New Pathologic Insights into Vulnerable Plaque

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Conflict of Interest Statement

Within the past 12 months, I or my spouse/partner have had a financial interest/arrangement or affiliation with the organization(s) listed below.

Physician Name: Renu Virmani, M.D.

Company/Relationship:Research Grants

Medtronic AVE; Guidant; Abbott; GE Healthcare; Takeda; Atrium Medical Corp.; ev3; Conor Medsystems; TopSpin Medical (Israel) Ltd.; Paracor Medical, Inc.; OrbusNeich; Terumo Corp.; Vascular Therapies, LLC; CardioKinetix; Osiris Therapeutics, Inc.; Edwards Life Sciences; Biomerix; Nitinol Device and Components; Sorin Biomedica Cardio S.r.l; 3F Therapeutics; Hancock Jaffee Labs, Inc.; Cardiovascular Device Design; Coaptus; Biotegra; Cardica, Inc.; Cordis Corp.; Cryo Vascular Systems, Inc.; CVRx, Inc.; diaDexus, Inc.; InfraReDx, Inc.; Kensey Nash Corp.; Medeikon Corp.; MedNova USA, Inc.; Microvention, Inc.; Oregon Medical Laser Center; Spectranetics Corp.; Takeda Pharmaceuticals North America; Toray Industries, Inc.; Vascular Concepts; Volcano Therapeutics, Inc.; BioSensors International; Alchimer S.A. and Relisys.

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Employment 25%: Cardiovascular Research Foundation

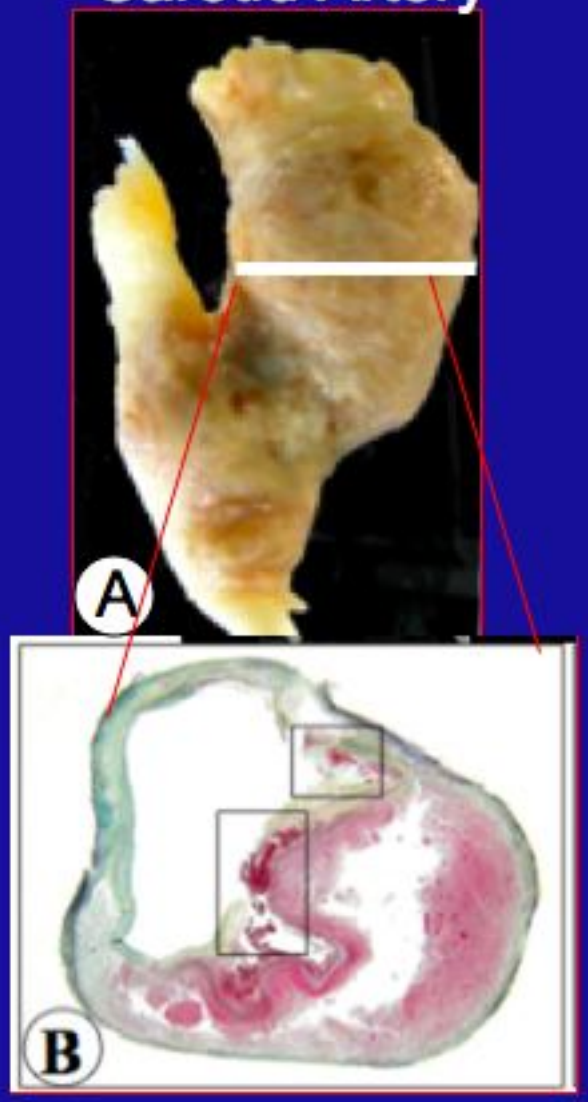
Do not own any stock in any company.

Natural History of Atherosclerosis

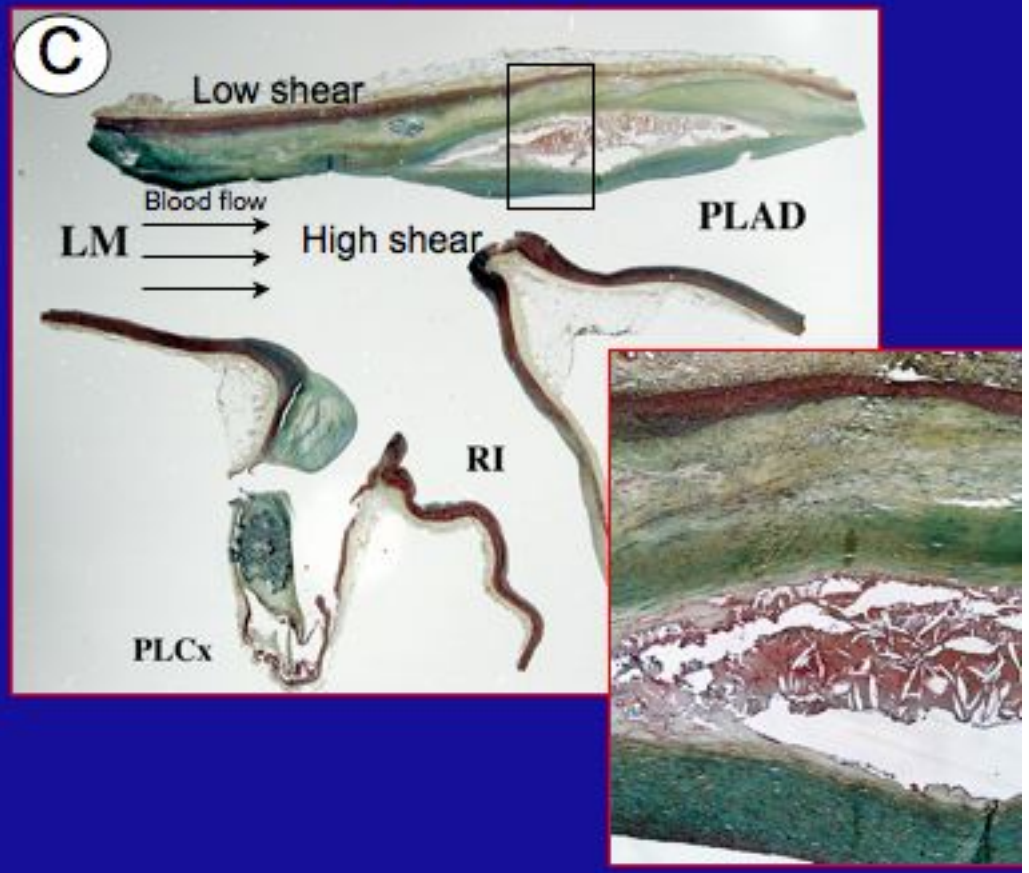
- Systemic factors - hyperlipidemia, diabetes mellitus, smoking, hypertension, age and sex, hsCRP, Lp-PLA_2, etc.
- Local factors: at branch points, e.g., carotid bifurcation, abdominal aorta just above bifurcation and coronary branch points, arch vessels at take off, are the sites of atherosclerosis manifestation
- 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 to precipitate thrombosis

Branch points are the sites of atherosclerosis and occur in areas of low shear

Carotid Artery



Left Coronary artery

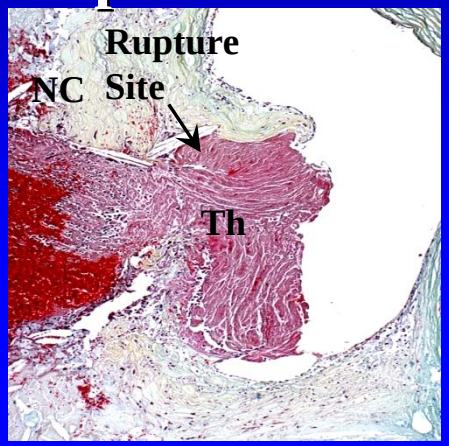


Lesions with Thrombi

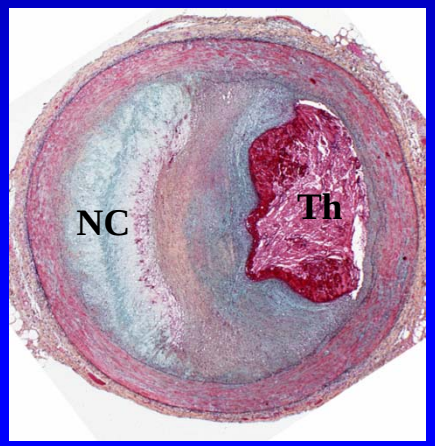
- *Plaque Rupture*
- *Plaque Erosion*
- *Calcified Nodule*

