



Athero-Express

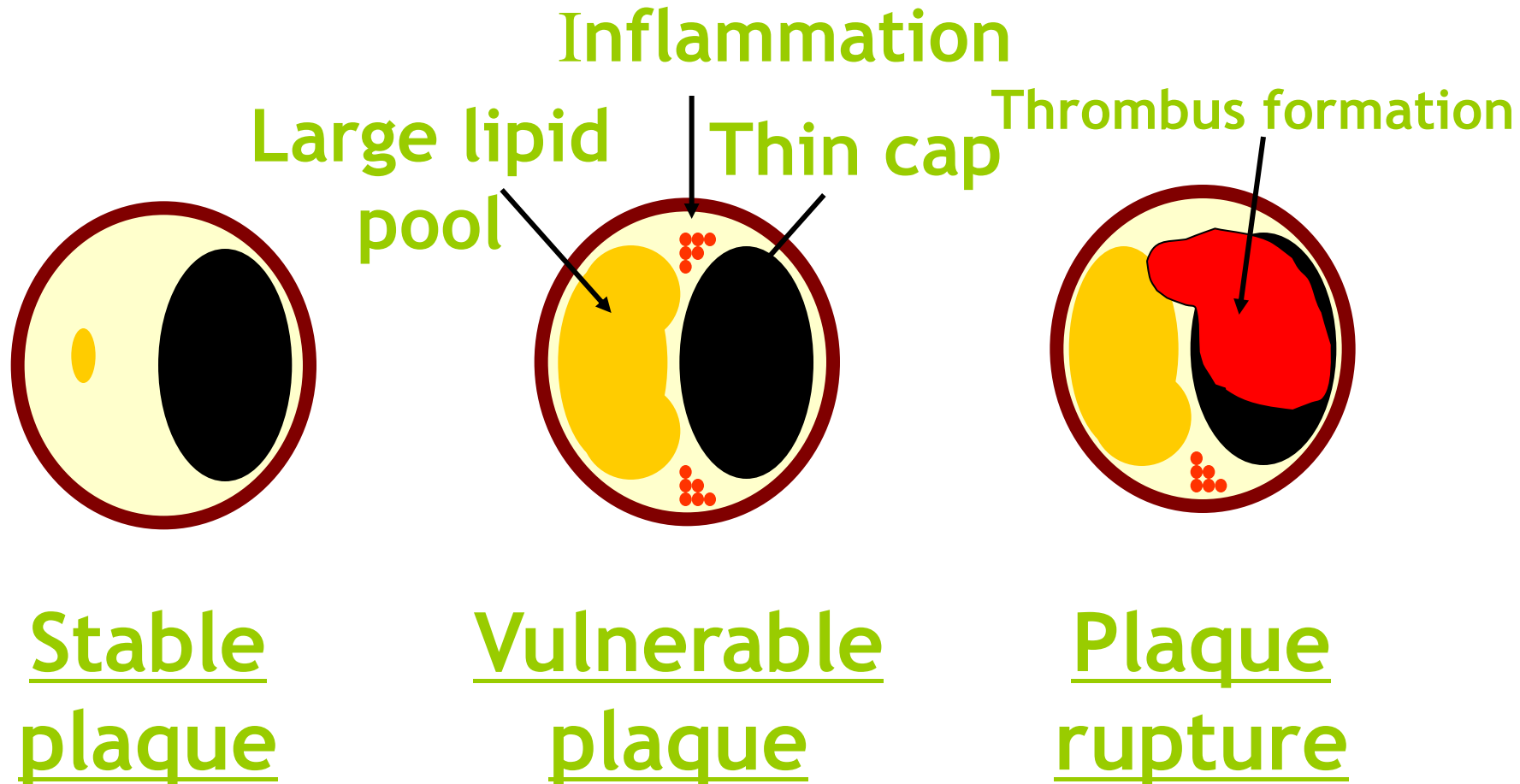
G.Pasterkamp UMCU

Nice 2013



University Medical Center
Utrecht

Atherosclerosis: current hypothesis

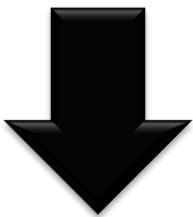


Atherosclerosis: current hypothesis

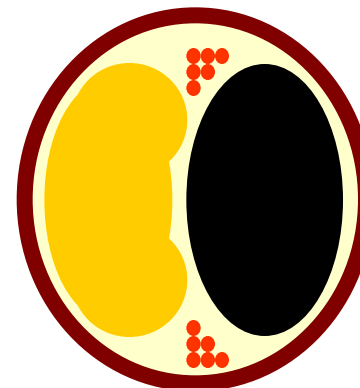
- Myocardial infarction



- Plaque rupture/erosion



- Histological features



- Collecting endarterectomy specimen (carotid and femoral) and blood (start 2002)
- Patient characteristics by questionnaire, clinical parameters
- Now >2800 patients included of which >1700 with CEA.
- **Follow up**: duplex and adverse cardiovascular events (hospital and phone)
- Objective: to discover local plaque characteristics predictive for systemic adverse events



Plaque

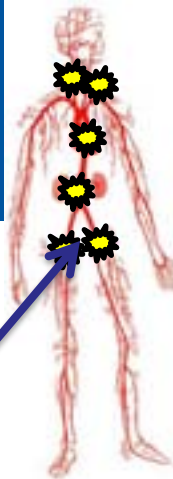


Baseline:
Carotid or Femoral
endarterectomy

3 year Follow
Up



No event



Event

- Coronary
- Peripheral
- CV death
- Cerebral

Baseline
plaque

- Histology
- Proteomics

Validation

Individual
risk
assessment

Fatty-Acid-Binding-Protein (FABP4)

- Fatty acid binding proteins (FABP) are involved in fatty acid metabolism and cellular lipid transport,
- Adipocyte FABP (aP2 (=FABP-4)) is also expressed in macrophages.
- Role FABP4:
 - *Reversibly bind saturated and unsaturated fatty-acids*
 - *Facilitate lipid transport to specific parts of the cell*
 - mitochondria for oxidation,
 - nucleus for lipid-mediated transcriptional regulation
 - outside the cell to signal in an autocrine or paracrine manner
 - *Role in insulin resistance*

Ref: 1 Perrella MA, FASEB, 2001

2 Makowski L, Nature, 2001

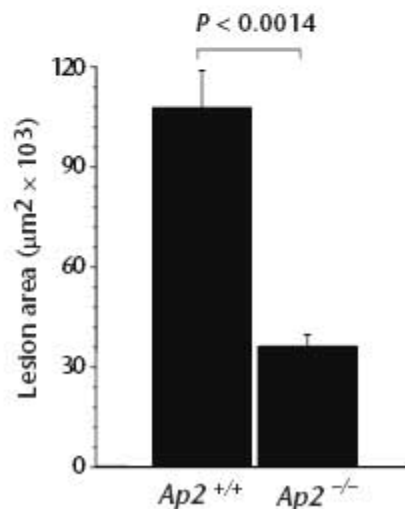
3 Layne MD, FASEB, 2001

Lack of macrophage fatty-acid-binding protein aP2 protects mice deficient in apolipoprotein E against atherosclerosis

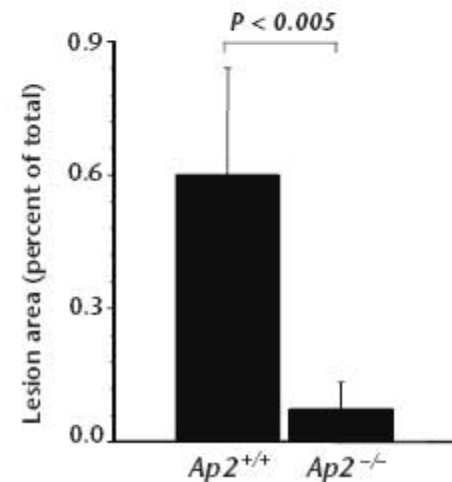
NATURE MEDICINE • VOLUME 7 • NUMBER 6 • JUNE 2001

LIZA MAKOWSKI¹, JEFFREY B. BOORD², KAZUHISA MAEDA¹, VLADIMIR R. BABAEV²,
K. TEOMAN UYSAL¹, MAUREEN A. MORGAN⁵, REX A. PARKER⁵, JILL SUTTLES⁶, SERGIO FAZIO^{2,3},
GÖKHAN S. HOTAMISLIGIL¹ & MACRAE F. LINTON^{2,4}

d



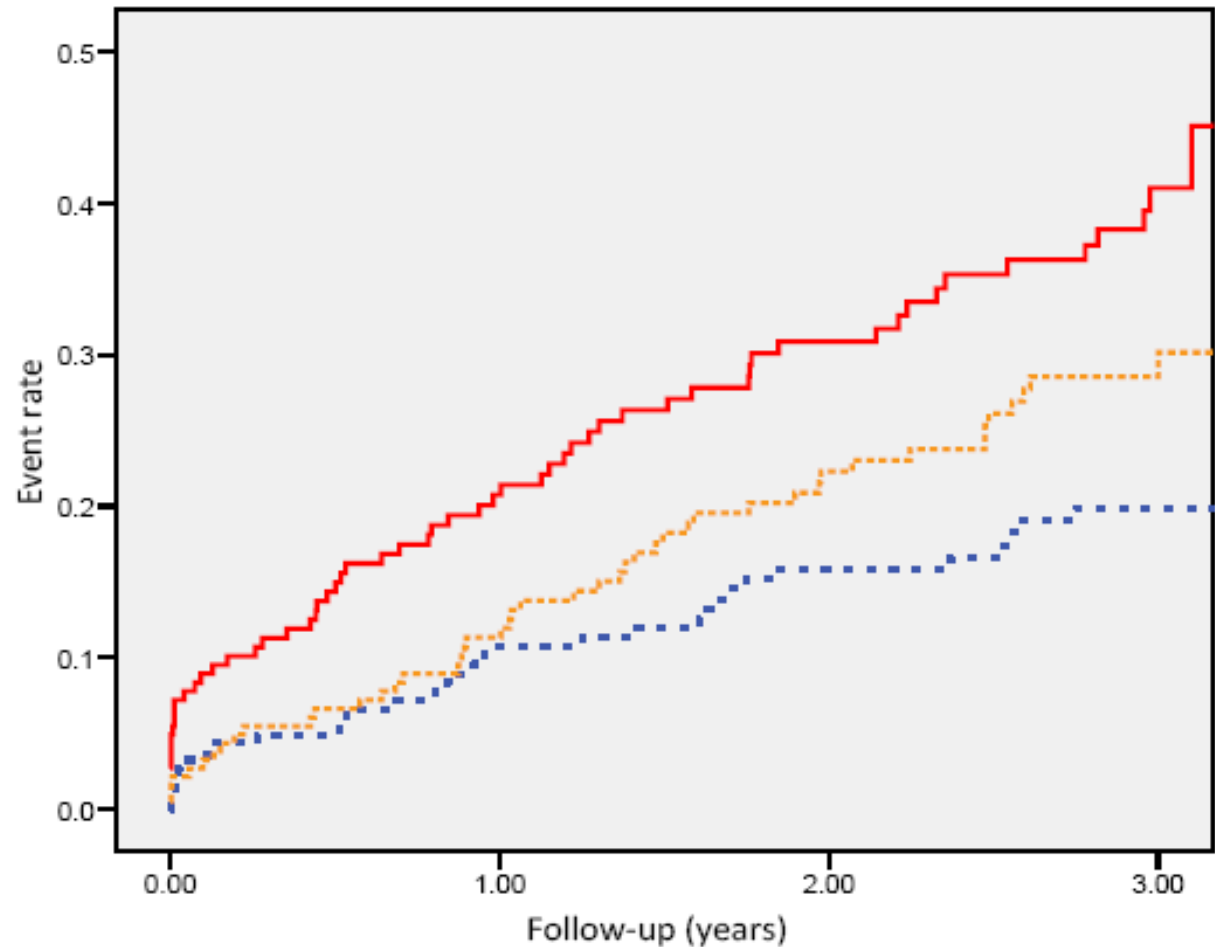
e



Survival analysis, Plaque FABP4 (Composite EP)

