

# ***Characteristics of Vulnerable Plaque: Structural Observations and Natural History Insights from the Core Pathology Laboratory***

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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

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**Consultant: Medtronic AVE; Guidant; W.L. Gore; CryoVascular Systems, Inc.; Volcano Therapeutics Inc.; Precient Medical; Medeikon; CardioMind, Inc.; Direct Flow; and Atrium Medical Corp.**

**Employment 25%: Cardiovascular Research Foundation  
Do not own any stock in any company.**

# Natural History of Atherosclerosis

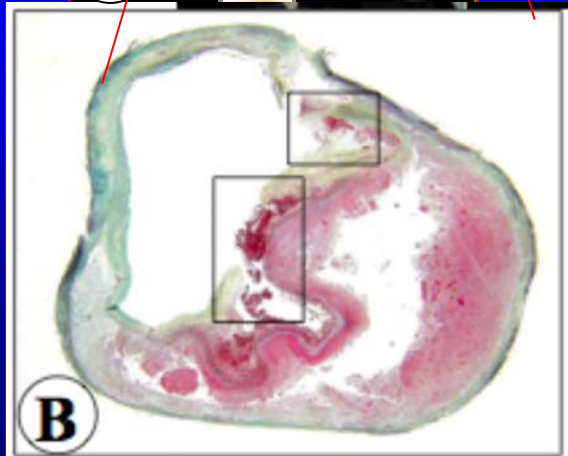
- Systemic factors - hyperlipidemia, diabetes mellitus, smoking, hypertension, age and sex, hsCRP, Lp-PLA<sub>2</sub>, etc.
- Local factors: at branch points, e.g., carotid bifurcation, abdominal aorta just above bifurcation coronary branch point, and arch vessels at take off, are the sites of atherosclerosis 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 that result in thrombosis

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

## Carotid Artery

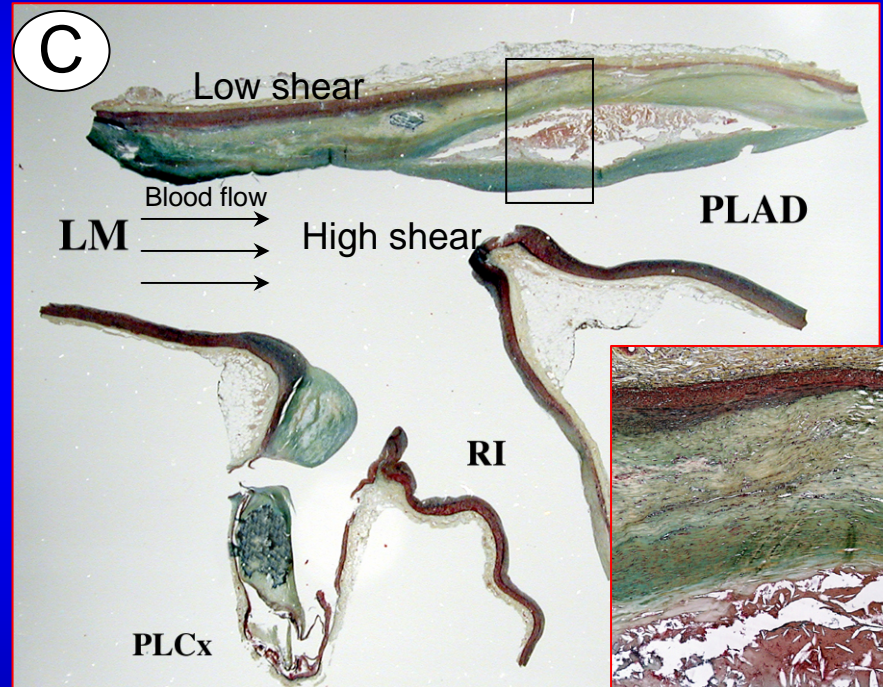


**A**



**B**

## Left Coronary artery



**C**

Low shear

Blood flow

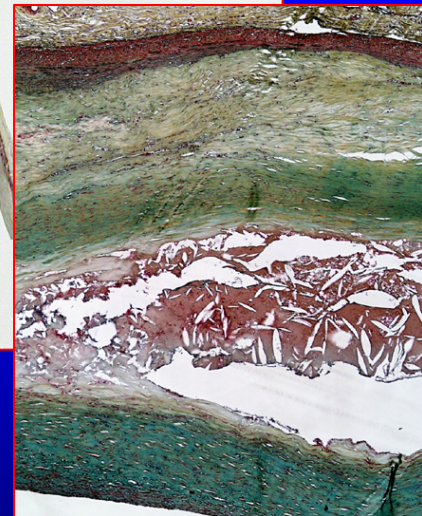
LM

High shear

PLAD

RI

PLCx



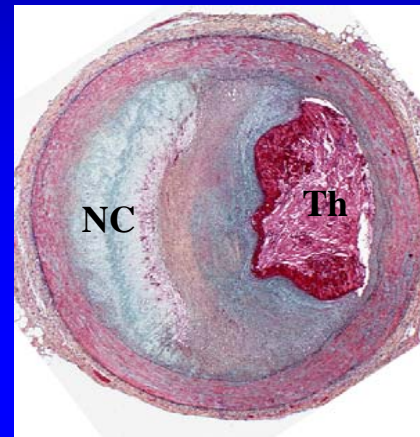
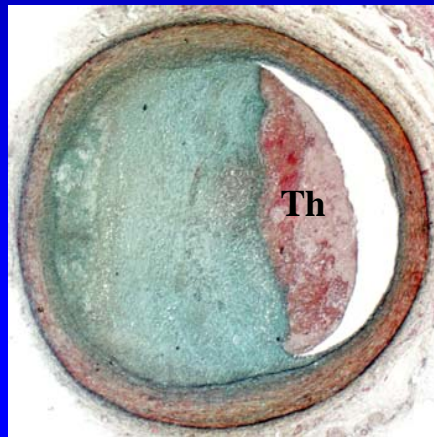
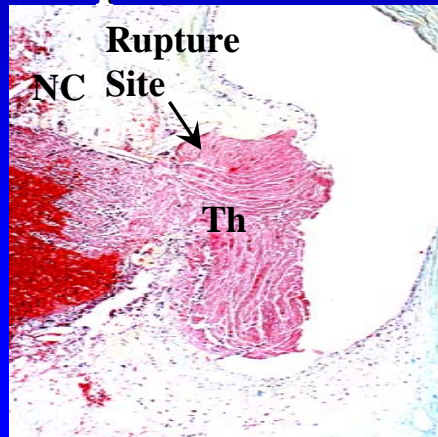
# Lesions with Thrombi

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

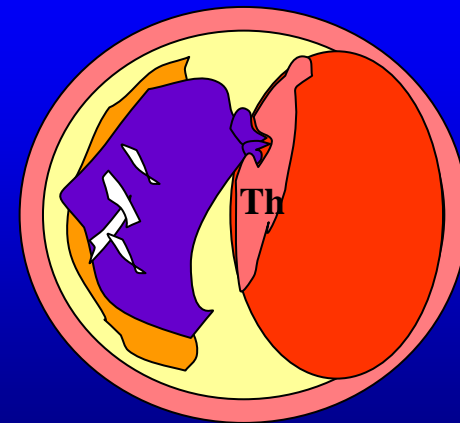
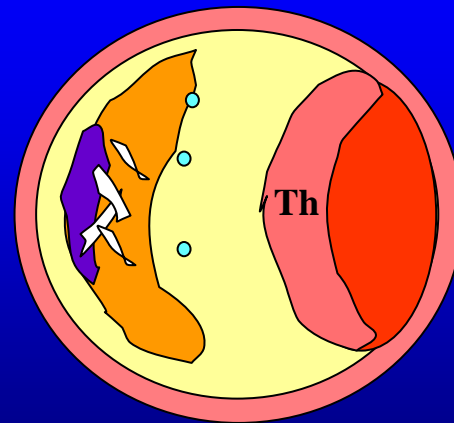
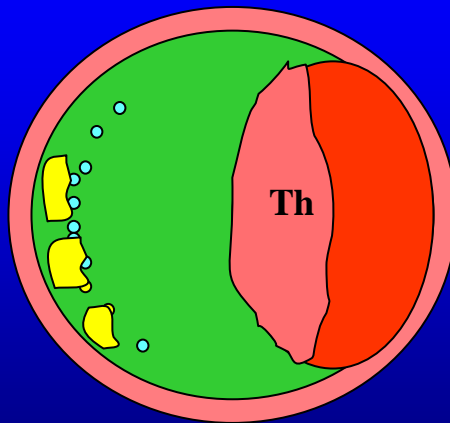
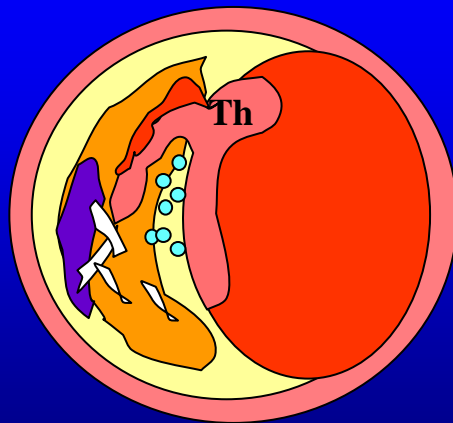
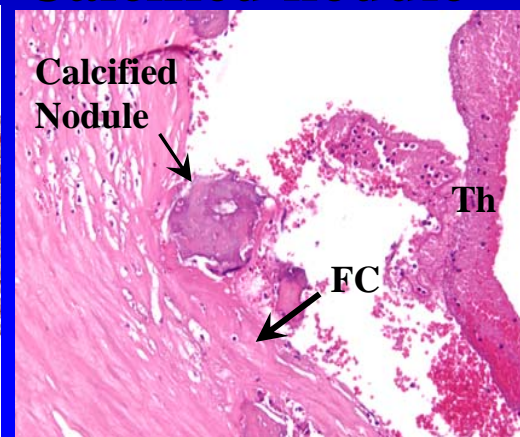
# Causes of Coronary Thrombosis