Causes of Coronary Thrombosis

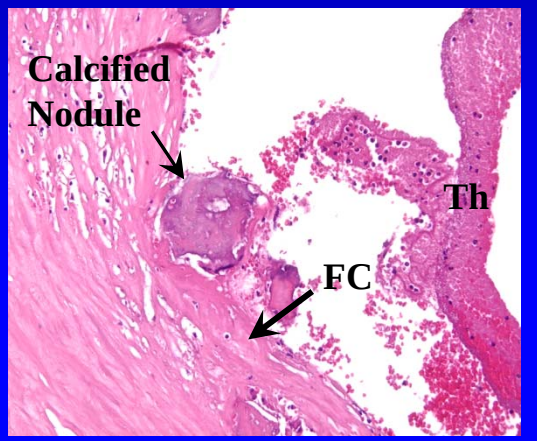
Rupture

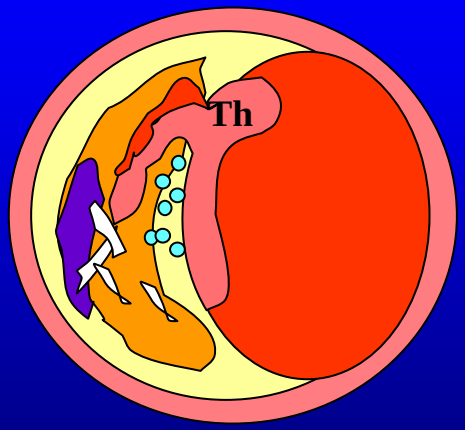


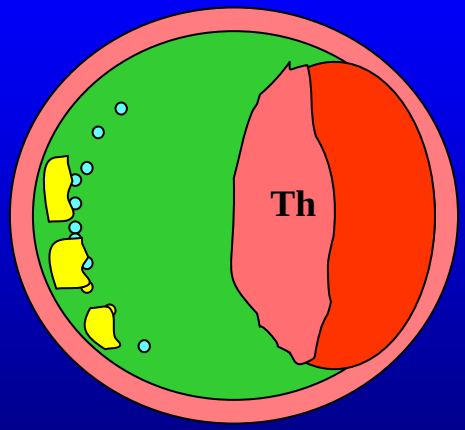
Erosion

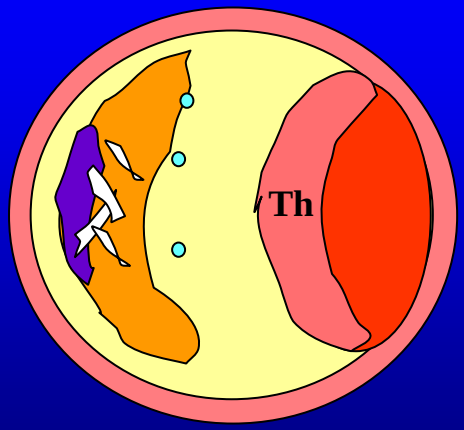


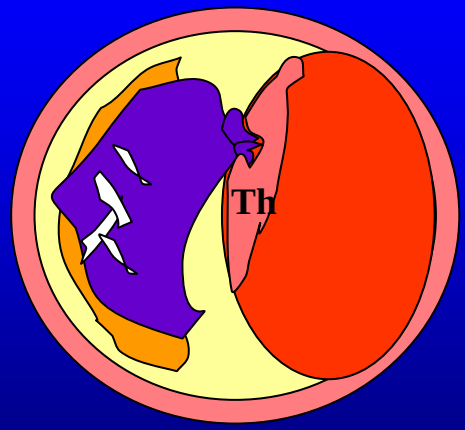
Calcified nodule





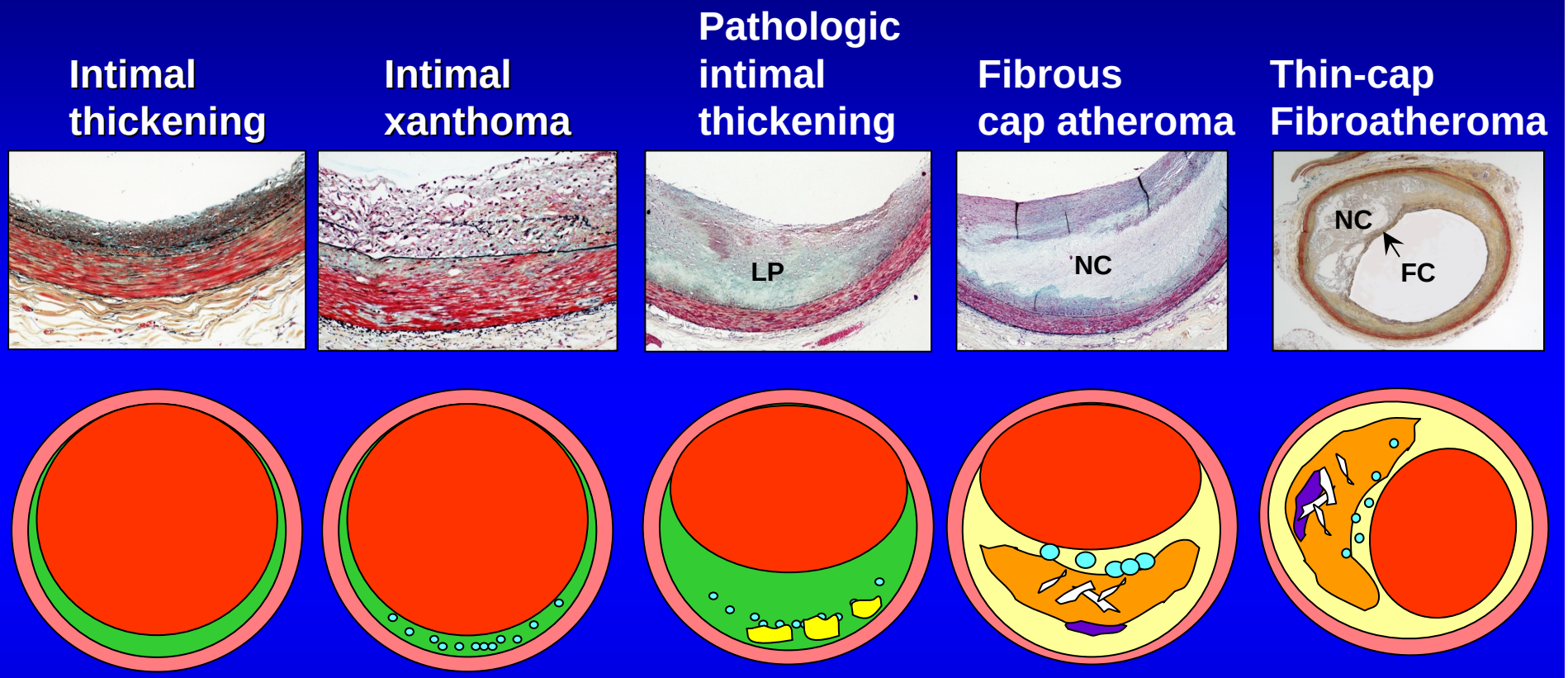






Virmani R, et al. Arterioscler Thromb Vasc Biol 2000;20:1262

Development of Human Coronary Atherosclerosis



FC = fibrous cap
LP = lipid pool
NC = necrotic core

Thin-Cap Atheroma (Vulnerable Plaque) Components

- Necrotic core
- Thin fibrous cap (< 65 μm)
- Cap infiltrated by macrophages and lymphocytes
- Cap composition – type 1 collagen and few smooth muscle cells

A Non-Hemodynamically Limiting Thin-cap Fibroatheroma

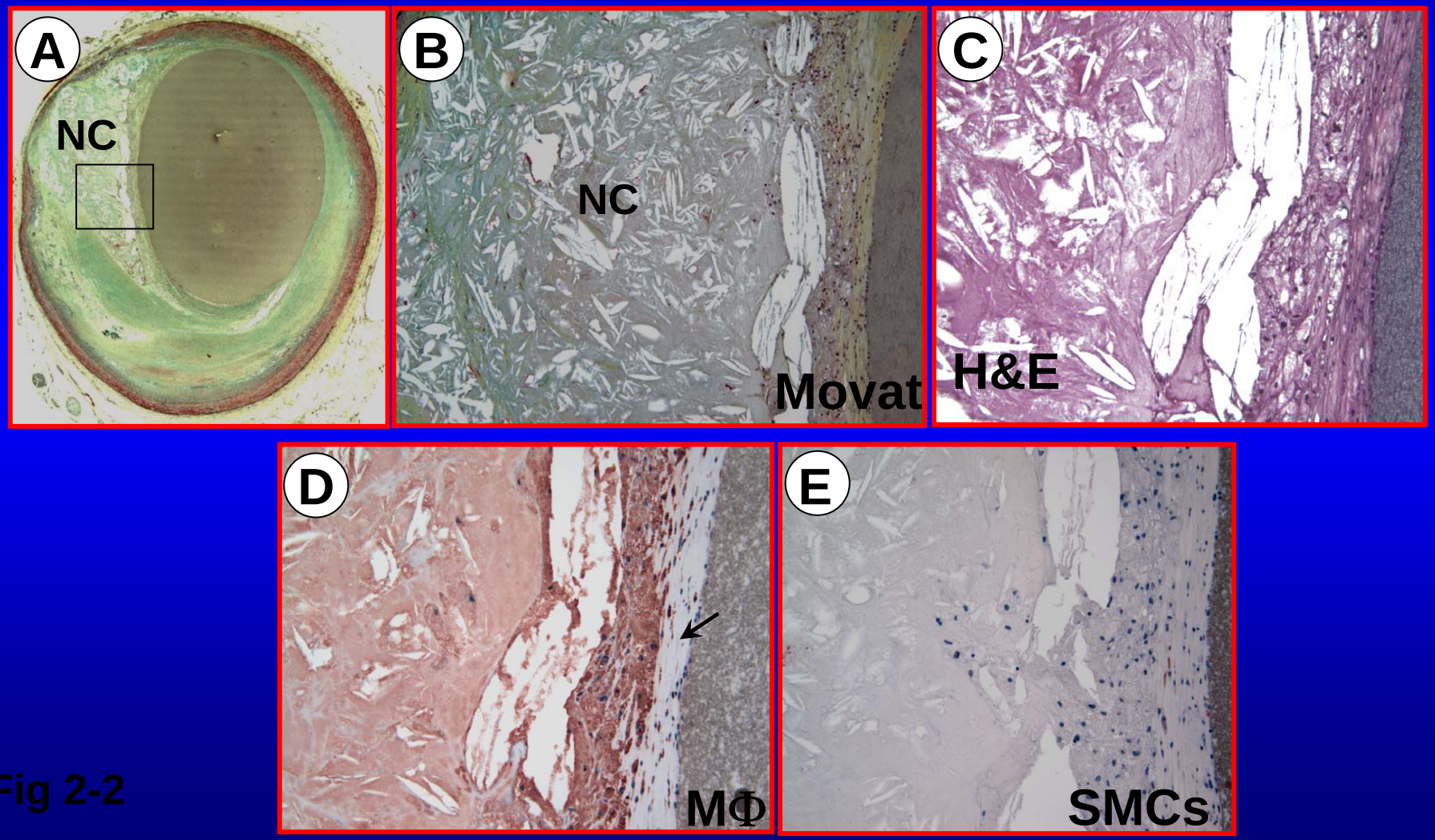


Fig 2-2

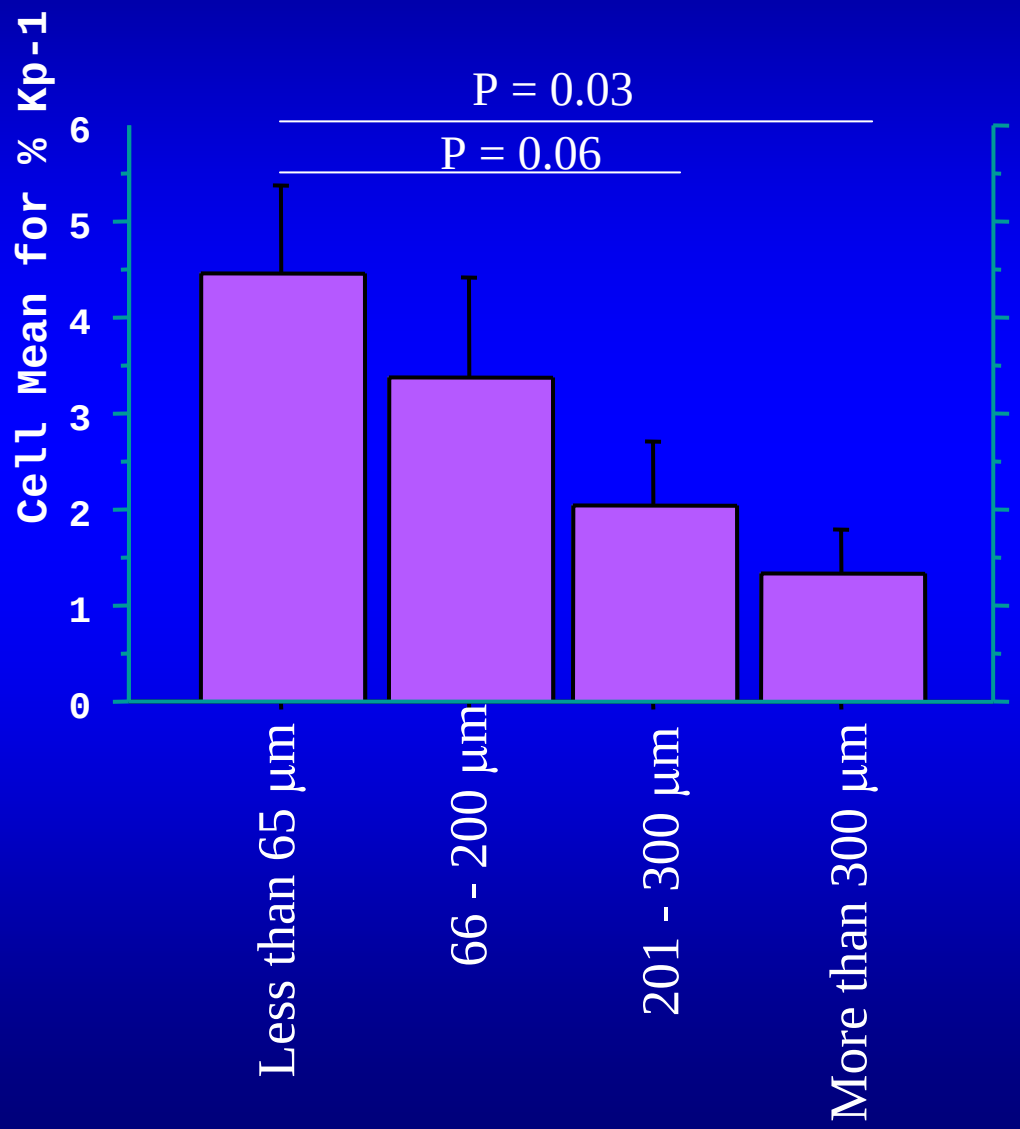
Morphologic Characteristics of Plaque Rupture and Thin-cap Fibroatheromas

