Plaque Progression and Instability: Critical Insights from Pathology

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Non-Progressive and Progressive Coronary Plaques

non-progressive → progressive

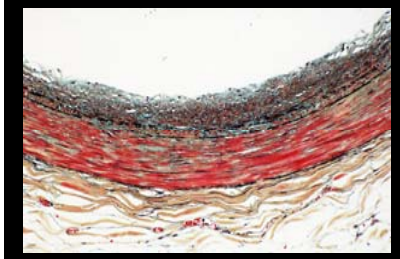
adaptive intimal thickening

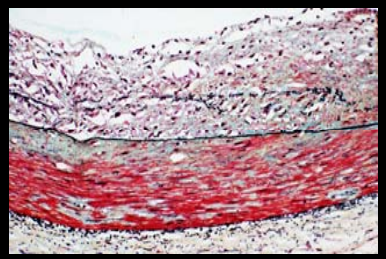
Intimal xanthoma

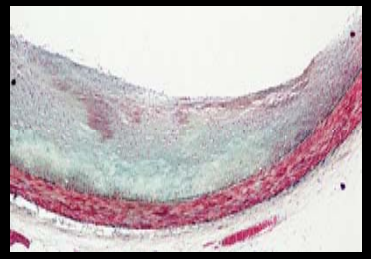
pathologic intimal thickening

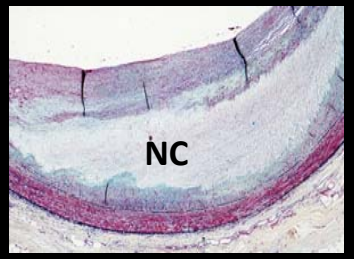
fibroatheroma

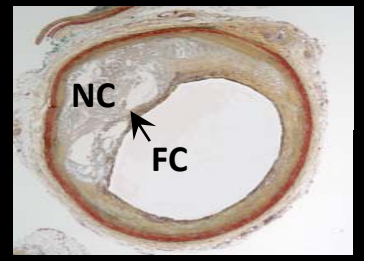
thin-cap fibroatheroma

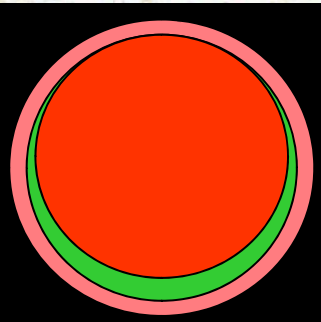


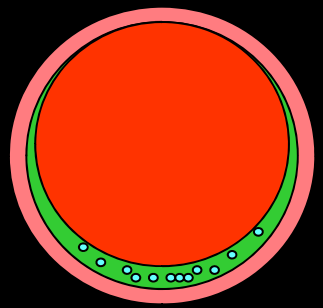


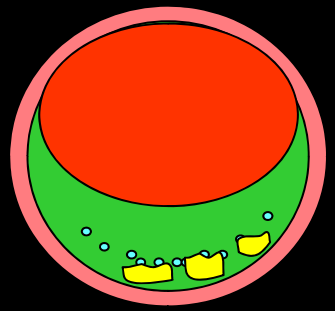


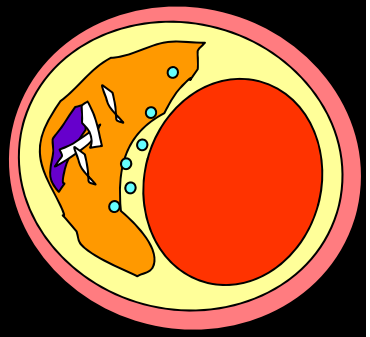












lipid pool

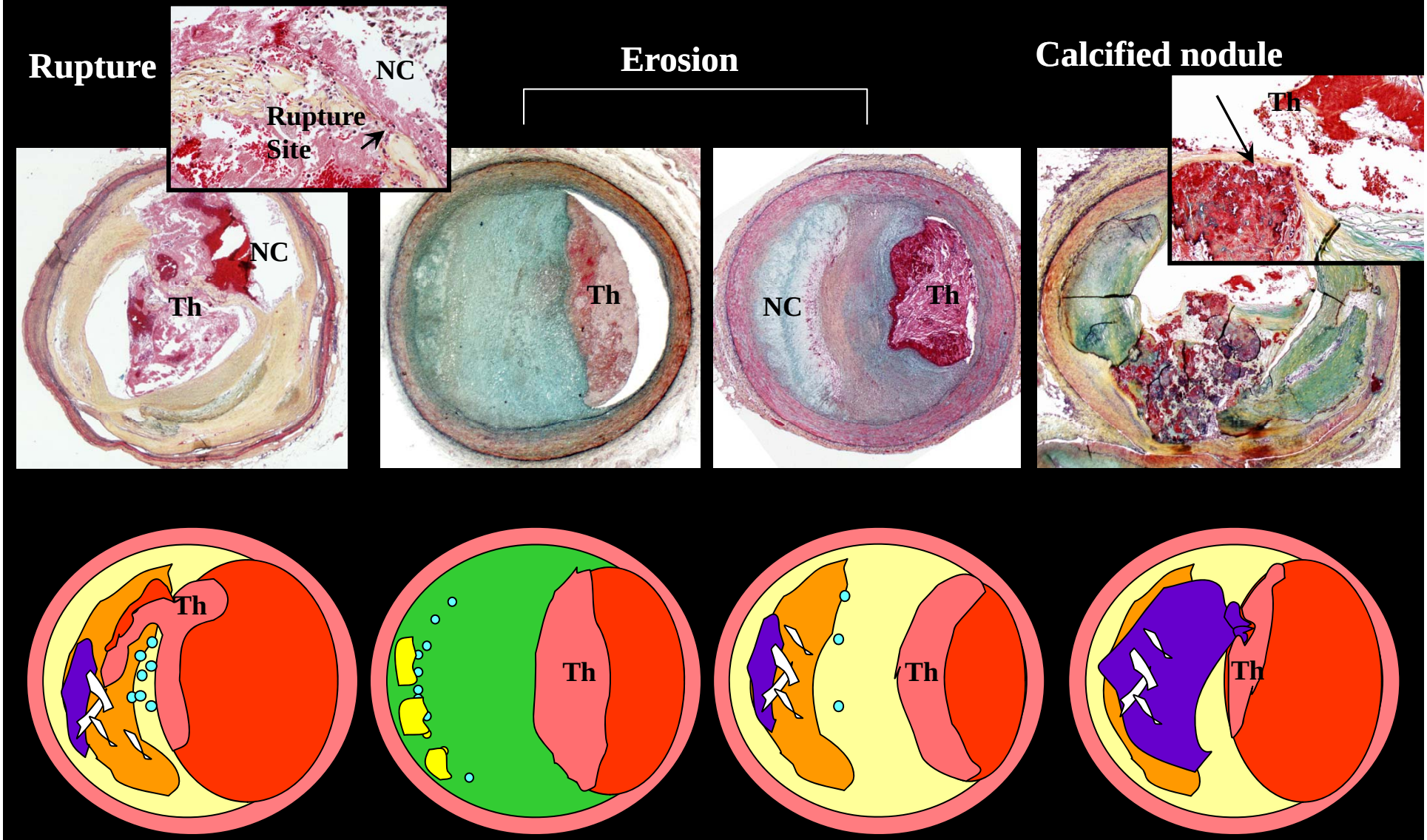
necrotic core

early → late necrosis

Lesions with Thrombi

- *Plaque Rupture*
- *Plaque Erosion*
- *Calcified Nodule*

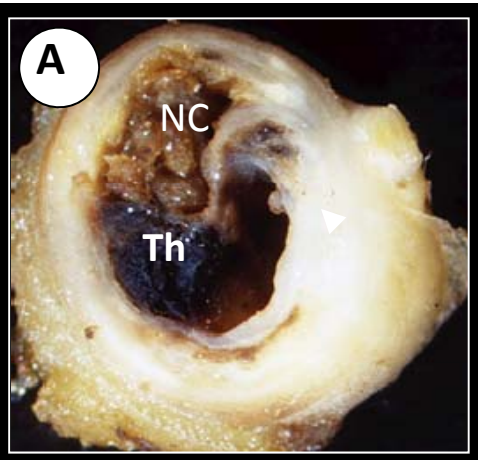
Causes of Coronary Thrombosis

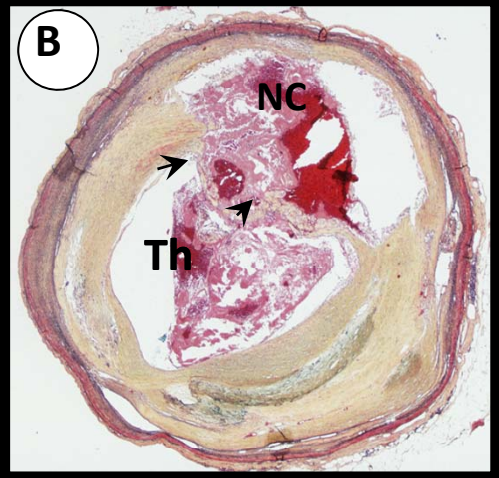


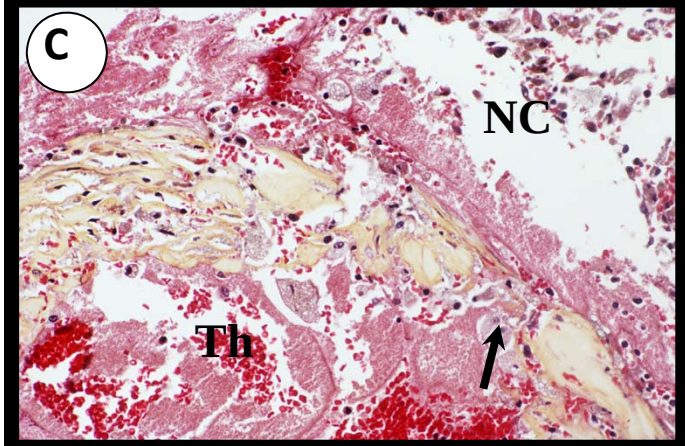
Virmani R, et al. Arterioscler Thromb Vasc Biol 2000;20:1262

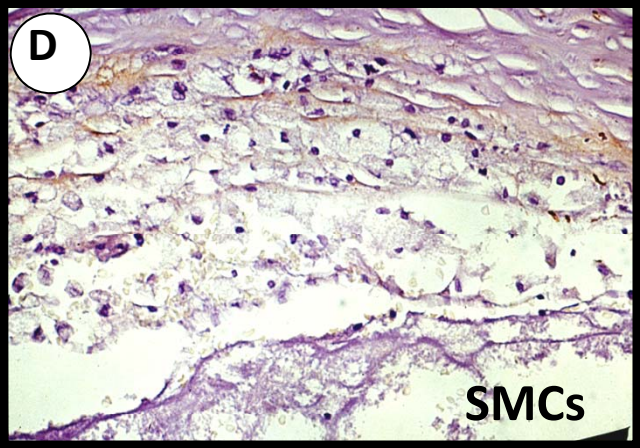
Gross and Light Microscopic Features of Plaque Rupture

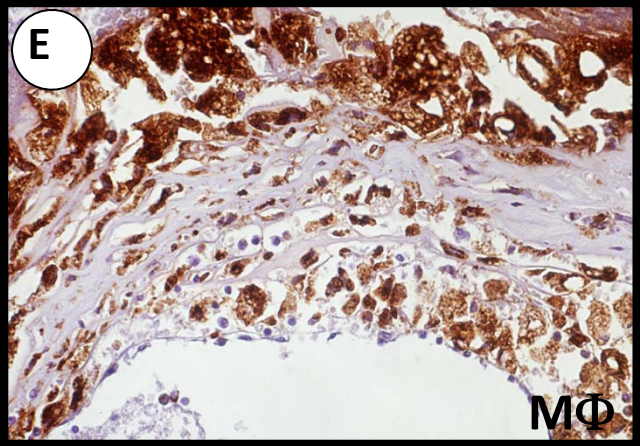
60-65% of Thrombi in Sudden Coronary Death occur form Plaque Rupture

