Thrombosis and Inflammation in Plaque Vulnerability: What is the Next Target for New Drugs?

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Natural History of Atherosclerosis

- Systemic factors –

  Dyslipidemia, diabetes mellitus, smoking, hypertension, age and gender, hsCRP, Lp-PLA_2, etc.

- Local factors –

  Branch points, e.g. carotid bifurcation, abdominal aorta just above bifurcation, coronary branch point, and arch vessels at take off, are the sites of early atherosclerosis .

- Thrombosis –

  Occurs in the coronary arteries at focal points and is most often seen in the proximal segments of the three main coronary arteries (systemic coagulation factors play a role), and occur at sites where there are underlying plaque characteristic that result in thrombosis.

Non-Progressive and Progressive Coronary Plaques

non-progressive → progressive

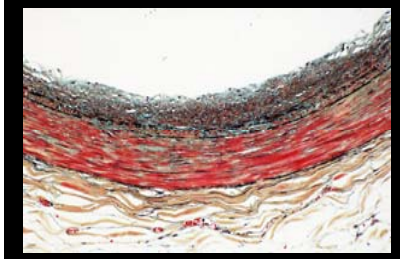
adaptive intimal thickening

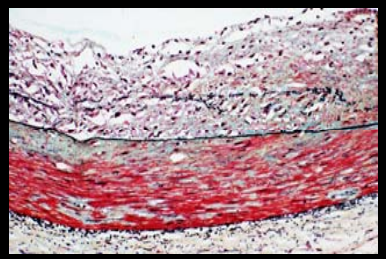
Intimal xanthoma

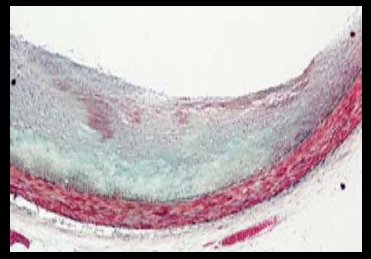
pathologic intimal thickening

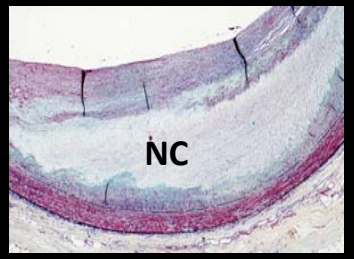
fibroatheroma

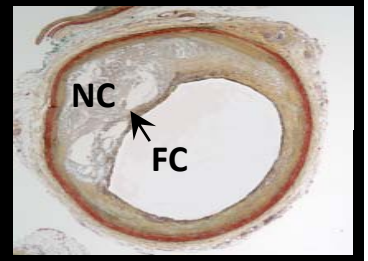
thin-cap fibroatheroma

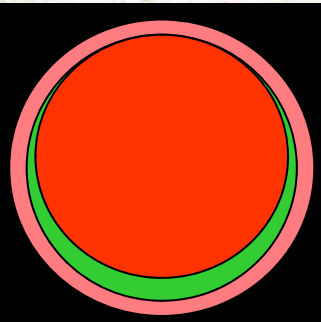


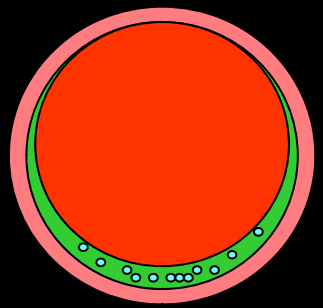


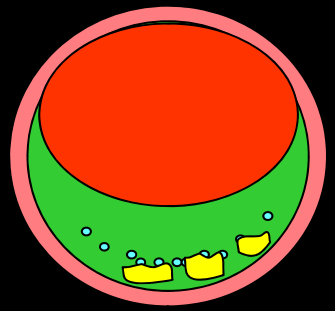


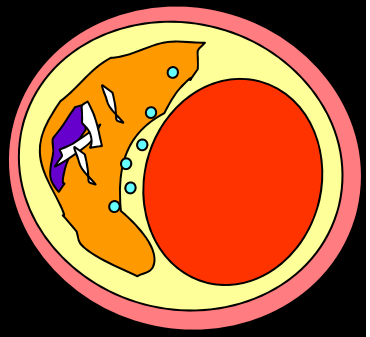










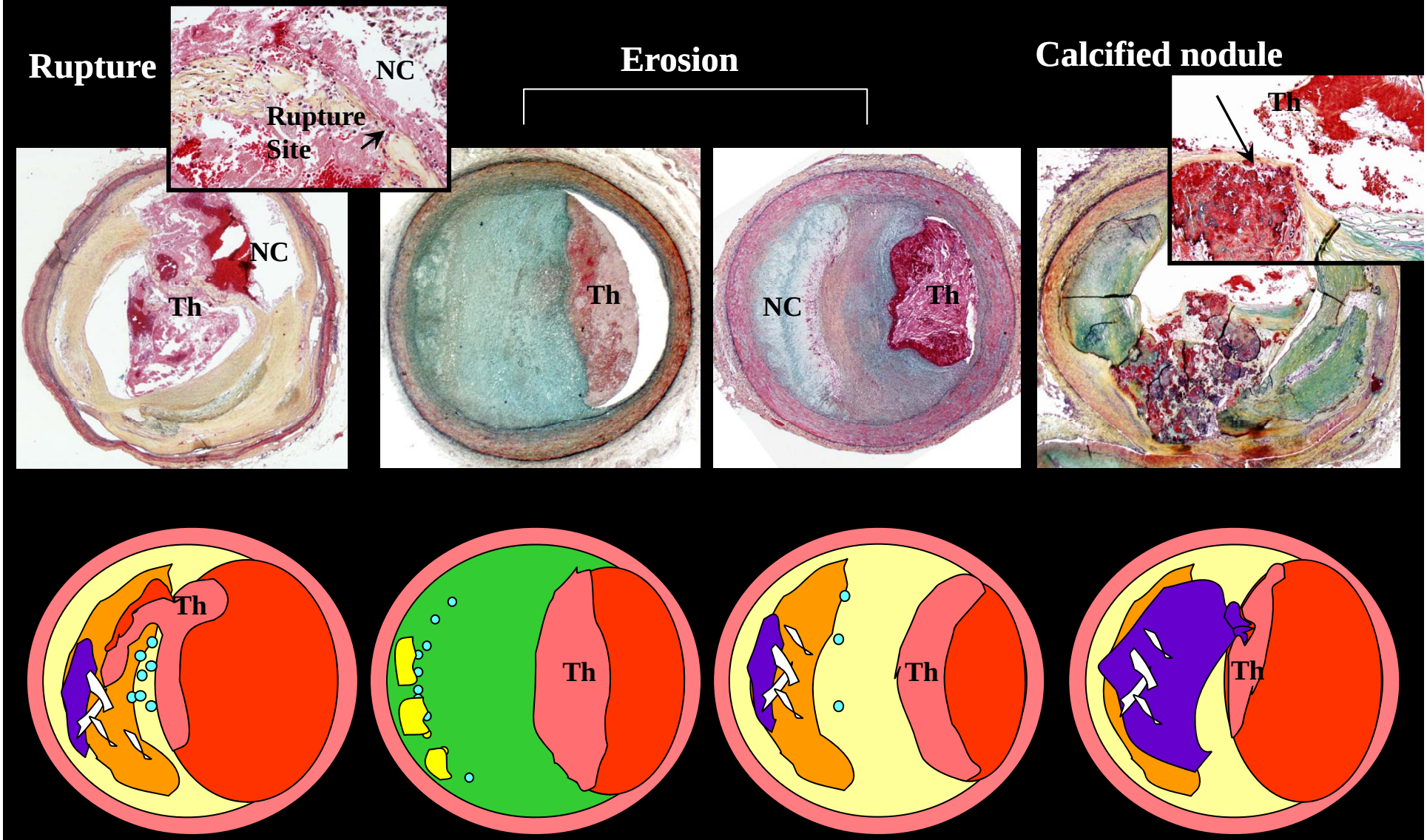


lipid pool

necrotic core

early → late necrosis

Causes of Coronary Thrombosis



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

The Role of Risk Factors in SCD patients

Sudden Coronary Death N=264

	Control N=163	Stable plaque n=131	Rupture n=88	Erosion n=45	P value (ANOVA)
Age	45.6	54.0*	48.8	45.0	***<0.0001***
Male	118 (72%)	102 (78%)	78 (90%)	29 (64%)	***0.0032***
Race %Black	75 (46%)	46 (35%)	21 (24%)	16 (36%)	***0.0060***
BMI	28.4	28.3	29.4	26.7	0.21
Hx of DM	8 (5%)	28 (21%)	6 (7%)	4 (9%)	***<0.0001***
Insulin use	5 (3%)	21 (16%)	6 (7%)	1 (2%)	***0.0002***
HbA1c	6.6	7.5*	7.2	7.0	***0.0043***

* Significantly different from Control by post-hoc analysis

Extent of Coronary Artery Disease in SCD patients

	Control N=163	Stable plaque N=131	Rupture N=88	Erosion N=45	P value (ANOVA)
Hx of HTN	35 (21%)	61 (47%)	27 (31%)	9 (20%)	***<0.0001***
Smoker	64 (39%)	63 (48%)	53 (60%)	33 (73%)	***<0.0001***
T. Chol	202.2	208.7	257.8*	210.8	***<0.0001***
HDL	45.9	41.4	36.5*	39.0	***0.0018***
T.Chol/HDL	5.3	5.8	7.7*	5.9	***<0.0001***
TC/HDL>5	69 (42%)	70 (53%)	77 (88%)	24 (53%)	***<0.0001***
Heart Wt	457.4	493.9*	495.1	428.0	***0.0014***
Healed MI	1 (1%)	76 (58%)	37 (42%)	11 (24%)	***<0.0001***
Plaque Burden	---	232.4	248.1	178.9	***<0.0001***

* Significantly different from Control by post-hoc analysis

Independent risk factors for SD from **Stable Plaque**

Attributes	Odds	CI	P value
HbA1c	1.26	1.10-1.45	***0.0007***
T. Chol/HDL	1.03	0.93-1.14	0.58
Hypertension	2.65	1.47-4.76	***0.0012***
Smoker	1.85	1.08-3.18	***0.026***

Independent risk factors for SD from **Plaque Rupture**

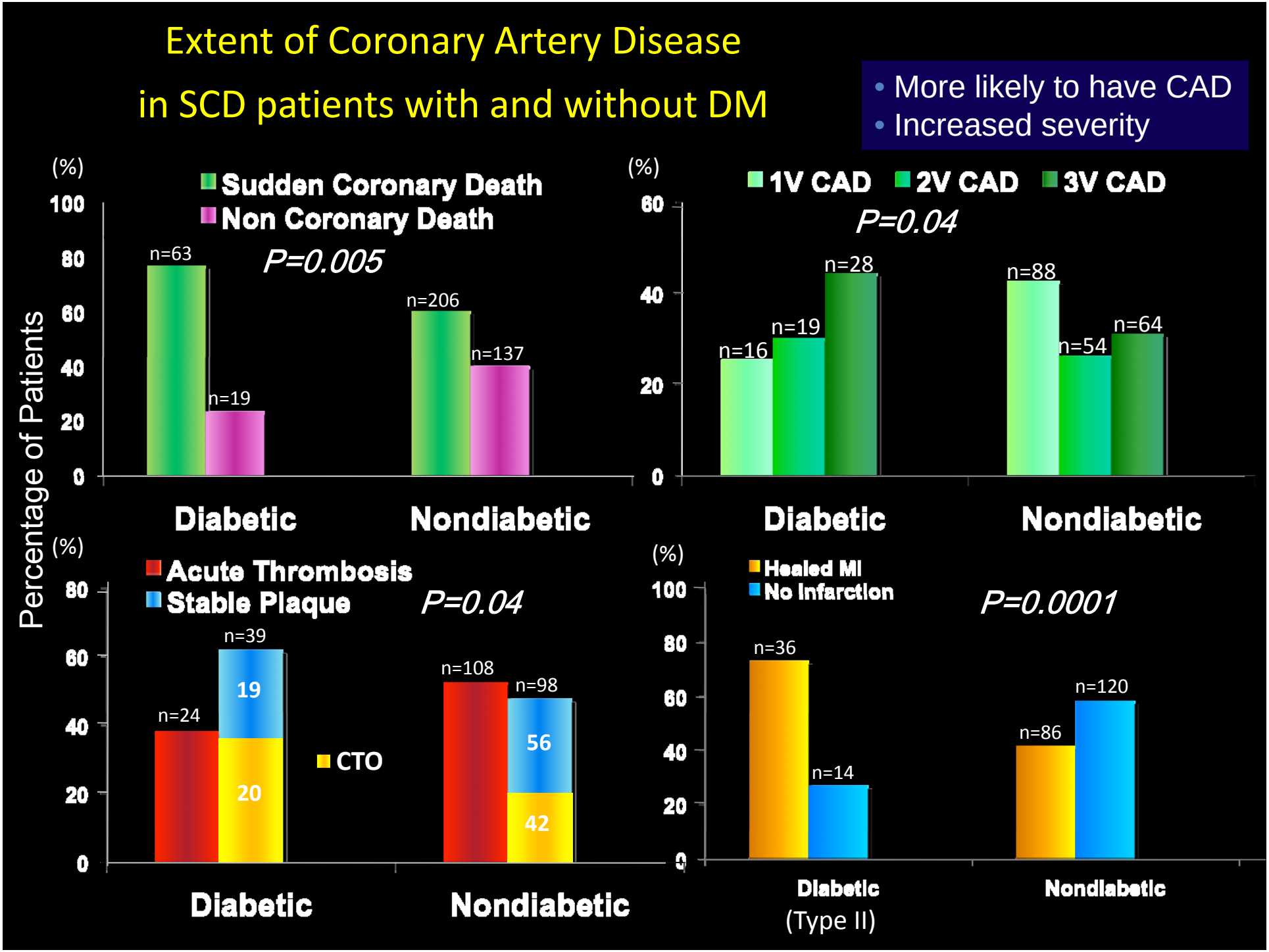
Attributes	Odds	CI	P value
HbA1c	1.16	1.01-1.34	***0.041***
T. Chol/HDL	1.29	1.15-1.45	***<0.0001***
Hypertension	1.69	0.83-3.44	0.15
Smoker	2.74	2.46-5.13	***0.0017***

Independent risk factors for SD from **Plaque erosion**

Attributes	Odds	CI	P value
HbA1c	1.13	0.95-1.36	0.17
T. Chol/HDL	1.07	0.94-1.22	0.29
Hypertension	1.34	0.47-2.78	0.78
Smoker	4.93	2.29-10.64	***<0.0001***