## Erosion

### Rupture



### Calcified nodule



# Gross and Light Microscopic Features of Plaque Rupture

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

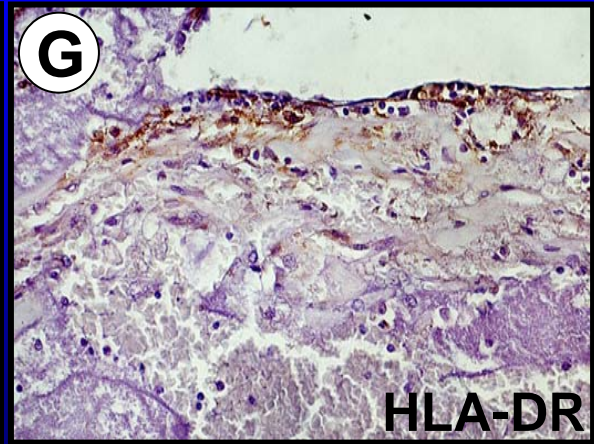
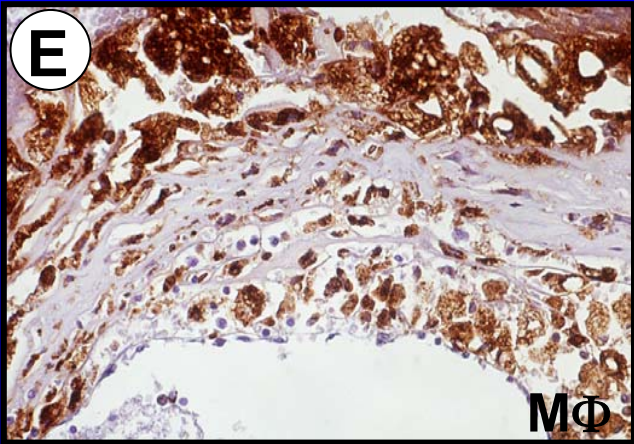
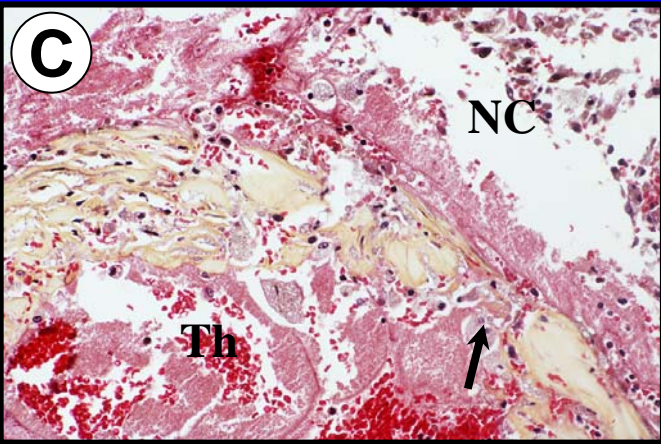
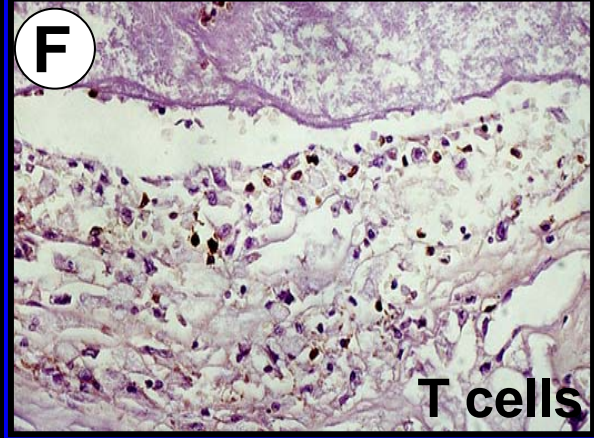
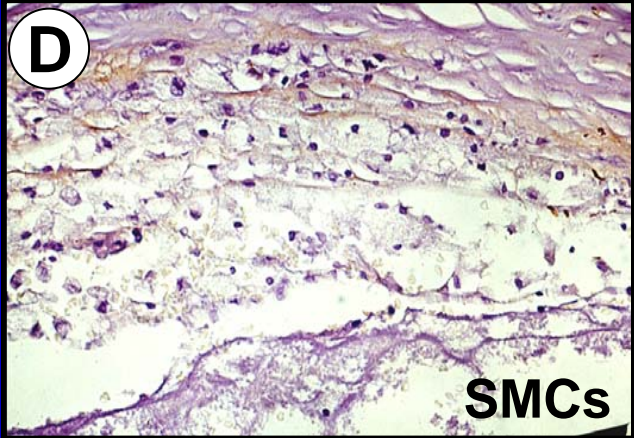
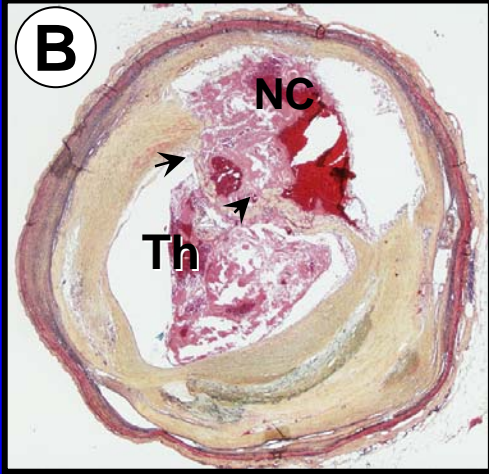
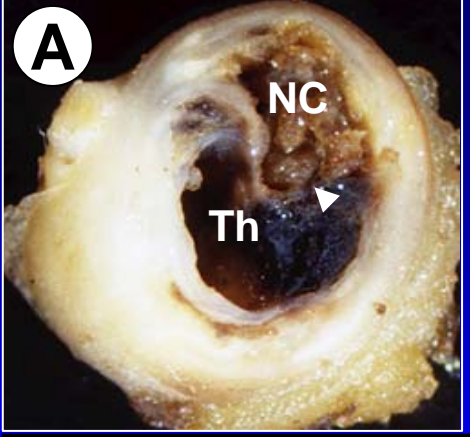
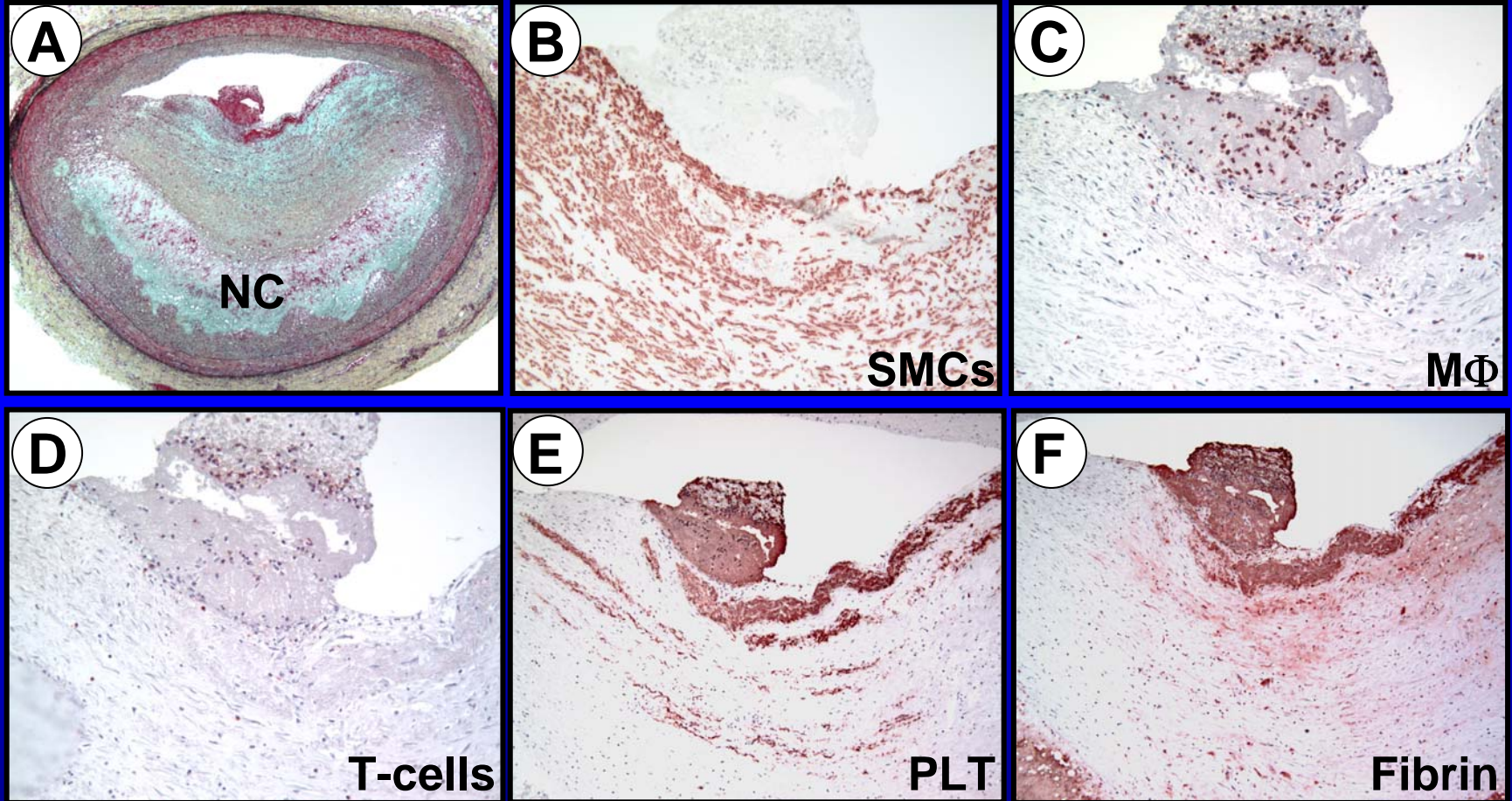


Fig 3-1

# Plaque Erosion: 30-35% of thrombi in SCD

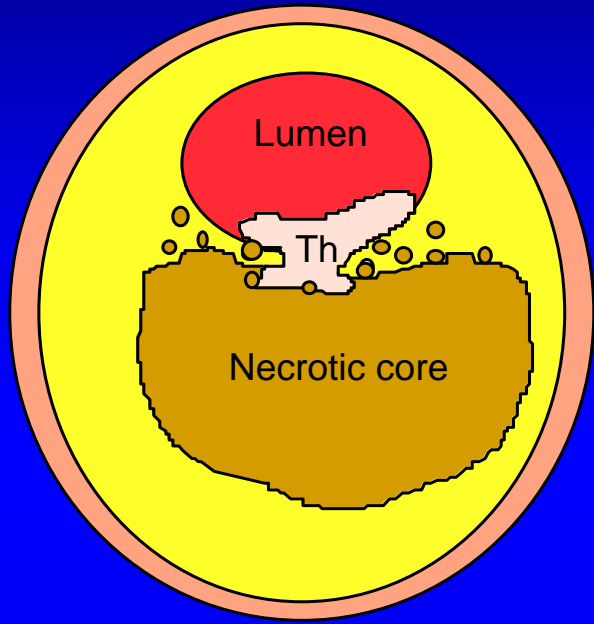
*Plaque erosion in a 33 year-old female complaining of chest pain for two-weeks and discharged from the emergency room with a diagnoses of anxiety.*



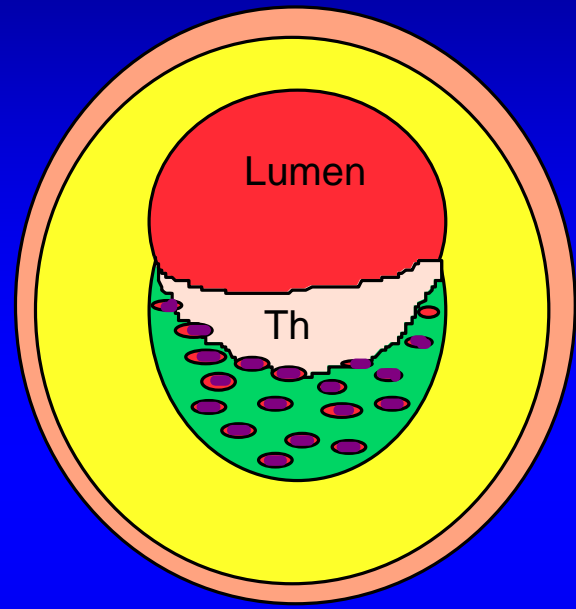


# Clinical and Morphologic Difference in Plaques Associated with Luminal Thrombi

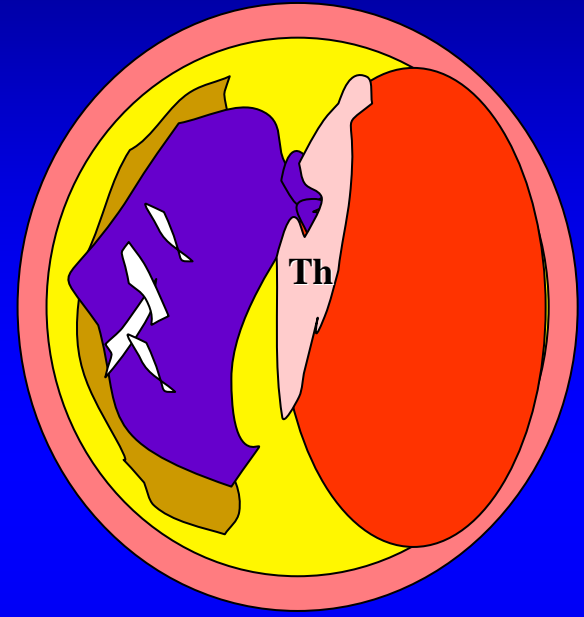
**Plaque rupture**



**Plaque erosion**



**Calcified nodule**



60% thrombi in SCD  
 M>F, Older, Ca<sup>++</sup>  
 Eccentric =  
 concentric  
 Greater % stenosis  
 Macs, T cells,HLADr

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

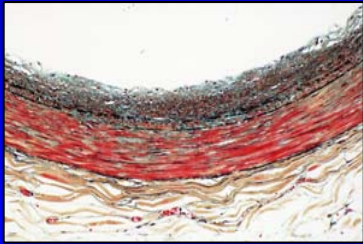
2-7% thrombi in SCD, calcified plates  
 M>F, older, mid RCA  
 Usually eccentric  
 Stenosis variable  
 Nodules of bone

# Acute Coronary Syndrome

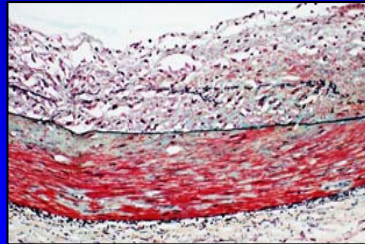
- **Acute Myocardial Infarction**
  - >90% have coronary thrombi, usually occlusive
  - 65-75% from plaque rupture
  - 25-35% from plaque erosion (Arbustini E, et al. Heart 1999)
- **Unstable Angina Pectoris**
  - 70% have thrombi, mostly non-occlusive (Mizuno K, et al. Lancet 1991. Ueda Y et al. JACC 1996)
  - Distribution whether rupture or erosion, unknown
- **Sudden Coronary Death**
  - 60% have thrombi- 60% rupture, 35% erosion, 2-5% calcified nodule)
  - 40% have stable plaques with >75% x-sectional narrowing (no thrombus)
  - 40% have HMI and 15% AMI (Virmani R, et al. ATVB 2000)

# Development of Human Coronary Atherosclerosis

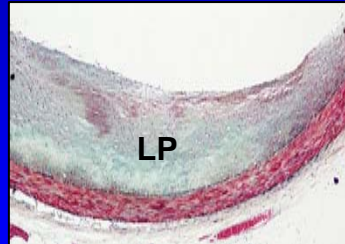
Intimal thickening



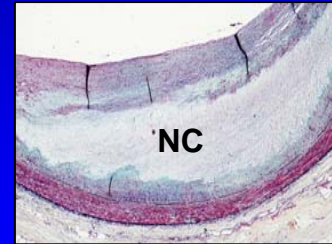
Intimal xanthoma



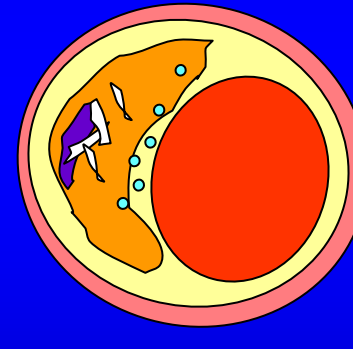
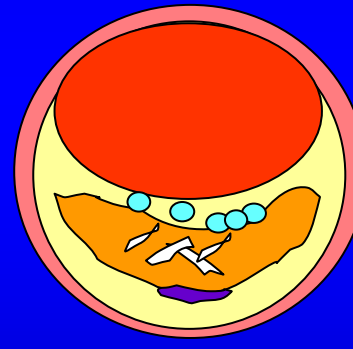
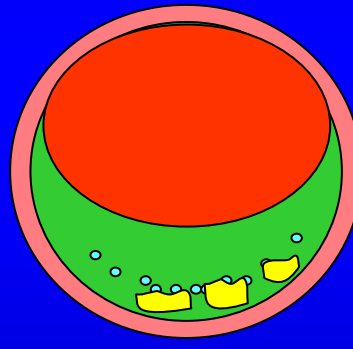
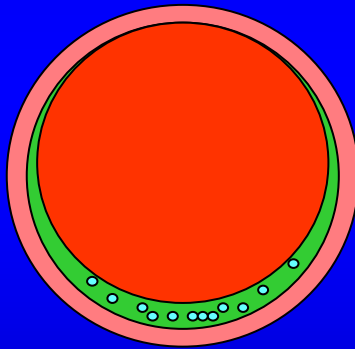
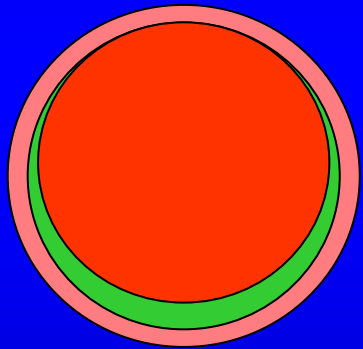
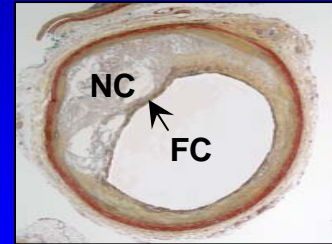
Pathologic intimal thickening