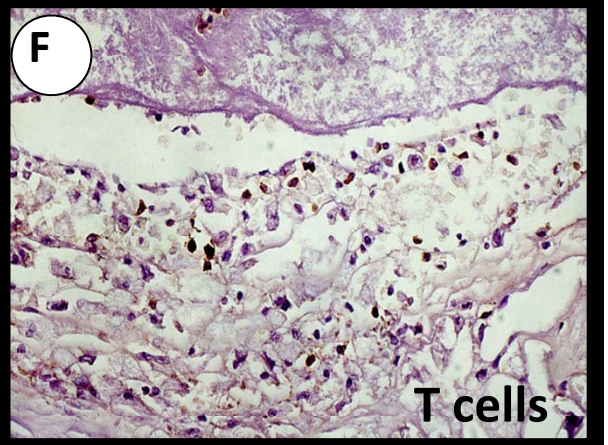


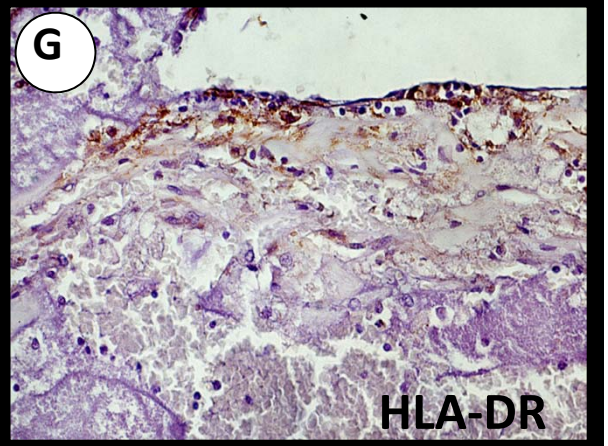






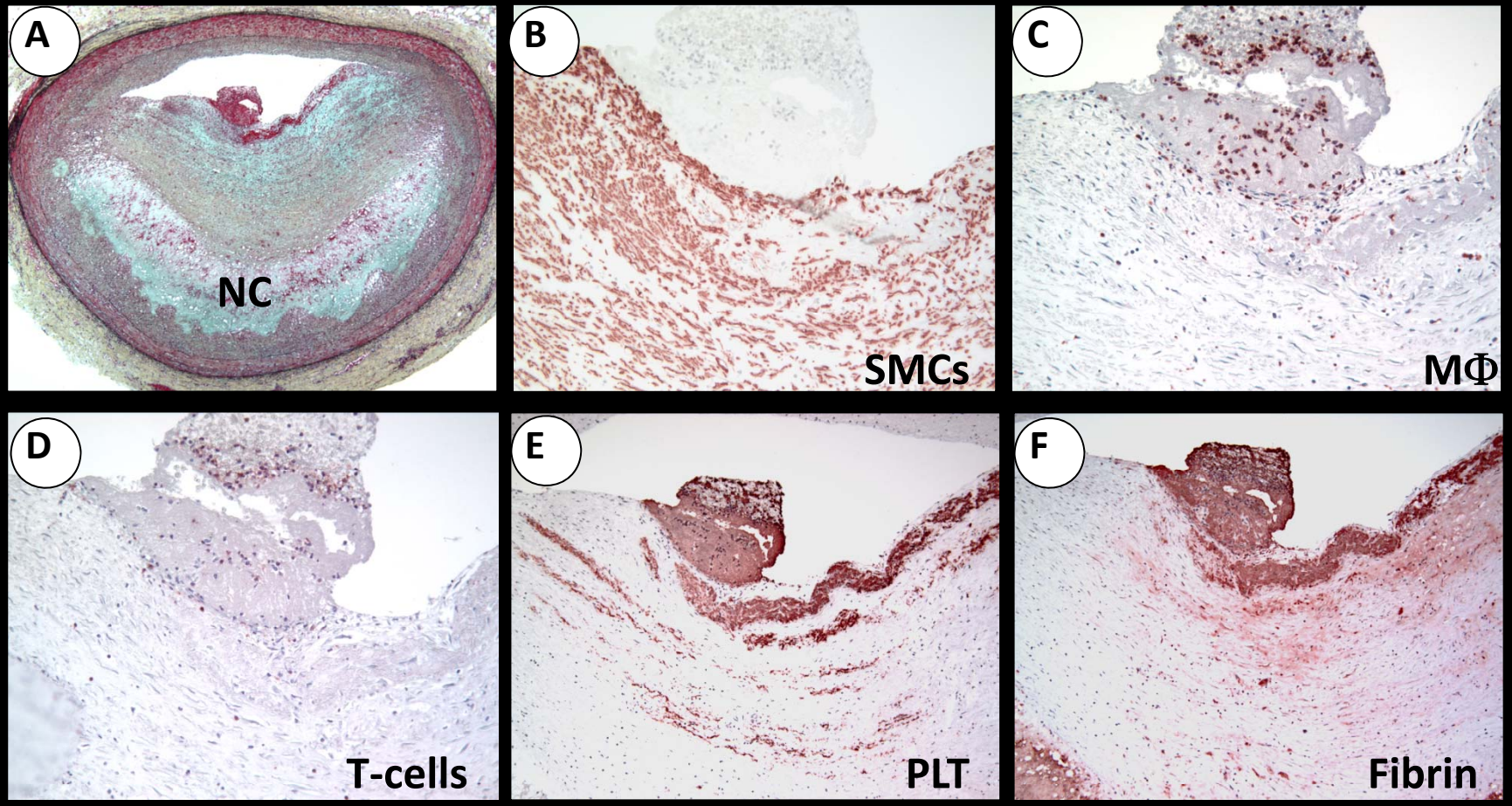




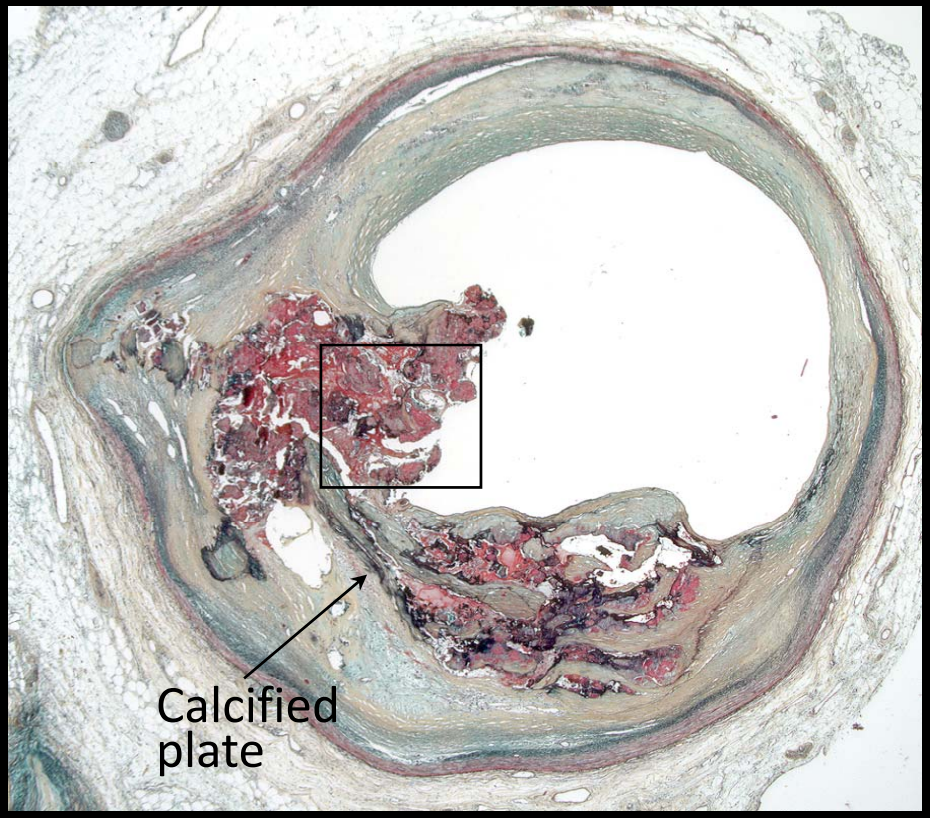


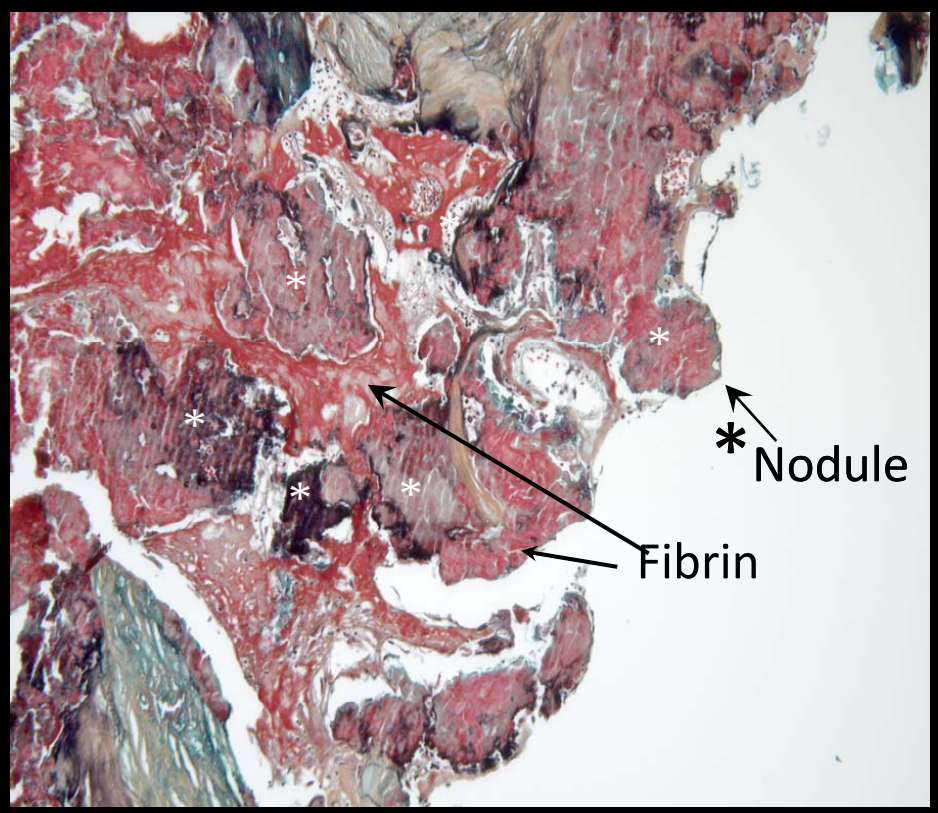
Plaque Erosion: 30-35% of thrombi in SCD

Plaque erosion in a 33 year-old female complaining of chest pain for two-weeks and discharged from the emergency room with a diagnoses of anxiety.



Calcified Nodule

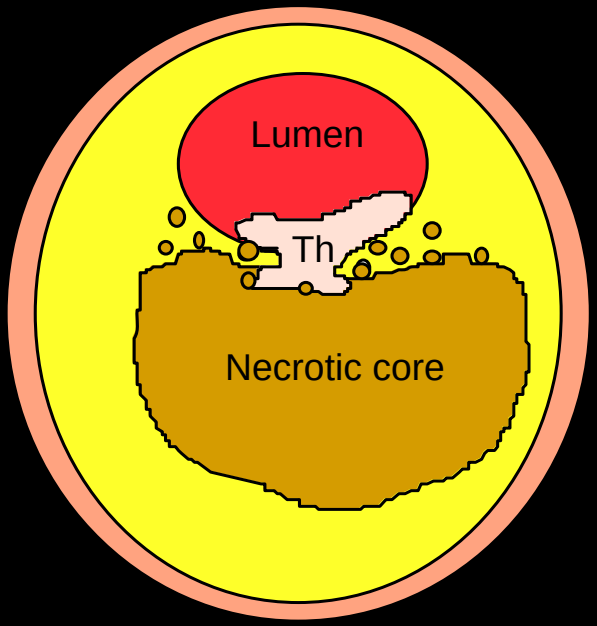




Frequency 2-7% of SCD, Older individuals, usually Men, T2D and Prediabetes, equally common in tortuous right and left coronary arteries

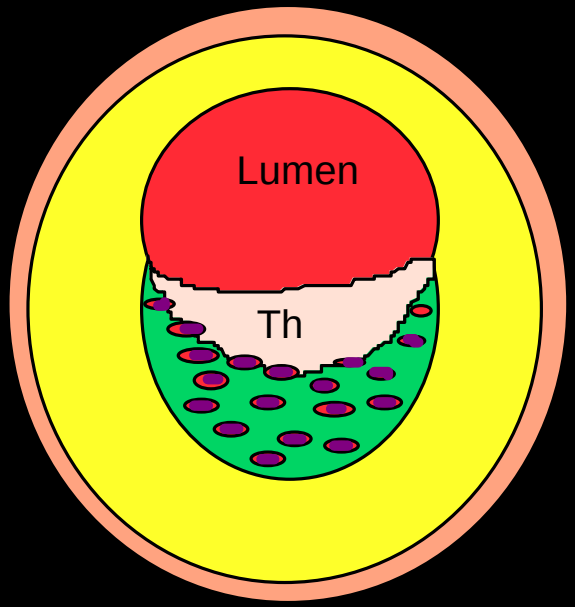
Clinical and Morphologic Difference in Plaques Associated with Luminal Thrombi

Plaque rupture



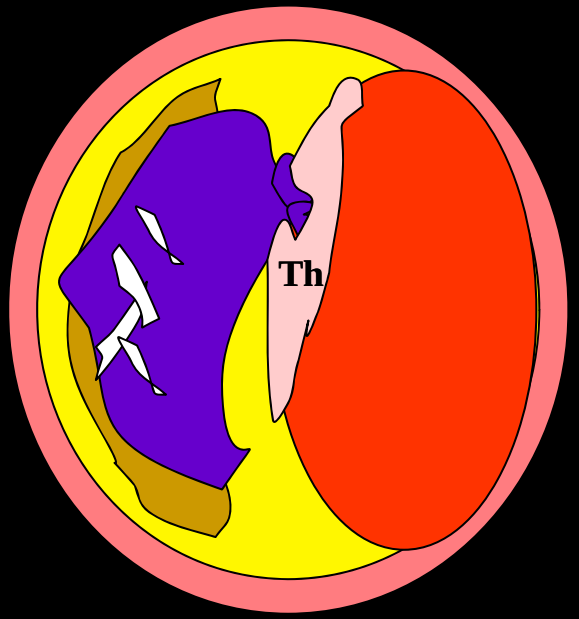
60% thrombi in SCD
M>F, Older, Ca^{++}
Eccentric = concentric
Greater % stenosis
Macs, T cells,
HLA-DR

Plaque erosion



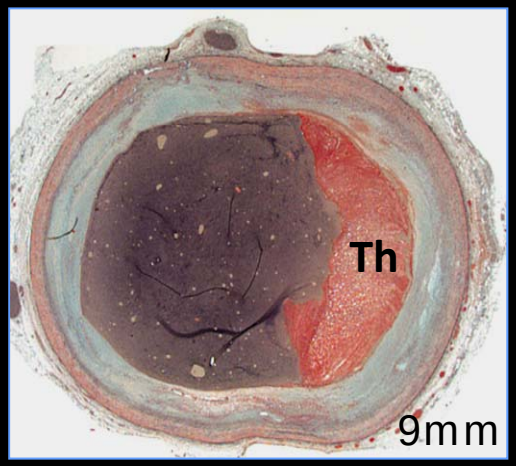
30-35% thrombi in SCD
M=F, younger
Usually eccentric
Lesser % stenosis
SMC rich, proteoglycans

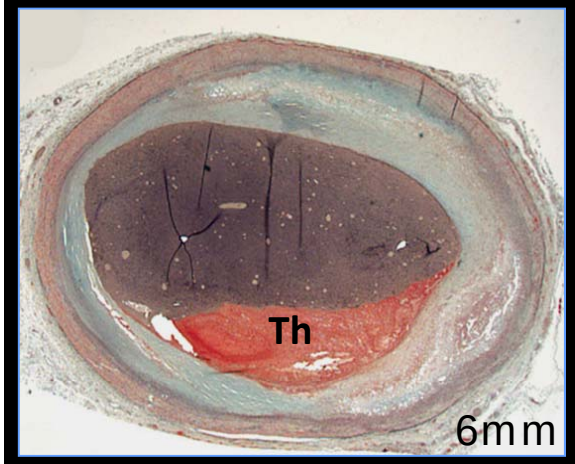
Calcified nodule

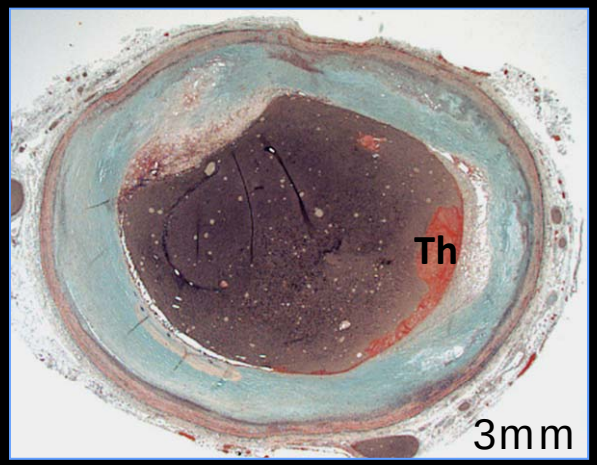


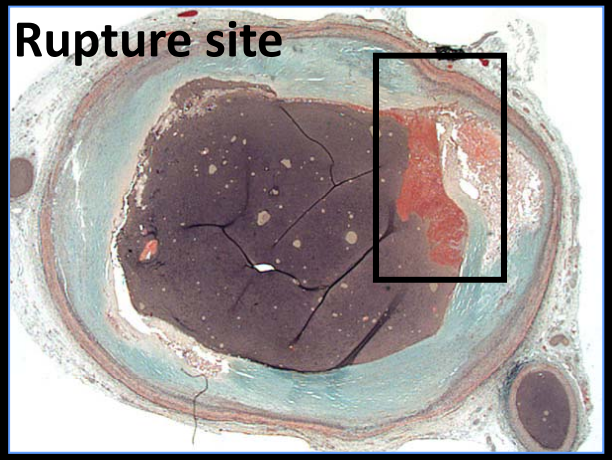
2-7% thrombi in SCD, calcified plates
M>F, older, mid RCA, LAD
Usually eccentric
Stenosis variable
Nodules of bone

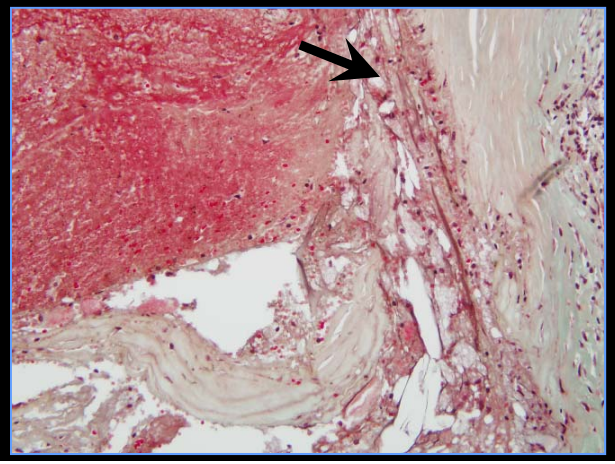
Plaque rupture with mild stenosis and non-occlusive thrombus: a mechanism by which plaques progress from an asymptomatic to symptomatic phase

