Pathogenesis of vulnerable plaque

Andrew Newby

Wei-chun Huang, Nick Jenkins, Sarah George, Jason Johnson, Karina di Gregoli, Buket Reel, Rebecca Salter, Graciela Sala-Newby

Questions

- **What is a vulnerable plaque?**

- **What effect does it have in patients?**

- **What are the mechanisms?**

- **Is there an animal model?**
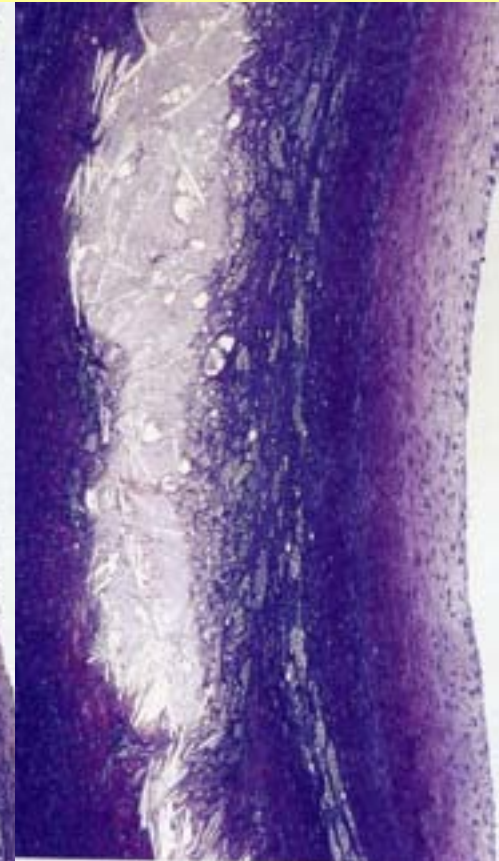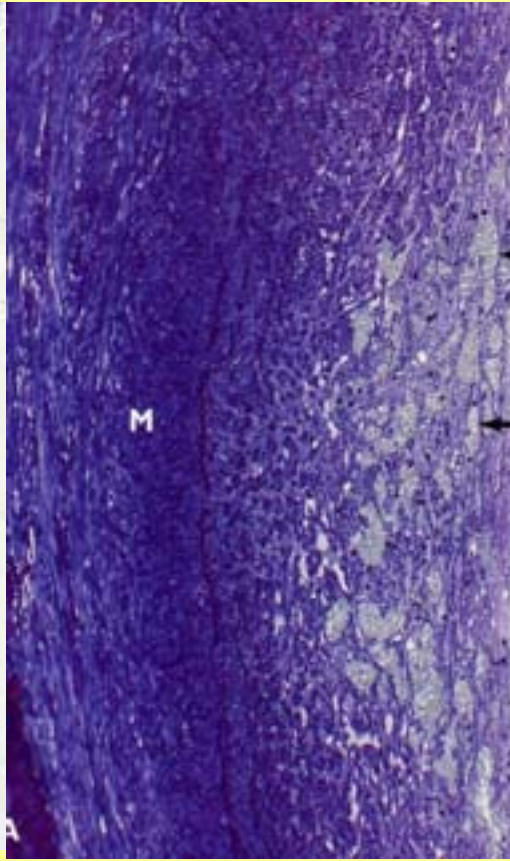
- **What can we do about it?**

Atherosclerotic plaque is a highly prevalent, slowly developing cause of angina pectoris and a risk factor for MI and stroke
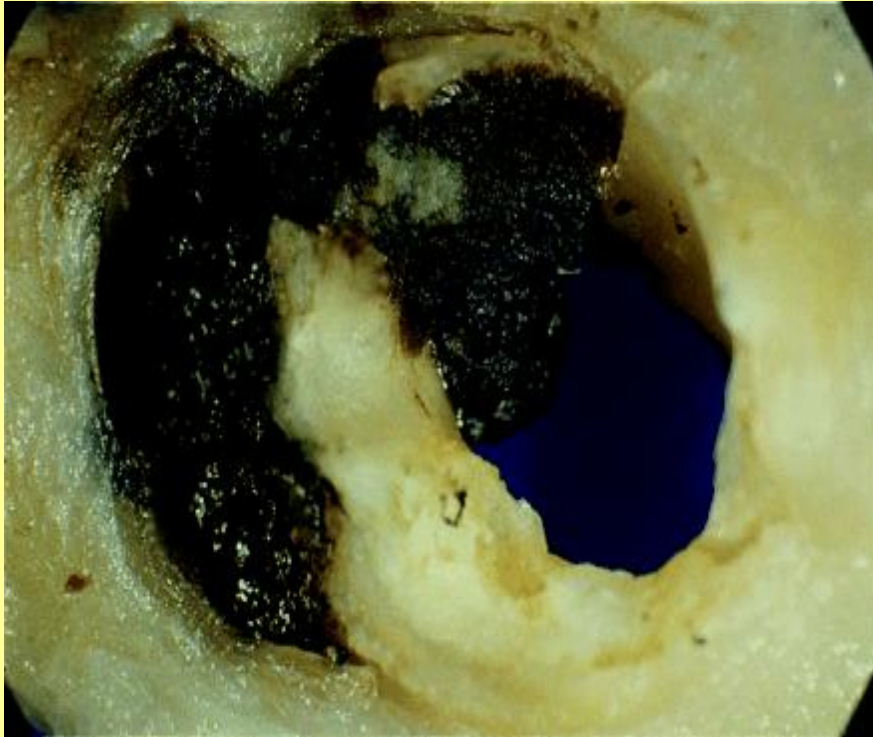

0	10	20	40+ years
I	Type II	Type III	Type IV

Stroke and MI (heart attack) are mainly caused by plaque rupture or endothelial erosion

**Plaque rupture
– 85% of male MI**

**Surface erosion
- 40% of female MI
- smokers**

Davies, MJ Heart 2000; 83:361-6

Vulnerable plaque - definitions and assumptions

- **Plaque with a high probability to rupture or erode hence provoke thrombosis**

- **Predictor of MI and stroke**

- **Similar structural characteristics as plaques with recent thrombus**

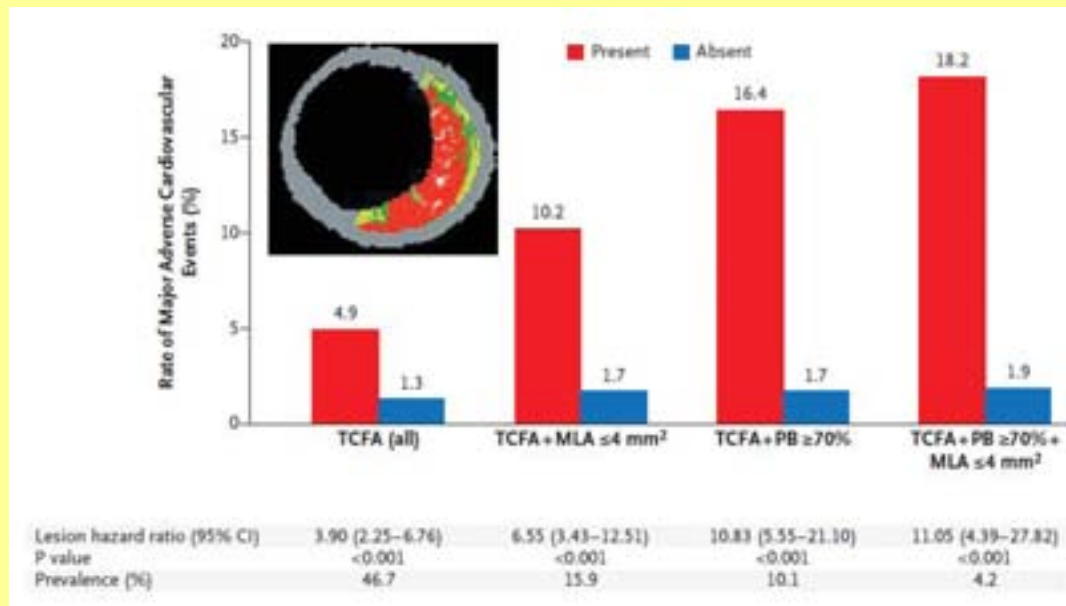- **Formed by similar mechanisms as other plaques only more so!**

Are these assumptions valid?
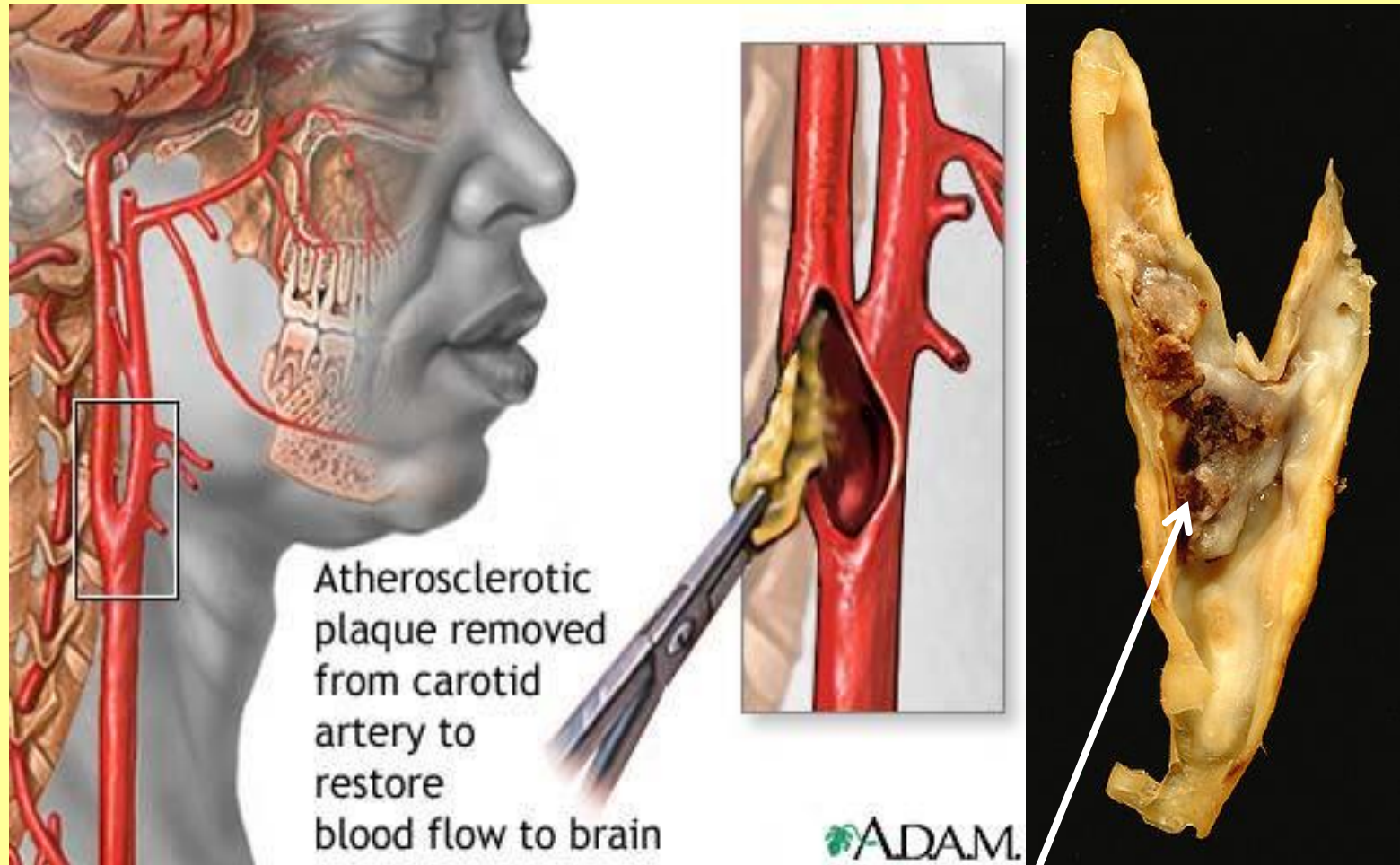
Does Vulnerable Plaque predict MI

In 697 STEMI patients, thin-cap fibroatheromas were more likely to give rise to MACE in next 3 years.

However, most MACE were new episodes of angina

MI was too infrequent for statistical power

Vulnerable plaque was a good predictor of subsequent MACE but the situation for MI is still unclear.

	TCFA (all)	TCFA+MLA ≤4 mm²	TCFA+PB ≥70%	TCFA+PB ≥70%+ MLA ≤4 mm²
Lesion hazard ratio (95% CI)	3.90 (2.25–6.76)	6.55 (3.43–12.51)	10.83 (5.55–21.10)	11.05 (4.39–27.82)
P value	<0.001	<0.001	<0.001	<0.001
Prevalence (%)	46.7	15.9	10.1	4.2

Stone, NEJM;364:226

Transient ischaemic attacks predicts stroke – carotid endarterectomy

Atherosclerotic plaque removed from carotid artery to restore blood flow to brain

A.D.A.M.

Ulcerated plaque with thrombus

What features of VP are predictive?

