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Histological Topographical Comparisons of Atherosclerosis Progression in Juveniles and Young Adults

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Abstract

Background—The histologically topographic comparisons on atherosclerosis progression among three anatomical sites, mid-thoracic and lower abdominal aorta and left anterior descending coronary artery (LAD) were performed using a young population (age 15-34) from the Pathobiological Determinants of Atherosclerosis in Youth (PDAY) study.

Methods and Results—The histological classification based on the American Heart Association grading scheme showed that in the thoracic aorta type 2 lesions (numerous macrophage foam cells with fine particles but no pools of extracellular lipid) appeared in the first ten year age group, with no significant change in prevalence in the next ten years. Lesions greater than type 2 were rarely seen in the thoracic aorta. Although type 2 lesions appeared later in the LAD than in the aorta, the lesions within the LAD progressed rapidly to more advanced lesions (types 4 and 5) or atheroma. Lesion development in the abdominal aorta was intermediate to lesion development in the thoracic aorta and the LAD.

Conclusions—The most striking topographic difference on lesion progression among the three anatomical sites was the vulnerability of type 2 lesions to progress into advanced lesions. The histology study, including immunohistochemistry limited to the type 2 lesions suggested that lesion progression was related to the intimal thickness and the amount of collagen but not to the number of macrophage foam cells.

Introduction

Although there has been numerous histological studies on atheromatous plaque rupture,¹ there has been no standardized criteria for histologically classifying the initial lesions of atherosclerosis since establishment of the histological grading system by the American Heart Association.²⁻³ The purpose of this study is to classify histologically the initial changes within the arterial wall that ultimately lead to the development of advanced lesions of atherosclerosis. This is essential for the prevention of atherosclerosis in children and young adults since atherosclerosis begins in the early years of life.⁴⁻⁷

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The Pathobiological Determinants of Atherosclerosis in Youth (PDAY) study was a multi-institutional project conducted to investigate atherosclerosis in 15-34 year old subjects.⁸ Findings from the PDAY study, based on gross evaluations of atherosclerotic lesions, include: (1) fatty streaks (FS) begin at an earlier age in the thoracic aorta (TA) and the abdominal aorta (AA) compared to the right coronary artery (RCA); (2) a higher prevalence of FS in the TA compared to the AA and the RCA; (3) the prevalence of FS is lowest in the RCA than either segment of the aorta in the younger age groups; and (4) the prevalence of raised lesions (RL) is highest in the RCA than either segment of the aorta in the older age groups.⁹ Although there is little difference in prevalence and extent of FS between the TA and the AA in either age group, there is a higher prevalence and extent of advanced lesions in the AA than in the TA in the older age group. These findings suggest that the vulnerability of FS to progress to advanced atheromatous lesions should differ according to these three arterial sites. To date, there have been no systematic histological studies to explore the progression of atherosclerosis based on topographical distributions.

In the present study, we use histology to investigate the progression of atherosclerotic lesions in three anatomical sites from the same subjects, specifically, the mid-thoracic aorta, the lower abdominal aorta and the left anterior descending (LAD) coronary artery. To our knowledge, the present study is the first analysis using the histological grading system of the AHA to compare the progression of atherosclerosis among these three arterial sites. In addition, we randomly selected twenty cases to focus on the histological components of the fatty streak at these three anatomical sites.

Methods

PDAY Project

This study was conducted using material derived from the multi-institutional PDAY project. PDAY subjects consist of forensic cases 15 - 34 years of age who died of external causes (accidents, homicides, suicides) within 72 hours after injury and were autopsied within 48 hours after death. Persons of race other than black or white and those with congenital heart disease, Down's syndrome, AIDS, or hepatitis were excluded.⁹ For the present study, 879 PDAY subjects with available samples from three anatomical sites (mid-TA, lower AA and LAD) were included in the analysis.

Preparation of Arteries for Microscopic Analyses

The methods for dissection and preservation of arteries have been described previously in detail.⁷ Briefly, the aortas were opened longitudinally on the dorsal surface midway between the orifices of the intercostal and lumbar arteries. Standardized core samples from two regions of the right half of the aorta were used for histological evaluation in the present study — a portion of the TA between the 8th and 9th intercostal arteries (PDAY core sample number 1), and aorta portion of the AA just above the common iliac bifurcation (PDAY core sample number 16). The left main and LAD coronary arteries were perfusion fixed with 10% neutral buffered formalin under 100 mmHg pressure. Standardized PDAY core samples included in the present study consist of a 5 mm length of artery located immediately distal to the bifurcation of the circumflex artery (PDAY core sample number 44) and a 5mm length of artery located adjacent and distal to the first region (PDAY core sample number 45).