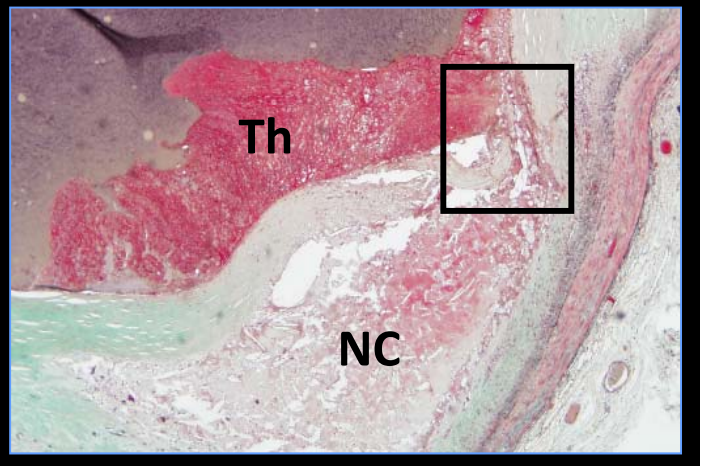






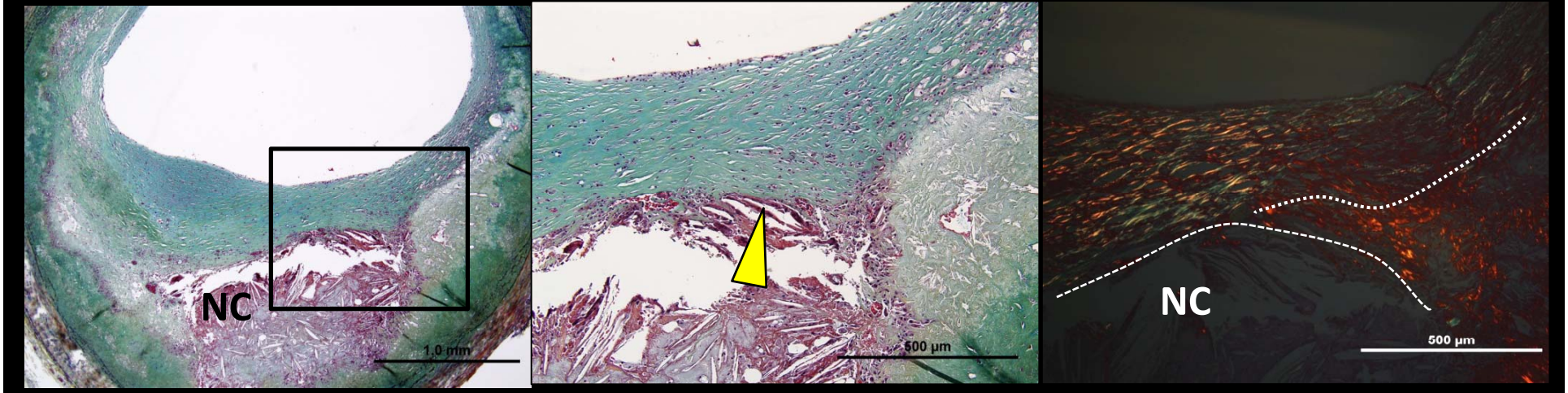




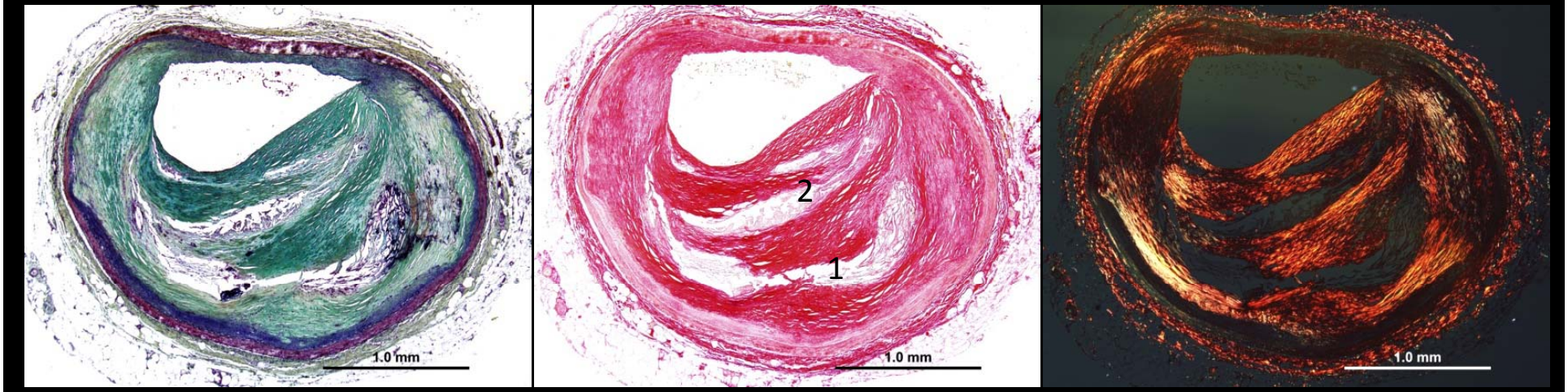


Healed lesions lead to plaque progression

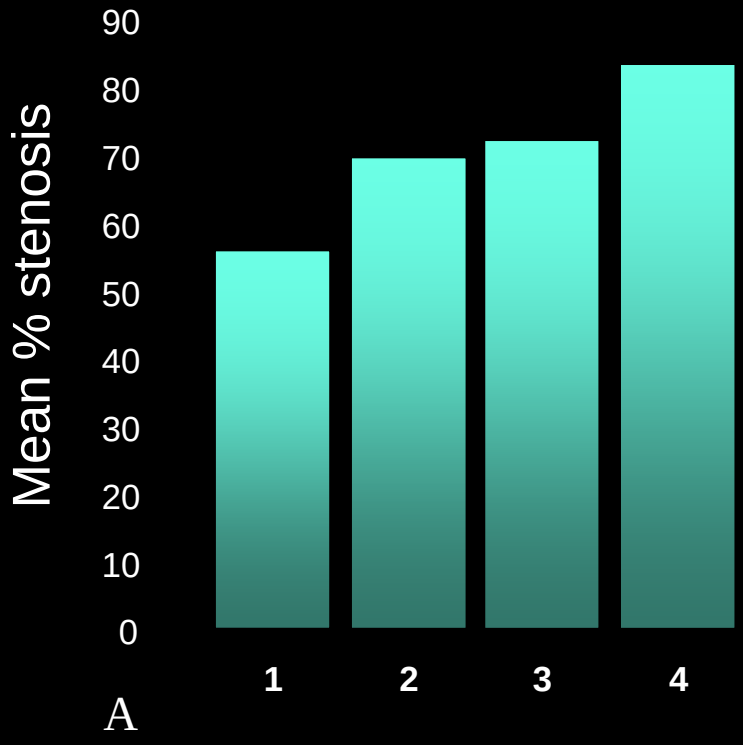
Healed plaque rupture

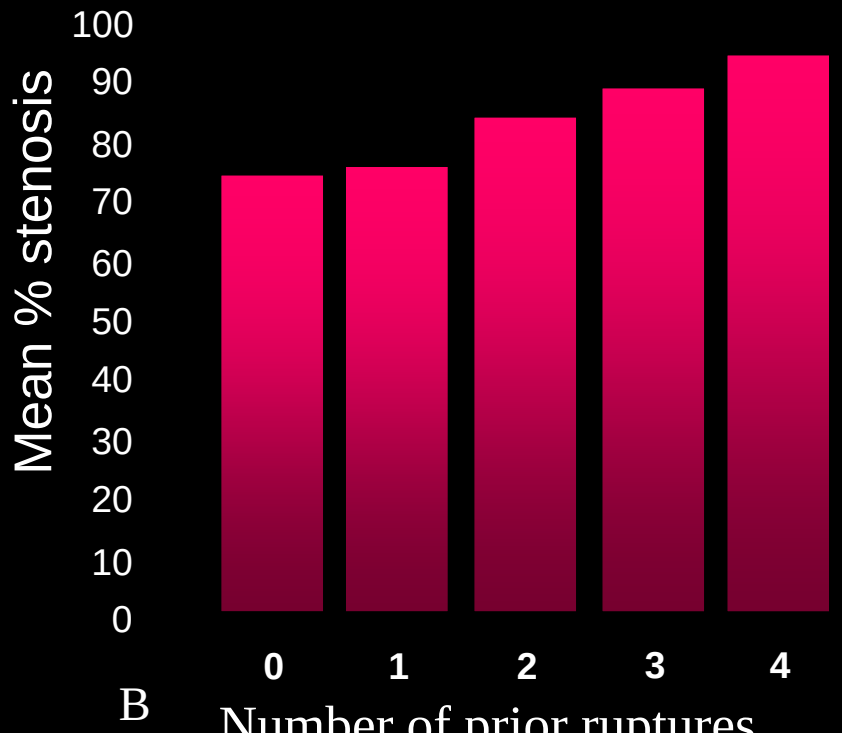


Multiple healed rupture



Mean % stenosis increases with number of prior rupture sites



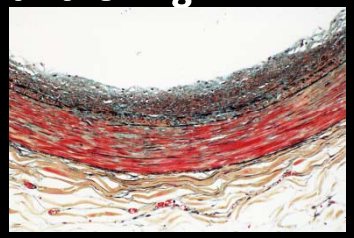


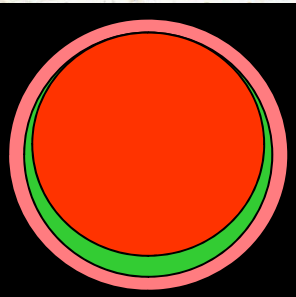
Burke, A P et al. Circulation 2001;103:9364-940

Progression of Human Coronary Atherosclerosis

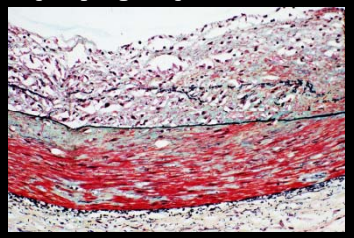
Virmani R, et al. ATVB2000;20:1262

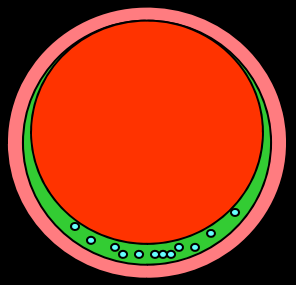
Intimal thickening



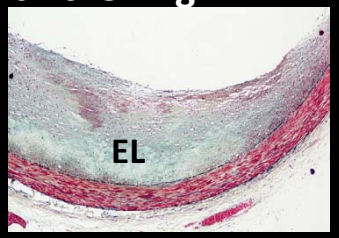


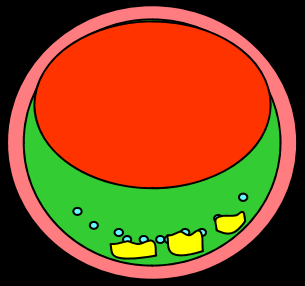
Intimal xanthoma



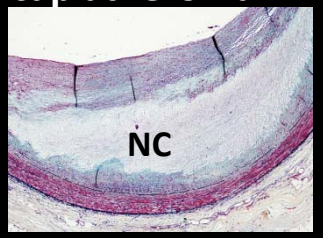


Pathologic intimal thickening

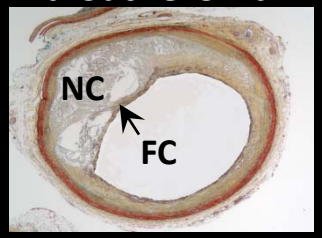


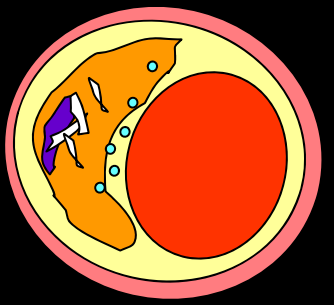


Fibrous cap atheroma

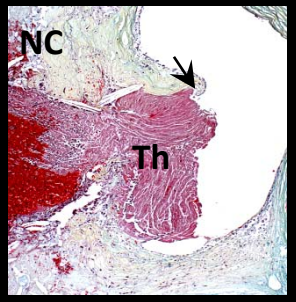


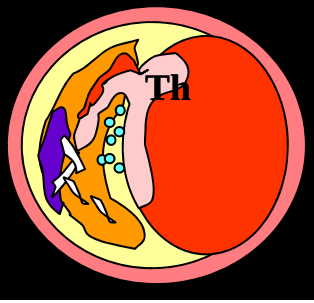
Thin-cap Fibroatheroma



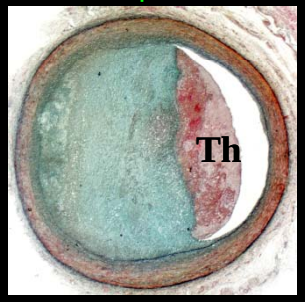


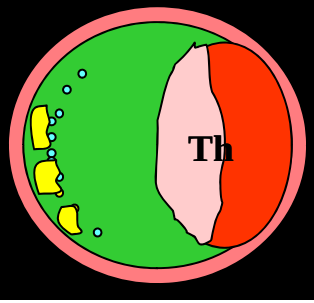
Rupture

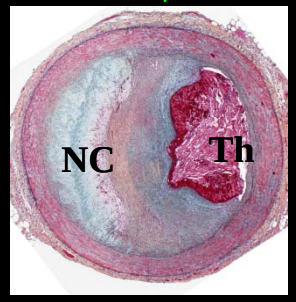


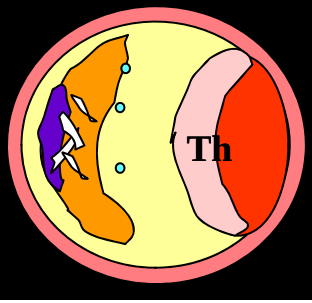


Erosion

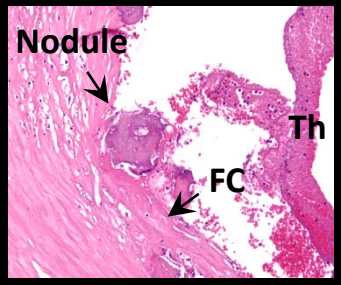


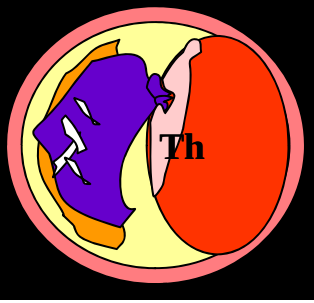




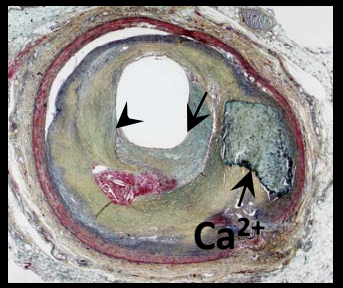


Calcified nodule



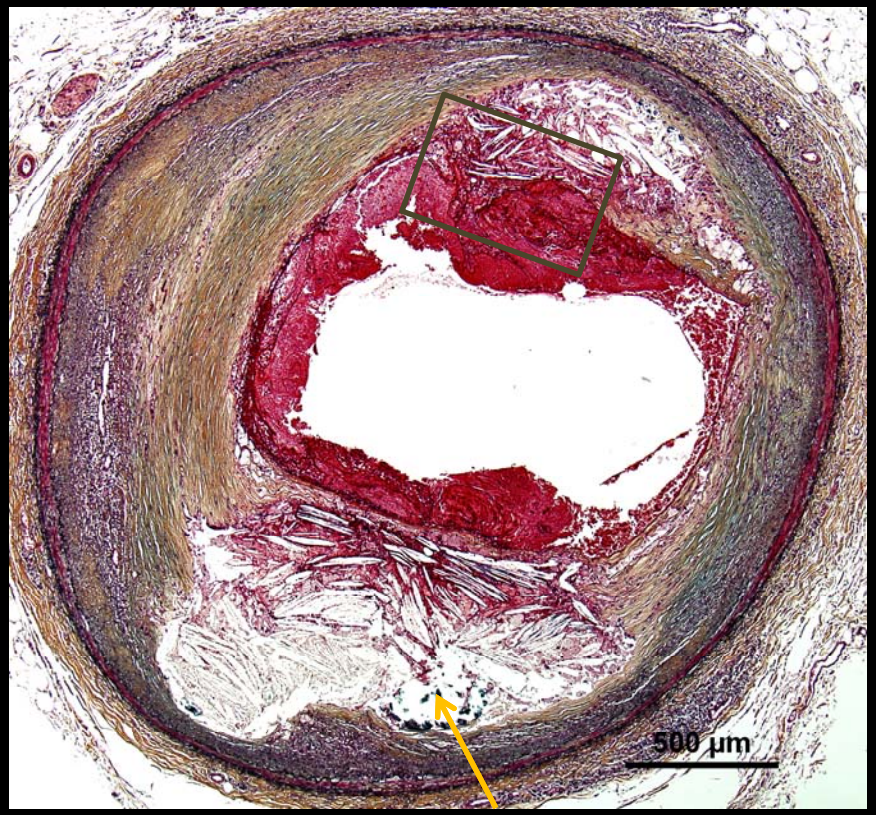


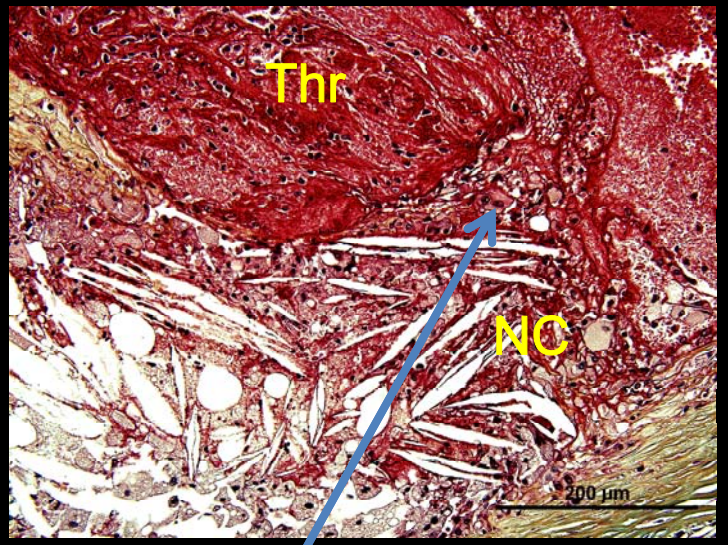
Healed Rupture



Development of Necrotic Core

The Necrotic Core
"graveyard of dead Mϕs"



