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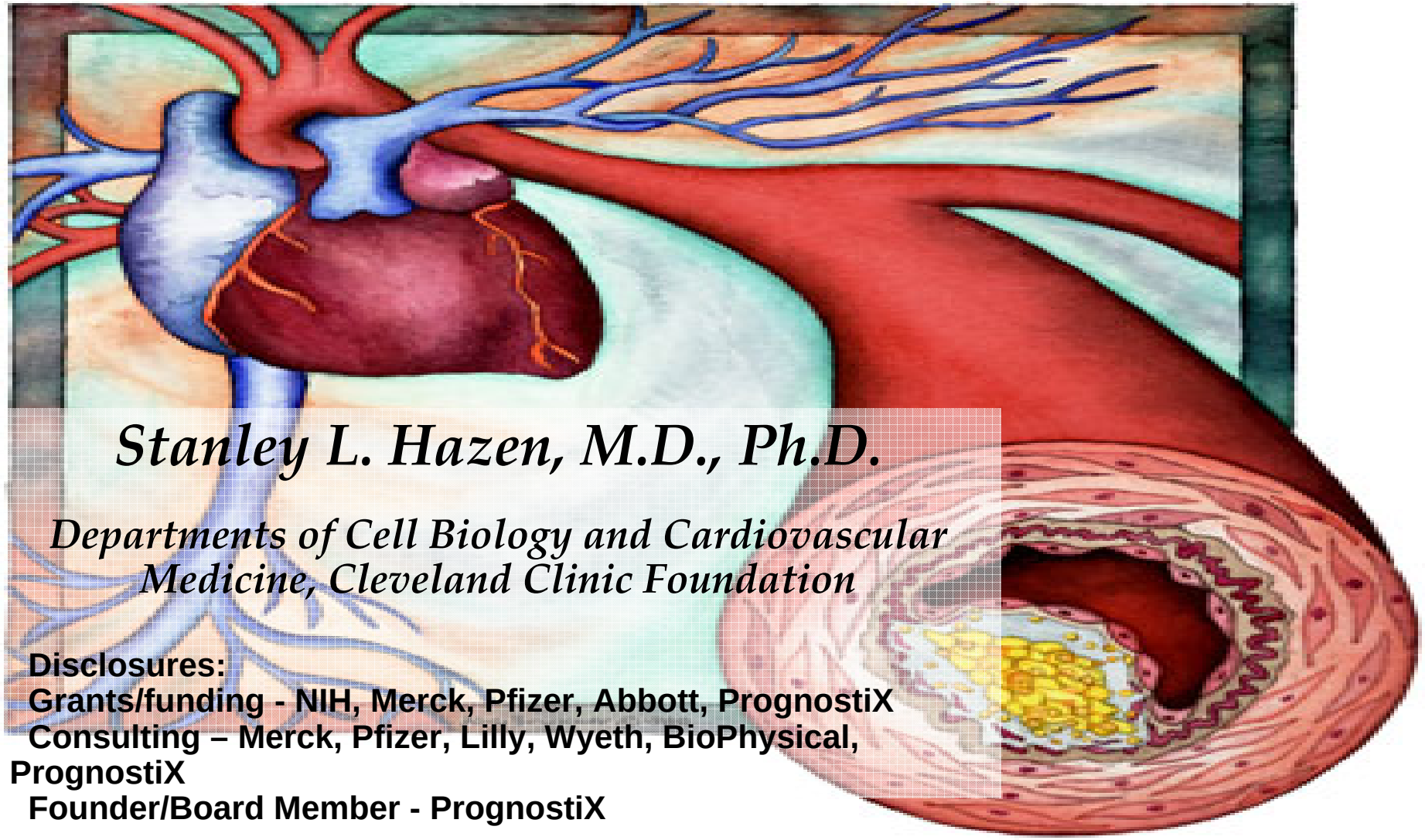
The Impact of Free Radical Research on Disease: The MPO Story

Stanley L. Hazen, M.D., Ph.D.

Departments of Cell Biology and Cardiovascular Medicine, Cleveland Clinic Foundation

Disclosures:
Grants/funding - NIH, Merck, Pfizer, Abbott, PrognostiX
Consulting – Merck, Pfizer, Lilly, Wyeth, BioPhysical, PrognostiX
Founder/Board Member - PrognostiX

The Impact of Free Radical Research on Disease: The MPO Story



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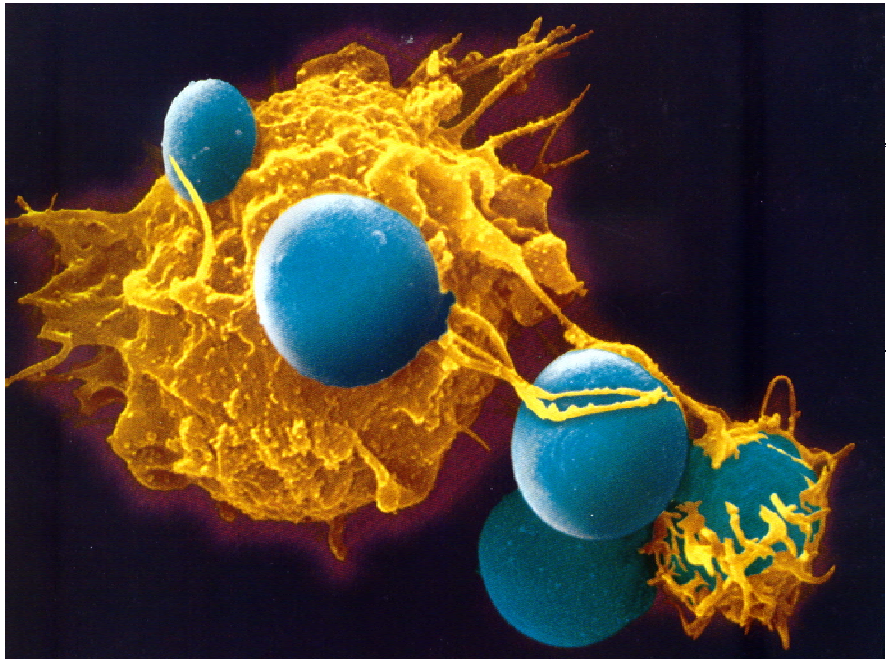
Neutrophils, Monocytes and some Macrophages Use MPO to Generate Cytotoxic Oxidants



O_2 Oxygen

NADPH Oxidase

$O_2^{\bullet}$ Superoxide

H_2O_2 Hydrogen peroxide

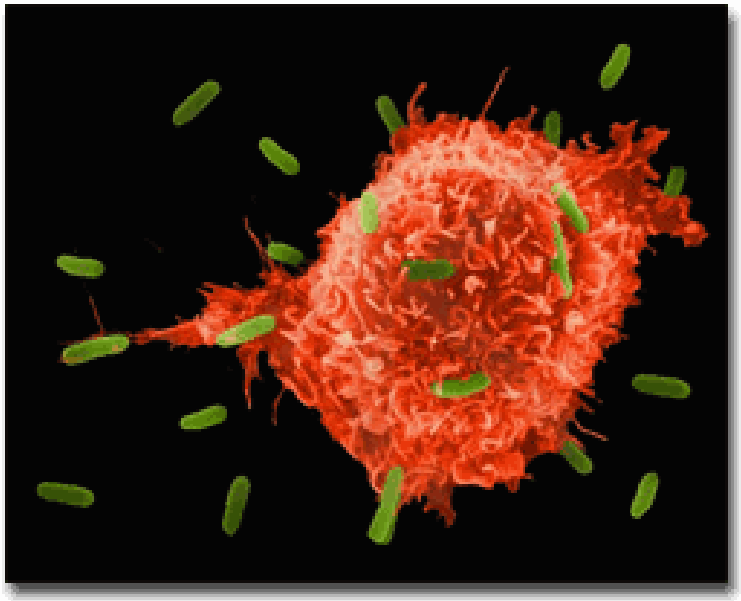
MPO

Array of cytotoxic oxidants & free radicals

Slide courtesy AJ Kettle

MPO as Friend in the Immune System

- MPO produces HOCl and other reactive chlorinating species
- HOCl is antimicrobicidal, killing invading parasites and pathogens

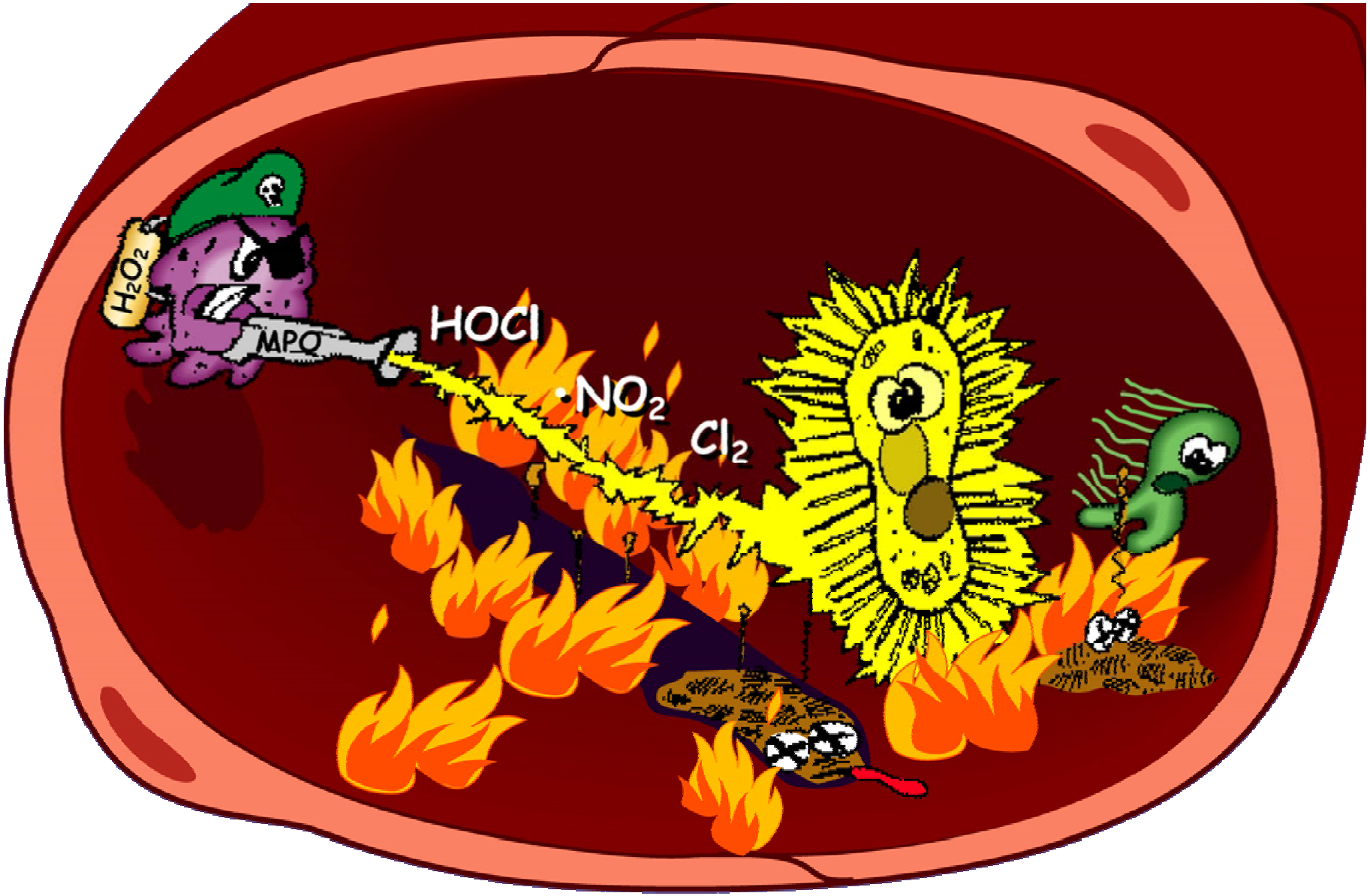


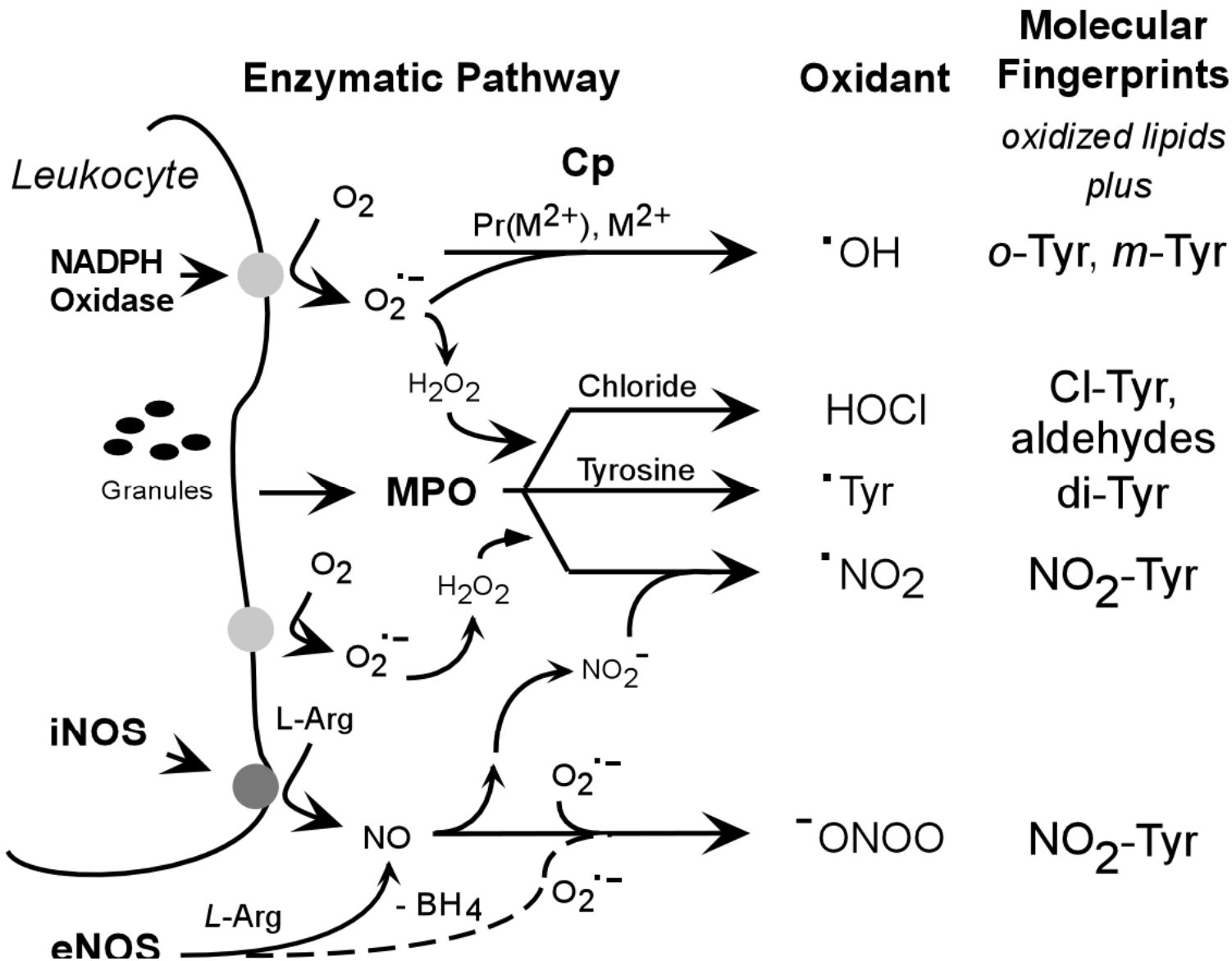
$$H_2O_2 + Cl^- + H^+ \xrightarrow{\text{Myeloperoxidase}} H_2O + HOCl$$