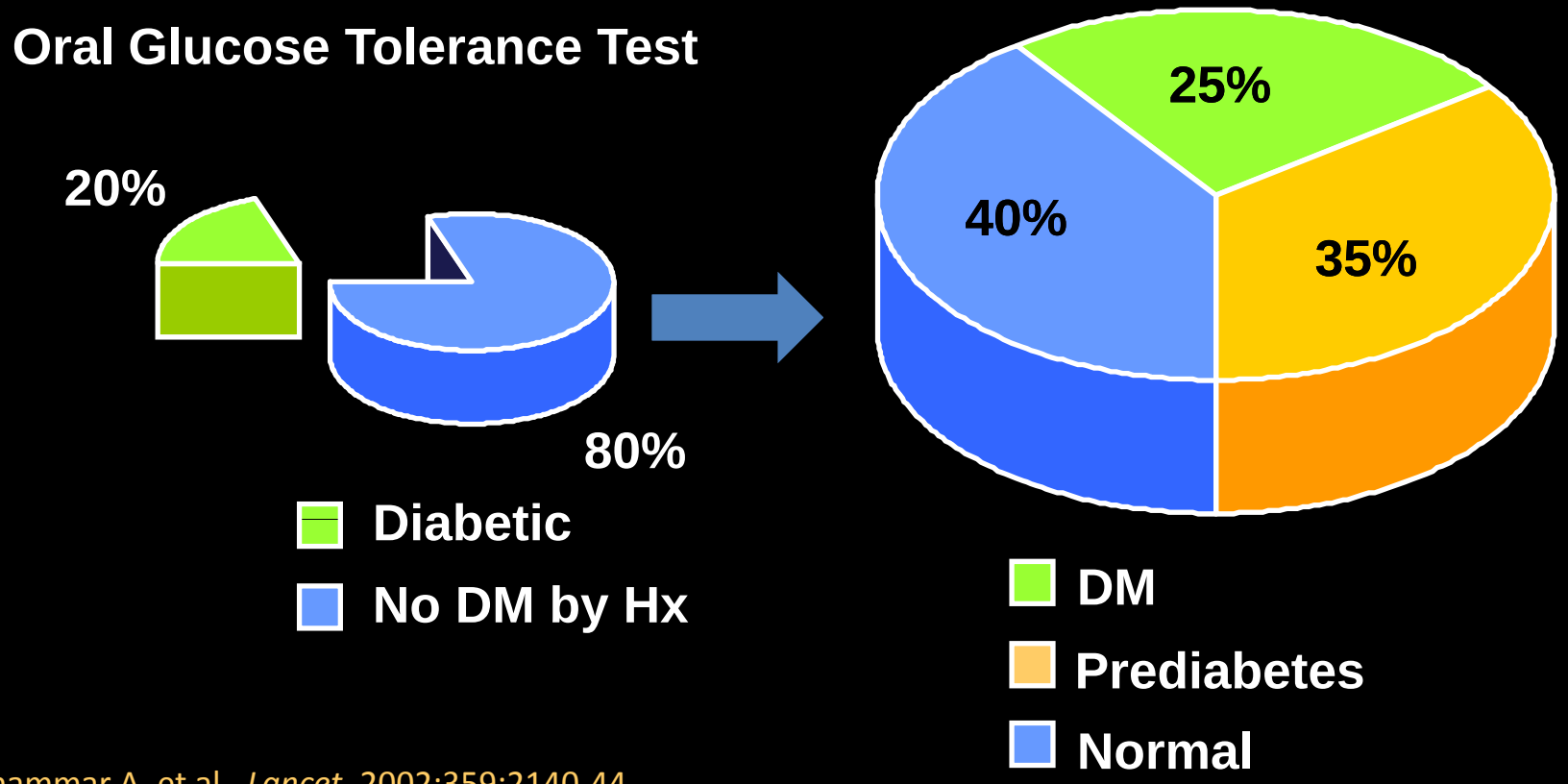
Extent of Coronary Artery Disease in SCD patients with and without DM

- More likely to have CAD
- Increased severity



Diabetes and Pre-Diabetes Are Present In Most MI patients

Consecutive patients presenting with an AMI, *n* = 181



Norhammar A, et al., *Lancet*. 2002;359:2140-44.

Undiagnosed Prediabetes and T2D

One-third of patients do not survive their first MI.

Exponential increase in obesity, pre-diabetes, T2D.

Increasing identification of undiagnosed T2D in CVD.

What is the prevalence of undiagnosed T2D and pre-diabetes in individuals having sudden death?

Schneiderman, Virmani, Plutzky et al, in review

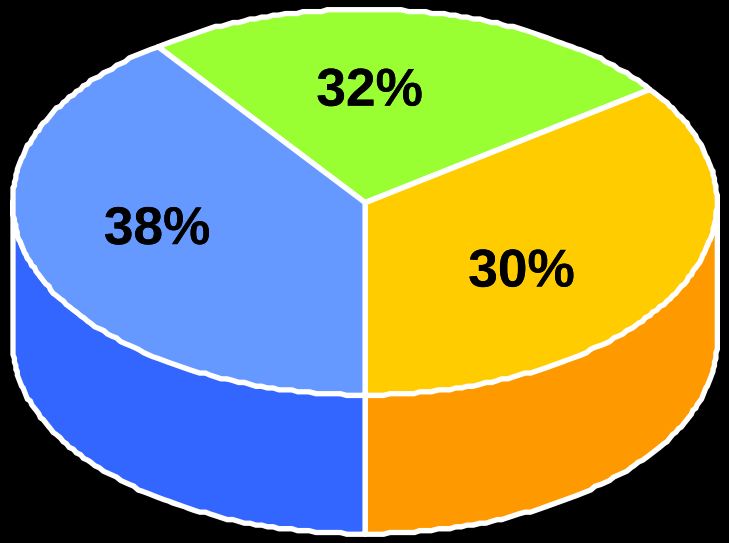
Incidence of Undiagnosed T2D in Sudden Coronary Death

~1/3 patients die from first heart attack

Incidence of undiagnosed T2D in sudden death?

ADA criteria for dx of T2D (≥6.5 HbA1c), pre-DM (≥5.7 to <6.5)

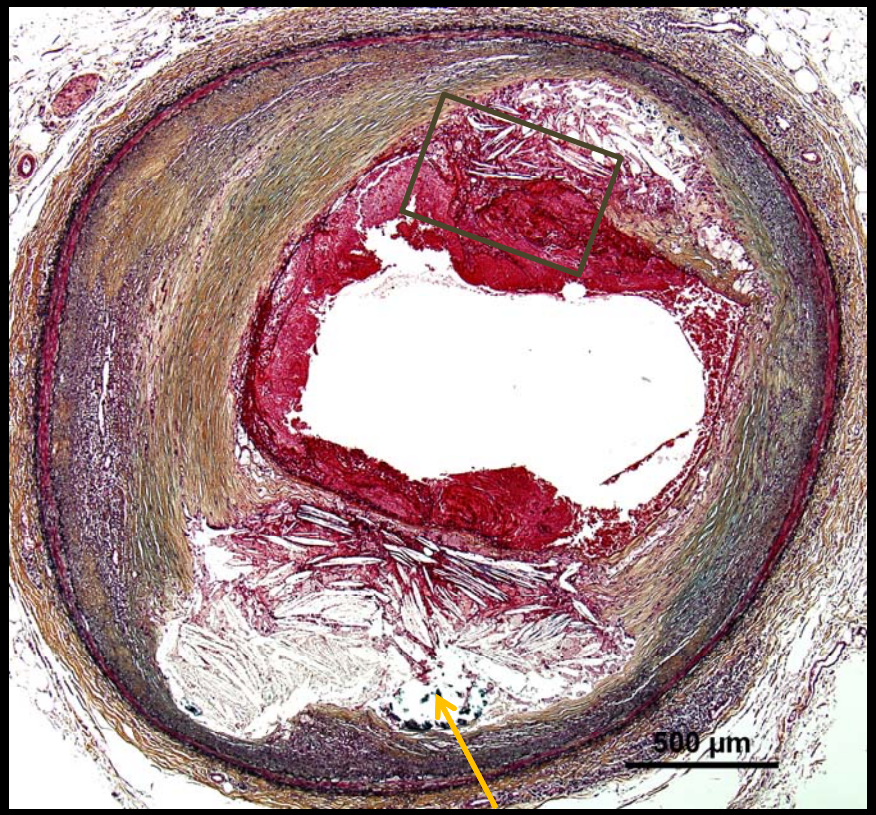
580 cases
Out of hospital deaths
Autopsy series
A1C

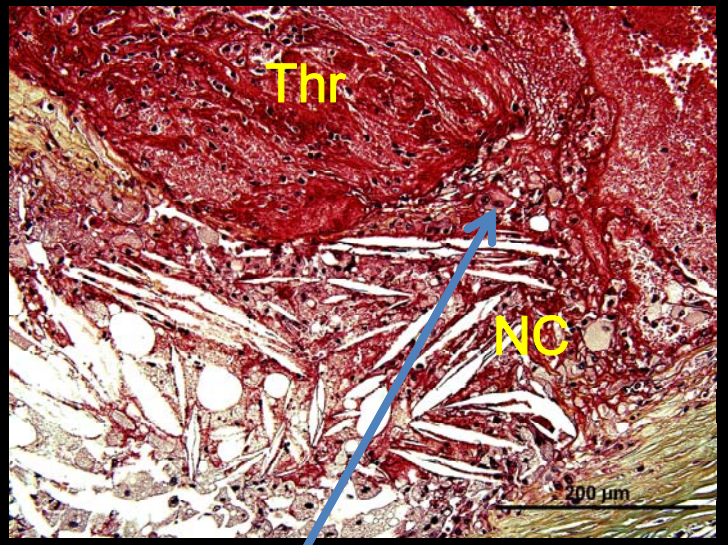


Schneiderman, Virmani, Plutzky et al

Development of Necrotic Core

The Necrotic Core
"graveyard of dead Mϕs"