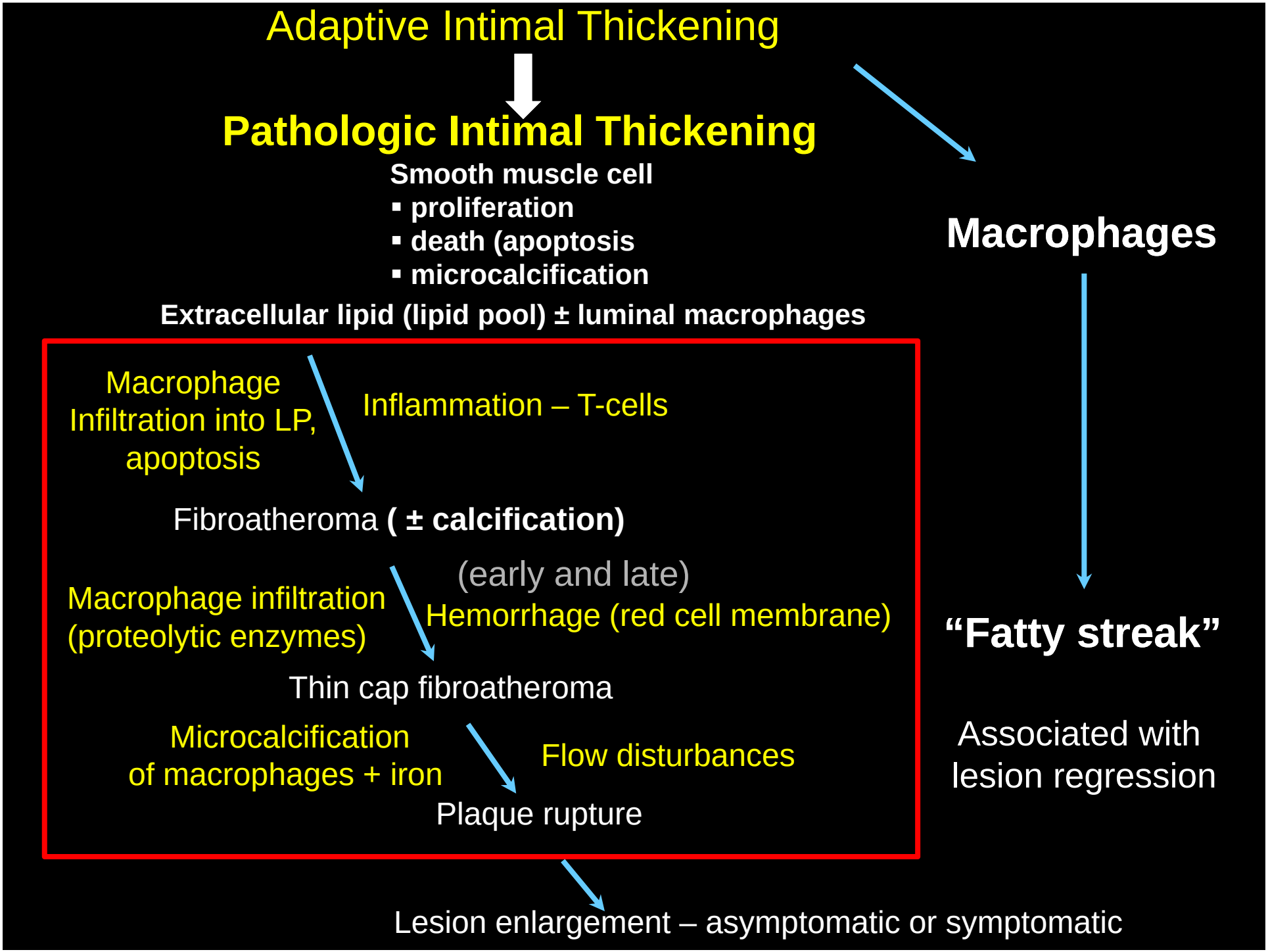


Ruptured plaque at area of thinned fibrous cap

Necrotic Core

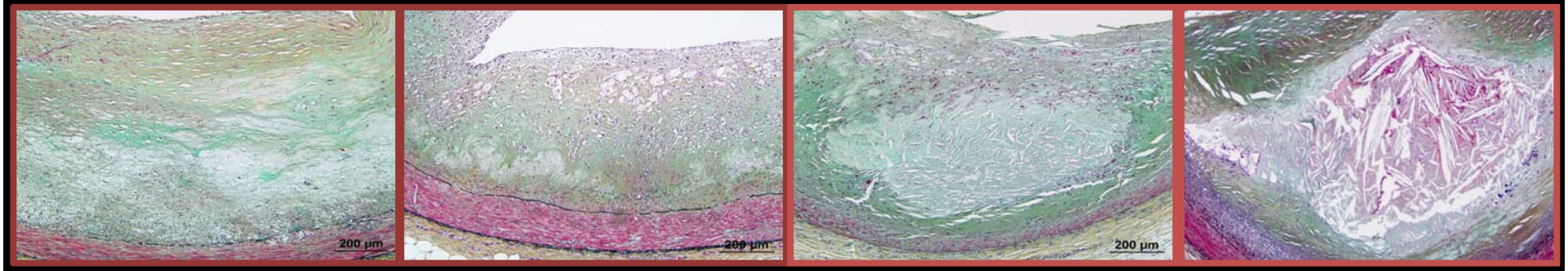
- inflammation
- Coagulation thrombosis
- proteases
- stress on fibrous cop

Thorpe and Tabas J Leukoc Biol 2009;86:1089-95


Adaptive Intimal Thickening
Pathologic Intimal Thickening
Smooth muscle cell
▪ proliferation
▪ death (apoptosis
▪ microcalcification
Extracellular lipid (lipid pool) ± luminal macrophages
Macrophages
Macrophage Infiltration into LP, apoptosis
Inflammation – T-cells
Fibroatheroma (± calcification)
(early and late)
Macrophage infiltration (proteolytic enzymes)
Hemorrhage (red cell membrane)
Thin cap fibroatheroma
Microcalcification of macrophages + iron
Flow disturbances
Plaque rupture
"Fatty streak"
Associated with lesion regression
Lesion enlargement – asymptomatic or symptomatic

Histomorphometric Analysis of Progressive Coronary Lesions

Pit (no Macs) Pit (+ Macs) Early FA Late FA



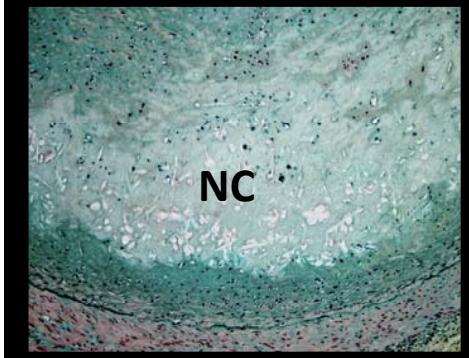
Lesion Type (n=61)	Plaque Area (mm2)	Stenosis (%)	NC Area (mm)	Macrophages (%)	Apoptotic cells/mm	No Vasa Vasorum
PIT - M (n=12)	3.3 ± 1.9	42 ± 10		0.7 ± 0.7	1.8 ± 1.3	
PIT + M (n=16)	2.5 ± 1.1	46 ± 14		1.8 ± 1.5	2.4 ± 1.6	4.6 ± 7.6
Early Fibroatheroma (n=19)	4.8 ± 2.5	60 ± 12	0.93 ± 0.92	3.1 ± 3.8	3.1 ± 2.1	10.1 ± 5.6
Late Fibroatheroma (n=14)	5.8 ± 2.7	70 ± 13	1.34 ± 0.70	4.3 ± 3.7	5.4 ± 3.3	31.1 ± 35.4
P value	<0.0001	<0.0001	0.16	<0.0001	0.004	0.02

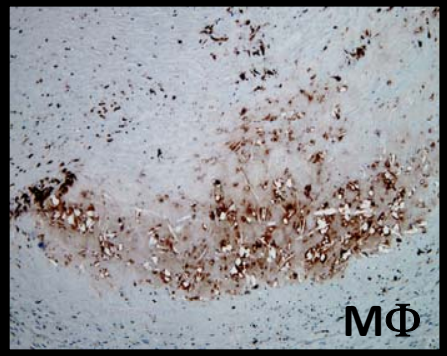
Differential Expression of Hyaluronan and Versican in the Developing Necrotic Core

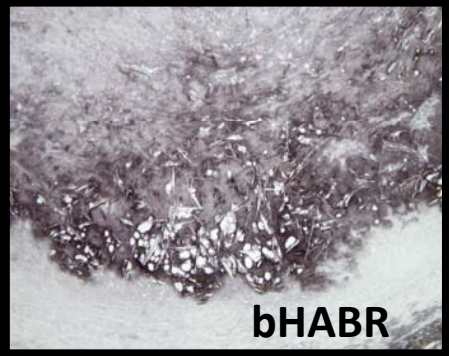
A

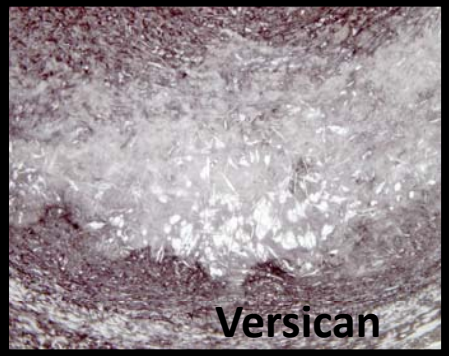
B

Fibroatheroma ‘Early’ Necrosis





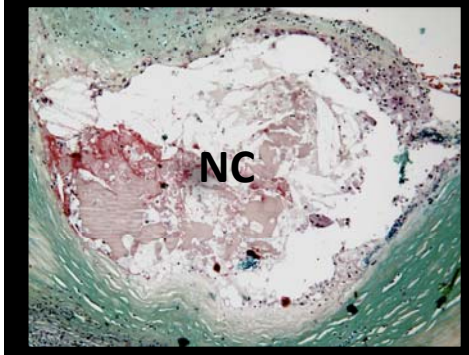


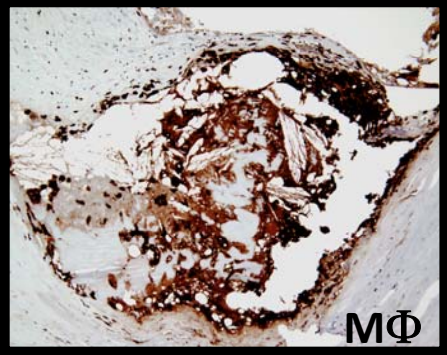


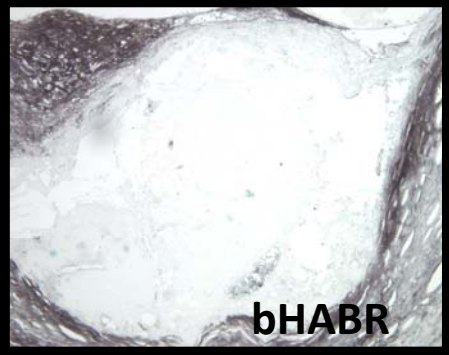
C

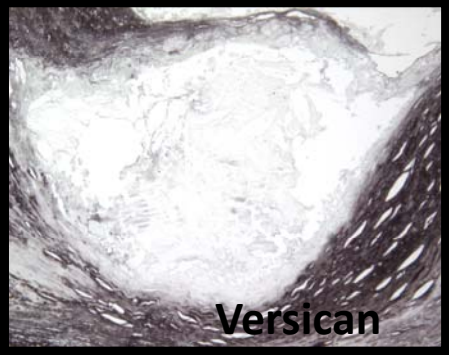
D

Thin-cap Fibroatheroma ‘Late’ Necrosis



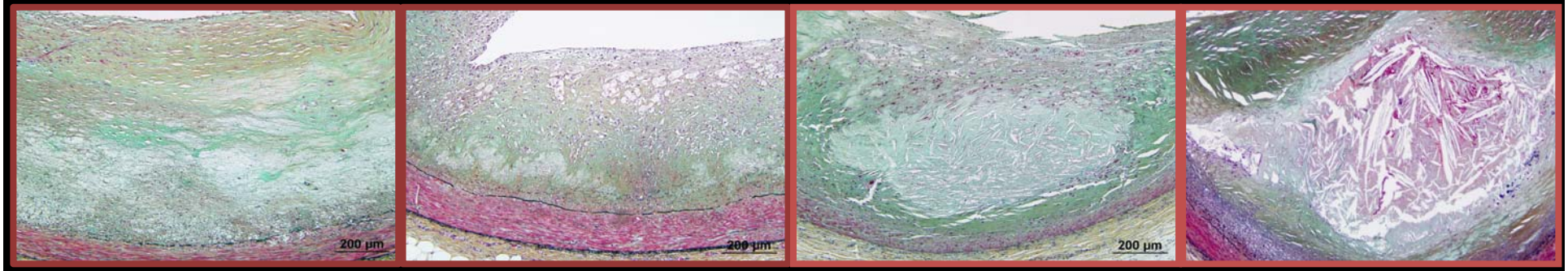




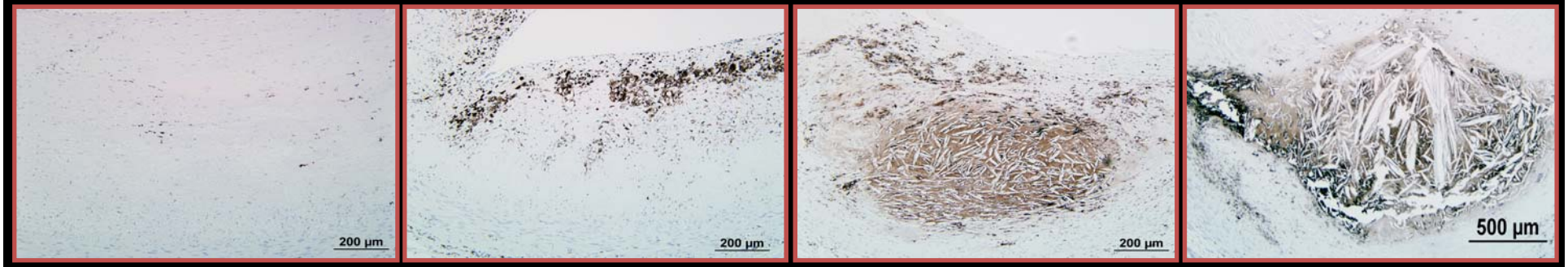


Histomorphometric Analysis of Plaque Component

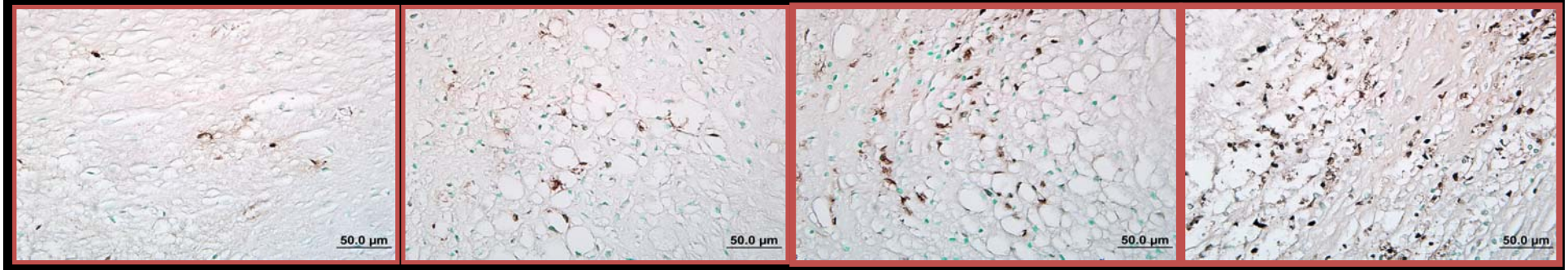
Pit (no Macs) | Pit (+ Macs) | Early FA | Late FA



Macrophage (KP-1.CD68)

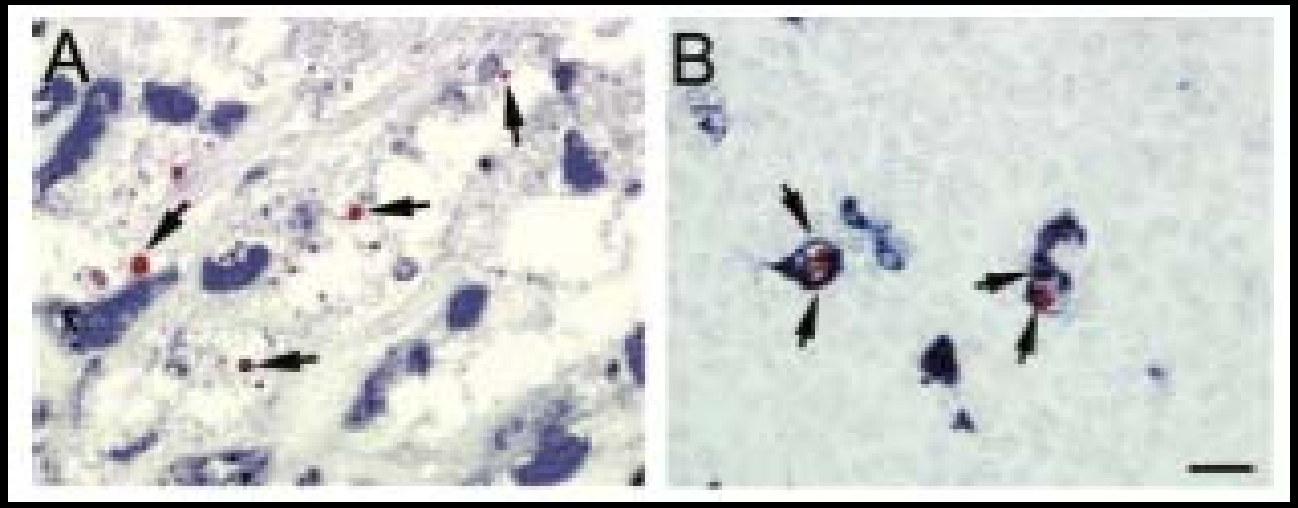


In-Situ End Labeling (DNA fragmentation, apoptosis)