$$HOCl + H^+ + Cl^- \rightleftharpoons Cl_2 + H_2O$$

Klebanoff SJ. *J Leuk Biol* 2005
Hazen SL et al, JCI, 1996

MPO as Foe – Oxidative tissue injury

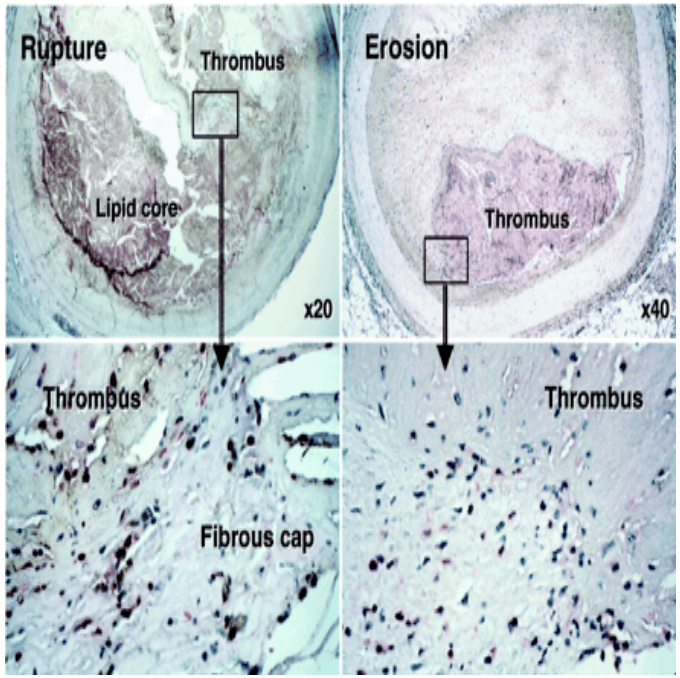




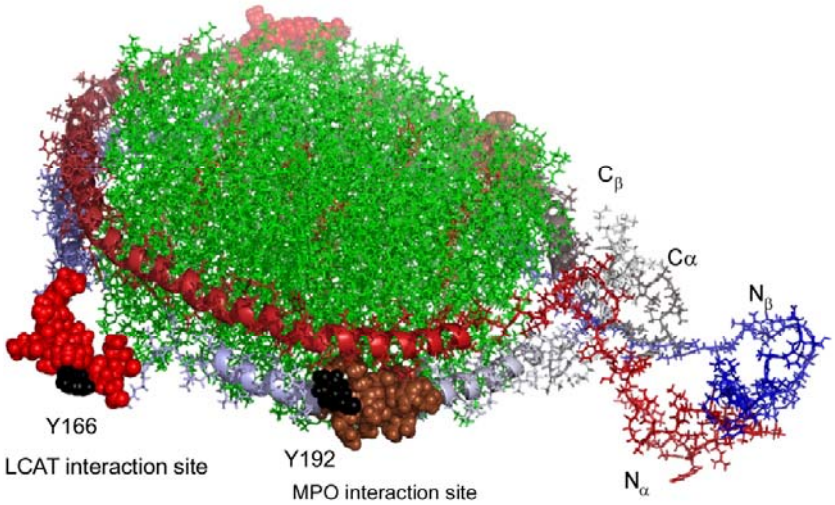
from: M.L. Brennan and S.L. Hazen. "Amino acid and protein oxidation and cardiovascular disease." 2003. In: *Amino Acids*. Volume Editors Francesco Galli and Earl Stadtman. Elsevier Press.

MPO as a Link to Heart Disease: Some Milestones

- MPO is found in atherosclerotic plaques
  (Daugherty et al, JCI (1994) 94:437)
- MPO oxidizes LDL in the artery wall
  (Hazen et al, JCI (1997) 994:2075)
- MPO oxidized LDL is atherogenic
  (Podrez et al, JCI (1999) 103:1547)
- MPO initiates lipid oxidation in vivo
  (Hazen et al, Circ Res (1999) 85:950)
- MPO consumes nitric oxide as substrate
  (Abu-Soud and Hazen, JBC (2000) 275:37524)
- MPO is enriched in culprit lesions
  (Sugiyama et al, Am J Path (2001) 994:20158)
- MPO promotes endothelial dysfunction
  (Eiserich et al, Science (2002) 296:2392)
  (Vita et al, Circulation (2004) 110:1134)
  (Baldus et al, FRBM (2004) 37:902)
- Unstable coronary plaque triggers MPO release
  (Buffon et al, NEJM (2002) 347:5)
  (Brennan et al, NEJM (2003) 345: 1595)
  (Baldus et al, Circulation (2004) 108:1440)
- MPO renders HDL "dysfunctional" in vivo
  (Zhang et al, JCI (2004) 114:529)
  (Shao et al, JBC (2005) 280:5983)
  (Wu et al, Nature SMB (2007) 14:861)
- MPO mediated carbamylation in human atherosclerotic plaque is proatherogenic
  (Wang et al, Nature Medicine (2007) 13:1176)



Sugiyama Am J Pathology 2001



Wu et al, Nature SMB, 2007

Human genetic studies suggest a role for MPO in atherosclerosis

MPO-/- subjects

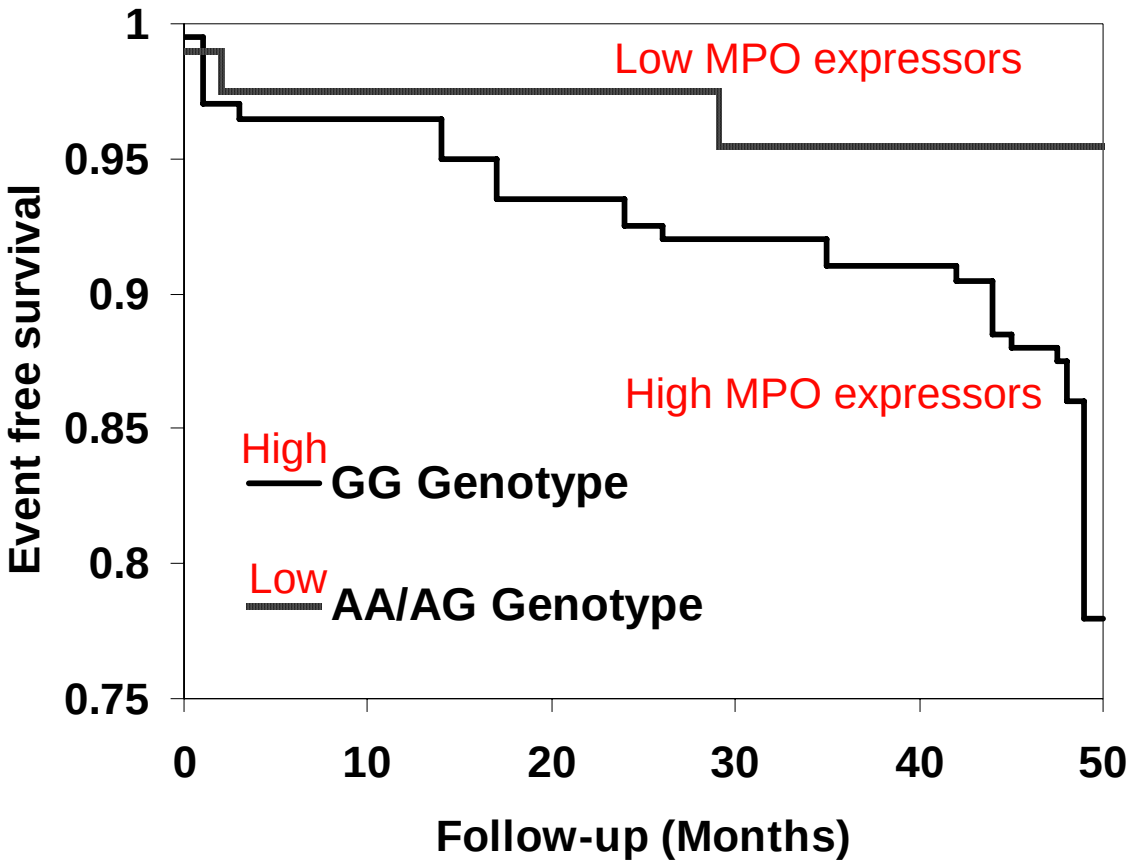
↓ Cardiovascular events
↑ Life-threatening infections

Kutter, D., et al. Acta Hematologica, 2000; 104:10-15.

(- 463 G/A) Promoter SNP

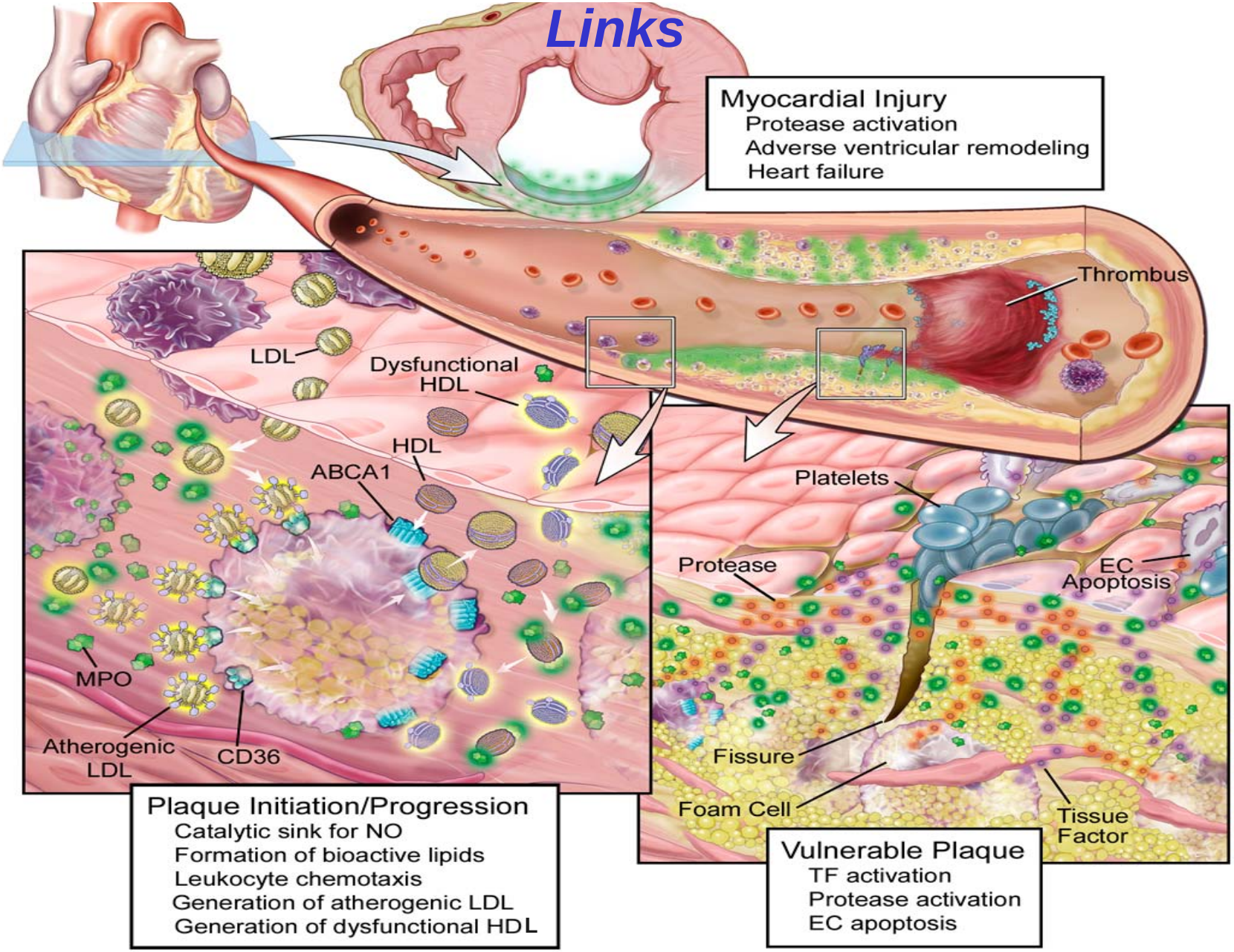
- Affects MPO transcription 2X↓
- Positive findings

- French (n=446)
- Dutch (n=200)



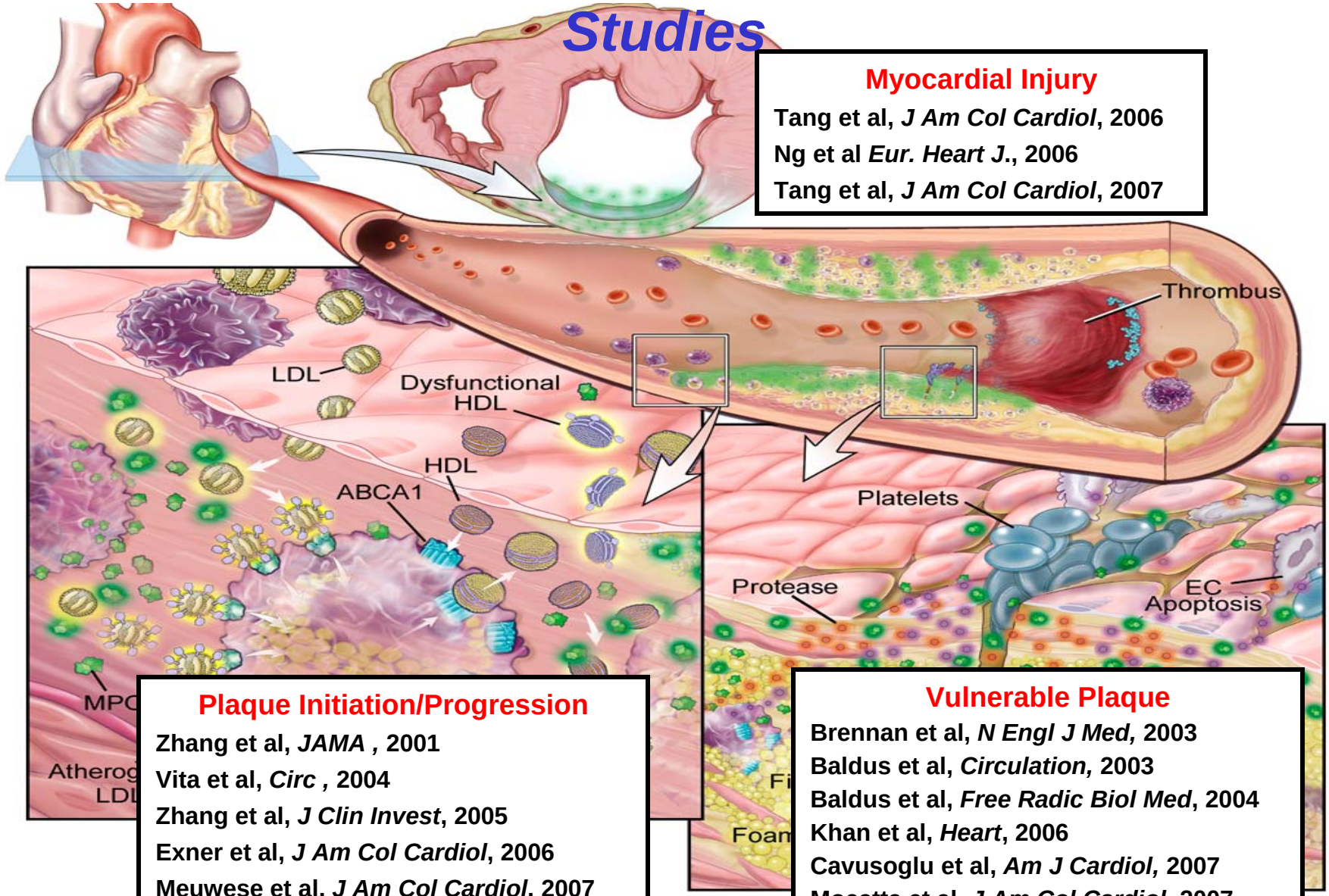
Asselbergs et al., NEJM 2004

MPO and the Evolution of CVD – Select Mechanistic Links



Hazen et al., ATVB 2005

MPO and the Evolution of CVD – Select Clinical Studies



Myocardial Injury

Tang et al, *J Am Col Cardiol*, 2006
Ng et al *Eur. Heart J.*, 2006
Tang et al, *J Am Col Cardiol*, 2007