In preparation for microscopic analysis, the proximal half of the standardized arterial segments were sectioned on a freezing microtome and stained with Oil Red O (ORO). The distal half was embedded in a paraffin block in preparation for special (Gomori-Trichrome Aldehyde Fuschin (GTAF)) and immunohistochemical analysis.⁸

Classification of Arterial Lesions

Eight hundred seventy-nine cases with samples from all the three anatomical sites (PDAY core samples 1, 16 and 44) were used for microscopic lesion classification. The racial and gender distributions of subjects were 372 black males (42.3%), 122 black females (13.9%), 281 white males (32.0%), and 104 white females (11.8%). Three pathologists blindly evaluated Oil-Red-O and trichrome stained sections independently. Each case was classified using a system developed by the Committee on Vascular Lesions of the Council of the American Heart Association.²⁻³ Differences were resolved by discussion and a consensus grade was determined. According to the AHA classification, Type 0 lesions are characterized by normal intima with no intimal lipid, with or without adaptive intimal thickening; type 1 lesions are characterized by isolated lipid-laden macrophages; type 2 lesions are characterized by numerous macrophage foam cells and fine particles of extracellular lipid, but no pools of extracellular lipid; type 3 lesions are characterized by one or more pools of extracellular lipid, but no well-defined core of lipid, and represent the intermediate or transitional lesion; type 4 lesions are characterized by a well-defined core of extracellular lipid covered by normal intima; type 5 lesions are characterized by one or more cores of extracellular lipid plus a reactive fibrous cap, vascularization, or calcification; type 6 lesions are characterized by an intimal surface defect. Type 6 lesions were not encountered in the PDAY samples.

Selection for Immunohistochemistry Study

Twenty cases demonstrating type 2 lesions on Oil Red O stained slides in all three anatomical sites (PDAY core samples #1, #16 and #44 or #45) were randomly selected for the immunohistological study. Of the twenty randomly selected cases, there were six whites, fourteen blacks, seventeen males and three females. The average age was 25.7 ± 4.0 years.

Immunohistochemical Staining

Four-micrometers-thick tissue sections were obtained from core samples 1, 16, and 44 or 45 on charged slides. The sections were deparaffinized and rehydrated in xylene and graded alcohols, incubated for 15 minutes in 3% aqueous hydrogen peroxide and rinsed in distilled water.

Two dual immunohistochemical stains were conducted for each core sample: (1) macrophages (HAM-56, Ventana, Tucson, AZ) detected with a peroxidase-diaminobenzidine (DAB) system (Ventana), and alpha smooth muscle actin (1A4, Dako, Carpinteria, CA) detected with an alkaline phosphatase (ALP)-fast red system (Ventana); and (2) T-lymphocytes (anti-CD3, PS1, Ventana) detected with the DAB system, and B-lymphocytes (anti-CD20, L265, Ventana) detected with the ALP system. Human tonsil was used as positive control for HAM-56, anti-CD3 and anti-CD20. Human colon was used as positive control of anti-alpha-smooth muscle actin antibody.

Immunohistochemical staining was conducted with the gen II automated immunohistochemistry slide stainer (Ventana). The sections were cooled in the retrieval solution at room temperature for 40 minutes and washed in distilled water for 5 minutes before being transferred into the immunostainer. The sections were incubated with 20% goat serum for 12 minutes and with the primary antibodies for 32 minutes. Next, the DAB and ALP detection systems were used following the vendor's instructions.

The sections were counterstained with hematoxylin for 4 minutes and then exposed to bluing reagent for 4 minutes, washed in distilled water, dehydrated, and coverslipped.