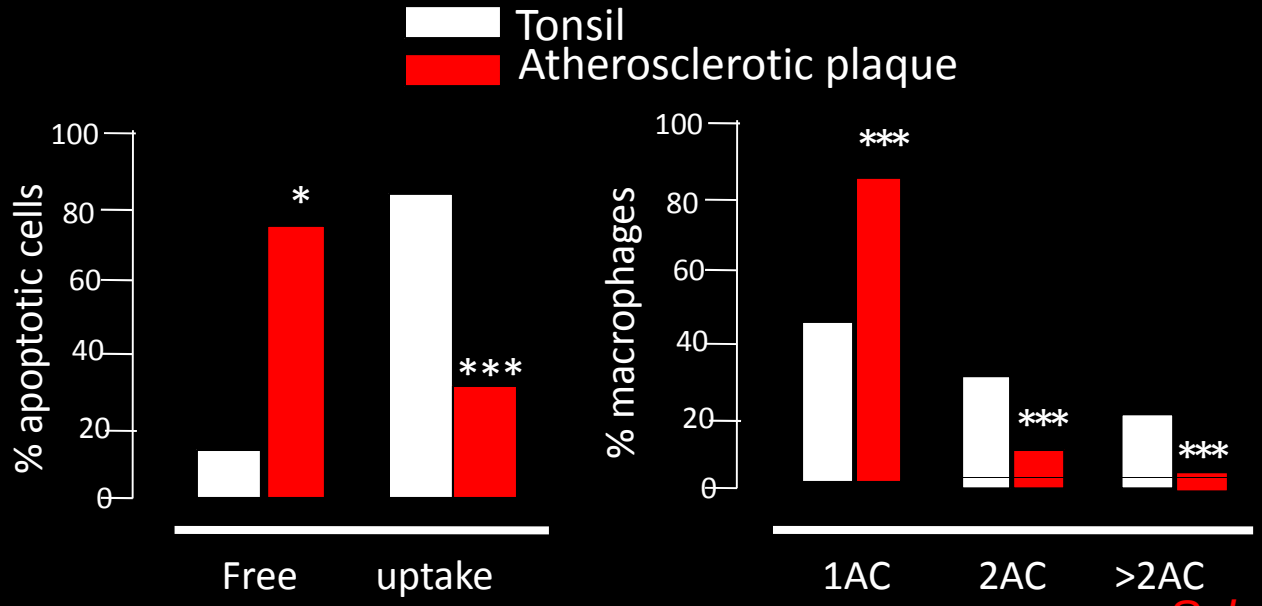
Phagocytosis efficiency of apoptotic cells (AC) in advanced atherosclerotic plaque and human tonsils

Carotid plaque **Tonsil**



TUNNEL (AC, red)

CD 68
(macrophages, blue)



The radio of free AC versus phagocytized AC was 19 times higher in athero-sclerotic plaques as compared to human tonsil

Schrijvers DM et al. ATVB,2007

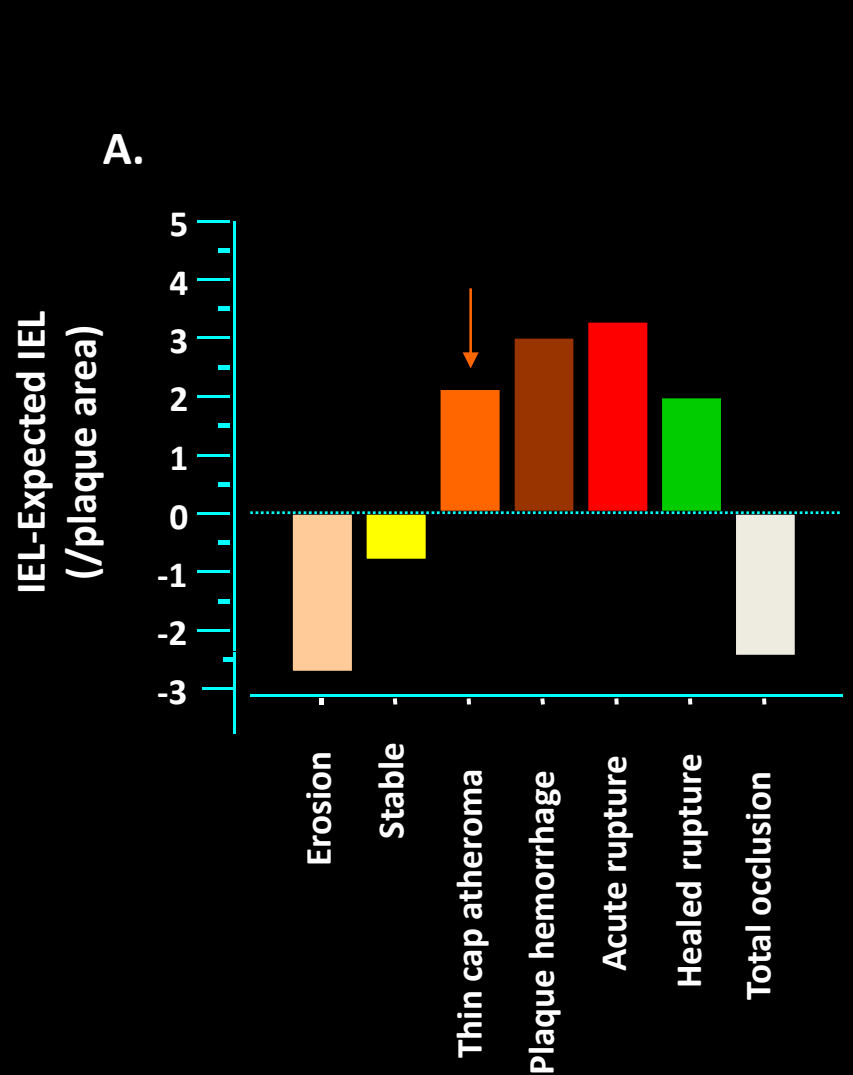
Morphologic Characteristics of Plaque Rupture and Thin-cap Fibroatheromas

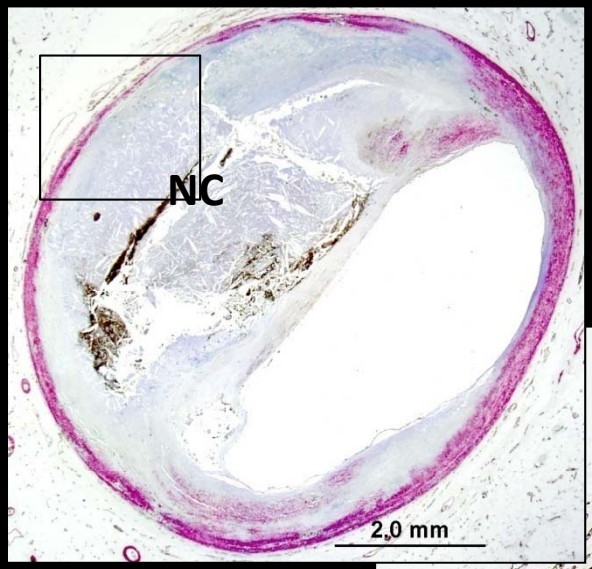
Plaque type	Necrotic Core (%)	Fibrous cap Thickness (μm)	MΦs (%)	SMCs (%)	T-lymph	Calcification Score
Rupture	34±17	23±19	26±20	0.002±0.004	4.9±4.3	1.53±1.03
Thin-cap Fibroatheroma	23±17	<65μm	14±10	6.6±10.4	6.6±10.4	0.97±1.1
P value	0.01		0.005	ns	ns	0.014

Mean values are represented ± standard deviation. Abbreviations: MΦs= macrophages, SMCs= smooth muscle cells, T-lymph= T-lymphocytes

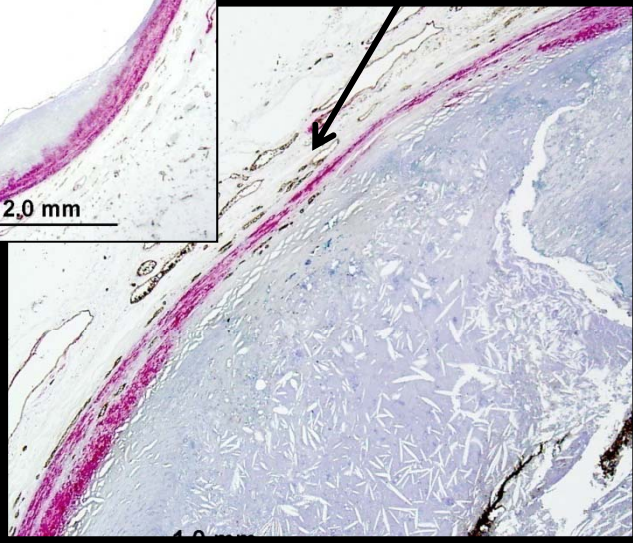
Kolodgie F, et al. Current Opinion in Cardiology 2001;16:285

Remodeling in Varying Coronary Lesion Morphologies

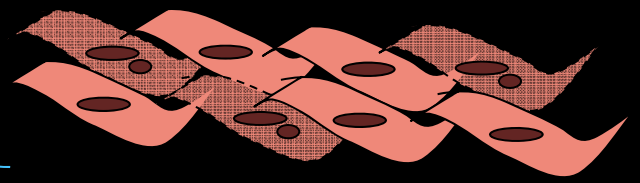




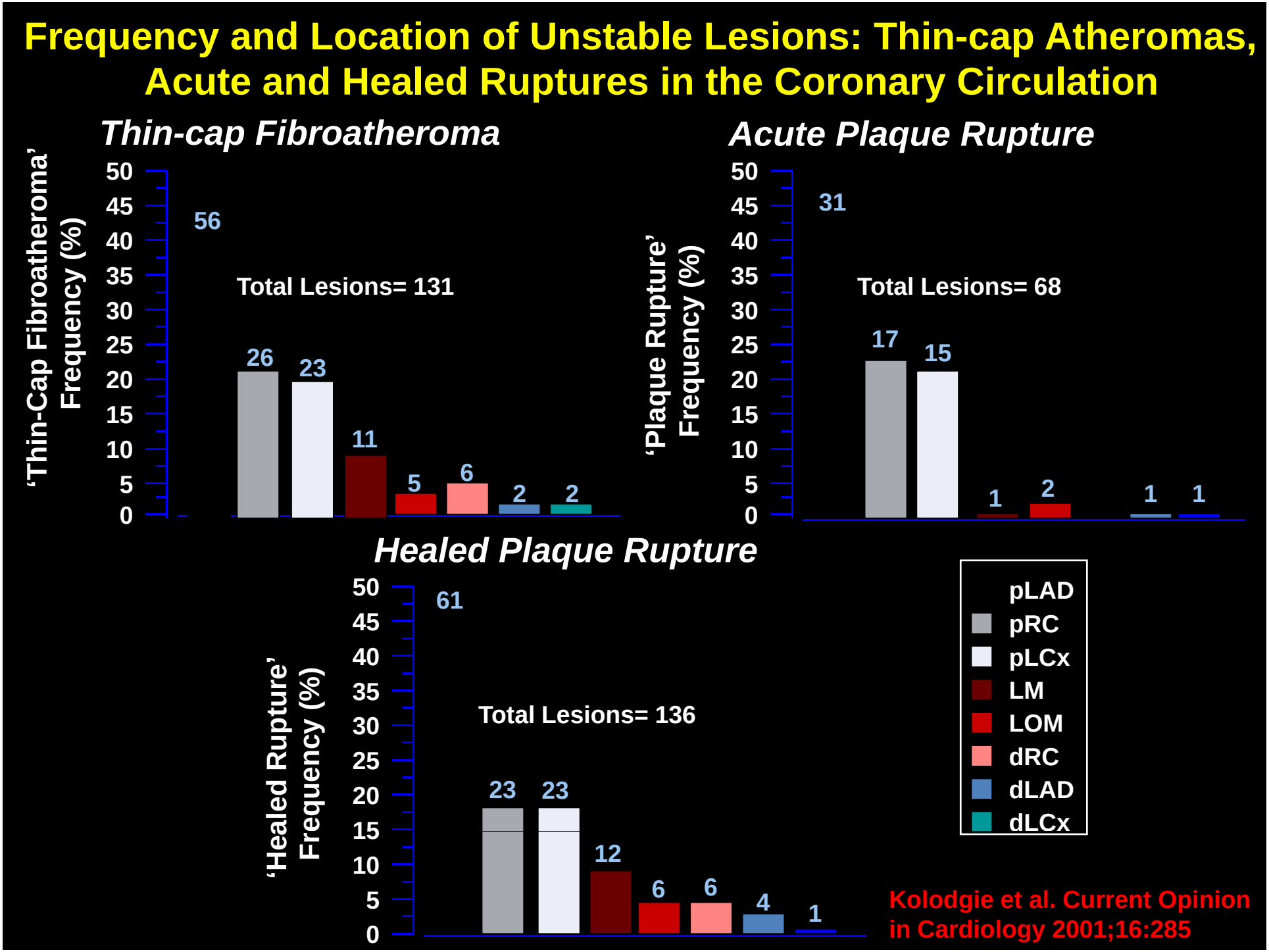
Medial SMC loss



Medial SMC apoptosis



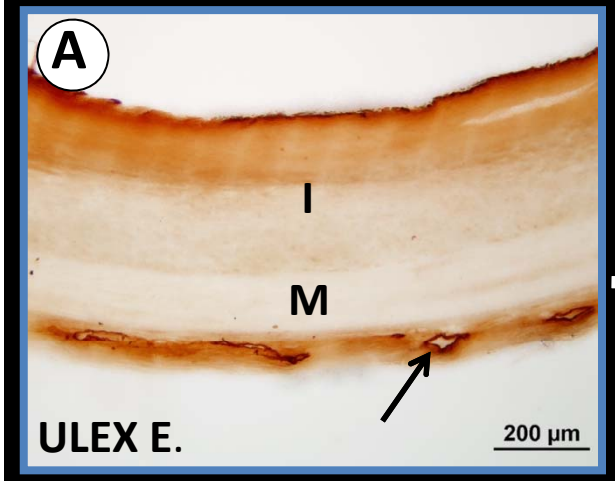
Frequency and Location of Unstable Lesions: Thin-cap Atheromas, Acute and Healed Ruptures in the Coronary Circulation



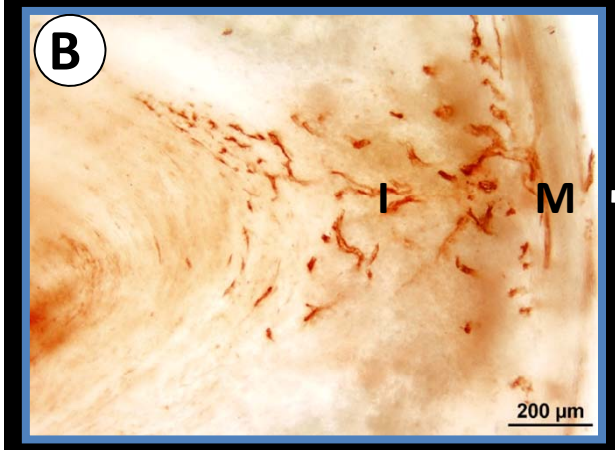
Kolodgie et al. Current Opinion in Cardiology 2001;16:285

Intraplaque Hemorrhage, Oxidant Stress & Plaque Vulnerability

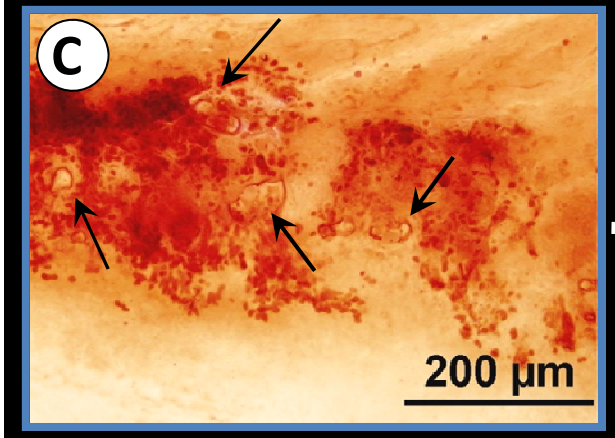
Evidence that Human Coronary Plaques Express a Latent Proangiogenic Phenotype