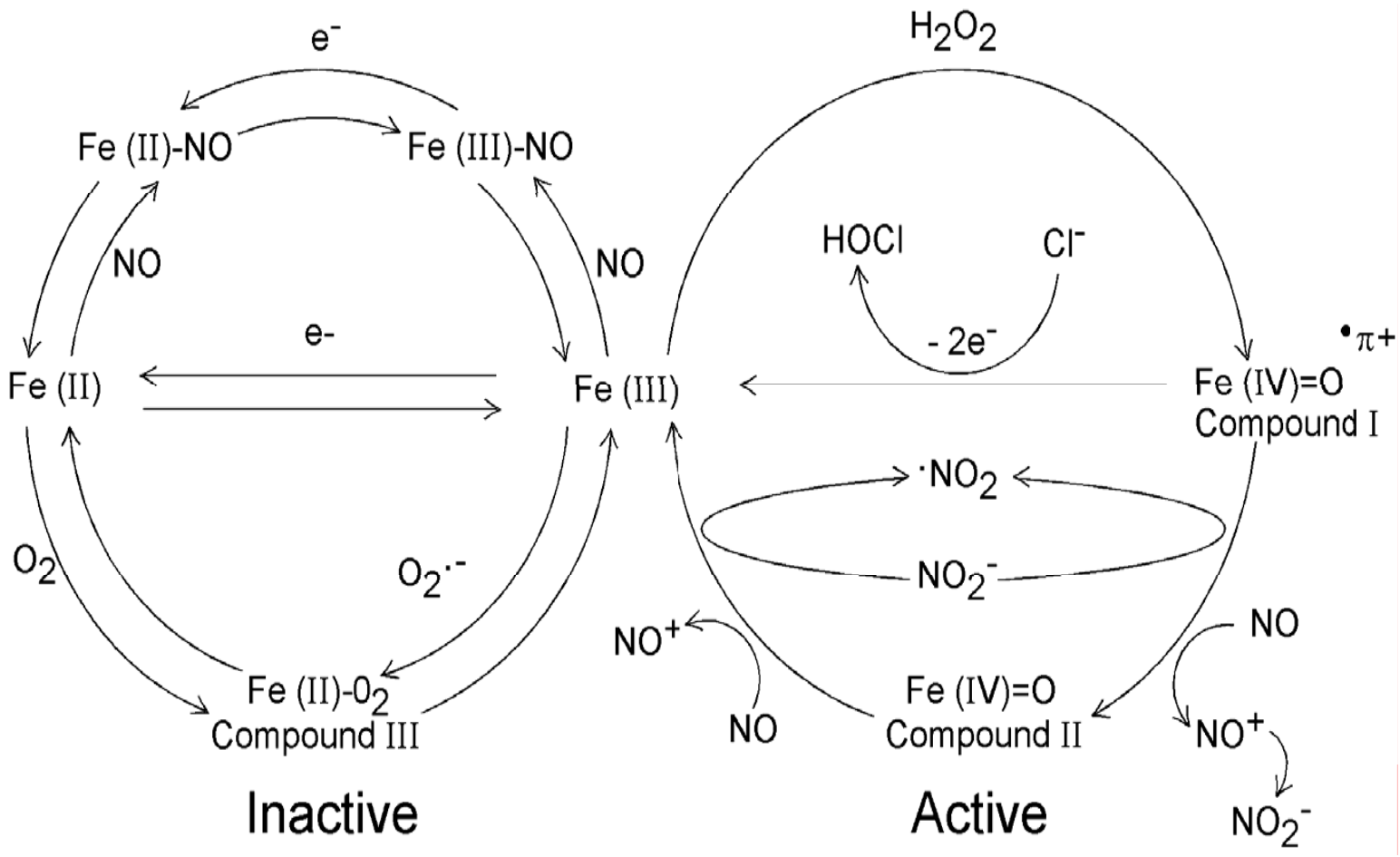
Plaque Initiation/Progression

Zhang et al, *JAMA* , 2001
Vita et al, *Circ* , 2004
Zhang et al, *J Clin Invest*, 2005
Exner et al, *J Am Col Cardiol*, 2006
Meuwese et al, *J Am Col Cardiol*, 2007
Wu et al, *Nature SMB*, 2007
Wang et al, *Nature Medicine*, 2007

Vulnerable Plaque

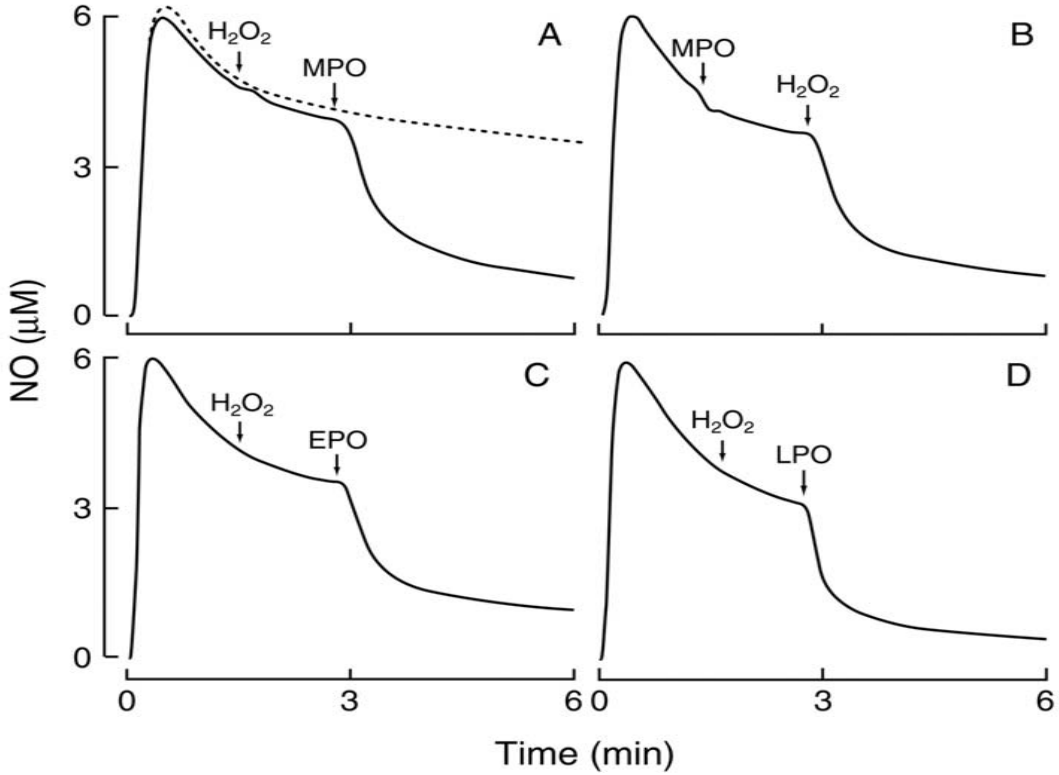
Brennan et al, *N Engl J Med,* 2003
Baldus et al, *Circulation,* 2003
Baldus et al, *Free Radic Biol Med*, 2004
Khan et al, *Heart*, 2006
Cavusoglu et al, *Am J Cardiol,* 2007
Mocatta et al, *J Am Col Cardiol*, 2007
Tang et al, *J Am Col Cardiol*, 2007
Podrez et al, *Nature Medicine*, 2007

•NO-Peroxidase Interactions



AbuSoud & Hazen, (1999), *JBC*
AbuSoud & Hazen, (2000), *JBC*
AbuSoud, et al. (2001), *Biochem*.

Mammalian Heme Peroxidases Catalytically Consume NO as a Physiological Substrate

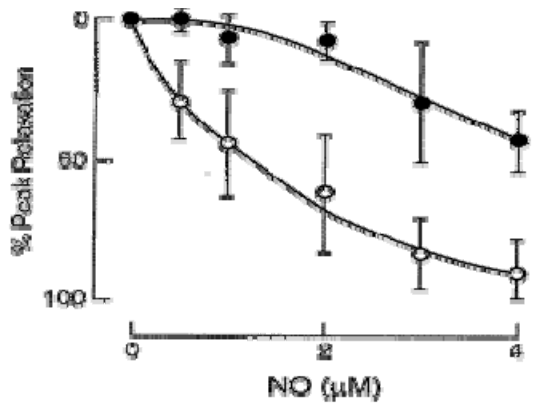


AbuSoud & Hazen, (1999), *JBC*

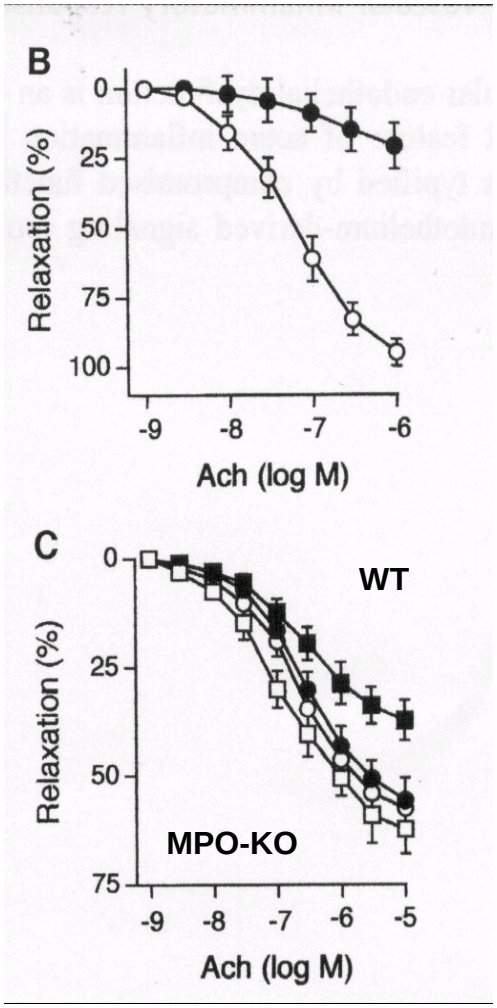
AbuSoud & Hazen, (2000), *JBC*

AbuSoud, et al. (2001), *Biochem*.

MPO, EPO or LPO



Septic Shock Model



LPS Tx

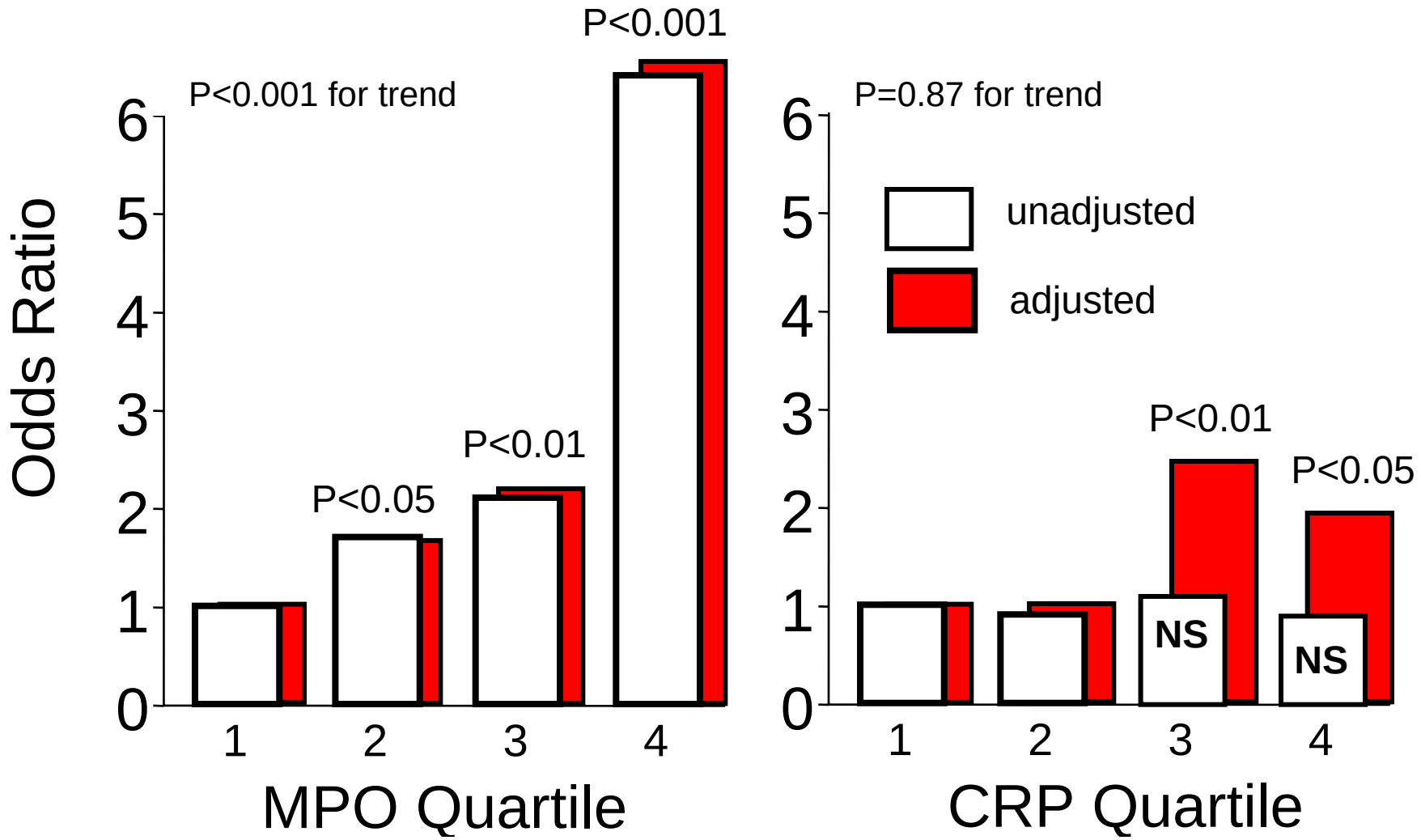
Normal

WT + LPS

WT
KO + LPS
KO

Eiserich, et al. (2002), Science

MPO Levels Predict Risk for Endothelial Dysfunction

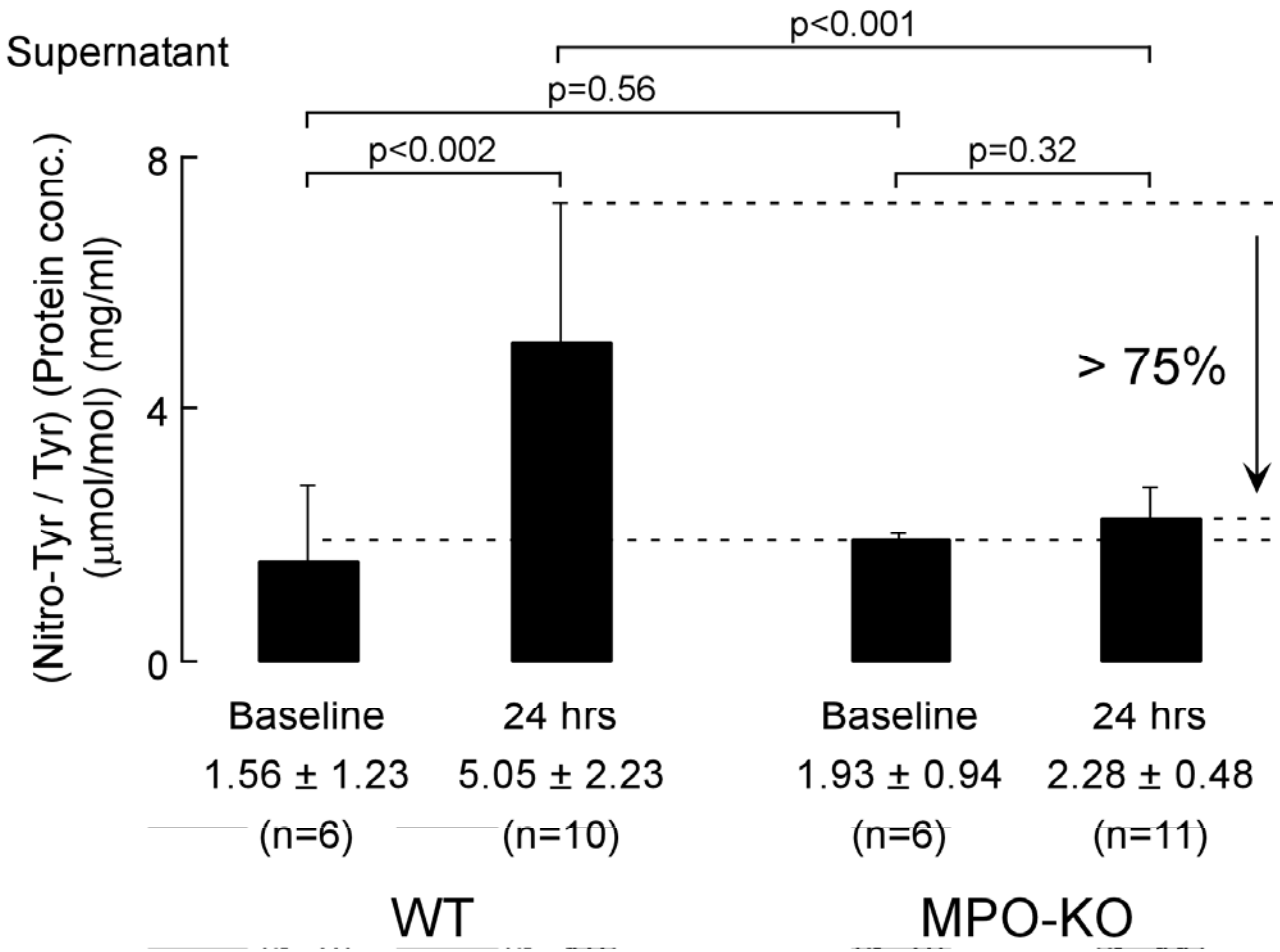


Adjusted for Framingham Risk Score, prevalent cardiovascular disease, cardiac medications, and the alternative marker of inflammation (CRP or MPO)