Peeters et al.
Eur Heart J 2010

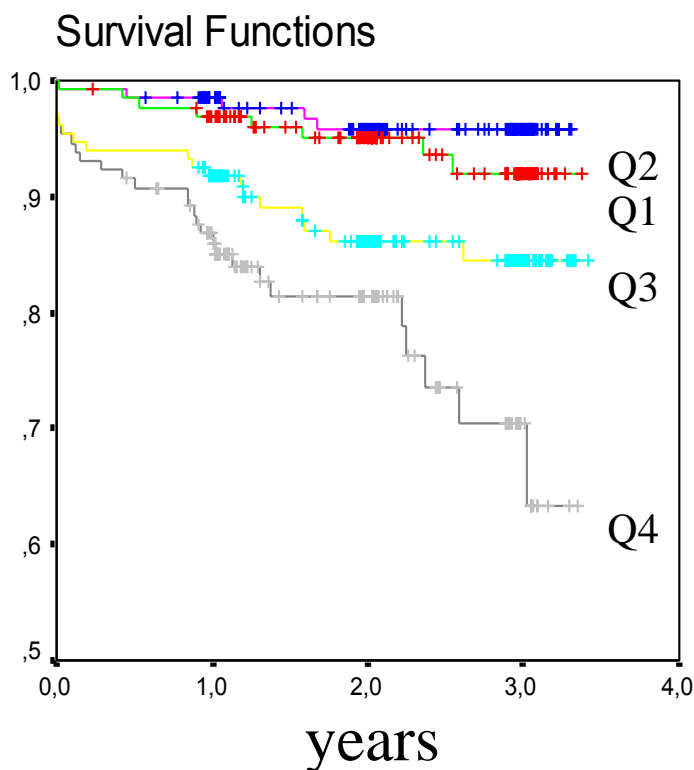
..... First tertile
..... Second tertile
—— Third tertile



1ST VS 3RD HR 1.99 – CI: 1.30 – 3.04

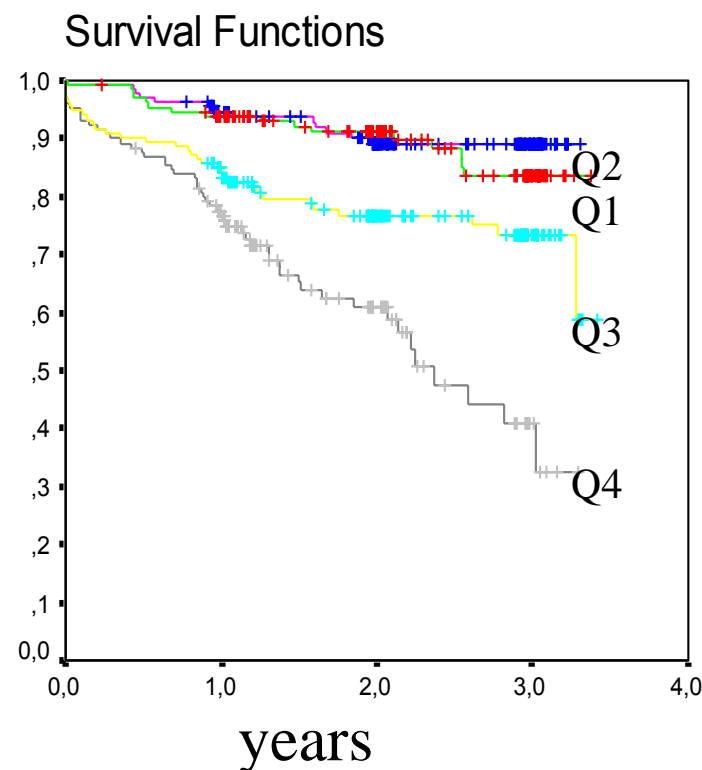
P=0.005

Osteopontin and endpoints



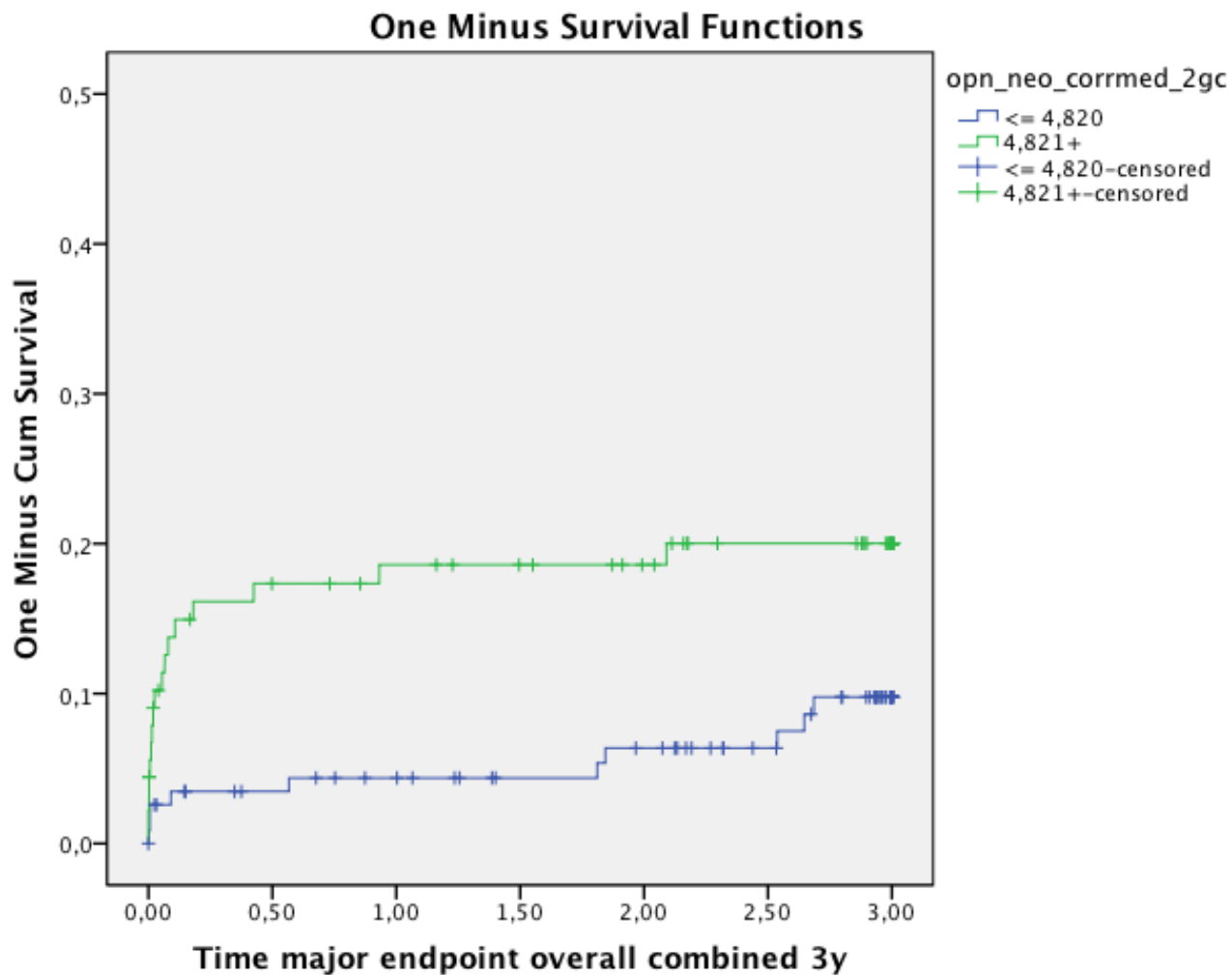
Combined endpoint:
clinical event

N=650



Combined endpoint: clinical
event + peripheral intervention

AAA, n=219



Is local IPH a marker for future risk?

Coronary artery from autopsy case: glycophorin A positive core.

Arbustini Heart 2002



University Medical Center
Utrecht

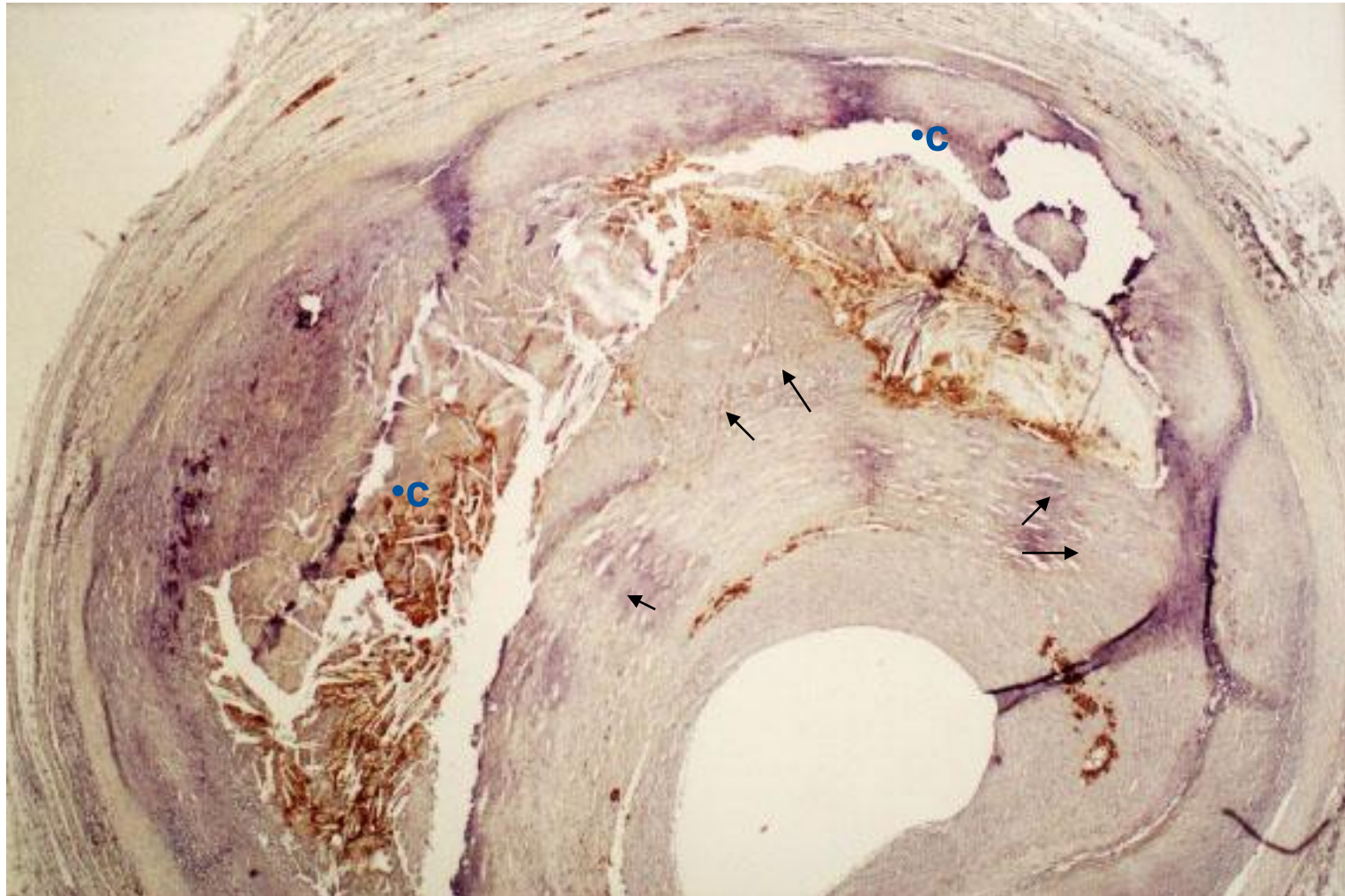


Table 1. Morphometric Analysis of 365 Plaques in Coronary Arteries from Patients Who Died Suddenly of Coronary Causes.*

Type of Plaque	No. of Plaques	Glycophorin A Score†	Iron Score†	Size of Necrotic Core <i>mm</i> ²	Extent of Macrophage Infiltration
Plaque with pathologic intimal thickening but with no necrotic core	129	0.09±0.04	0.07±0.05	—	0.002±0.001
Fibroatheroma					
Core in early stage of necrosis	79	0.23±0.07	0.17±0.08	0.06±0.02	0.018±0.004
Core in late stage of necrosis	105	0.94±0.11‡	0.41±0.09‡	0.84±0.08‡	0.059±0.007‡
Thin-cap fibroatheroma	52	1.60±0.20‡	1.24±0.24‡	1.95±0.30‡	0.142±0.016‡

* Plus-minus values are means ±SE.