Image Analysis

A 50 μ m-length area with the greatest number of total foam cells along the intimal surface was selected for microscopic analysis of macrophages and smooth muscle cells on the dual immunohistochemical stained slides. The same area was analyzed on the GTAF slides and another dual immunohistochemical stained slides for T- and B-lymphocytes. The area captured for image analysis included the 50 μ m-length target area along with an adjacent 50 μ m-length area on each side. JPEG images (1280 $\times$ 1024 pixels) were captured using a light microscope (Olympus BX40) at 200 $\times$ magnification with a digital camera (Olympus DP11), transferred to a Gateway personal computer (E-3600), and stored on a 650 MB CD-Rom for archival storage. For use as a guide for trimming of the images, images were also taken at 100 $\times$ and 40 $\times$.

The image analysis methods used in the present study were previously described by Taatjes.¹⁰ The images were imported and merged as 24-BMP mode into Adobe Photoshop (Version 8.0; Adobe Systems; San Jose, AC). The area of interest along intimal surface of each slide was isolated. The cell counts, intimal area (mm²) and intimal thickness (mm) were determined. The intimal thickness was defined as the maximum length from the intimal surface to the internal elastic lamina. The total count and density of each cell including macrophages, SMC, T- and B-lymphocytes were calculated and expressed as a percent of the total area. The percent of area involved with collagen was also determined by image analysis. To calculate the percent of collagen, the number of pixels corresponding to the colored area of interest was expressed as a ratio of the total number of pixels within the 150 μ m-length area along intimal surface.

Statistical Analyses

Tests for associations of AHA lesion progression in the TA (#1), AA (#16) and LAD (#44) and for correlations between histological components were performed using simple regression analyses. The comparisons of histological elements between the three anatomical sites were examined by Student's t-test.

Results

AHA classification of lesions in the TA, AA and LAD by 10 year age groups

Figure 1 shows a preponderance of type 2 lesions (early fatty streaks) in both segments of the aorta in both age groups while the LAD shows a preponderance of type 0 or type 1 (normal intima or adaptive intimal thickening, respectively) in both age groups. The TA shows little tendency for type 3 (transition lesions) in either age group, while the AA shows type 3 in addition to types 4 and 5 (advanced lesions) in the 25-34 year old age group. The LAD, on the other hand, had more type 3, 4 and 5 lesions in both age groups than either segment of the aorta. AHA grades in the LAD showed a stronger correlation with the AA ($R=0.183$, $p<0.0001$) than the TA ($R=0.078$, $p<0.020$). AHA grades in the TA was significantly associated with ones in the AA ($R=0.212$, $p<0.0001$).

Table 1 shows the prevalence of AHA grades for the three arterial segments in the two age groups by gender. There were no significant gender differences in lesion progression in the TA. In the AA, the prevalence of type 0 and 1 lesions was less in women than in men in both age groups and more type 2 lesions were present in women than in men in both age groups. In the LAD, both age groups demonstrated a greater prevalence of type 0 and 1 lesions in women than men. In the 25-34 year old age group, the prevalence of type 3, 4 and 5 lesions was less in women than in men ($p<0.05$).

Table 2 shows the prevalence of AHA grades for the three arterial segments in the two age groups by race. Although there were no significant differences between blacks and whites, the

prevalence of type 2 lesions was greater in blacks than whites in the TA and greater in whites than blacks in the AA. There were no notable differences between blacks and whites in the LAD.

Comparison of Histological Components

Table 3 shows the comparisons of histological and cellular components among the three arterial segments for the twenty randomly selected subjects. The LAD demonstrates the greatest mean intimal thickness and intimal area compared to the TA ($p<0.01$) and the AA. The area of collagen is least in the TA compared to the AA ($p<0.01$) and the LAD ($p<0.01$). The density of collagen is also least in the TA compared to the AA ($p<0.01$) and the LAD ($p<0.1$).

The total number of SMC is least in the TA compared to the AA ($p<0.01$) or the LAD ($p<0.01$). Although there are no differences in the density of total SMC (which includes SMC foam cells), the density of SMC foam cells is greater in the TA than the AA ($p<0.01$) and greater in the AA than the LAD ($p<0.01$). There are no significant differences in the total number of SMC foam cells or density of SMC foam cells between the TA and the AA; however, they are greater in the TA and AA than in the LAD ($p<0.01$). The ratio of SMC foam cells to the total number of SMC is greatest in TA than the AA or the LAD ($p<0.01$).

There are no significant differences among the three arterial sites in the number of macrophage foam cells. The TA contains the greatest density of macrophage foam cells when compared to the AA ($p<0.01$) or the LAD ($p<0.01$).