In 818 carotid plaques from AtheroExpress

- **Haemorrhage and micro-vessels were predictors of MACE**

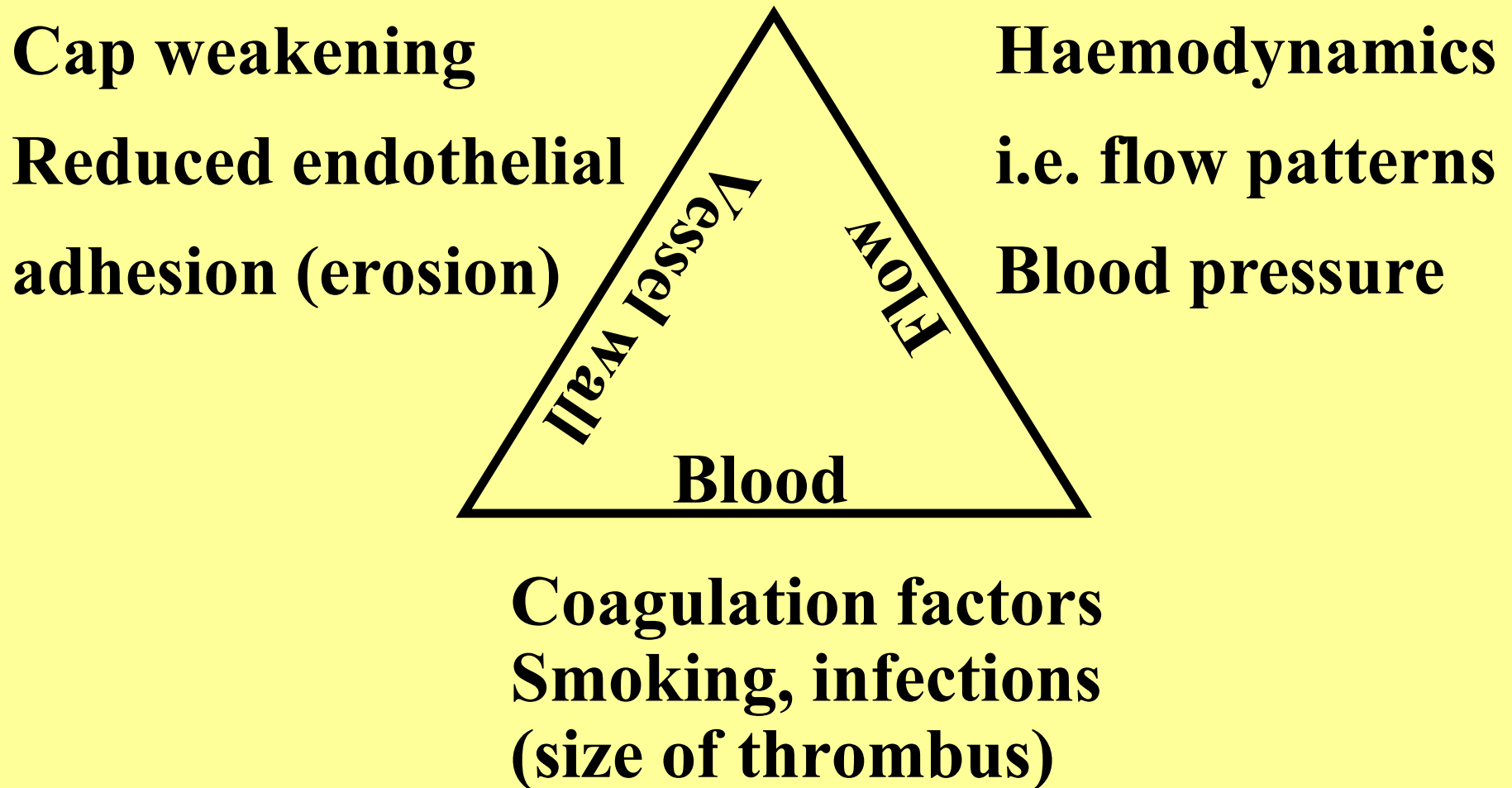- **Lipid core size, macrophages, smooth muscle cells and collagen were not predictive**

Hellings, Circulation 2010;121:1941
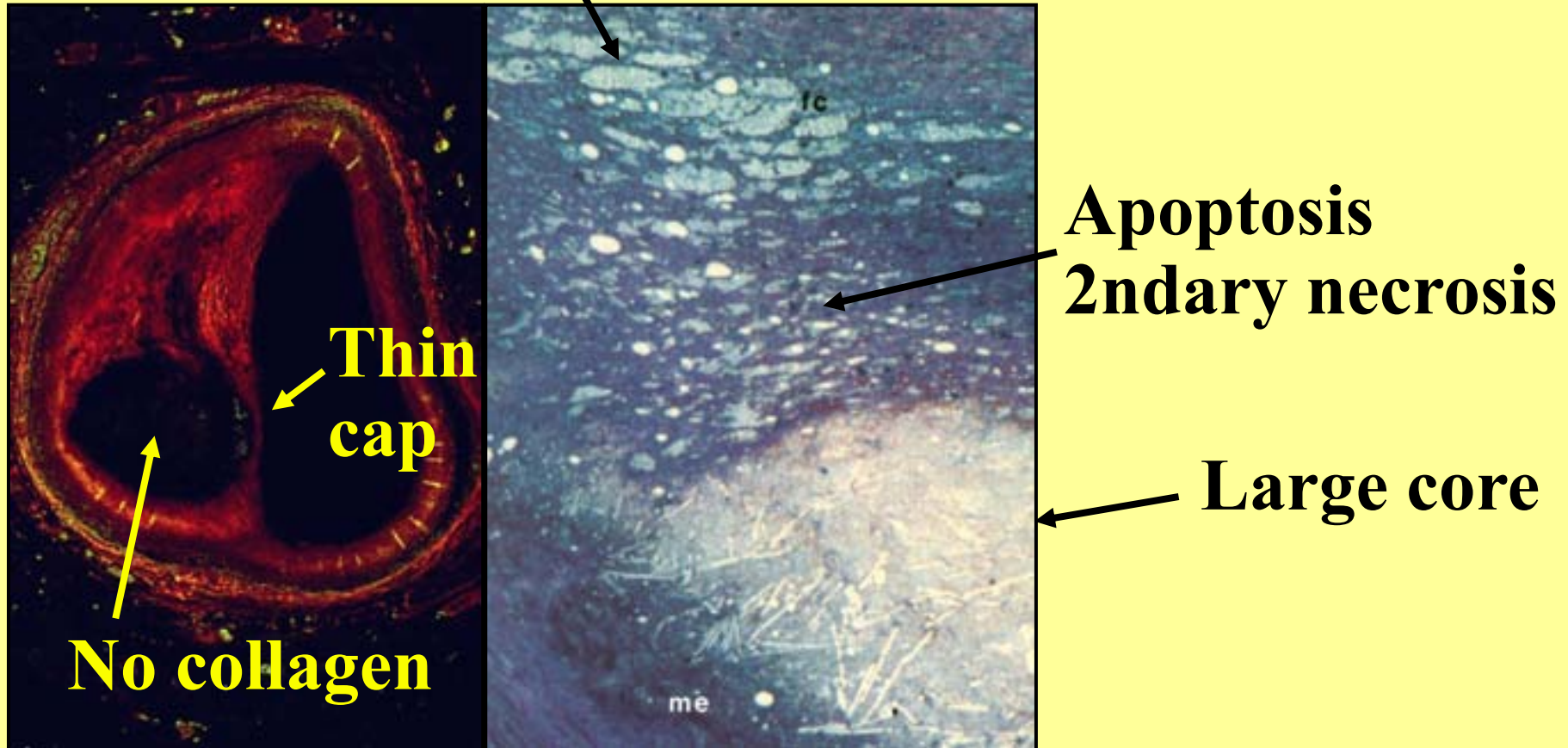
Why does plaque rupture occur?

Plaque rupture like bridge collapse results from the chance interplay of intrinsic weakness and external forces

Factors that influence MI and stroke

Cap weakening

Reduced endothelial

adhesion (erosion)

Haemodynamics

i.e. flow patterns

Blood pressure

Vessel wall

Flow

Blood

Coagulation factors
Smoking, infections
(size of thrombus)

What makes plaque likely to rupture?

Foamy macrophages

Apoptosis 2ndary necrosis

Large core

Thin cap

No collagen

Thin fibrous cap (<65 μm), large lipid core, lots of foam cells, loss of collagen from the cap or core, microvessels
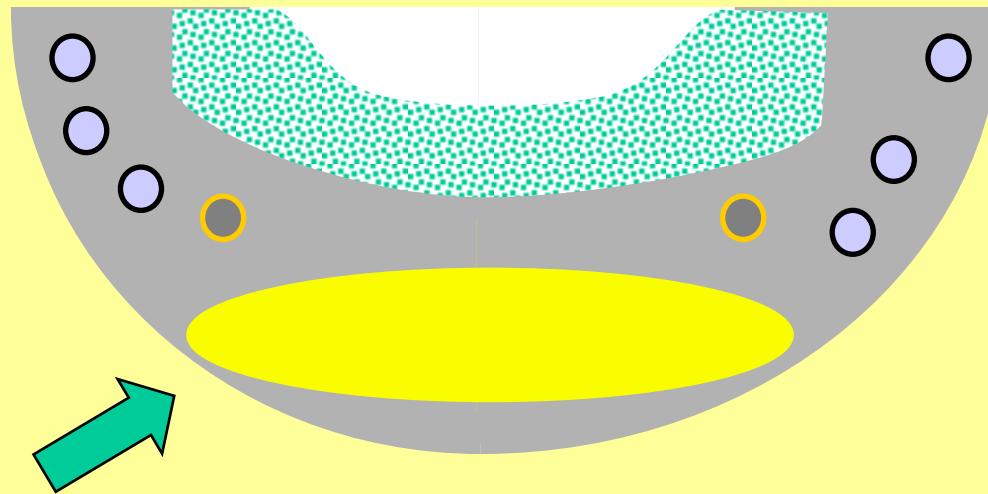
Davies, M Circulation 1996; Falk, E JACC 2006;47:C7-12

How do mechanical factors influence MI?

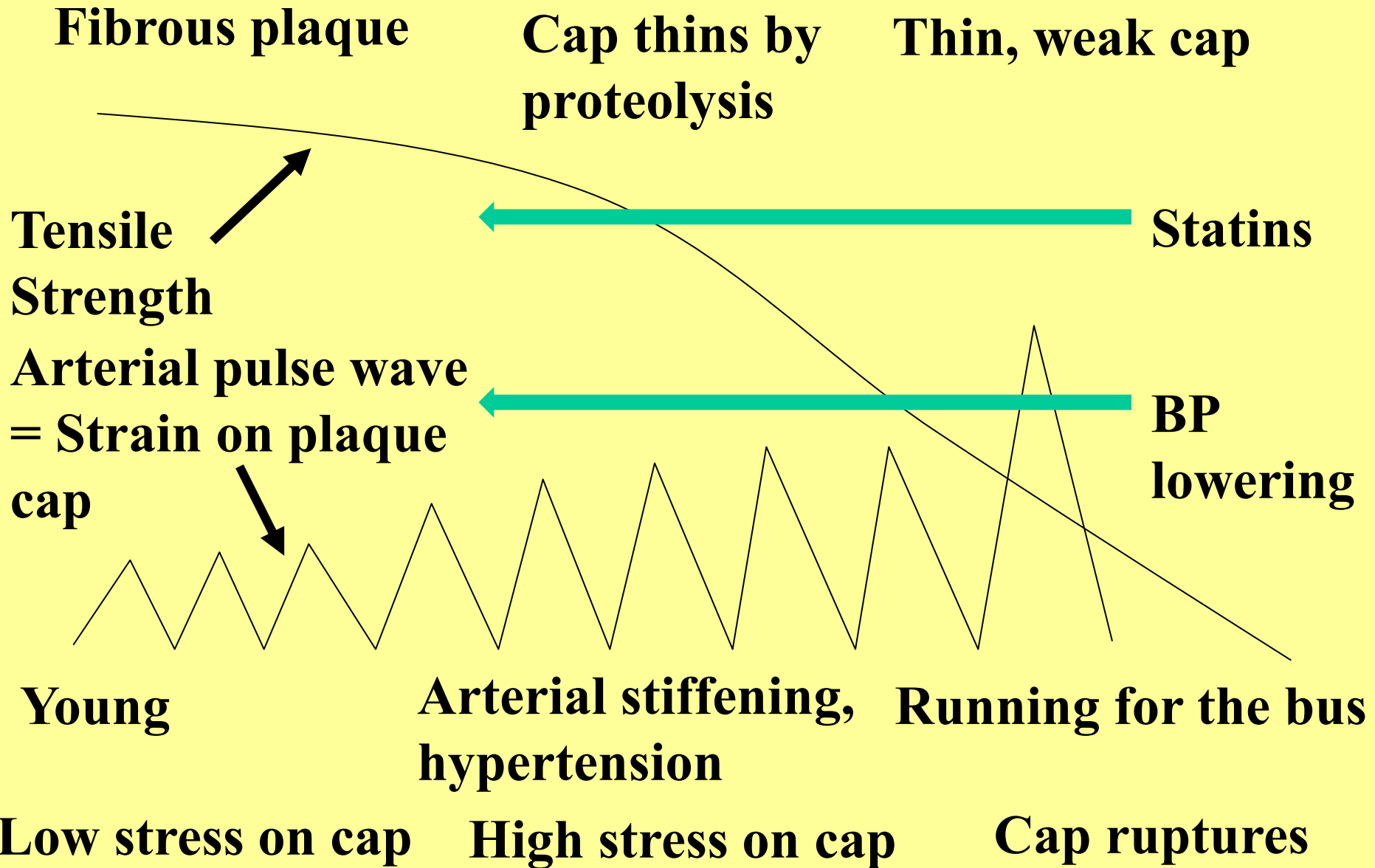**Hypertension, arterial stiffness = increased pressure wave = high stress**
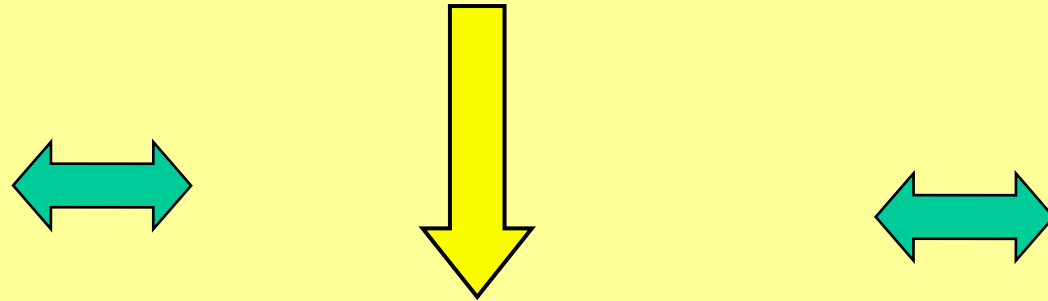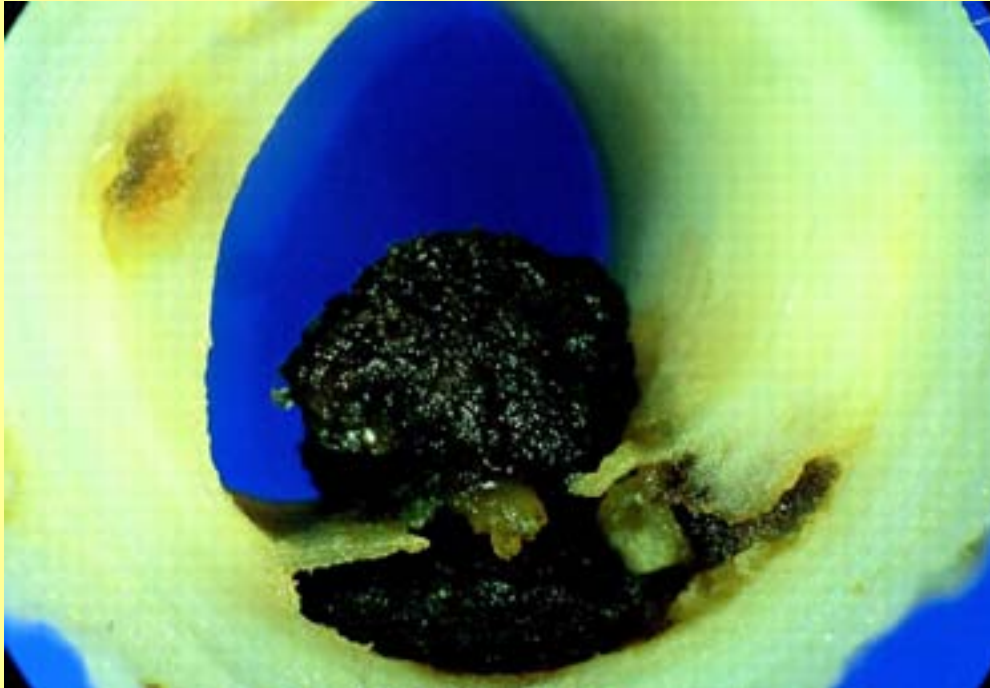
Weak shoulder = High strain
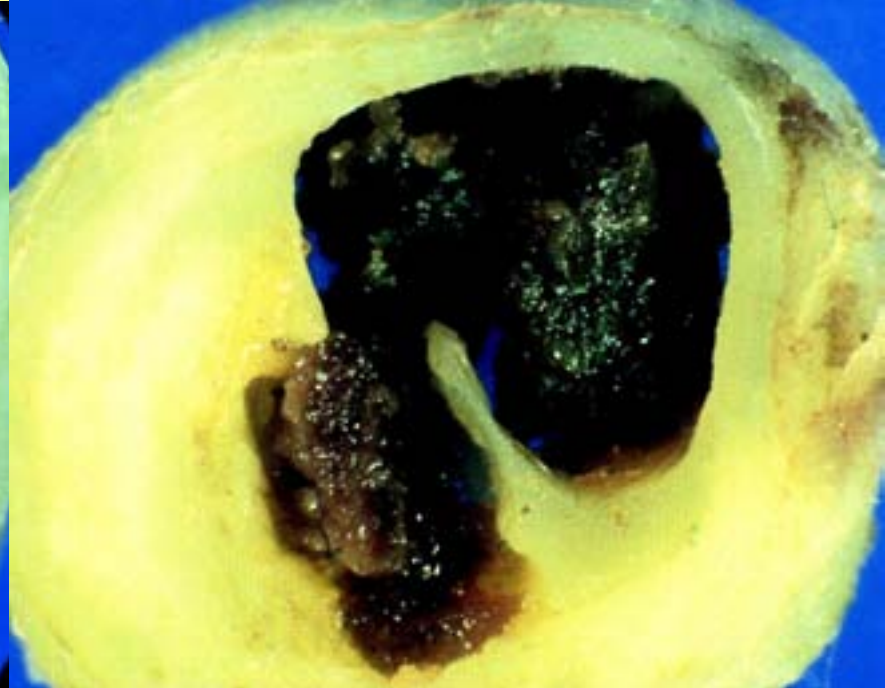
Big lipid core = high strain