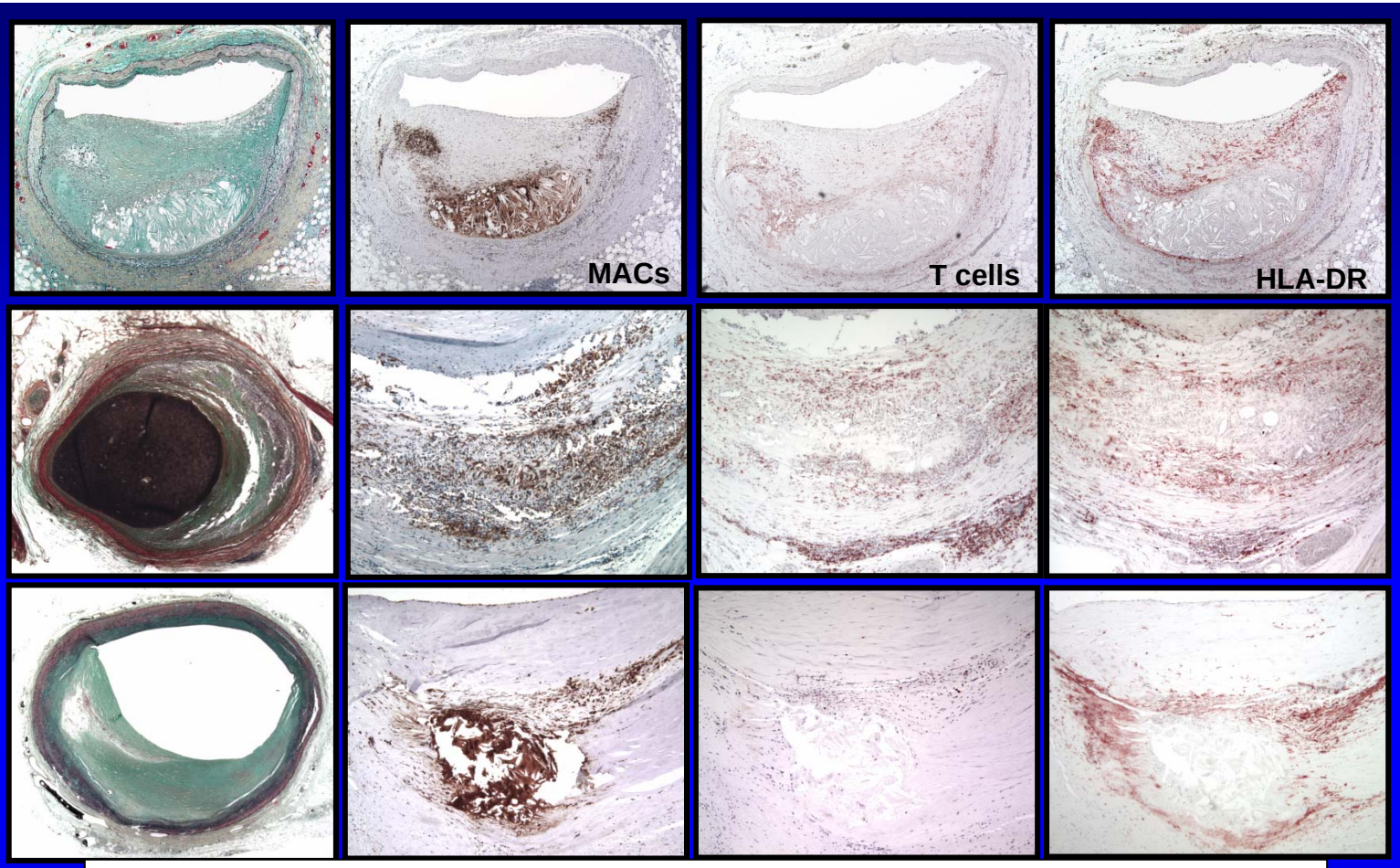
Plaque type	Necrotic Core (%)	Fibrous cap Thickness (μm)	MΦs (%)	SMCs (%)	T-lymph	Calcification Score
Rupture	34±17	23±19	26±20	0.002±0.004	4.9±4.3	1.53±1.03
Thin-cap Fibroatheroma	23±17	<65μm	14±10	6.6±10.4	6.6±10.4	0.97±1.1
P value	0.01		0.005	ns	ns	0.014

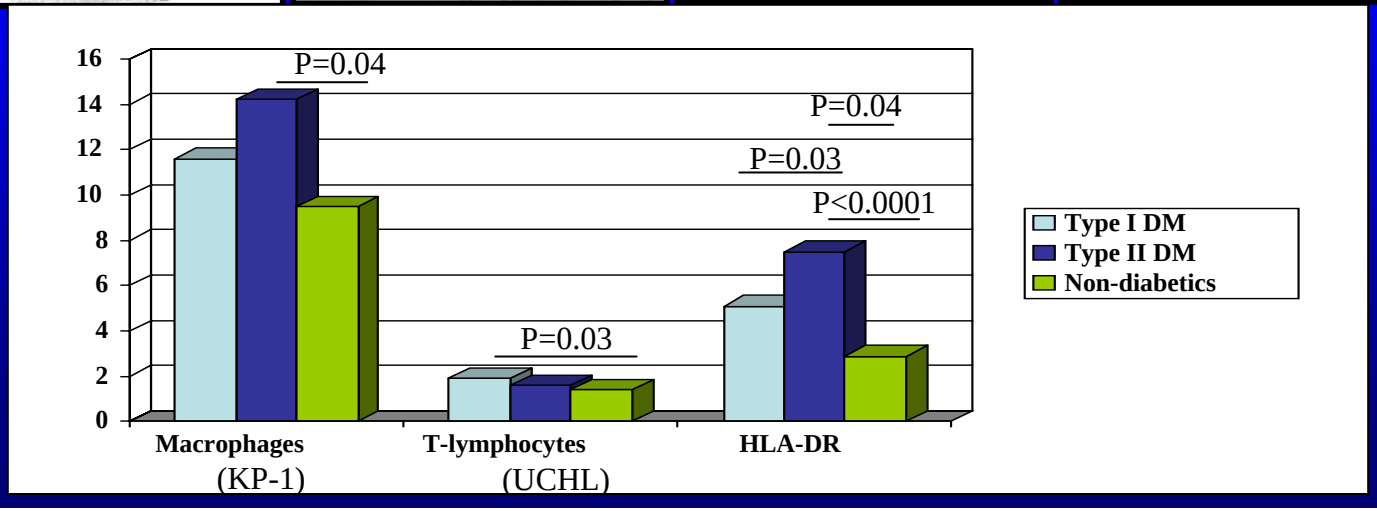
Mean values are represented ± standard deviation. Abbreviations: MΦs= macrophages, SMCs= smooth muscle cells, T-lymph= T-lymphocytes

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

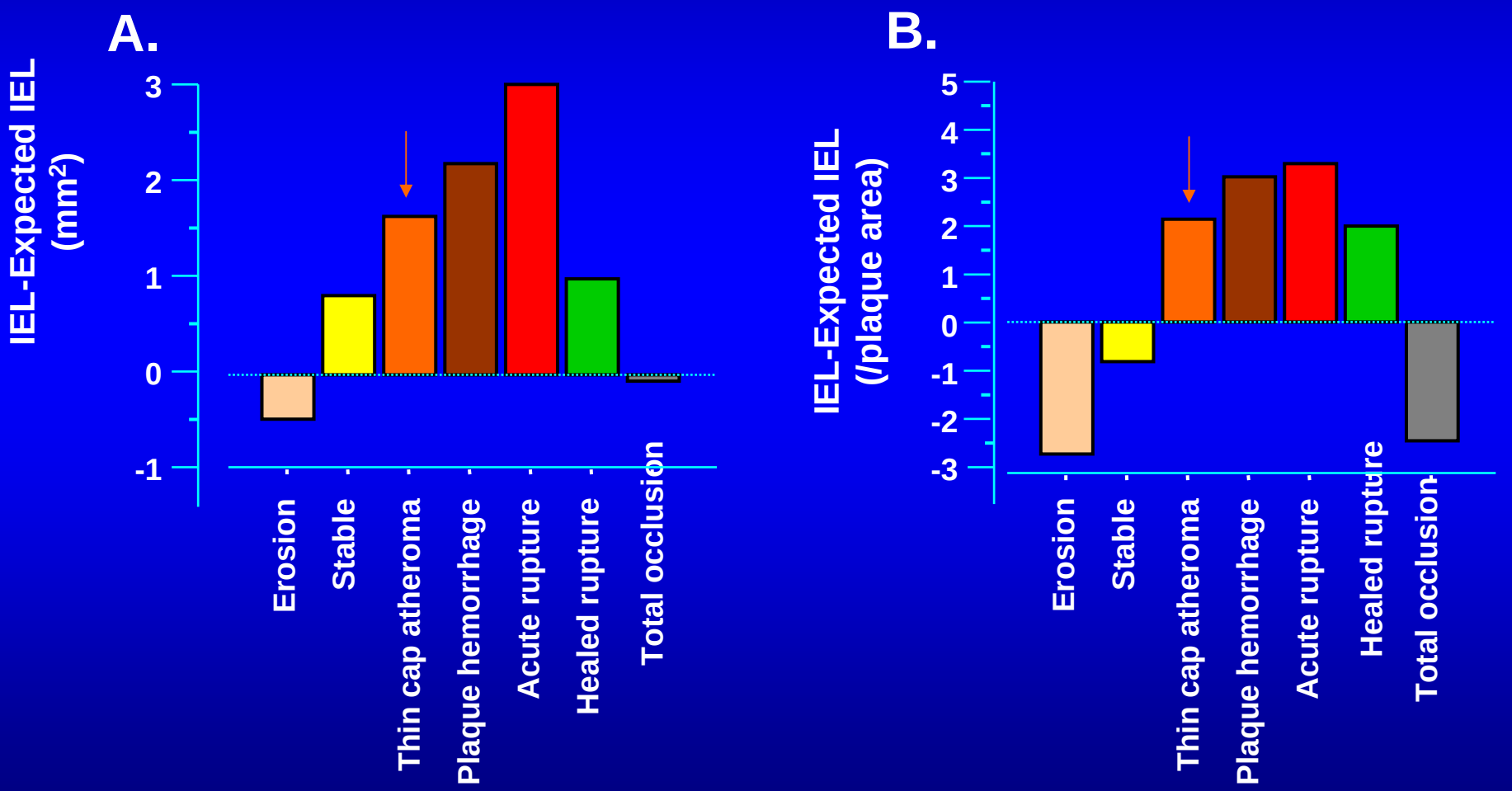
Relationship of Fibrous Cap Thickness to Macrophage Infiltration