The number of T-lymphocytes is least in the AA compared to the TA ($p<0.05$) and the LAD ($p<0.05$). B-lymphocytes were rarely seen in any of the three arterial segments.

Associations among histological components

The area of collagen was associated with the intimal area more significantly in LAD ($p=0.0001$) than in the TA ($p=0.0145$) or the AA ($p=0.0128$). The intimal area was significantly associated with the number of macrophage foam cells ($p=0.0046$) and SMC foam cells ($p=0.0161$) in the TA, but not in the LAD. In the AA, only the number of macrophage foam cells was associated with intimal area ($p=0.0279$). T-lymphocytes were not significantly associated with other histological components.

Discussion

The histological classification by AHA grading demonstrates that atherosclerosis progresses at different rates among these three arterial segments. The gross evaluation of lesions in the PDAY study reveals that most of raised lesions in the older age groups appears in the area of previous fatty streaks in the younger age groups.⁹ Thus, fatty streak should be a precursor lesion of advanced raised lesion, but some intimal lesions remain as fatty streaks under certain conditions and at certain anatomical sites.⁹ These gross fatty streaks correspond mostly to AHA type 2 lesions with some type 3 lesions.

In this study, the most striking topographic difference in lesion progression is between the TA and the LAD and the vulnerability of type 2 lesions to progress into more advanced lesions. Most of the type 2 lesions in the TA seems to remain type 2 lesions without regression to type 1 and 0 lesions. In contrast, the LAD demonstrated a delay in the progression of type 0 and type 1 lesions to type 2 lesions. However, once type 2 lesions appear in the LAD, they progressed more rapidly to the more advanced lesions [type 3 (transition) and types 4 and 5 (atheroma)]. The AA demonstrated intermediate results between the TA and the LAD. The fact that progression from type 2 to type 3 lesions, which corresponds to the appearance of

pools of lipid, is a key event for atherosclerosis progression has been supported by many previous histological⁶⁻⁷ and chemical¹¹ studies.

This study demonstrates gender differences in the initiation and progression of lesions among these three arterial segments. There are no gender differences in lesion initiation or progression in the TA. In the AA, the initiation of lesion progression from type 0 and 1 to type 2 begins earlier in women than in men. However, the prevalence of more advanced lesions (types 3, 4 and 5) is not significantly greater in women than in men. In the LAD, the initiation of lesions, as well as the course of lesion progression, appears to be delayed in women compared to men. This delay appears to account for the gender differences in the prevalence of advanced lesions in the older age group.

The histological composition of the LAD where the most progression-prone type 2 lesions appeared could be characterized by greater intimal thickness and area when compared to that of the TA. The greater intimal thickness and area could be explained by greater volume of collagen and number of SMC. Thus, the vulnerability of type 2 lesions to progress into type 3 or more advanced lesions appears to be associated with an increase in intimal area and collagen. Stary found that type 2 lesions developed in areas of diffuse intimal thickening composed predominantly of SMCs in the LAD.¹²⁻¹³ Rao et al¹⁴, using PDAY materials, revealed that the density of synthetic SMC in the dorsal AA, which had been phenotypically changed from the contractile SMC, was related to collagen production. The density of synthetic SMC was greater in the intimal layer of the dorsal AA than the ventral TA. In a histological study, including electron microscopic observations, fatty streaks with cholesterol clefts and an increase in extracellular lipid pools tended to associate with collagen tissue proliferation and an increase in intimal thickness.¹⁵ Then, lipid pools are formed by extracellular lipid in the deep intima, but not by foam cell death in the layer just under endothelium.¹⁵ The study using PDAY materials by Miller et al¹⁶ showed that there were significant associations between the amount of collagen and esterified cholesterol per unit surface in three of the four sites studied, namely, the dorsal and ventral TA and the dorsal AA. In the ventral AA, the amount of collagen increased without an associated increase in esterified cholesterol. On the basis of their findings, they speculated that connective tissue proliferation precedes lipid deposition. Indeed, type 2 lesions as well as type 5 lesions, in which fibrous caps on atheroma are formed, are the most active stages for proliferation of synthetic SMC.¹⁷

