LIPID Study 20th Anniversary Meeting
26th October 2018 12-5pm Sydney University

LIPID Biomarker Studies

Major findings guiding future research and practice

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Disclosure Statement of Financial Interest

Within the past 12 months, I or my spouse/partner have had a financial interest/ arrangement or affiliation with the healthcare related company listed below

Relationship	Company
Research Grants	Sanofi Aventis; Eli Lilly; NIH; Omthera Pharmaceuticals, Pfizer, Elsai Inc.
Lecture fees	AstraZeneca; DalCor Pharmaceuticals, CSL Boehring Sanofi Aventis
Advisory Boards	Acetelion, Sirtex

Academic Disclosures

Executive committees:

LIPID, ODYSSEY

Steering committees:

A- Z, Dal outcomes, IMPROVE –IT, SPIRE 1 and SPIRE 2 Trials, ACELERATE, STRENGTH, Dal-GenE, AEGIS-II, CLEAR OUTCOMES

Member:

Cholesterol Treatment Trialists (CTT) Collaboration, 1995 - now looking at adverse events in 160,000 patients

Guideline committees:

National Heart Foundation of Australia Lipid Guidelines, New Zealand Cardiovascular Risk Guidelines, Australian New LIPID Guidelines

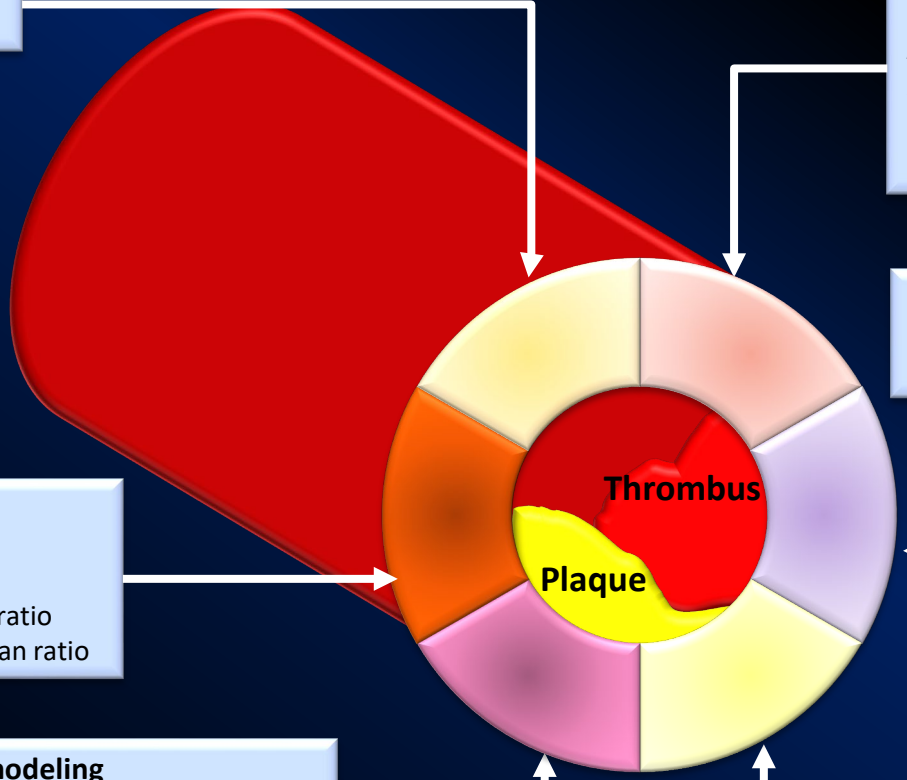
LIPID

Long-Term
Intervention with
Pravastatin in
Ischaemic
Disease

Selected Cardiovascular biomarkers associated with prognosis in stable ischemic heart disease

Novel biomarkers
microRNAs

Metabolic
Homocysteine
Adiponectin
S-RAGE
Ceramides, dimethylglycine, choline
Lipoprotein (a)



Myocardial injury
High sensitivity cardiac troponin (hs-cTnI)
High sensitivity cardiac troponin (hs-CTnT)

Renal/Urine
Serum creatinine
Cystatin-C
Urine albumin:creatinine ratio
Urine kynurenin:tryptophan ratio

Myocardial stress and remodeling
B-type natriuretic peptide (BNP)
N-terminal pro-B-type natriuretic peptide (NT-proBNP)
Mid-regional pro-A-type natriuretic peptide (mr-proANP)
Mid-regional pro-adrenomedullin (mr-prADM)
Growth differentiation factor 15 (GDF-15)
Soluble suppression of tumorigenicity 2 (sST2)
Renin
Copeptin
Pro-endothelin-1

Markers of matrix and cellular remodelling
Including collagen pro-peptides and degradation products, matrix metalloproteinases and their inhibitors

Inflammatory markers
C-reactive protein
White blood count
Pentraxin 3
Growth differentiation factor-15
Soluble suppression of tumorigenicity (sSST2)
Myeloperoxidase
Lipoprotein-associated phospholipase A2
Osteoprotegerin
Interleukin-6
Neopterin
Kynurenin:tryptophan ratio

White HD.Future Med Ltd; 2012.p18-29 / Omland & White. Clin Chem. 2017;63(1):165-176

Selected Cardiovascular biomarkers associated with prognosis in stable ischemic heart disease

Novel biomarkers
microRNAs

In LIPID

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Homocysteine
Adiponectin
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Ceramides, dimethylglycine, choline
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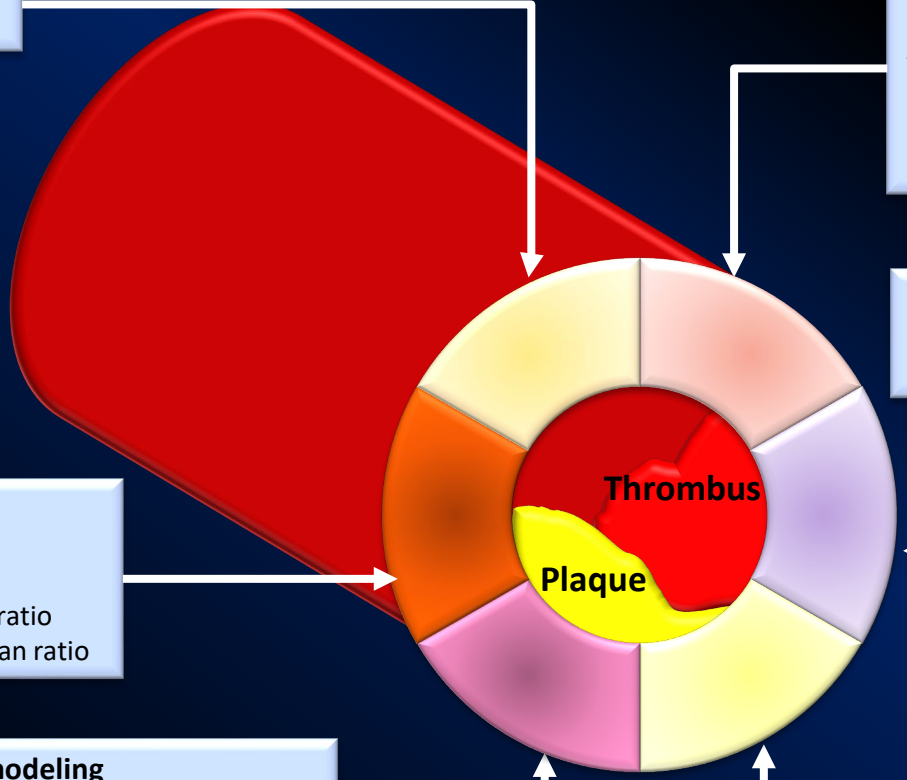