From: Vita et al, Circulation, 2004
See also: Baldus et al, FRBM, 2004; and Baldus et al, Circulation, 2006

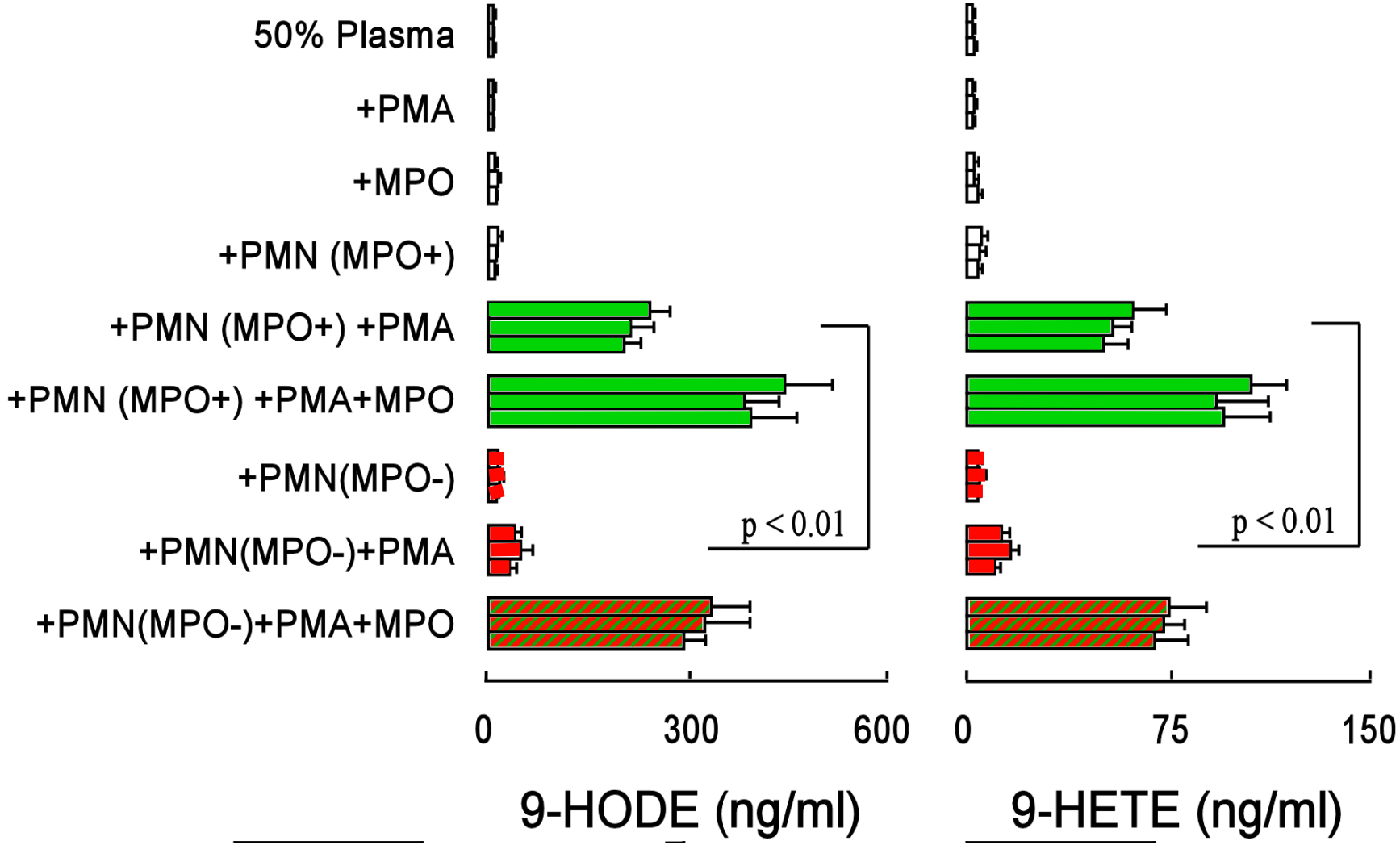
Myeloperoxidase Plays a Major Role in Nitration of Extracellular Proteins *in vivo*

Peritonitis model



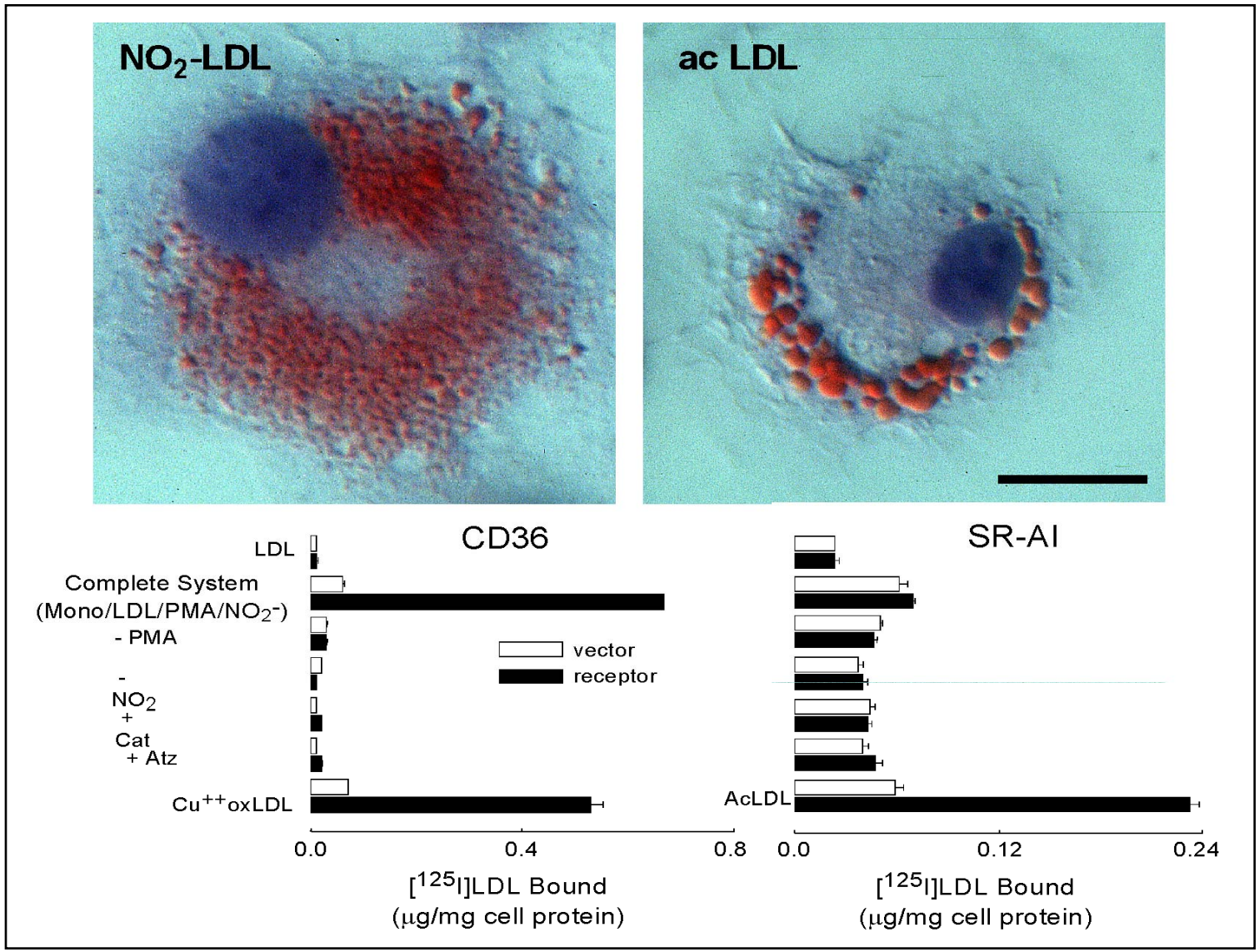
From : Brennan et al, (2002) *J. Biol.Chem.* 277:17415
See also : Gaut et al, (2002) *J. Clin. Invest.* 109:1287
Baldus et al, (2001) *J. Clin. Invest.* 108:1759

MPO is a Physiologic Catalyst for the Peroxidation of Endogenous Plasma Lipids by Human Neutrophils



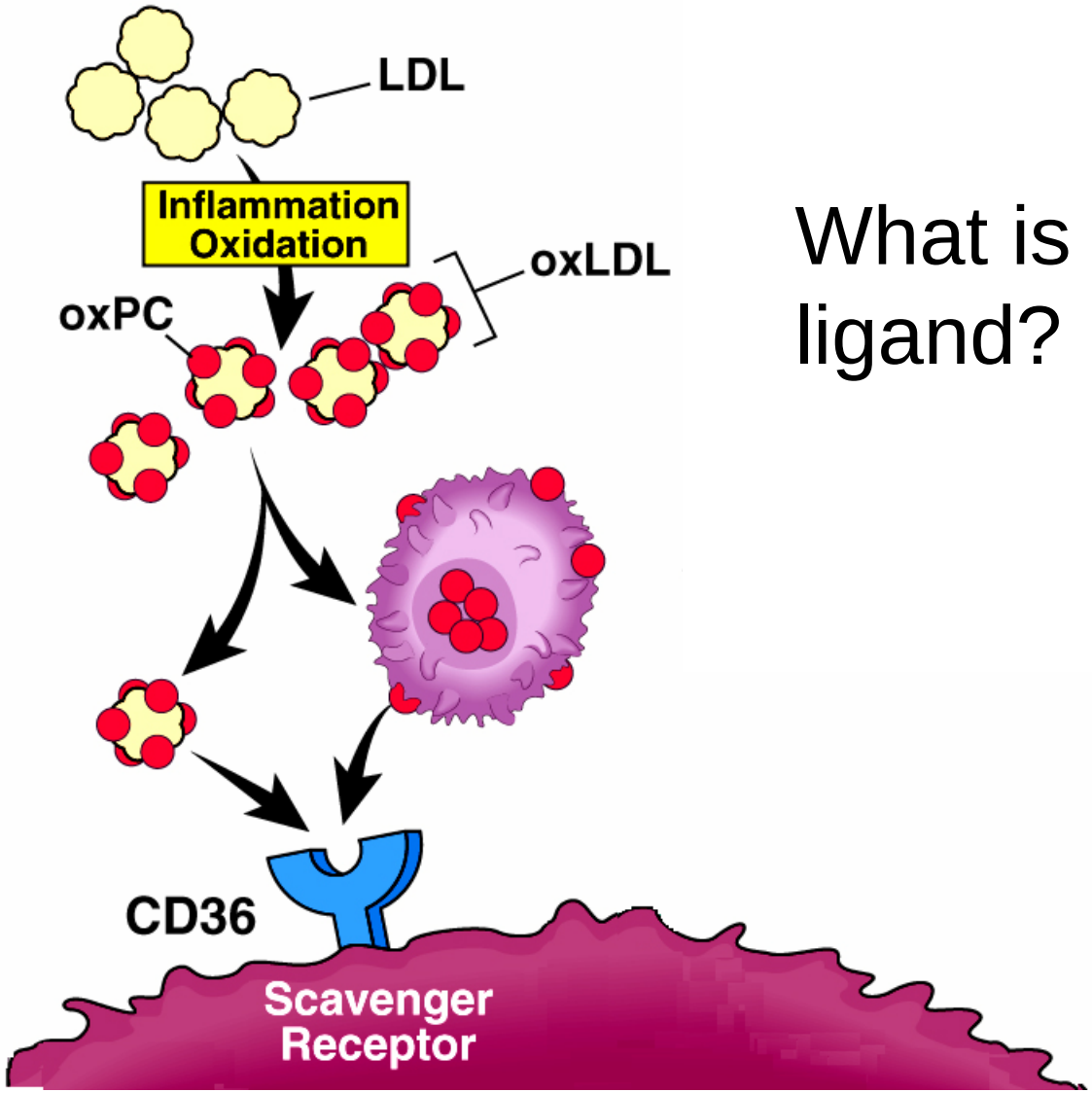
From: Zhang et al, Blood, 2003
See also: Hazen et al, Circ Res, 1999; Zhang et al, J Biol Chem, 2002

Monocytes Use the MPO/H_2O_2/Nitrite System to Convert LDL into a High Uptake Form



Podrez et al. (2000) *J. Clin. Invest.*

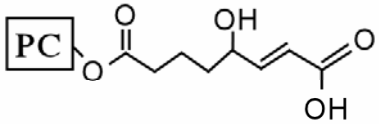
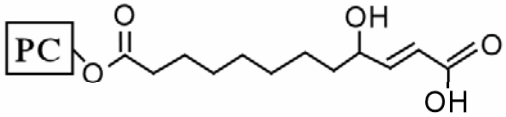
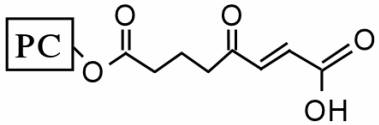
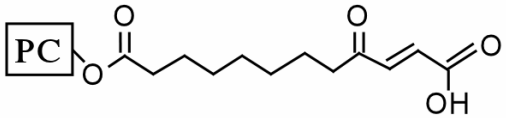
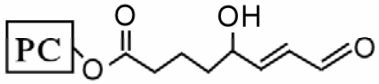
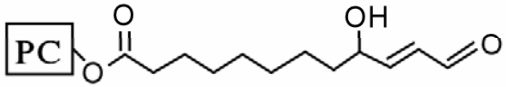
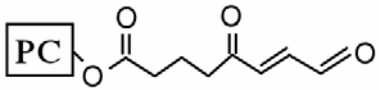
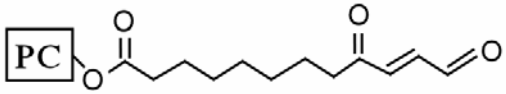
How do macrophages recognize oxLDL?

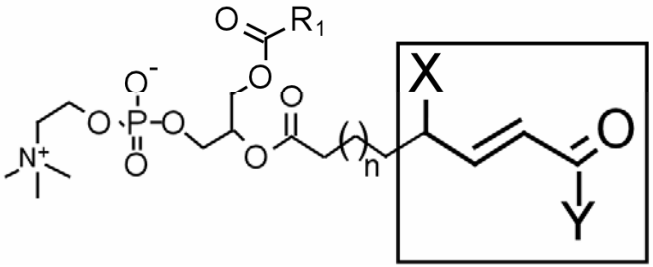


What is the ligand?