Fibrous cap atheroma



Thin-cap Fibroatheroma



- Smooth muscle cells
- Macrophage foam cells
- Extracellular lipid
- Cholesterol clefts
- Necrotic core

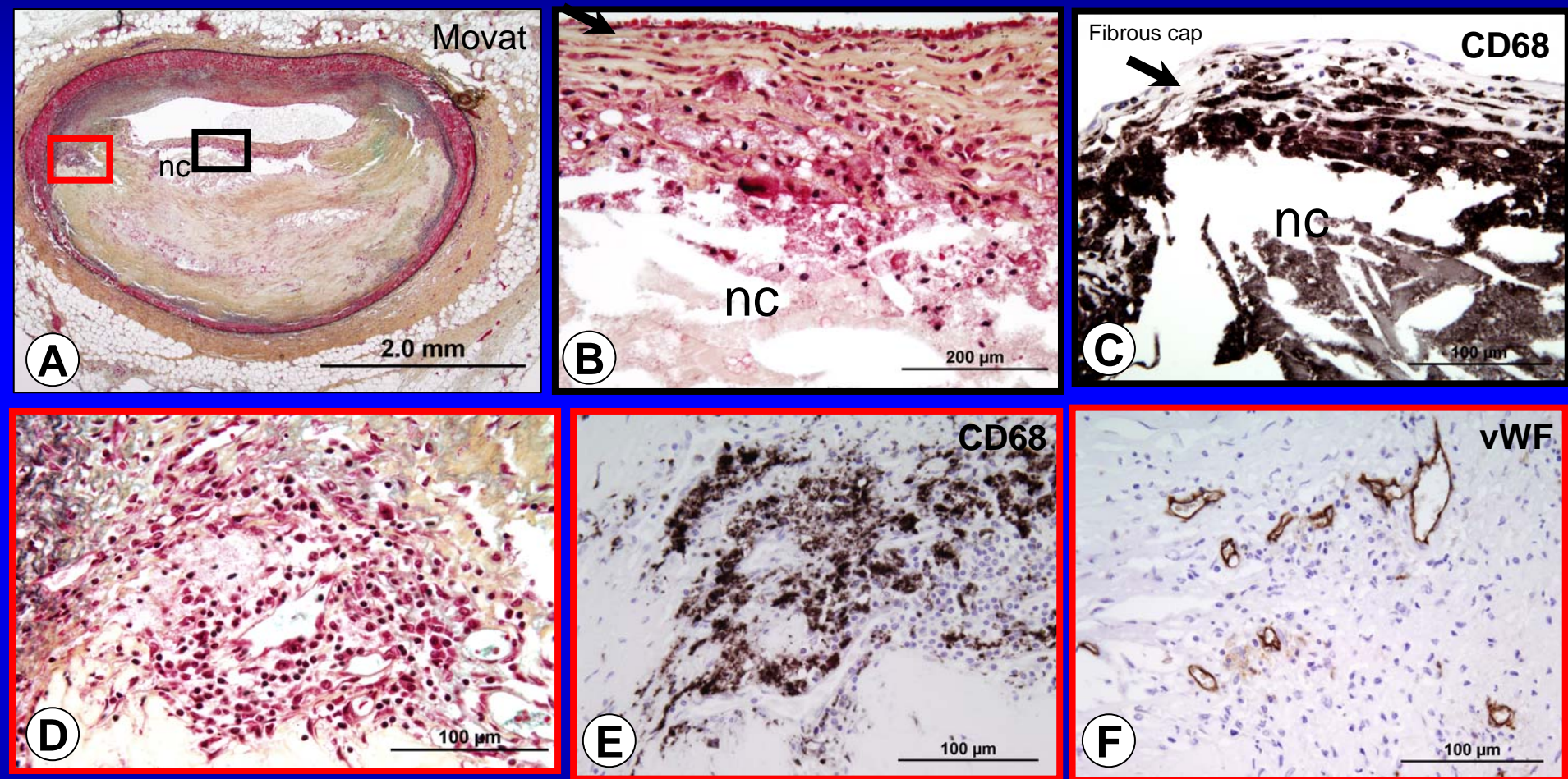
- Calcified plaque
- Hemorrhage
- Thrombus
- Healed thrombus
- Collagen

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

# Thin-Cap Atheroma (Vulnerable Plaque) Components

- Necrotic core
- Thin fibrous cap (< 65  $\mu\text{m}$ )
- Cap infiltrated by macrophages and lymphocytes
- Cap composition – type 1 collagen and few smooth muscle cells

# Thin cap Fibroatheroma (Vulnerable Plaque)

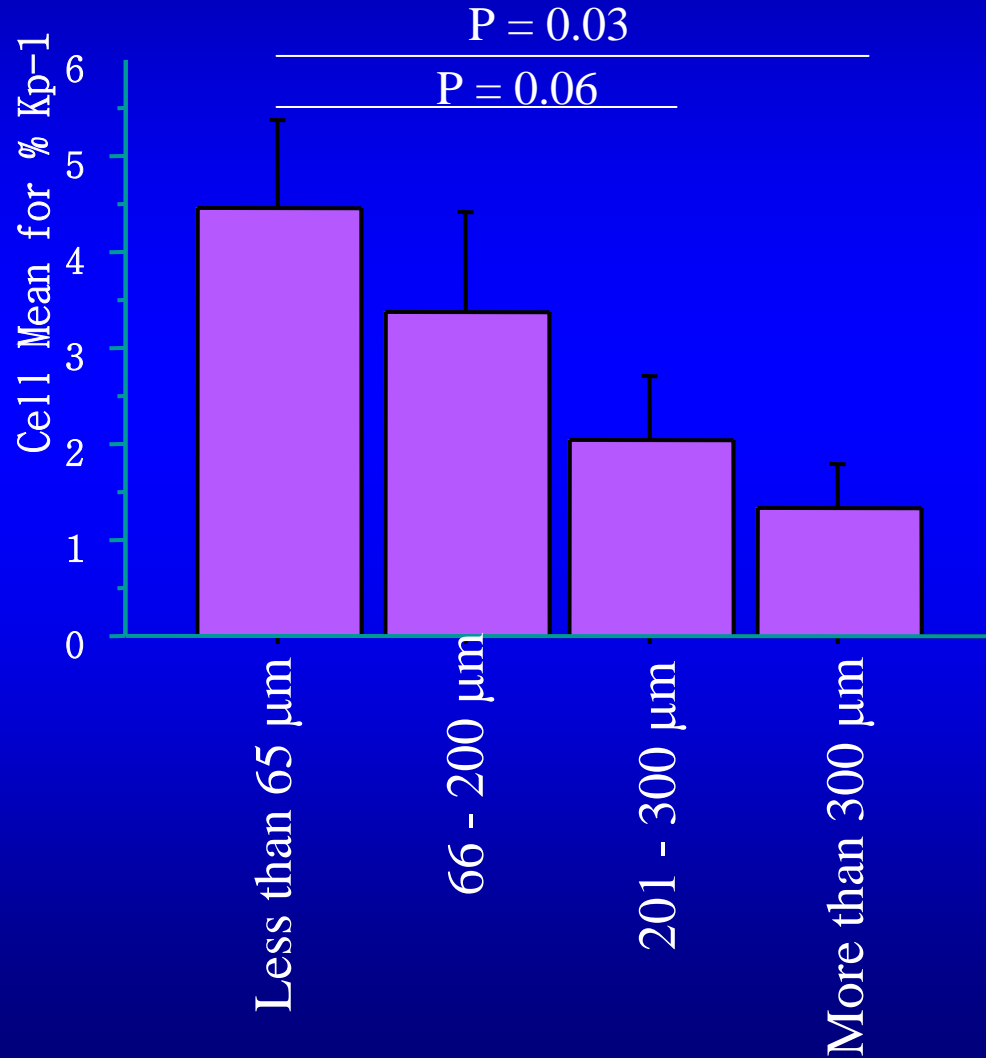


# Morphologic Characteristics of Plaque Rupture and Thin-cap Fibroatheromas

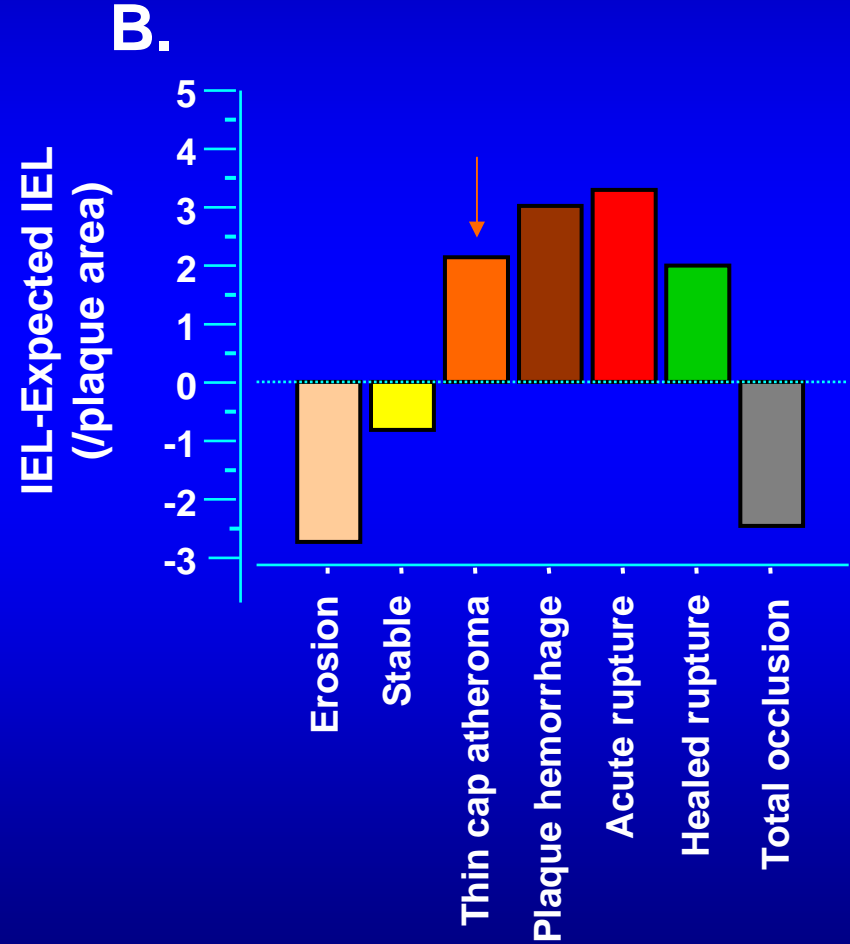
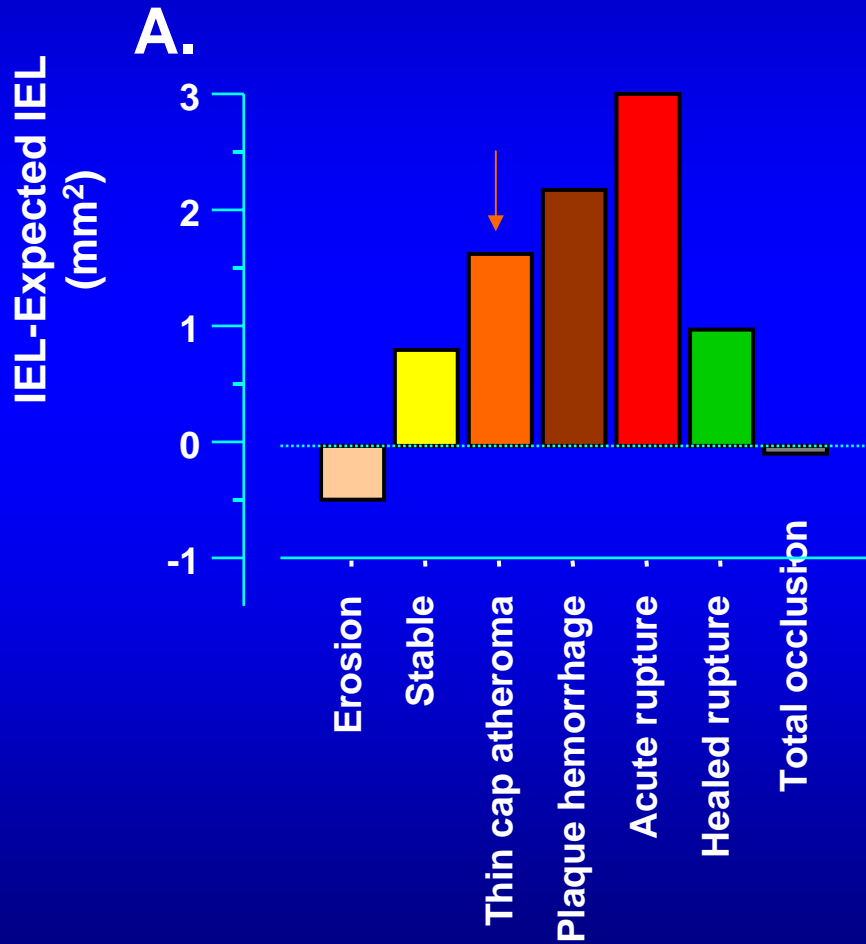
Plaque type	Necrotic Core (%)	Fibrous cap Thickness ( $\mu\text{m}$ )	M $\Phi$ s (%)	SMCs (%)	T-lymph	Calcification Score
Rupture	34 $\pm$ 17	23 $\pm$ 19	26 $\pm$ 20	0.002 $\pm$ 0.004	4.9 $\pm$ 4.3	1.53 $\pm$ 1.03
Thin-cap Fibroatheroma	23 $\pm$ 17	<65 $\mu\text{m}$	14 $\pm$ 10	6.6 $\pm$ 10.4	6.6 $\pm$ 10.4	0.97 $\pm$ 1.1
P value	0.01		0.005	ns	ns	0.014