† Scores can range from 0 to 4, with higher scores indicating greater proportions of the analyte.

‡ P<0.001 for the comparison with fibroatheromas whose cores were in an early stage of necrosis.

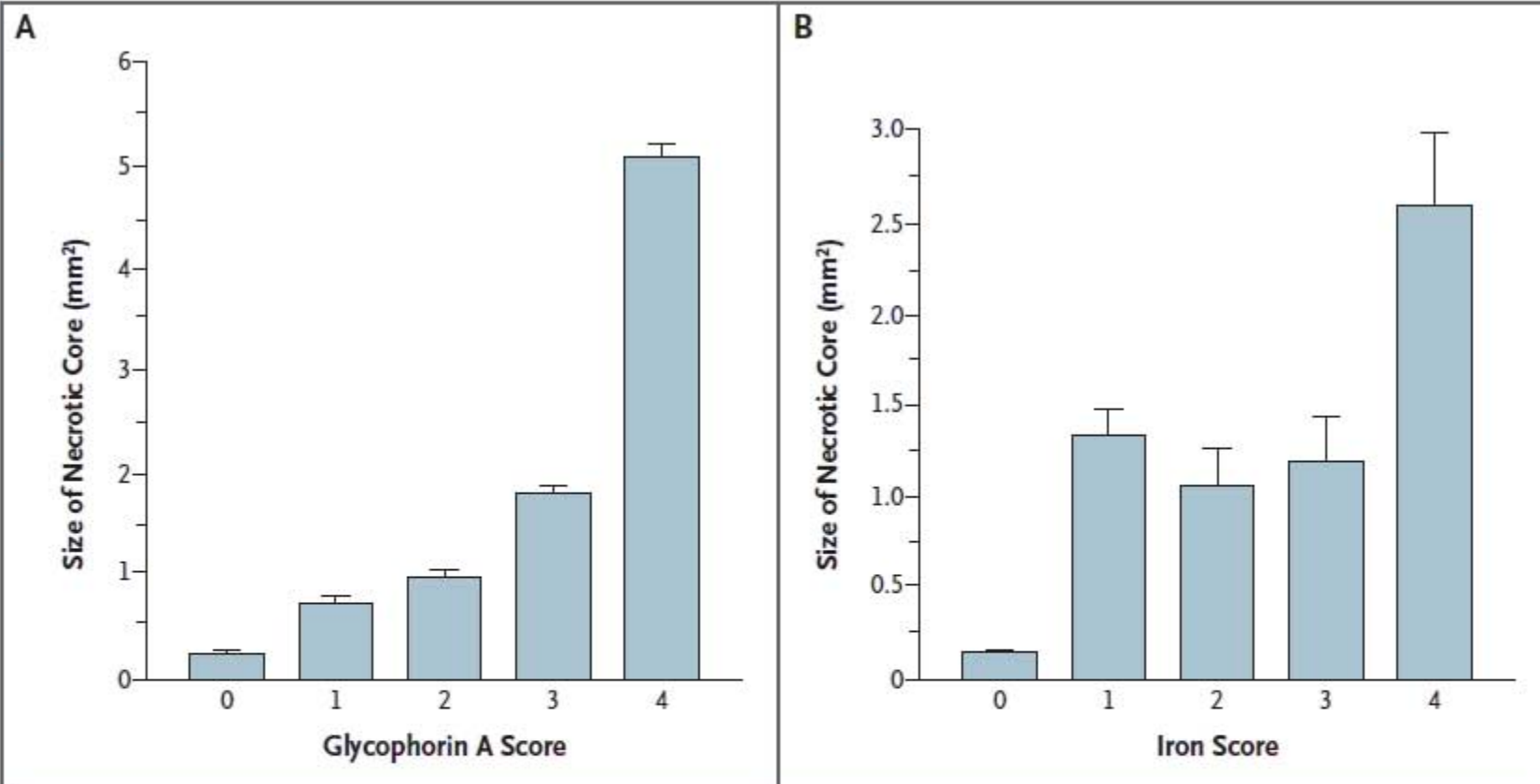
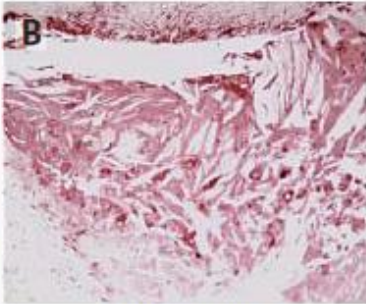
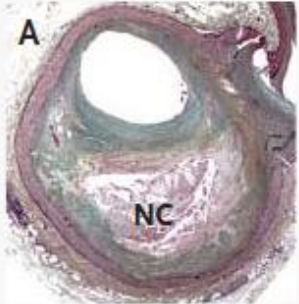


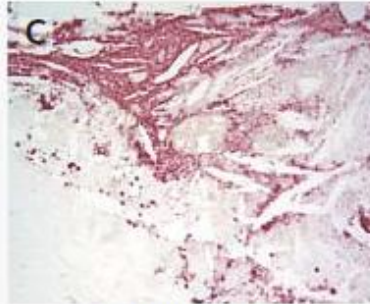
Figure 2. Relation of Glycophorin A Scores (Panel A) and Iron Scores (Panel B) to the Mean (\pm SE) Size of the Necrotic Core.

The amounts of glycophorin A and iron in plaque are predictive of the size of the necrotic core. Glycophorin A scores are as follows: 0 indicates no detectable staining, 1 indicates focal granular staining in less than 5 percent of the plaque, 2 indicates mild granular staining in 5 to 10 percent of the plaque, 3 indicates moderate granular staining in 11 to 25 percent of the plaque, and 4 indicates marked granular staining in more than 25 percent of the plaque. Only lesions with staining of erythrocyte fragments were included in the analysis. Iron staining was scored in a similar manner: 0 indicates no detectable staining, 1 indicates trace staining (1 to 2 macrophages), 2 indicates mild staining (3 to 5 macrophages), 3 indicates moderate staining (6 to 20 macrophages), and 4 indicates marked staining (more than 20 macrophages).

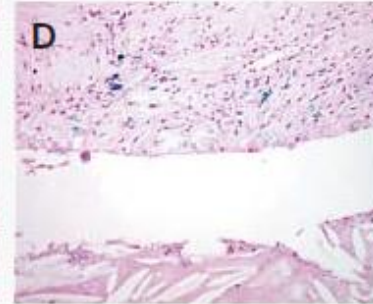
Fibroatheroma, Late-Stage Necrosis



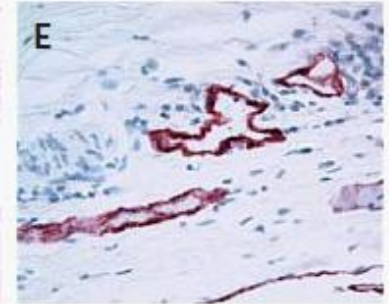
Macrophages



Glycophorin A

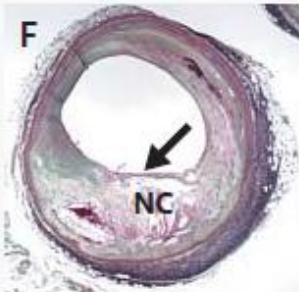


Iron

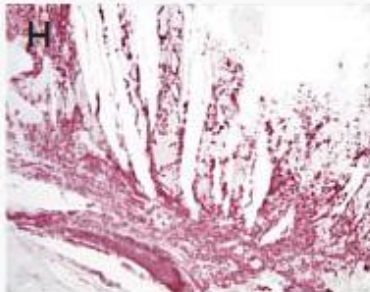


vWF

Thin-Cap Fibroatheroma



Macrophages



Glycophorin A



Iron



vWF

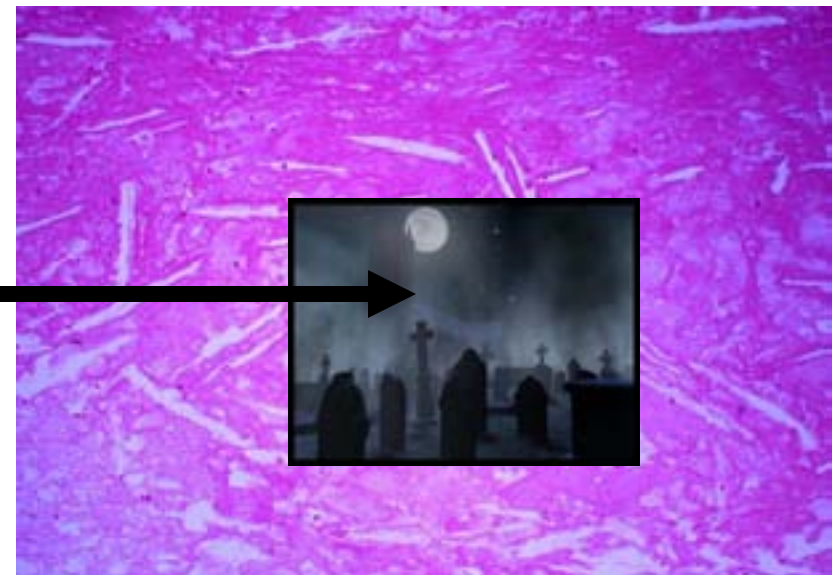
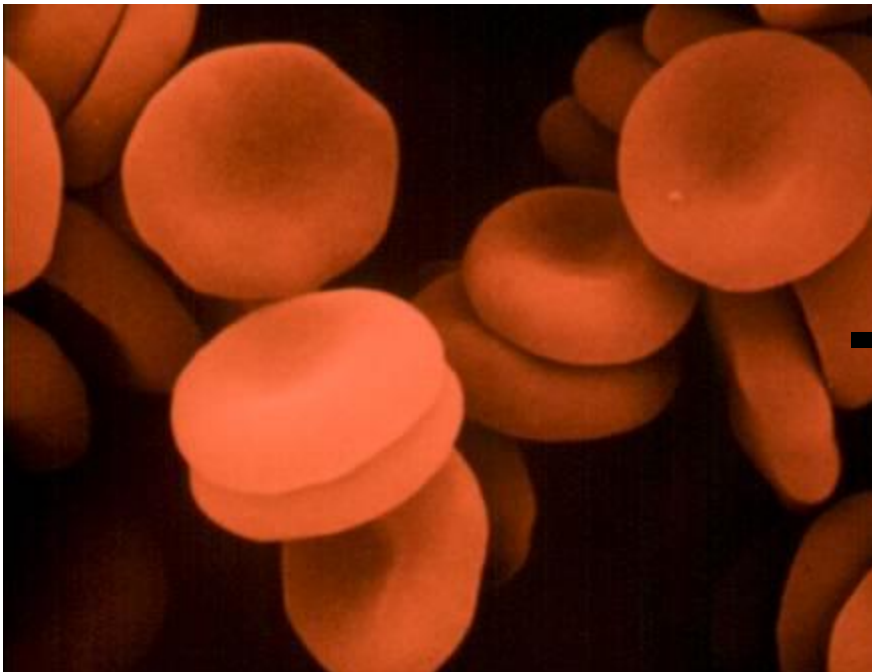


CONCLUSIONS

By contributing to the deposition of free cholesterol, macrophage infiltration, and enlargement of the necrotic core, the accumulation of erythrocyte membranes within an atherosclerotic plaque may represent a potent atherogenic stimulus. These factors may increase the risk of plaque destabilization.