The increase in intimal thickness causes tissue hypoxia in the deep intima by reduced passive transfusion. This hypoxia should induce the release of extracellular lipid¹⁸ caused by foam cell necrosis and apoptosis.¹⁹ Furthermore, because of the reduction in cell population, supplement of extracellular lipid should exceed the efflux of lipid by macrophages in the deep intima.^{15,20} The fatty streak is the first stage in atherosclerosis progression characterized by the appearance of the apoptosis marker, BAX.²¹ Therefore, the increase in intimal thickness by collagen proliferation should be at least one of the prerequisites for the formation of lipid pools. In contrast, the accumulation of macrophage foam cells characteristically seen in the TA is not associated with lesion progression. It was also concerned with the findings that initial extracellular lipid deposition appeared independently of foam cell death.²²

The reason why there are differences in the amount of collagen synthesis and intimal thickness among the three anatomical sites cannot be determined. In both the coronary artery and the aorta, a negative association between shear stress and intimal thickness was indicated.²³⁻²⁴ Unlike the TA, the AA contains the renal, mesenteric and iliac artery bifurcations. The coronary arteries are of smaller caliber than the aorta. Furthermore, the histological compositions differ between elastic arteries such as the aorta and muscular arteries such as coronary artery. These complicating factors, including rheological and anatomical factors, may explain the topographical differences in histological composition among the three arterial segments.

It is often difficult to discriminate the border of type 2 lesions from that of other atheromatous lesion types. Although the size of type 2 lesions vary among the subjects, we identified a 50µm-length area that contained the greatest number of foam cells as the area of interest and the adjacent 50µm-length area on either side. The total cell numbers in the present study in the TA was compatible with that reported by Orekhov et al²⁵ (1320 ± 60 per unit length of the subendothelial intima in type 2 lesion of thoracic aorta). However, in the contrast with our results, Katsuda et al²⁶ described that the number of SMC-derived foam cells per lesion (55.4 ± 49.6) were greater than that of macrophage-derived foam cells (29.4 ± 22.2) in the area of grossly appearing fatty streaks of the posterior ascending aorta. When many foam cells were seen in the subendothelial location, the majority of foam cells reacted with anti-macrophage antibody.²⁶⁻²⁷ In this study, we looked at the area showing the greatest density of foam cells including both the subendothelial space and the deep intima. Therefore, this may explain the differences between our study and previous studies of Katsuda et al.²⁶

The AA demonstrated intermediate properties in comparison to the TA and the LAD in the composition of cellular components with the exception of the T-cell population. The T-cell density was least in the AA. Our observations suggest that the T-cell population correlates with the macrophage population in the type 2 lesions in more than 50 TA (data not shown). Emeson et al²⁸ demonstrated that an increase in macrophages was not always associated with an increase in T-cells, although the lesion with a large population of T-cells was generally accompanied by an increase in macrophages. There was correlation between T-cells activation and proliferation of T-cells and proliferation of other intimal cells.^{20,29-30} The lack of association between lesion progression and the number of T-cells may be due to the proportion of two types of T-cells such as Th1 and Th2.³¹ B-cells were rarely seen in any of the three arterial segments; this is consistent with previous studies.¹³

The present study reveals that the number of macrophage foam cells and T-lymphocytes are not always related to lesion progression. In addition, collagen proliferation must be important for the progression from type 2 lesions into the more advanced lesions, including lesions with lipid pools and lipid cores. Thus, inhibition of collagen proliferation should be one of the targets for various treatments for prevention of early lesion progression. Collagen proliferation also plays a protective role by stabilizing type 4 lesions, which contain plaques with a thin fibrous cap, and therefore, vulnerable to rupture. The future of prevention depends on the ability to design novel individual treatment plans based on the stage of atherosclerosis.

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Figure 1.
Distribution (percent) of AHA grades within the Thoracic Aorta, Abdominal Aorta and Left Anterior Descending Coronary Artery by 10 year age groups (N=879)

Table 1

Distribution (percent) of the American Heart Association (AHA) grades within the Thoracic Aorta, Abdominal Aorta and Left Anterior Descending Coronary Artery (LAD) by gender and 10 year age groups (N=879)