Mean values are represented  $\pm$  standard deviation. Abbreviations: M $\Phi$ s= macrophages, SMCs= smooth muscle cells, T-lymph= T-lymphocytes

# Relationship of Fibrous Cap Thickness to Macrophage Infiltration



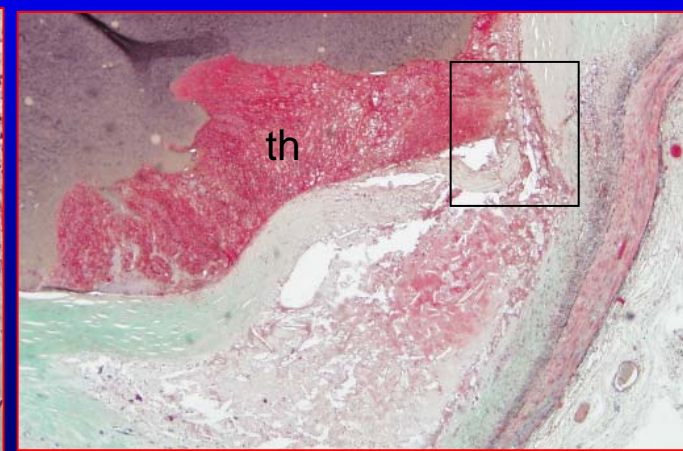
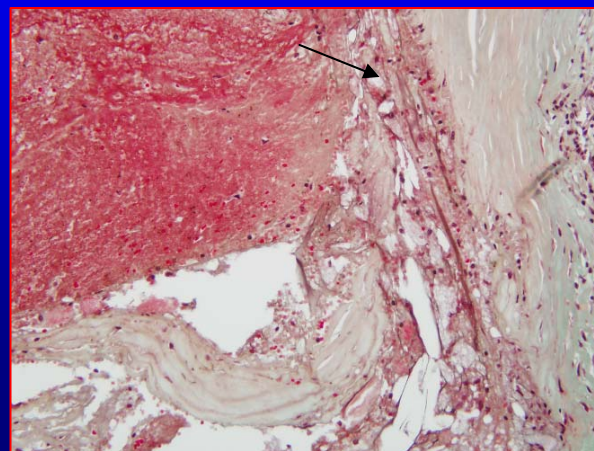
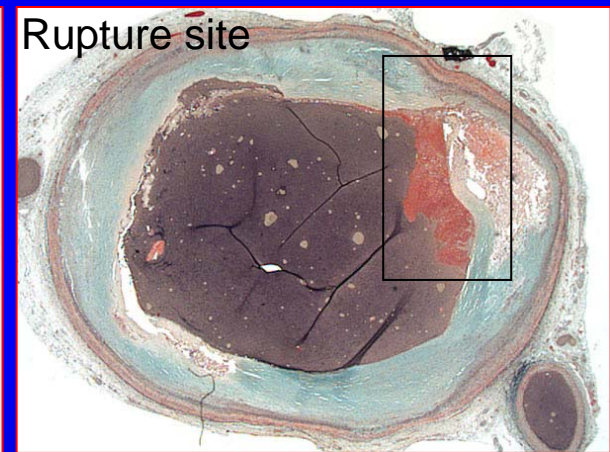
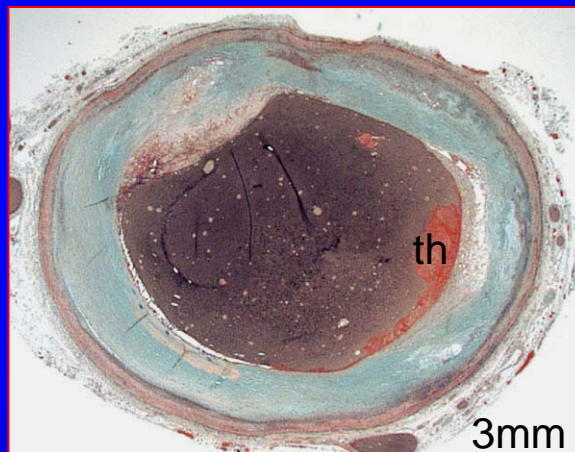
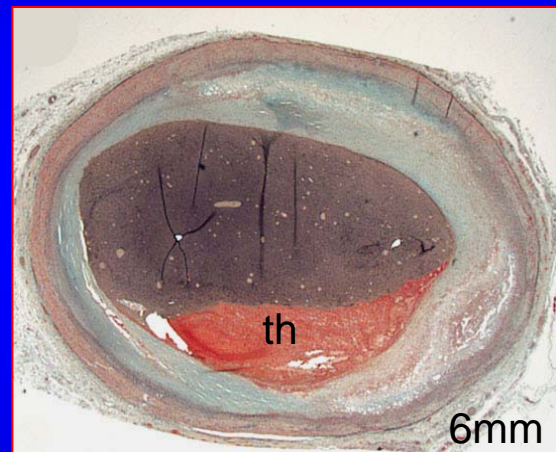
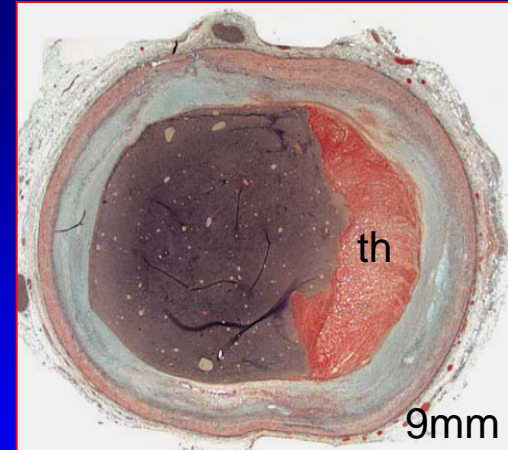
# Remodeling in Varying Coronary Lesion Morphologies





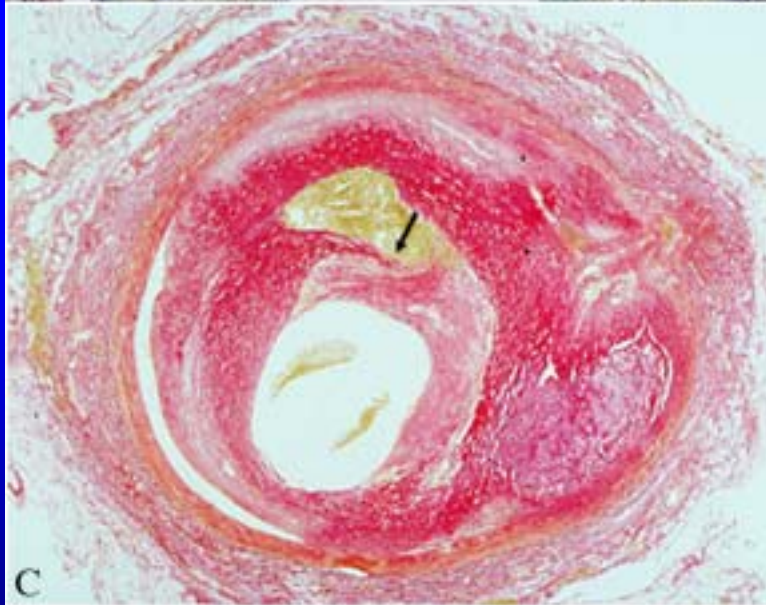
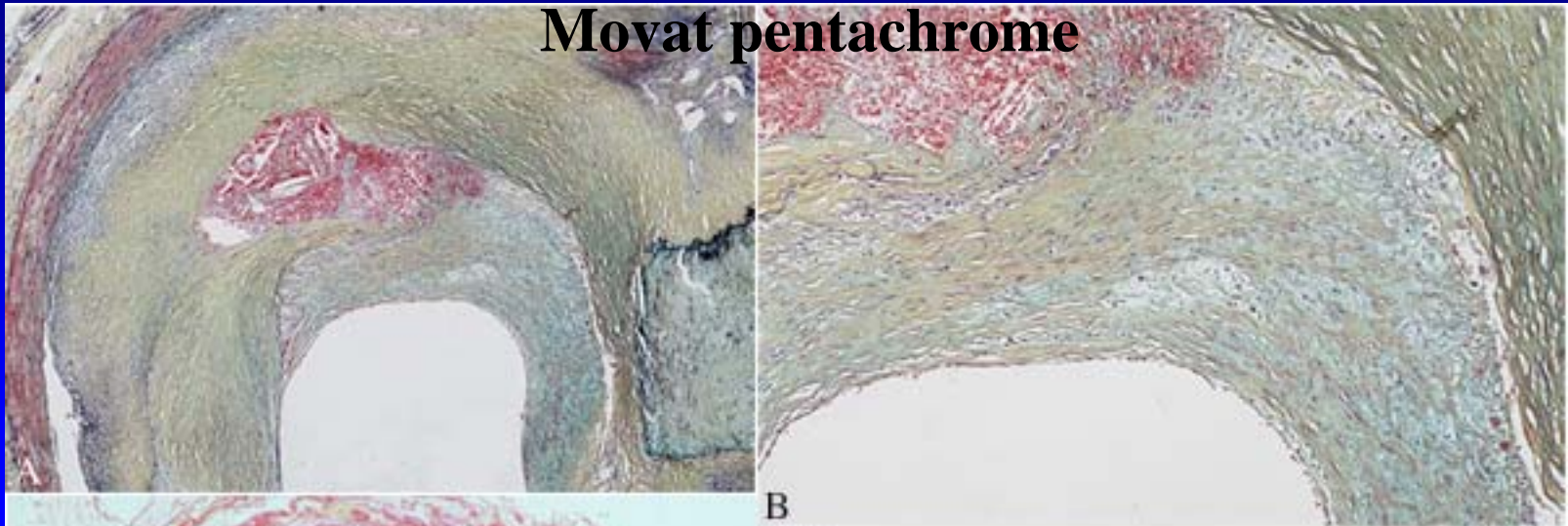
# Plaque rupture with mild non occlusive thrombus: mechanism by which plaques progress