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White HD.Future Med Ltd; 2012.p18-29 / Omland & White. Clin Chem. 2017;63(1):165-176

LIPID Study

The LIPID study was conducted over 20 years ago

- However the cohort has ongoing major relevance to current clinical management
- The study was undertaken in patients who were at least three months after their qualifying myocardial infarction or hospitalisation for unstable angina. Therefore subsequent major developments in the management of acute coronary syndromes are not pertinent

LIPID Study

- There was very high background usage of present evidence-based therapies for ongoing prevention in randomised patients
- Furthermore patients had a broad range of cholesterol levels reflecting those in usual clinical practice
- The cohort is one of the most well-characterised in research trials in CHD with ascertainment of vital status in all but one patient and adjudication of major cardiovascular events, the endpoints in the biomarker studies, in all

Methods

- 9,014 patients randomized
- with cholesterol levels 4.0 – 7.0 mmol/L (155-217 mg/dL)
- 3 – 36 months after MI or admission with unstable angina
- receive placebo or pravastatin 40mg/day

All analyses were pre-specified in a biomarker protocol with CHD death/MI as primary endpoint

LIPID: Biomarkers Studies

- White blood cell count predicts reduction in coronary heart disease mortality with pravastatin. Stewart RAH et al. Circulation 2005; 111: pp. 1756-62
- The value of N-terminal fragment of brain natriuretic peptide and tissue inhibitor of metalloproteinase-1 levels as predictors of cardiovascular outcome in the LIPID study. West MJ et al. Eur Heart J 2008; 29(7):923-31
- Changes in lipoprotein-associated phospholipase A2 activity predict coronary events and partly account for the treatment effect of pravastatin results from the Long-Term Intervention with Pravastatin in Ischemic Disease study. White HD. et al. J. Am. Heart Assoc. 2013; 2: pp. e000360
- Plasma lipoprotein(a) concentration predicts future coronary and cardiovascular events in patients with stable coronary heart disease. Nestel PL et al. Arterioscler. Thromb. Vasc. Biol. 2013; 33: pp. 2902-8
- Association of contemporary sensitive troponin I levels at baseline and change at 1 year with long-term coronary events following myocardial infarction or unstable angina: results from the LIPD study. White HD. et al. J. Am. Coll. Cardiol. 2014; 63: pp. 345-54
- Midregional proadrenomedullin and its change predicts recurrent major coronary events and heart failure in stable coronary heart disease patients: the LIPID study. Funke-Kaiser A. et al. Int. J. Cardiol. 2014; 172: pp. 411-8
- Biomarkers in stable coronary heart disease, their modulation and cardiovascular risk: The LIPID biomarker study. Tonkin AM et al., Int J Cardiol. 2015; 201:499-507
- D-dimer Predicts Long-Term Cause-Specific Mortality, Cardiovascular Events and Cancer in Stable Coronary Heart Disease Patients: The LIPID study. Simes J et al. Circulation. 2018; 138:712-23

Effect of Biomarker and and their Change for Risk Prediction in the LIPID Study

Stefan Blankenberg, Andrew Tonkin, Adrienne Kirby, David Colquhoun, David Hunt, Anthony Keech, Paul Nestel, Paul Glasziou, David Sullivan, Peter Thompson, Malcolm West, Harvey White, Tanja Zeller, Wendy Hague, Kristy Mann, John Simes for the LIPID Study Group

University Heart Center, Hamburg, Germany
Monash University, Melbourne, Australia
Clinical Trial Center, Sydney, Australia

Orlando, November 14th, 2011

Background

- Biomarkers reflecting mechanisms of inflammation, micronecrosis, hemodynamics, coagulation, lipid metabolism and renal function predict risk in both, primary and secondary prevention setting
- Risk estimation might be improved by the observation of biomarker changes over time
- Statin therapy might impact the level of various biomarkers
- Although the effect of statins on CHD is largely explained by LDL lowering, the treatment effect of statins might act beyond this action by influencing multiple biomarkers.

Aims

To assess

- the association between multiple biomarkers and coronary events (CHD death + MI) in the LIPID study
- the impact of pravastatin treatment on biomarker levels
- the extent of risk prediction and treatment effect explained by changes in biomarkers

Methods

post ACS pts (n=9 014)

cholesterol levels 4.0-7.0 mmol/L

Placebo (n=4 502)	Pravastatin (n=4 512)
Biomarker assessment	Biomarker assessment
Year 1 f/u	Year 1 f/u
Biomarker assessment	Biomarker assessment
CHD Endpoint (n=3 218)	CHD Endpoint (n=3 230)
5.9 years	5.9 years