AHA Type	Thoracic Aorta (PDAY Core Sample #1)		Abdominal Aorta (PDAY Core Sample #16)		LAD (PDAY Core Sample #44)	
	Women	Men	Women	Men	Women	Men
15-24 Year Old						
0	29 (27.6%)	86 (25.3%)	35 (33.3%)	150 (44.1%)	75 (71.4%)	206 (60.6%)
1	13 (12.4%)	35 (10.3%)	11 (10.5%)	44 (12.9%)	19 (18.1%)	59 (17.4%)
2	63 (60.0%)	217 (63.8%)	57 (54.3%)	145 (42.6%)	10 (9.5%)	51 (15.0%)
3	0 (0%)	2 (0.6%)	1 (1.0%)	0 (0%)	0 (0%)	16 (4.7%)
4	0 (0%)	0 (0%)	1 (1.0%)	0 (0%)	1 (1.0%)	5 (1.5%)
5	0 (0%)	0 (0%)	0 (0%)	1 (0.3%)	0 (0%)	3 (0.9%)
Total	105	340	105	340	105	340
25-34 Year Old						
0	28 (23.1%)	81 (25.9%)	17 (14.0%)	95 (30.4%)	53 (43.8%)	113 (36.1%)
1	8 (6.6%)	19 (6.1%)	3 (2.5%)	32 (10.2%)	27 (22.3%)	50 (16.0%)
2	82 (67.8%)	208 (66.5%)	89 (73.6%)	167 (53.4%)	27 (22.3%)	81 (25.9%)
3	2 (1.7%)	5 (1.6%)	9 (7.4%)	13 (4.2%)	7 (5.8%)	35 (11.2%)
4	0 (0%)	0 (0%)	1 (0.8%)	2 (0.6%)	3 (2.5%)	18 (5.8%)
5	1 (0.8%)	0 (0%)	2 (1.7%)	4 (1.3%)	4 (3.3%)	16 (5.1%)

AHA Type	Thoracic Aorta (PDAY Core Sample #1)		Abdominal Aorta (PDAY Core Sample #16)		LAD (PDAY Core Sample #44)	
	Women	Men	Women	Men	Women	Men
Total	121	313	121	313	121	313

Table 2
Distribution (percent) of the American Heart Association (AHA) grades within the Thoracic Aorta, Abdominal Aorta and Left Anterior Descending Coronary Artery (LAD) by race and 10 year age groups (N=879)

AHA Type	Thoracic Aorta (PDAY Core Sample #1)		Abdominal Aorta (PDAY Core Sample #16)		LAD (PDAY Core Sample #44)	
	Blacks	Whites	Blacks	Whites	Blacks	Whites
15-24 Year Old						
0	58 (22.9%)	57 (29.7%)	114 (45.1%)	71 (40.0%)	160 (63.2%)	121 (63.0%)
1	26 (10.3%)	22 (11.5%)	28 (11.1%)	27 (14.1%)	46 (18.2%)	32 (16.7%)
2	168 (66.4%)	112 (58.3%)	111 (43.9%)	91 (47.4%)	35 (13.8%)	26 (13.5%)
3	1 (0.4%)	1 (0.5%)	0 (0%)	1 (0.5%)	9 (3.6%)	7 (3.6%)
4	0 (0%)	0 (0%)	0 (0%)	1 (0.5%)	2 (0.8%)	4 (2.1%)
5	0 (0%)	0 (0%)	0 (0%)	1 (0.5%)	1 (0.4%)	2 (1.0%)
Total	253	192	253	192	253	192
25-34 Year Old						
0	42 (17.4%)	67 (34.7%)	68 (28.2%)	44 (47.3%)	100 (41.5%)	66 (34.2%)
1	22 (9.1%)	5 (2.6%)	22 (9.1%)	13 (6.7%)	41 (17.0%)	36 (18.7%)
2	173 (71.8%)	117 (60.6%)	133 (55.2%)	123 (63.7%)	59 (24.5%)	49 (25.4%)
3	3 (1.2%)	4 (2.1%)	11 (4.6%)	11 (5.7%)	21 (8.7%)	21 (10.9%)
4	0 (0%)	0 (0%)	2 (0.8%)	1 (0.5%)	8 (3.3%)	13 (6.7%)
5	1 (0.4%)	0 (0%)	5 (2.1%)	1 (0.5%)	12 (5.0%)	8 (8.6%)

AHA Type	Thoracic Aorta (PDAY Core Sample #1)		Abdominal Aorta (PDAY Core Sample #16)		LAD (PDAY Core Sample #44)	
	Blacks	Whites	Blacks	Whites	Blacks	Whites
Total	241	193	241	193	241	193