Proximal

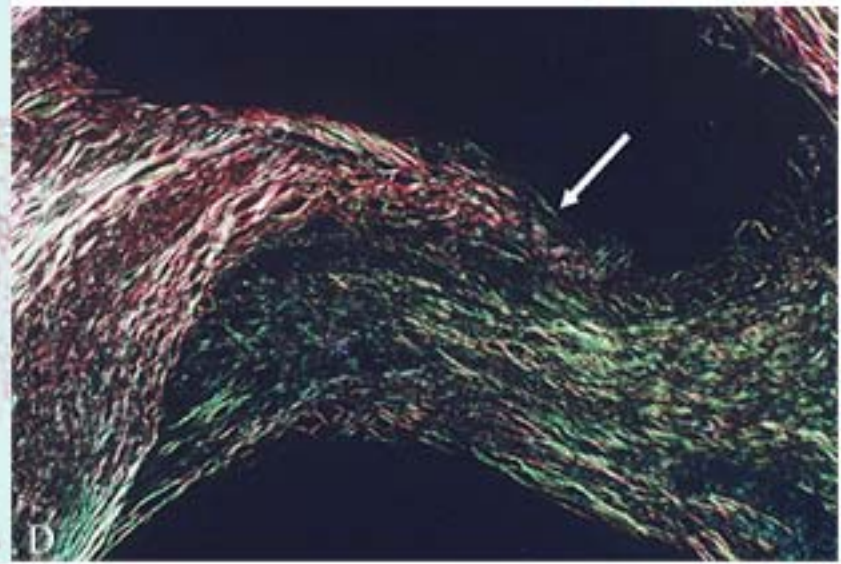


# Do TCFAs lead to plaque progression ?

Movat pentachrome

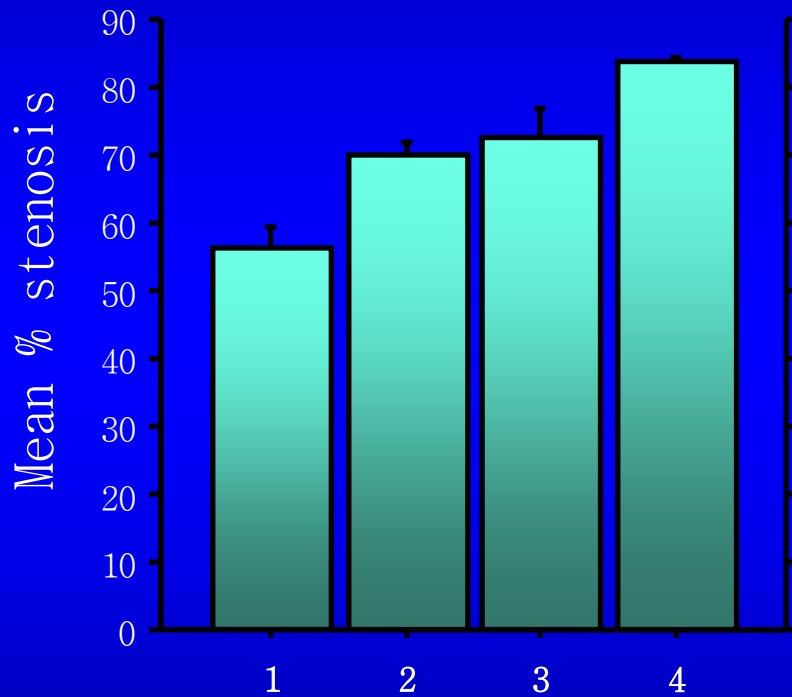


Sirius red

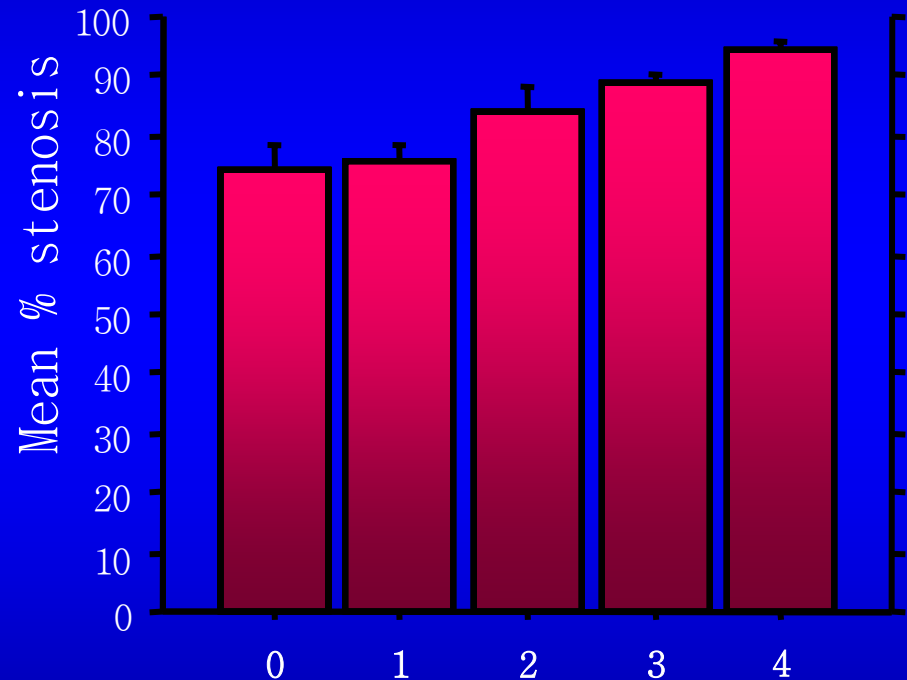


Sirius red with polarized light

# Mean % stenosis increases with number of prior rupture sites



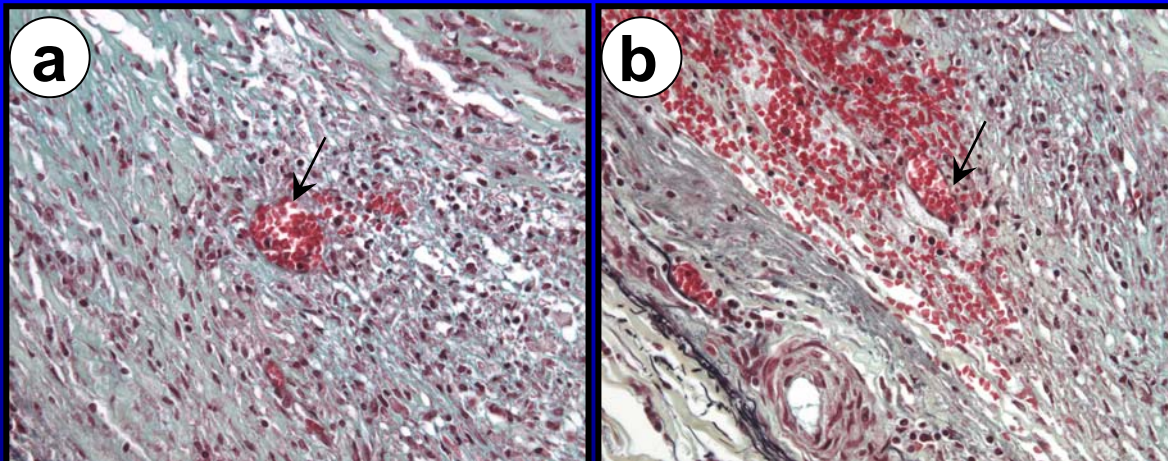
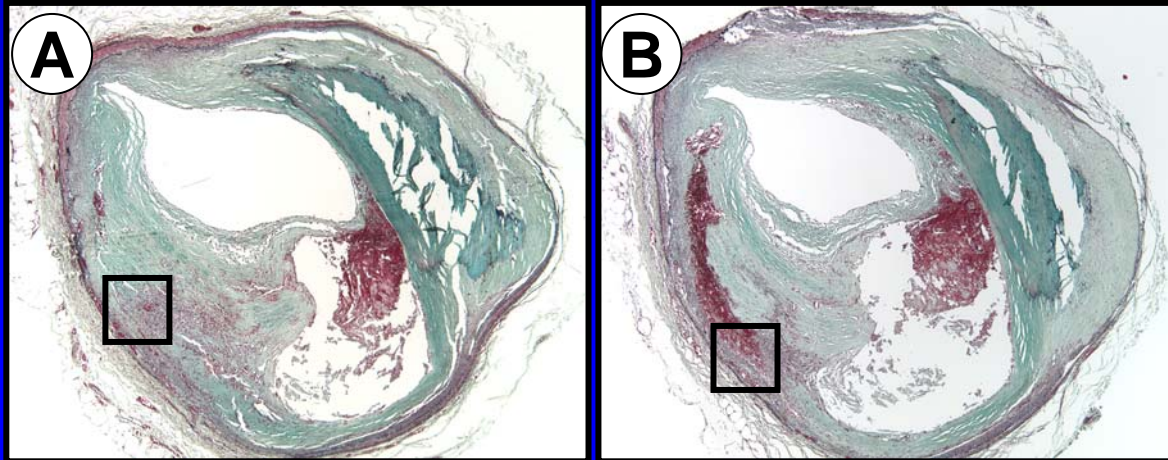
**A** Number of prior ruptures, healed rupture sites



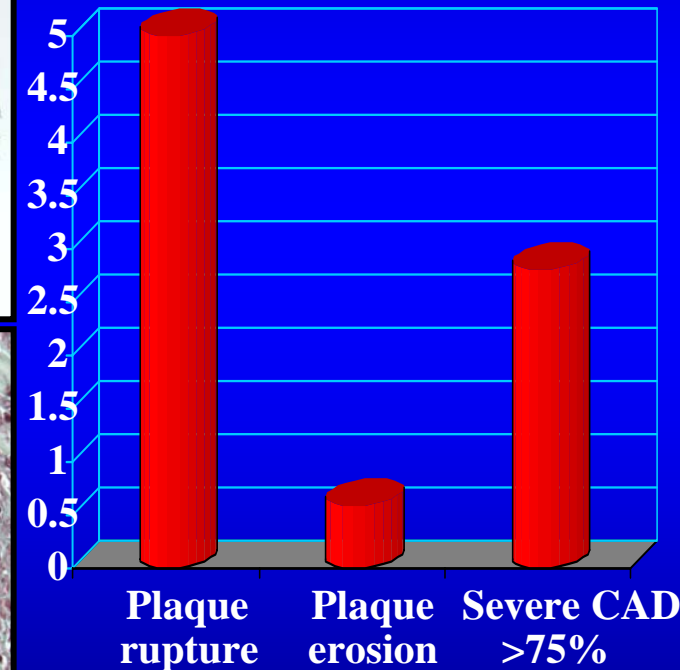
**B** Number of prior ruptures, acute rupture sites

# Thin-cap Fibroatheroma

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



## Plaque Hemorrhage



# Consequence of Extravasated Erythrocytes Outside the Vasculature

- Free cholesterol content of erythrocyte membrane exceeds that of all other cells in the body, with lipid constituting 40% of the weight
- Yeagle in 1985 showed that extravasated erythrocytes contain free cholesterol and Arbustini et al. in 2002 showed macrophage infiltration in intimal plaques in pulmonary trunk of patients with pulmonary hypertension at sites containing erythrocyte membranes

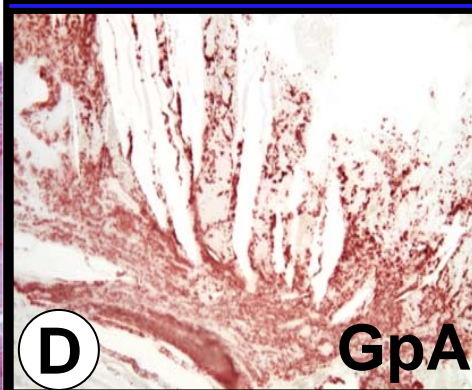
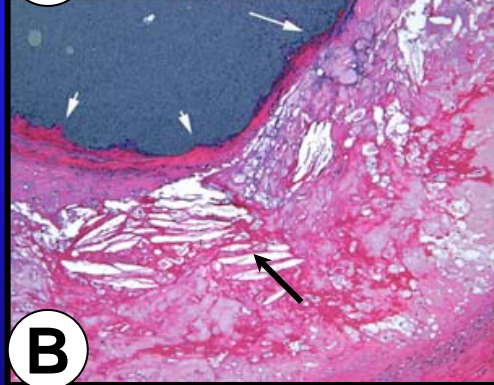
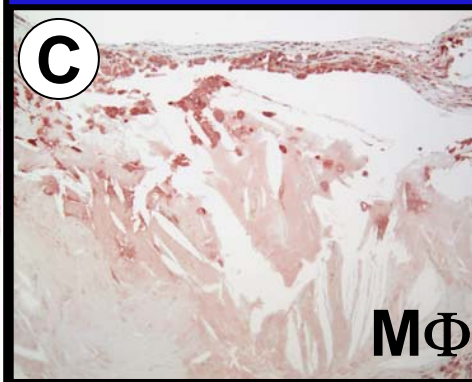
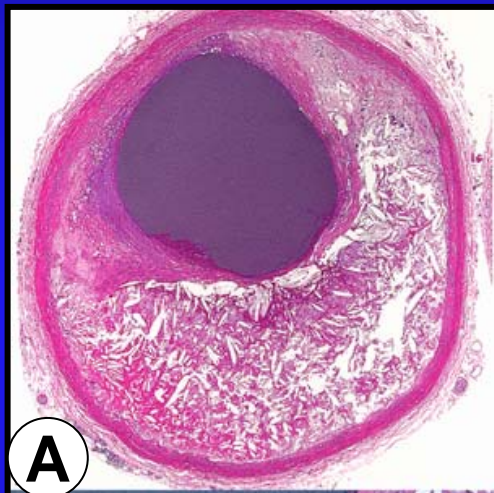
We examined tissues from nonvascular location to determine the effect of hemorrhage

- Pericardial hemorrhage
- Intratumor hemorrhage (atrial hemangiomas, papillary carcinoma of kidney etc)

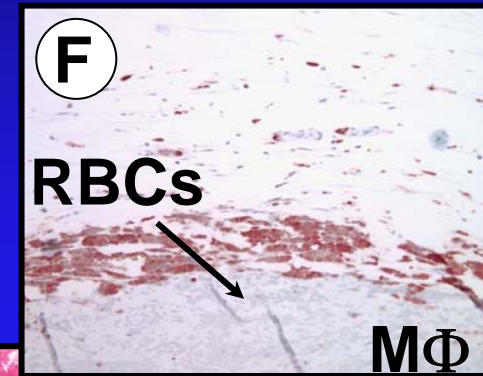
# Phase Separation of Erythrocyte-Derived Cholesterol in Coronary and Non-Coronary Diseases

Thin Fibrous Cap Atheroma

Hemorrhagic Pericarditis



Hemorrhage  
Periphery



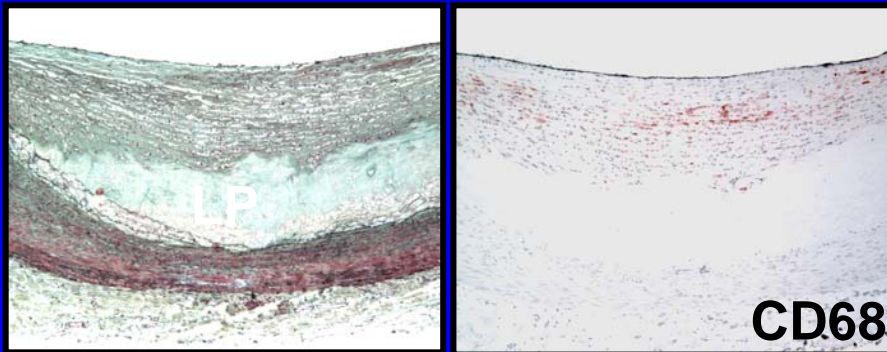
Hemorrhage  
Core



# Plaque Types Studied

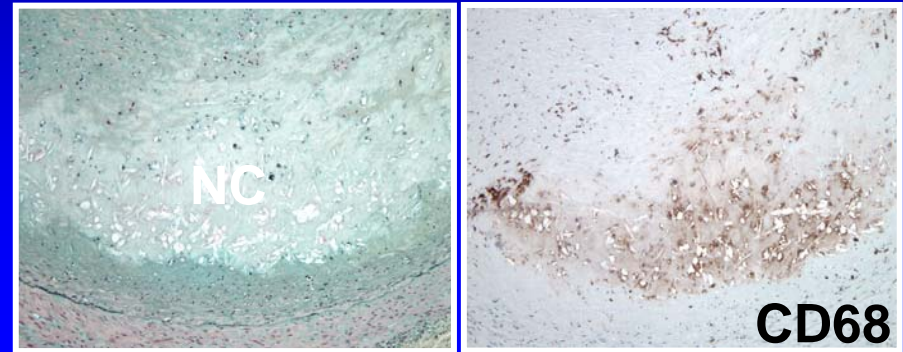
A.