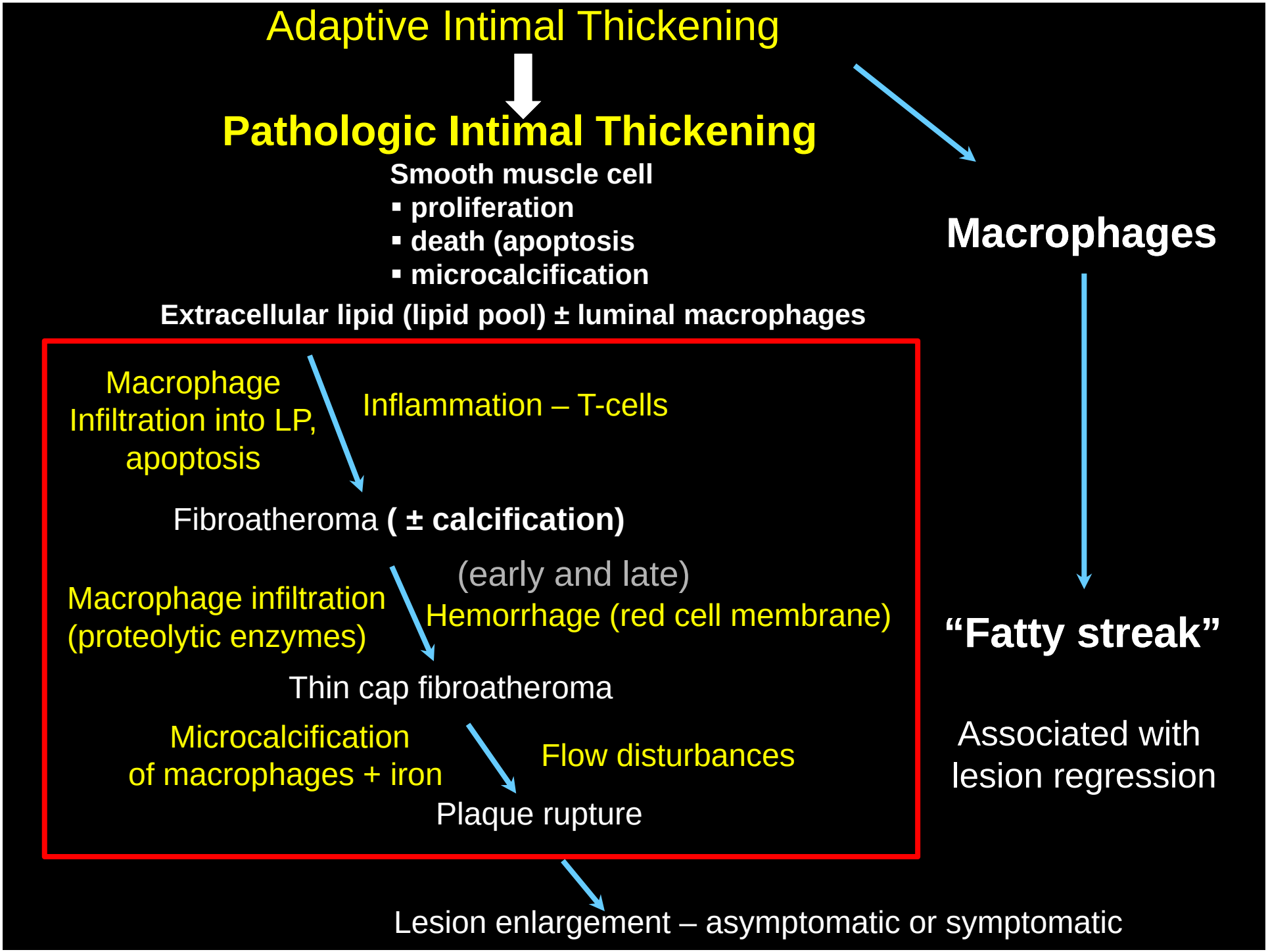


Ruptured plaque at area of thinned fibrous cap

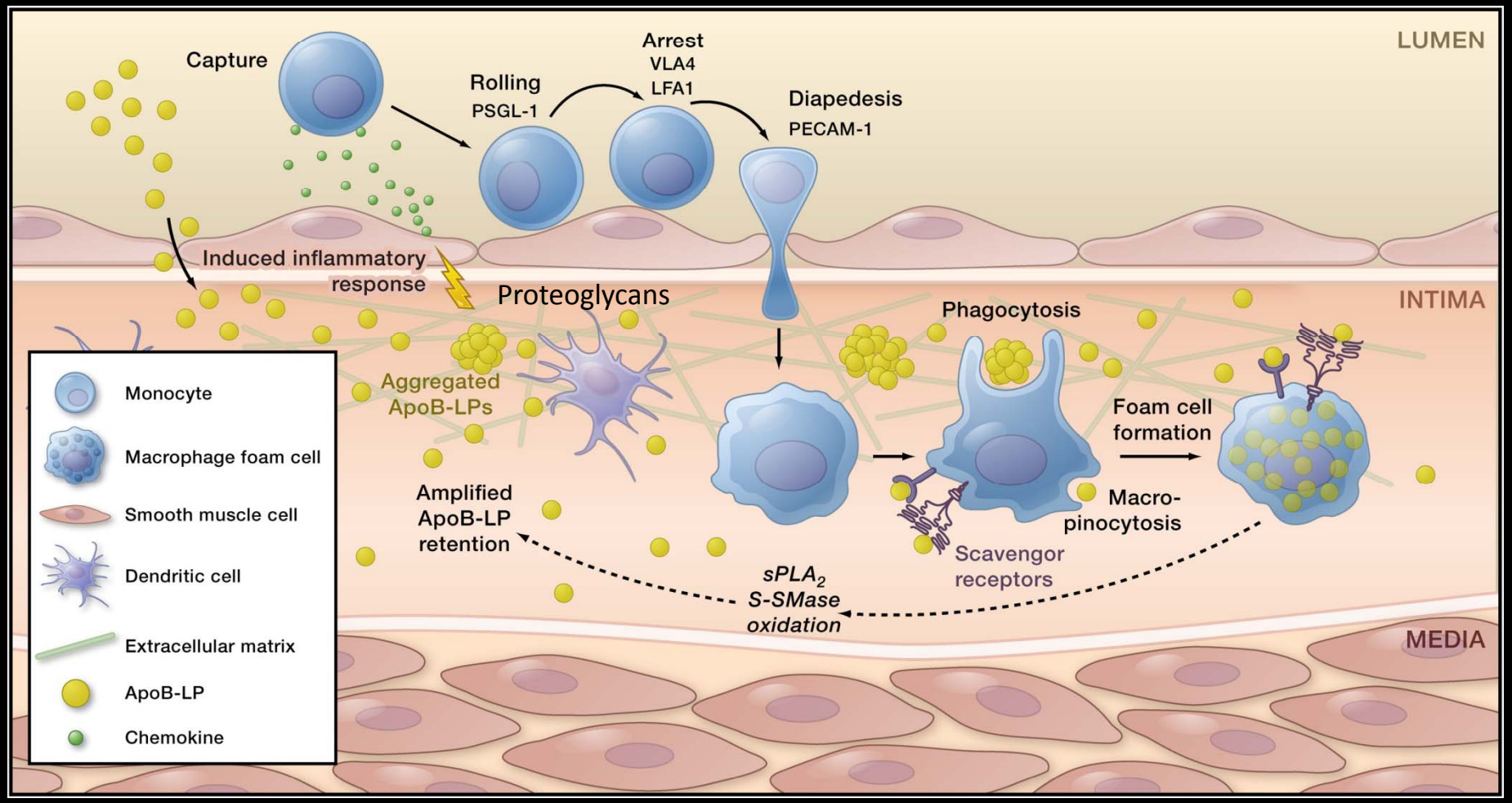
Necrotic Core

- 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

ApoB-lipoprotein (ApoB-LP) Promotes Monocyte Recruitment and Subsequent Foam Cell Formation



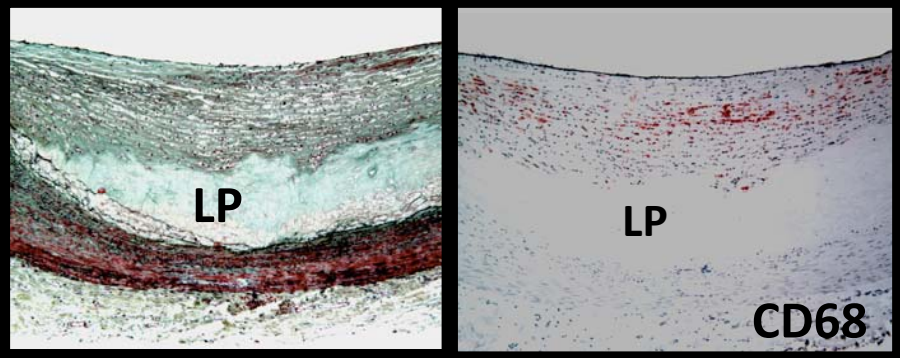
$sPLA_2$ = secretory phospholipase A_2
S-SMase = secretory sphingomylinase

Moore, Tabas . Cell. 2011;145:341-355

Plaque Progression

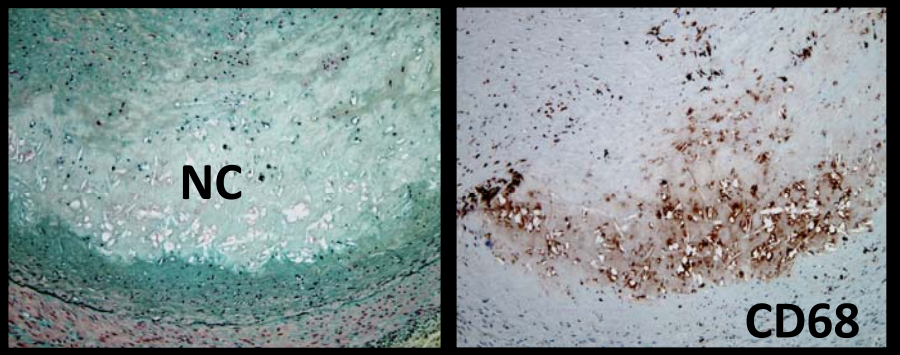
A.

Pathologic Intima Thickening



B.

Fibroatheroma ‘Early’ Core

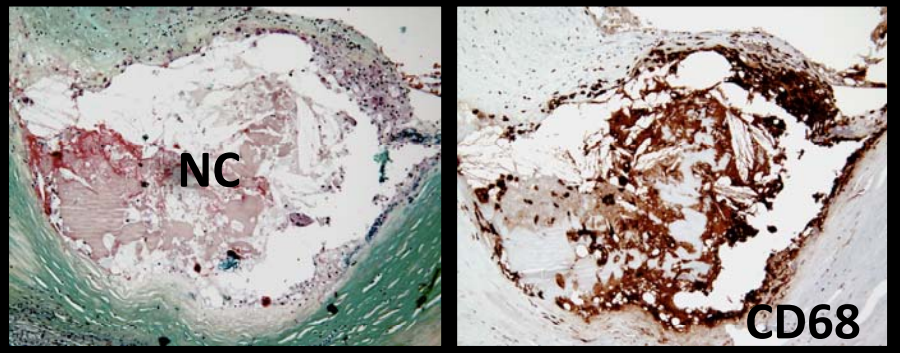


C.

Fibroatheroma ‘Late’ Core

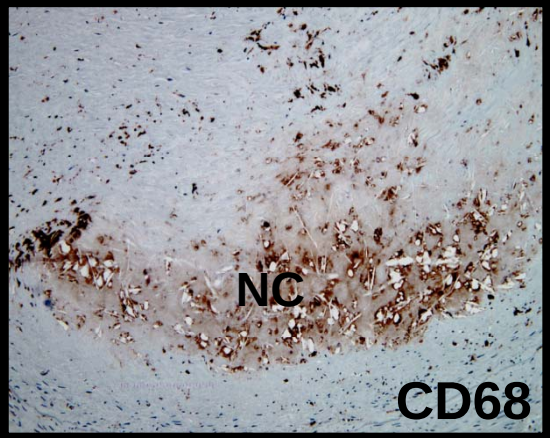
D.