Table 3

Comparison of Gomori's Trichrome Aldehyde Fuschin (GTAF) stained histological components and immunohistochemically (IHC) stained cellular components among the Thoracic Aorta, Abdominal Aorta and Left Anterior Descending Coronary Artery (LAD) (N=20)

GTAF Stained Histological Components					
	Thoracic Aorta (PDAY Core Sample #1)	Abdominal Aorta (PDAY Core Sample #16)	LAD (PDAY Core Sample #44 or #45)	Associations #1 vs #16	#16 vs #44 or #45
Mean Area (mm ²)	0.605 ± 0.223(0.050)	0.848 ± 0.317(0.071)	1.011 ± 0.677(0.151)	0.0012**	0.0069**
Intimal Thickness	0.402 ± 0.137(0.031)	0.555 ± 0.202(0.045)	0.666 ± 0.398(0.089)	0.0032**	0.0051**
Collagen	Area (mm ²)	0.136 ± 0.063(0.014)	0.173 ± 0.129(0.029)	0.0002**	0.0027**
	Density	0.113 ± 0.050(0.011)	0.165 ± 0.073(0.016)	0.184 ± 0.137(0.031)	0.0022**
IHC Cellular Components					
Mean Area (mm ²)	0.569 ± 0.218 (0.049)	0.797 ± 0.327 (0.073)	0.924 ± 0.560 (0.125)	0.0067**	0.0053**
Smooth Muscle Cells (including foam cells)	Total	159.55 ± 75.59(16.9)	252.1 ± 119.2(26.7)	0.0004**	0.0026**
	Density	298.2 ± 115.8(25.9)	320.8 ± 94.8(21.2)	0.2442	0.2675
Smooth Muscle Foam Cells	Total	52.00 ± 40.63(9.09)	55.05 ± 36.08(8.07)	0.7430	0.0021**
	Density	89.8 ± 59.6(13.3)	72.9 ± 52.1(11.6)	0.2099	0.0003**
Macrophage Foam Cells	Total	55.35 ± 31.00(6.93)	44.2 ± 42.84(9.58)	0.3066	0.1288
	Density	96.2 ± 46.6(10.4)	58.9 ± 41.1(9.2)	0.0023**	0.0024**
All Foam Cells (MΦ + SMC foam cells)	Total	107.35 ± 58.08(12.99)	99.25 ± 58.84(13.16)	0.6234	0.0052*
	Density	186.0 ± 73.9(16.5)	131.8 ± 63.1(14.1)	0.0036**	<0.0001**
Ratios	MΦ / SMC foam cells	1.428 ± 1.299(0.291)	1.268 ± 1.498(0.335)	0.6073	0.2349
	SMC foam cells/ SMC	0.314 ± 0.190(0.042)	0.239 ± 0.159(0.036)	0.0705 ⁺	<0.0001**
IHC Cellular Components - Lymphocytes					
Mean Area (mm ²)	0.587 ± 0.211 (0.047)	0.822 ± 0.319 (0.071)	0.968 ± 0.611 (0.137)	0.0026**	0.0056**

0.1798

		Thoracic Aorta (PDAY Core Sample #1)	Abdominal Aorta (PDAY Core Sample #16)	LAD (PDAY Core Sample #44 or #45)	Associations	
					#1 vs #16	#16 vs #44 or #45
T-Lymphocytes	Total	22.85 ± 25.05(5.60)	11.05 ± 9.88(2.21)	26.25 ± 32.63(7.30)	0.0216 [*]	0.6787
	Density	37.0 ± 38.9(8.7)	15.0 ± 13.4(3.0)	27.3 ± 28.7(6.4)	0.0065 ^{**}	0.3130
B-Lymphocytes	Total	0.150 ± 0.366(0.082)	0.050 ± 0.224(0.050)	0.900 ± 2.245(0.502)	0.3299	0.1266
	Density	0.3 ± 0.7(0.2)	0.1 ± 0.3(0.1)	0.9 ± 2.2(0.5)	0.2556	0.2065

±S.D. (S.E.)
MΦ - macrophage
SMC —Smooth Muscle Cell

** p<0.01
* p<0.05
+ p<0.1