Why do plaques rupture when they do?

Fibrous plaque

Cap thins by proteolysis

Thin, weak cap

Tensile Strength

Statins

Arterial pulse wave = Strain on plaque cap

BP lowering

Young

Arterial stiffening, hypertension

Running for the bus

Low stress on cap

High stress on cap

Cap ruptures

Extent of thrombosis determines severity of outcome

Partial occlusion – NSTEMI   **Total occlusion – STEMI**

Davies, MJ Heart 2000; 83:361-6

Autoantigens promote T-cell activation

LUMEN

CD16 +/-

HSP60

Mo

Foam cell

F C

INTIMA

ox-LDL

CX3CL CCL5

(-)

++

SMC

Th1

IFNγ

MEDIA

(-)

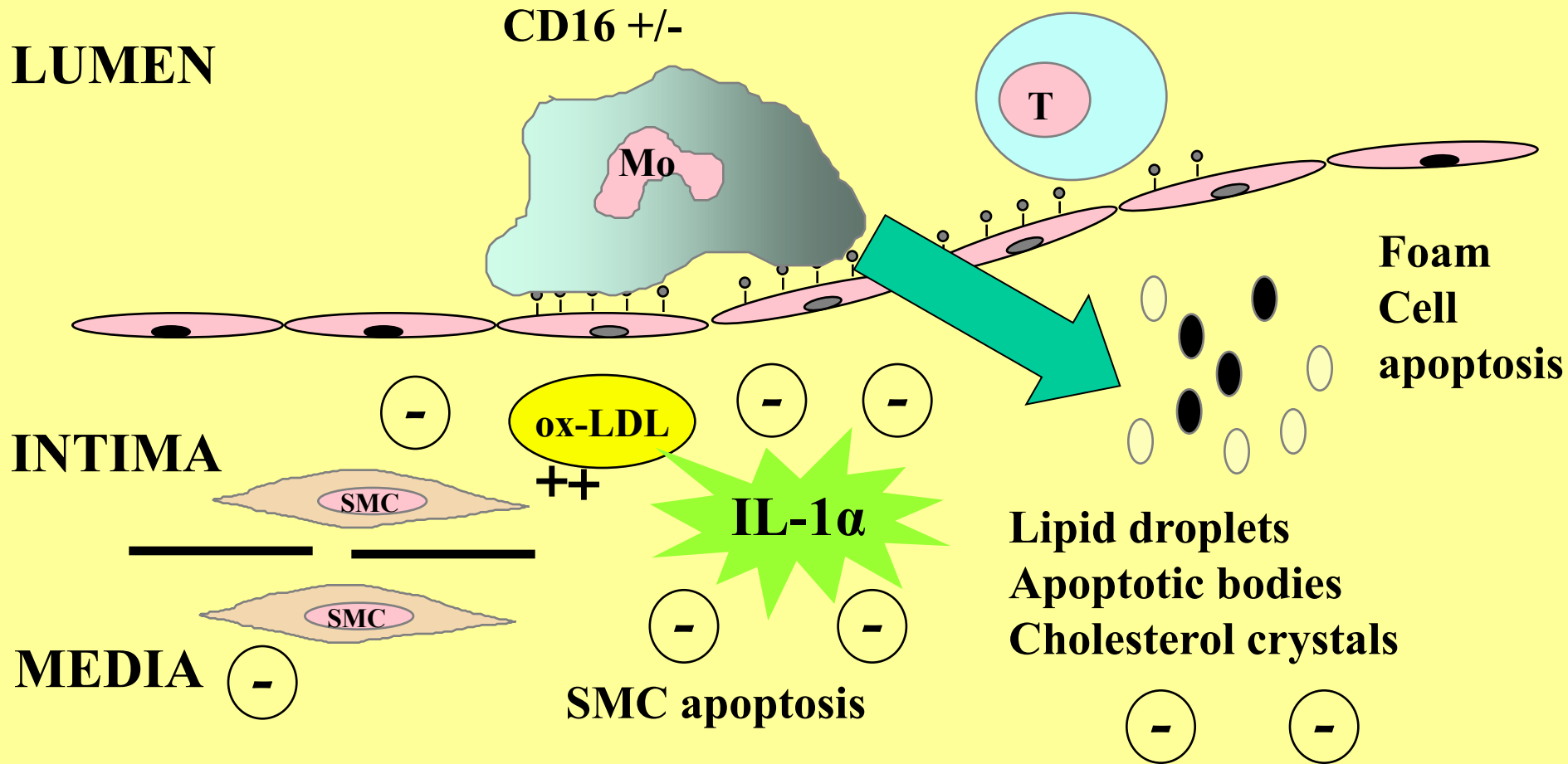**SMC**

(-)

(-)

SMC

SMC

(-)

HSP60

(-)
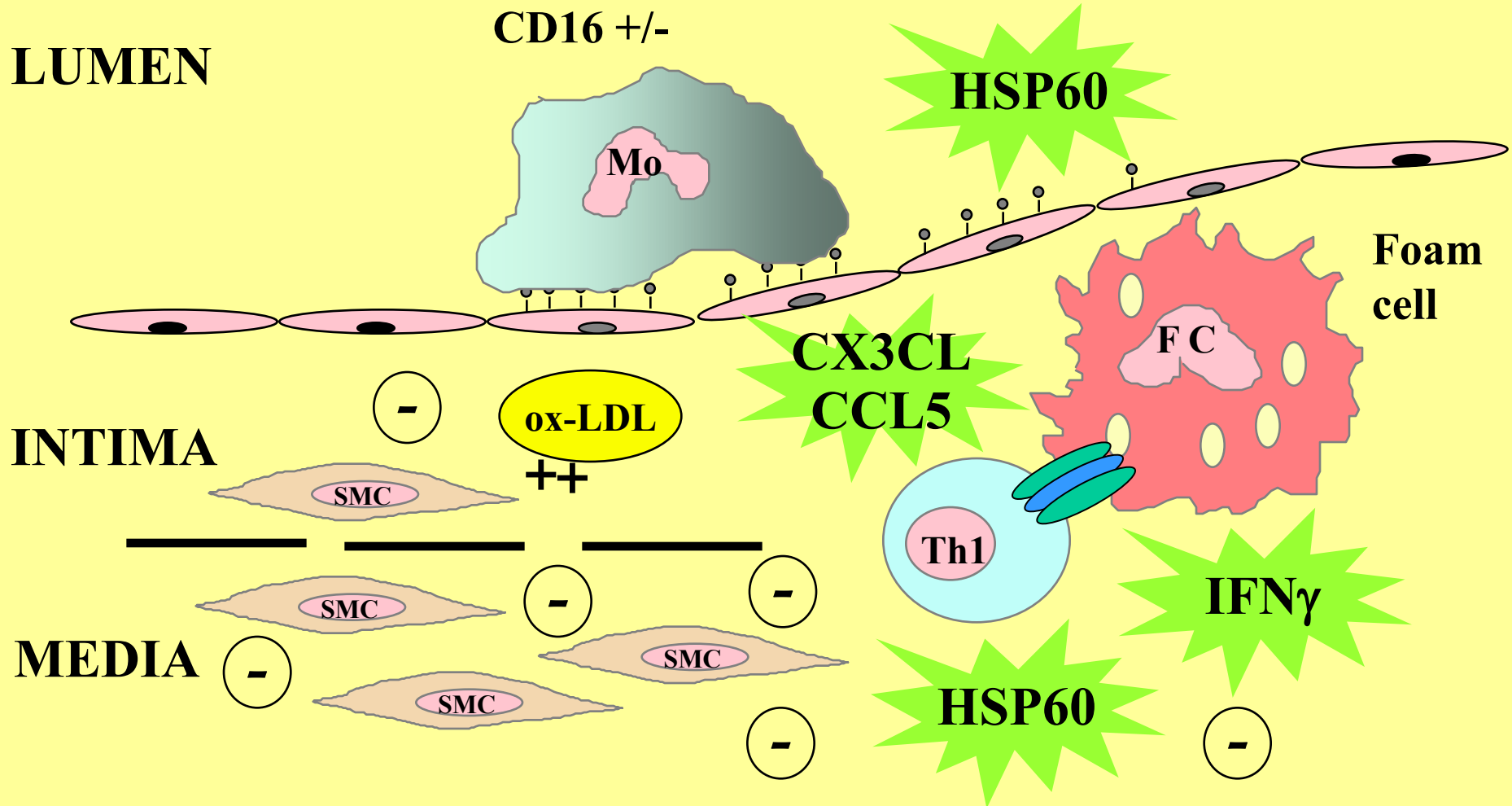
TCC, T-cell chemokines, CXCL9, 10, 11, CD40L, OX40L

Various damage associated molecular patterns are recognised by Toll-like receptors

CD16 +/-

LUMEN

Mo

INTIMA

-

ox-LDL

++

SMC

TLL

F C

Foam Cell (M1)