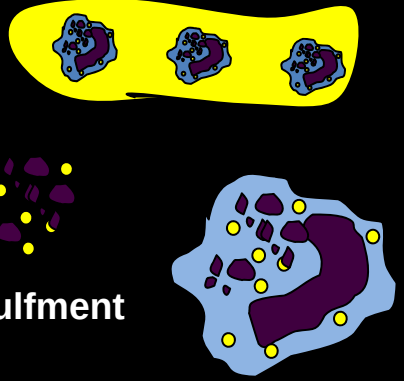
Thin Cap Fibroatheroma

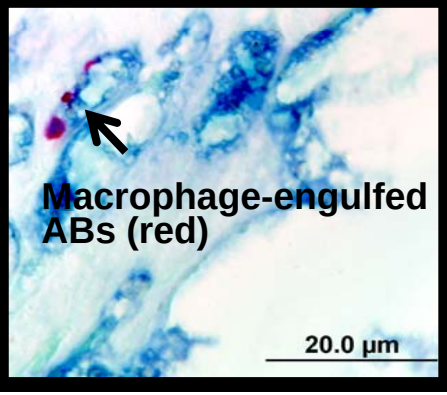


Mechanism of Necrotic Core Progression

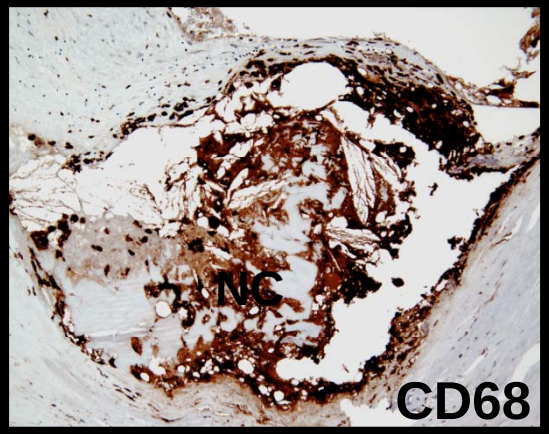
A. Early

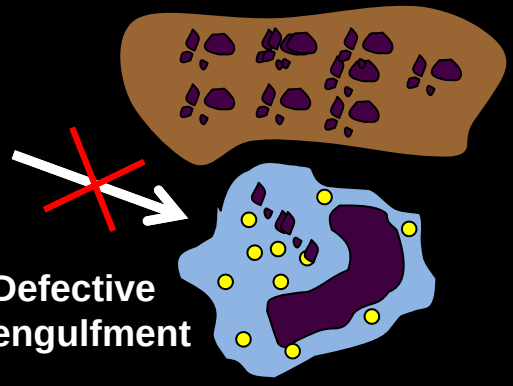


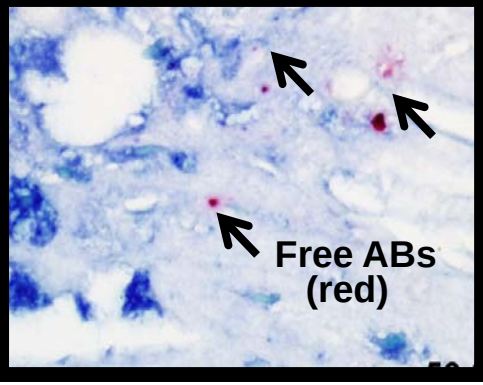




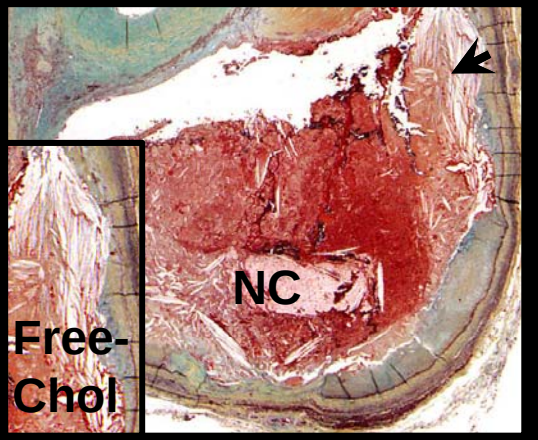
B. Late

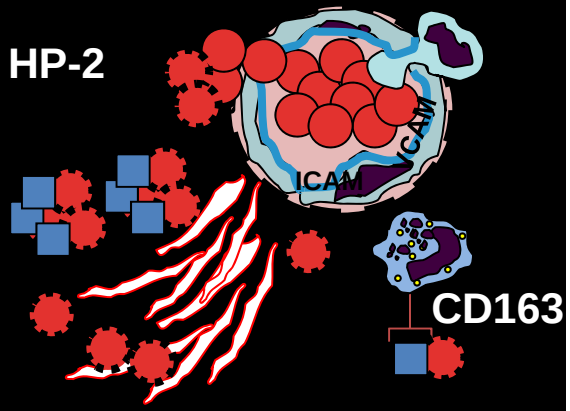


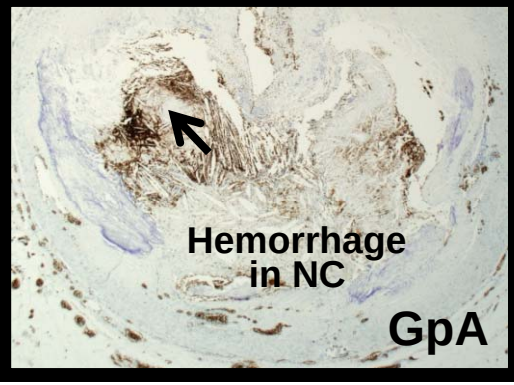




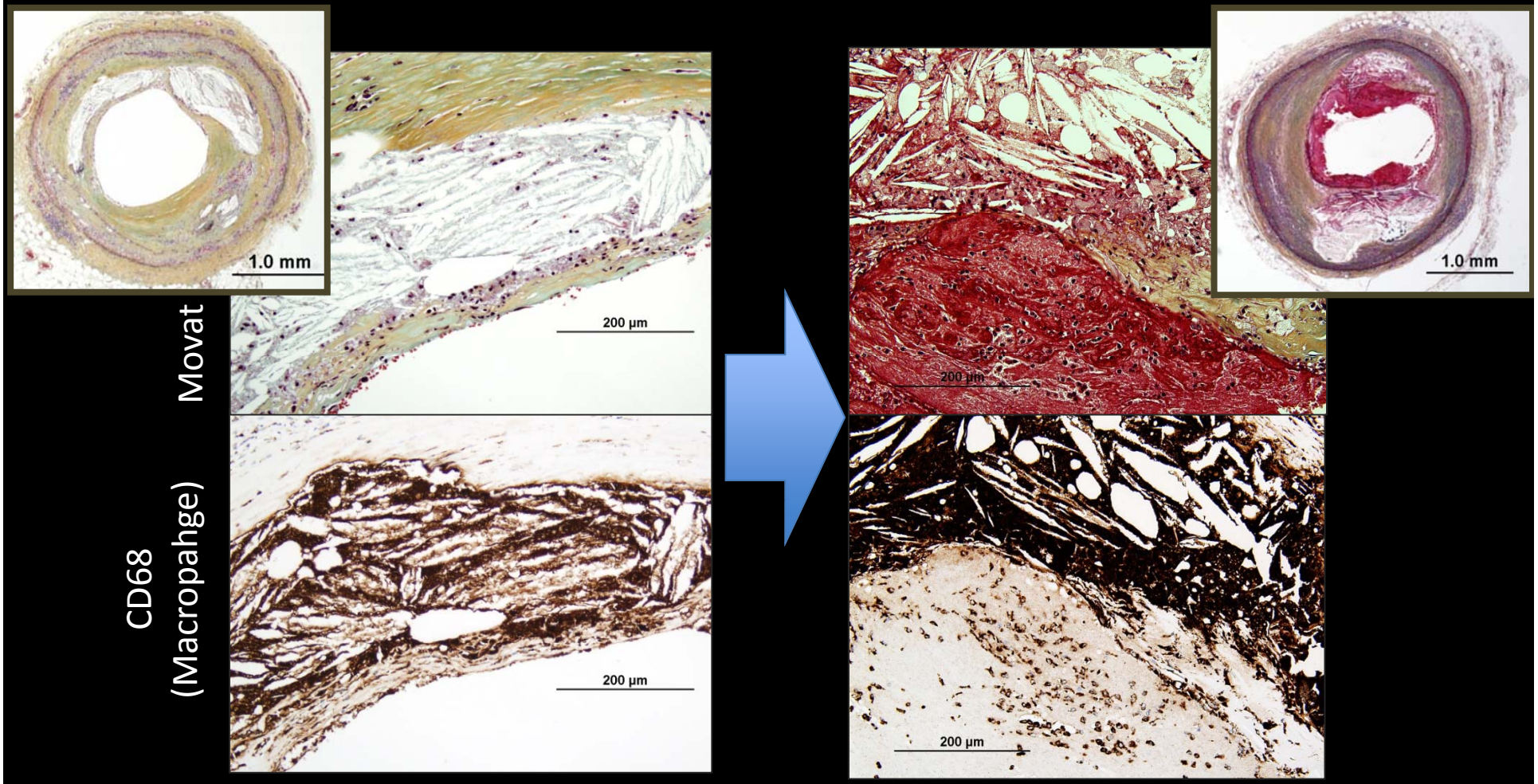
C. Hemorrhagic







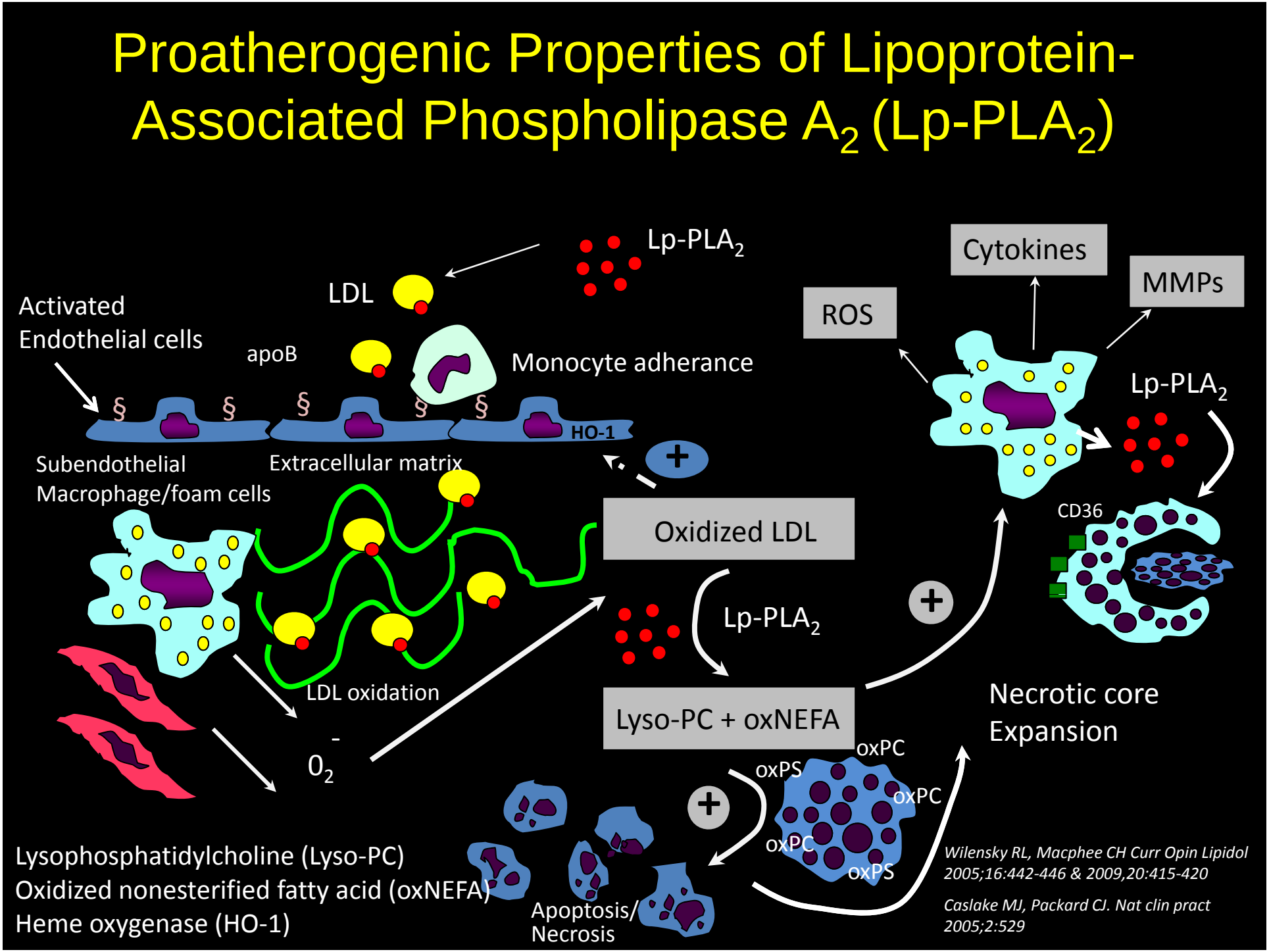
Independent Morphological Predictor of Rupture vs. TCFA



	P Value	Odds Ratio*	95% CI
NC Area	0.02	2.73	1.14 – 6.30
Cap thickness	0.02	0.44	0.23 – 0.82
Macrophage Area	0.07	1.71	0.96 – 3.03

*adjusted by S.D.

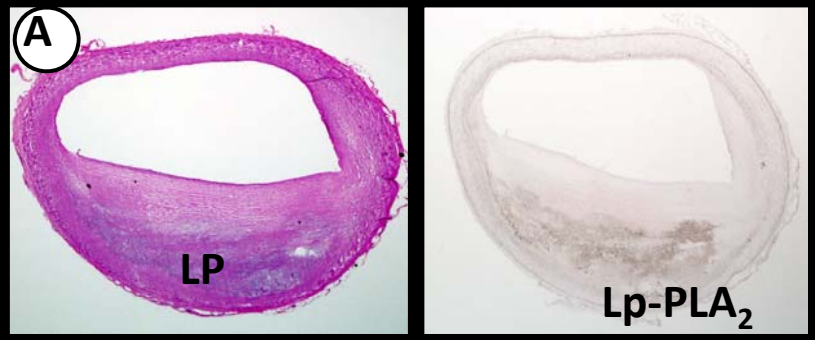
Proatherogenic Properties of Lipoprotein-Associated Phospholipase A_2 ($Lp\text{-}PLA_2$)



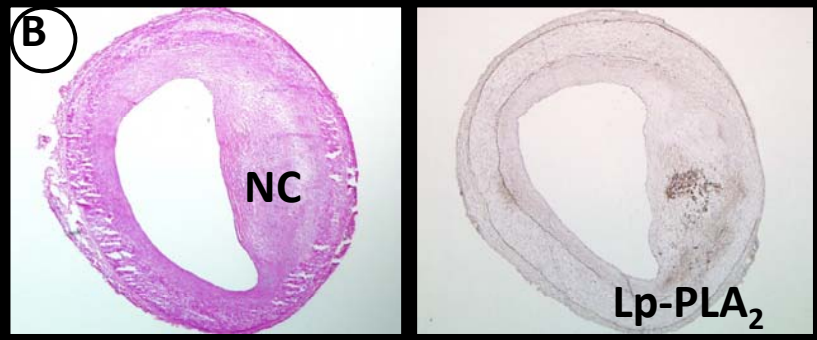
Wilensky RL, Macphee CH Curr Opin Lipidol 2005;16:442-446 & 2009,20:415-420

Caslake MJ, Packard CJ. Nat clin pract 2005;2:529

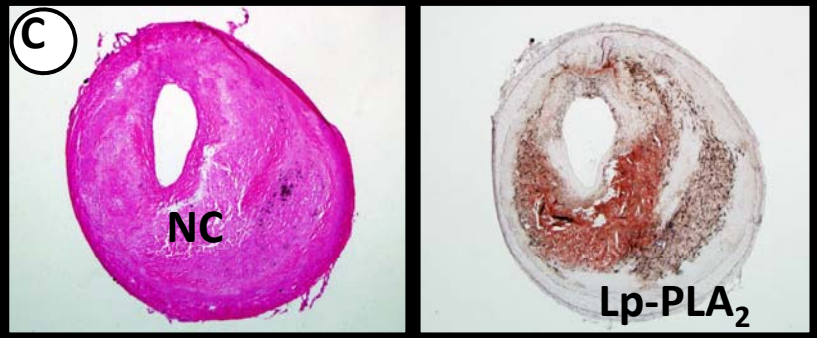
Pathologic Intimal thickening



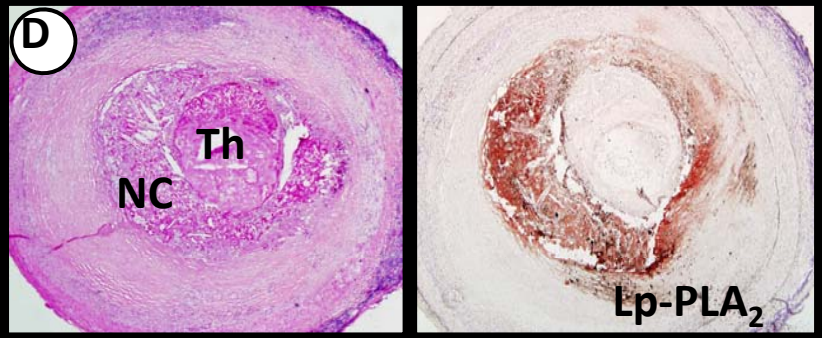
Fibroatheroma

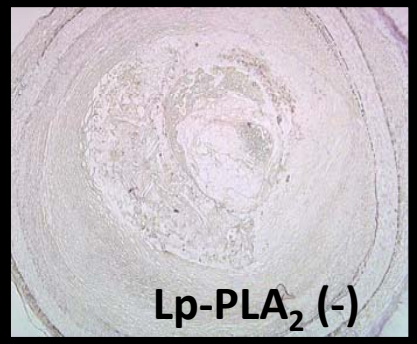


Thin Cap Fibroatheroma

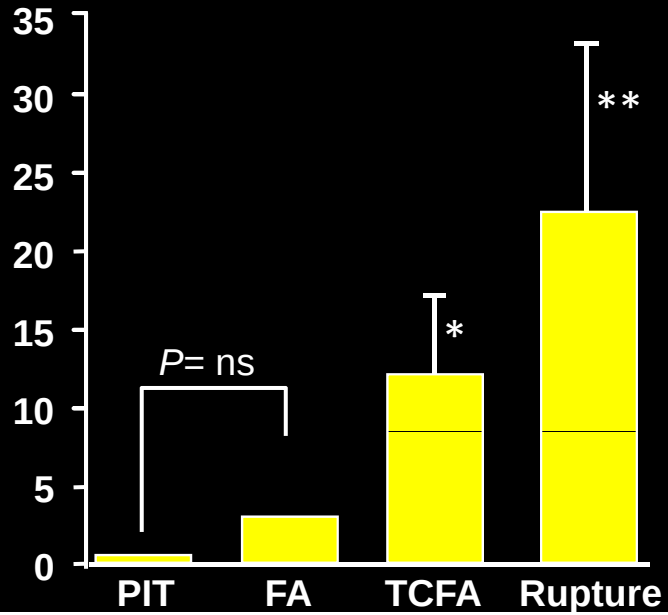


Plaque Rupture





E.

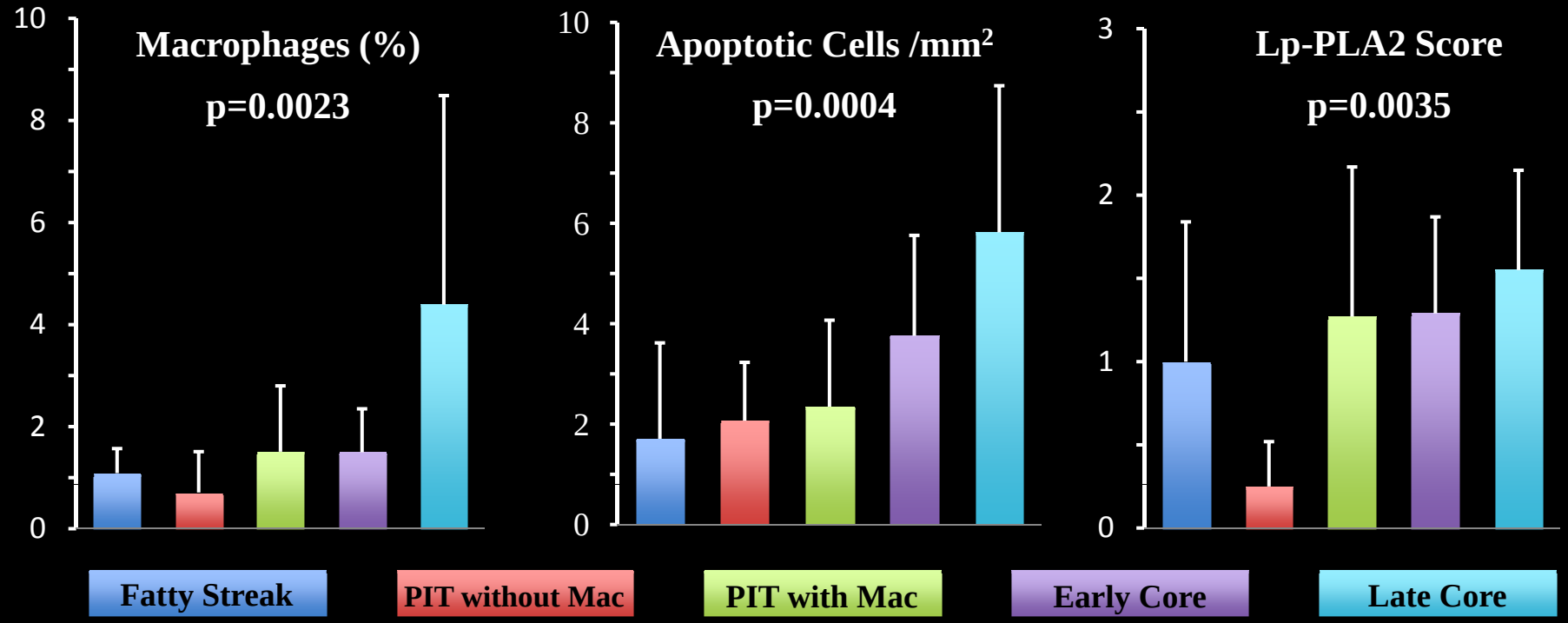


% Lp-PLA$_2$ Staining in Varying Coronary Plaque Morphologies

Lp-PLA$_2$ is strongly expressed within the necrotic core and surrounding macrophages of vulnerable and ruptured plaques.