Pathologic Intima Thickening



B.

Fibroatheroma 'Early' Core



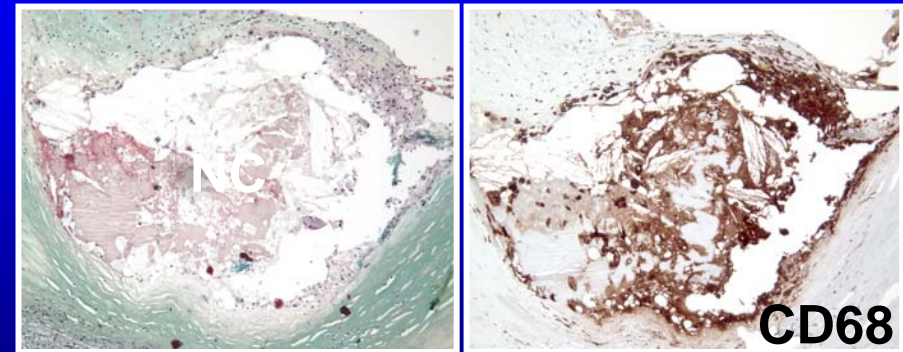
C.

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 <sup>2</sup> )	MΦ (mm <sup>2</sup> )
<b>PIT no core</b> (n=129)	0.09±0.04	0.07±0.05	0.0	0.002±0.001
<b>FA early core</b> (n=79)	0.23±0.07	0.17±0.08	0.06±0.02	0.018±0.004
<b>FA late core</b> (n=105)	*0.94±0.11	*0.41±0.09	*0.84±0.08	*0.059±0.007
<b>TCFA</b> (n=52)	*1.60±0.20	*1.24±0.24	*1.95±0.30	*0.142±0.016

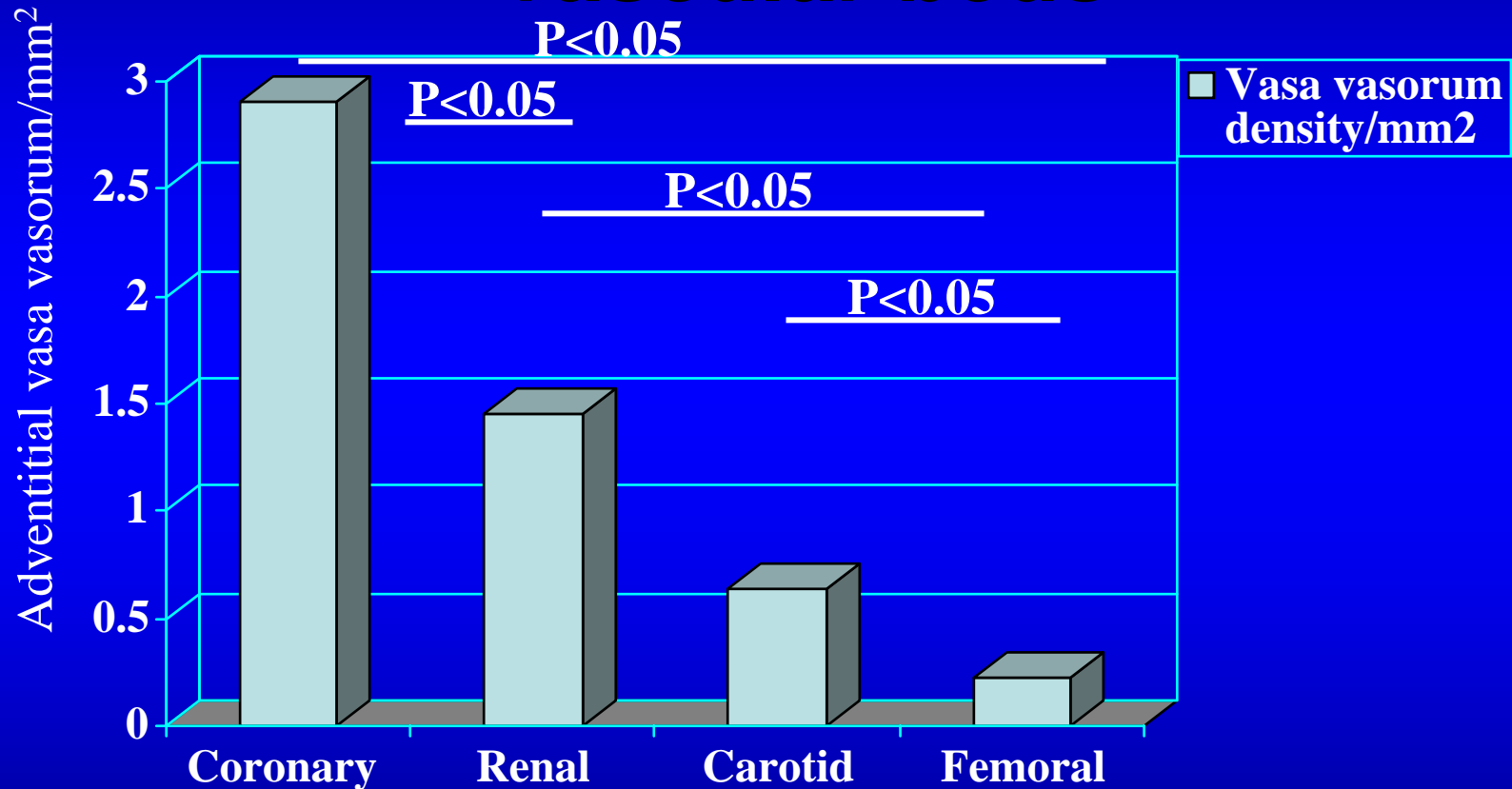
Values are reported as the means±SE, \*p<0.001 versus early core. The number in parenthesis represent the number of lesions examined;the total number= 365. MΦ = macrophages



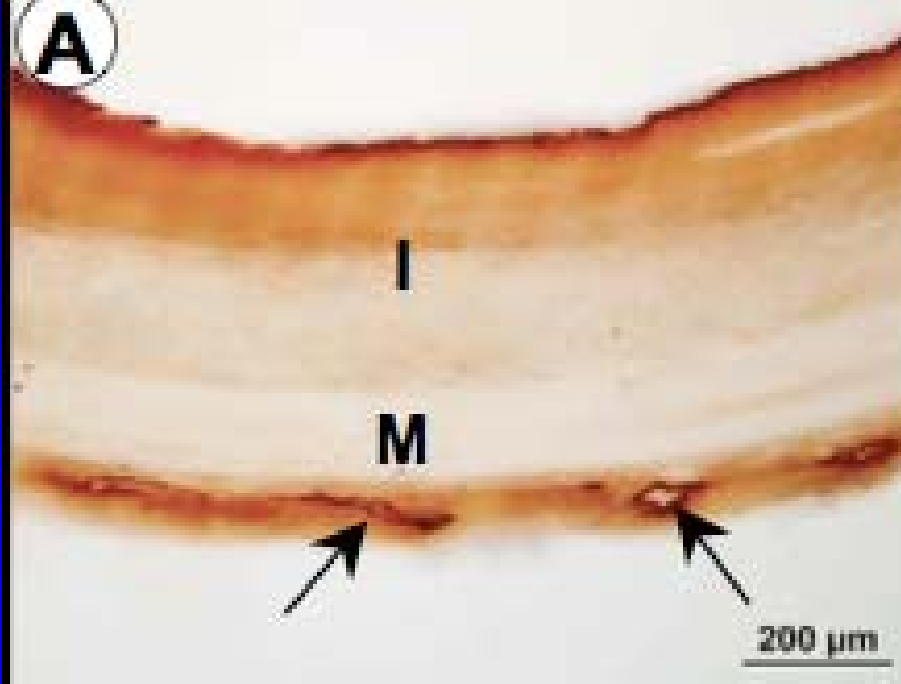
# Plaque Vasa Vasorum

- 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

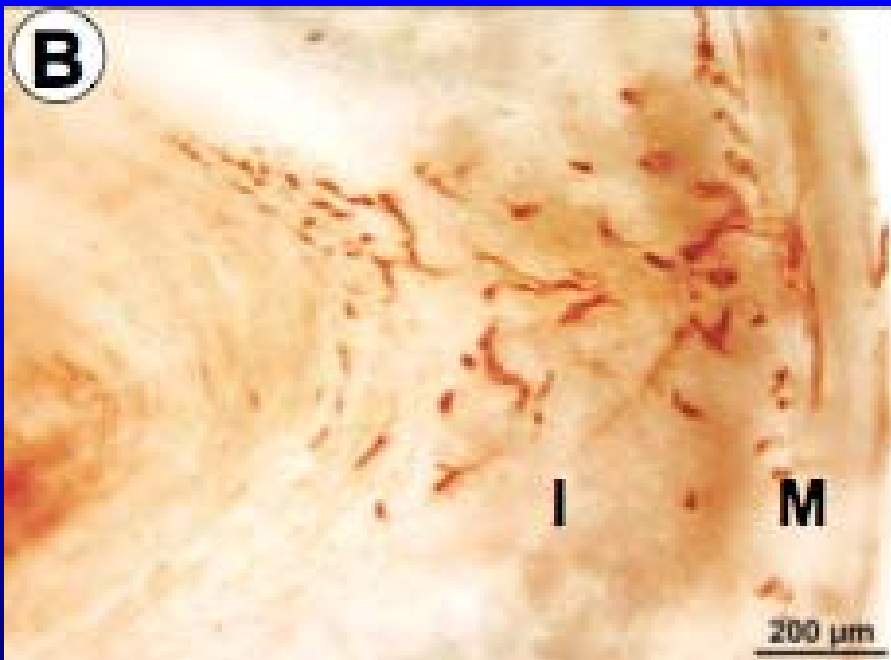


Low vasa vasorum density in internal thoracic artery may be responsible for the low incidence of atherosclerosis

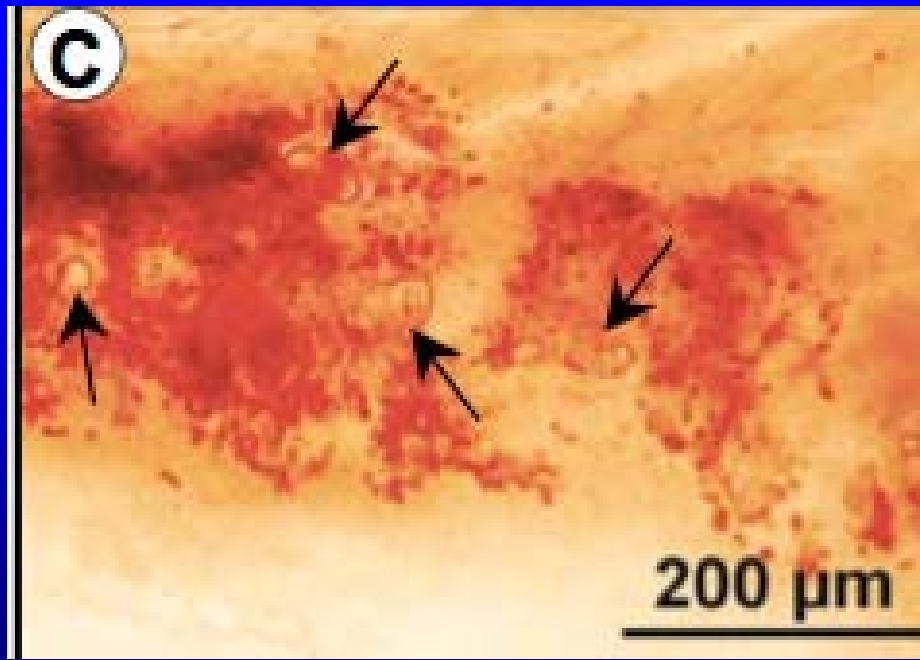
**A**

# Vasa vasorum at various stages of plaque development

Adaptive Intimal thickening  
Vv in adventitia

**B**

Fibroatherma Abnormal Vv

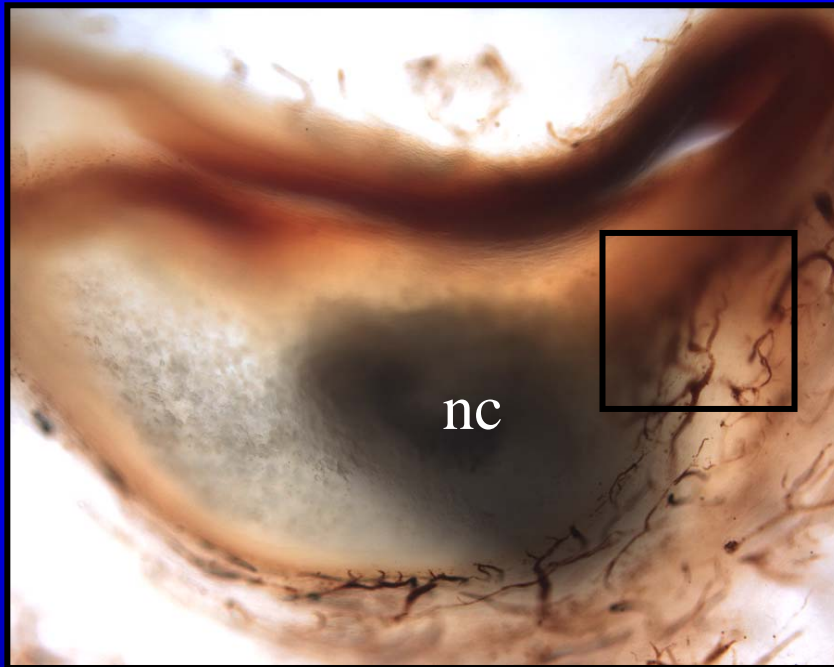
**C**

Fibroatherma with leaky Vv

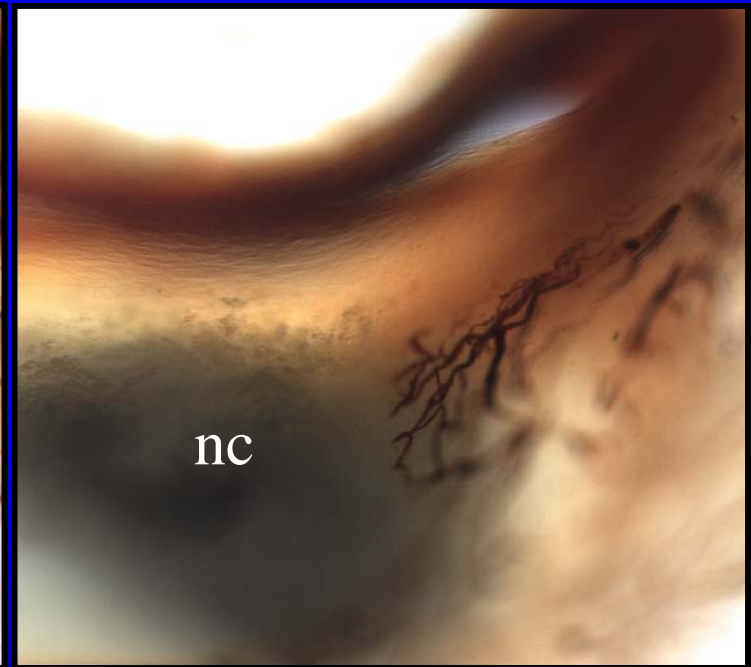
# Intraplaque Vasa Vasorum in Coronary Plaques with a Necrotic Core