MEDIA

-

SMC
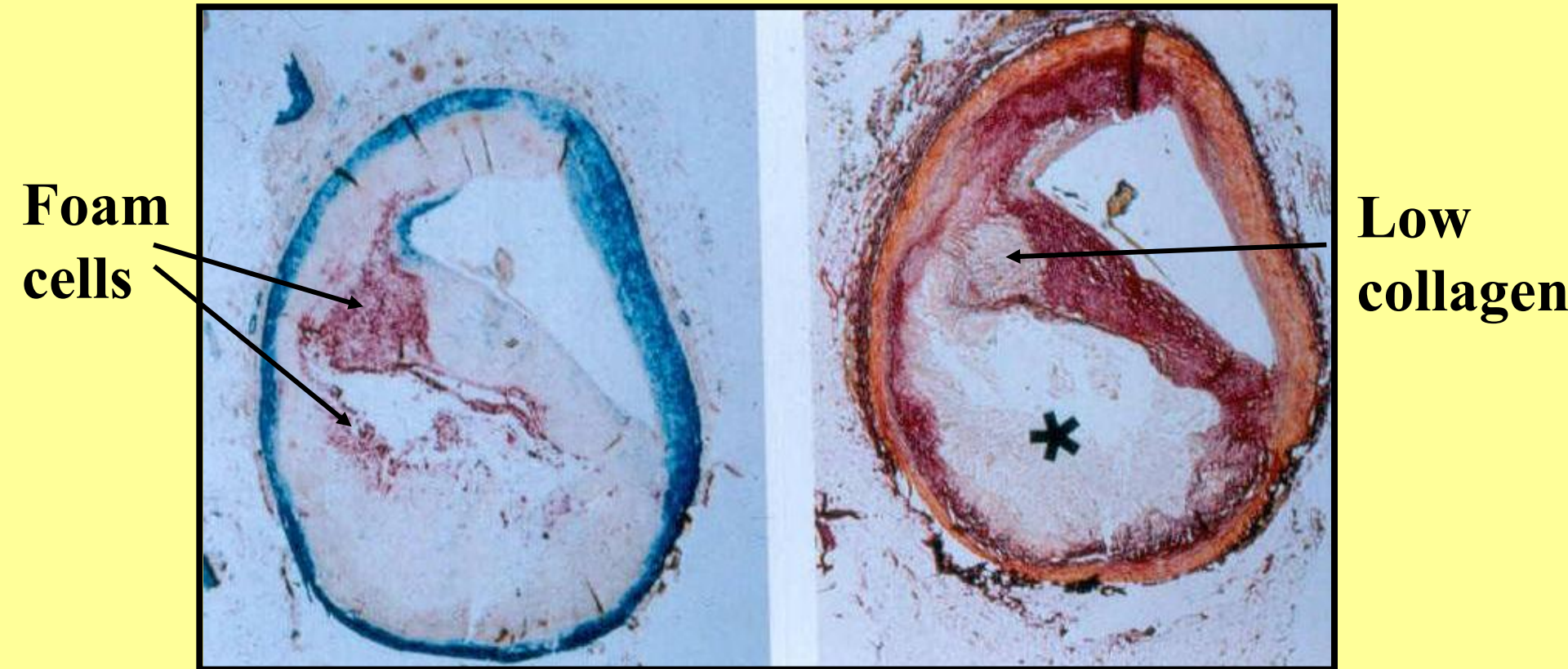
Cell injury, altered matrix components
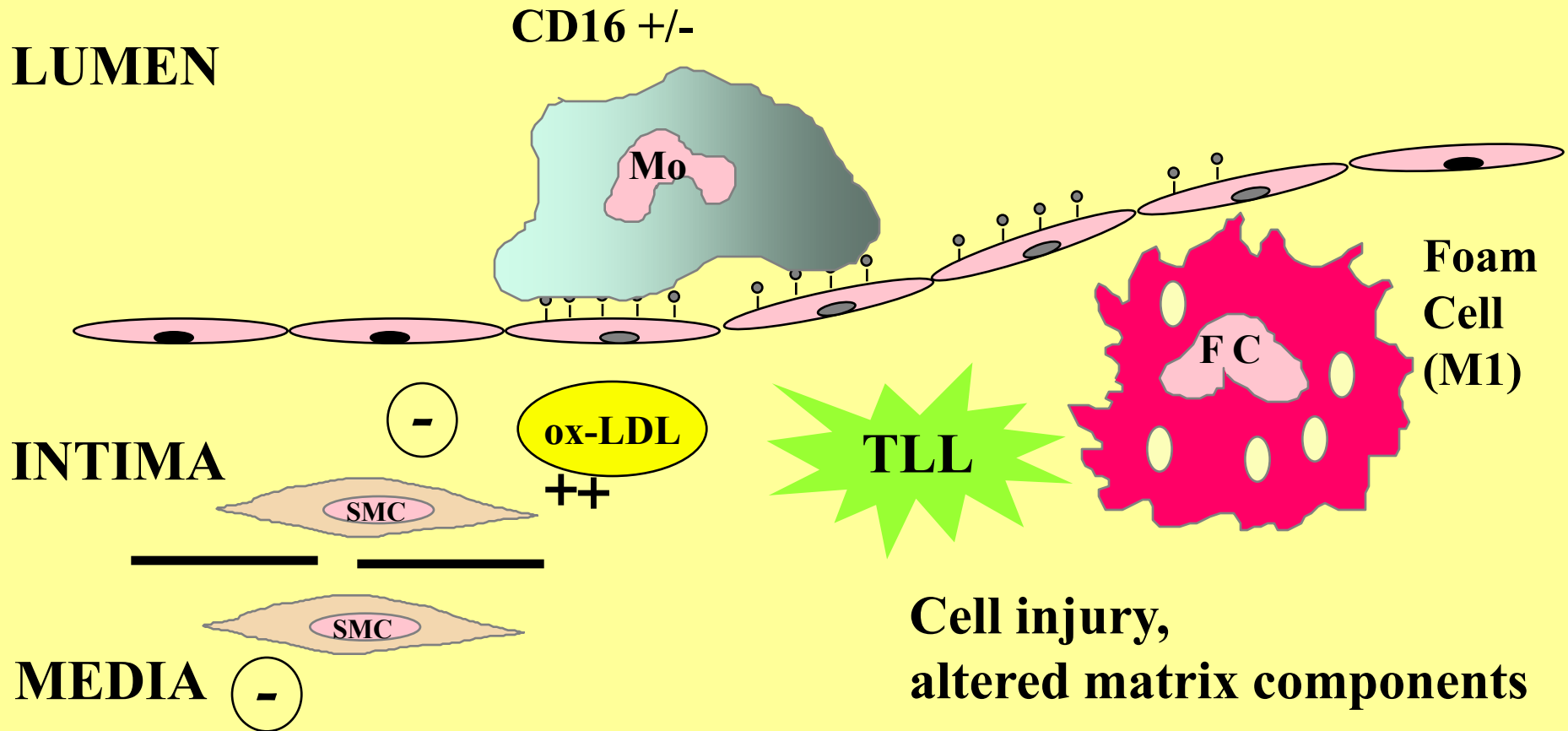
TLL, Toll-like ligands esp TLR2, Monaco C. Circulation. 2009;120(24):2462-2469.

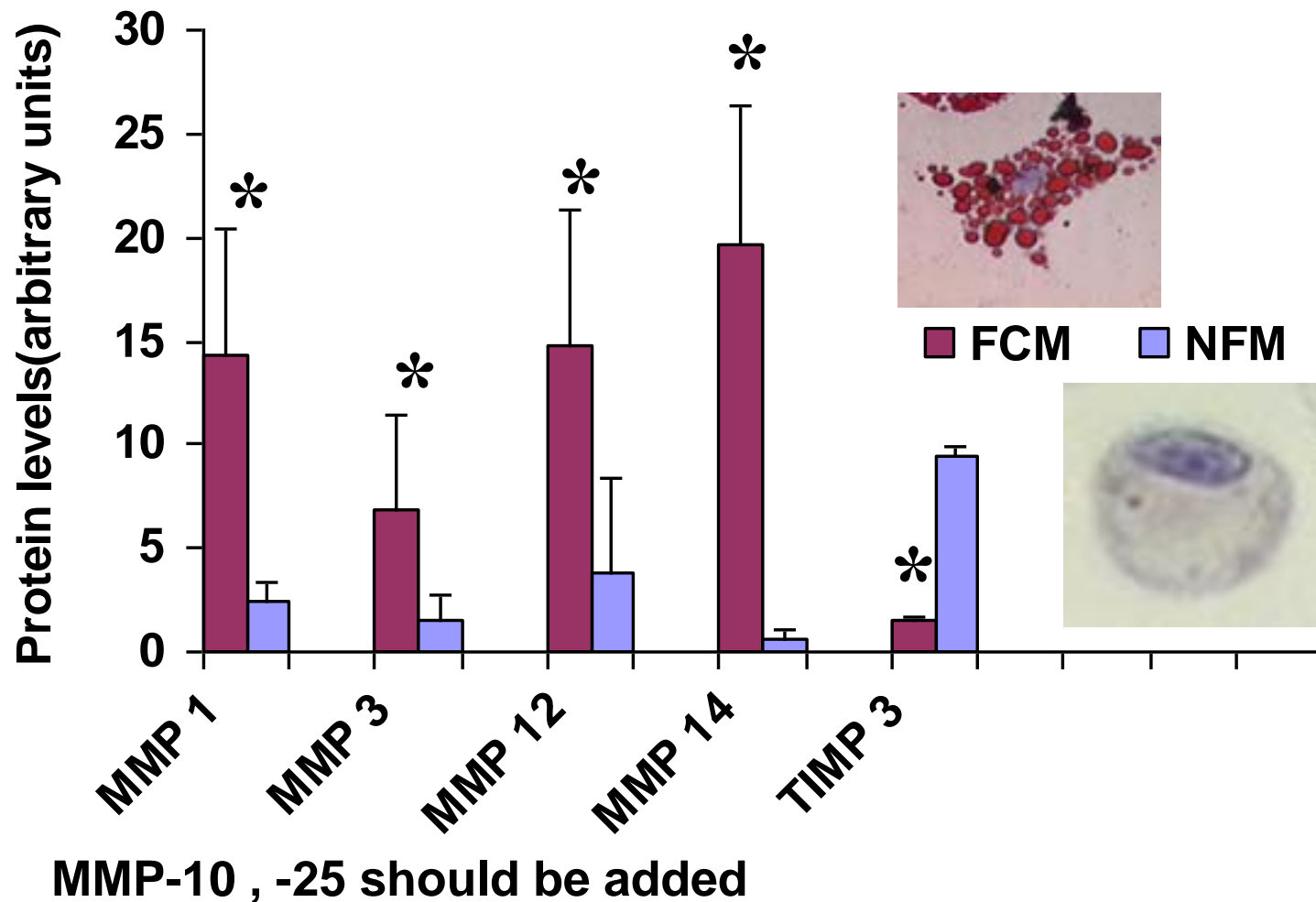# Foam cell and smooth muscle apoptosis and matrix remodelling ensues

LUMEN

CD16 +/-

Mo

T

INTIMA

ox-LDL

-

++

SMC

IL-1α

-

-

Foam Cell apoptosis

Lipid droplets
Apoptotic bodies
Cholesterol crystals

MEDIA

-

SMC

-

-

SMC apoptosis

-

-

Free cholesterol activates the inflammasome
Duewell, P et al. Nature, 2010;464:1357-61

BHI
Bristol Heart Institute

Foam cells make proteases that destroy collagen

Foam cells

Low collagen

van der Wal Cardiovasc Res 1999;41:334-344

Which proteases are important - rabbit foam cells

Chase et al. ATVB 2002;22:765-771

What about MMP-12, MMP-14 and TIMP-3 in man?