Adapted from Hazen and Chisolm, PNAS, 2004

Structure of $oxPC_{CD36}$ Species

Peak	PAPC series	PLPC series
I	 **HOdiA-PC**	 **HDdiA-PC**
I	 **KOdiA-PC**	 **KDdiA-PC**
II	 **HOOA-PC**	 **HODA-PC**
III	 **KOOA-PC**	 **KODA-PC**

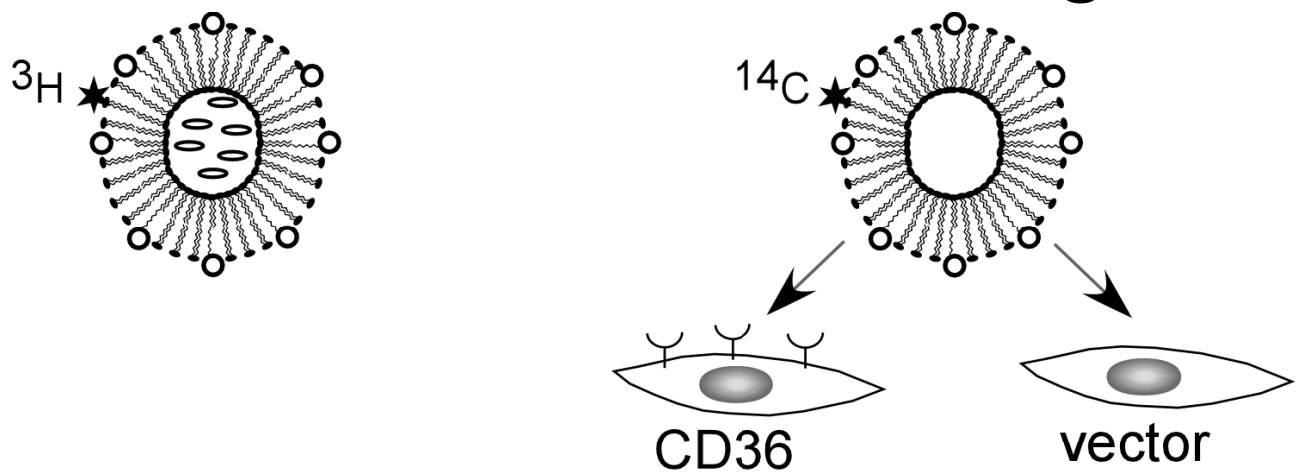


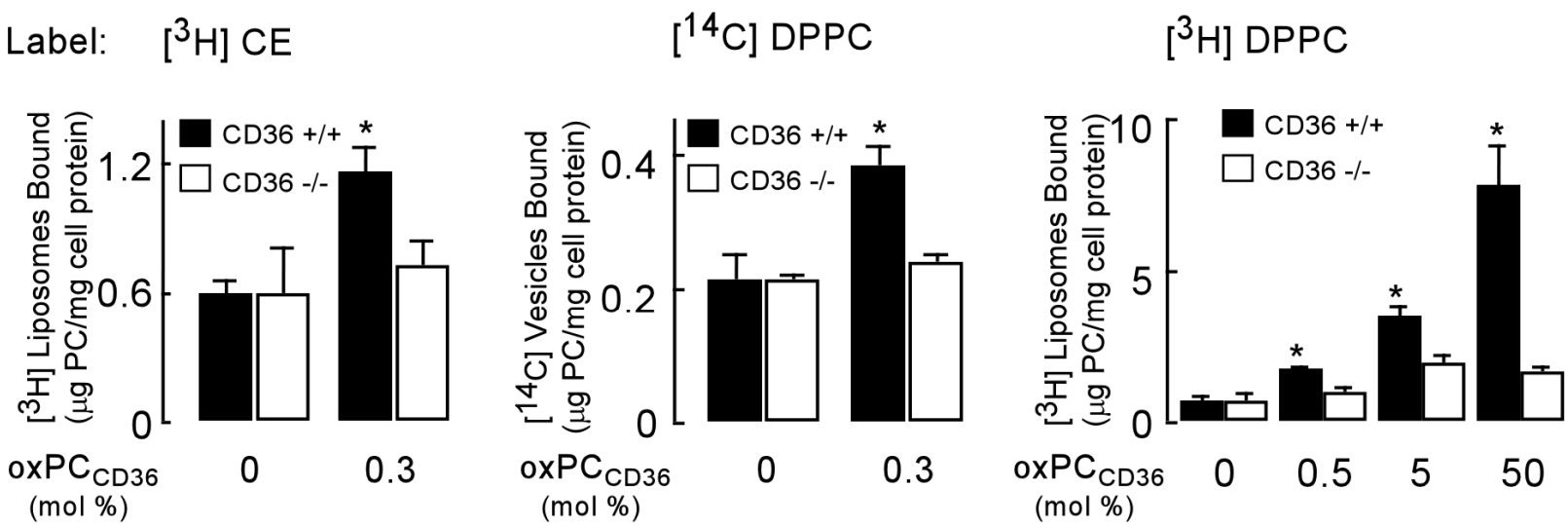
X = OH or =O

Y = OH or H

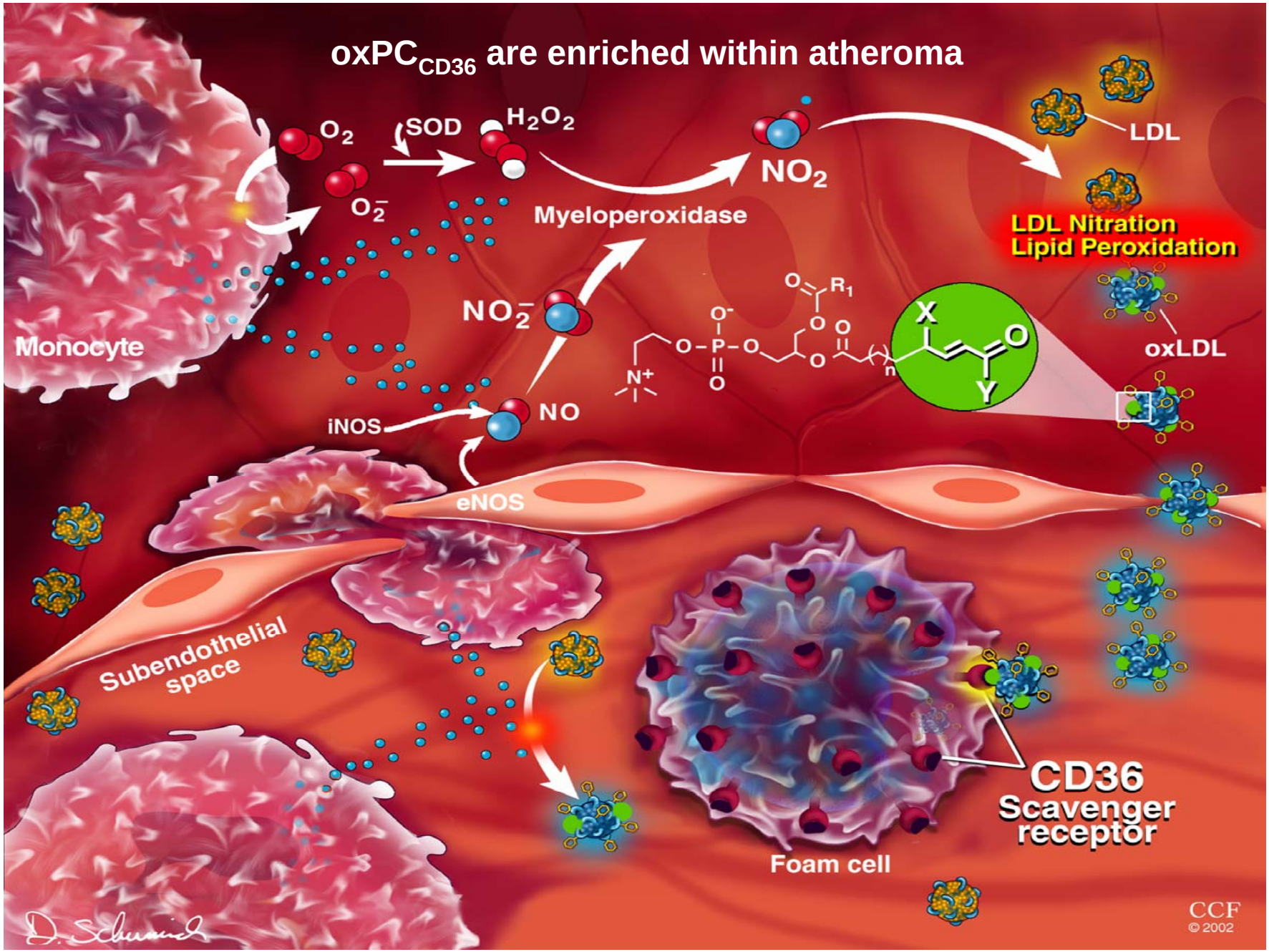
Podrez et al (2002) *J. Biol. Chem*. 277:38503-16

Only a few molecules of $oxPC_{CD36}$ per particle confer CD36 binding activity





Podrez et al (2002) *J. Biol. Chem*. 277:38517-23



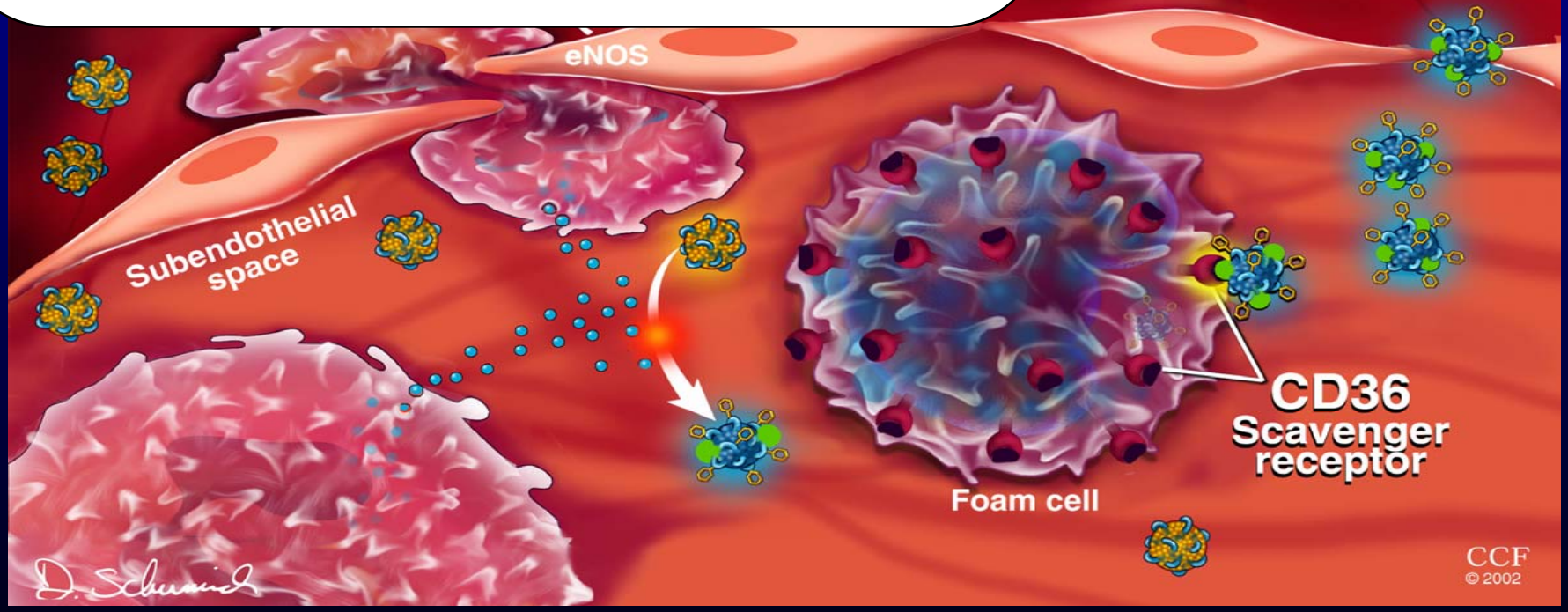
Podrez et al (2002) *J. Biol. Chem*. 277:38503-16
Podrez et al (2002) *J. Biol. Chem*. 277:38517-23

MPO and CVD risk

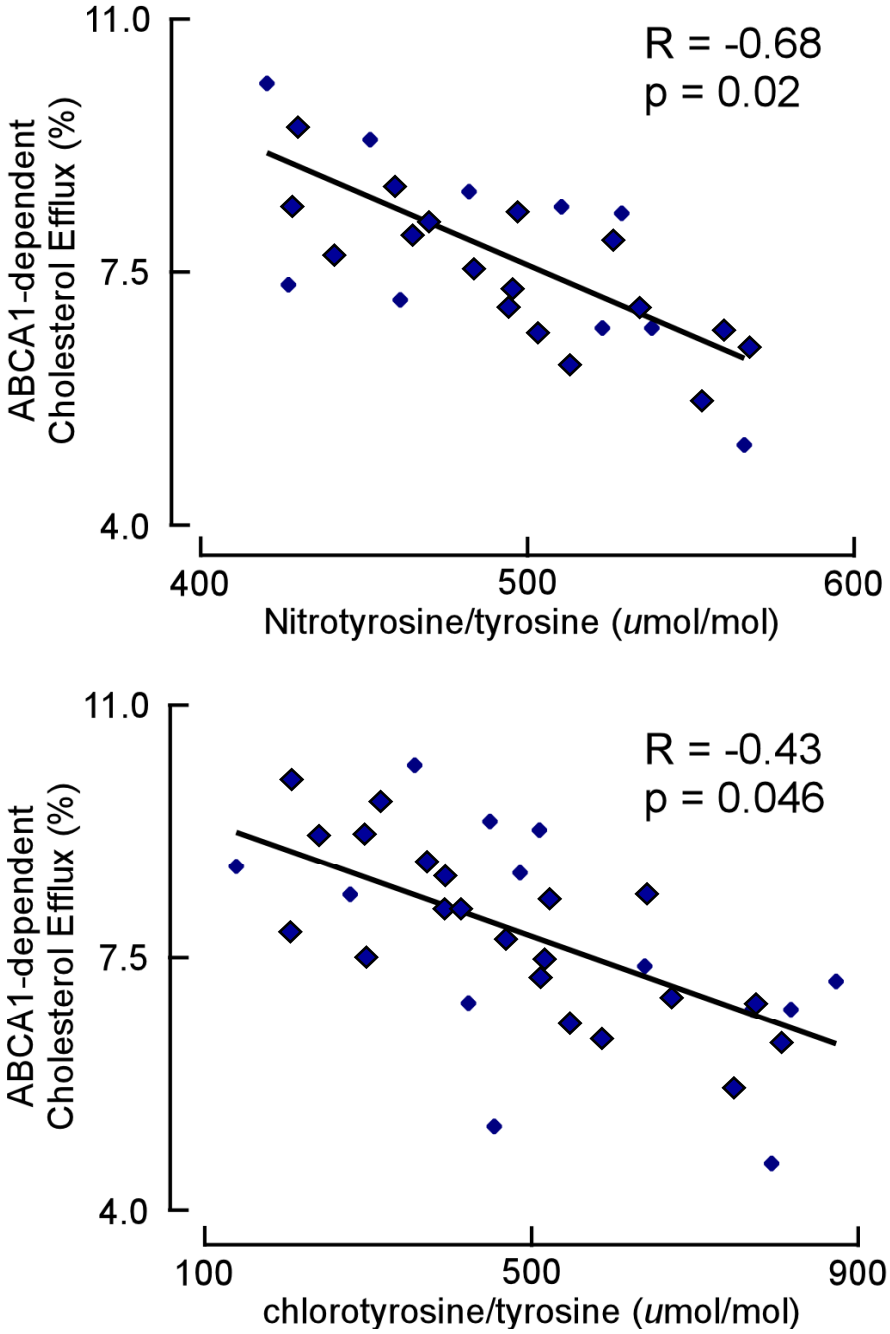
Zhang et al, 2001, *JAMA*; 281:2136
Brennan et al, 2003, *NEJM* ; 349:1595
Baldus et al, 2003, *Circ;* 108:1440
Baldus et al, 2004, *FRBM;* 37:902
Vita et al, 2004, *Circ ;* 110:134
Asselbergs, 2004, *NEJM*; 350:516
Hakonarson et al, 2005, *JAMA*; 293:2245
Tang et al, 2006, *Am J Cardiol*; 98:796
Kallantar et al, 2006, *Am J Kidn Dis;* 48:59
Ng et al, 2006, *Am Heart J*; 94:101
Exner et al, 2006*, JACC*; 47:2212
Mocatta et al, 2007, *JACC*; 49:1993
Covusoglu et al, 2007, *Am J Cardiol ;* 99:1364
Meuwese et al, 2007, *JACC*; 50:159
Tang et al, 2007, *JACC*; 49:2364
Wang et al, 2007, *Nature Med*; in press
Tardif et al, 2007, *Atheroscler.*; in press