Predictive Value of Baseline Biomarkers on CHD events

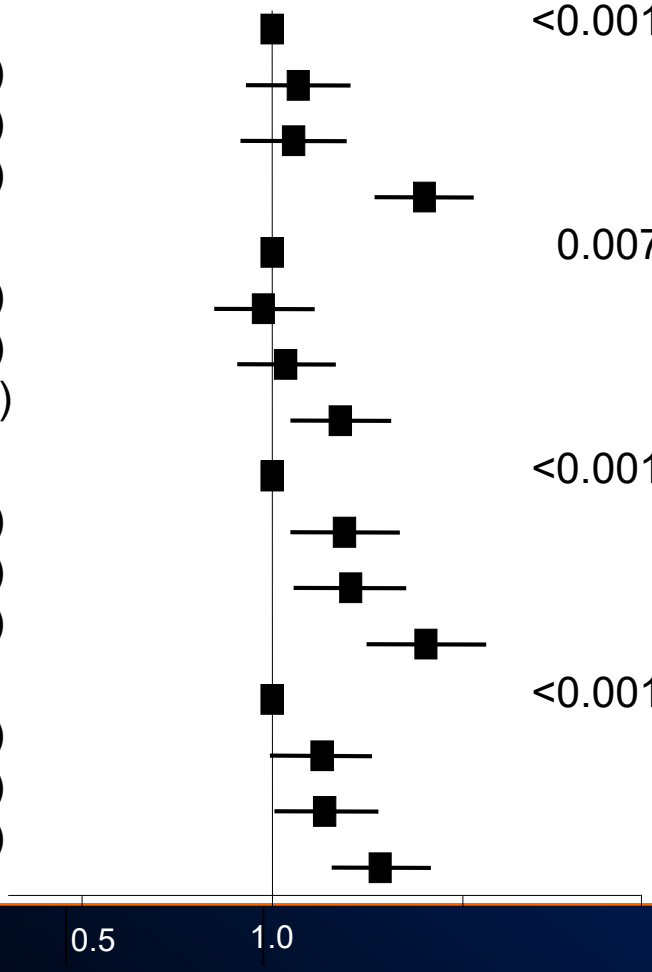
Baseline model includes:

treatment with Pravastatin, prior stroke, diabetes mellitus, current smoker, history of hypertension, total cholesterol, HDL, age, sex, type of prior acute coronary syndrome, timing of coronary revascularisation, SBP, atrial fibrillation, eGFR, BMI, dyspnoea grade, angina grade, WBC, peripheral vascular disease, aspirin, fasting glucose, triglycerides, Apo B, Apo A1

Each biomarker is added to this baseline model

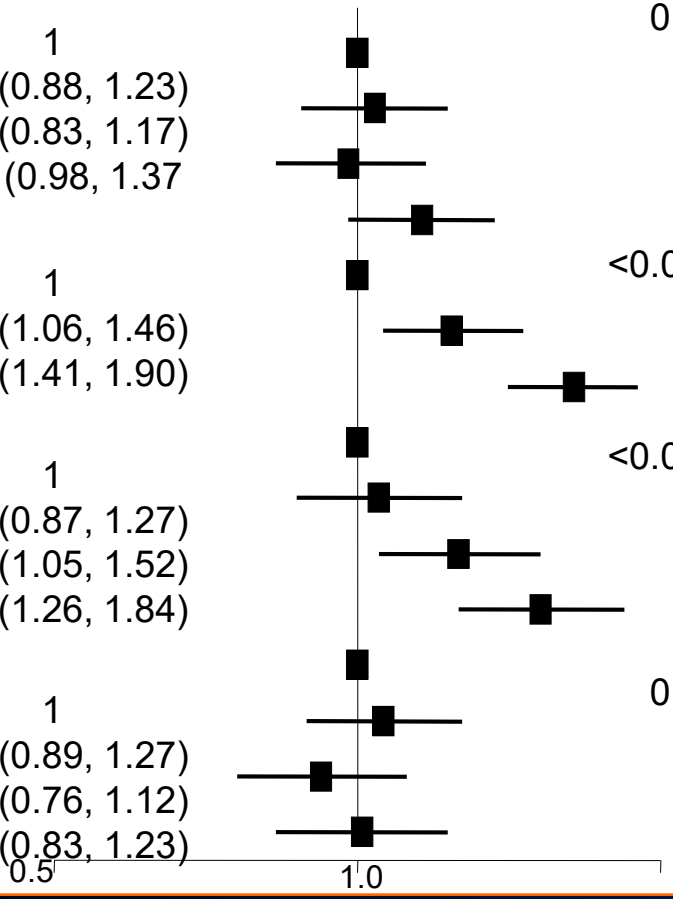
Effect of baseline levels of biomarker levels on coronary events adjusted for baseline model

Biomarker	Quartile levels	HR (95% CI)	P-value (trend)	P-value (interaction)
BNP (pg/ml)	≤9.73	1	<0.001	0.46
	9.73-23.36	1.10 (0.91, 1.33)		
	23.36-50.29	1.08 (0.89, 1.31)		
	>50.29	1.74 (1.45, 2.08)		
CRP (mg/L)	≤ 1.22	1	0.007	0.72
	1.22-2.43	0.97 (0.81, 1.17)		
	2.43-4.78	1.05 (0.88, 1.26)		
	> 4.78	1.28 (1.07, 1.54))		
Cystatin C (mg/L)	≤ 0.72	1	<0.001	0.56
	0.72-0.81	1.30 (1.07, 1.59)		
	0.81-0.93	1.33 (1.08, 1.63)		
	> 0.93	1.75 (1.41, 2.18)		
D dimer (ng/ml)	≤ 112	1	<0.001	0.99
	112-173	1.20 (0.99, 1.44)		
	173-273	1.21 (1.01, 1.47)		
	> 273	1.48 (1.24, 1.78)		



Effect of baseline levels of biomarker levels on coronary events adjusted for baseline model

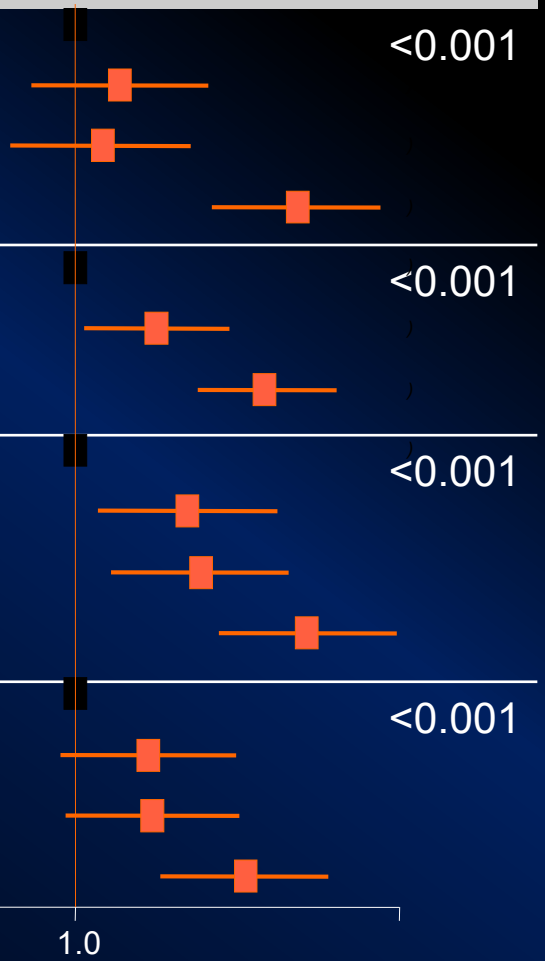
Biomarker	Quartile levels	HR (95% CI)	P-value (trend)	P-value (interaction)
LP a (mg/dL)	≤ 6.6	1	0.09	0.19
	6.6-13.9	1.04 (0.88, 1.23)		
	13.9-44.05	0.98 (0.83, 1.17)		
	> 44.05	1.16 (0.98, 1.37		
sen TnI (ng/ml)	nd	1	<0.001	0.19
	<0.018	1.24 (1.06, 1.46)		
	≥0.018	1.64 (1.41, 1.90)		
Mid-Regional pro-Adrenomedullin (nmol/l)	≤0.381	1	<0.001	0.93
	0.381-0.474	1.05 (0.87, 1.27)		
	0.474-0.578	1.26 (1.05, 1.52)		
	> 0.578	1.52 (1.26, 1.84)		
Lp-PLA_2 (nmol/min/ml)	≤229.142	1	0.91	0.42
	229.142-261.028	1.06 (0.89, 1.27)		
	261.028-293.767	0.92 (0.76, 1.12)		
	> 293.767	1.01 (0.83, 1.23)		