150  $\mu\text{m}$  thick sections stained with Ulex

A

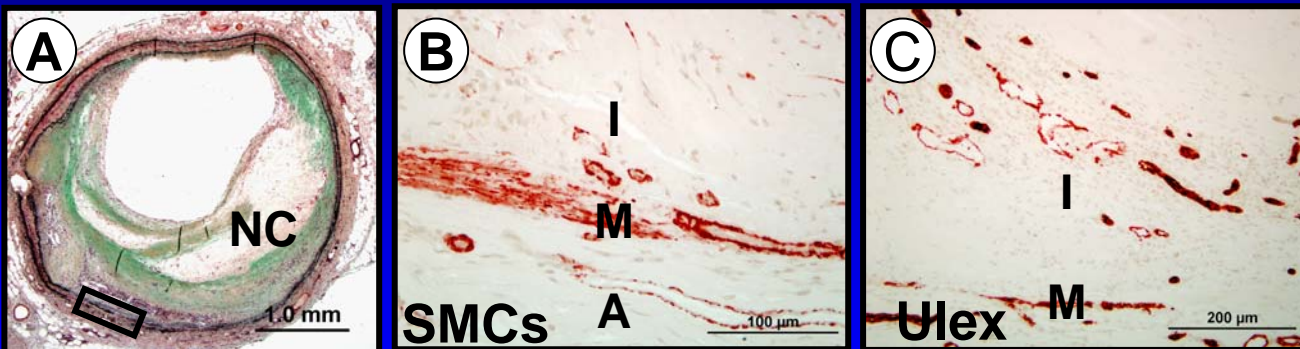


B

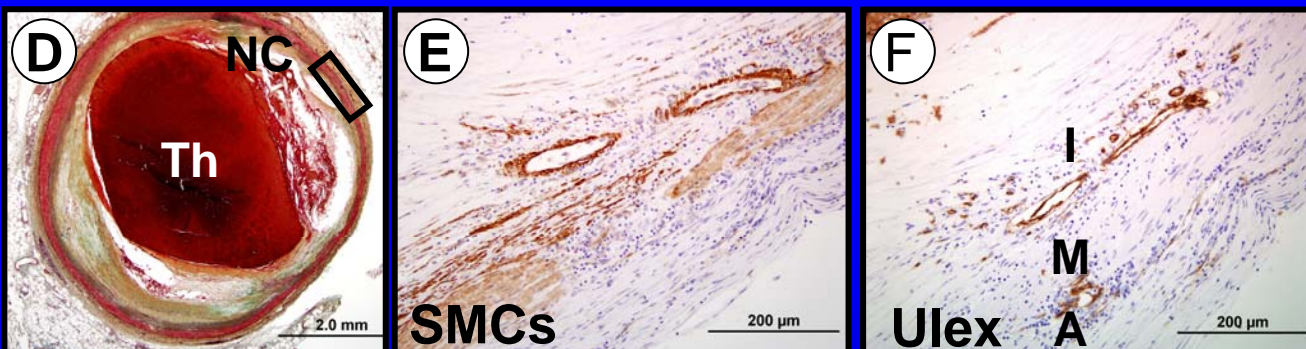


# Vasa Vasorum by Plaque Type

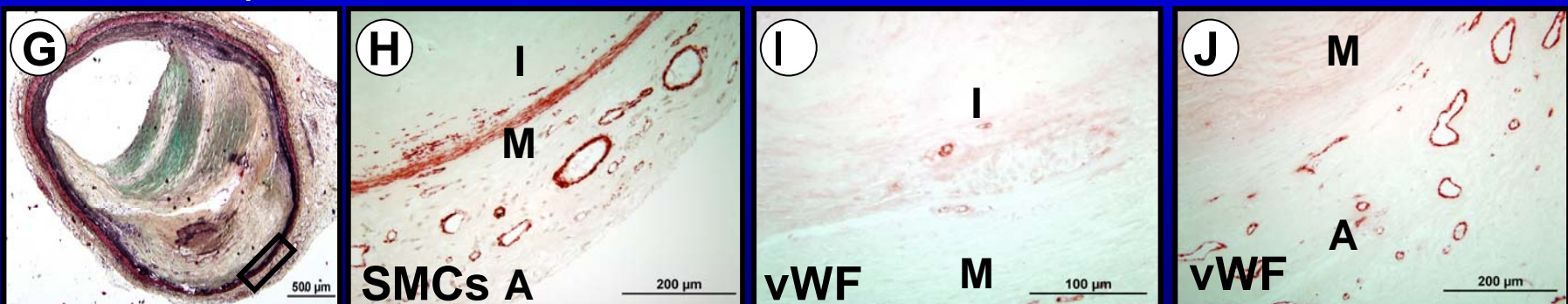
## Thin-Cap Fibroatheroma



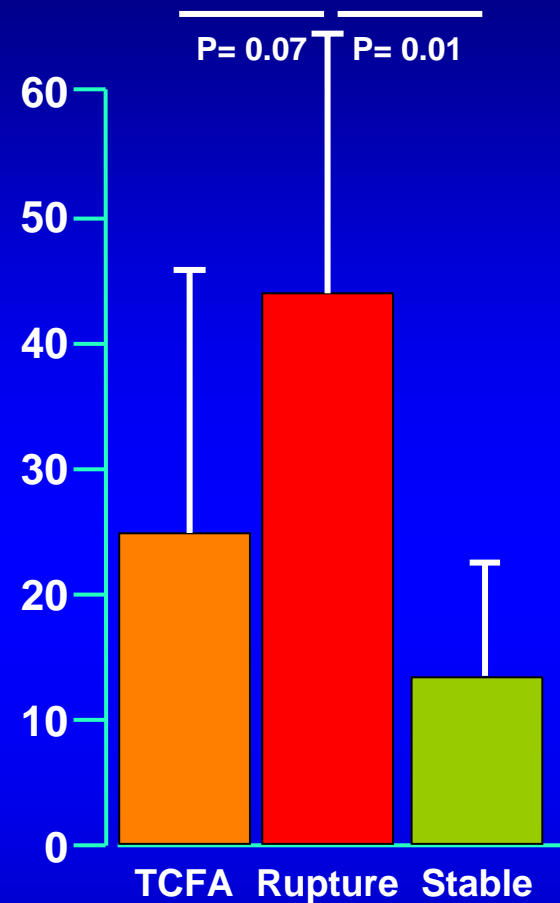
## Plaque Rupture



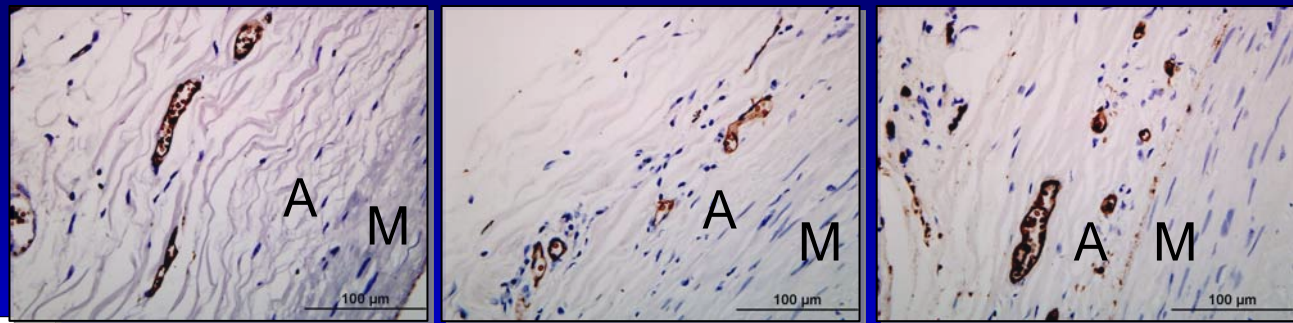
## Stable Plaque



## K Mean No. of Vasa Vasorum by Plaque Type



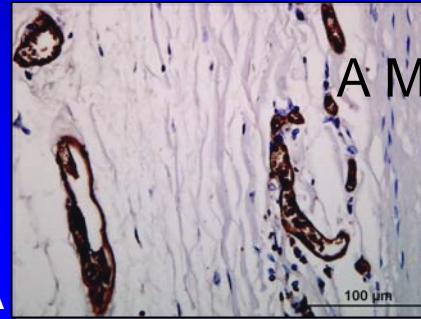
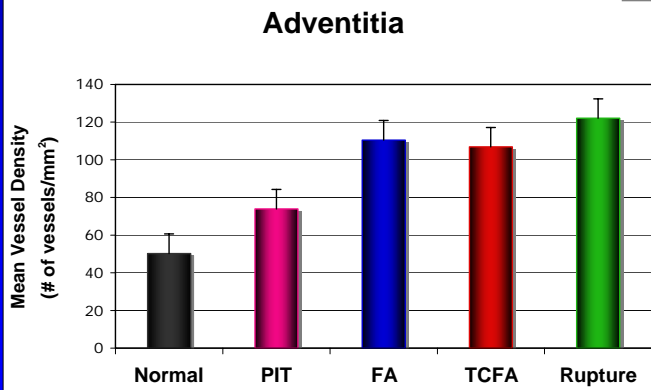
# Adventitial Vasa Vasorum In Varying Plaque Morphologies (Ulex Europaeus)



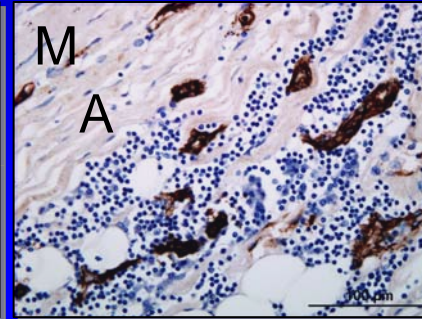
Normal

PIT

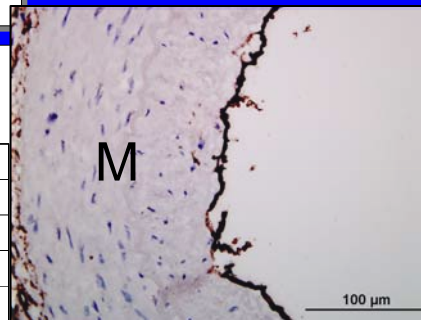
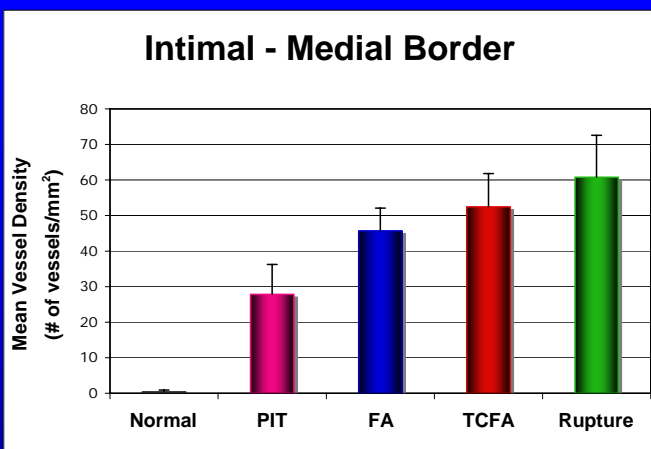
FA