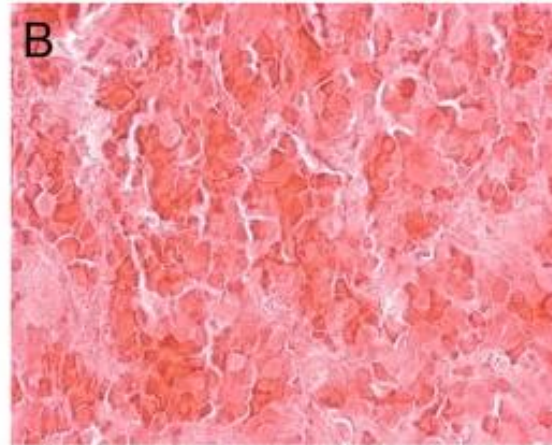
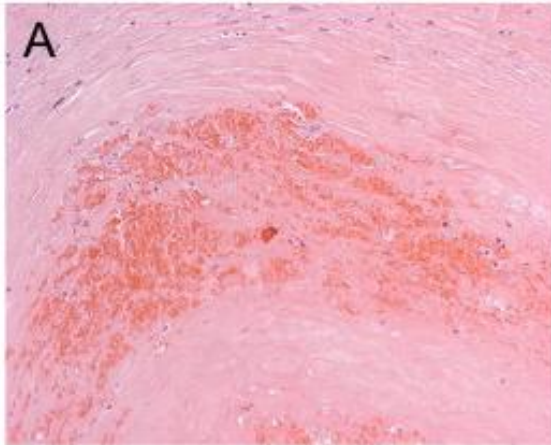
The necrotic lipid core: graveyard of the red blood cell?

- The red cell membrane is 1.5–2.0 times richer in cholesterol than any other cell.
- About 40% of the weight of the erythrocyte is composed of lipid

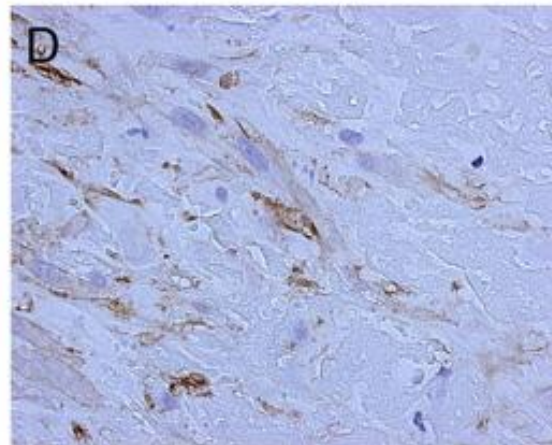
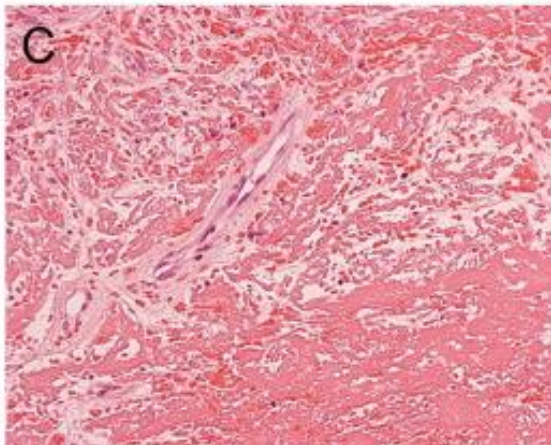


Atherosclerosis 2011 Derksen et al. Carotid plaques

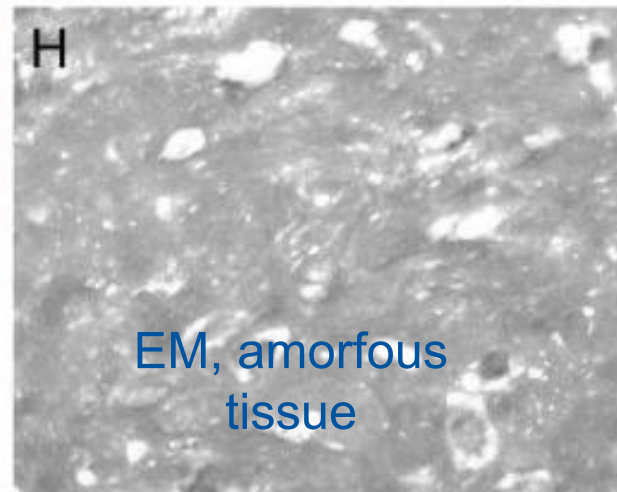
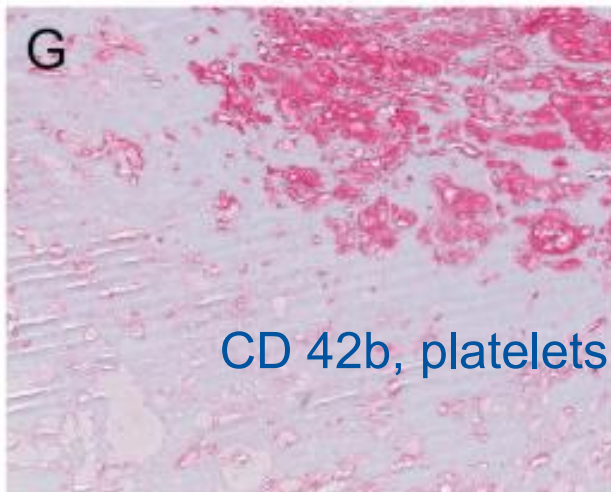
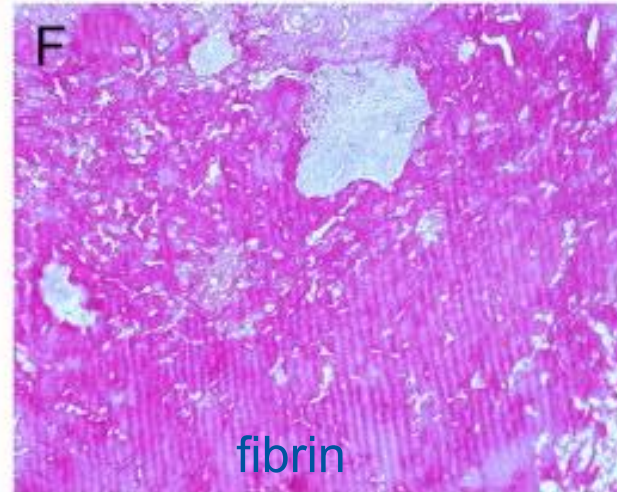
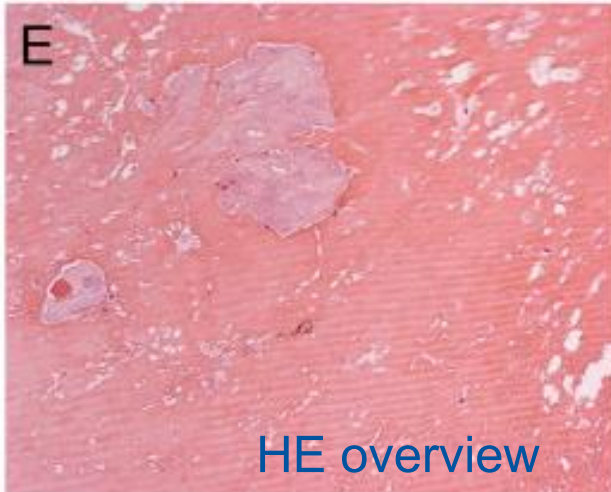
Recent