Atherosclerotic plaque removed from carotid artery to restore blood flow to brain
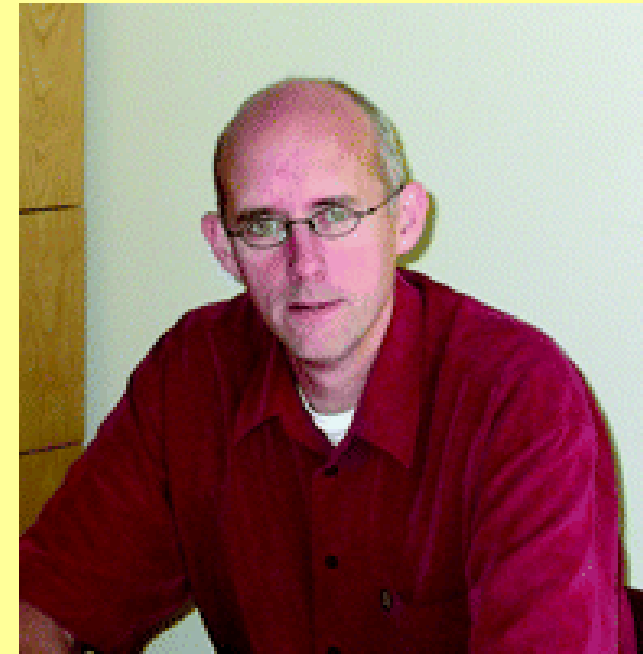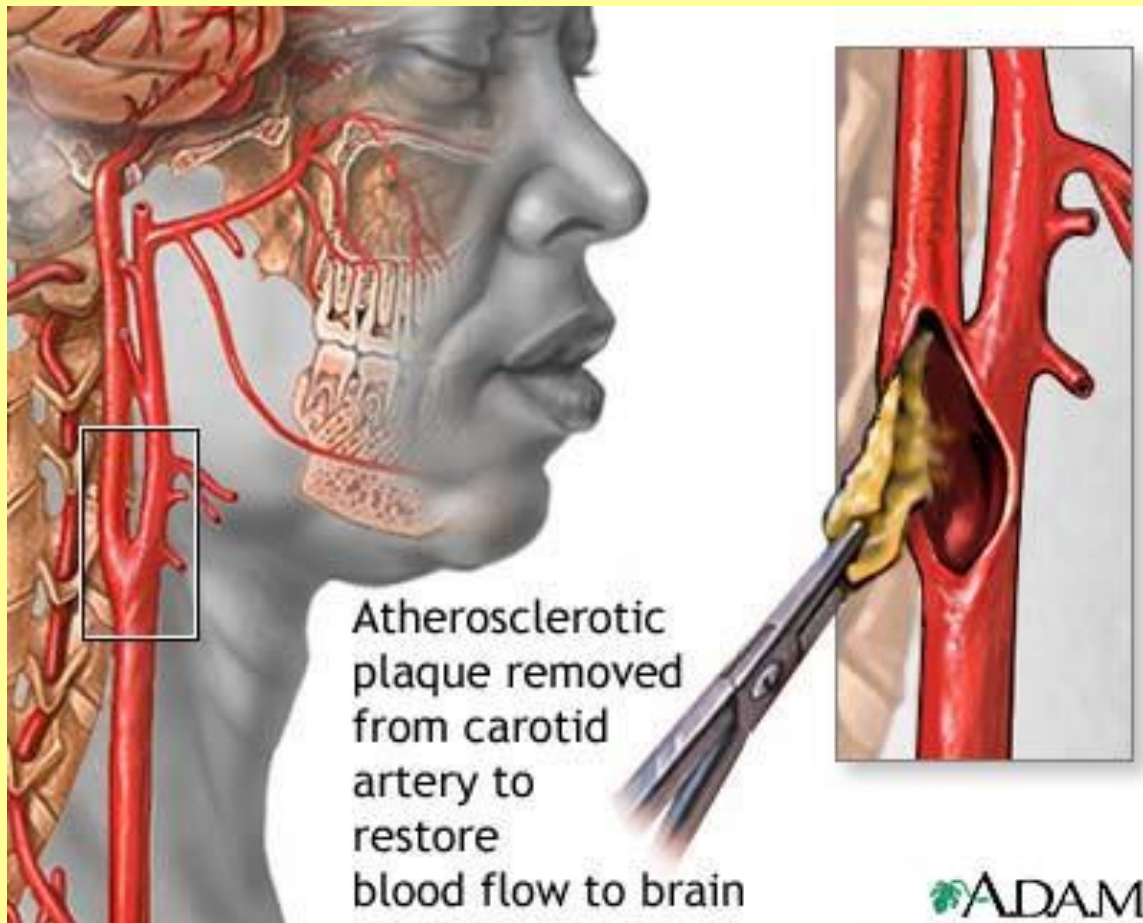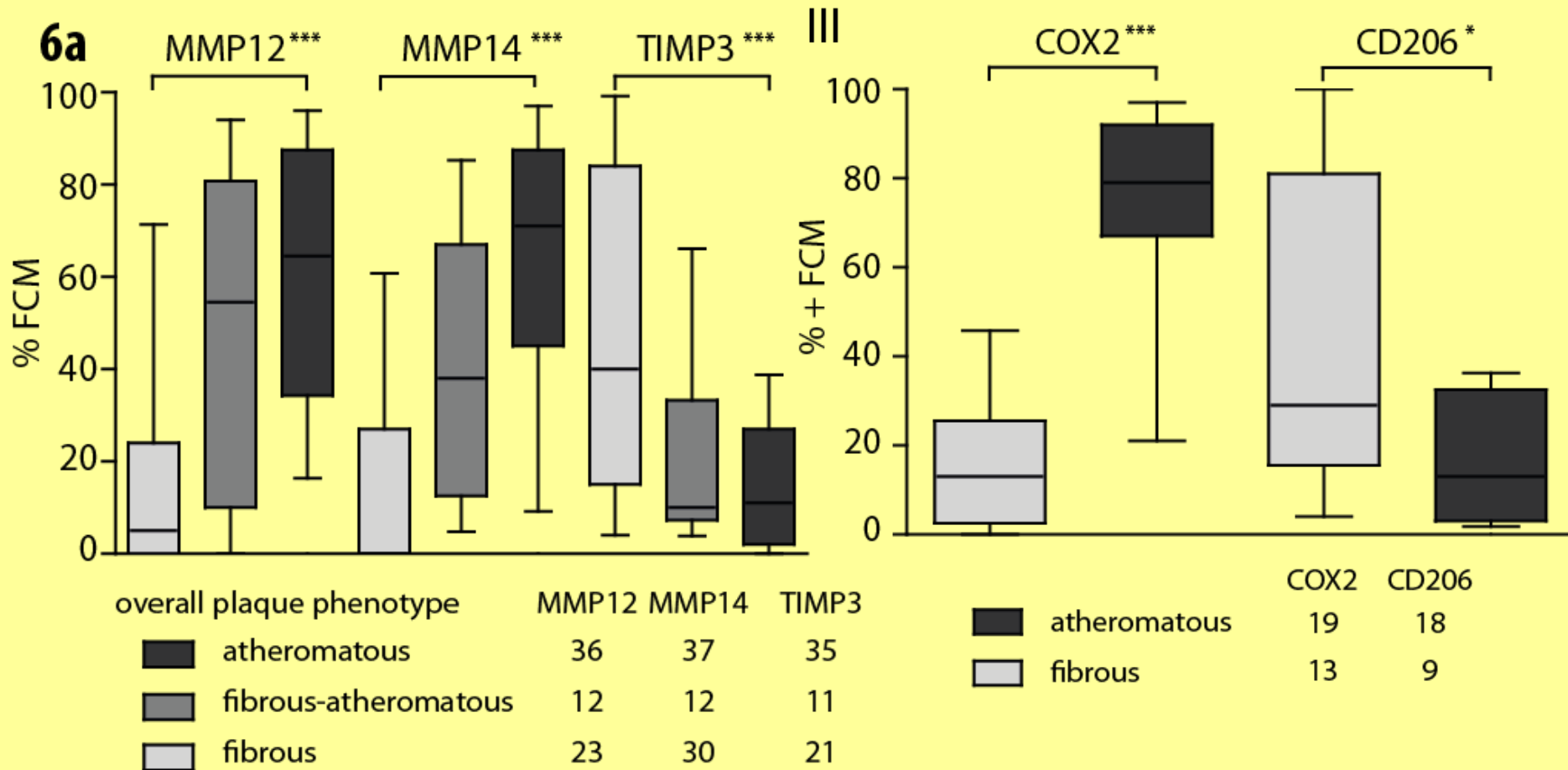
Gerard Pasterkamp
Vincent Scholtes

AtheroExpress Biobank (n>2000)

Staining and controls
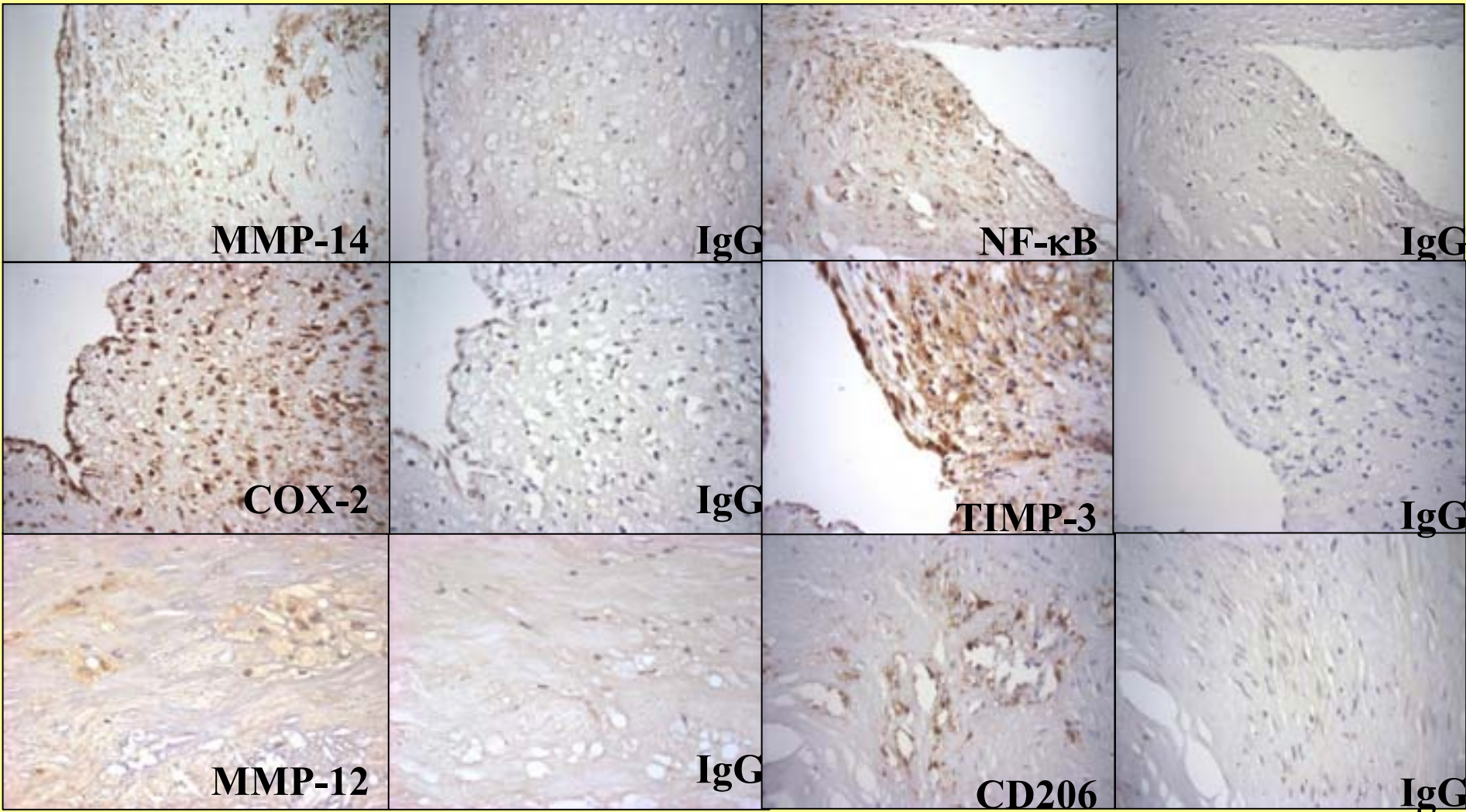
# Histological correlates of macrophage MMP-12, MMP-14 and TIMP-3 expression

6a

MMP12***   MMP14***   TIMP3***   III   COX2***   CD206*

% FCM

% + FCM

overall plaque phenotype	MMP12	MMP14	TIMP3
atheromatous	36	37	35
fibrous-atheromatous	12	12	11
fibrous	23	30	21

	COX2	CD206
atheromatous	19	18
fibrous	13	9

Correlation between parameters

	MMP-14		TIMP-3	
	P value	**rho**	**P value**	**rho**
Collagen semiquantitative	*0.000*	*-.409*	.019	.283
SMC semiquantitative	*0.000*	*-.623*	.000	.475
SMC quantitative	*0.000*	*-.466*	.021	.283
% fat / atheroma	0.000	.665	*0.001*	*-.402*
Macrophage semiquantitative	0.000	.567	*0.000*	*-.441*
Macrophage quantitative	0.026	.453	*0.009*	*-.316*
Thrombus	NS		*.018*	*-.286*

Stable { Collagen semiquantitative, SMC semiquantitative, SMC quantitative

Un-stable { % fat / atheroma, Macrophage semiquantitative, Macrophage quantitative, Thrombus

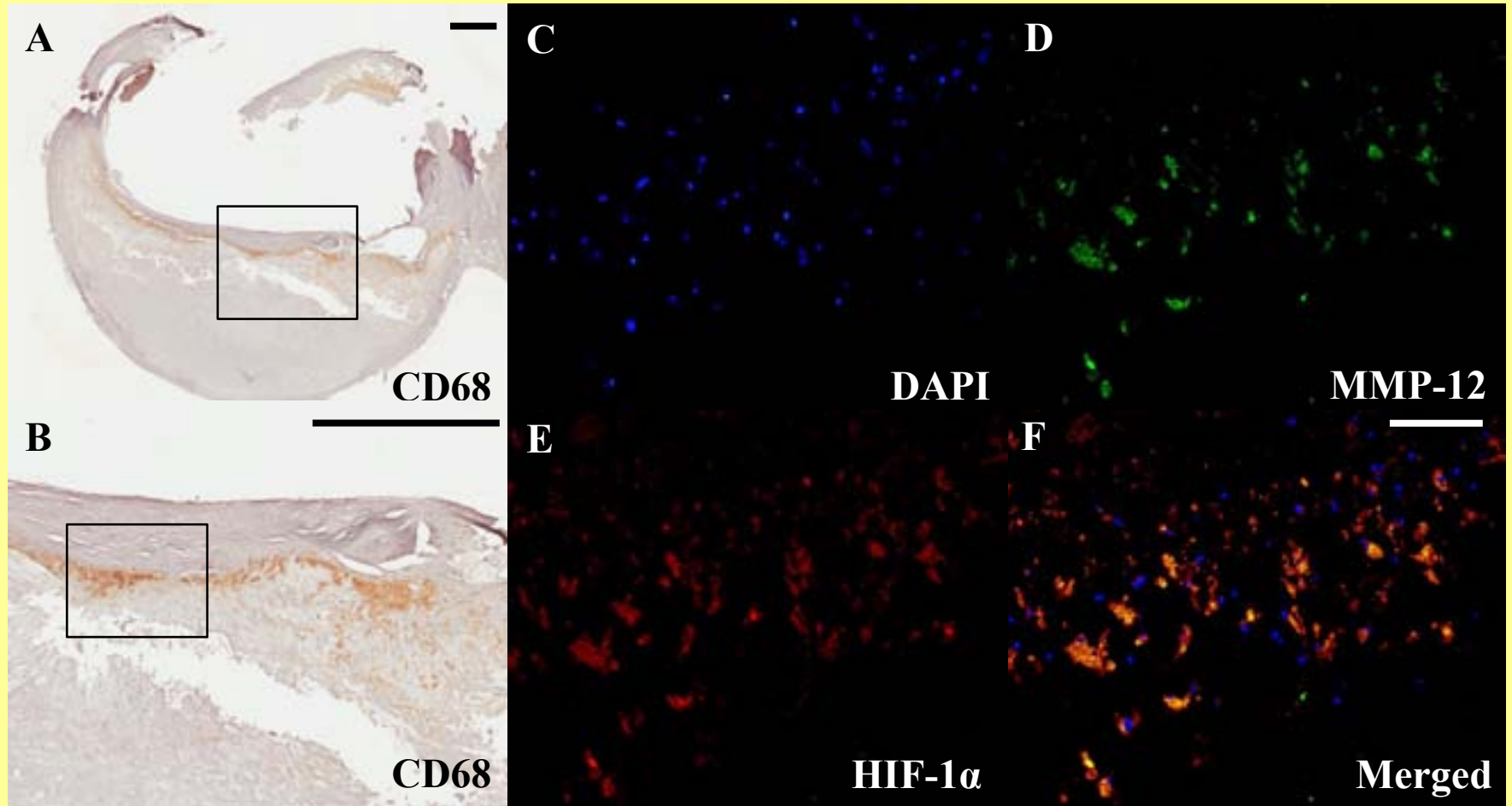**Collaboration with Vincent Scholtes and Gerard Pasterkamp**