NO_2Tyr and CVD risk

Shishehbohr, 2003, *JAMA* ; 289:1675
Zheng et al, 2004, *JCI* ; 114:529
Pennathur et al, 2004, *JBC ;* 279:42977
Hayashi et al, 2004, *Atheroscler*; 176:255
Zheng et al, 2005, *JBC* ; 279:42977
Shishehbor et al, 2006, *FRBM;* 41:1678
Parastatidis et al, 2007, *Circ Res;* 101:368
Nicholls et al, 2007, *Circulation*; in press



Podrez et al (2002) *J. Biol. Chem*. 277:38503-16
Podrez et al (2002) *J. Biol. Chem*. 277:38517-23



Serum apoA1 nitration and chlorination are correlated with impaired HDL mediated ABCA1-dependent cholesterol efflux function in vivo

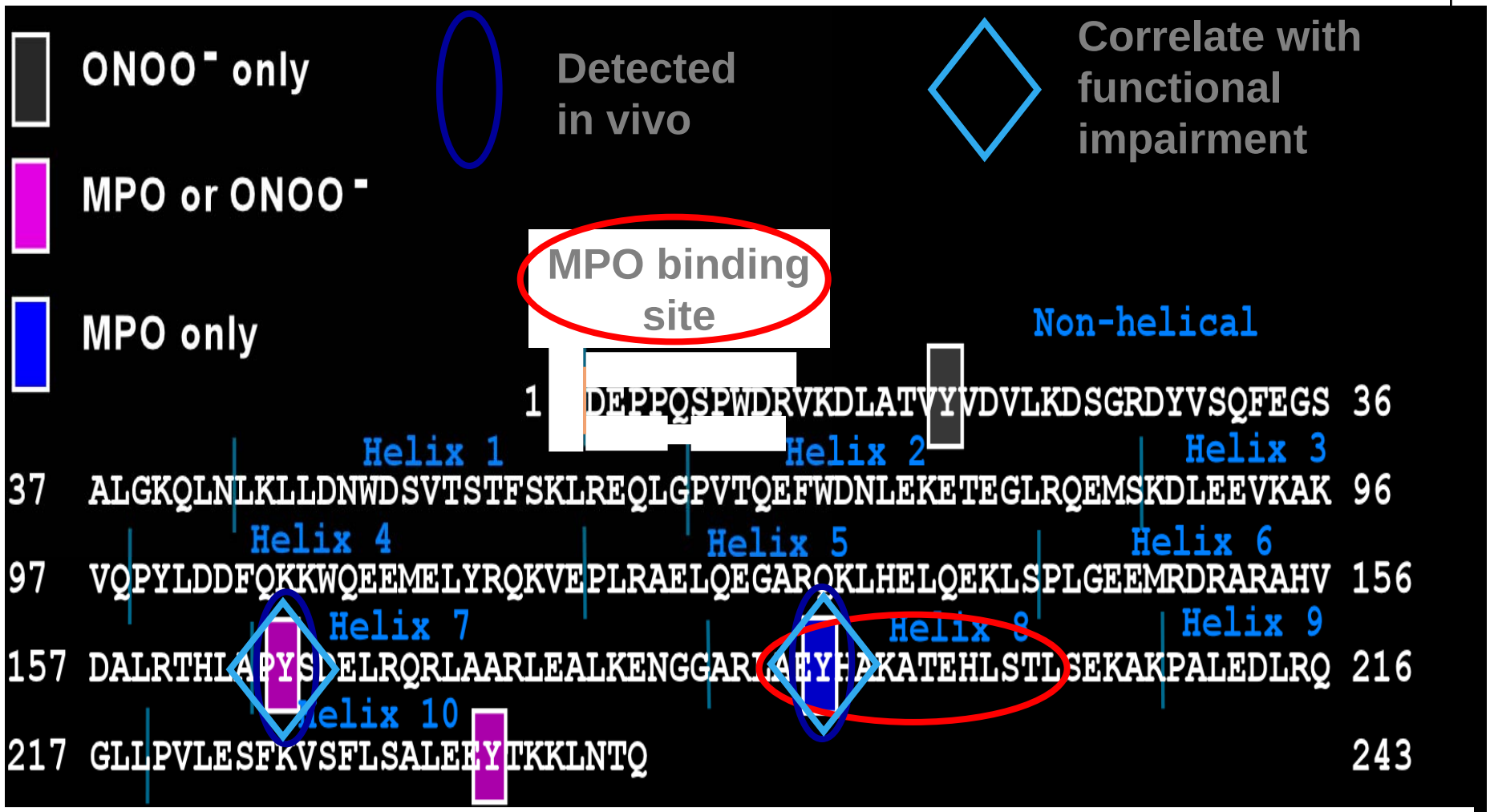
Case:Study (n=100)

Top tertile ClApoA1

16-fold risk for CVD

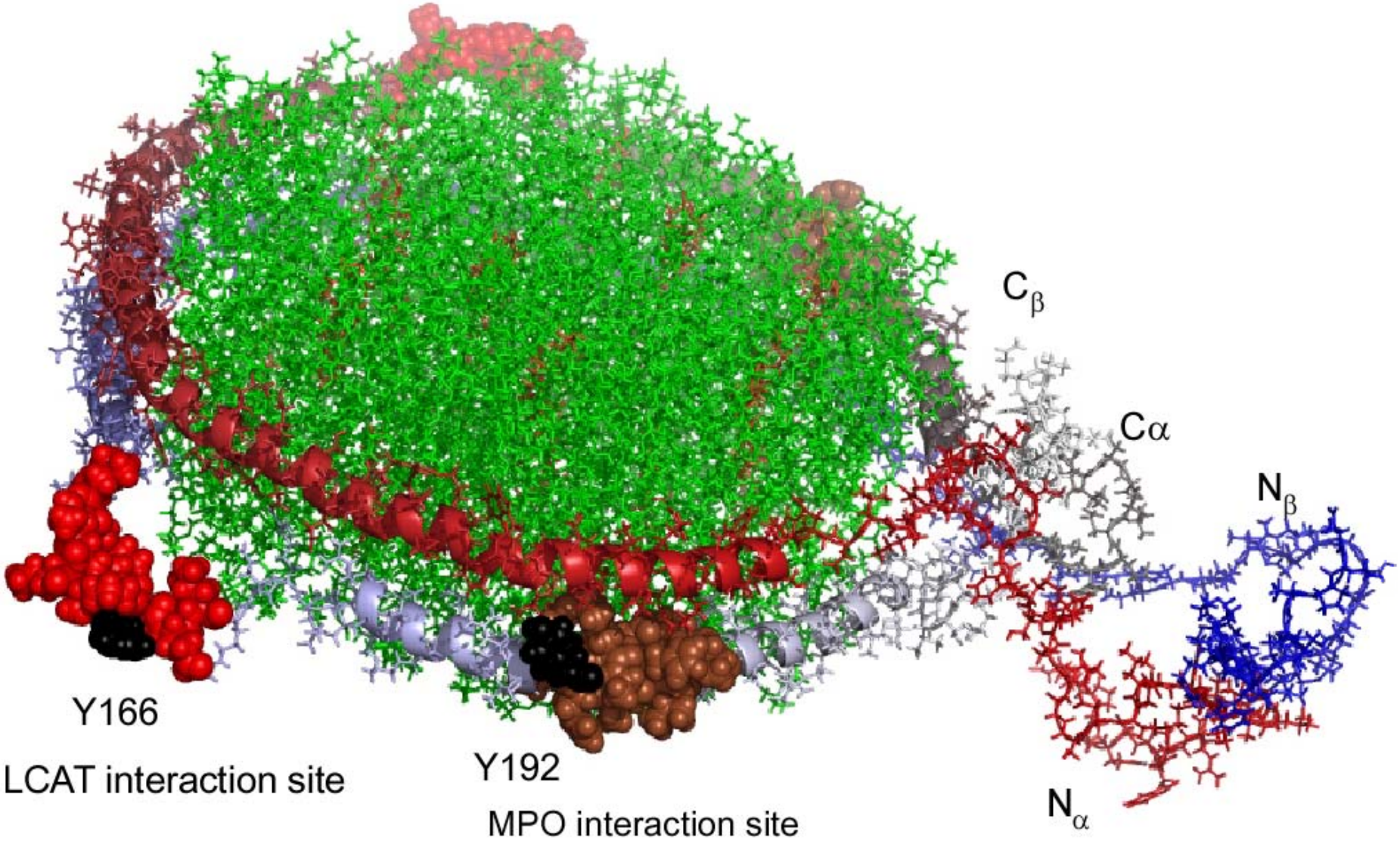
Adapted from Zheng et al, JCI, 2004

Map of apoAI Sites of Modification and MPO Interaction



Adapted from: Zheng et al, JCI, 2004; Zheng et al, JBC, 2005; Peng et al, JBC, 2005
See also: Bergt et al, PNAS, 2004; Shao et al, JBC 2005

The Solar Flares model of nascent HDL

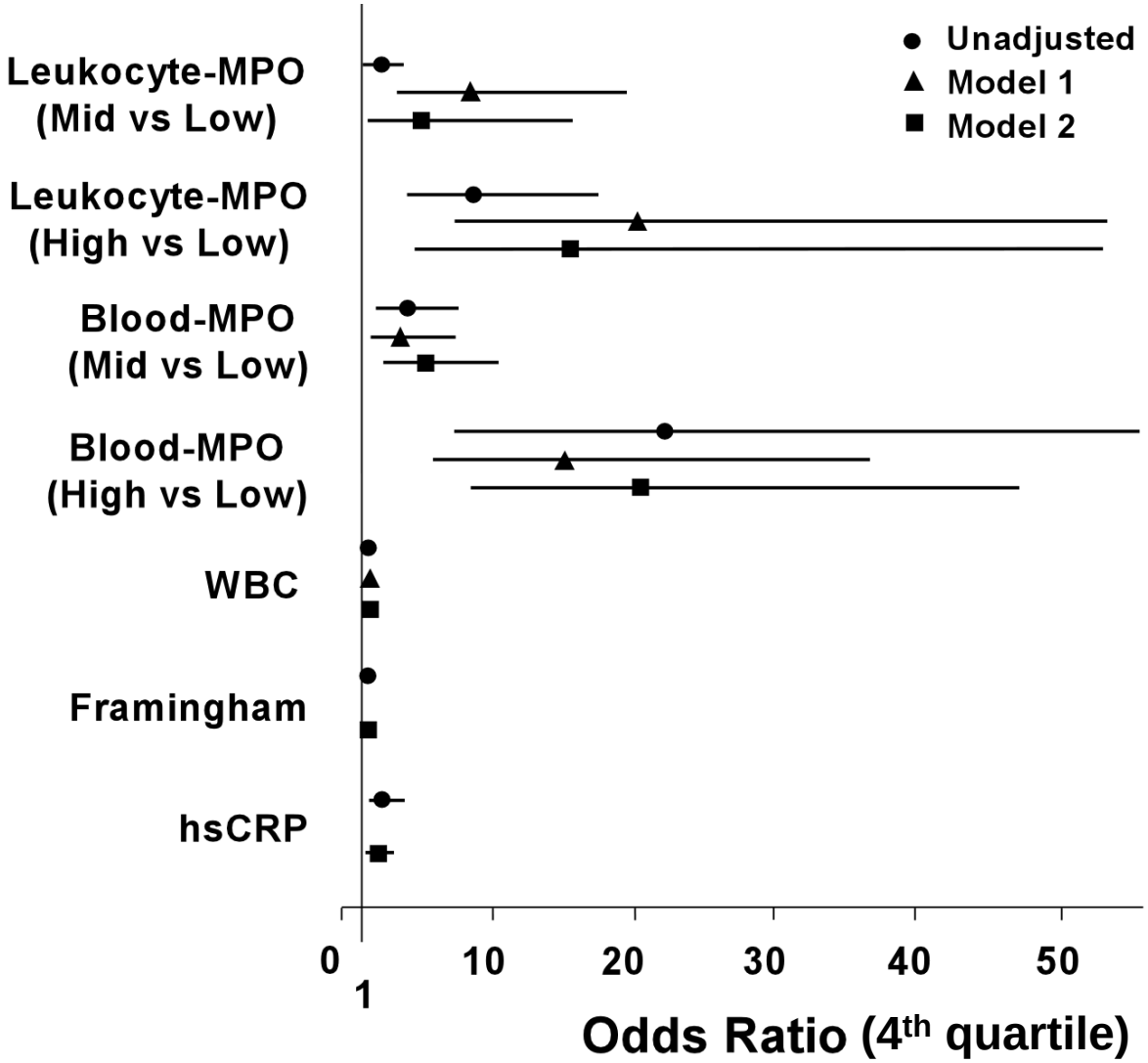


Wu et al, *Nature Structural & Molecular Biology*, 2007

Selected Published Clinical Literature

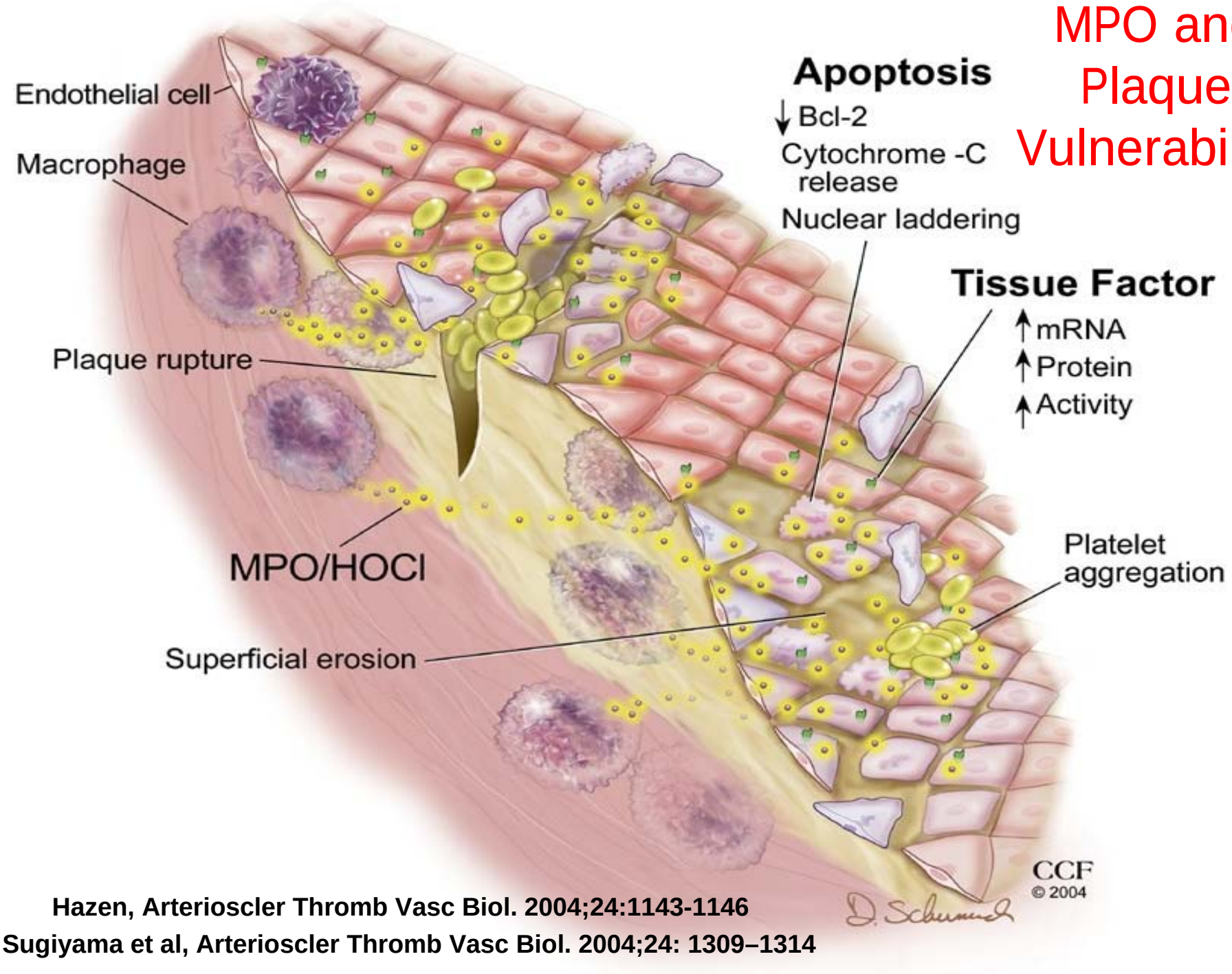
MPO : An inflammatory marker pathogenically linked to CVD