Normal artery with adventitial Vv

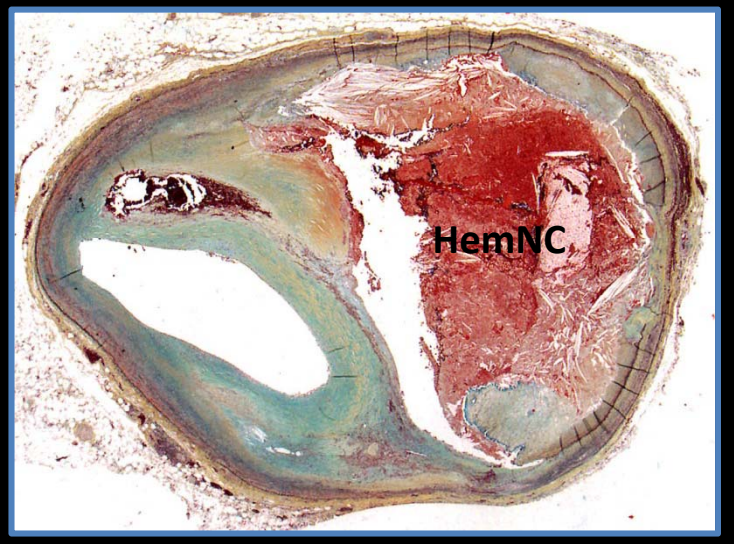


Fibroatheroma with Tortuous and Abnormal Vv



Fibroatheroma with Leaky Vv (peri-vascular hemorrhage)

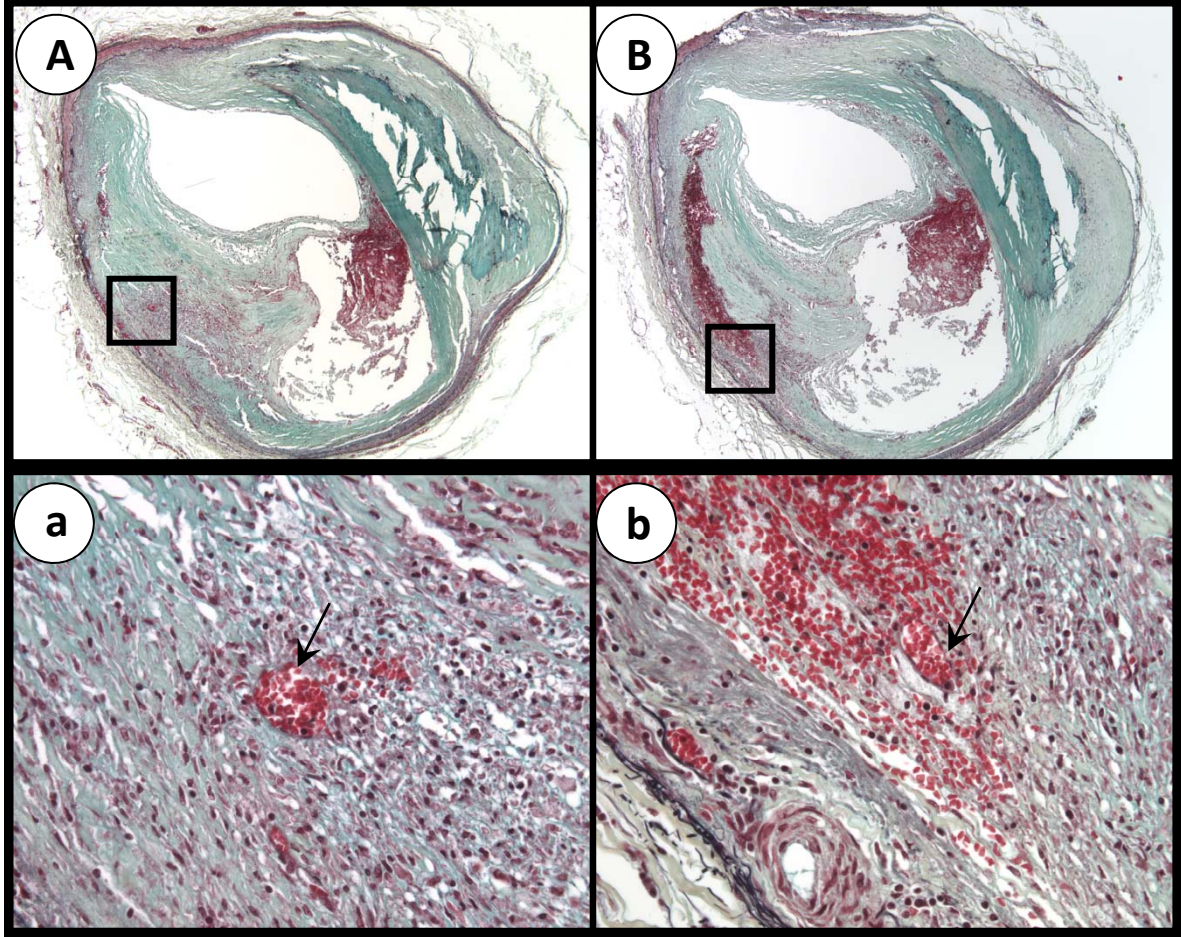
Fibroatheroma with severe Intraplaque hemorrhage

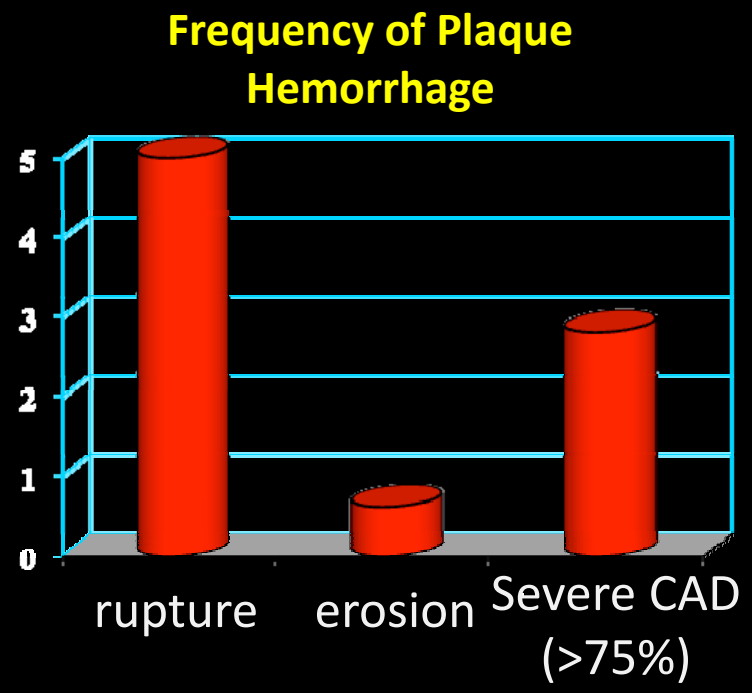


Modified from Jain et al., Nat Clin Pract Cardiovasc Med, 2007)

Thin-cap Fibroatheroma

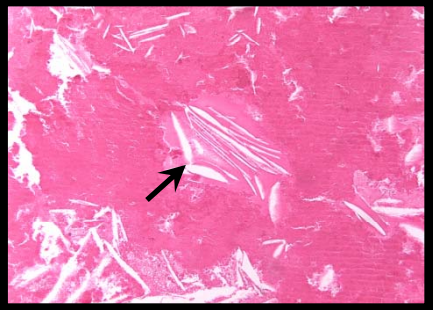
Recent Intraplaque Hemorrhage is seen at Multiple sites in Patients Dying SCD

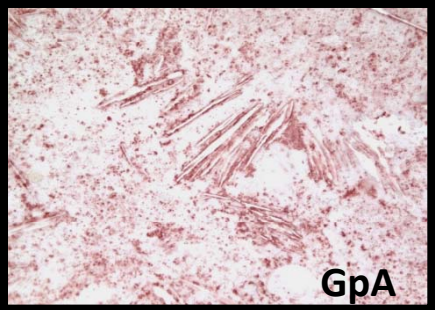


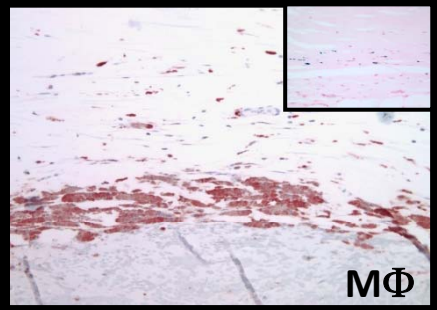


Morphometric Analysis of Hemorrhagic Events in Human

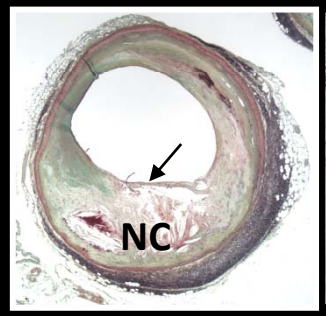
Hemorrhagic Pericarditis

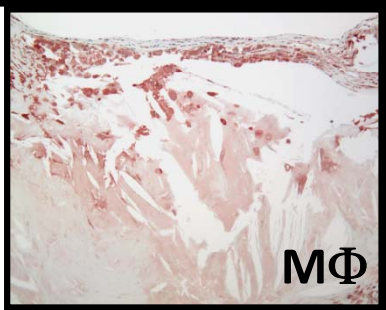


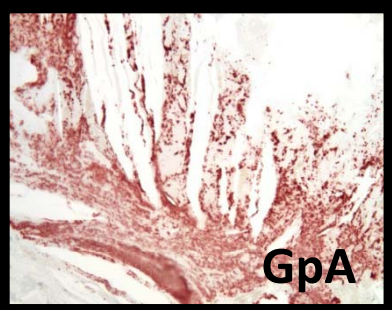


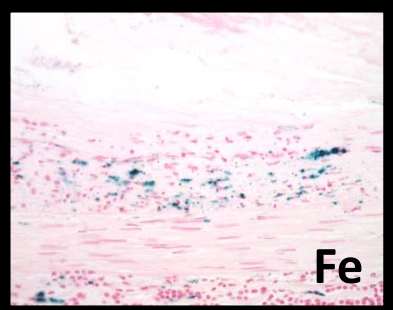


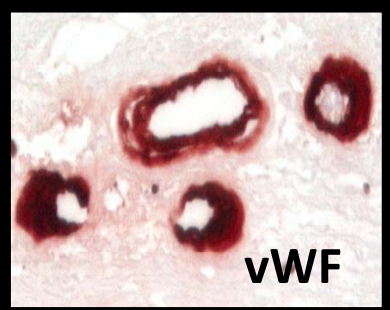
Vulnerable Plaque











Plaque Type	GpA Score	Iron	Necrotic Core (mm^2)	MΦ (mm^2)
PIT ***no*** **core** *(n=129)*	0.09±0.04	0.07±0.05	0.0	0.002±0.001
FA *early* core *(n=79)*	0.23±0.07	0.17±0.08	0.06±0.02	0.018±0.004
FA *late* core *(n=105)*	*0.94±0.11	*0.41±0.09	*0.84±0.08	*0.059±0.007
TCFA *(n=52)*	*1.60±0.20	*1.24±0.24	*1.95±0.30	*0.142±0.016

Values are reported as the means±SE, *p<0.001 versus early core. The number in parenthesis represent the number of lesions examined;the total number= 365. MΦ = macrophages

Kolodgie FD, et al. New Engl J Med 2003

Necrotic Core Formation

Mechanism(s) and Molecules

Early Necrosis

engulfment:

- Early foam cell apoptosis (via ER stress path
- Clearance by phagocytosis (efferocytosis)

defective engulfment: (molecular genetic causation studies in mice)

Late Necrosis

Excess foam cell apoptosis

Defective efferocytosis

1) Fas ligand (apoptosis stimulating fragment)
2) transglutaminase-2
3) lactadherin
4) Mertk (Mer receptor tyrosine kinase)

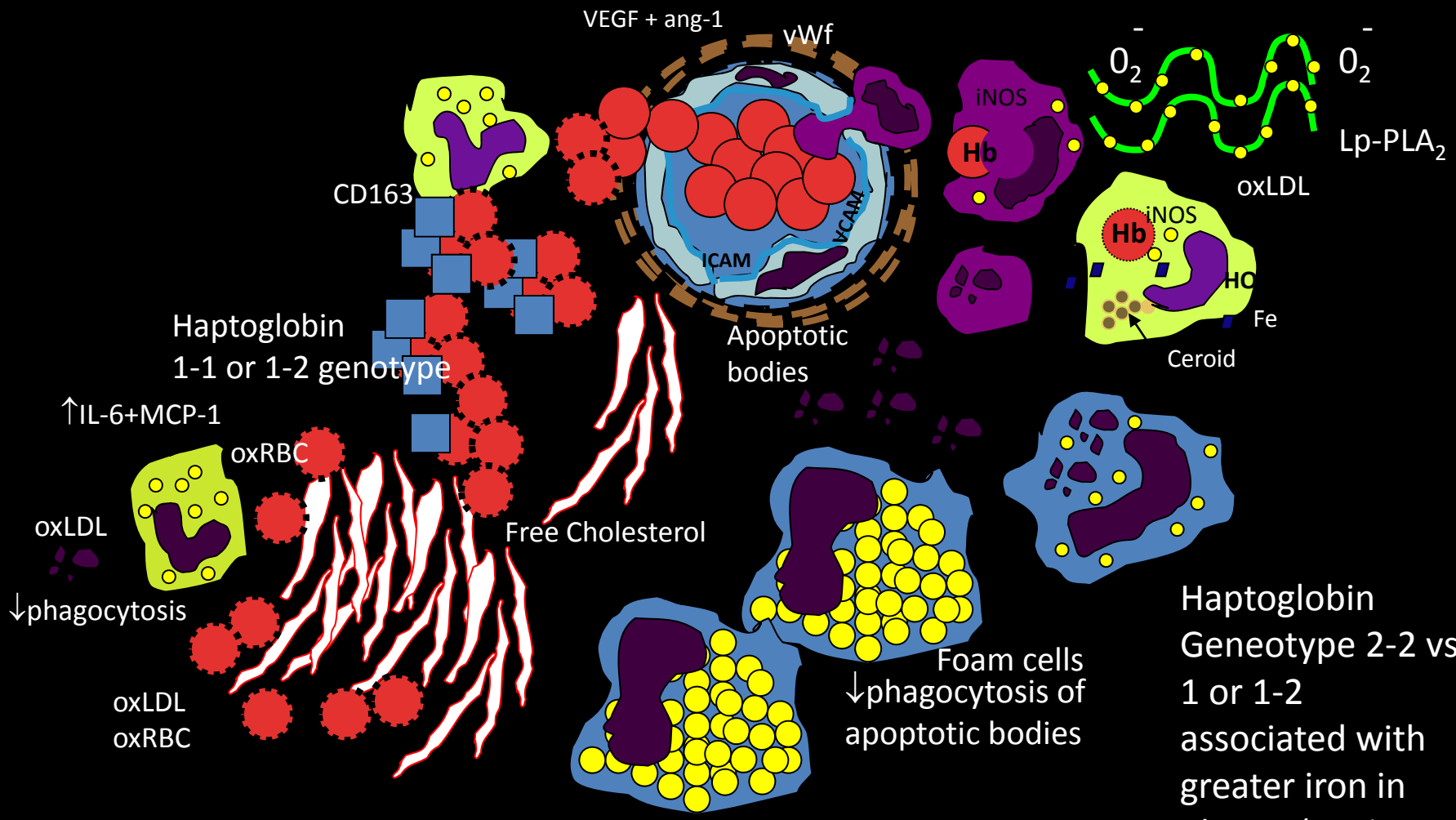
Hemorrhagic Necrosis

Excess

- free cholesterol
- free hemoglobin (Hb)
- macrophages

efferocytosis

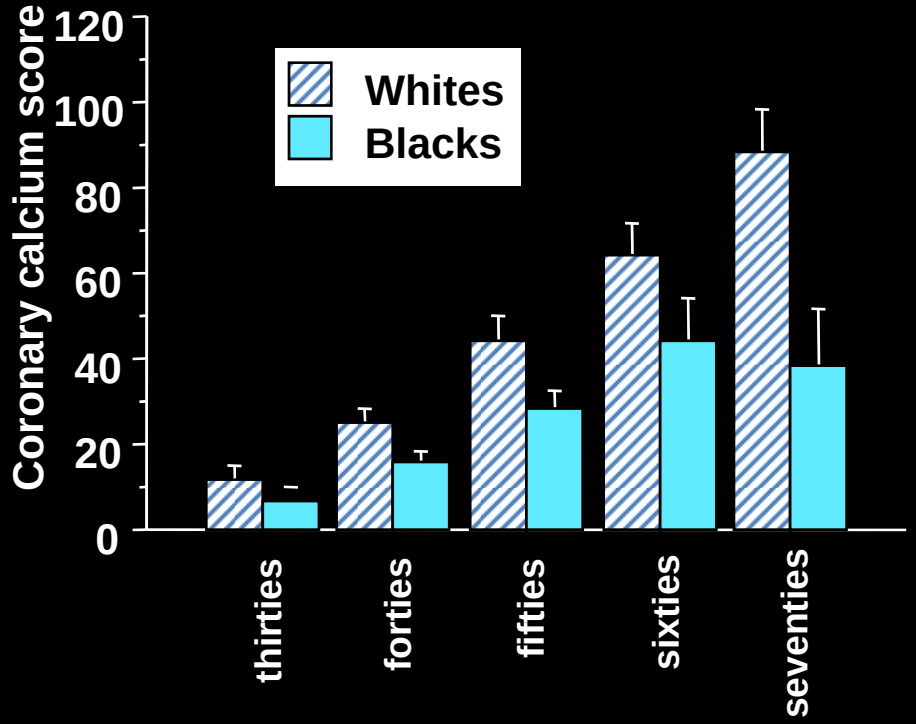
Role of Vasa Vasorum in the leakage of RBC into the plaque and macrophage activation