Organized

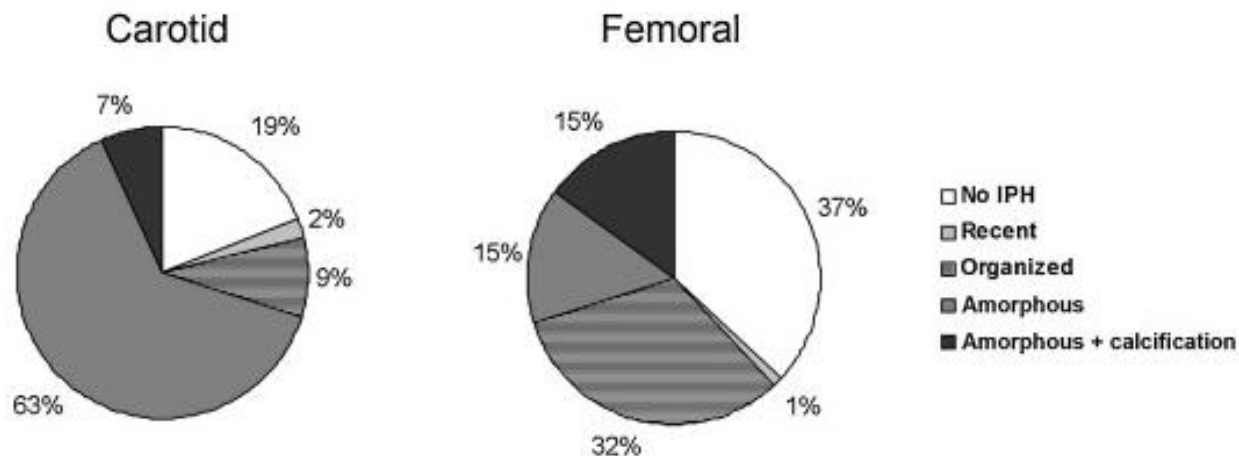


Amorphous

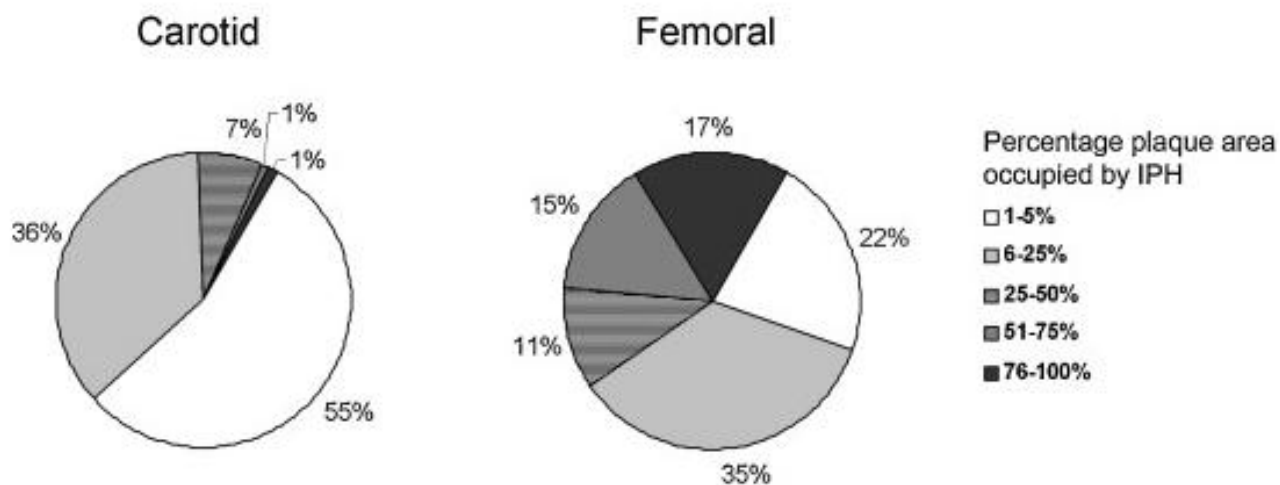


A

Distribution of IPH types



B Distribution of percentage plaque occupied by IPH



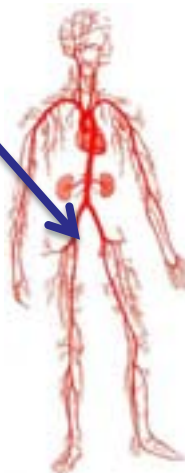


Plaque

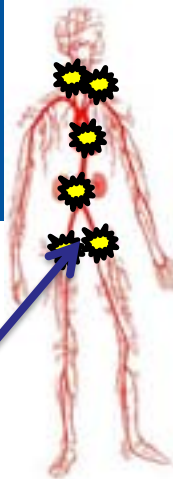


Baseline:
Carotid or Femoral
endarterectomy

3 year Follow
Up



No event



Event

- Coronary
- Peripheral
- CV death
- Cerebral

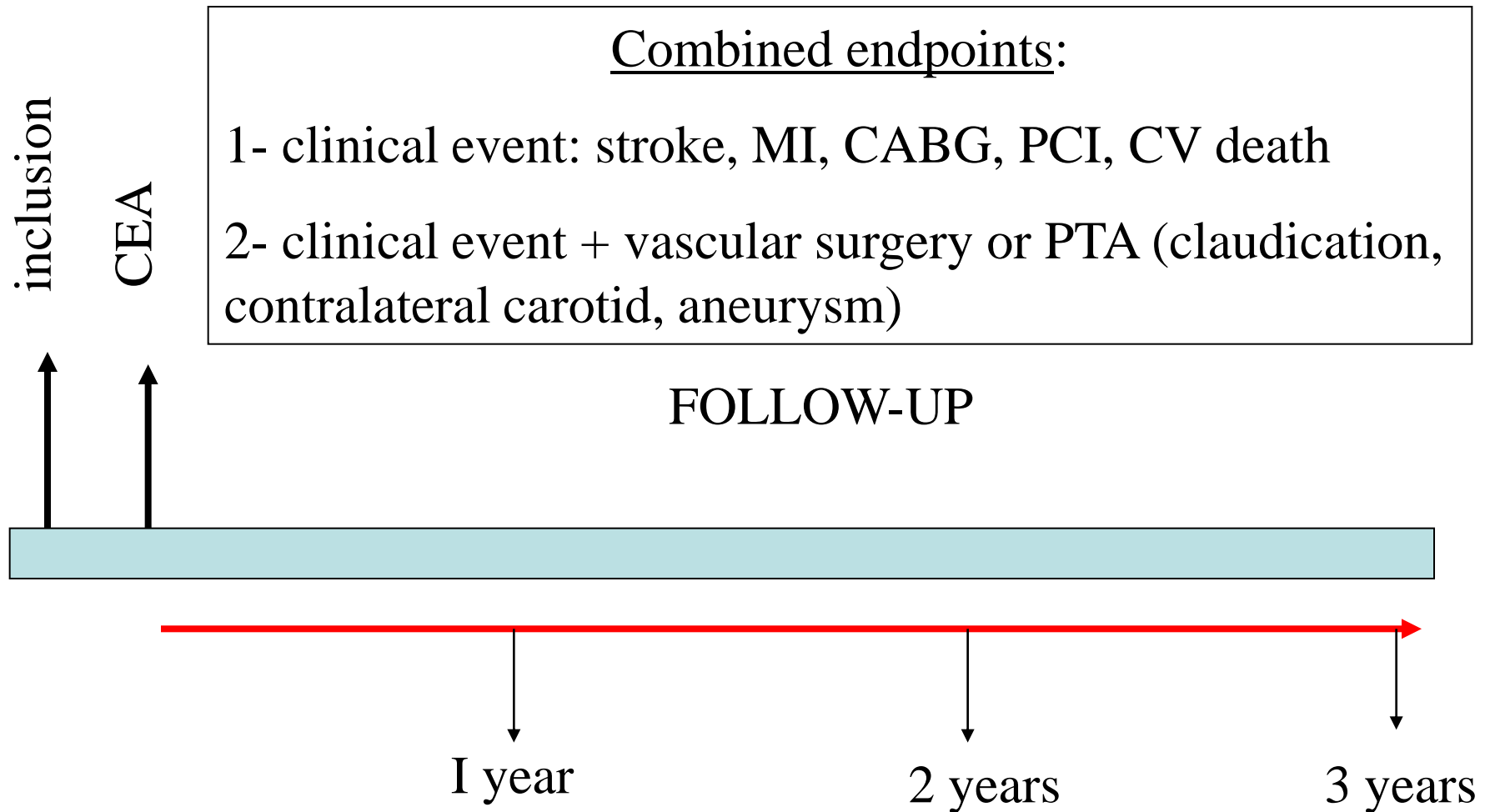
Baseline
plaque

- Histology
- Proteomics

Validation

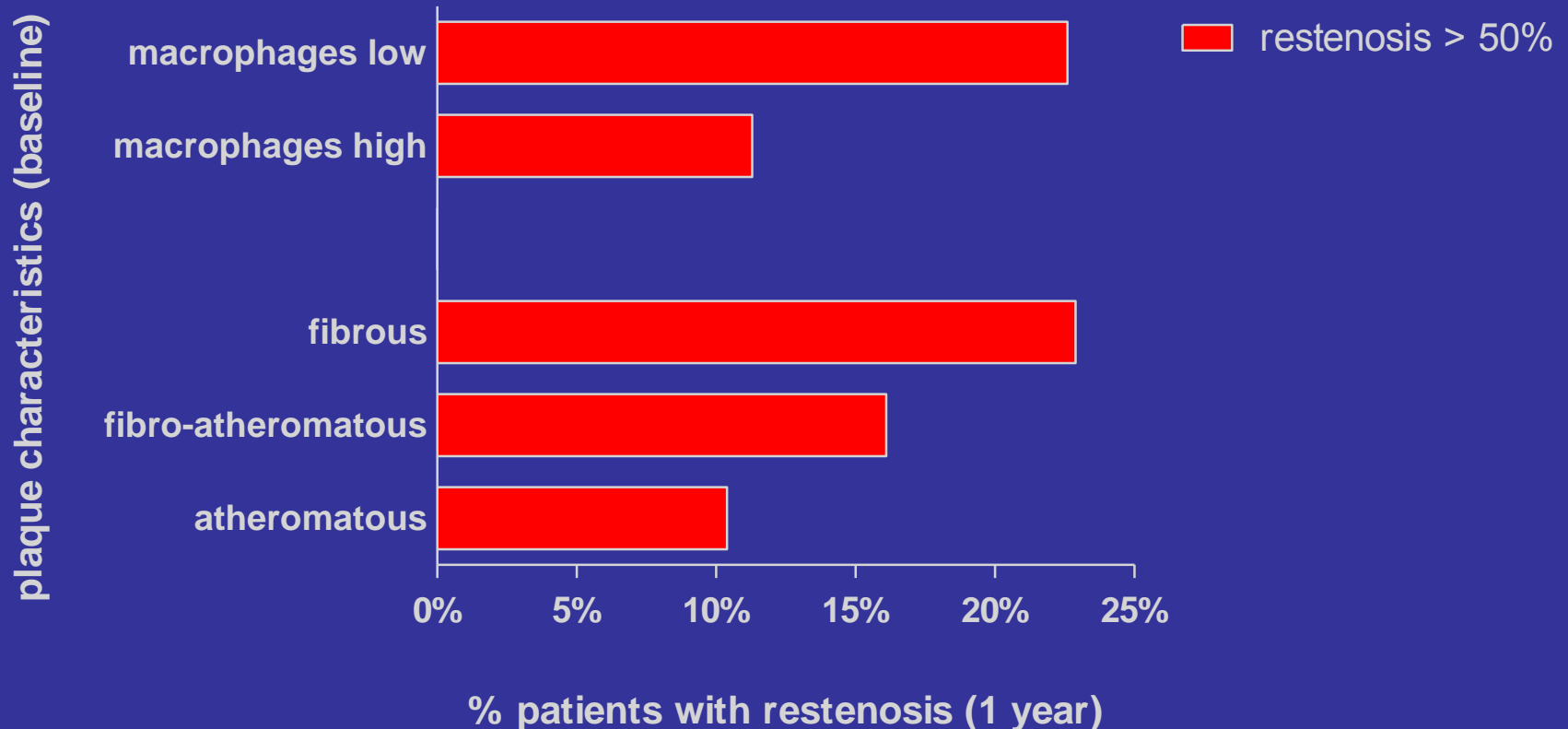
Individual
risk
assessment

Study design



Local predictive value

Plaque histology vs. restenosis



N = 500

* $p < 0.0005$; $p < 0.0005$

Hellings et al. JAMA 2008

The classical definition of the vulnerable plaque and predictive value for systemic outcome

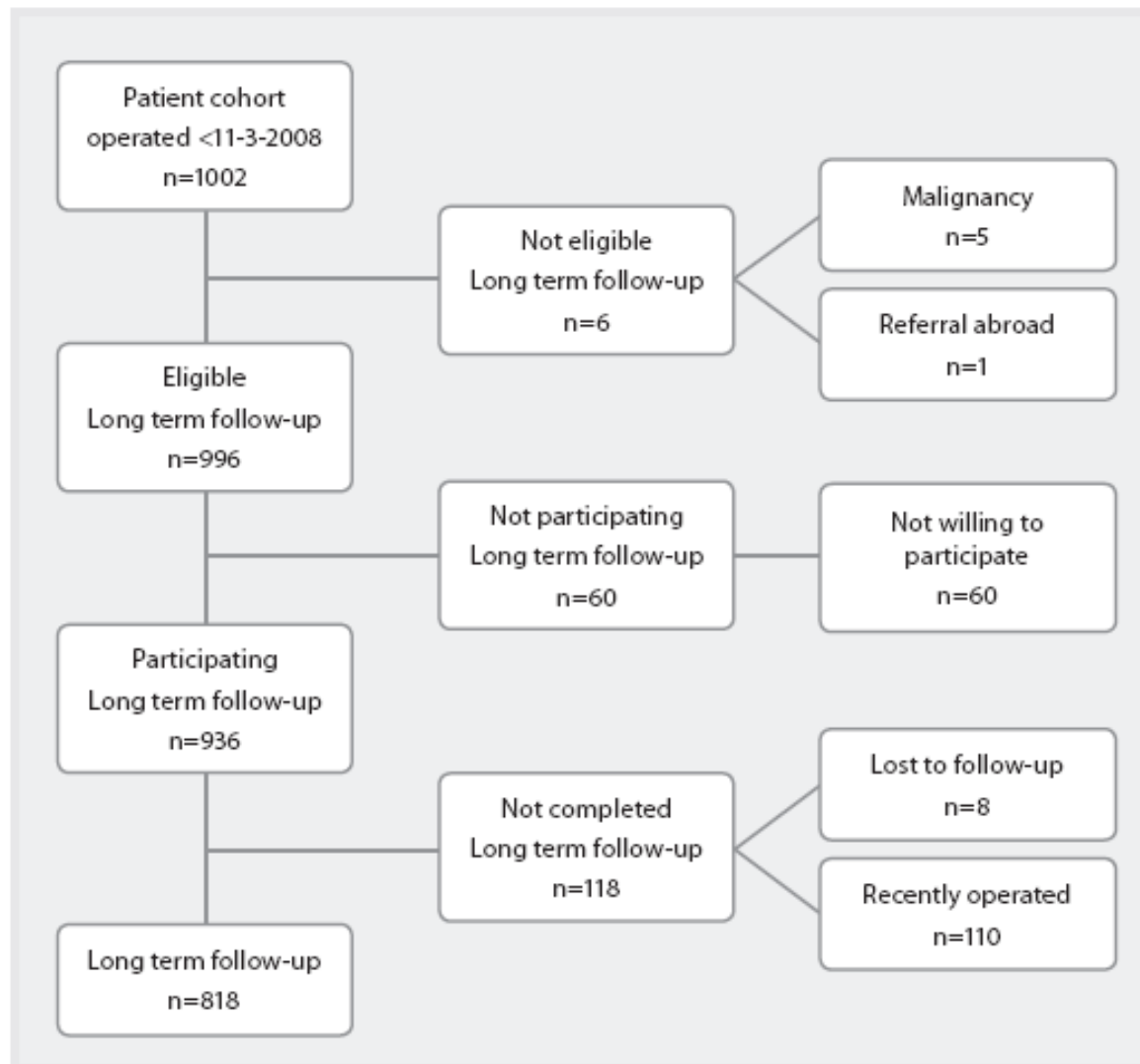
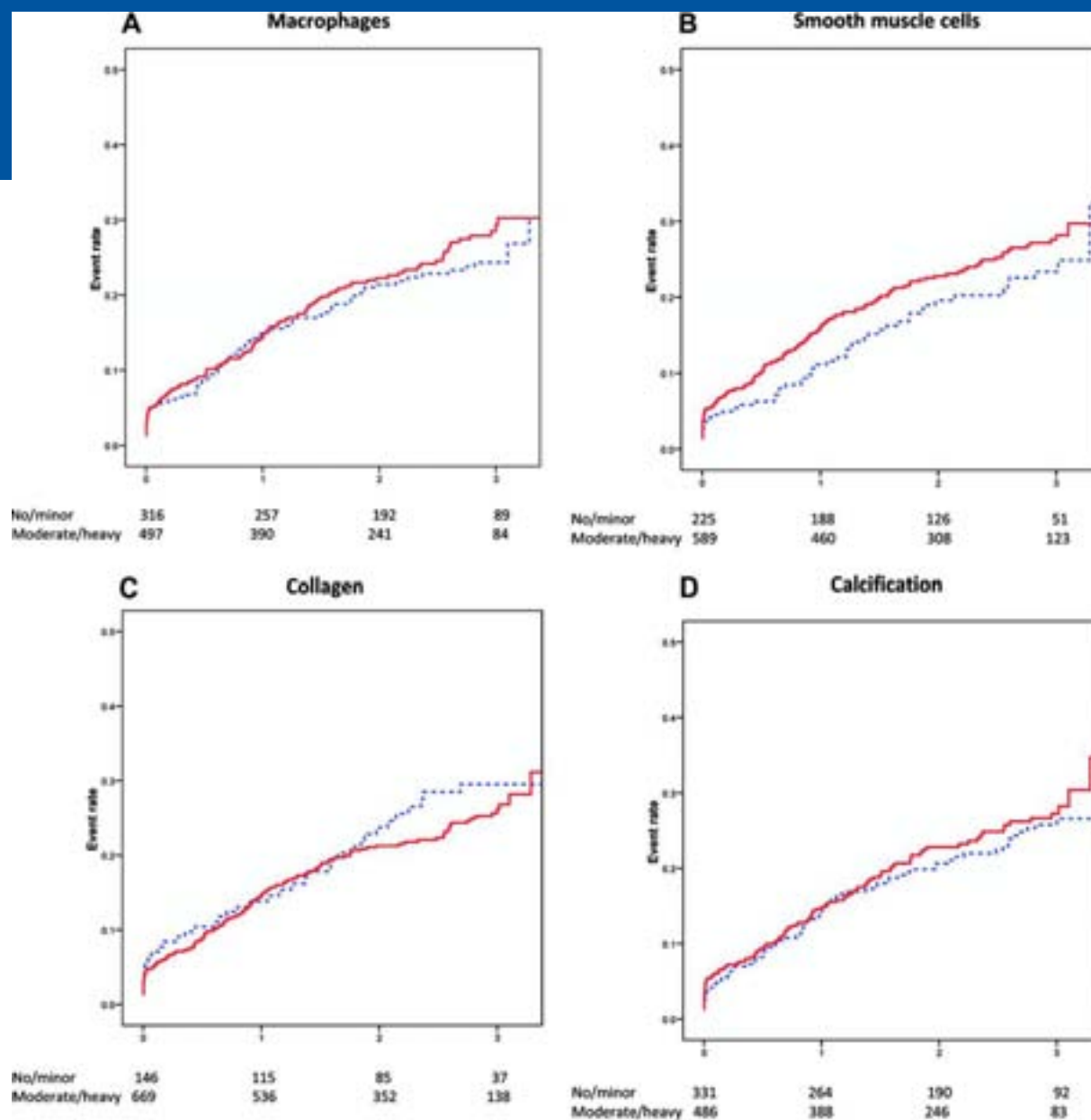