MACs
T cells
HLA-DR
Type II DM
Type I DM
Non-Diabetic


16
14
12
10
8
6
4
2
0
P=0.04
P=0.04
P=0.03
P<0.0001
P=0.03
Macrophages
(KP-1)
T-lymphocytes
(UCHL)
HLA-DR
Type I DM
Type II DM
Non-diabetics

Remodeling in Varying Coronary Lesion Morphologies



Predictors Remodeling Score* Independent of Age, Sex, and Distance from Ostium

Plaque Parameters	T	P
% Macrophages	5.3	<0.0001
Fibrous calcium	4.5	<0.0001
% Lipid Core	4.3	<0.0001
% Fibrous tissue	-3.8	<0.0001
% Calcified lipid core	3.0	0.002
Medial atrophy	2.0	0.05
Adventitial thickness	-1.6	0.11

*IEL area – expected IEL area/plaque area

Inflammation plays a primary role in progression of human atheroma

- Key role of inflammation in atherosclerosis is evident in many epidemiology studies indication an association between inflammatory markers: CRP, interleukin-6, oxLDL and risk of cardiovascular events.
- Lp-PLA_2 is a novel inflammatory marker that has been the recent focus of several epidemiology studies showing that plasma levels predict cardiovascular events
- Lp-PLA_2 mRNA and protein have been detected in macrophages in man and rabbit non-coronary atherosclerotic lesions

Lp-PLA_2

- Lipoprotein-associated phospholipase A_2(Lp-PLA_2), an enzyme bound mainly to LDL-cholesterol, results in the formation of pro-inflammatory lysophosphatidylcholine and oxidized fatty acids
- Lp-PLA_2 induces cell death of human monocytes/macrophages in the presence of mildly oxidized LDL *(Curr Opin Lipidol 2005;16:442-446)*
- Hydrolysis of platelet activating factor and other phospholipids by Lp-PLA_2 may however reduce inflammation
- The pro- or anti-inflammatory role of Lp-PLA_2 in humans coronary atherosclerosis has not been established

Morphometric assessment of vessel area, stenosis, necrotic core size, and macrophage density from 25 pts with SCD (frozen sections)

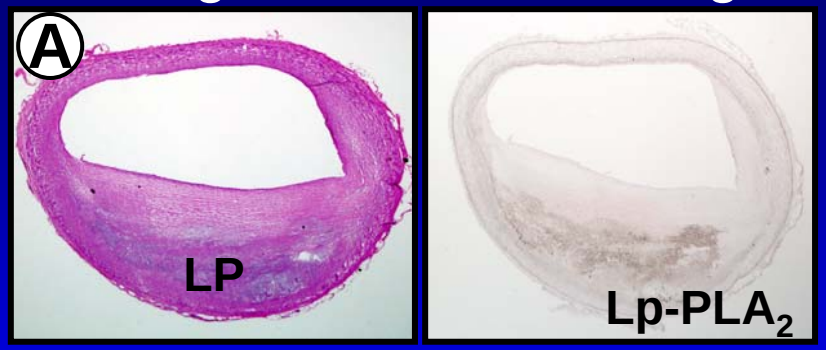
Plaque Type	IEL mm^2	Stenosis %	Necrotic core %	Macrophage (%CD68)
Pathologic intimal thickening (n=7)	10.4±2.5	45.8±18.8	0	3.1±3.2
Fibroatheroma (n=8)	10.0±4.4	70.8±14.7	14.5±8.6	7.4±5.4
Thin-cap Fibro-atheroma(n=8)	9.0±1.7	82.4±8.8	32.1±18.3	8.2±4.1
Plaque rupture (n=7)	13.2±6.4	84.6±7.5	36.5±14.0	11.4±3.7
P value	ns	<0.0001*	<0.0001**	<0.01***

Lipoprotein-associated phospholipase A_2 (Lp-PLA_2), apoptosis by cell type, and cell density in macrophages and SMC rich regions

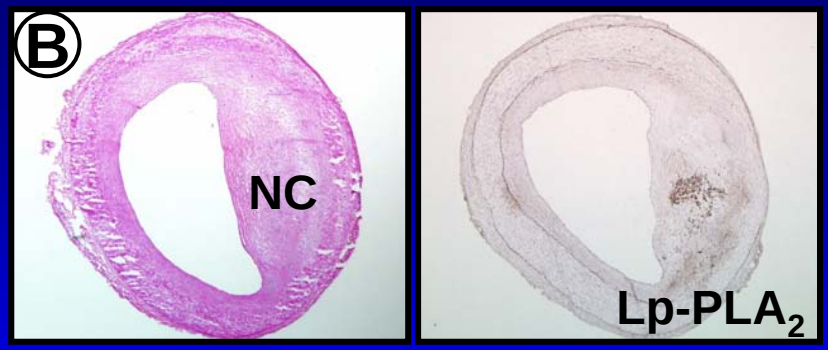
Plaque Type	Lp-PLA2	Apoptosis~		Cell Density(cells/mm2)	
		Macrophages	SMC's	Macrophages	SMC's
PIT (n=7)	0.4±0.5	45.8±18.8	0	1005±374 858±286	
FA (n=8)	2.5±2.6	70.8±14.7	14.5±8.6	1247±493 1114±376	
TCFA(n=8	11.8±5.4	82.4±8.8	32.1±18.3	1661±495 1183±257	
PR(n=7)	22.9±13.8	84.6±7.5	36.5±14.0	1796±430 1074±355	
P value	0.0001*	<0.0001*	<.0001**	0.03**	ns

* Significant differences between rupture vs. thin cap fibroatheroma, fibroatheroma, and pathologic intimal thickening. ** significant difference between rupture and pathologic intimal thickening. ~Apoptosis and cell density measurements were done in the same region.

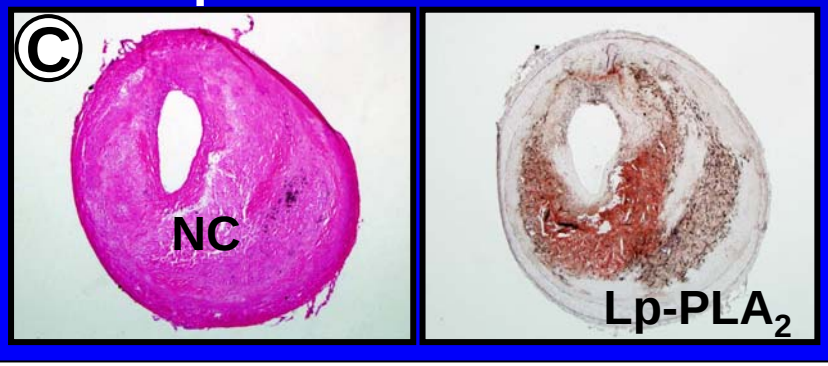
Pathologic Intimal thickening



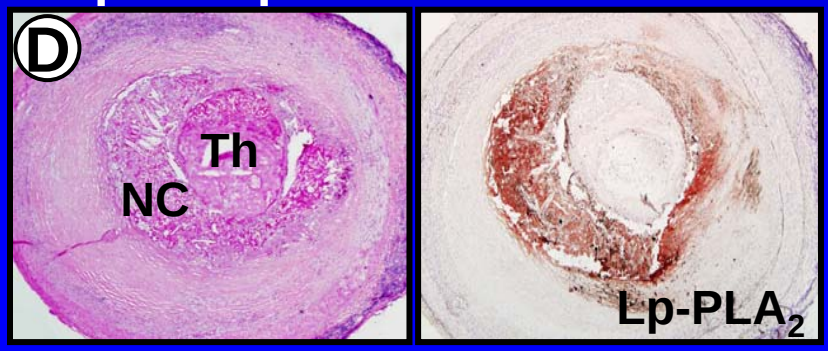
Fibroatheroma



Thin Cap Fibroatheroma



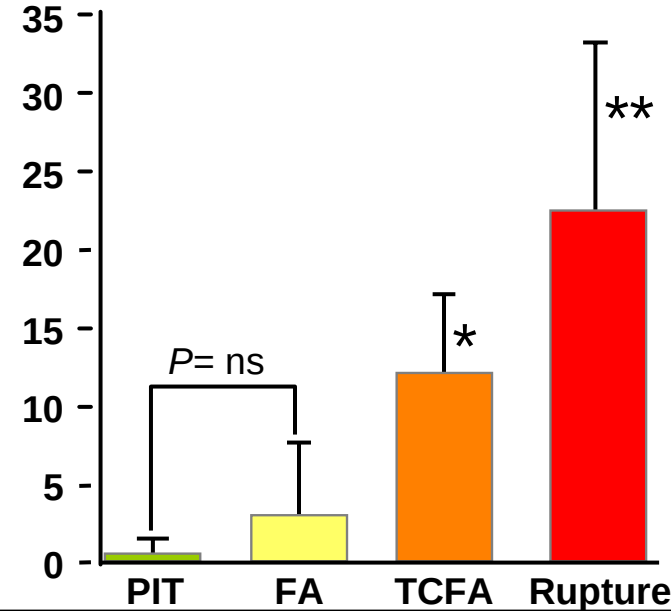
Plaque Rupture



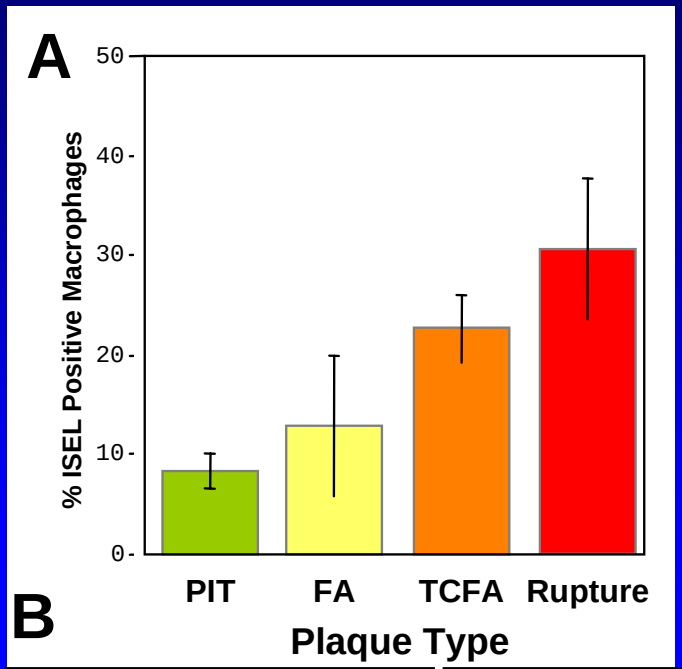
E.