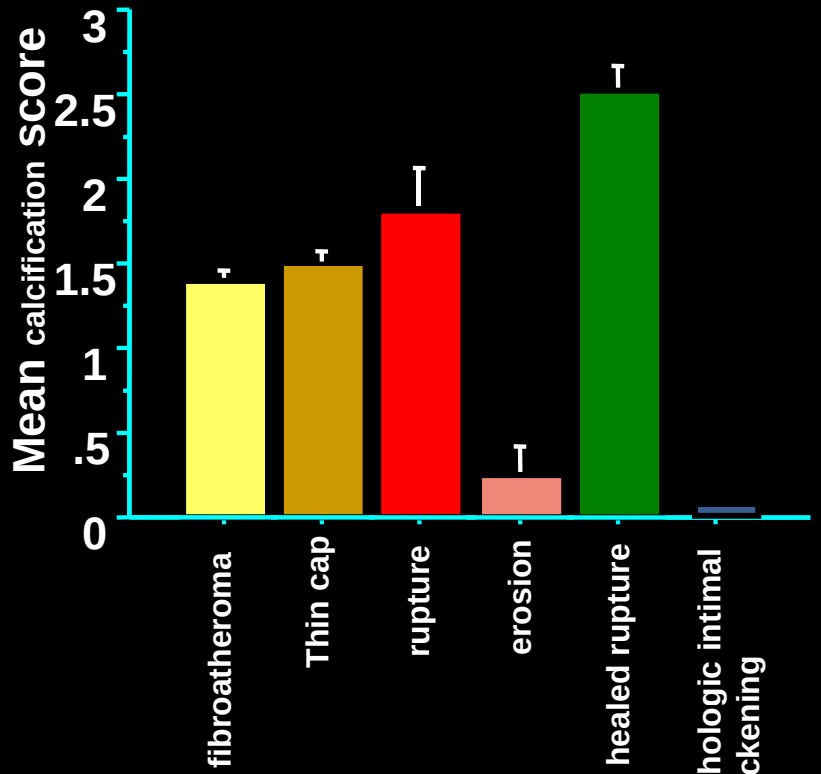


Haptoglobin Geneotype 2-2 vs.1-1 or 1-2 associated with greater iron in plaque (46% vs.12%) among T2D

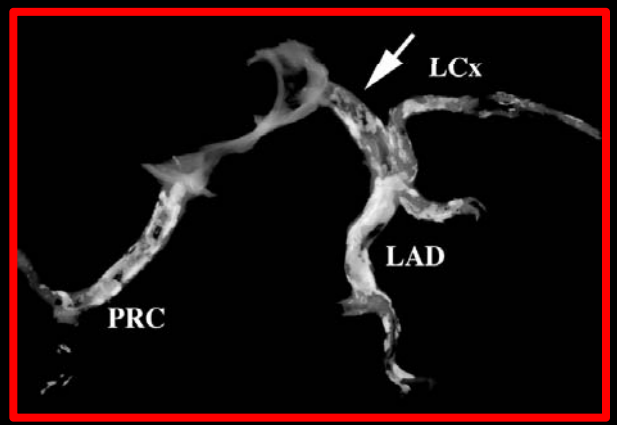
Development of Calcified Nodule

Patients dying from Sudden coronary Death: Extent of Coronary calcification by decades

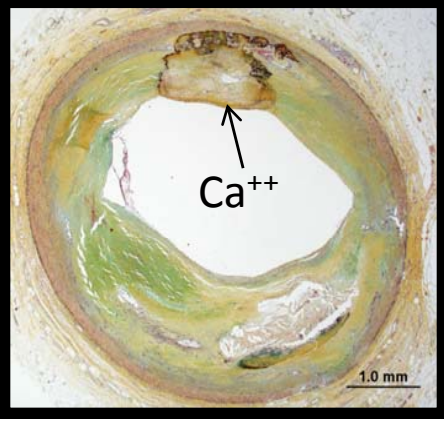




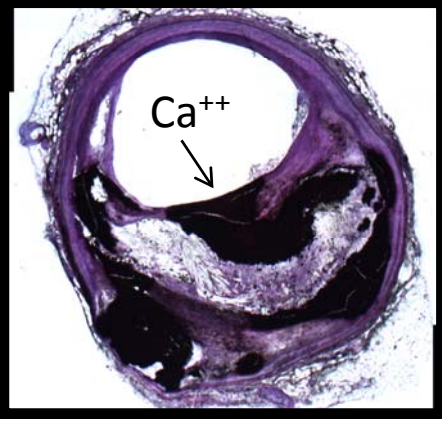
Radiograph



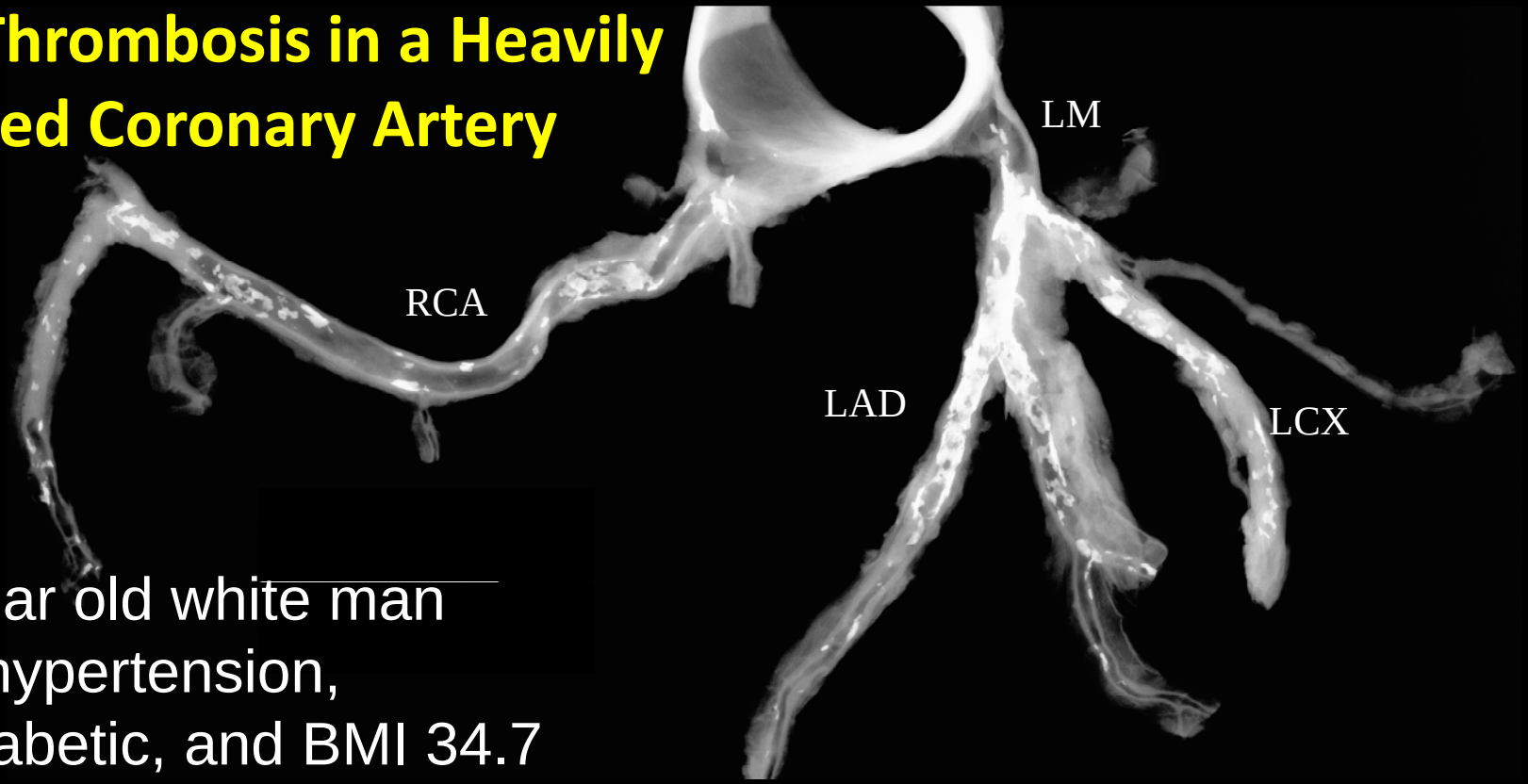
Movat Stain



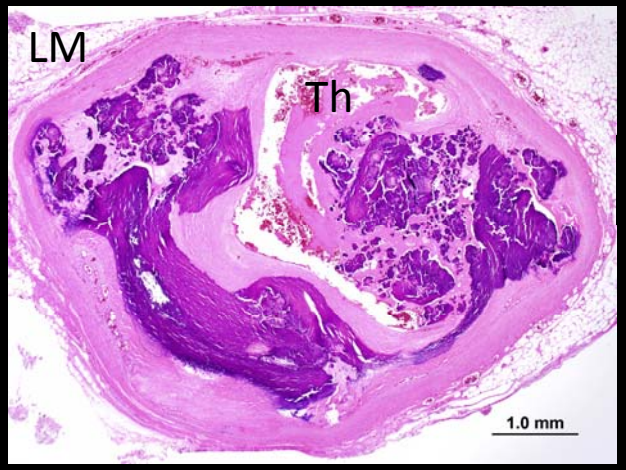
Ground Section (40 µm)

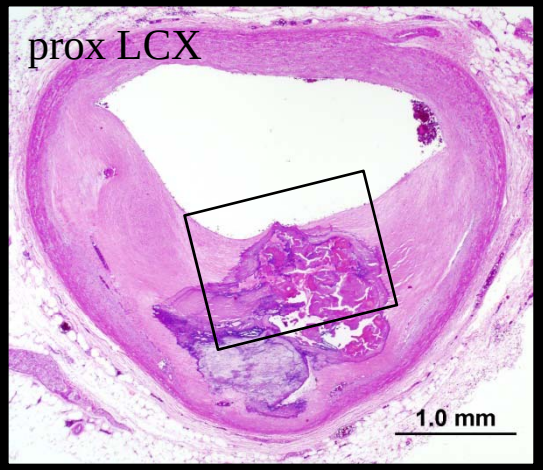


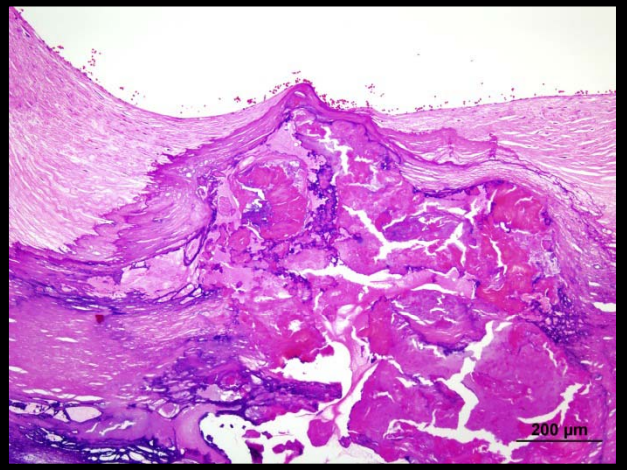
Eruptive Calcified Nodule with Thrombosis in a Heavily Calcified Coronary Artery



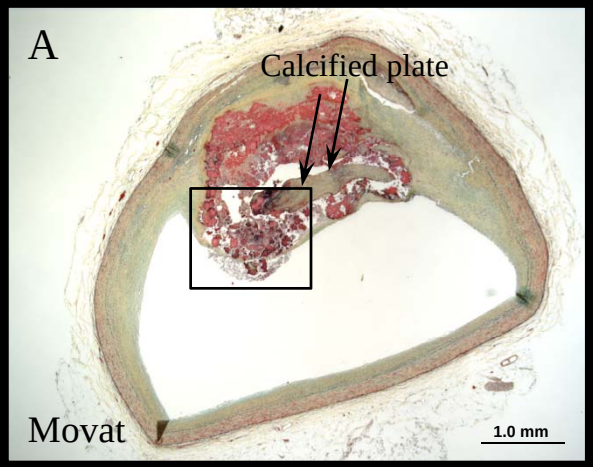
66 year old white man
H/O hypertension,
prediabetic, and BMI 34.7

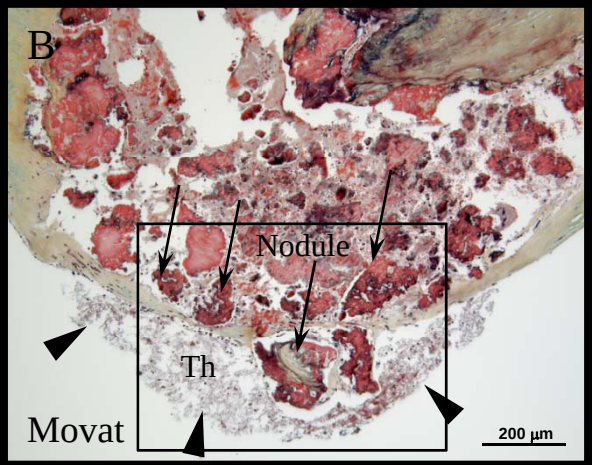


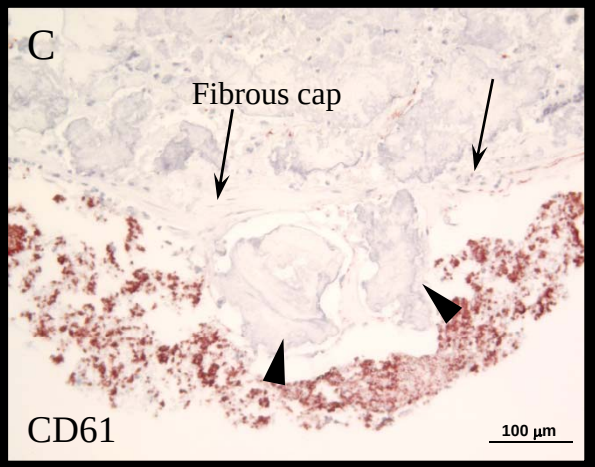


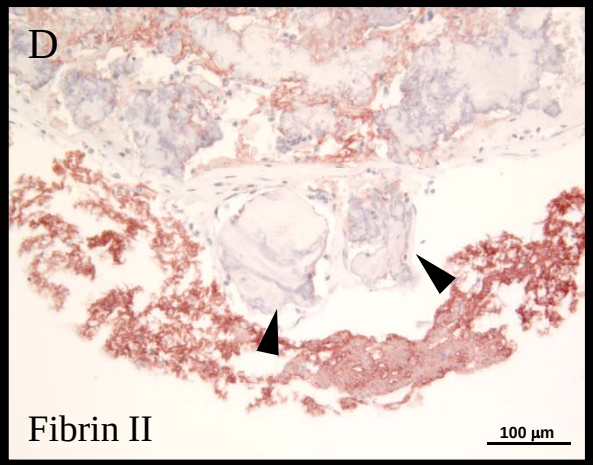


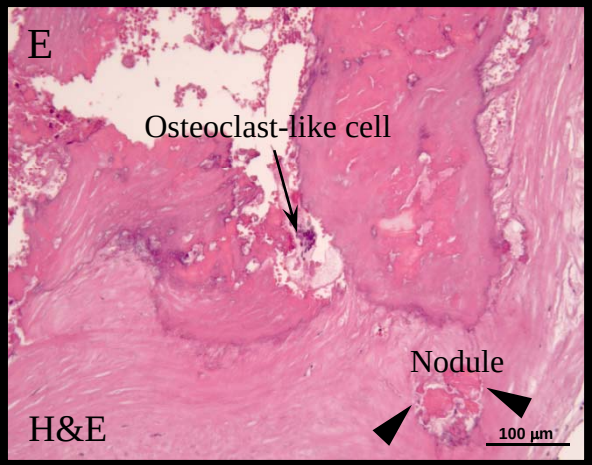
Eruptive Calcified Nodule with Thrombosis