Figure I Flow chart describing flow of patients included in the study



•Hellings W E et al. Circulation 2010;121:1941-1950

Thrombus presence [intraplaque/intraluminal] and endpoints

P = 0.004
Hazard ratio = 1.7 [1.1 – 2.4]

— no
— yes

years

Number of neovessels in plaque.

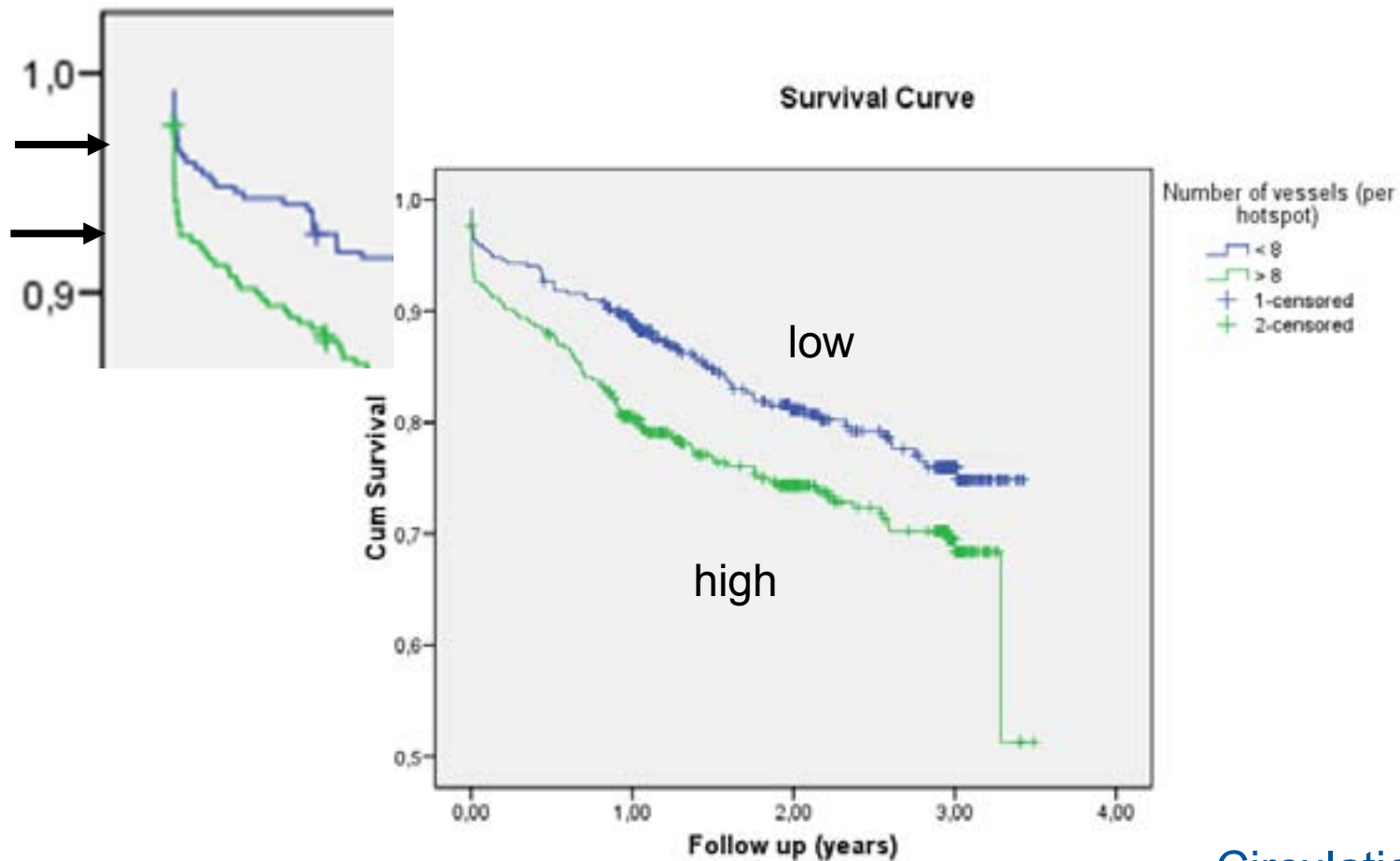




Table 3. Multivariable Analysis

	Risk of Primary Outcome	
	Univariate, HR (95% CI)	Multivariate, HR (95% CI)
Plaque hemorrhage	1.7 (1.2–2.5)	2.2 (1.2–3.8)
Plaque vessel density	1.4 (1.1–1.9)	1.5 (1.1–2.2)
Age ≥ 70 y	1.3 (0.9–1.7)	NS
Male sex, %	1.6 (1.1–2.2)	NS
Current smoker, %	1.2 (0.9–1.7)	
Diabetes mellitus, %	1.0 (0.7–1.5)	
Hypertension, %	1.2 (0.9–1.6)	NS
Hypercholesterolemia, %	1.4 (0.9–1.7)	
History of vascular intervention	1.7 (1.3–2.2)	1.6 (1.1–2.3)
History of myocardial infarction	1.5 (1.1–2.1)	NS
Body mass index ≥ 25 kg/m ²	0.8 (0.6–1.2)	
C-reactive protein	1.0 (1.0–1.0)	NS
High-density lipoprotein	0.4 (0.2–0.7)	0.5 (0.3–0.9)
Statin use	1.5 (1.1–2.2)	1.8 (1.1–2.9)
Aspirin use	0.6 (0.4–0.8)	NS
Oral anticoagulant use	1.6 (1.1–2.3)	NS
Dipyridamole use, n (%)	0.9 (0.6–1.1)	NS
Bilateral carotid stenosis	1.7 (1.3–2.3)	1.5 (1.1–2.2)
Symptomatic carotid stenosis	1.1 (0.7–1.6)	

Femoral plaques, composite endpoint

Table 2. Plaque characteristics and relation with primary outcome*

Plaque Characteristics	All patients N=320	Number of patients with event ^A	3 years cumulative risk (KM estimate)	3 years risk difference	Risk of primary outcome HR [95% CI] [#]	P-value
Lipid core						
Absent	235 (73%)	88/ 235 (37%)	41%	-		
Present	85 (27%)	40/ 85 (47%)	49%	+8%	1.29 [0.89-1.88]	0.18
Macrophages						
no/ minor	246 (77%)	97/ 246 (40%)	43%	-		
moderate/heavy	74 (23%)	31/ 74 (42%)	45%	+2 %	1.09 [0.73-1.64]	0.67
Macrophages (QA)	0.6 (1.4)	0.6 (1.2)	-	-	1.03[0.91-1.17]	0.64
Collagen						
no/ minor	45 (14%)	17/ 45 (38%)	43%	-		
moderate/heavy	275 (86%)	111/ 275 (40%)	43%	+0%	1.02 [0.61-1.71]	0.93
Smooth muscle cells						
no/ minor	74 (23%)	32/ 74 (43%)	49%	-		
moderate/heavy	246 (77%)	96/ 246 (39%)	42%	-7%	0.80 [0.54-1.20]	0.28
Smooth muscle cells (QA)	3(3.9)	2.9 (3.2)	-	-	0.97 [0.93-1.02]	0.26
Calcification						
no/ minor	106 (33%)	35/ 106 (33%)	35%	-		
moderate/heavy	214 (67%)	93/ 214 (44%)	48%	+13%	1.50 [1.01-2.21]	0.04 [†]
Intraplaque hemorrhage						
Absent	113 (35%)	36/ 113 (32%)	36%	-		
Present	207 (65%)	92/ 207 (44%)	49%	+13%	1.56 [1.06-2.29]	0.02 [†]
Vessel density						
no/low density	98 (52%)	45/ 98 (46%)	49%	-		

Table 3. Multivariate analysis



	Risk of primary outcome HR [95% CI]	P-value
Age	N.S.	-
Gender: male	N.S.	-
Current smoker	1.52 [1.04-2.23]	0.03
Diabetes Mellitus	1.77 [1.21-2.57]	0.03
Hypertension	1.63 [1.04-2.55]	0.03
Hypercholesterolemia	1.83 [1.18-2.83]	0.007
History: myocardial infarction	N.S.	-
History: leg amputation	N.S.	-
Serum Creatinin	N.S.	-
Critical ischemia (Fontain class III-IV)	1.50 [1.00-2.21]	0.049
Calcification	N.S.	-
Intra- plaque hemorrhage	1.67 [1.12-2.49]	0.01



N.S.= removed from the multivariate model based on the backward stepwise likelihood ratio including 1 in the confidence interval.