MPO levels are strongly correlated with cardiovascular disease prevalence



adapted from Zhang et al (2001) JAMA 286:2136-42

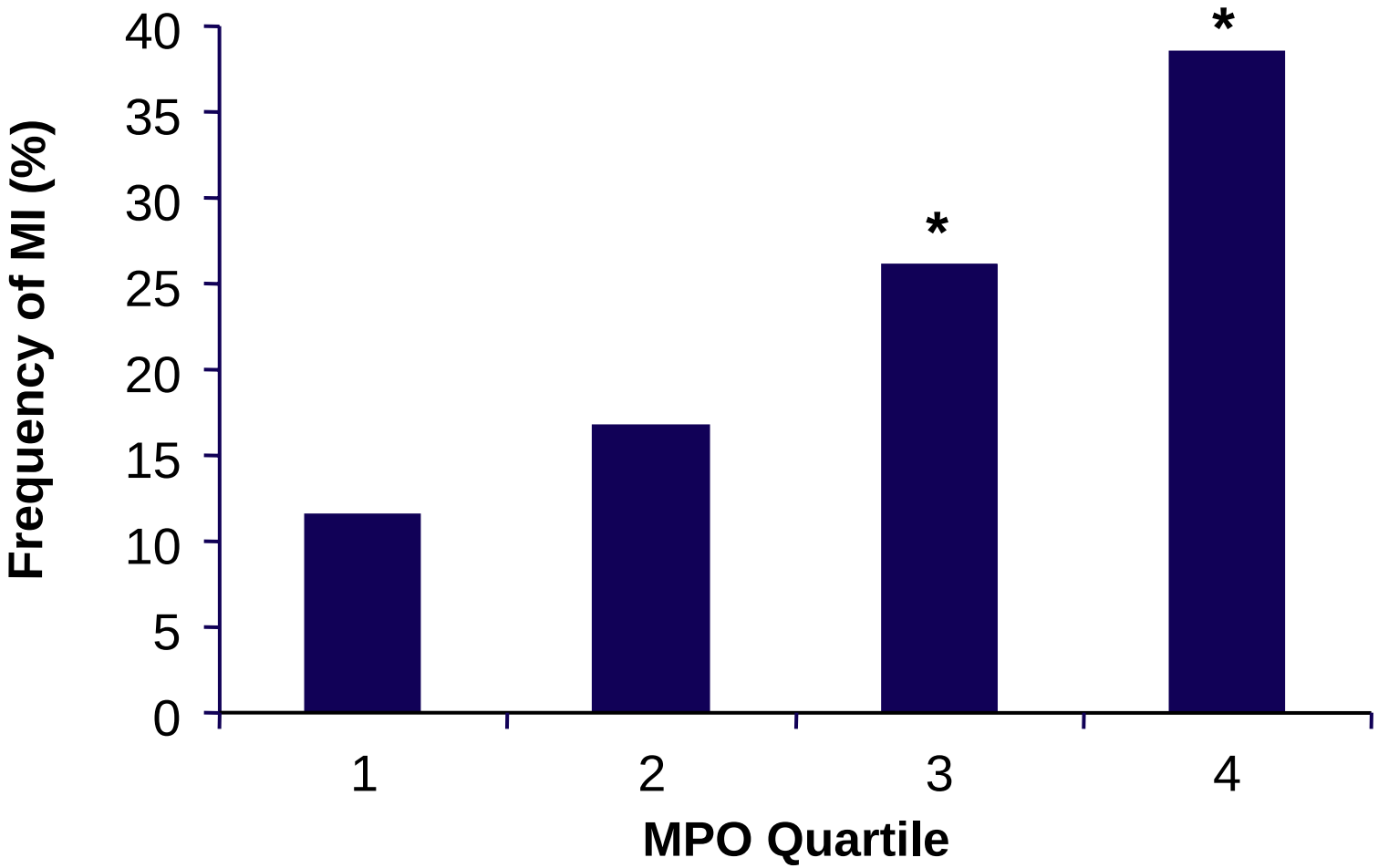
MPO and Plaque Vulnerability



Hazen, Arterioscler Thromb Vasc Biol. 2004;24:1143-1146

Sugiyama et al, Arterioscler Thromb Vasc Biol. 2004;24: 1309–1314

Initial baseline MPO level predicts risk for myocardial infarction in subjects presenting with chest pain



(pM)	<119	119 – 198	198 – 394	> 394

Brennan et al, NEJM, (2003) 349:1595-604

Relative Risk Associated with Major Adverse Cardiac Events per MPO Quartile

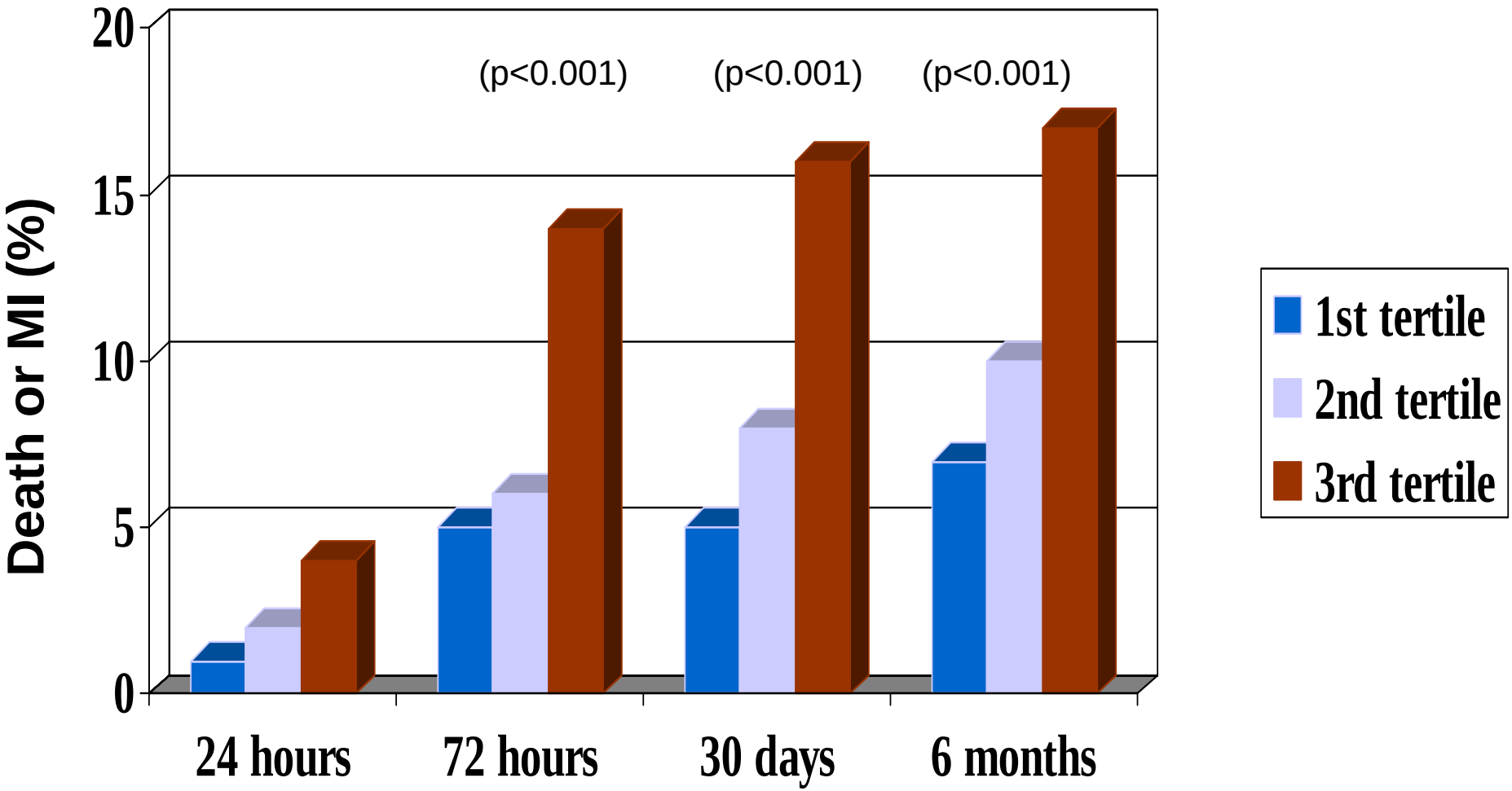
Sequential patients presenting to ER with chest pain

	MPO Quartile			
	Q1	**Q2**	**Q3**	**Q4**
(pM)	<119	119 – 198	198 – 394	> 394
MI at Evaluation				
All patients	1.0	1.2 (0.7-2.3)	2.1 (1.2-3.8)*	3.9 (2.2-6.8)**
MACE, 30 days				
All patients	1.0	1.7 (1.1-2.9)*	3.3 (2.0-5.5)**	4.9 (3.0-8.1)**
Troponin negative	**1.0**	**2.2 (1.5 – 3.1)****	**3.7 (2.5 – 6.1)****	**5.7 (2.9 – 8.6)****
MACE, 6 months				
All patients	1.0	1.6 (1.0-2.7)*	3.6 (2.2-5.8)**	4.7 (2.9-7.7)**
Troponin negative	**1.0**	**2.5 (1.7 – 3.6)****	**3.8 (2.4 – 6.5)****	**5.8 (2.4 – 9.3)****

*** p<0.05 relative to Q1**
**** p<0.01 relative to Q1**

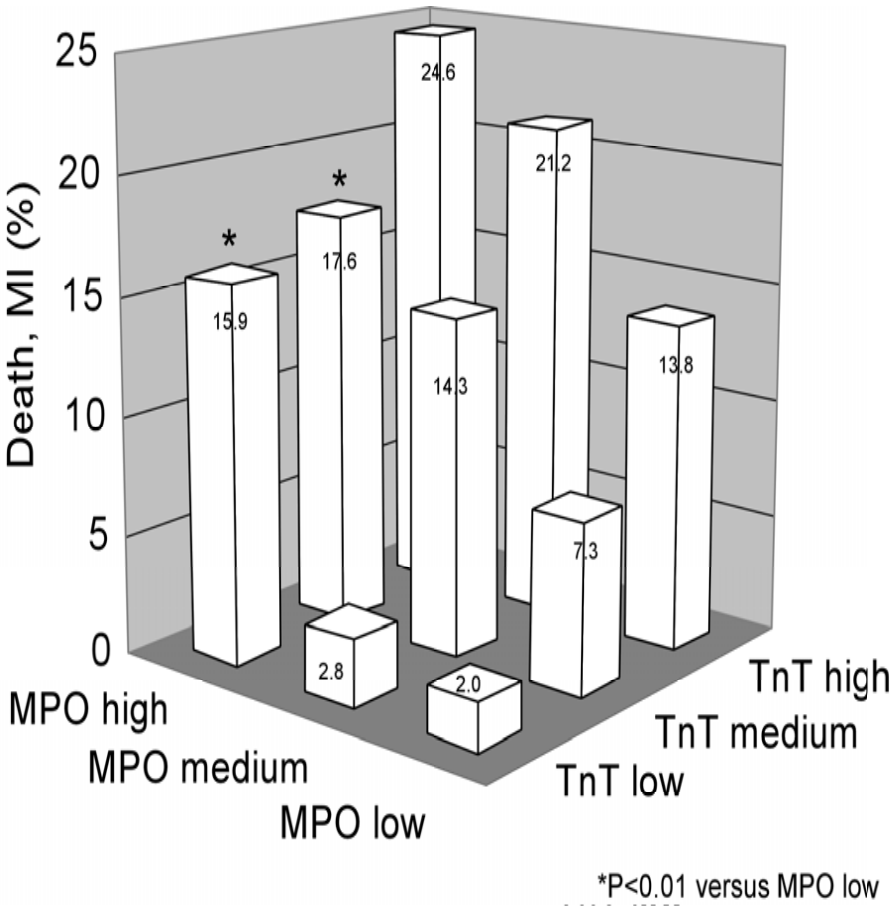
Adapted from Brennan et al, NEJM, (2003)

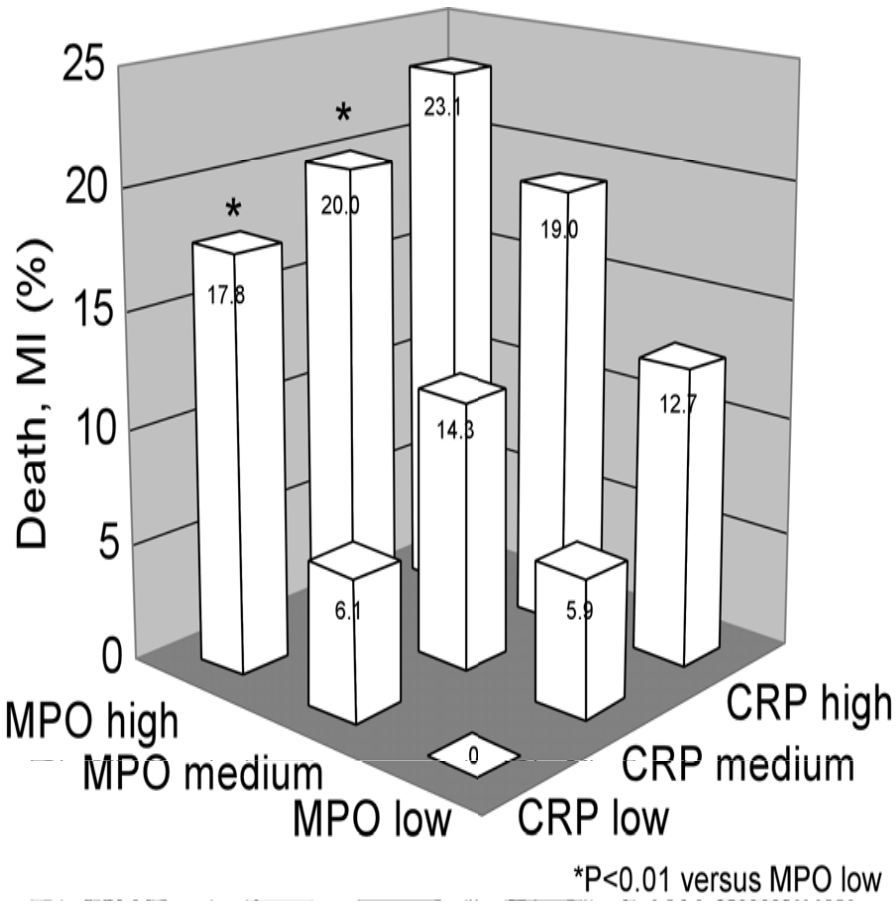
Results: Elevated MPO predicts increased short and long term risks of MI and death



Baldus, et al. Circulation 2003;108: 1440-5.