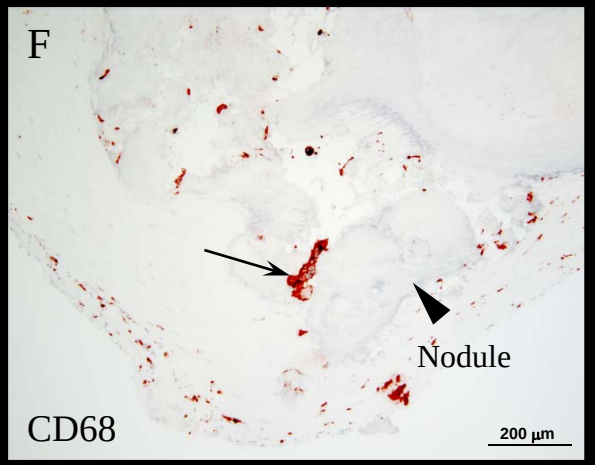










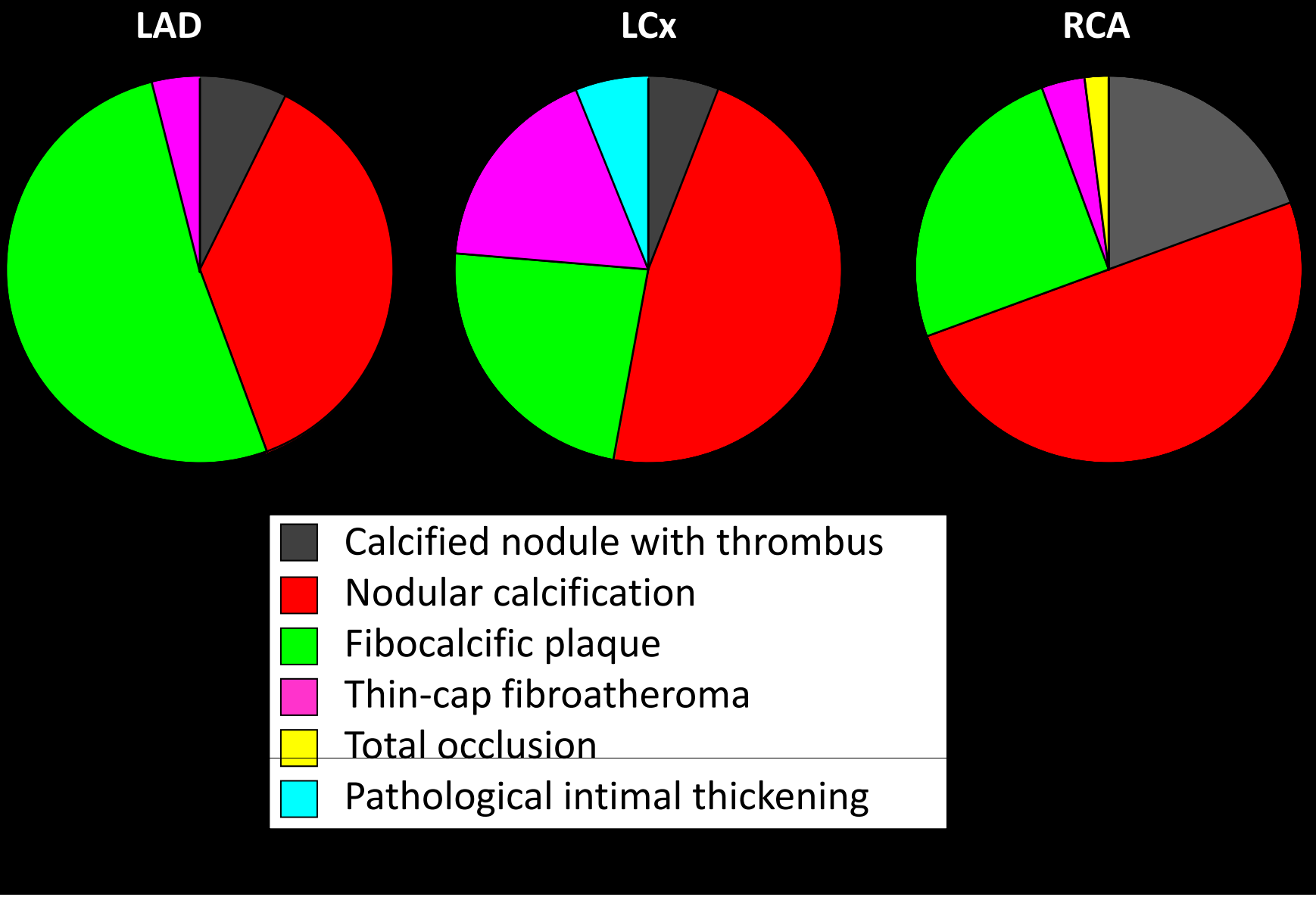


The Role of Risk Factors in SCD patients

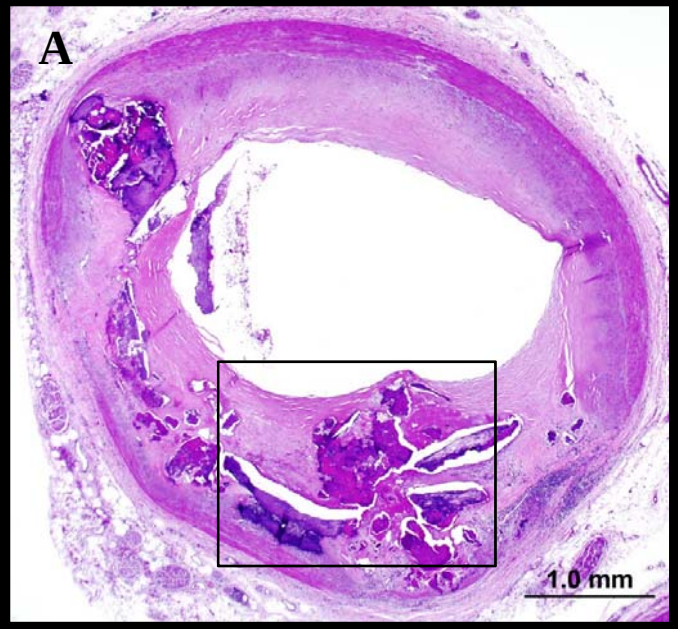
	Control N=163	Stable plaque n=131	Rupture n=84	Erosion n=45	Calcified nodules N=8
Age	46	54	49	45	**57**
Male	118 (72%)	102 (78%)	78 (90%)	29 (64%)	6 (88%)
Race %Black	75 (46%)	46 (35%)	21 (24%)	16 (36%)	2 (25%)
BMI	28.4	28.3	29.4	26.7	**31.8**
HbA1c	6.6	7.5	7.2	7.0	7.0*
Hx of HTN	35 (21%)	61 (47%)	27 (31%)	9 (20%)	**5 (63%)**
Smoker	64 (39%)	63 (48%)	53 (60%)	33 (73%)	**7 (88%)**
T. Chol	202	208	258	211	208
HDL	46	41	37	39	42
T.Chol/HDL	5.3	5.8	7.7	5.9	**5.4**
Healed MI	1 (1%)	76 (58%)	37 (42%)	11 (24%)	4 (50%)
Plaque Burden	---	232.4	248.1	178.9	**258**

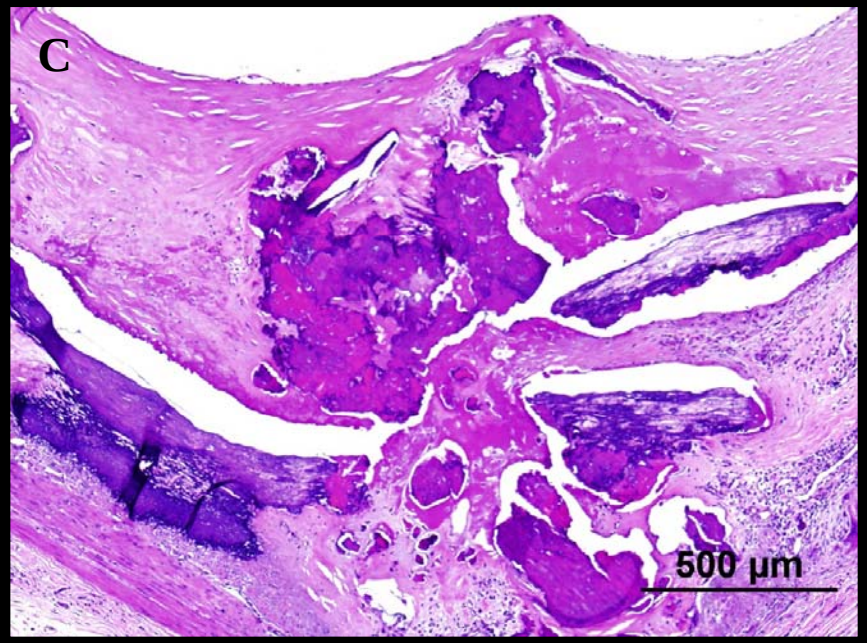
*** Out of 8 case, 4 were DM, and 2 were Pre-DM.**

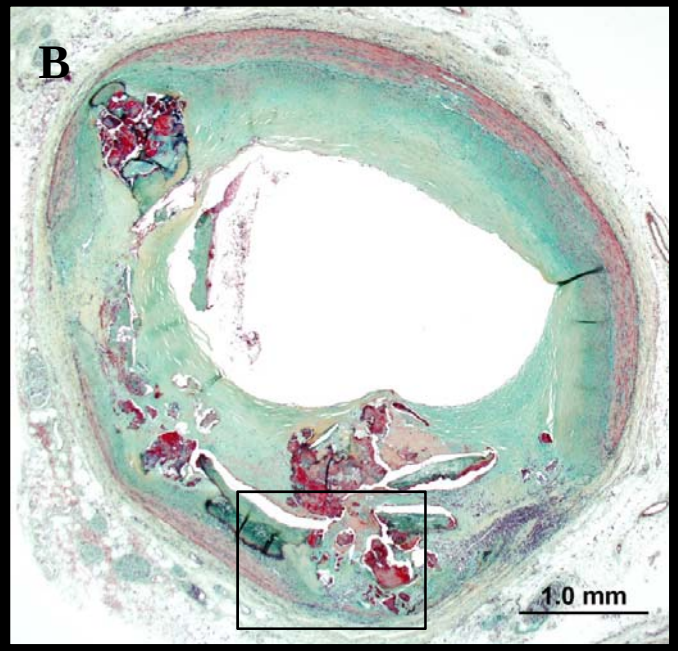
Distribution of Atherosclerotic Coronary Disease

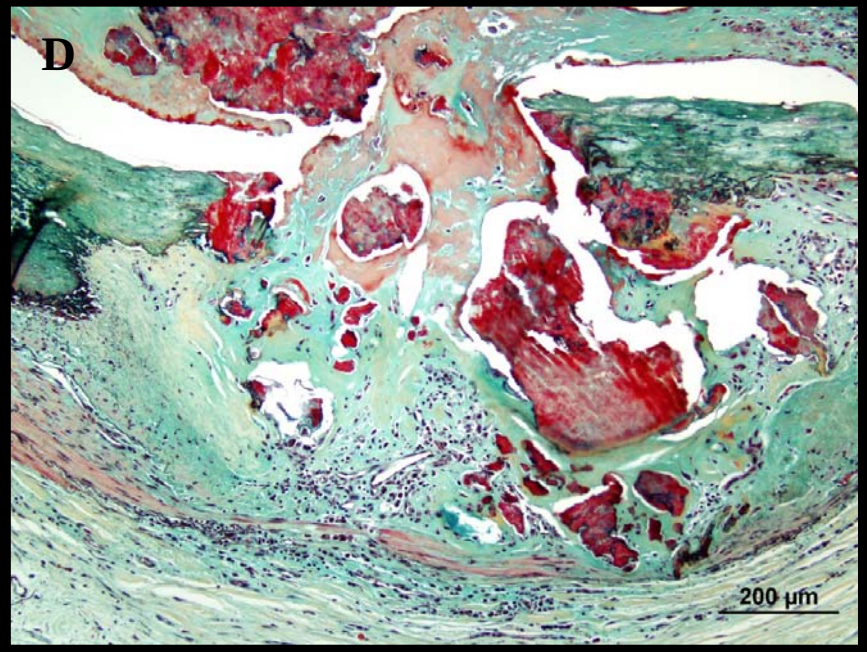


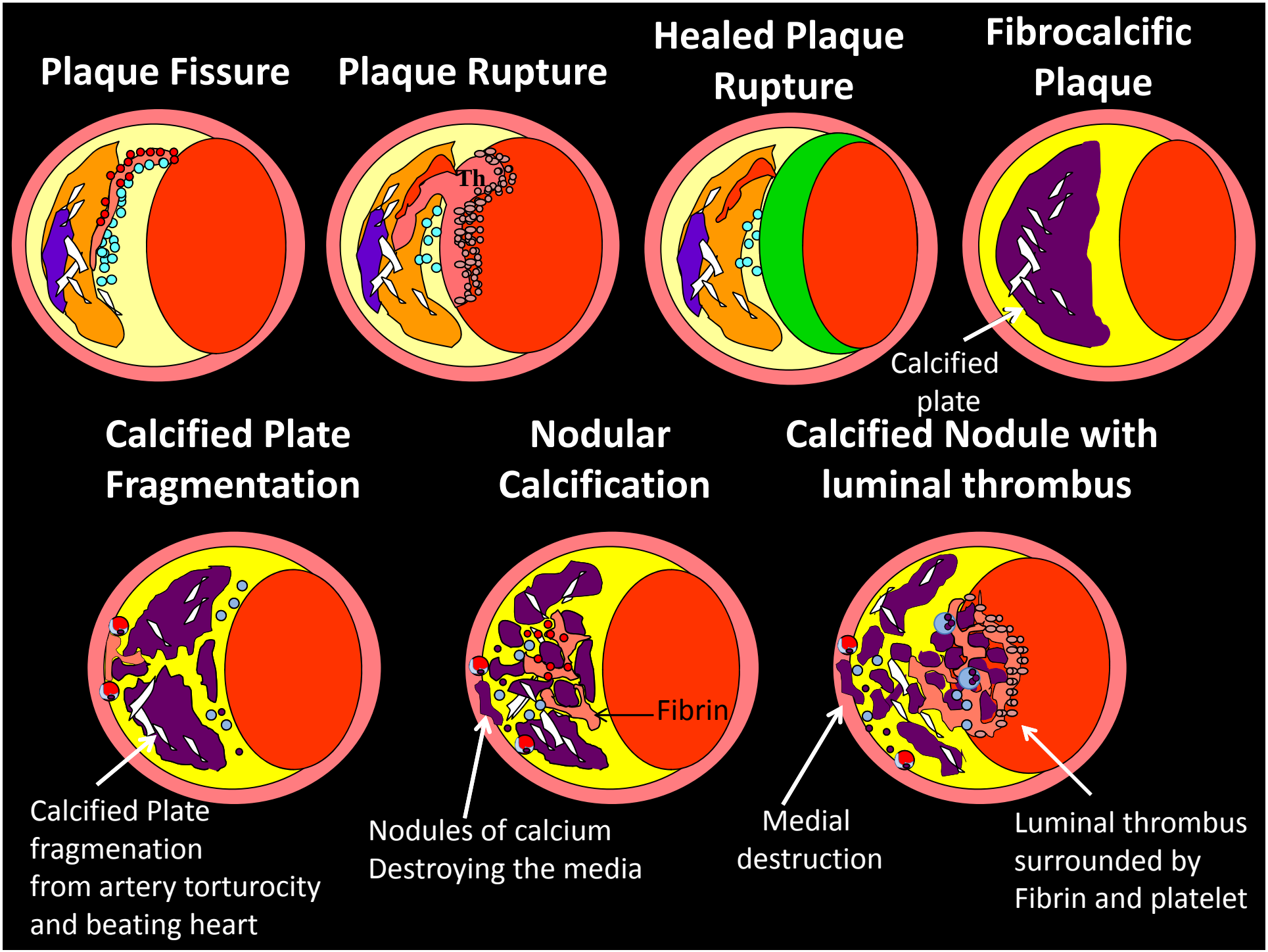
Nodular Calcification without Thrombosis










Plaque Fissure
Plaque Rupture
Th
Healed Plaque Rupture
Fibrocalcific Plaque
Calcified plate
Calcified Plate Fragmentation
Nodular Calcification
Calcified Nodule with luminal thrombus
Fibrin
Calcified Plate fragmenation from artery torturocity and beating heart
Nodules of calcium Destroying the media
Medial destruction
Luminal thrombus surrounded by Fibrin and platelet

Inflammation: The Road to Plaque Progression

Summary:

- The earliest lesion of plaque progression is Pathologic Intimal thickening.
- PIT, lipid pool is converted to necrotic core from macrophages infiltration and apoptosis leading to early necrotic core formation.
- Late necrotic core is likely the result of defective efferocytosis as well as plaque hemorrhage which contribute to free cholesterol within necrotic core
- Inflammation continues to increase as plaques progress and is maximum in plaque rupture.

Summary: Thrombosis

- Plaque rupture is a main cause of thrombosis (65-70%), while other minor causes include erosion (30%) and calcified nodule (4-7%).
- Vulnerable plaques (TCFA) is a likely precursor lesions of rupture. Macrophage infiltration play an important role in modification of plaque vulnerability.
- Plaque hemorrhage from “leaky” vasa vasorum is an important contributor to the enlargement of the necrotic core.
- Calcified nodules is another substrate for thrombosis, especially in elderly male individuals with high plaque burden, tortuous arteries, diabetes , metabolic syndrome, hypertension, and smoking.

Acknowledgments

- Masataka Nakano, M.D.
- Fumiyuki Otsuka, M.D.
- Frank Kolodgie, Ph.D.
- Aloke V. Finn, M.D.
- Elena Ladich, M.D.
- Ed Acampado, D.V.M.
- Robert Kutz, M.S.
- You-hui Liang, M.D.
- Erica Pacheco, M.S.
- Hedwig Avallone
- Lila Adams
- Russ Jones
- Abebe Atiso
- Rosalind Mathew

CVPath Institute, Inc.