Kolodgie et al, ATVB, 2006;26:2523-2529

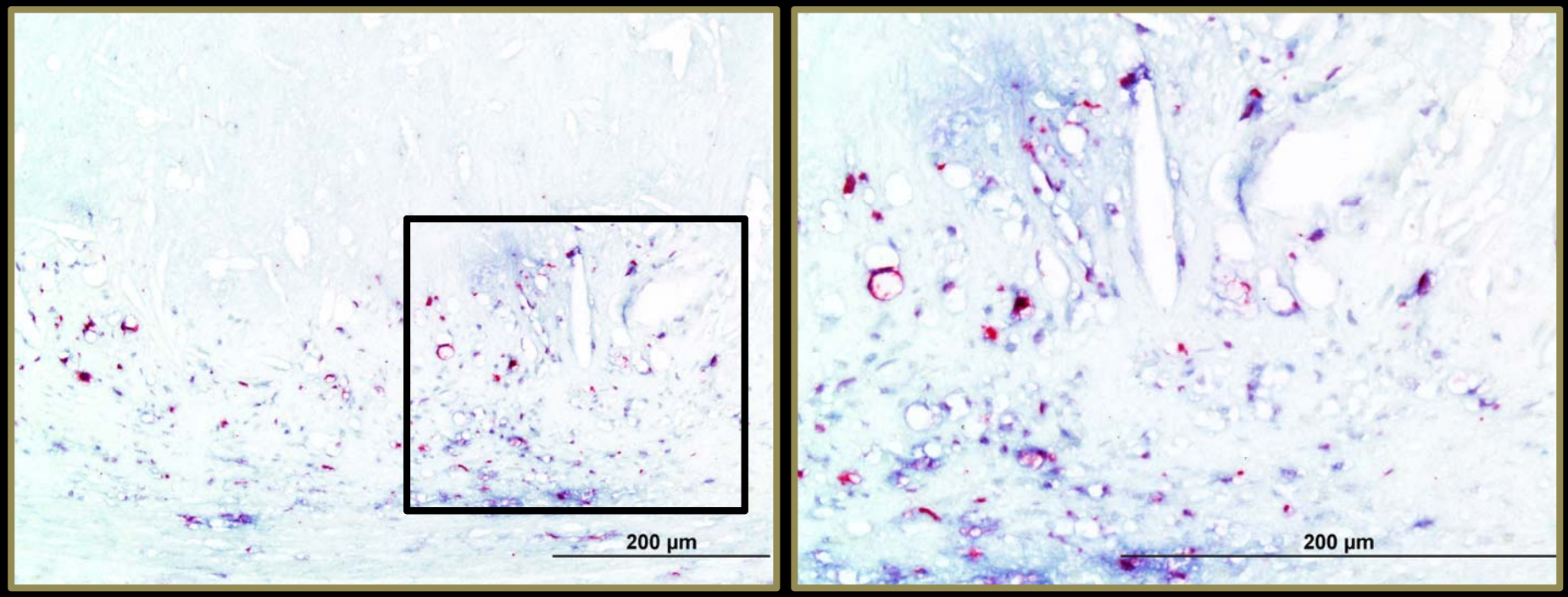
Histomorphometric Analysis Stratified by Lesion Type



Lesion Type (n=53)	Plaque Area (mm^2)	Stenosis (%)	NC Area (mm^2)	Macrophages (%)	Apoptotic Cells /mm^2	Lp-PLA2 Score
Fatty Streak (n=6)	1.45±0.78	41.3±22.2		1.09±0.48	1.71±1.91	1.00±0.84
PIT without Mac (n=8)	3.86±2.12	41.9±12.3		0.71±0.80	2.07±1.16	0.25±0.27
PIT with Mac (n=15)	2.42±0.96	45.2±14.6		1.51±1.29	2.35±1.72	1.27±0.90
Early Fibroatheroma (n=14)	4.39±1.79	60.3±12.4	1.03±1.03	1.50±0.85	3.76±2.00	1.29±0.58
Late Fibroatheroma (n=10)	4.77±1.69	69.7±14.5	1.24±0.67	4.39±4.10	5.82±2.92	1.55±0.60
P value	0.0002	0.0002	0.5961	0.0023	0.0004	0.0035

Co-Expression of LP-PLA$_2$ with Apoptotic Macrophages in Early Necrotic Core

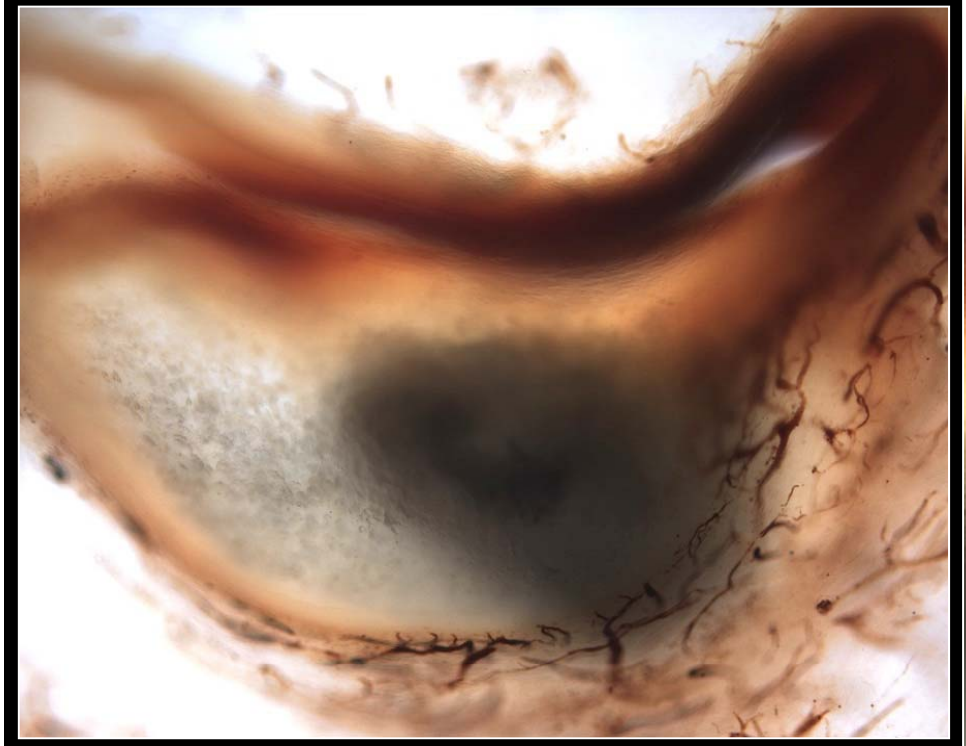
LP-PLA$_2$ Immunostaining (Bluish-Grey) plus TUNEL (Red)

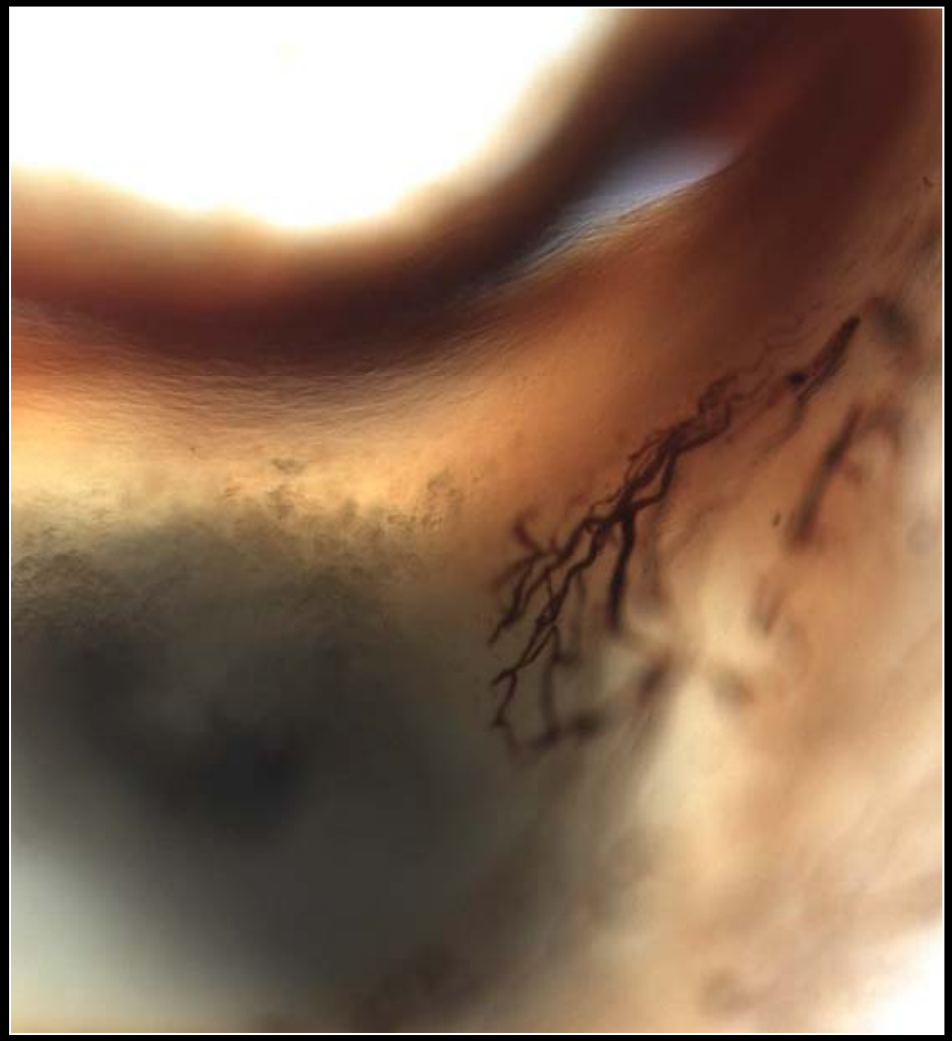


Angiogenesis and inflammation in the progressive enlargement of the necrotic core

Adventitia & Intraplaque Vasa Vasorum

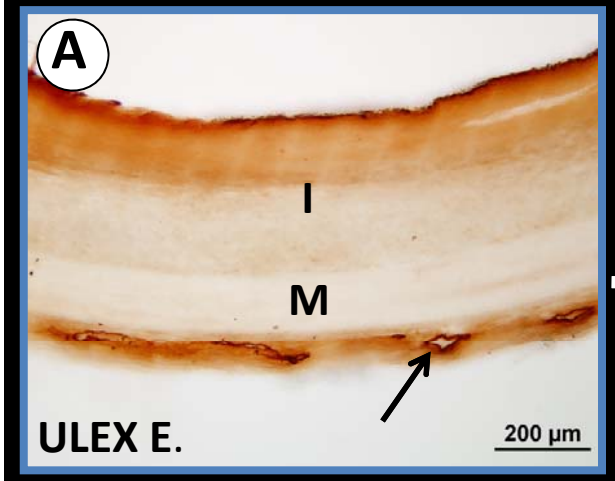
150 μm thick sections stained with Ulex



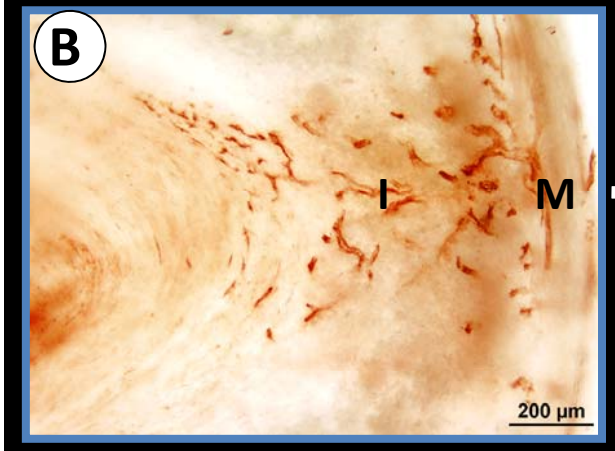


Finn AV, et al. Arteriosclero Thrombosis Vascular Biology 2008

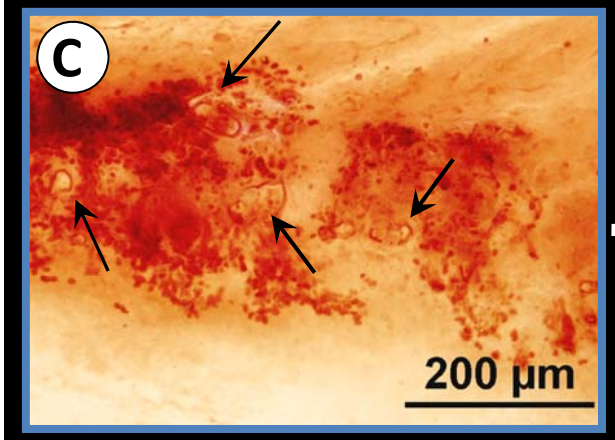
Evidence that Human Coronary Plaques Express a Latent Proangiogenic Phenotype



Normal artery with adventitial Vv

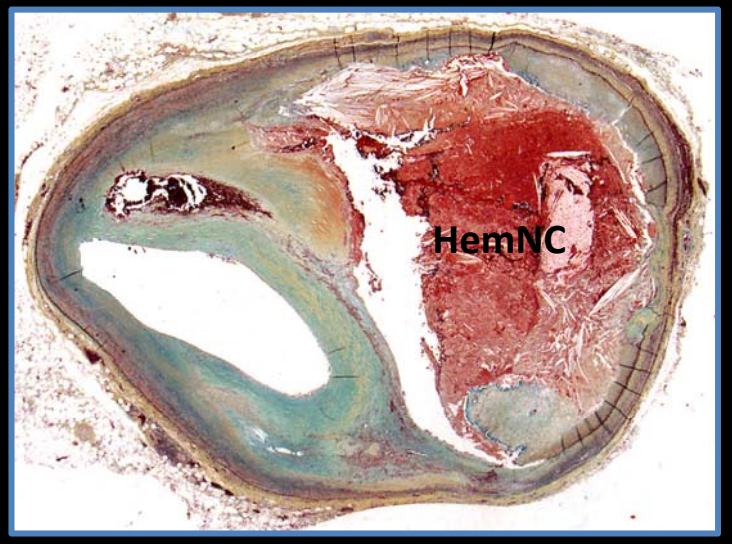


Fibroatheroma with Tortuous and Abnormal Vv



Fibroatheroma with Leaky Vv (peri-vascular hemorrhage)