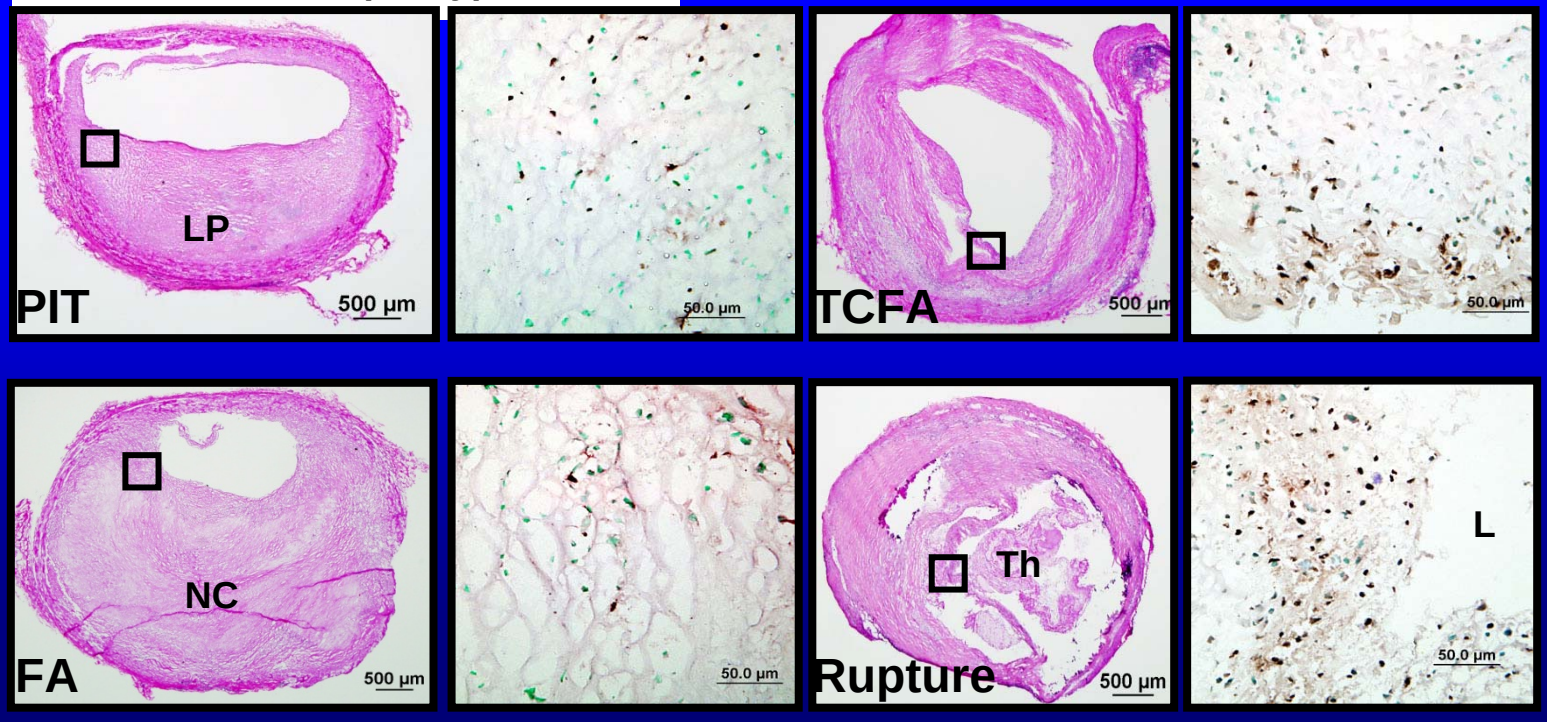
% Lp-PLA$_2$ Staining in Varying Coronary Plaque Morphologies

* significant differences between



Macrophage Apoptosis in Various Human Coronary Plaque Morphologies





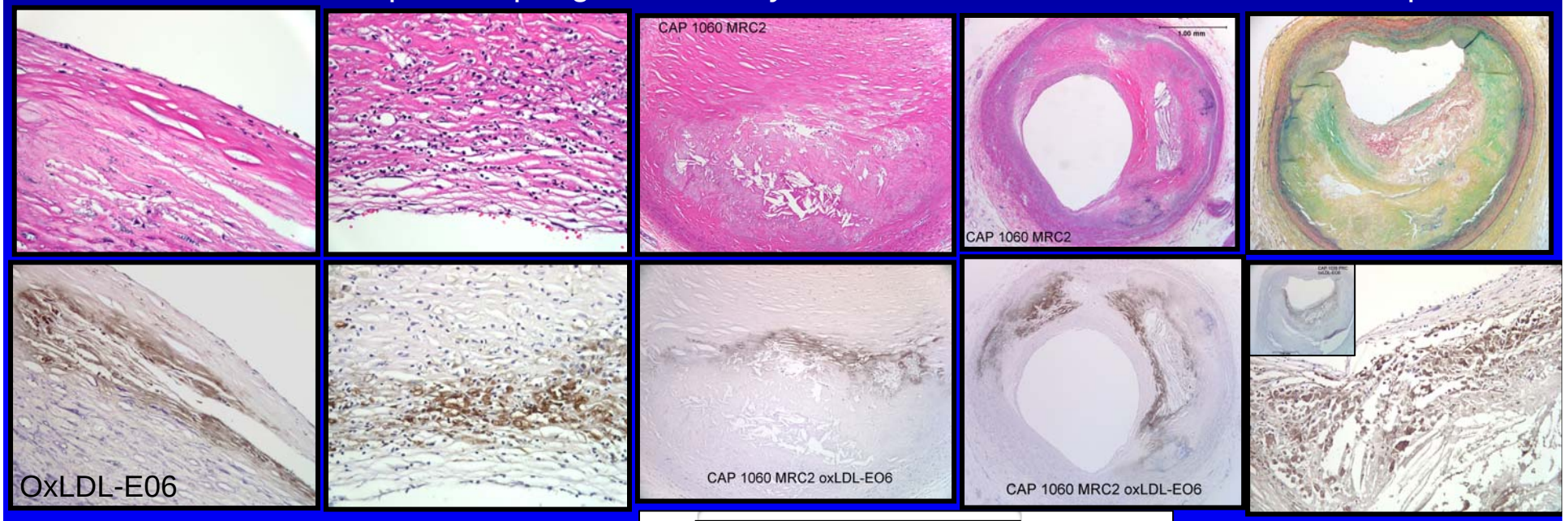
Lipoprotein-Associated A_2 Protein Expression in natural progression of Human Coronary Atherosclerosis

Summary:

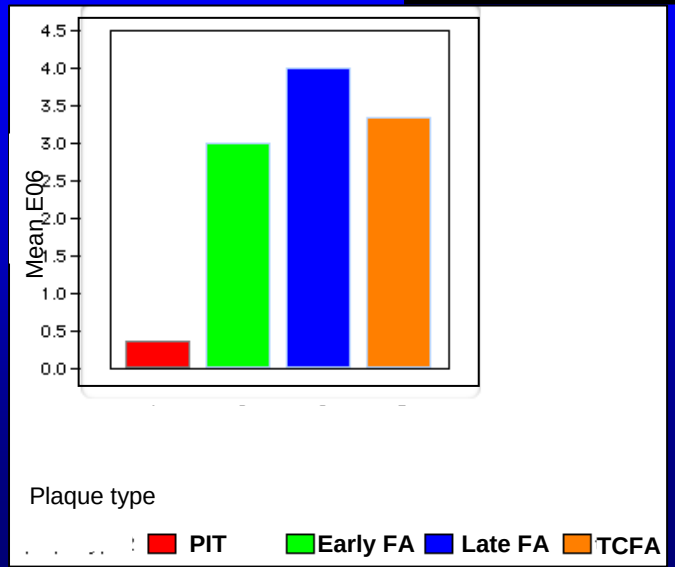
- First study to characterize the expression of Lp-PLA_2 protein within human coronary atheroma of various morphologies or phenotypes.
- Lp-PLA_2 was expressed by macrophages within fibrous cap region of rupture-prone and ruptured lesions.
- Lp-PLA_2 staining co-localized with apoptotic macrophages
- Lp-PLA_2 staining was also intense in regions abundant in lipids and oxidative products (e.g., necrotic core)
- Lp-PLA_2 and its enzymatic products may play a role in promoting plaque instability.