MMP-14 and TIMP-3 don't co-localise
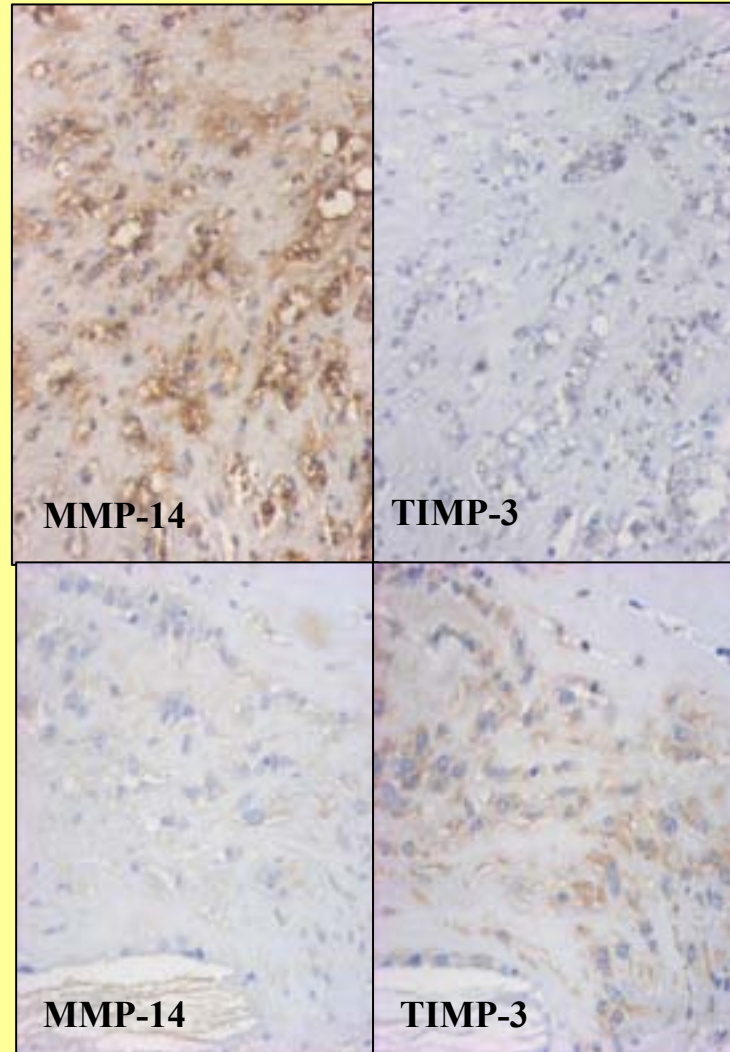
MMP-12 and MMP-14 don't co-localise
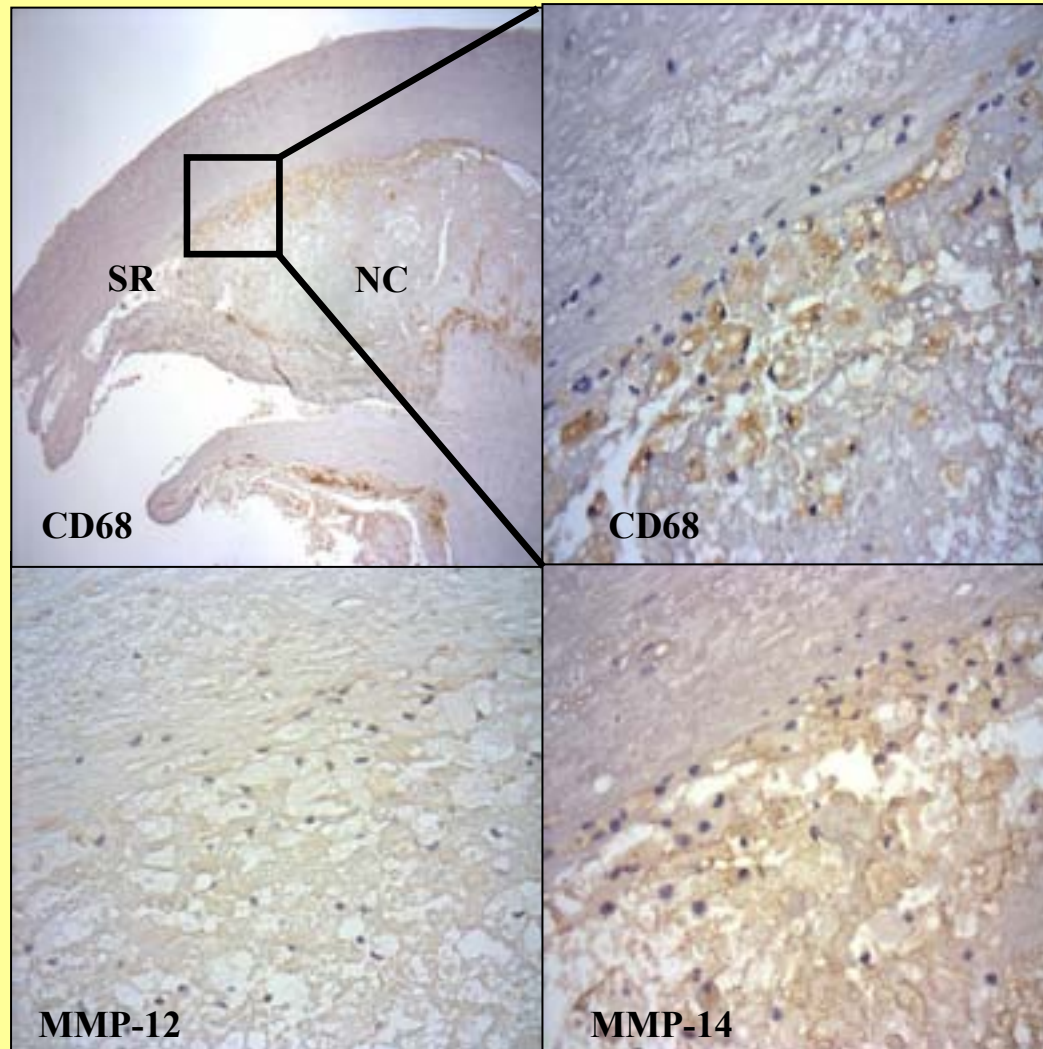
Hypoxia and MMP-12 expression *in vivo*

A — CD68

B — CD68

C — DAPI

D — MMP-12

E — HIF-1α
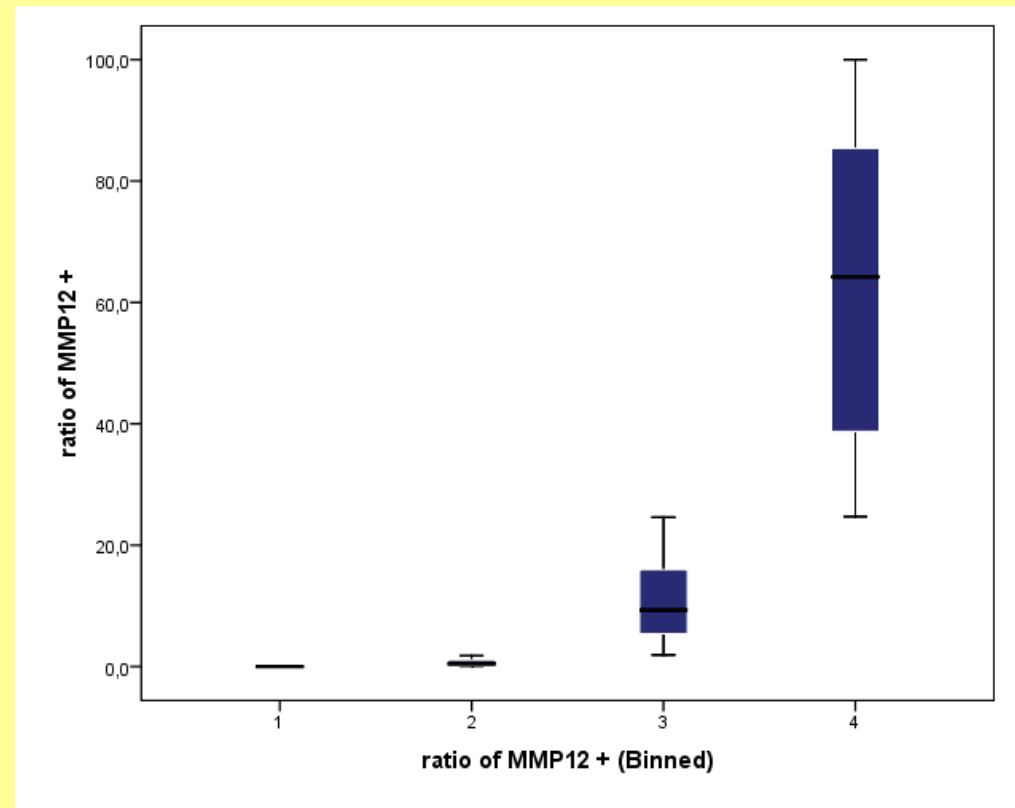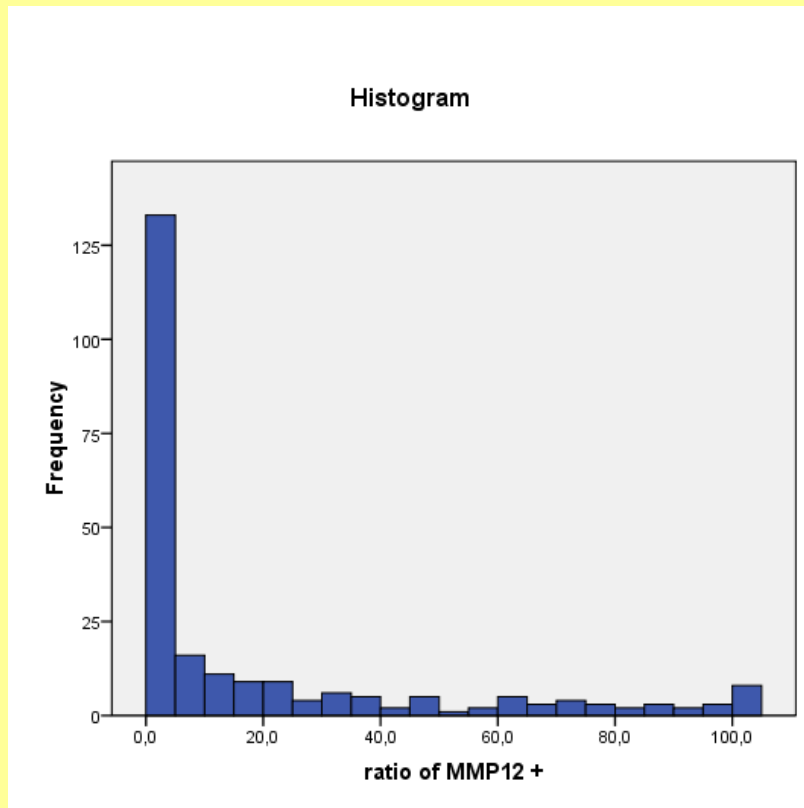
F — Merged

Outcome studies

- **Plaques from 236 patients following endarterectomy.**

- **3 year follow up.**

- **Primary outcome was any vascular event or vascular intervention.**

- **Sections stained for MMP-12, MMP-14 or TIMP-3 and CD68.**

- **Ratio positive for MMP-12, MMP-14 or TIMP-3 was quantified.**

Areas of interest show a wide variation or MMP-12 positivity

Scholtes, et al, JAHA, 2012

Macrophage MMP-12 expression associates with poor prognosis

Scholtes, et al, JAHA, 2012
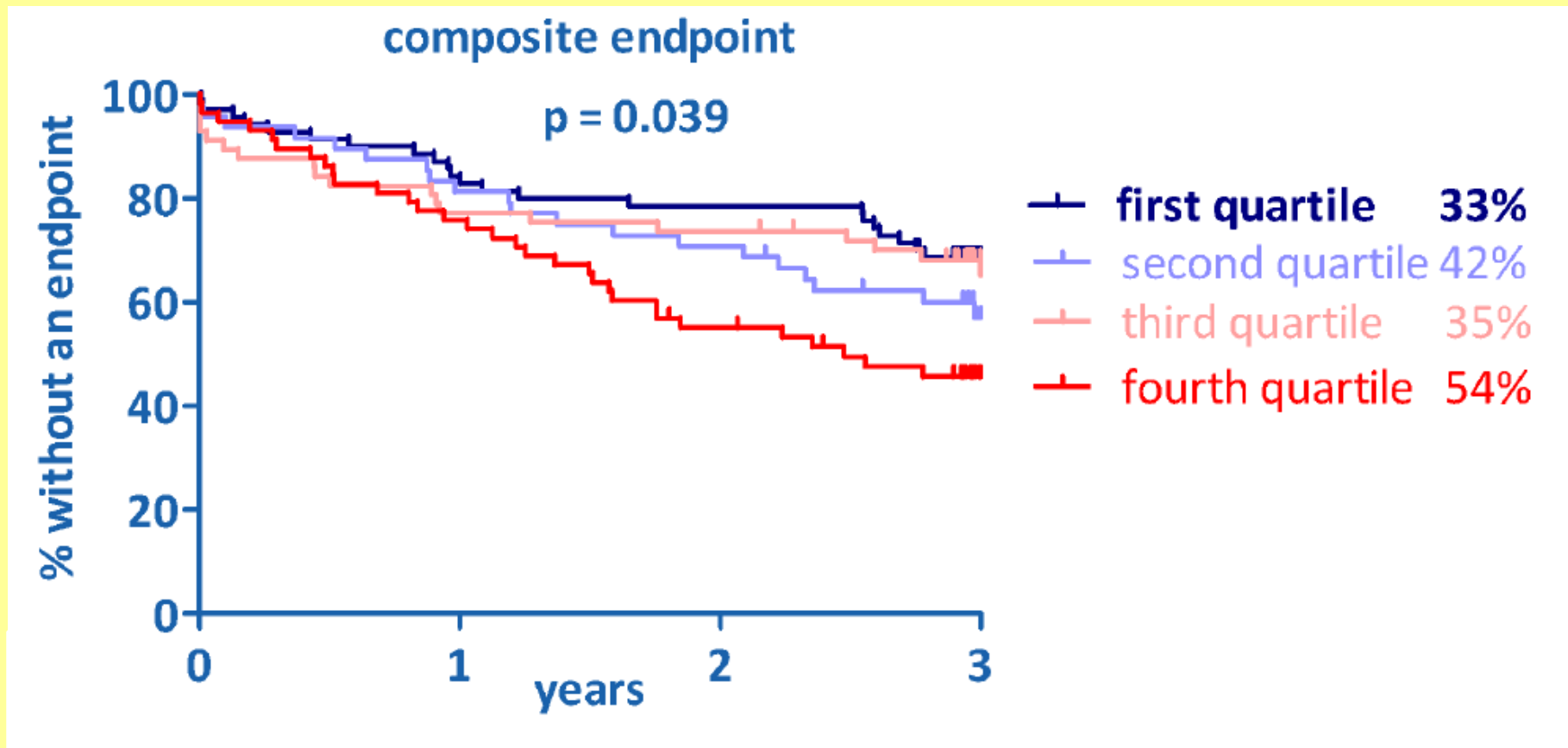
Macrophage MMP-12 expression associates with poor prognosis

MMP12 ratio	Major endpoint	Stroke endpoint
1e quartile	16%	7%
2e quartile	25%	15%
3e quartile	24%	14%
4e quartile	31%	20%
overall	23%	14%
	p = 0.021	p = 0.018

Too much MMP-12 can kill you!