Fibroatheroma with severe Intraplaque hemorrhage



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

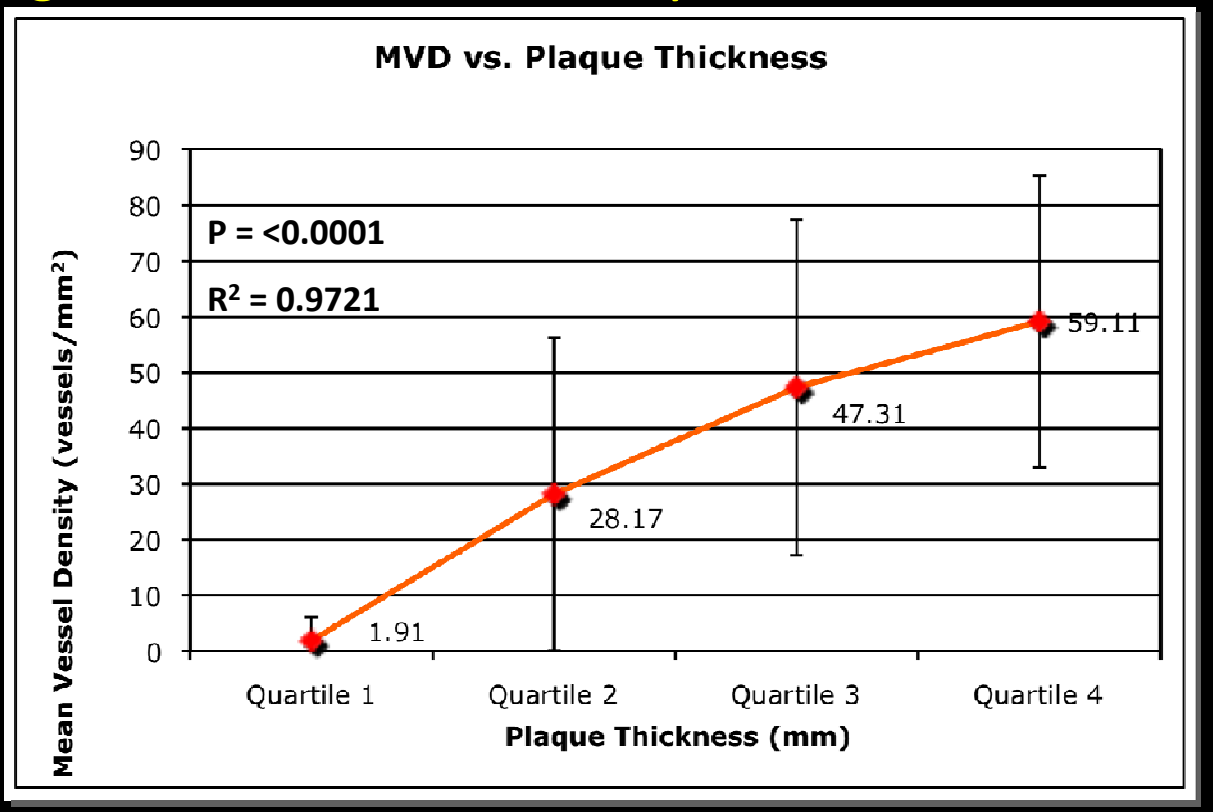
The Relationship of Plaque Thickness and Vasa Vasorum Density in Progressive Human Coronary Lesions

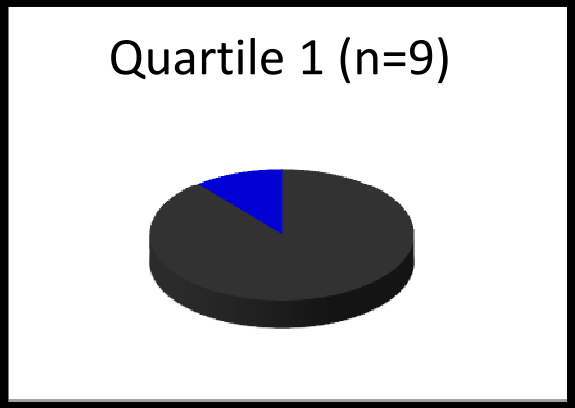
Quartile 1 (n=9) <0.50 mm

Quartile 2 (n=15) 0.50 mm - 0.99 mm

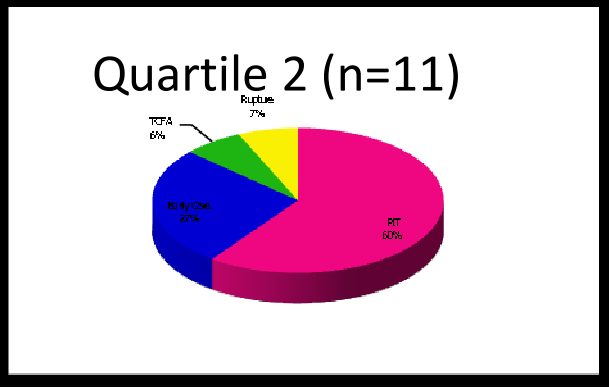
Quartile 3 (n=26) 1.00 mm - 1.50 mm

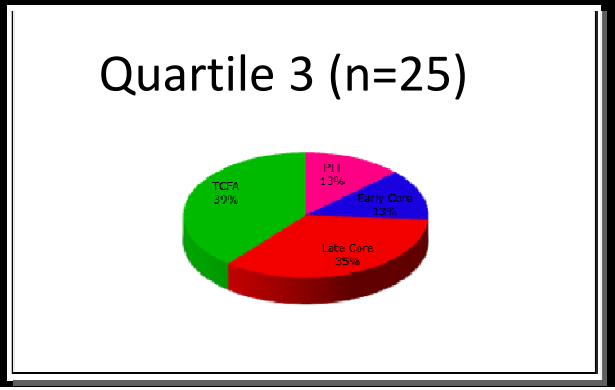
Quartile 4 (n=23) >1.50 mm

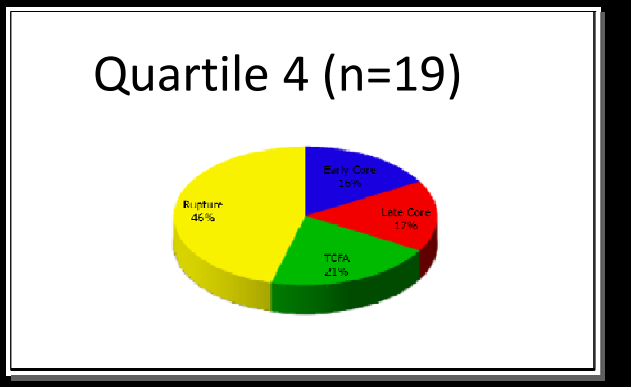




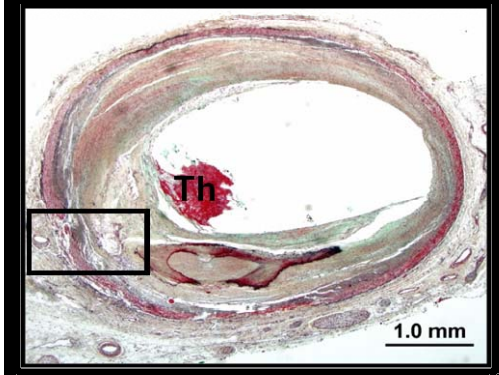
AIT PIT FA (early core) FA (late core) TCFA Plaque Rupture

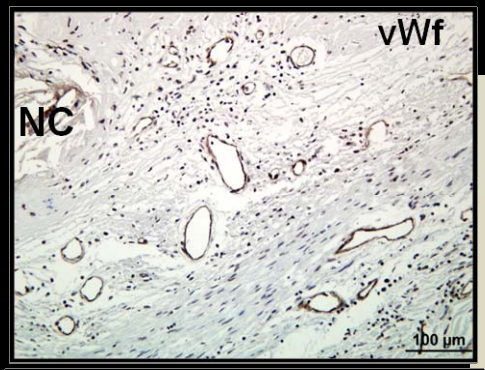


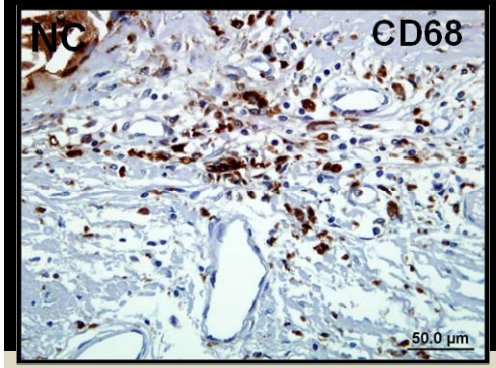


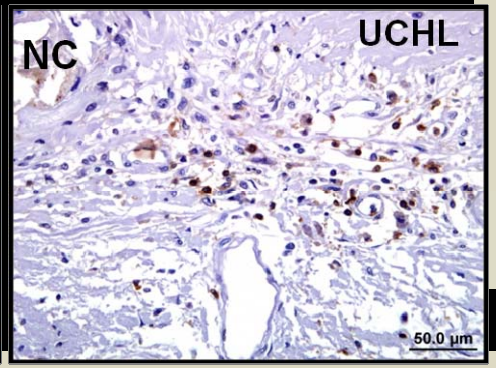


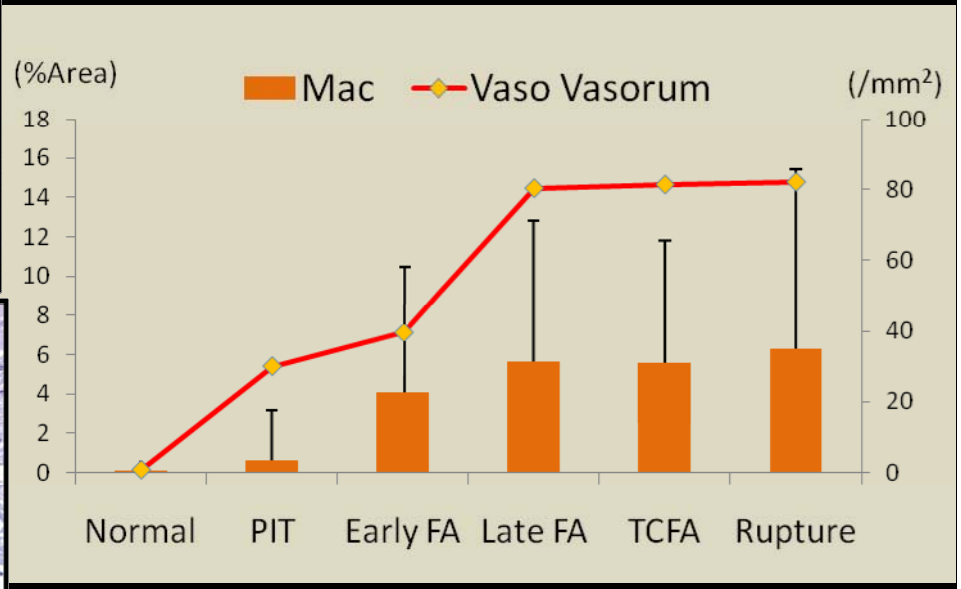
Vaso Vasorum Density and Inflammatory Cells