Effect of baseline levels of multiple biomarkers on coronary events

Multivariate model of all biomarkers that remain significant

Biomarker	Quartile levels	HR (95% CI)	P-value (trend)
BNP (pg/ml)	≤9.73	1	<0.001
	9.73-23.36	1.10 (0.91, 1.33)	
	23.36-50.29	1.06 (0.87, 1.28)	
	>50.29	1.61 (1.34, 1.92)	
sen TnI (ng/ml)	nd	1	<0.001
	<0.018	1.19 (1.02, 1.39)	
	≥0.018	1.50 (1.30, 1.75)	
Cystatin C (mg/L)	≤ 0.72	1	<0.001
	0.72-0.81	1.27 (1.05, 1.54)	
	0.81-0.93	1.31 (1.08, 1.58)	
	> 0.93	1.64 (1.36, 1.99)	
D dimer (ng/ml)	≤ 112	1	<0.001
	112-173	1.17 (0.97, 1.41)	
	173-273	1.18 (0.98, 1.42)	
	> 273	1.44 (1.20, 1.72)	



Predictive Value of Baseline Biomarkers

	Net Reclassification Index		Integrated Discrimination Index		C-statistics	
Biomarker added	NRI (%)	p-value	IDI	p-value	Without novel biomarker	With novel biomarker
BNP	5.96	0.001	0.0072	<.001	0.662	0.670
CRP	1.90	0.15	0.0021	<.001	0.662	0.664
Cystatin C	4.61	0.005	0.0031	<.001	0.662	0.667
D Dimer	1.63	0.27	0.0034	<.001	0.662	0.663
Troponin	6.42	<.001	0.0073	<.001	0.662	0.672
LP(a)	0.95	0.34	0.0006	0.04	0.662	0.662
Mid Regional Pro-Adrenomedullin	4.86	0.001	0.0025	<.001	0.662	0.666
PLA Activity	0.78	0.44	0.0005	0.03	0.662	0.662

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D Dimer	1.63	0.27	0.0034	<.001	0.662	0.663
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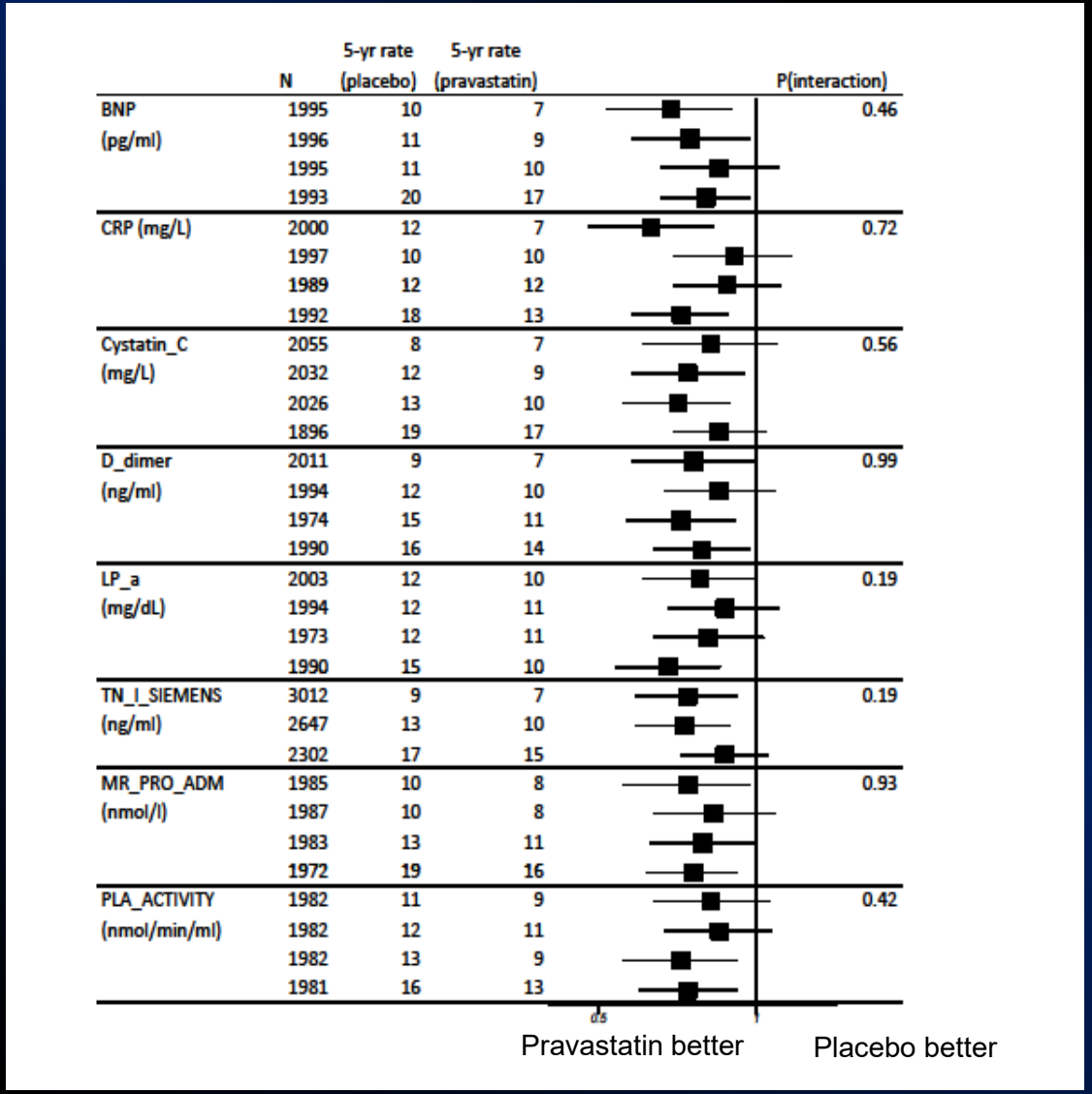
Mid Regional Pro-Adrenomedullin	4.86	0.001	0.0025	<.001	0.662	0.666
PLA Activity	0.78	0.44	0.0005	0.03	0.662	0.662