Scholtes, et al, JAHA, 2012

Is there an animal model?

Reproducible

Quick

Easy

Cheap

Publishable

Near to human

Highly predictive

Sites of predilection are similar

Cheng Circulation.2006;113:2744 –2753
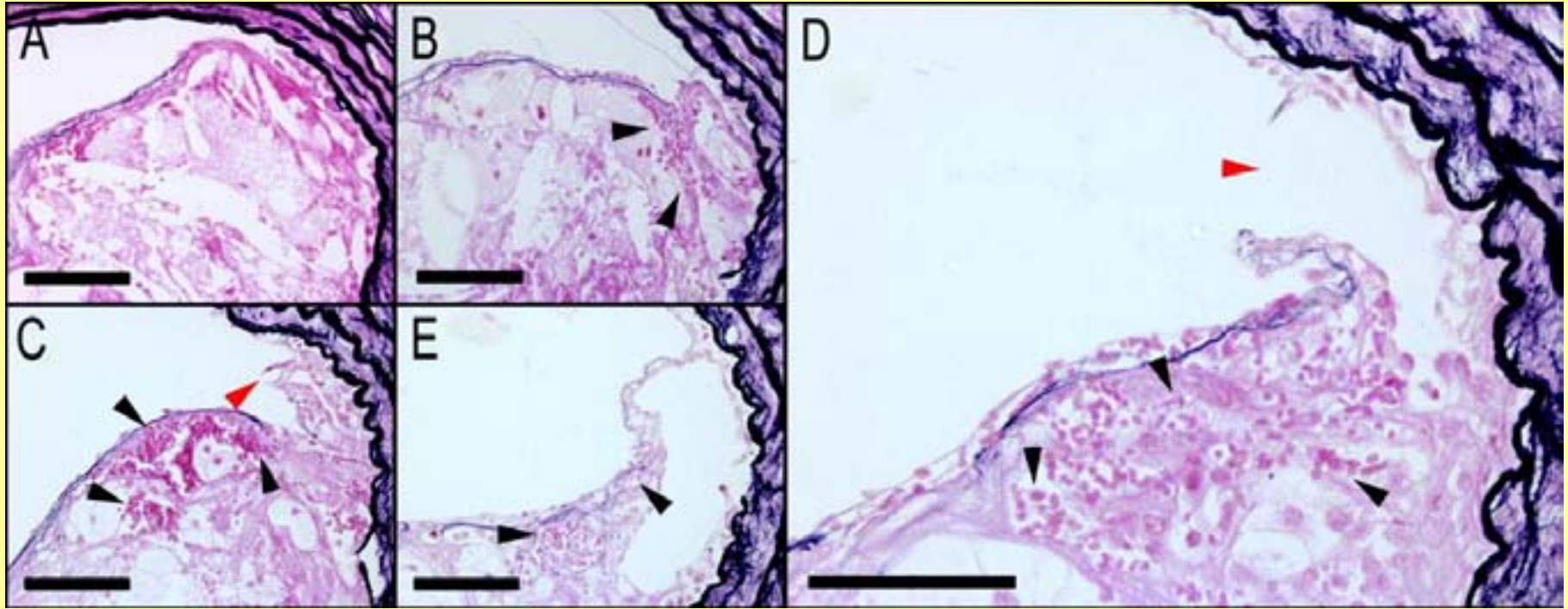
Size matters!

3.5 mm

0.5 mm

< 10 µ

< 65 µm

< 10 µm
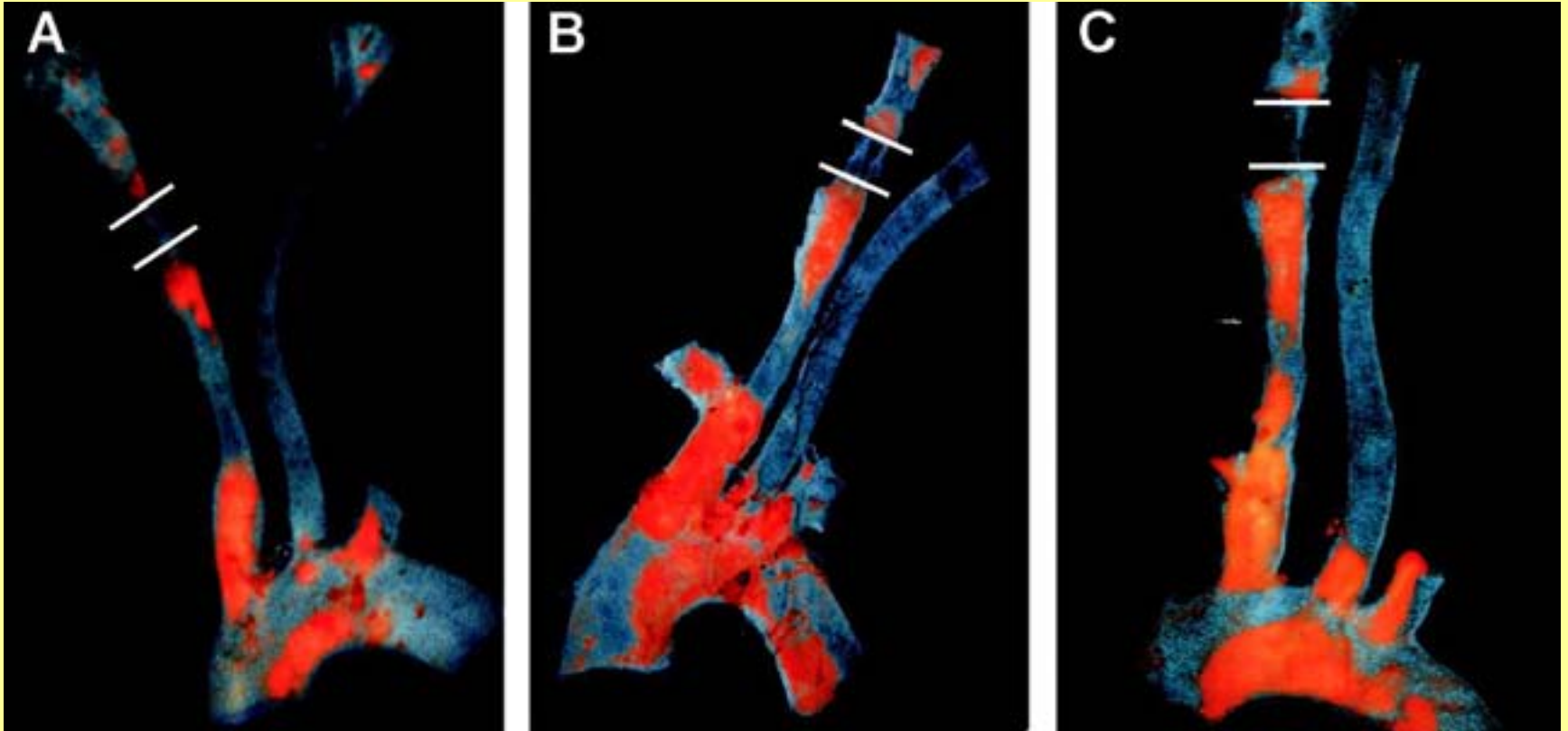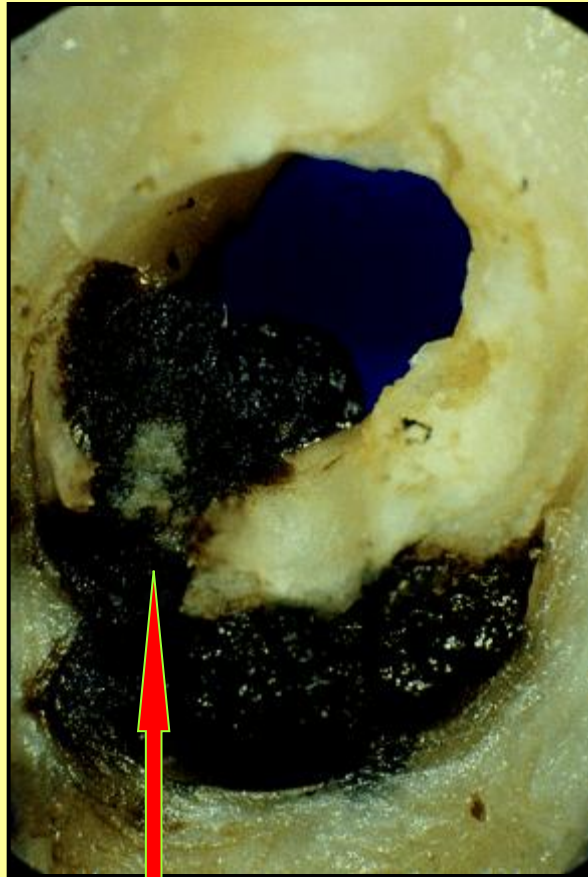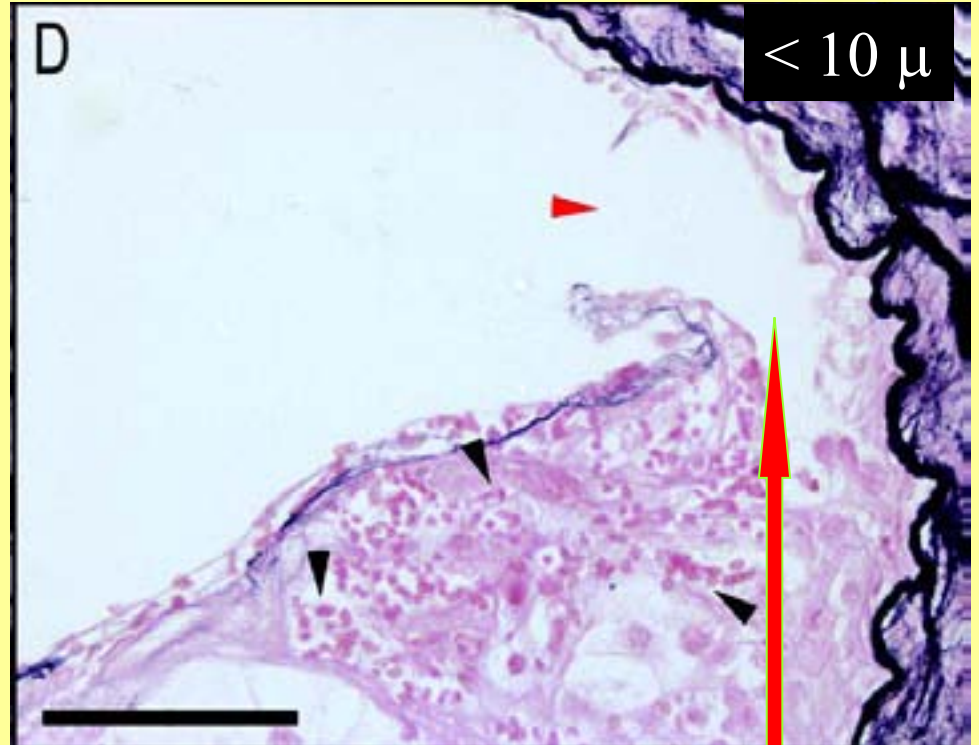
Thombus volume 1000 times less
**Jackson et al ATVB 2007; 27:714**

Plaque rupture or intraplaque haemorrhage?