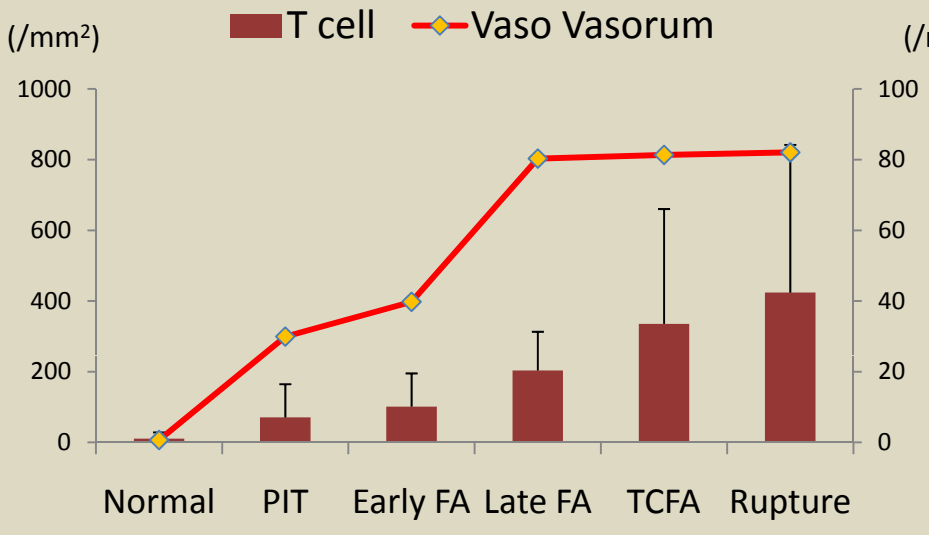


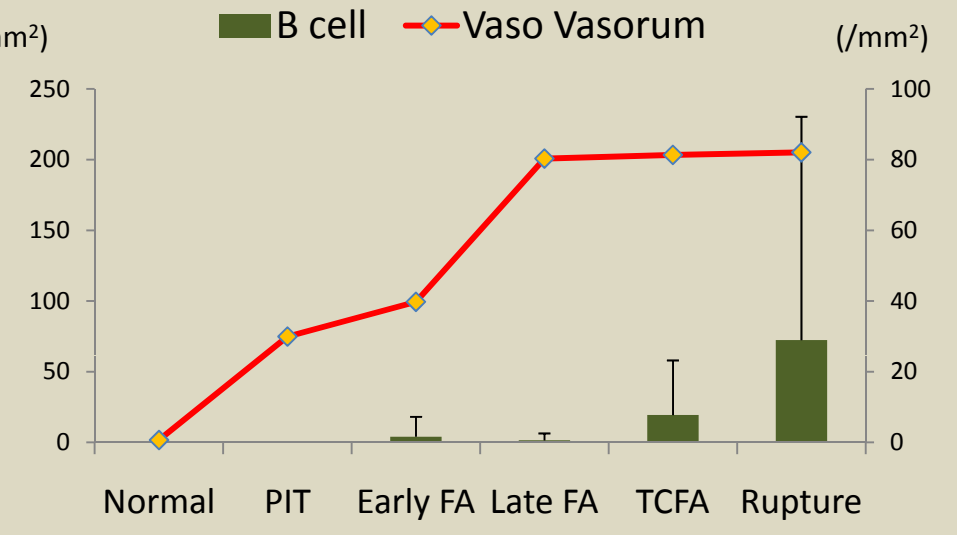




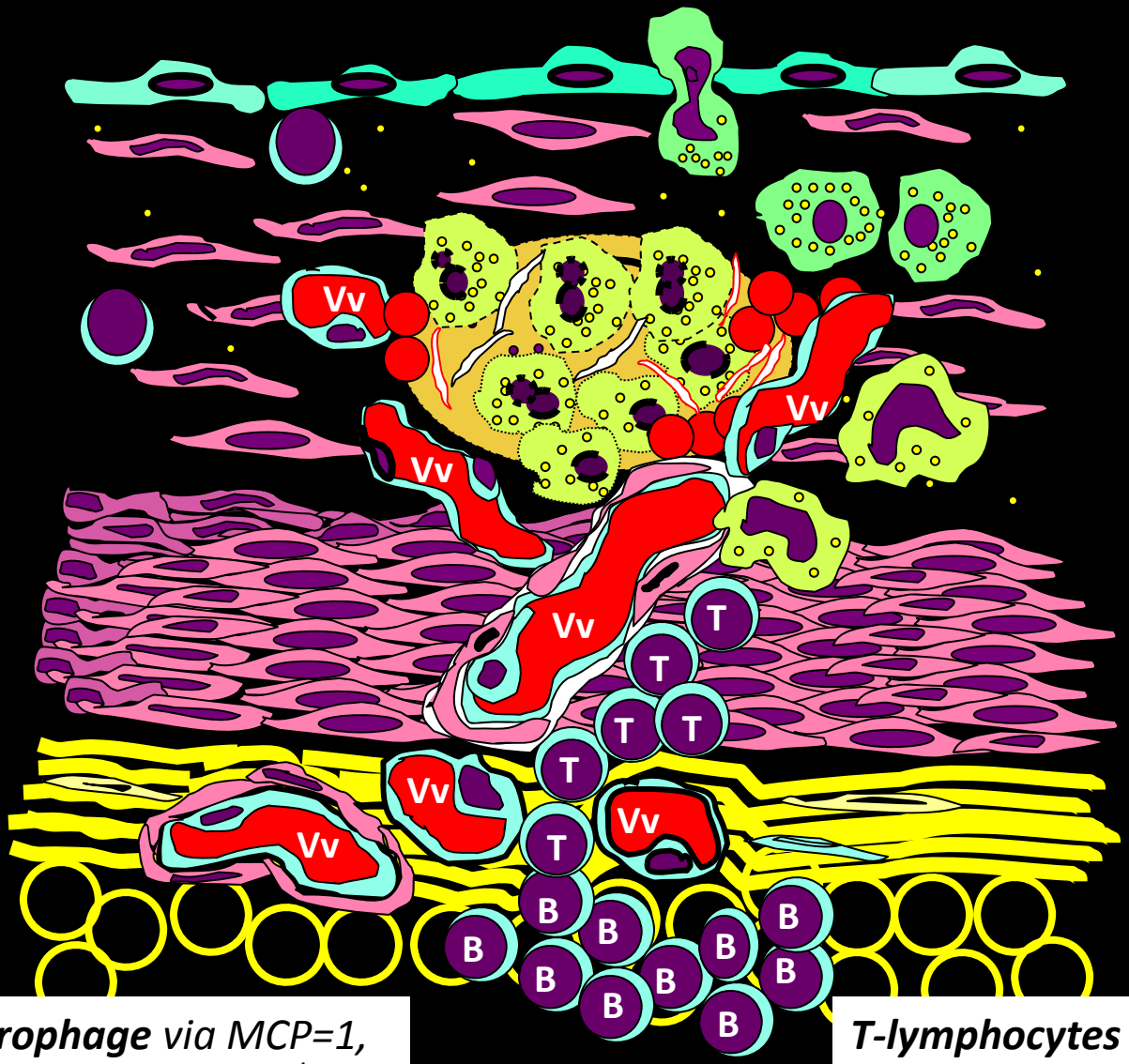








Mechanisms of coordinated angiogenesis and inflammation in the progressive enlargement of the necrotic core

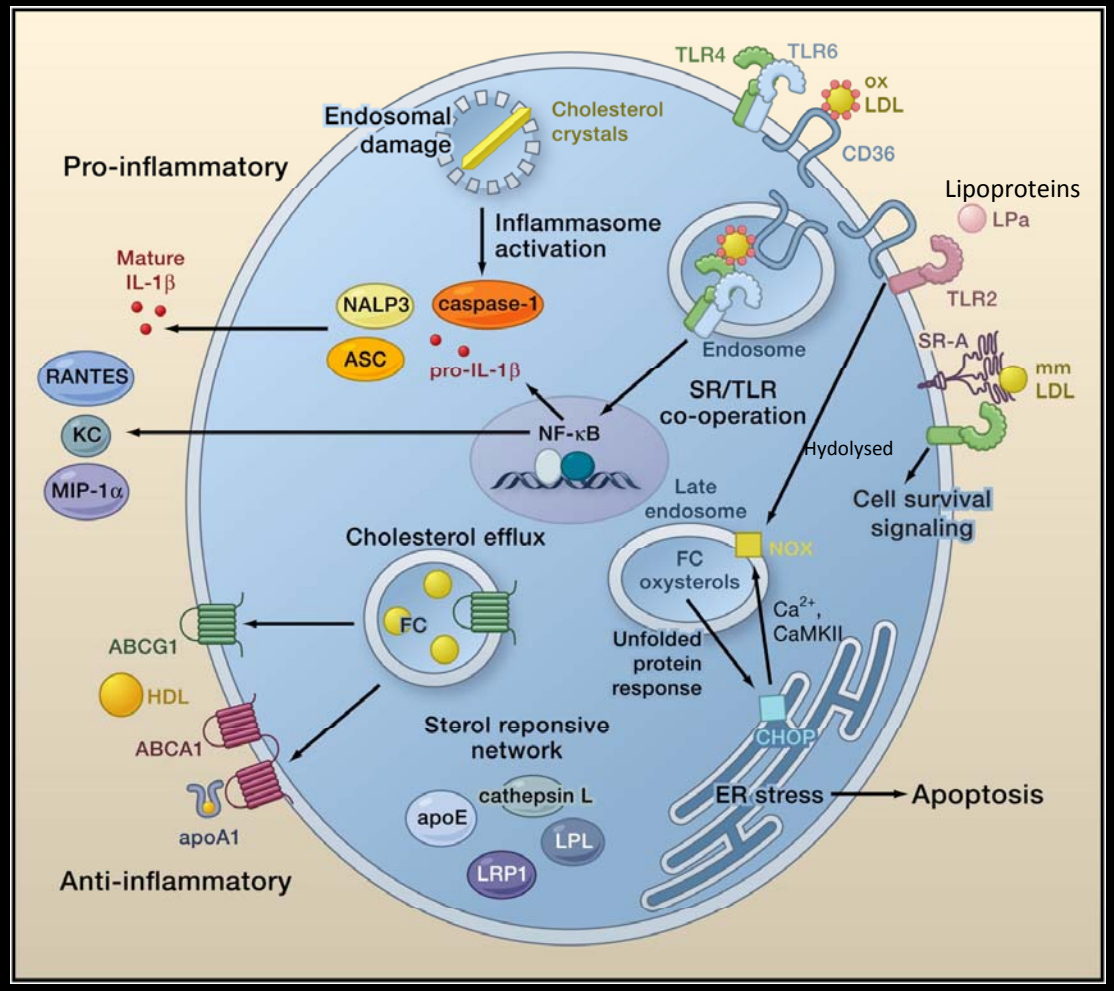


↑***Macrophage*** *via MCP=1, M-CSF within plaque -* ↑ *VEGF =* ↑*angiogenesis*

T-lymphocytes - ↑***angiogenesis via*** *Toll-like receptors (TLR) 2 and 4, CD40/CD40L*

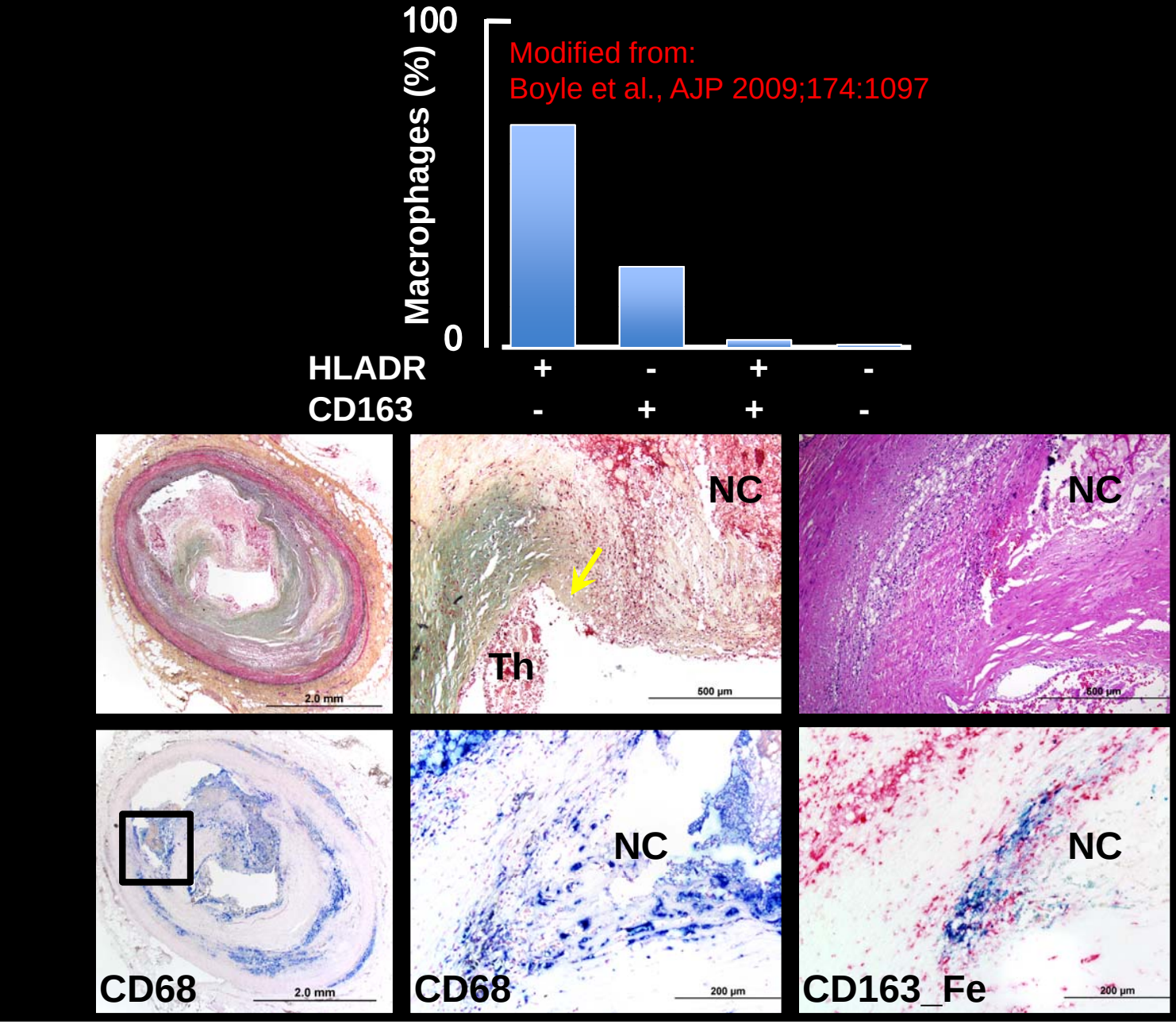
Macrophage Diversity around Angiogenesis, Plaque Hemorrhage and Iron deposits

Signaling Pathways in Macrophage Atherosclerosis

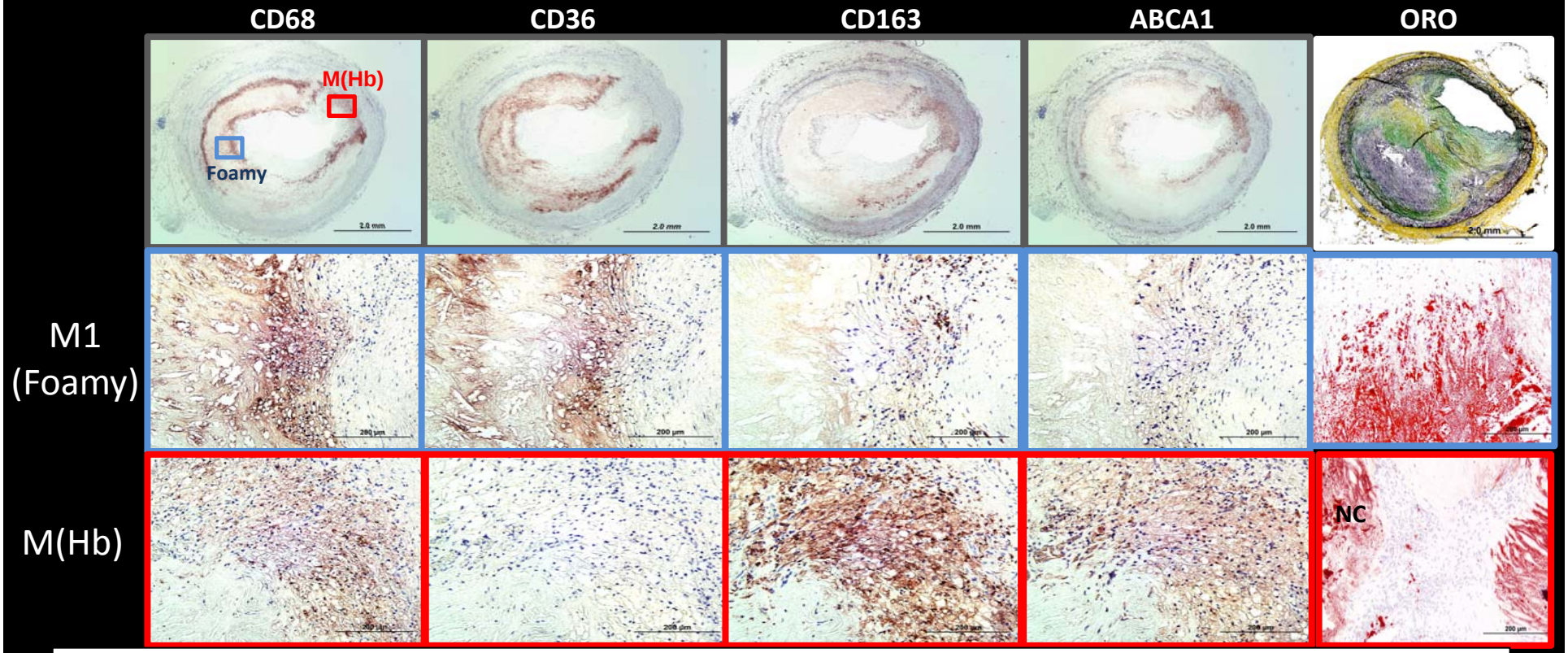


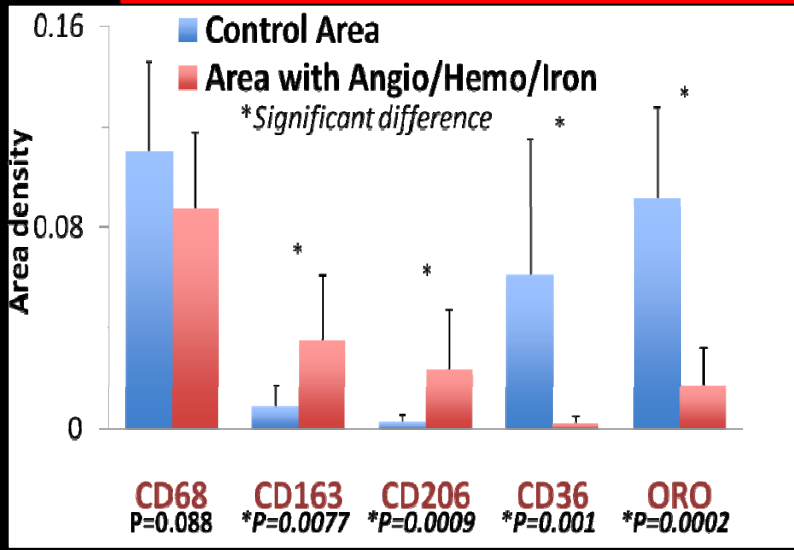
Moore et al. Cell. 2011;145:341-355

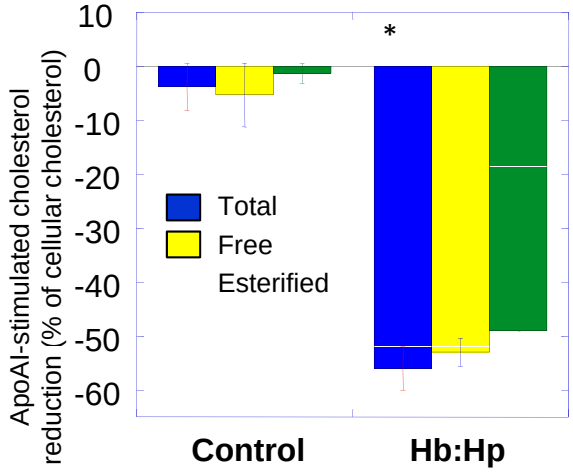
Expression of Haptoglobin Receptor (CD163) on a Subset of Macrophages in Human Coronary Plaque Rupture