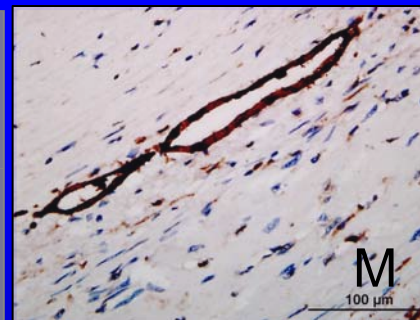
TCFA



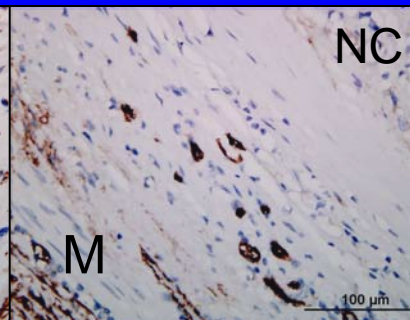
Plaque Rupture



Normal



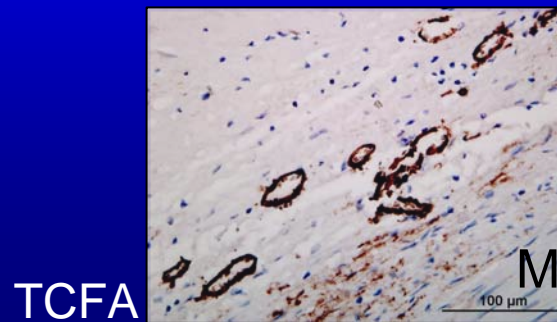
PIT



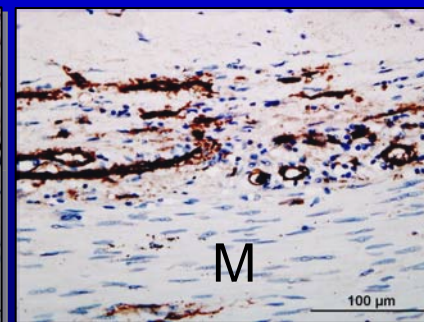
FA

NC

# Intimal-Medial Border Vasa Vasorum in Varying Plaque Morphologies (CD31/CD34)



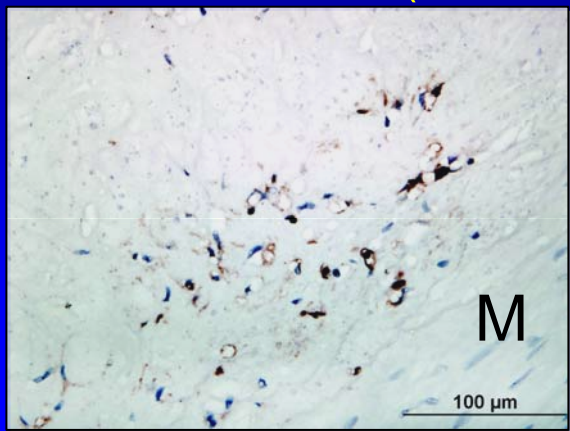
TCFA



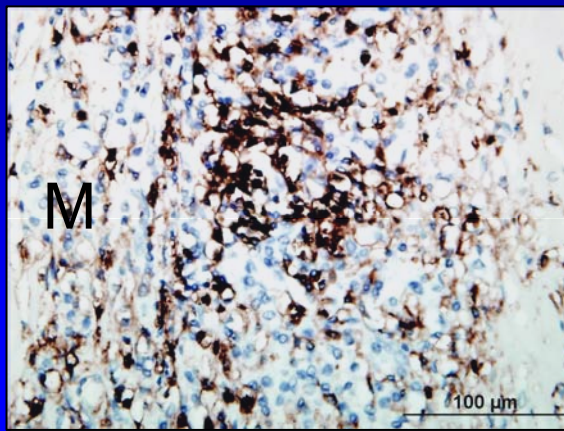
Plaque Rupture

# Intimal-Medial Border T Cell Densities and % Macrophage Infiltration at Vaso Vasorum Hotspots in Varying Plaque Morphologies

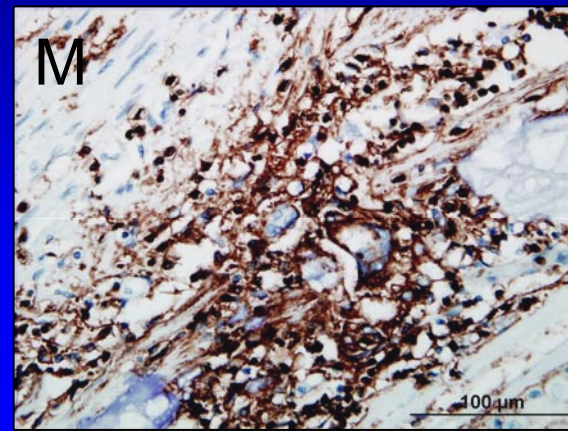
## T Cell Densities (UCHL-1)



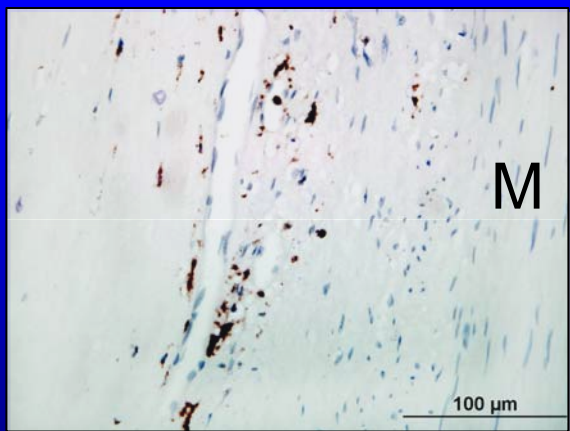
PIT



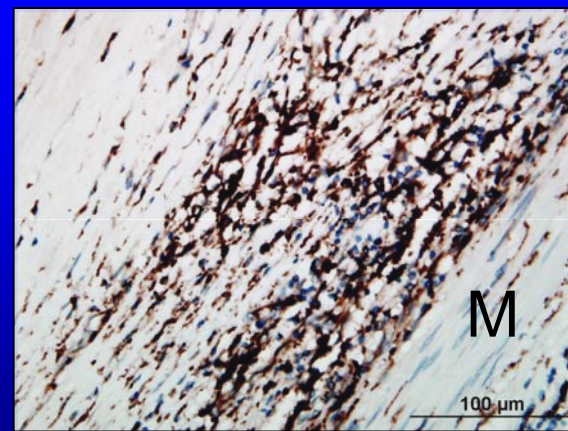
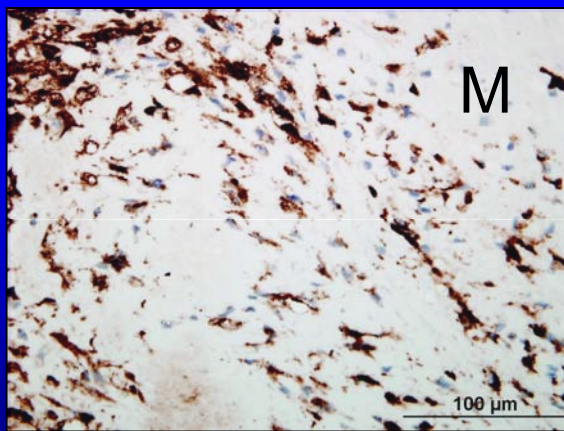
Late FA



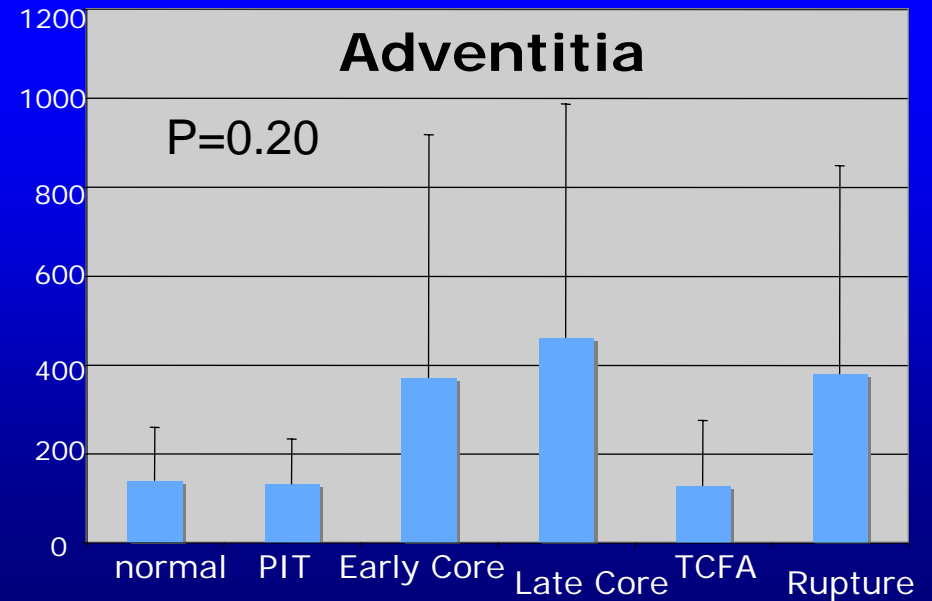
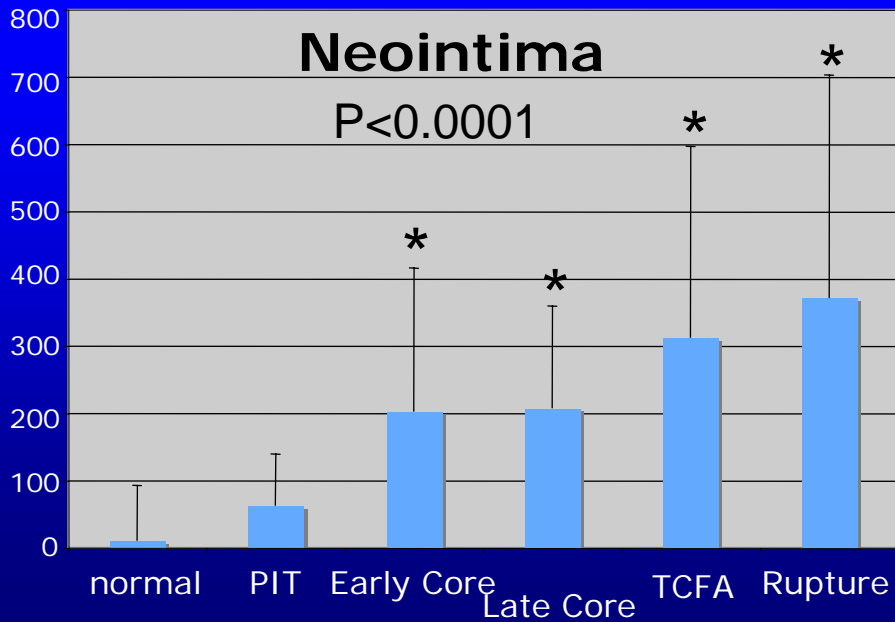
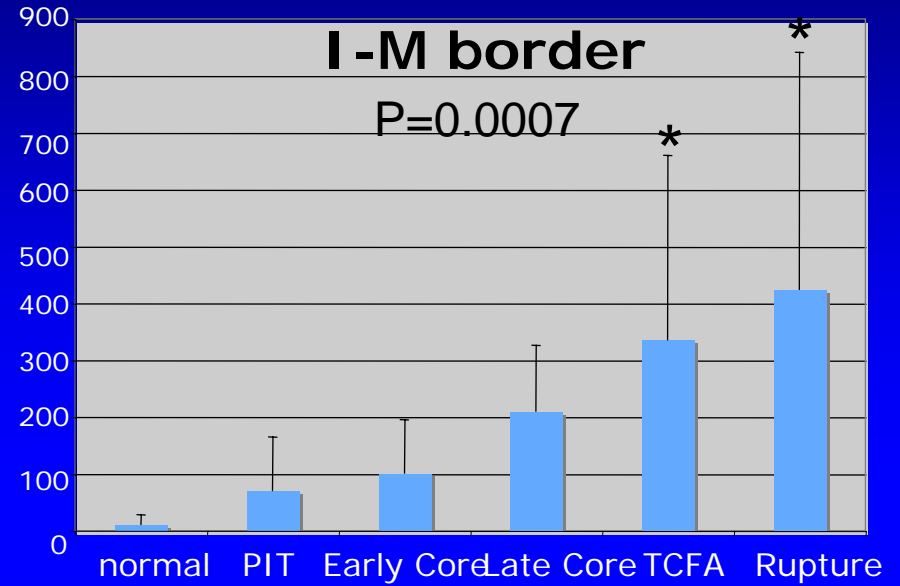
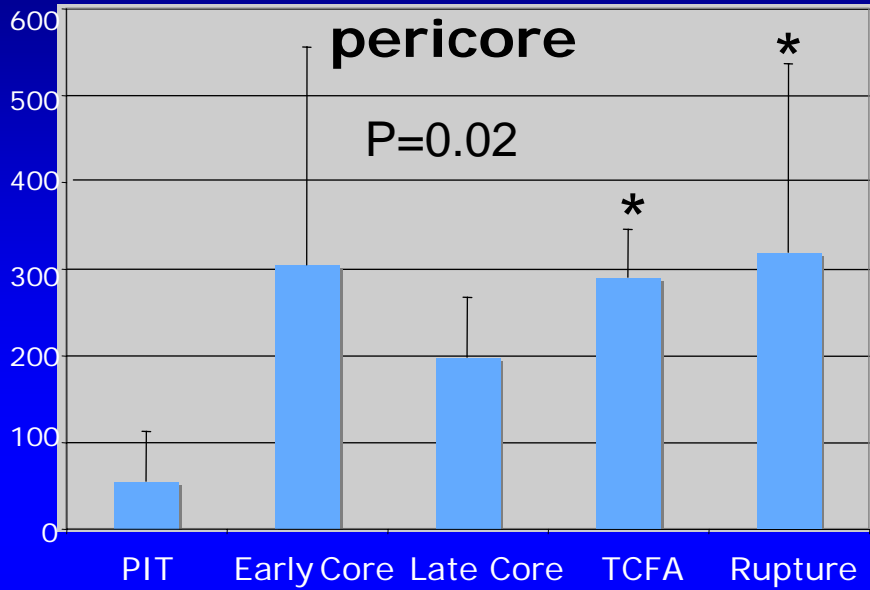
Rupture



Macrophage Infiltration  
(CD68)



# T cell



\*=significant VS. normal



# 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 to a lesser extent macrophage infiltration.
- ❑ Intra plaque hemorrhages from leaky vv are 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.

# Development of atherosclerosis and Progression to Thrombosis