'The most worrisome difference between the pathology in the mouse and the pathology of human disease is the absence of fibrin formation either within the lesion or within the lumen'. Rosenfeld ATVB 2000;20:2587

200 times smaller, less PAI-1= quicker thrombolysis
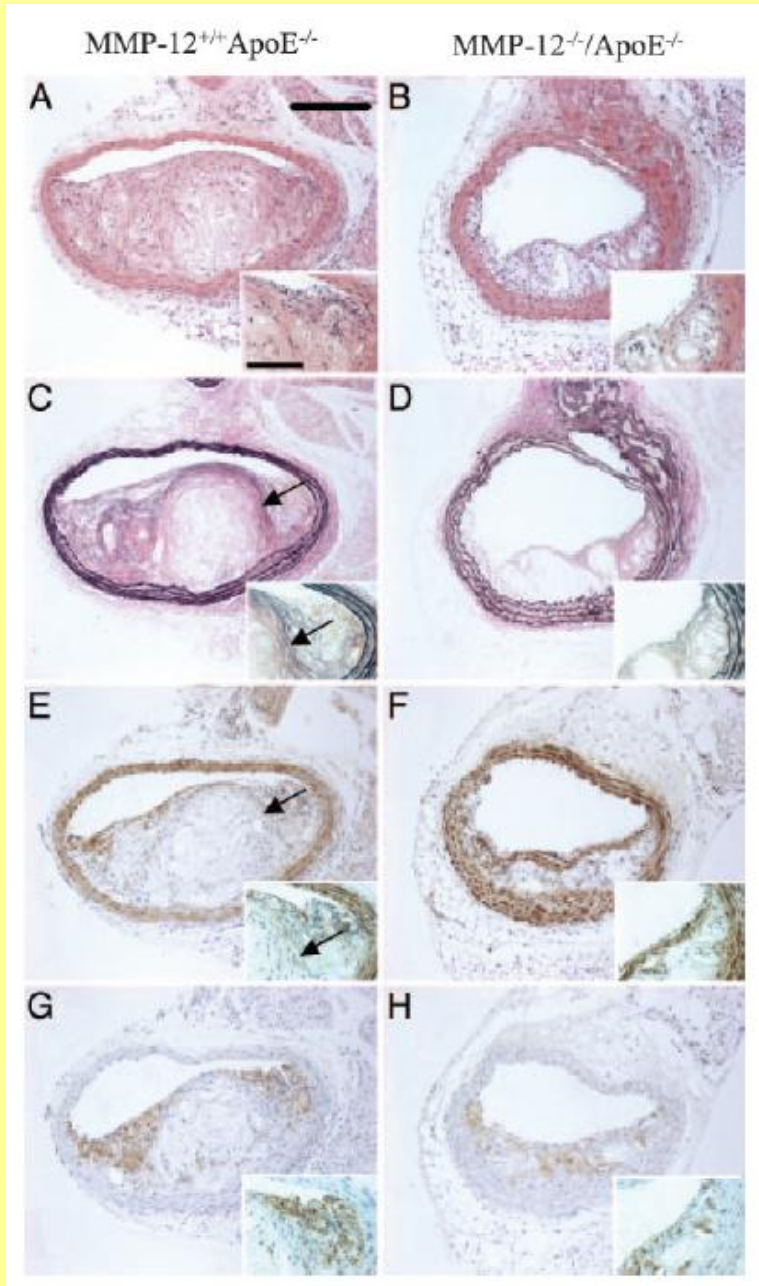Jackson et al ATVB 2007; 27:714

BCA, 8 week high-fat diet
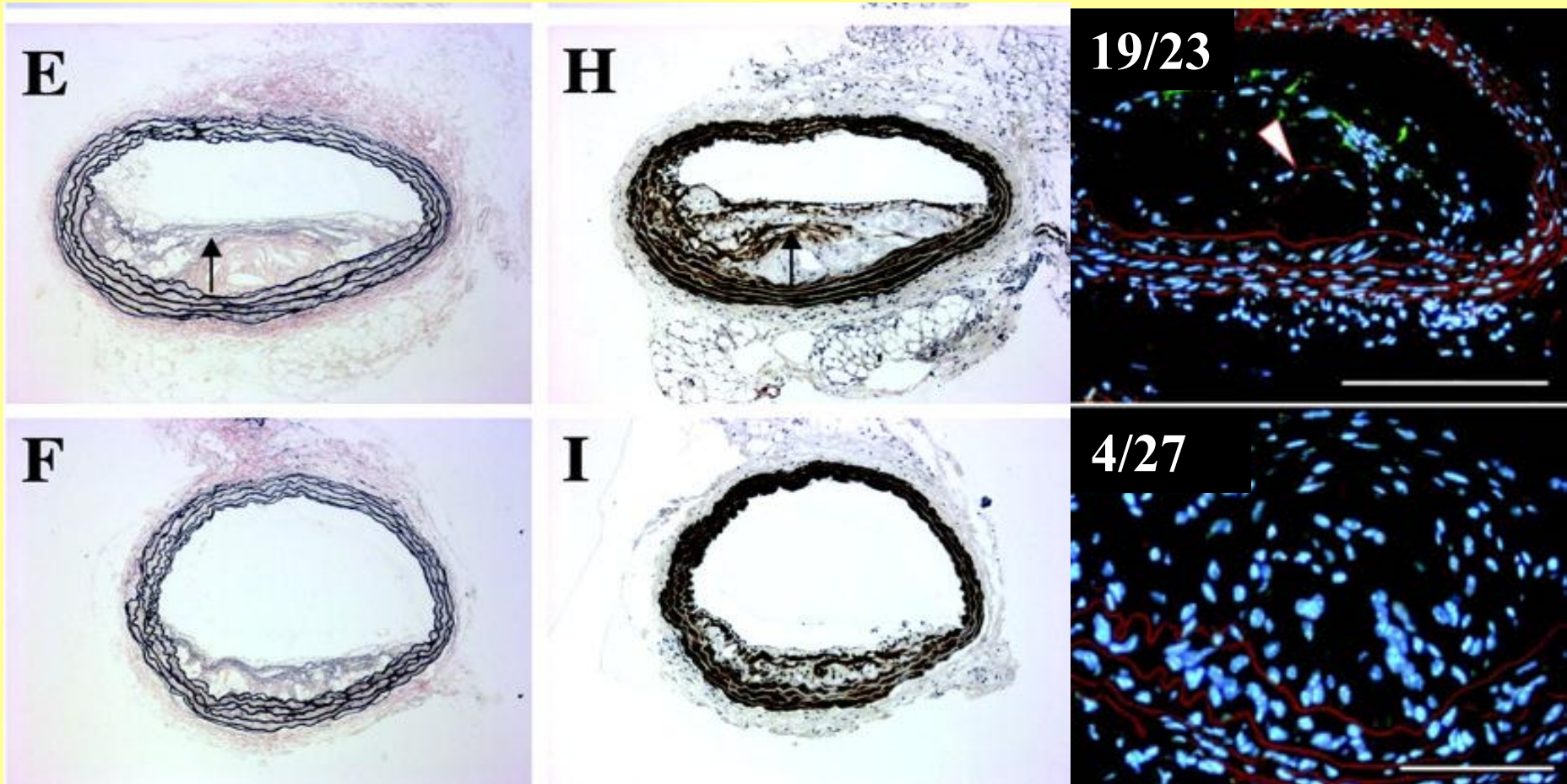
62% of all animals: 1.05 buried caps/plaque
Neither occur in aortic sinus

p<.000001

Johnson JL et al, Circulation 2005;111:1422.

MMP-12^{+/+}ApoE^{-/-}   MMP-12^{-/-}/ApoE^{-/-}

MMP-12 may reduce plaque stability by increased macrophage migration

	ApoE-/-: MMP-12+/+	ApoE-/-: MMP-12-/-
Plaque area (x10³ mm²)	116 ± 12	56 ± 7*
Buried fibrous layers	1.33 ± 0.21	0.55 ± 0.14*
SMC (%)	9 ± 1	23 ± 3*
Macrophage (%)	32 ± 4	15 ± 4*

Johnson et. al. PNAS 2005;102:15575-80

BHI
Bristol Heart Institute

Development of Selective Inhibitors and Substrate of Matrix Metalloproteinase-12*
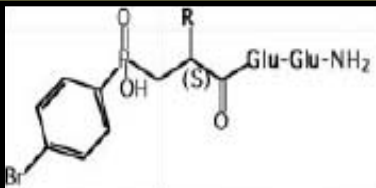
Laurent Devel[‡], Vassilis Rogakos[§], Arnaud David[‡], Anastasios Makaritis[§], Fabrice Beau[‡], Philippe Cuniasse[‡], Athanasios Yiotakis[§], and Vincent Dive[‡1]

RXP470.1

P$_1$'
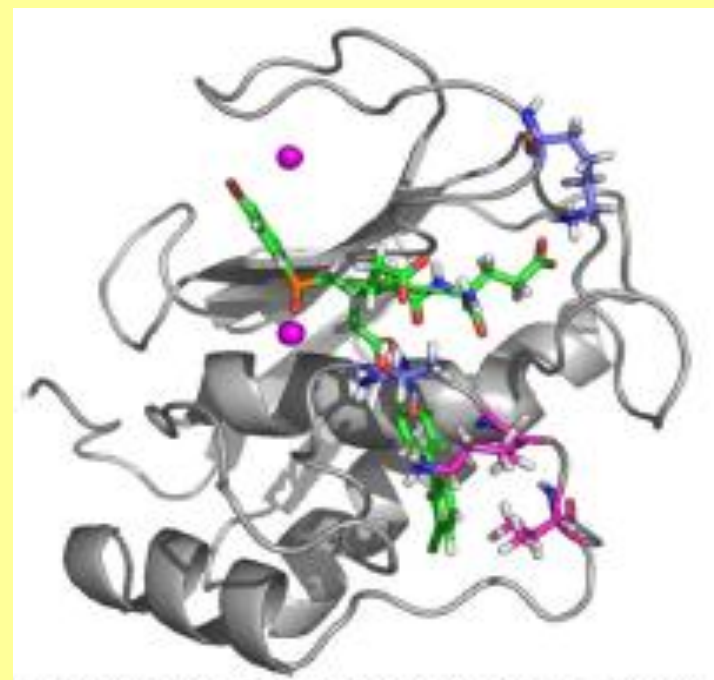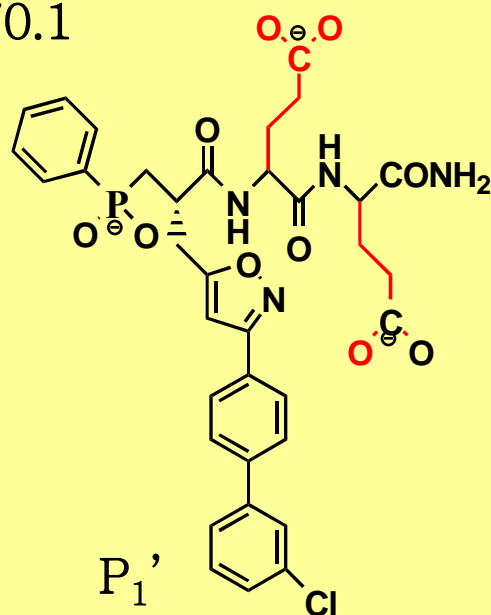
Jason Johnson
BHF, IRF

		MMP-1	MMP-2	MMP-3	MMP-7	MMP-8	MMP-9	MMP-11	MMP-12	MMP-13	MMP-14	ACE	NEP	TACE
Compound 1 R = 3	Ki (nM)	67000	192	40	626	271	1265	18400	0,19	49	140	>100000	>100000	>100000
	Selectivity/MMP-12	350000	1011	211	3295	1426	6658	96842	1	258	737	>500000	>500000	>500000

Effect on plaque progression

Johnson JL et. al. *Arterioscler Thromb Vasc Biol*. 2011;31:528-535
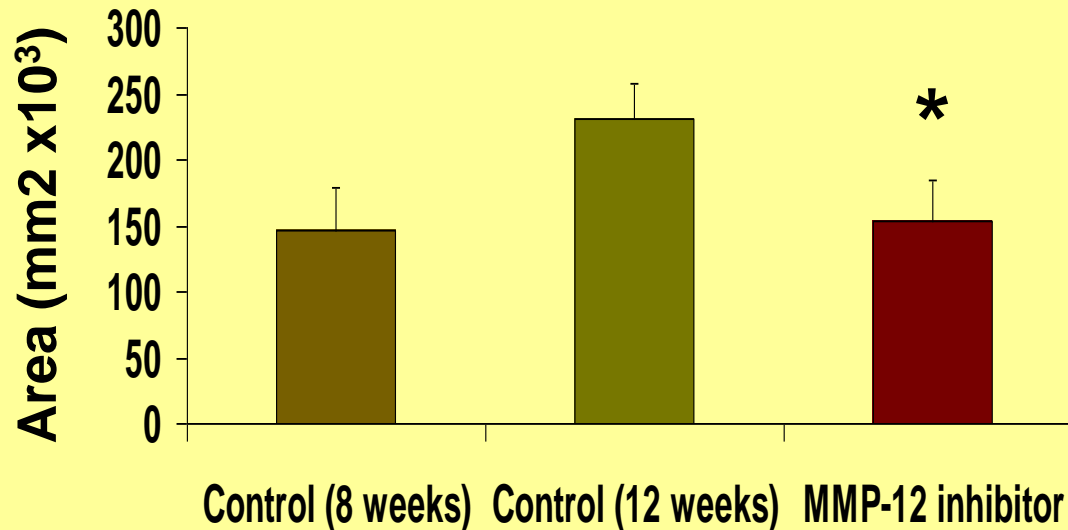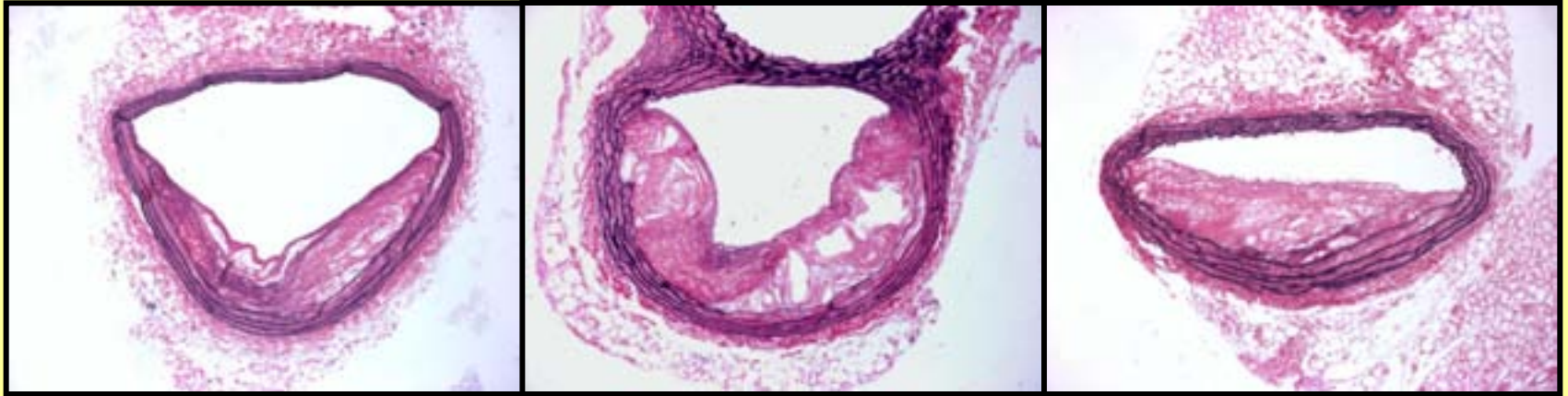
Vulnerable plaque – what can we do about it?

- **Reduce all known risk factors (life style changes, quit smoking, statin, BP lowering)**
- **Reduce thrombotic consequences (aspirin)**
- **Prophylactic angioplasty?**
- **Decrease plaque inflammation (NHR, cytokine/ chemokine inhibitors, immunotherapy)**
- **Prevent apoptosis (?)**
- **Prevent loss of collagen (protease inhibitor)**