Effect of Pravastatin on CHD Events according to biomarker level



Effect of change of Biomarkers on Incident CHD added to baseline model

	Baseline Model*		Change in biomarker Model*	
Biomarker	HR (95% CI)	p value*	HR (95% CI)	p value**
BNP	1.74 (1.45, 2.08)	<0.001	1.37 (1.11, 1.69)	0.003
C-reactive protein	1.28 (1.07, 1.54)	0.007	1.11 (0.90, 1.37)	0.34
Cystatin C	1.75 (1.41, 2.18)	<0.001	1.21 (0.98, 1.49)	0.07
D-dimer	1.48 (1.24, 1.78)	<0.001	1.00 (0.81, 1.24)	0.96
Lp(a)	1.16 (0.98, 1.37)	0.09	1.11 (0.90, 1.36)	0.33
sensitive Troponin I	1.64 (1.41, 1.90)	<0.001	1.42 (1.11, 1.82)	0.01
Mid-Regional pro-ADRM	1.52 (1.26, 1.84)	<0.001	1.34 (1.08, 1.66)	0.007
Lp-PLA_2 Activity	1.01 (0.83, 1.23)	0.91	1.54 (1.17, 2.02)	0.002
LDL cholesterol	1.19 (0.93, 1.53)	0.16	1.18 (0.90, 1.56)	0.23

*Model adjusted for all baseline risk factors; **Model adjusted for baseline risk factors and baseline levels of biomarker

Net reclassification improvement and C statistic for each baseline biomarker and the most important clinical variables (adjusted for all baseline variables).

	Net reclassification improvement		*C* statistic	
Variable added	NRI (%)	*P*	Without variable	With variable
Biomarkers				
Sensitive troponin I (ng/L)	5.49	0.003	0.66	0.67
BNP (pg/mL)	4.33	0.02	0.66	0.67
Cystatin C (mg/L)	2.51	0.11	0.66	0.67
Midregional pro-adrenomedullin (nmol/L)	1.51	0.29	0.66	0.67
Lp-PLA $_2$ activity (nmol/min/mL)	0.70	0.45	0.66	0.66
D-dimer (mg/L)	0.59	0.71	0.66	0.67
C-reactive protein (mg/L)	0.28	0.84	0.66	0.67
Lp (a) (mg/dL)	− 0.70	0.52	0.66	0.66
Risk factors [a]				
History of myocardial infarction	6.98	< 0.001	0.65	0.67
History of coronary revascularisation	4.31	0.02	0.66	0.67
Sex	3.19	0.005	0.66	0.67
Age	2.91	0.07	0.66	0.67

BNP, brain natriuretic peptide; and Lp-PLA $_2$, lipoprotein-associated phospholipase A2.

a Clinical variables remaining in the model: sex, treatment, nature of qualifying event, coronary revascularisation, stroke, diabetes, current smoking, angina grade > 0, dyspnoea class > 1, and white blood cell count.

Tonkin AM et al. Int J Cardiol. 2015;201:499-507

Summary

- BNP and sensitive Troponin I followed by Cystatin C and D-Dimer have the strongest predictive value for recurrent CHD event in the LIPID study
- Both, baseline and change of sensitive Troponin I and BNP concentration are associated with recurrent CHD event
- Pravastatin therapy lowers levels of PLA2 activity, CRP and D-Dimer after one year, other biomarkers remain largely unaffected
- Of those, only change of PLA2 activity is associated with outcome

Conclusion

- All baseline biomarkers except Lp-PLA_2 activity and Lp(a) were associated with outcome
- Strongest prediction was observed for BNP and sensitive troponin I baseline concentrations. The prediction strength of these biomarkers was also strong compared with classical risk factors and other clinical features
- Of all variables assessed, only a history of MI was a stronger predictor than troponin I or BNP
- Changes in concentrations of troponin I and BNP in addition to their baseline concentration predicted higher or lower CHD risk

Tonkin AM et al. Int J Cardiol. 2015;201:499-507

Conclusion

- The new findings are that:
  - Biomarkers of inflammation, thrombosis, cardiac injury, renal function, and neurohumoral activity each independently add to conventional risk factors in predicting outcomes in patients with stable CHD
  - Changes in concentrations of BNP, troponin, and Lp-PLA_2 activity each provide significant independent risk prediction
  - The improvement in net reclassification is appreciable when measured against the value of currently recommended conventional risk factor such as age

- Future risk model apps will more often include these in prognostication and clinical decision making