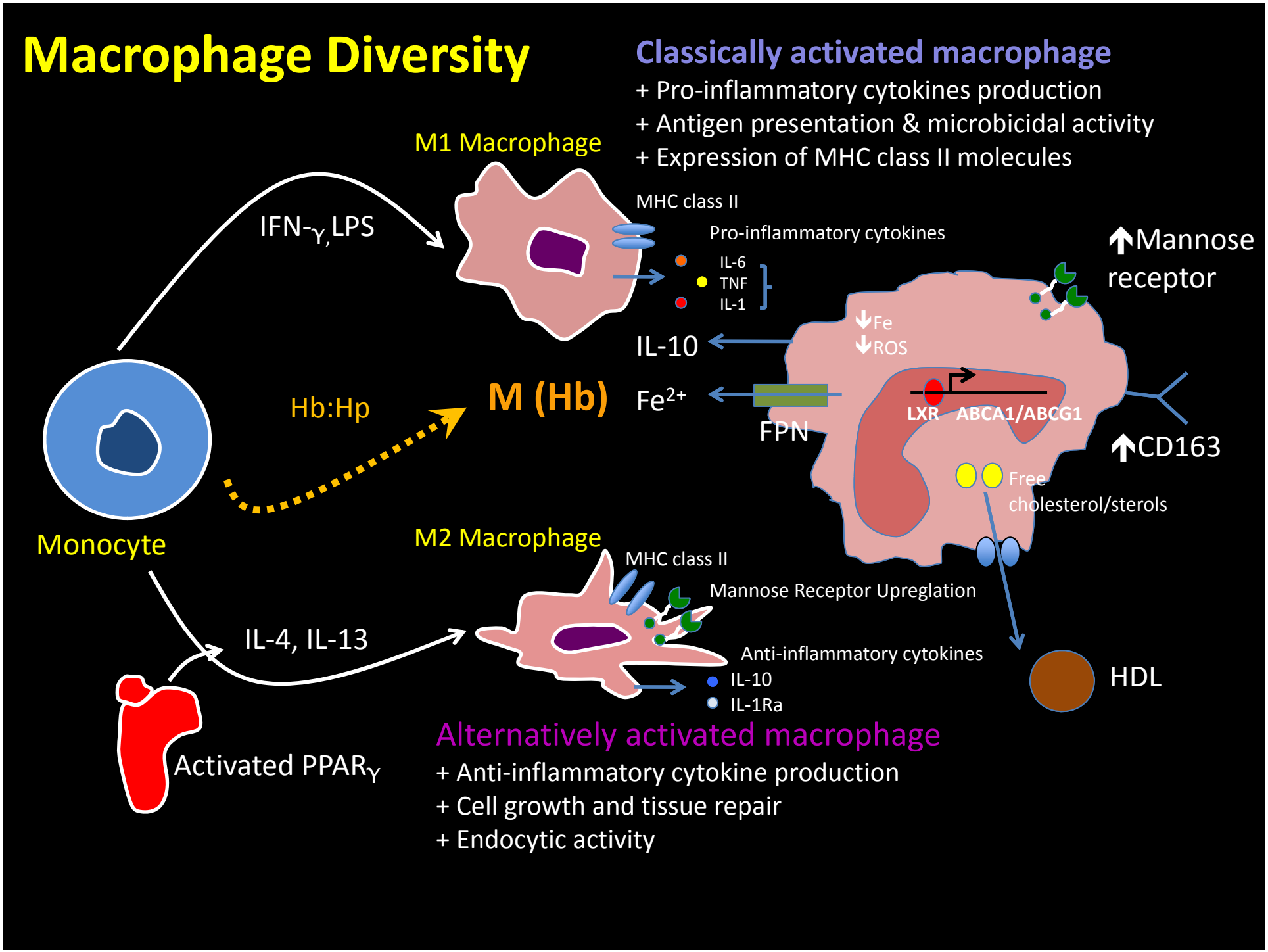


Different Phenotype of Macrophage Subclass

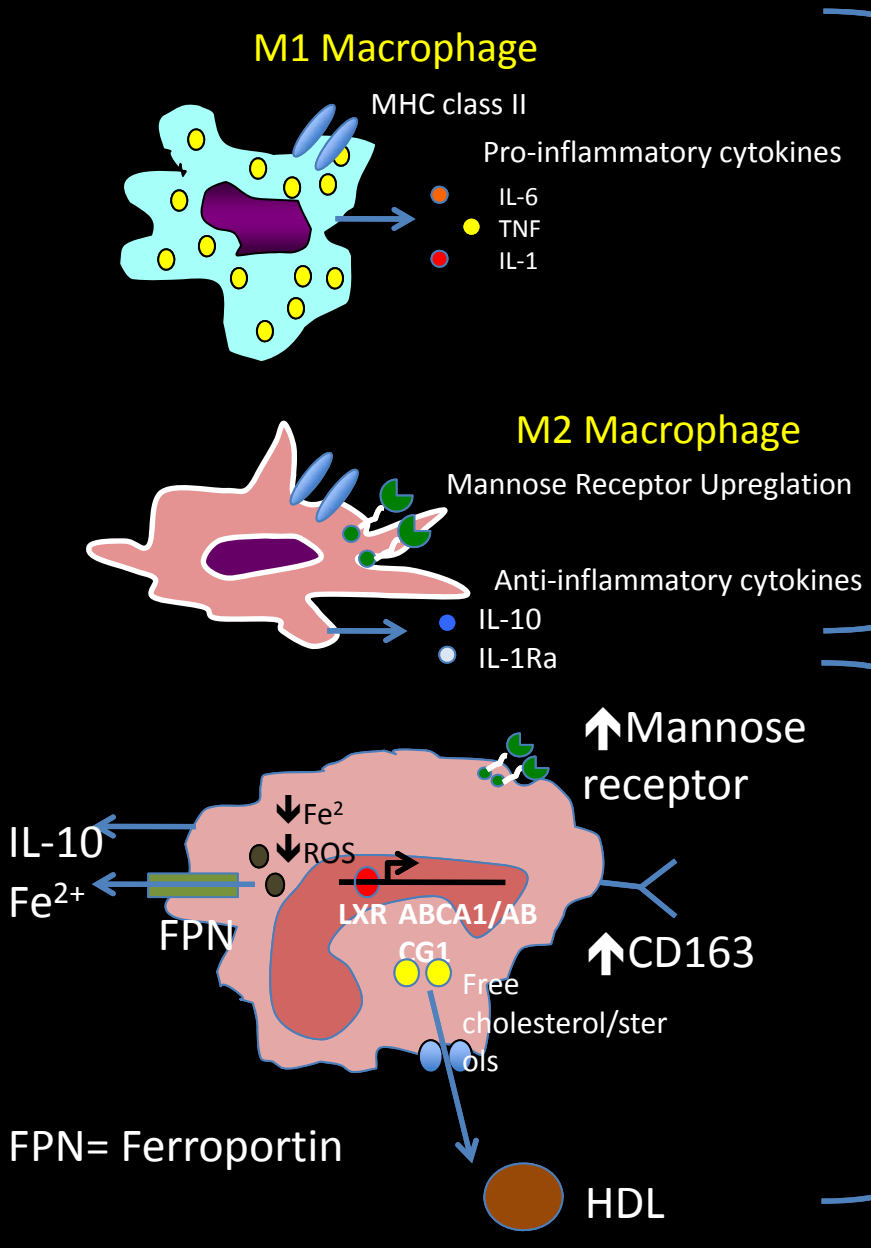







Macrophage Diversity
Classically activated macrophage
+ Pro-inflammatory cytokines production
+ Antigen presentation & microbicidal activity
+ Expression of MHC class II molecules
M1 Macrophage
IFN-γ, LPS
MHC class II
Pro-inflammatory cytokines
IL-6
TNF
IL-1
Mannose receptor
Fe
ROS
IL-10
Hb:Hp
M (Hb)
Fe2+
FPN
LXR
ABCA1/ABCG1
CD163
Free cholesterol/sterols
Monocyte
M2 Macrophage
MHC class II
Mannose Receptor Upreglation
IL-4, IL-13
Anti-inflammatory cytokines
IL-10
IL-1Ra
HDL
Activated PPARγ
Alternatively activated macrophage
+ Anti-inflammatory cytokine production
+ Cell growth and tissue repair
+ Endocytic activity

Possible Next Targets for New Drugs



- Statins, CETP inhibitors
- Darapladib – $LpPLA_2$ inhibitor
- Antiinflammatory drugs: Methotrexate, low dose; and IL1β antibody.

- LDN 193189 (LDN) inhibits BMP signaling to prevent activation of hepcidin transcription factors, leading to reduced hepcidin and maintenance of FPN in macrophages, thereby reducing intracellular iron and ROS and increasing the expression of ABCA1 and ABCG1 to promote lipid efflux and reduce foam cell formation.

Factors and Conditions Associated with Increased Risk for Acute Coronary Events (Circulation 2012)

Coronary Plaque Characteristics	Coronary Blood Flow Dynamics	Intrinsic Hemostasis Factors	Metabliic and Inflammatory Conditions	Neuroharmonal Imbalance	Enviornmental Factors and Drugs
Plaque Burden	Blood viscosity	Platelet function/volume	Diabetes Mellitus	Stress	Somking
Lumen encroachmen	Shear stress	Circadian variation	Obesity	Catecholamine surges	Pollution
Lesion location	Reduced blood flow/ low CO	Factor V Leiden def	Dyslipidemia	Depression	Climate
Plaque composition	Vascular tone and reactivity	vonWilliebrand Factor def	Connective tissue diseases	Exertion	Legal drugs
Plaque biology	Arterial hypertension	Antiphospho-lipid syndrome	Infections	Autonomic dysfunction	Illegal drugs
Plaque configuration and remodeling			Renal Disease	Endocrine imbalance	Diet
Endothelial dysfunction					Sedentary life

Summary: Thrombosis and Inflammation

- Plaque rupture is a main cause of thrombosis (65-70%), while other minor causes include erosion (30%) and calcified nodule (2-5%). (Rivaroxaban a Xa inhibitor was effective in reducing cummulative CV event rate in patients presenting with recent ACS (STEMI, NSTEMI, or UA)
- Risk factors are predictive of specific plaque types.
- Diabetes and metabolic syndrome play an important role in CAD. (Better control)
- Vulnerable plaques (TCFA) is a likely precursor lesions of rupture. Macrophage infiltration play an important role in modification of plaque vulnerability
- Extent of macrophages infiltration and necrotic core size are predictors of plaque vulnerability. (Antiinflammatory drugs: Methotrexate, low dose; and IL1β antibody).
- Lp-PLA_2 may be involved in plaque progression and necrotic core expansion, therefore the inhibition of this enzyme is a potential therapeutic approach to prevent plaque progression and stabilize atheromatous plaques. (Darapladib)
- Intraplaque hemorrhage from “leaky” vasa vasorum is an important contributor to necrotic core expansion and potential lesion instability.
- Macrophage subtypes may help us better understand the role of plaque hemorrhage and plaque stabilization vs. plaque rupture (LDN supresses hepcidin increases macrophage cholesterol efflux and reduces foam cells).

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Lila Adams, HT
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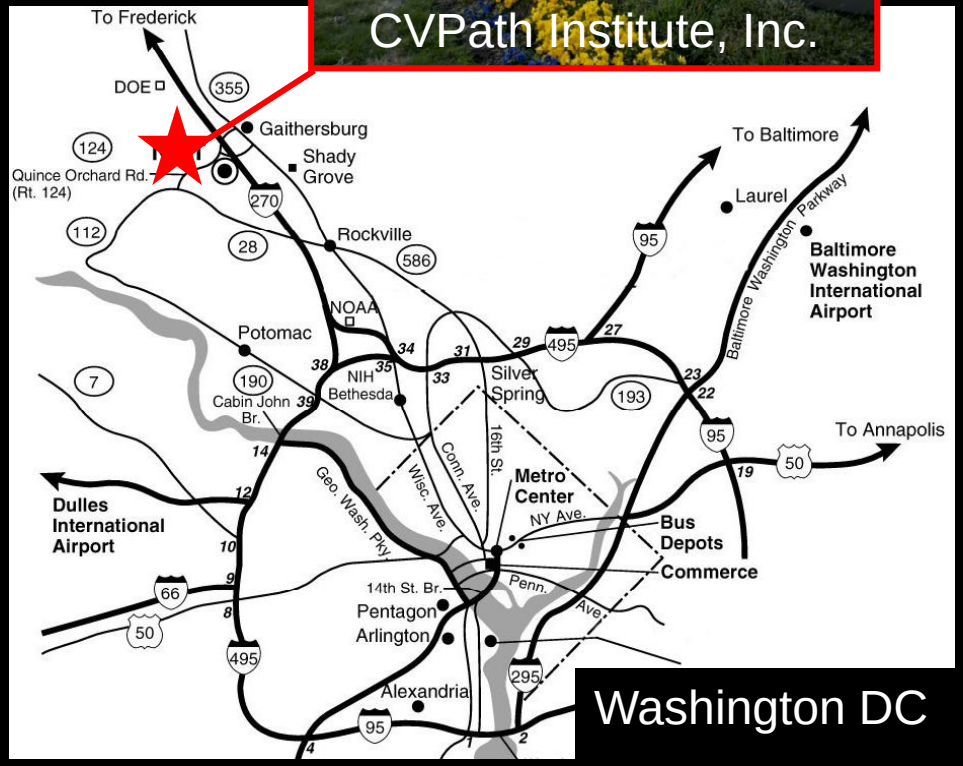

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