Plaque Development and Expression of OxLDL-E06

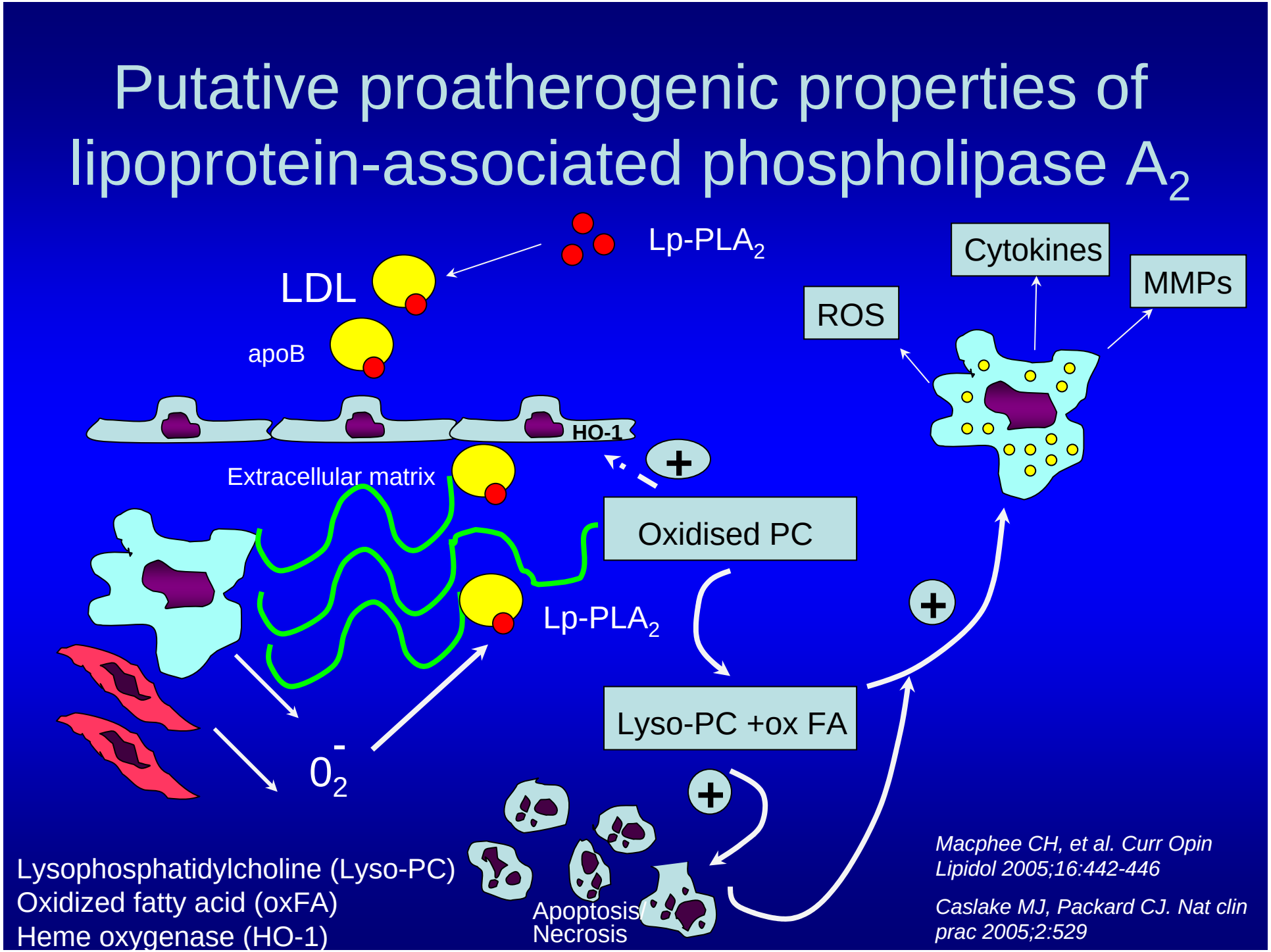
PIT | Cap Macrophages | Early FA | Late FA | Thin-cap FA



PIT - pathologic intimal thickening
Early FA - early fibroatheroma
Late FA - late fibroatheroma
TCFA - thin-cap fibroatheroma

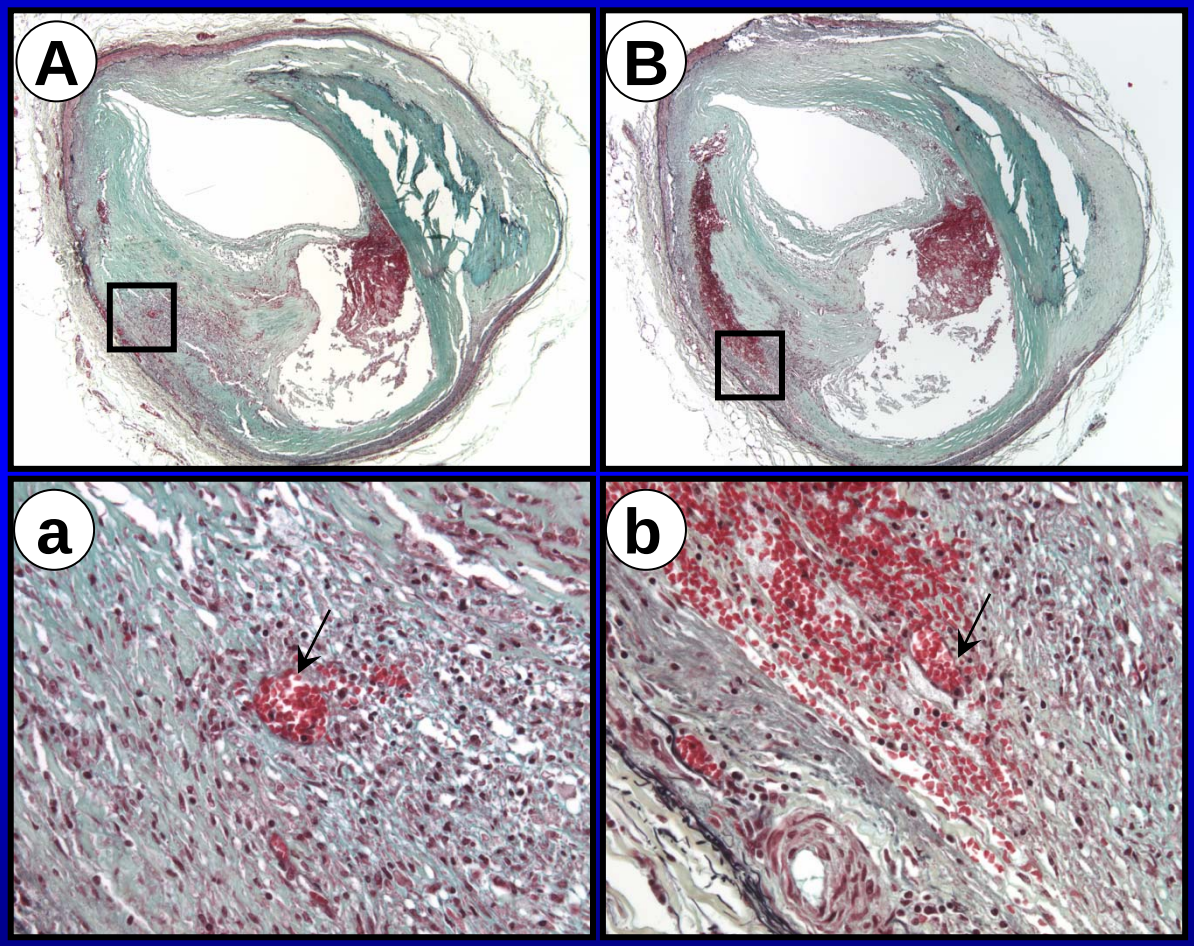


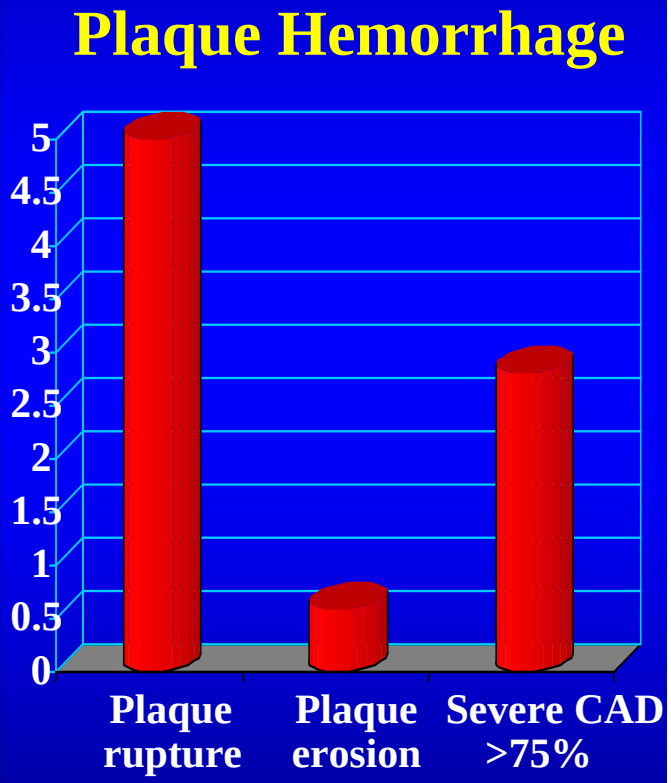
Putative proatherogenic properties of lipoprotein-associated phospholipase A_2



Thin-cap Fibroatheroma

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

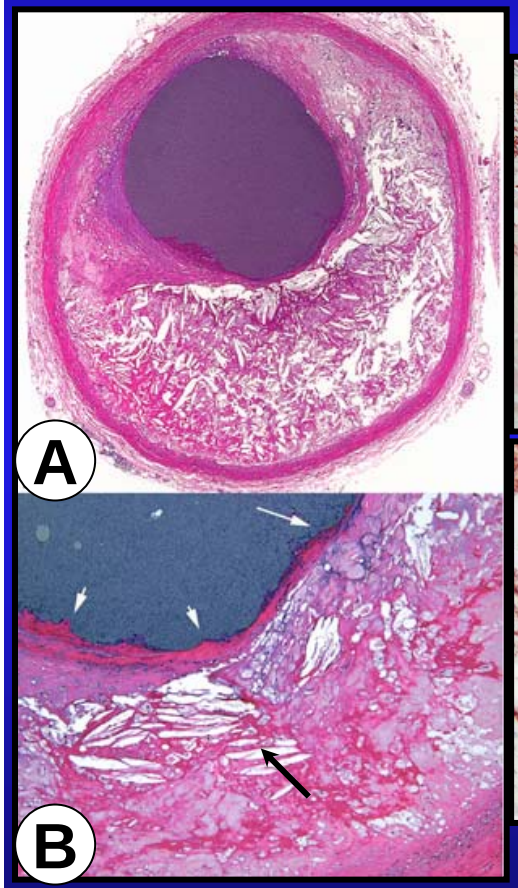


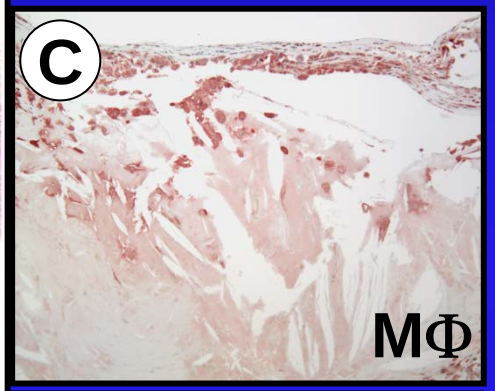


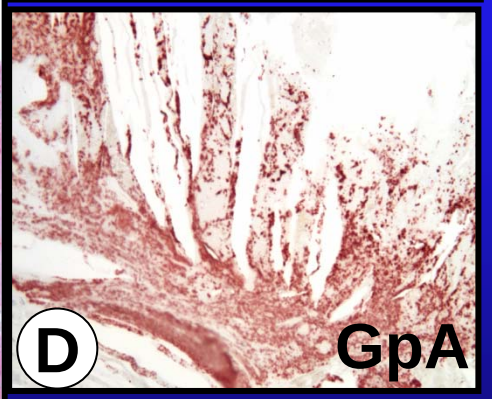
Phase Separation of Erythrocyte-Derived Cholesterol in Coronary and Non-Coronary Diseases