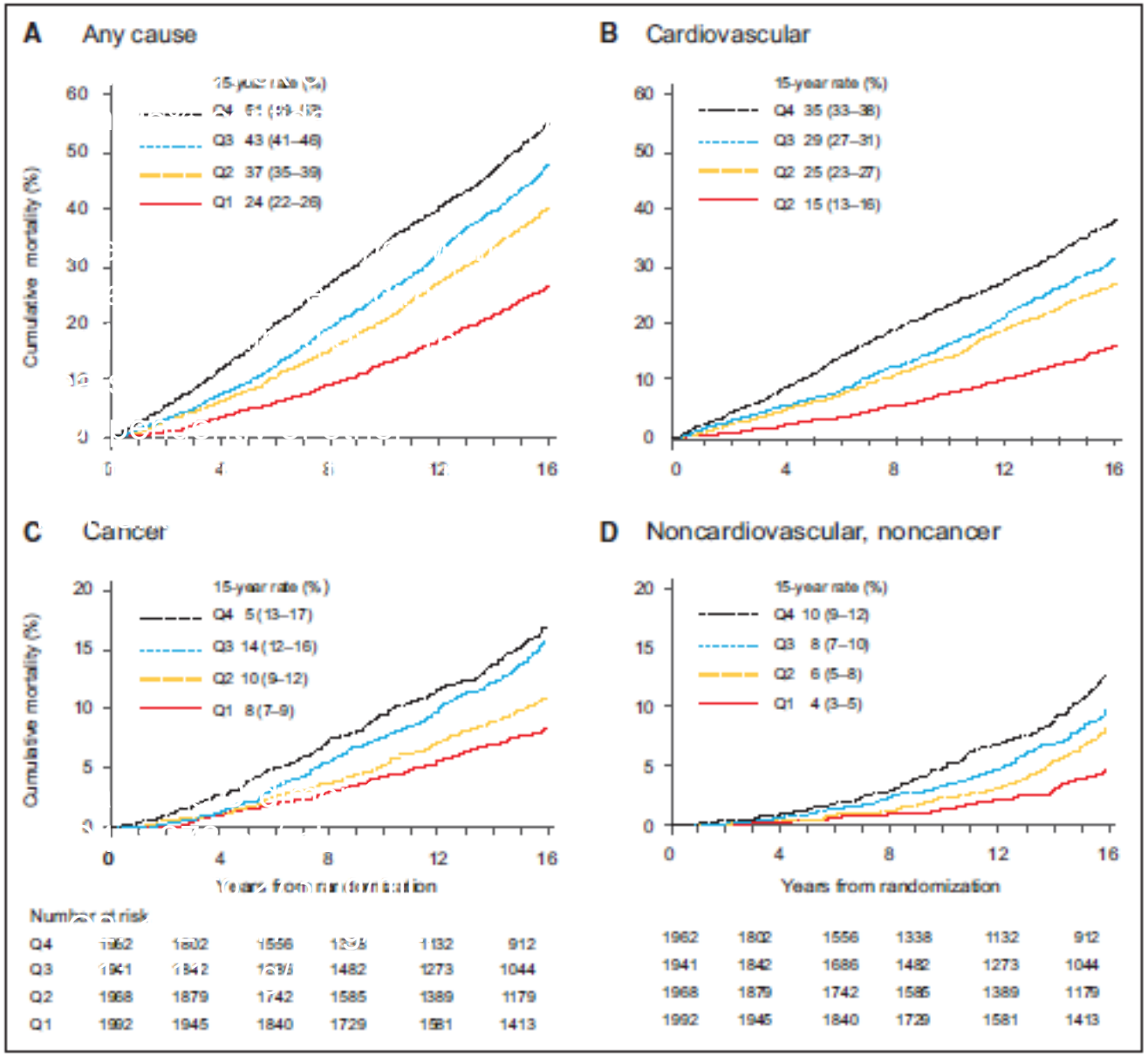
Tonkin AM et al. Int J Cardiol. 2015;201:499-507

D-dimer Predicts Long-Term Cause-Specific Mortality, Cardiovascular Events and Cancer in Stable Coronary Heart Disease Patients: The LIPID Study.

John Simes, Kristy P. Robledo, Harvey D. White, David Espinoza, Ralph A. Stewart, David R. Sullivan, Tanja Zeller, Wendy Hague, Paul J. Nestel, Paul P. Glasziou, Anthony C. Keech, John Elliott, Stefan Blankenberg, Andrew M. Tonkin
For the LIPID Study Investigators

Circulation. 2018; 138:712-23

Baseline D-dimer Levels and Cause-Specific Mortality in the LIPID Trial



Cumulative risk over 15 years (with 95% confidence intervals) each $p<0.001$

Increased mortality risk with elevated baseline D dimer levels maintained over 16 years
Independently of other traditional risk factors or novel biomarkers

Baseline D-dimer Quartiles:
Q4: >273 ng/mL;
Q3: 173 to 273 ng/mL;
Q2: 112 to 173 ng/mL;
Q1: < 112 ng/mL.

Simes J et al. Circulation. 2018; 138:712-723

D-Dimer Levels and Cancer Risk over 16 years – the LIPID Trial

Endpoint and D-Dimer level (ng/mL)	Events, n/N	15-y Event Rate ǂ	Adjusted I* HR (95% CI)	*P* for Trend	Adjusted II† HR (95% CI)	*P* for Trend
Cancer incident ǂ						
≤112	416/1992	19.8 (18.0-21.5)	1	<0.001	1	0.02
112-173	471/1968	23.1 (21.2-25.8)	1.33 (1.18-1.49)		1.09 (0.97-1.23)	
173-273	492/1941	23.9 (22.0-25.8)	1.44 (1.28-1.62)		1.08 (0.96-1.22)	
>273	502/1962	24.5 (22.6-26.4)	1.59 (1.42-1.79)		1.16 (1.03-1.31)	
Cancer mortality						
≤112	151/1992	7.7 (6.5-9.0)	1	<0.001	1	<0.001
112-173	181/1968	9.9 (8.6-11.5)	1.33 (1.18-1.49)		1.07 (0.86-1.33)	
173-273	244/1941	13.7 (12.0-15.5)	1.44 (1.28-1.62)		1.39 (1.13-1.72)	
>273	254/1962	15.2 (13.4-17.1)	1.59 (1.42-1.79)		1.54 (1.25-1.91)	

*HR, 95% CI, and P value adjusted for D-dimer quartile, trial treatment, sex, and anticoagulant treatment.

†HR, 95% CI, and P value adjusted for D-dimer quartile, trial treatment, anticoagulant treatment, and risk factors of cancer incidence that remained significant after backward selection: age, sex, baseline high-sensitivity C-reactive protein, white blood cell count, history of smoking, dyspnea, and aspirin use.

ǂThe event rates for cancer incidence are Fine and Gray estimates, accounting for competing risks

Simes J et al. Circulation. 2018; 138:712-723

D-Dimer Levels and Vascular Events over 6 years – the LIPID Trial

Endpoint and D-Dimer level (ng/mL)	Events, n/N	5-y Event Rate (95% CI),%	Adjusted I* HR (95% CI)	*P* for Trend	Adjusted II† HR (95% CI)	*P* for Trend
CHD events‡						
≤112	203/1992	8.2 (7.0-9.5)	1	<0.001	1	<0.001
112-173	266/1968	11.2 (9.92-12.7)	1.41 (1.17-1.69)		1.18 (0.98-1.42)	
173-273	280/1941	12.6 (11.2-14.1)	1.55 (1.29-1.86)		1.20 (0.99-1.44)	
>273	351/1962	15.0 (13.5-16.7)	1.97 (1.66-2.35)		1.45 (1.21-1.74)	
Strokes						
≤112	52/1992	2.0 (1.5-2.7)	1	<0.001	1	0.08
112-173	58/1968	2.6 (2.0-3.4)	1.17 (0.80-1.70)		0.88 (0.60-1.28)	
173-273	83/1941	3.3 (2.6-4.2)	1.73 (1.22-2.45)		1.08 (0.75-1.56)	
>273	117/1962	5.3 (4.3-6.4)	2.54 (1.83-3.52)		1.37 (0.96-1.95)	

- Increased Baseline D-Dimer Levels associated with increased risk of arterial and venous thrombotic events
- Independent of other traditional risk factors and novel biomarkers

*HR, 95% CI, and P value adjusted for D-dimer quartile, trial treatment, sex, and anticoagulant treatment.

