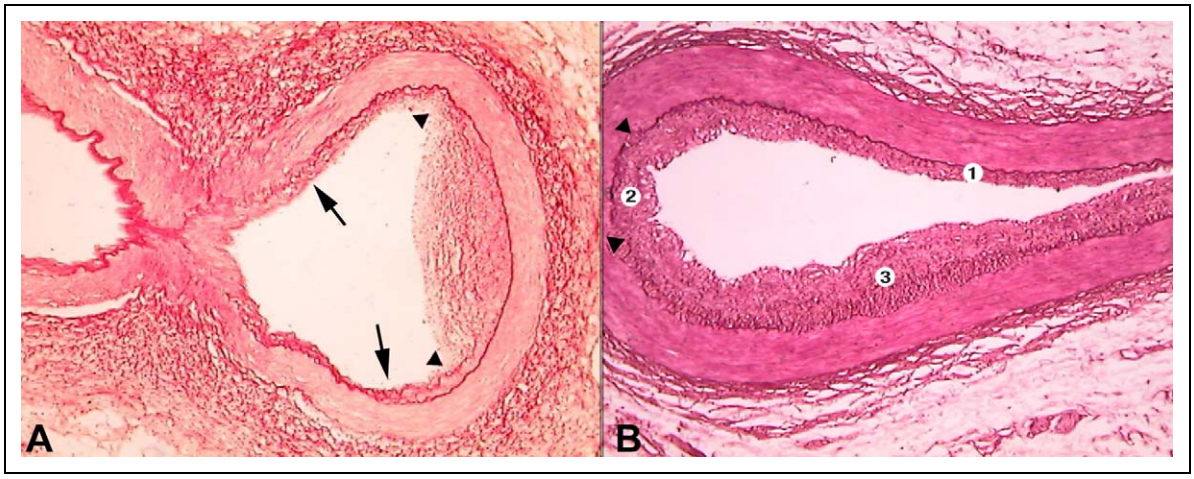


**Figure 4.** A, Bifurcation of the anterior descending coronary artery, showing at the left the first diagonal branch. A loose fibrous plaque partially occluding the artery lumen (50%) with large amounts of ground substance can be observed in the subendothelial connective tissue. Remnants of fragmented elastic fibers are seen at this level. In some areas, splitting of the internal elastic membrane are pointed (arrows), taken from a female of 11 months of age, who died of sudden infant death syndrome (SIDS). Orceine ×40. B, Right coronary artery giving rise to the acute marginal branch (right) with its first segment severely obstructed. In the upper half of the vessel, intimal proliferation could be observed (1), while in the lower half, a loose fibrous plaque is seen (3). An intermingled lesion is pointed out as (2). Between arrowheads, the internal elastic membrane is severely interrupted and fragmented, taken from a male of 8 days of age, who died of traumatic brain injury. Orceine ×40.

normally have segments of varying thickness. Accordingly, "thick segments" can be seen at and near bifurcations of arteries and at the ostium of even small vessels, where they tend to be focal and eccentric. A thick intima can also be found at some sites that are not obviously related to a branch vessel (Figure 5). It is also possible that an initially "adaptive" thickening may evolve into a pathologic lesion. In accordance with the response-to-retention hypothesis of atherogenesis,[31] the earliest lesion would be the "diffuse intimal thickening" or grade 0 of Nakashima et al[22] (ie, musculoelastic thickening in the current paper). These proteoglycan-rich thickenings have the ability to bind apoB-containing lipoproteins,[22] thus progressing to grade 1-2 lesion, or "fatty streaks" or "pathologic intimal thickening,"[27] and subsequently to the formation of plaques with defined necrotic cores. Very recently, Mayr et al[32] have proposed an attractive explanation for the proatherosclerotic role of intimal thickening. They surmise that oxygen diffusion is reduced in "thick" segments (because of increased diffusion distance), to the point that this may trigger local hypoxic damage and at the same time "compensatory" neoangiogenesis in the adventitia.

The potential proatherosclerotic role of intimal thickening already present very early in life must be viewed against (and perhaps tempered by) the clinical observations, indicating that

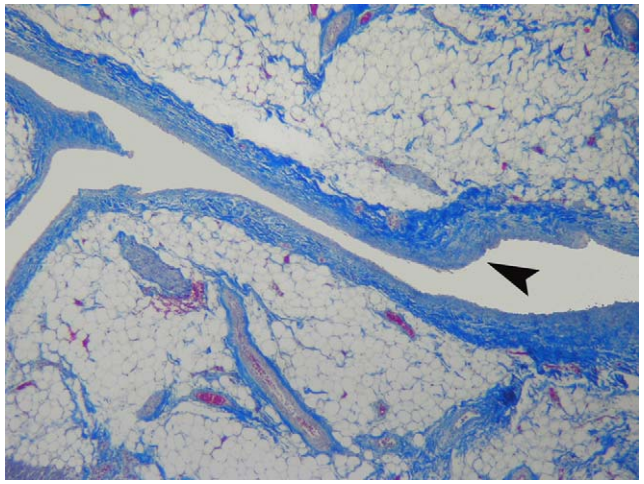

**Figure 5.** Longitudinal section of an anterior descendent coronary artery from a male of 10 months of age, showing its bifurcation in the first diagonal branch. Of note, the branch site is devoid of intimal thickening, while the arrowhead points out a thick segment far from the bifurcation. Masson trichome ×45.

ischemic symptoms may only occur many decades afterward, or not at all. Future studies will be needed to understand this discrepancy and/or to ascertain whether these lesions may regress, or stop progressing and be functionally minimized, through arterial remodeling and apoptosis.[27,33]

This proposed course of events attaches particular importance to SMCs and proteoglycans in atherogenesis as early players, followed by lipids and later by macrophages. In our series, virtually all specimens showing alterations were characterized by prominent proliferation of SMCs. It is well known that SMCs play a central role in established atherosclerosis,[17,18] as they represent 50% of the cellular components of chronic plaques and nearly 90% in early plaques.[22] Migration, proliferation, and differentiation of SMCs are pathological responses to different injuries (ie, vascular risk factors), which may contribute to the development and progression of early atherosclerotic lesions,[14] possibly reinstating their embryonic gene expression programs[23,31]; it is conceivable that cytokines and other inflammatory mediators activate transcription factors such as nuclear factor-κB, or protooncogenes such as c-fos and c-myc, which regulate the expression of genes involved in the inflammatory/proliferative response of preatherosclerotic lesions.[34-36] Consistent with this view, we have recently observed in coronary arteries of infants that SMCs present in the media do show *c-fos* gene activation,[37] suggesting that *c-fos* overexpression may promote proliferation, as evidenced by proliferating cell nuclear antigen-positive cells.

### *Study Limitations*

Because specimens were collected from forensic autopsies, it was not possible to perfuse-fix the arteries at physiologic pressure. For the same reason, it was not possible to obtain a complete clinical record of familial diseases or the genetic background.

In conclusion, intimal thickening is common in coronary arteries in the first months of life. Smooth muscle cells seem to play an essential role in these lesions, as they were characterized by SMC proliferation and migration from the media to the intima. These findings support the hypothesis that myointimal thickening can be an early event in the process of human coronary artery atherosclerosis.

## Authors' Note

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## Declaration of Conflicting Interests

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

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