Thin Fibrous Cap Atheroma

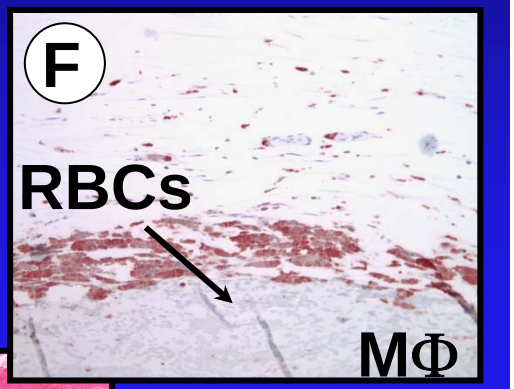
Hemorrhagic Pericarditis



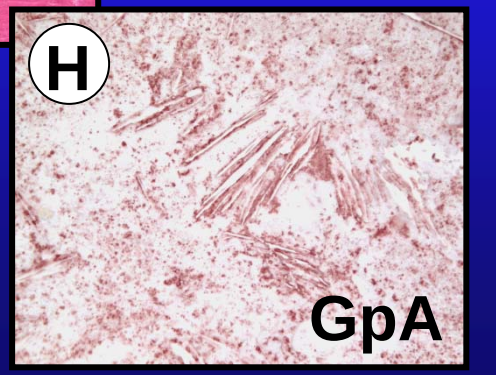




Hemorrhage Periphery →



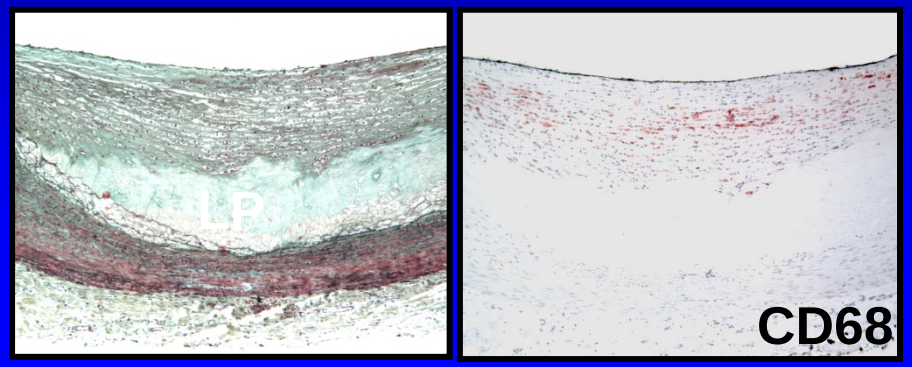
Hemorrhage Core →



Plaque Types Studied

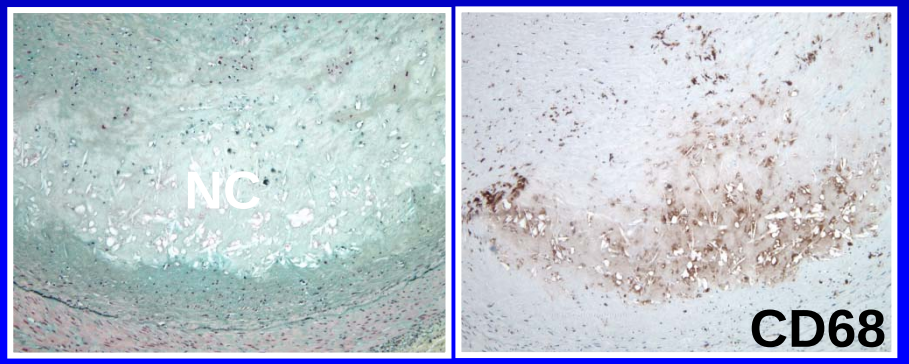
A.

Pathologic Intima Thickening



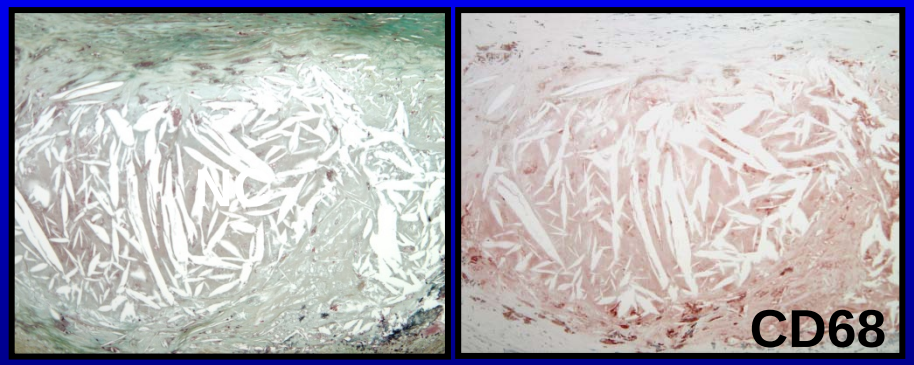
B.

Fibroatheroma ‘Early’ Core



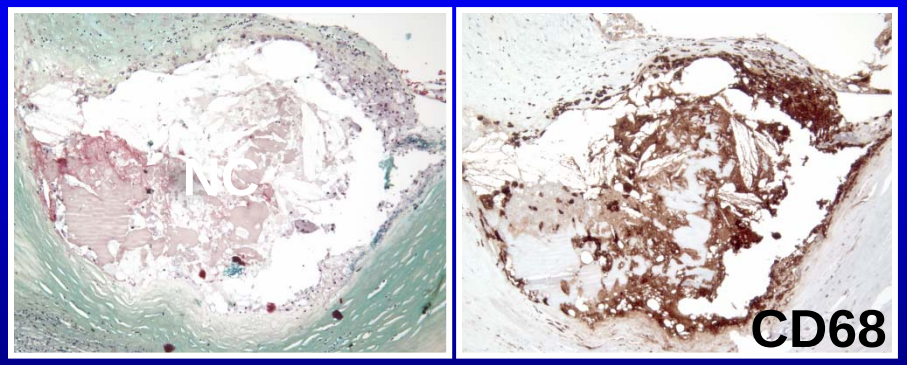
C.

Fibroatheroma ‘Late’ Core



D.

Thin Cap Fibroatheroma



Morphometric Analysis of Hemorrhagic Events in Human Coronary Plaques from Sudden Death Victims

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

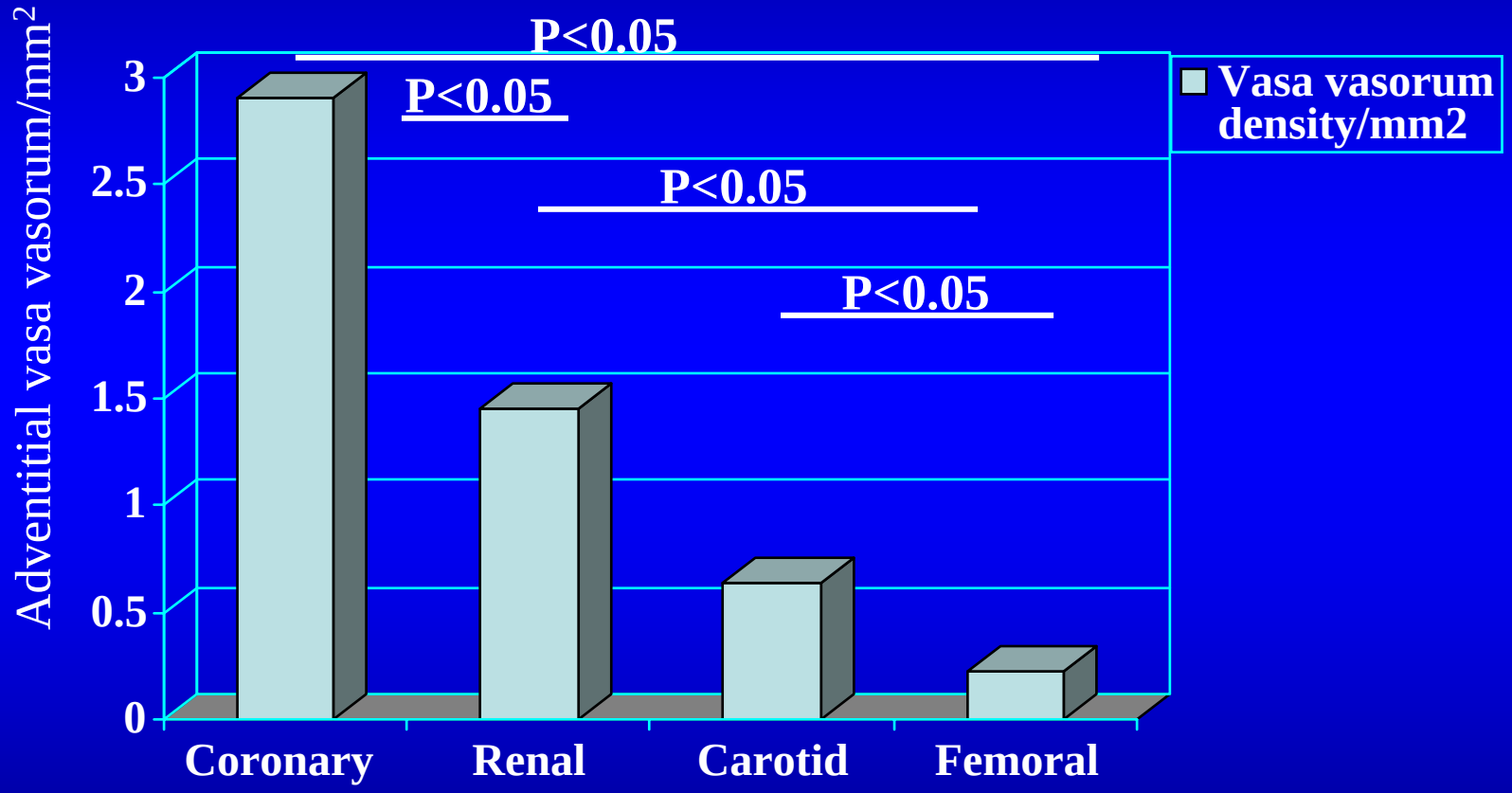
Plaque hemorrhage contributes to enlargement of the necrotic core

- Importance has been shown in human plaques - red cell membrane contributes to free cholesterol and larger necrotic cores.
- Macrophage accumulation is triggered by crystallization of cholesterol from erythrocyte membrane and foreign body reaction as seen in cholesterol granulomas and e.g., receptors on erythrocytes bind a wide array of chemokines, MCP-1; lipid oxidation from senescent RBCs or iron-catalyzed reactions may liberate potent chemoattractants

Plaque Vasa Vasorum

- Plaque capillaries are observed in atherosclerotic plaques with plaque thickness > 0.5 mm, suggesting that wall ischemia may be a determinant of neovascularization.
- Heistead and Armstrong reported a 5 fold increase in intimal/medial blood flow from proliferating micro vessels in monkeys fed a high cholesterol diet for 17 months. (Arteriosclerosis 1986)
- Plaque Vv may be a potential source of inflammation within the plaque [expression of VCAM-1, ICAM-1 and E-selectin has been shown in plaque Vv (O'Brian, et al. AJP 1994).
- Inflammation and matrix composition of atherosclerotic plaques may also influence angiogenesis.