†HR, 95% CI, and P value adjusted for D-dimer quartile, trial treatment, anticoagulant treatment, and risk factors of cancer incidence that remained significant after backward selection: age, sex, baseline high-sensitivity C-reactive protein, white blood cell count, history of smoking, dyspnea, and aspirin use.‡

‡CHD death or nonfatal myocardial infarction, or stroke

Simes J et al. Circulation. 2018; 138:712-723

D-Dimer Levels and Vascular Events over 6 years – the LIPID Trial

Endpoint and D-Dimer level (ng/mL)	Events, n/N	5-y Event Rate (95% CI),%	Adjusted I* HR (95% CI)	*P* for Trend	Adjusted II† HR (95% CI)	*P* for Trend
Major CVD events§						
≤112	252/1992	10.1 (8.8-11.5)	1	<0.001	1	<0.001
112-173	311/1968	13.1 (11.7-14.7)	1.32 (1.12-1.56)		1.08 (0.91-1.28)	
173-273	352/1941	15.4 (13.9-17.1)	1.57 (1.33-1.84)		1.14 (0.96-1.36)	
>273	471/1962	20.4 (18.6-22.3)	2.16 (1.85-2.51)		1.45 (1.23-1.71)	
VTEs						
≤112	16/1992	0.6 (0.4-1.1)	1	<0.001	1	<0.001
112-173	25/1968	0.8 (0.5-1.4)	1.67 (0.89-3.13)		1.60 (0.85-3.00)	
173-273	30/1941	1.3 (0.9-2.0)	2.06 (1.12-3.79)		1.91 (1.04-3.52)	
>273	62/1962	2.9 (2.2-3.8)	4.36 (2.51-7.57)		4.03 (2.31-7.03)	

- Increased Baseline D-Dimer Levels associated with increased risk of arterial and venous thrombotic events
- Independent of other traditional risk factors and novel biomarkers

*HR, 95% CI, and P value adjusted for D-dimer quartile, trial treatment, sex, and anticoagulant treatment.

†HR, 95% CI, and P value adjusted for D-dimer quartile, trial treatment, anticoagulant treatment, and risk factors of cancer incidence that remained significant after backward selection: age, sex, baseline high-sensitivity C-reactive protein, white blood cell count, history of smoking, dyspnea, and aspirin use.

§CHD death, nonfatal myocardial infarction, or stroke

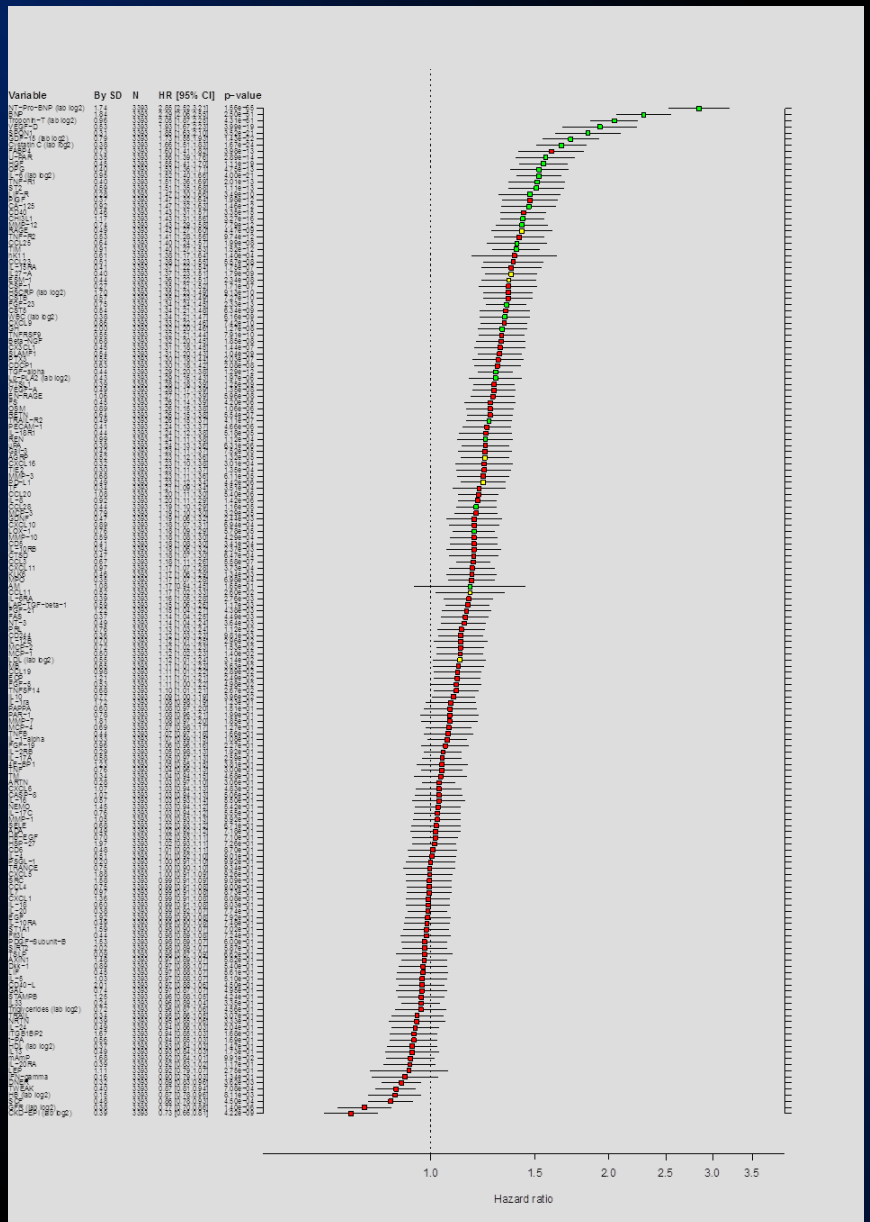
Simes J et al. Circulation. 2018; 138:712-723

Screening multiple biomarkers for associations with cardiovascular death in patients with stable coronary heart disease

- New analytical technologies allow simultaneous measurements of hundreds of protein with Proximity Extention Assay (PEA) technology, allowing simultaneous measurements of proteins in a 1.0 µl plasma sample by PCR amplification of DNA strands from DNA-labeled antibody pairs

- We explored and compared the associations between 157 cardiovascular (CV) and inflammatory biomarkers and CV death using PEA in two cohorts of patients with stable CHD: STABILITY and LURIC

Cox regression analyses in the STABILITY cohort of associations between biomarkers and cardiovascular death with adjustment for baseline characteristics