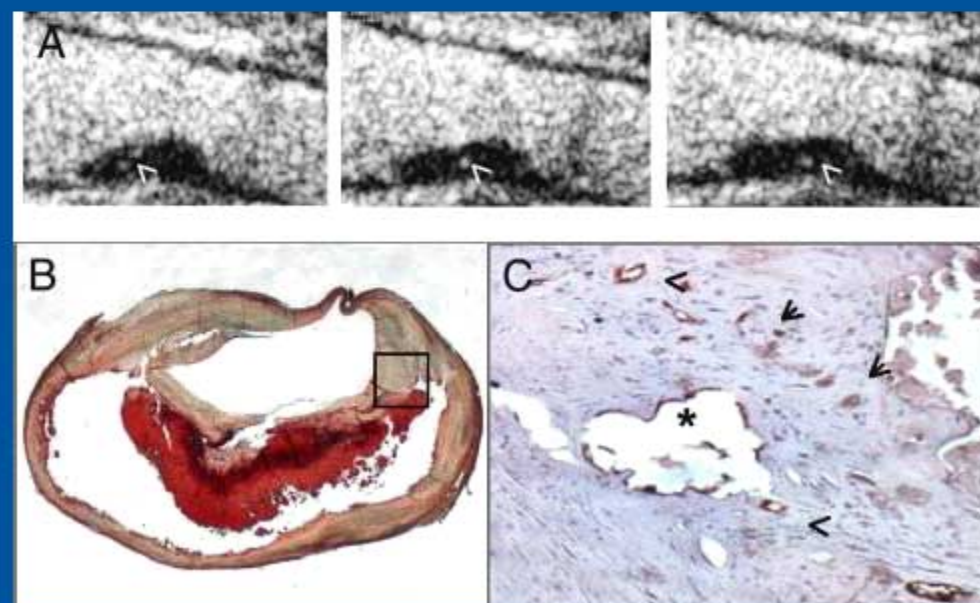
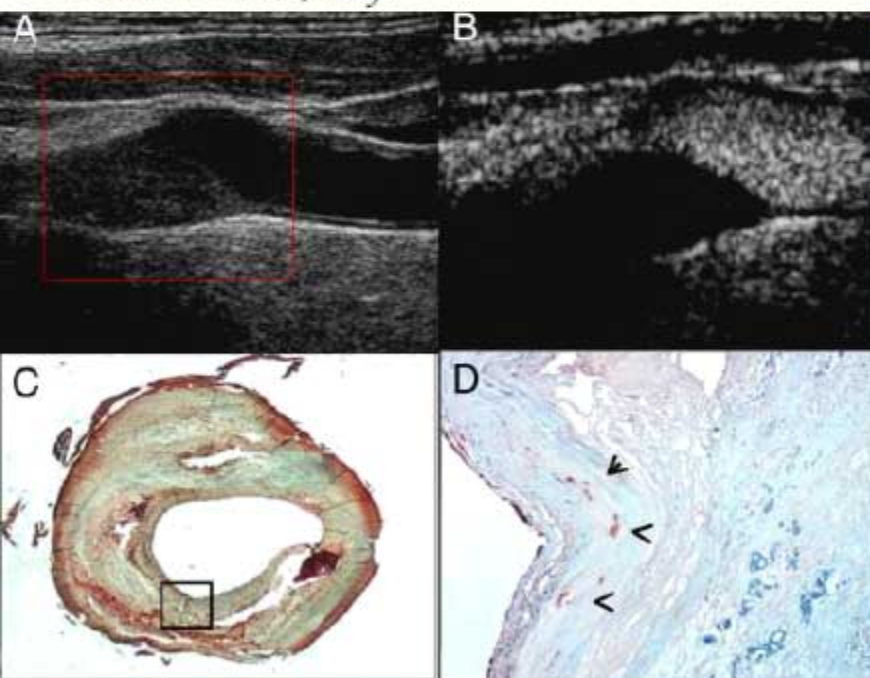


Contrast-Enhanced Ultrasound Imaging of Intraplaque Neovascularization in Carotid Arteries

Correlation With Histology and Plaque Echogenicity

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Massimiliano M. Marrocco-Trischitta, MD,* Giulio Melisurgo, MD,* Alessandro Mauriello, MD,‡
Luigi Spagnoli, MD,‡ Roberto Chiesa, MD,* Domenico Cianflone, MD, FESC,*
Attilio Maseri, MD, FACC*

Milan and Rome, Italy

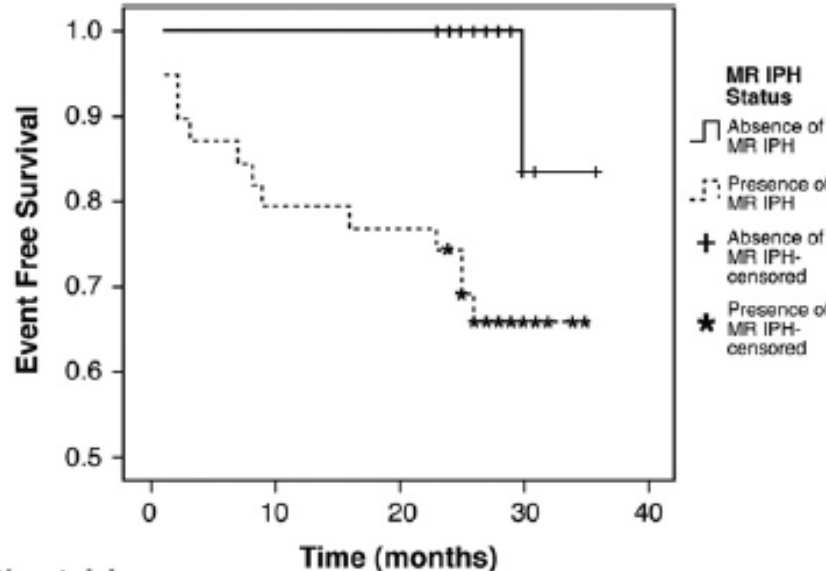


Staub D et al. Stroke 2010

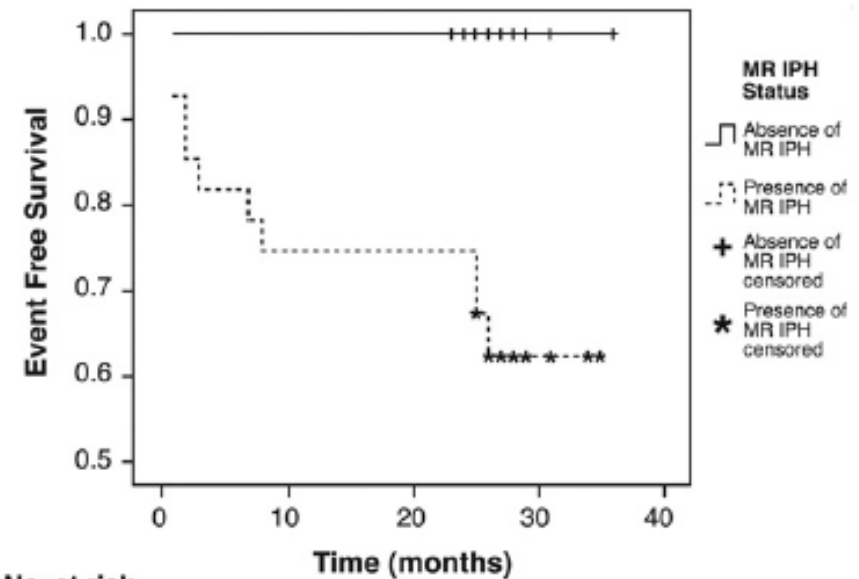
“Vasa vasorum and plaque neovascularization on contrast-enhanced carotid ultrasound imaging correlates with cardiovascular disease and past cardiovascular events.”

Detection of intraplaque hemorrhage by magnetic resonance imaging in symptomatic patients with mild to moderate carotid stenosis predicts recurrent neurological events

Nishath Altaf, MRCS,^{a,b} Lucy Daniels, MRCP,^{b,c} Paul S. Morgan, PhD,^b Dorothee Auer, PhD,^b
 Shane T. MacSweeney, FRCS,^a Alan R. Moody, FRCR,^d and John R. Gladman, FRCP,^c
Nottingham, United Kingdom; and Toronto, Ontario, Canada



No. at risk				
MR IPH absent	25	25	25	3
MR IPH present	39	31	30	6



No. at risk				
MR IPH absent	15	15	15	3
MR IPH present	27	20	20	3

All symptomatic patients

All symptomatic patients
with significant stenosis

Plaque type and stroke risk

Stroke risk can be calculated using a carotid stenosis risk prediction model, which has been described in detail previously and was validated against the NASCET patient database with a c-statistic of 0.67, 95% CI 0.63-0.72 ($p < 0.0001$).



Table 3. Odds-ratios for the presence of individual plaque characteristics in the highest versus lowest quartile of stroke risk.

Plaque characteristic	Oxford Plaque Study (n=481)			Athero-Express (n=1159)			Pooled Data (n=1640)		
	OR	95% CI	P-value	OR	95% CI	P-value	OR	95% CI	P-value
1 YEAR STROKE RISK									
Overall plaque instability	1.24	0.74-2.06	0.41	1.52	1.07-2.15	0.02	1.42	1.07-1.90	0.02
Thrombus	1.47	0.85-2.52	0.17	1.37	0.98-1.92	0.06	1.40	1.05-1.86	0.02
Heavy macrophage staining	1.78	1.05-3.00	0.03	1.19	0.85-1.67	0.30	1.39	1.04-1.84	0.03
High micro-vessel density	1.43	0.86-2.38	0.17	1.49	0.92-2.41	0.11	1.46	1.03-2.07	0.03
Large lipid core	1.22	0.74-2.02	0.44	1.34	0.93-1.97	0.11	1.31	0.97-1.76	0.08
Plaque haemorrhage	1.39	0.83-2.33	0.22	1.06	0.71-1.59	0.78	1.18	0.85-1.62	0.32
Fibrous plaque	0.63	0.37-1.07	0.09	0.66	0.47-0.93	0.02	0.65	0.49-0.87	0.004
Heavy calcification	0.91	0.56-1.49	0.71	0.82	0.59-1.14	0.24	0.84	0.64-1.11	0.23
5 YEAR STROKE RISK									
Overall plaque instability	1.29	0.78-2.12	0.32	1.47	1.03-2.09	0.03	1.40	1.05-1.87	0.02
Thrombus	1.44	0.85-2.45	0.17	1.41	1.00-1.98	0.05	1.42	1.11-1.89	0.02
Heavy macrophage staining	1.83	1.10-3.05	0.02	1.26	0.89-1.76	0.19	1.41	1.05-1.90	0.02
High micro-vessel density	1.42	0.87-2.33	0.17	1.57	0.96-2.56	0.07	1.49	1.05-2.11	0.03
Large lipid core	1.26	0.77-2.07	0.35	1.31	0.90-1.92	0.16	1.29	0.96-1.75	0.09
Plaque haemorrhage	1.40	0.84-2.32	0.19	1.02	0.67-1.53	0.94	1.15	0.84-1.59	0.38
Fibrous plaque	0.58	0.35-0.98	0.04	0.68	0.48-0.97	0.03	0.65	0.49-0.87	0.004
Heavy calcification	1.03	0.64-1.67	0.90	0.81	0.58-1.13	0.22	0.88	0.67-1.16	0.35

Abbreviations: OR, odds ratio; CI, confidence interval. Pooled data stratified by study cohort.

No significant heterogeneity was found between the study groups for any individual plaque features



In the field of CVD, successful biobanking in the past does not provide a guarantee for successful biomarker research in the future.