Adventitial Vasa Vasorum Heterogeneity among different vascular beds



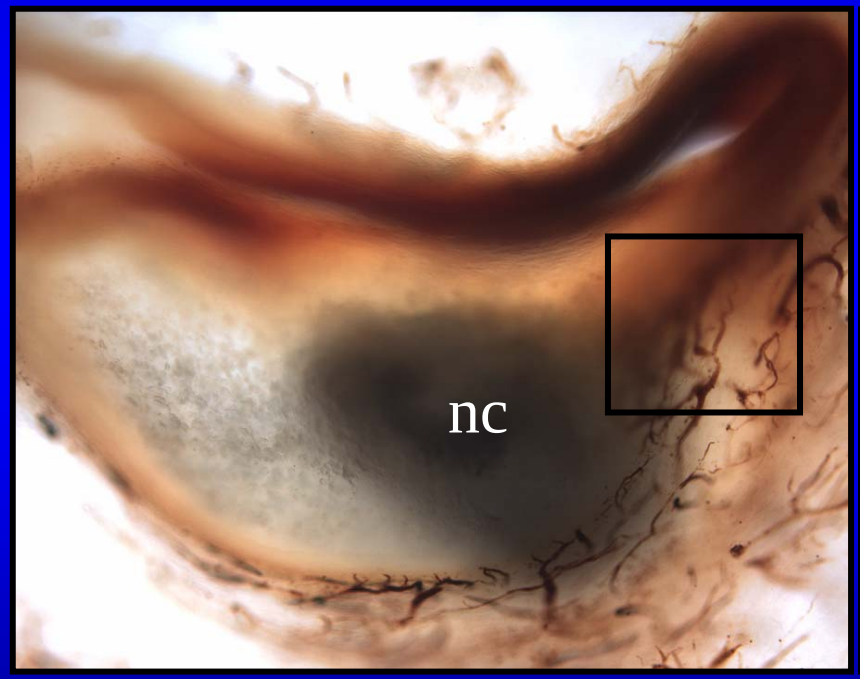
Low vasa vasorum density in internal thoracic artery may be responsible for the low incidence of atherosclerosis

Gallili et al. J Vasc Surg 2004;40:529 and J Thorac Cardiovasc Surg 2005;129:767

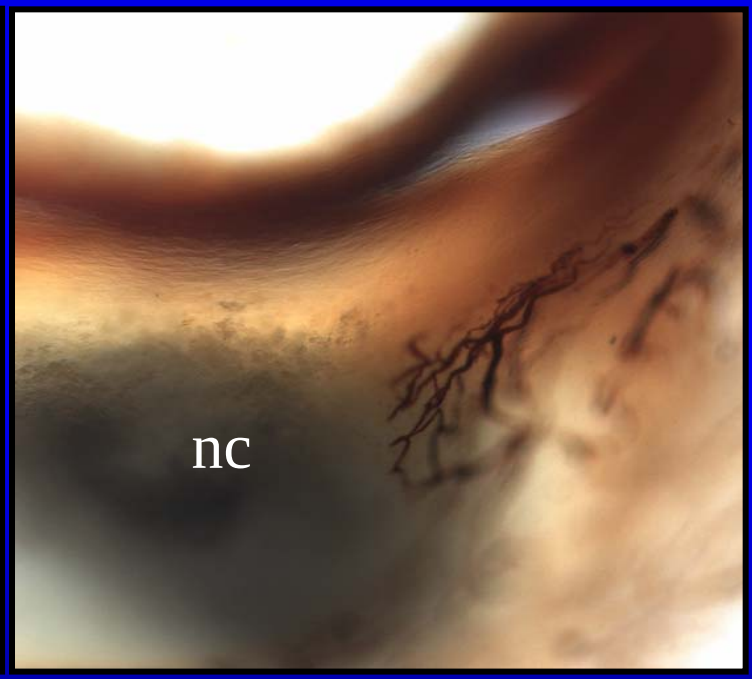
Intraplaque Vasa Vasorum in Coronary Plaques with a Necrotic Core

150 μm thick sections stained with Ulex

A



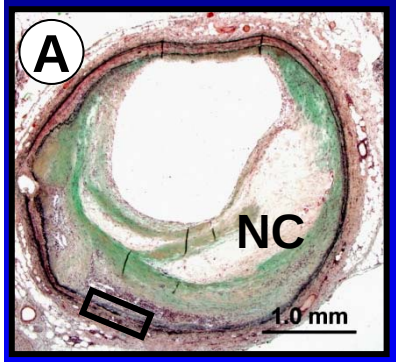
B

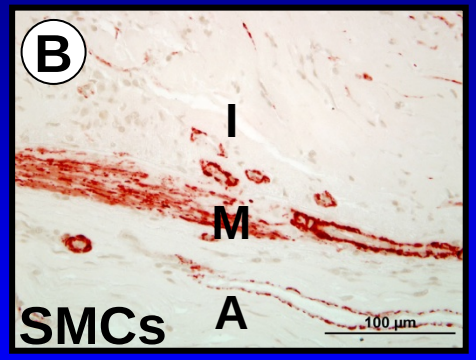


Virmani R, et al., Arteriosclero Throm Vac Biol 2000;20:1262

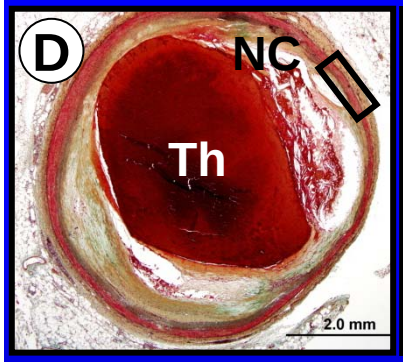
Vasa Vasorum by Plaque Type

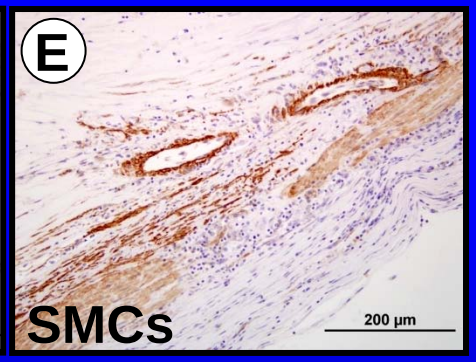
Thin-Cap Fibroatheroma

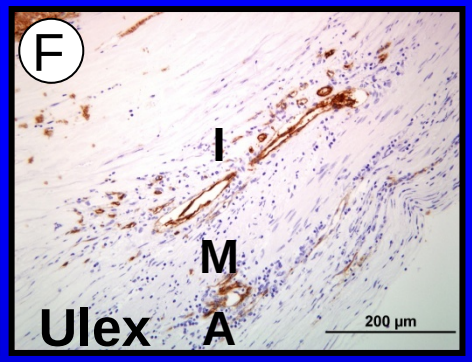




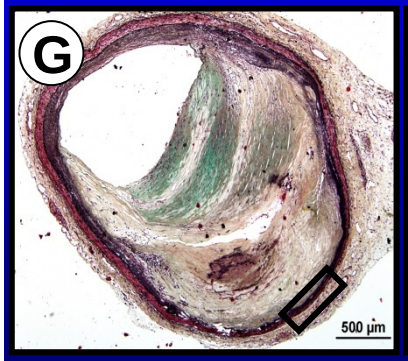
Plaque Rupture

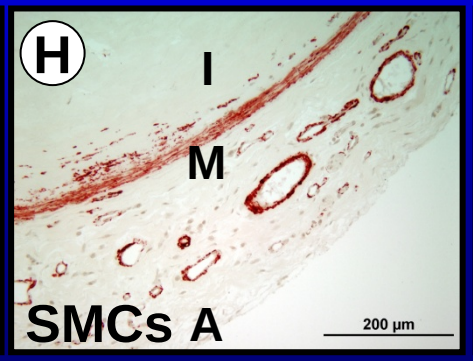


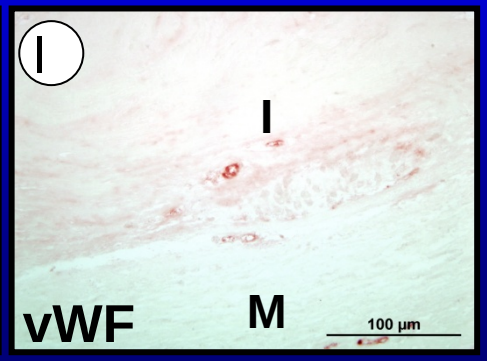


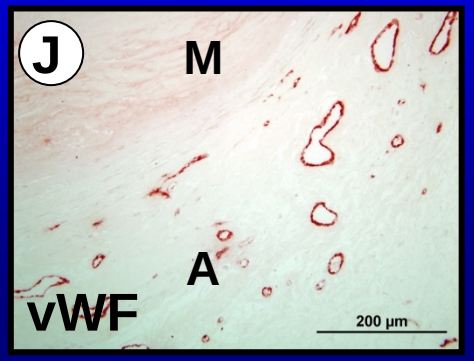


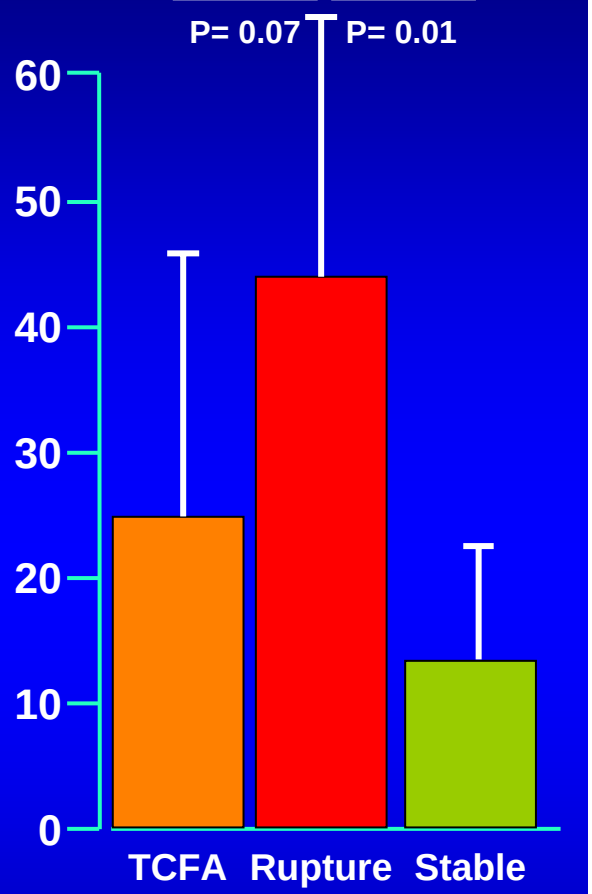
Stable Plaque











Conclusions

- Plaques occur focally at branch points in the presence of systemic risk factors
- The morphologic characteristics most predictive for the presence of unstable vs. stable plaque is necrotic core size, plaque area and extent of macrophage infiltration in the fibrous cap.
- Intra plaque hemorrhage is responsible for enlargement of necrotic core, macrophage infiltration and progressive luminal narrowing
- Non invasive detection of vulnerable plaques is the only mechanism through which morbidity and mortality for CAD can be reduced or eliminated.