Additive predictive value of elevated MPO levels for cumulative (6mo) incidence of death or non-fatal MI

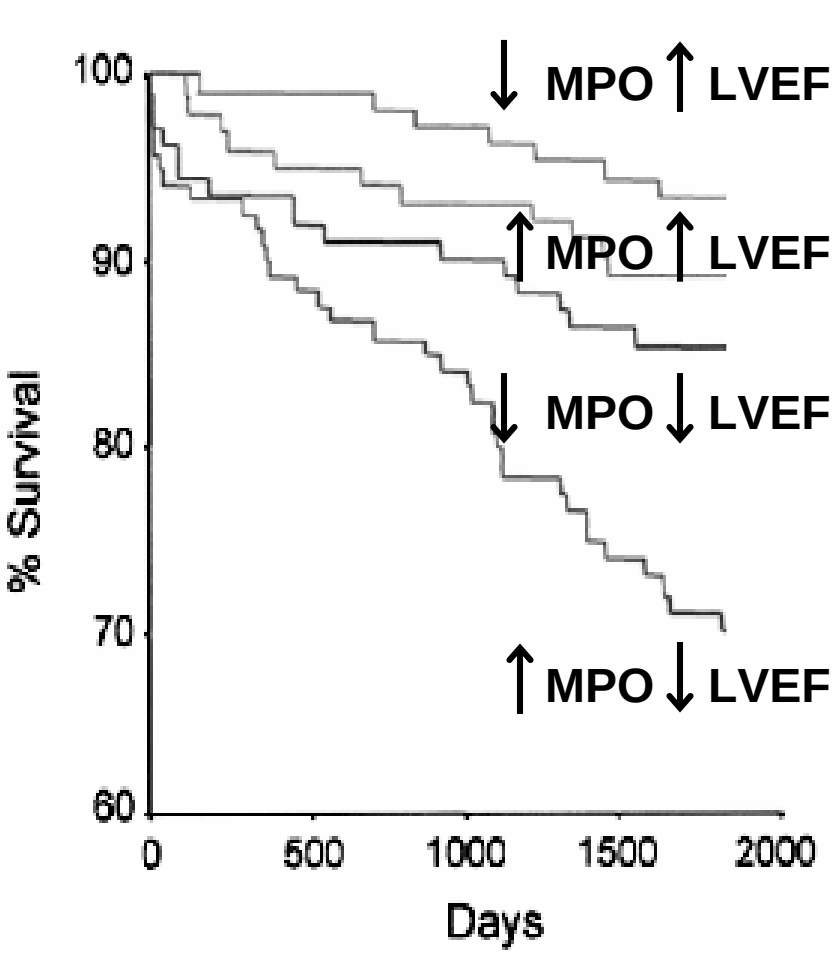


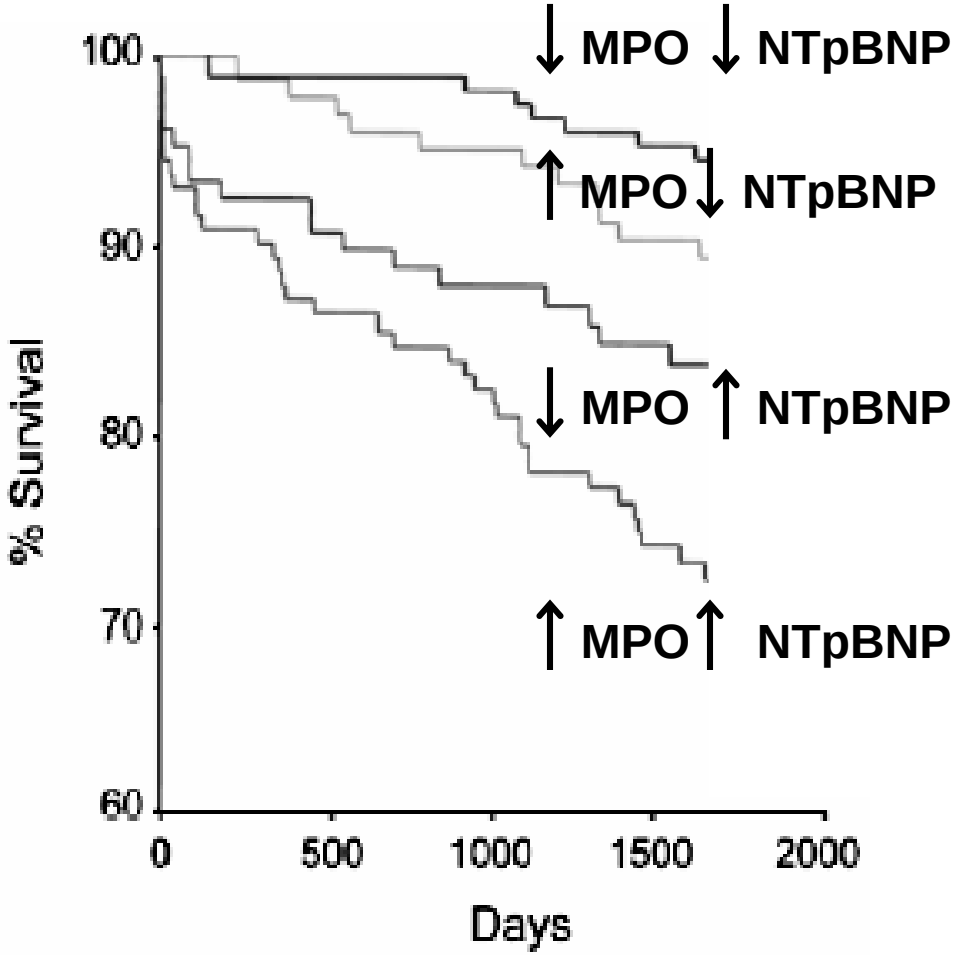


Baldus et al, Circulation (2003) 108:1440

Plasma Concentrations of Myeloperoxidase Predict Mortality After Myocardial Infarction. (Mocatta et al, J Am Coll Cardiol 2007; 49:1993.)

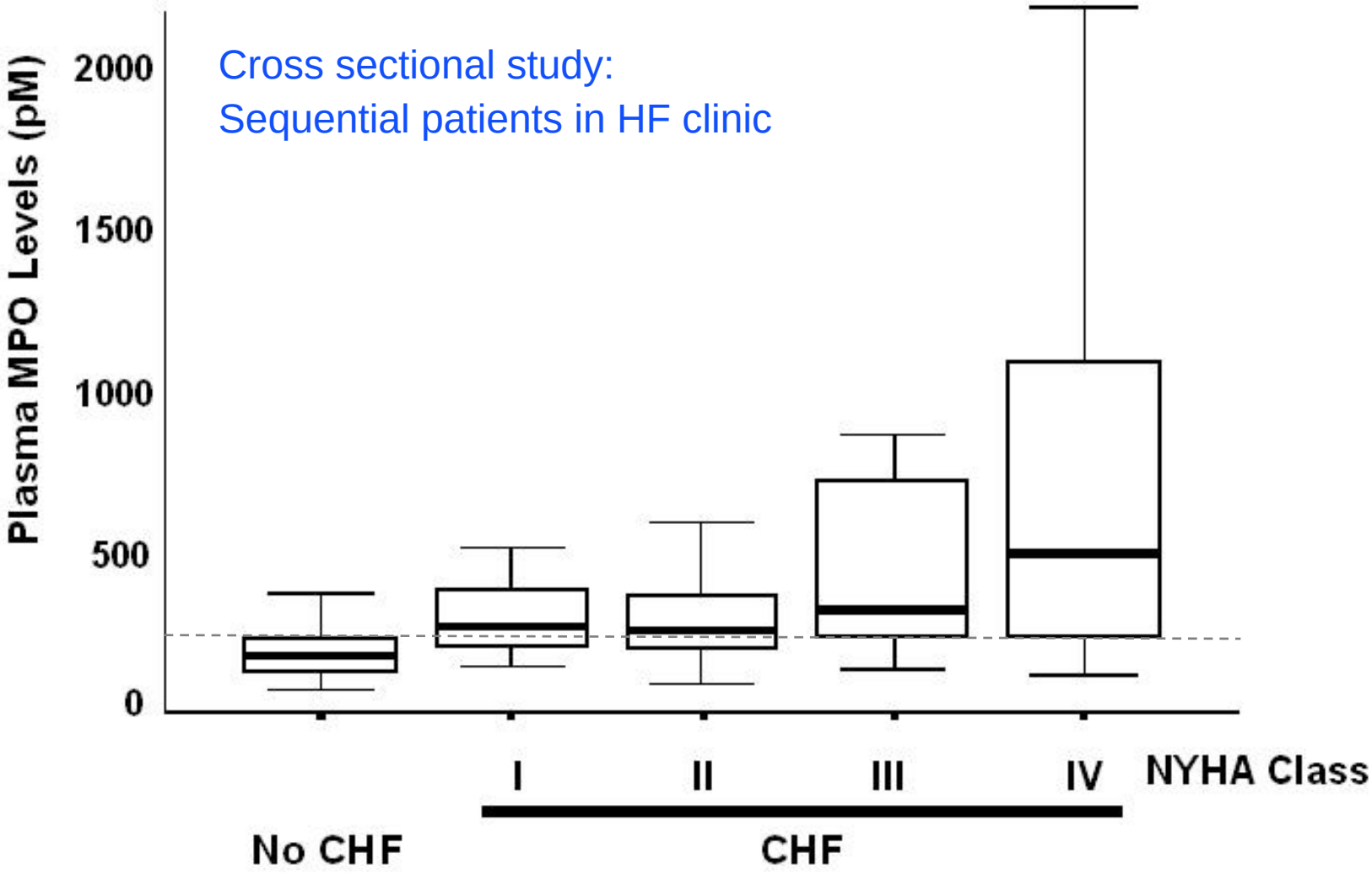
N=512, <80yo with MI and survived ≥ 24hr, 5yr survival monitored





Elevated MPO levels are associated with heart failure

(n=50 each group)

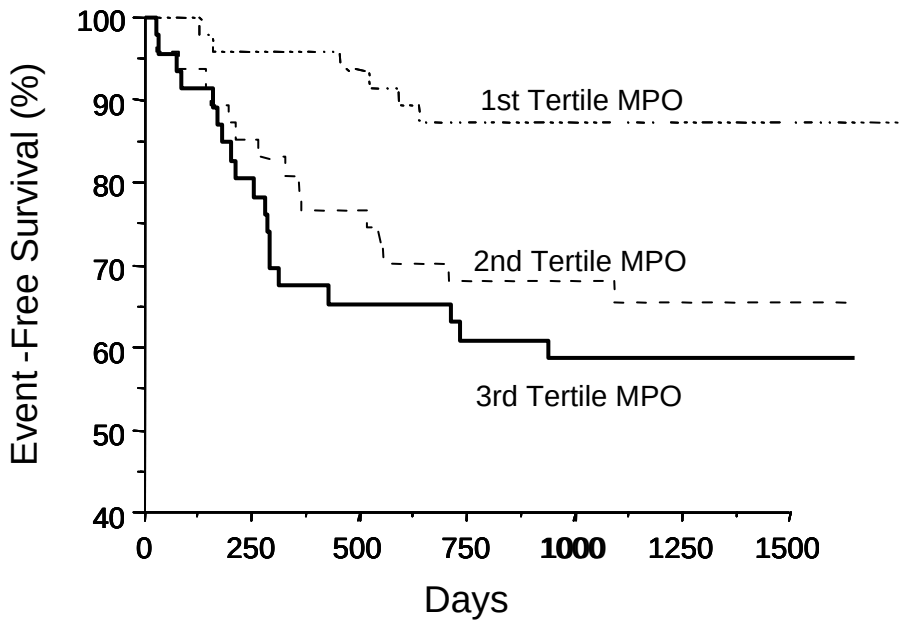


WH Tang et al, 2006

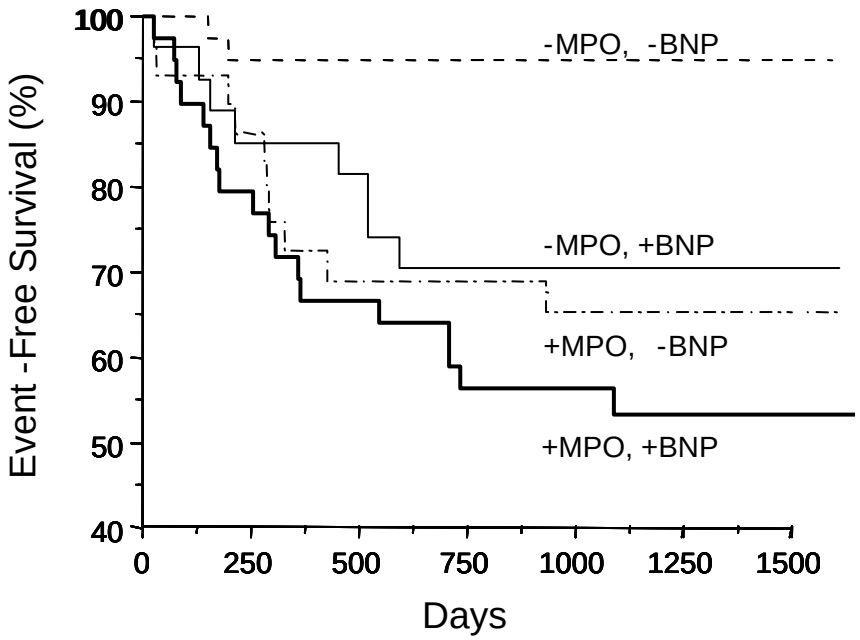
Kaplan-Meier analysis of all-cause death, cardiac transplantation, or heart failure hospitalizations stratified according to baseline plasma MPO and BNP levels.

N=140, EF <35%

Log -rank p=0.007



Log -rank p=0.002



–/+MPO = below/above median MPO
–/+BNP = below/above median BNP

WH Tang et al, Prognostic Value of Plasma Myeloperoxidase Levels Across the Spectrum of Systolic and Diastolic Dysfunction in Patients with Chronic Heart Failure, *J Am Col Cardiol*, 2007.

EPIC/NORFOLK study: Community screen of apparently healthy subjects monitored of 6 yrs Controls (N=2,237) : Cases (N=1,138)

M Meuwese, E Stroes, SL Hazen, J van Miert, J Kuivenhoven, R Schaub, N Wareham, R Luben, J Kastelein, K Khaw, S Boekholdt. (JACC 2007)

MPO predicts risk in subgroups otherwise associated with low risk

	Future CAD Events (MI and Death)	
	MPO < 600 pM	MPO ≥600 pM
LDL < 130 mg/dL	1	1.77 (1.24 - 2.53)
LDL > 130 mg/dL	1.71 (1.25 - 2.34)	2.14 (1.58 - 2.90)
HDL > 50 mg/dL	1	1.57 (1.26 - 1.97)
HDL < 50 mg/dL	1.98 (1.54 - 2.55)	2.21 (1.75 - 2.78)
CRP < 2 mg/dL	1	1.24 (1.00 - 1.53)
CRP > 2 mg/dL	1.89 (1.49 - 2.41)	2.39 (1.95 - 2.93)

Development of in vitro diagnostic assays for MPO

- PrognostiX Inc. – 2005, CardioMPO becomes first FDA cleared in vitro diagnostic test for MPO in clinical use.
- Abbott and Dade - developing MPO assays for automated high throughout instrument platforms.
- BioSite - developing MPO assay for point of care platform.
- Dade – high throughput assay submitted to FDA (2007)

Standardization

- PrognostiX CardioMPO serving as gold standard
- PrognostiX, Abbott and Dade have assigned calibrator values spectrophotometrically using the molar absorptivity (178,000 $M^{-1}cm^{-1}$) of the MPO Soret band (430 nm).
- Abbott, PrognostiX, Dade and BioSite have agreed to uniform reporting of units (pmol/L).

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14th Annual Meeting

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