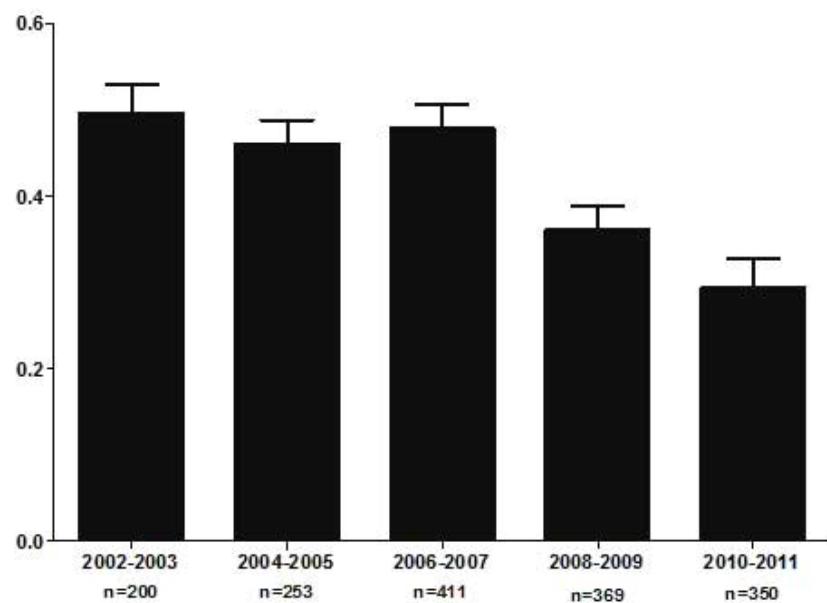
Inclusion number /year (carotid plaques)

Year of CEA	Frequency (n)	%
2002	83	5,5
2003	167	11
2004	199	13,1
2005	207	13,6
2006	198	13
2007	166	10,9
2008	156	10,3
2009	185	12,2
2010	145	9,5
Total	1506	100



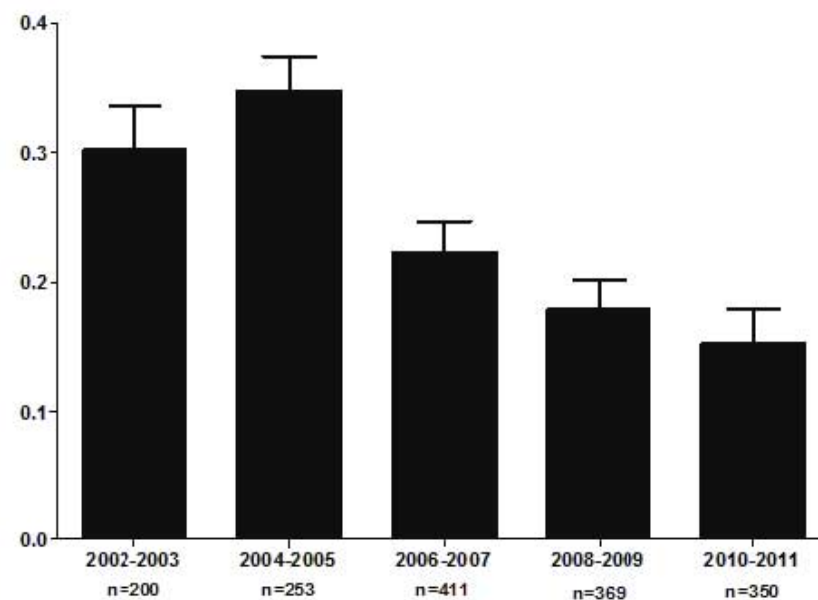
Luminal thrombus

Adjusted $P < 0.001$



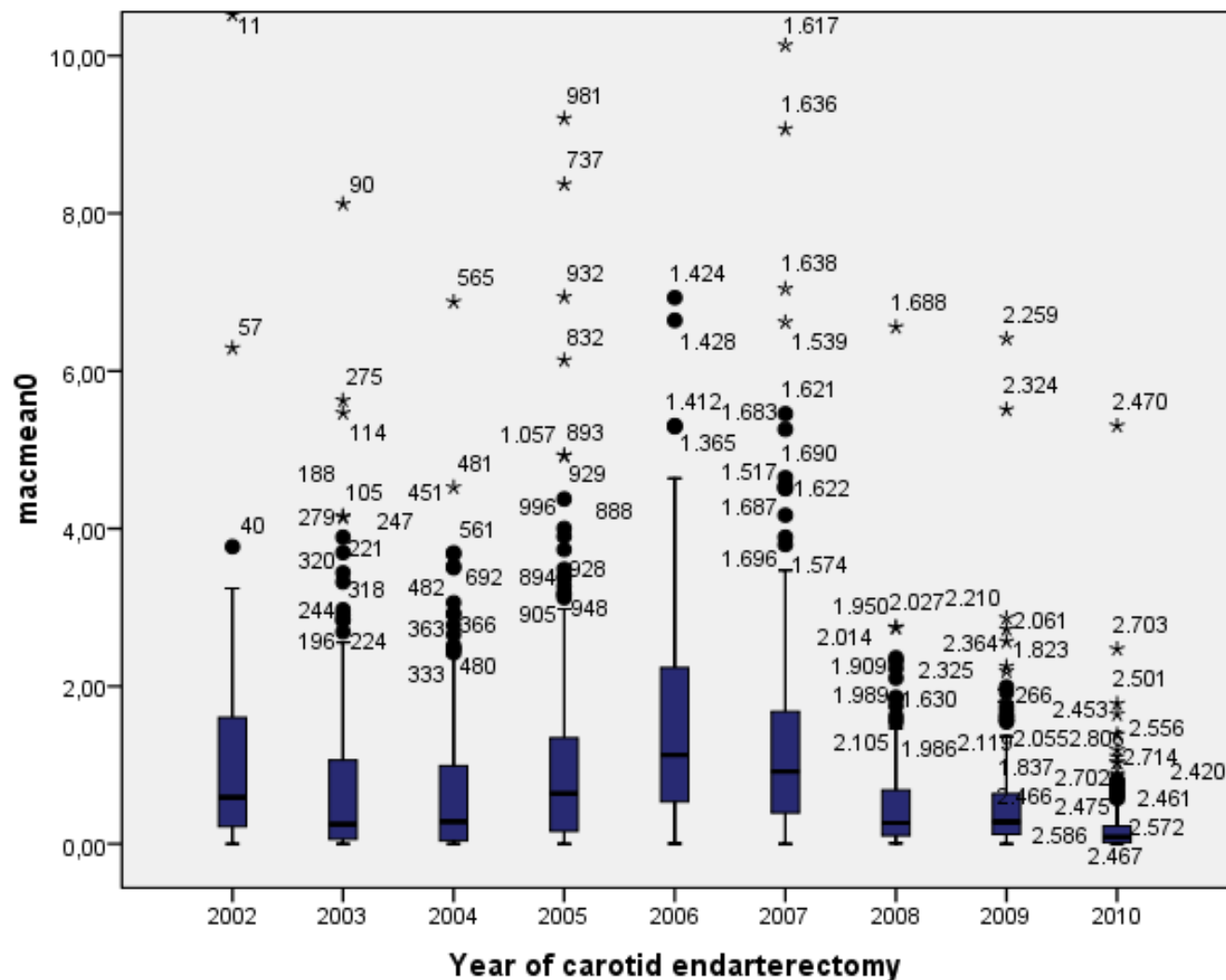
Lipid rich plaques

Adjusted $P < 0.001$



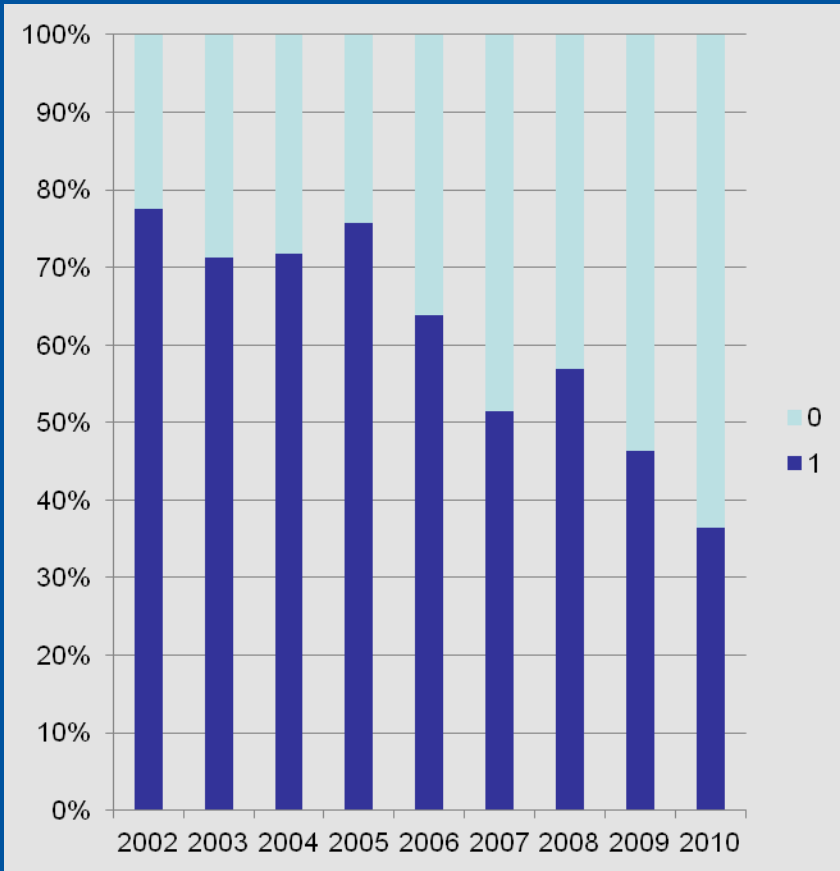


Quantitative Macrophages over time

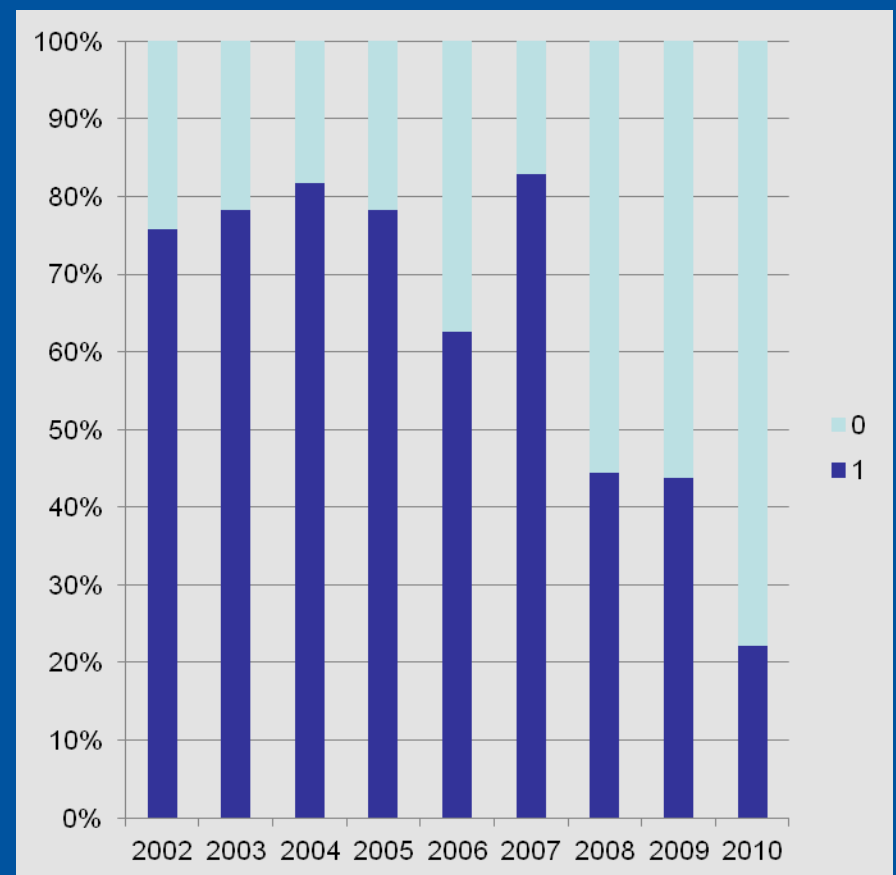


Spearman's
 $\rho = -0.127$;
 $P < 0.001$

IPH over time



patients treated with statins (n=1089)



patients not treated with statins (n=389)



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