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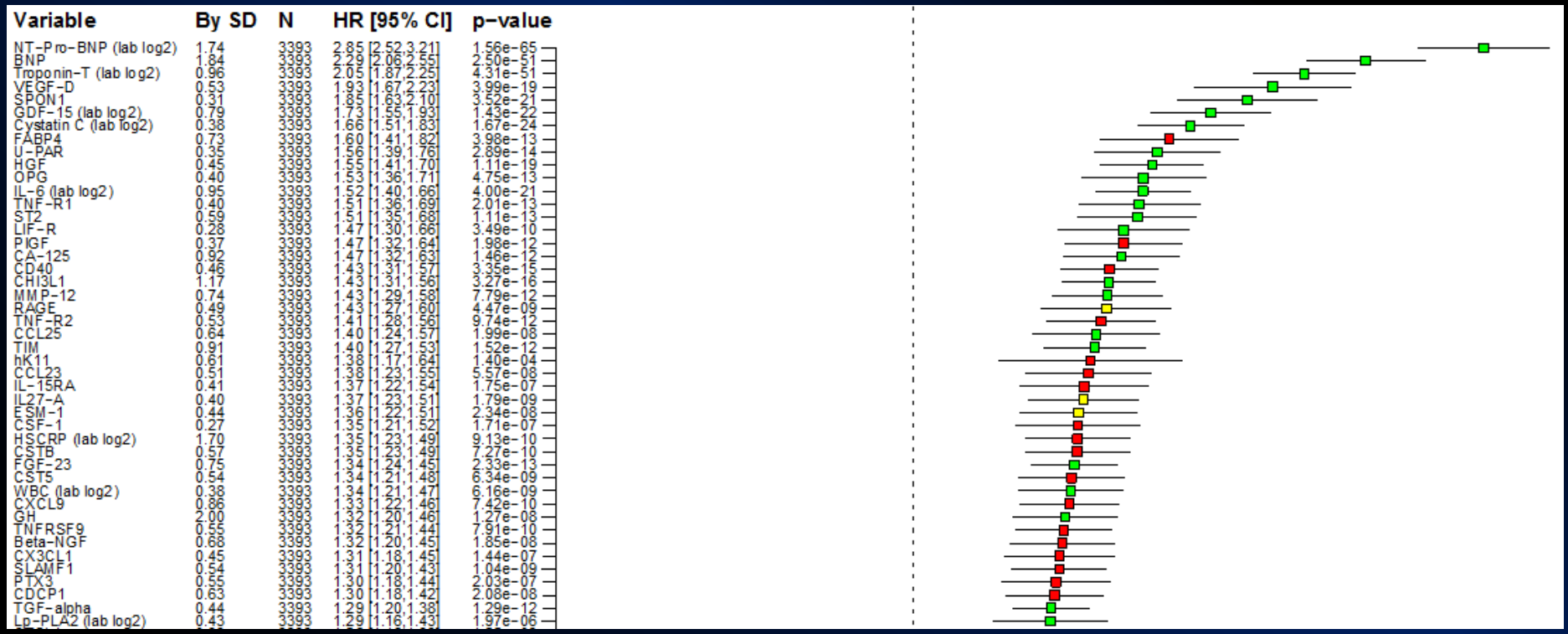
Cox regression analyses in the STABILITY cohort of associations between biomarkers and cardiovascular death with adjustment for baseline characteristics



Variable	By SD	N	HR [95% CI]	p-value
NT-Pro-BNP (lab log2)	1.74	3393	2.85 [2.52,3.21]	1.56e-65
BNP	1.84	3393	2.29 [2.06,2.55]	2.50e-51
Troponin-T (lab log2)	0.96	3393	2.05 [1.87,2.25]	4.31e-51
VEGF-D	0.53	3393	1.93 [1.67,2.23]	3.99e-19
SPON1	0.31	3393	1.85 [1.63,2.10]	3.52e-21
GDF-15 (lab log2)	0.79	3393	1.73 [1.55,1.93]	1.43e-22
Cystatin C (lab log2)	0.38	3393	1.66 [1.51,1.83]	1.67e-24
FABP4	0.73	3393	1.60 [1.41,1.82]	3.98e-13
U-PAR	0.35	3393	1.56 [1.39,1.76]	2.89e-14
HGF	0.45	3393	1.55 [1.41,1.70]	1.11e-19
OPG	0.40	3393	1.53 [1.36,1.71]	4.75e-13
IL-6 (lab log2)	0.95	3393	1.52 [1.40,1.66]	4.00e-21
TNF-R1	0.40	3393	1.51 [1.36,1.69]	2.01e-13
ST2	0.59	3393	1.51 [1.35,1.68]	1.11e-13
LIF-R	0.28	3393	1.47 [1.30,1.66]	3.49e-10
PlGF	0.37	3393	1.47 [1.32,1.64]	1.98e-12
CA-125	0.92	3393	1.47 [1.32,1.63]	1.46e-12
CD40	0.46	3393	1.43 [1.31,1.57]	3.35e-15
CHI3L1	1.17	3393	1.43 [1.31,1.56]	3.27e-16
MMP-12	0.74	3393	1.43 [1.29,1.58]	7.79e-12
RAGE	0.49	3393	1.43 [1.27,1.60]	4.47e-09
TNF-R2	0.53	3393	1.41 [1.28,1.56]	9.74e-12
CCL25	0.64	3393	1.40 [1.24,1.57]	1.99e-08
TIM	0.91	3393	1.40 [1.27,1.53]	1.52e-12
hK11	0.61	3393	1.38 [1.17,1.64]	1.40e-04
CCL23	0.51	3393	1.38 [1.23,1.55]	5.57e-08
IL-15RA	0.41	3393	1.37 [1.22,1.54]	1.75e-07
IL27-A	0.40	3393	1.37 [1.23,1.51]	1.79e-09
ESM-1	0.44	3393	1.36 [1.22,1.51]	2.34e-08
CSF-1	0.27	3393	1.35 [1.21,1.52]	1.71e-07
HSCRP (lab log2)	1.70	3393	1.35 [1.23,1.49]	9.13e-10
CSTB	0.57	3393	1.35 [1.23,1.49]	7.27e-10
FGF-23	0.75	3393	1.34 [1.24,1.45]	2.33e-13
CST5	0.54	3393	1.34 [1.21,1.48]	6.34e-09
WBC (lab log2)	0.38	3393	1.34 [1.21,1.47]	6.16e-09
CXCL9	0.86	3393	1.33 [1.22,1.46]	7.42e-10
GH	2.00	3393	1.32 [1.20,1.46]	1.27e-08
TNFRSF9	0.55	3393	1.32 [1.21,1.44]	7.91e-10
Beta-NGF	0.68	3393	1.32 [1.20,1.45]	1.85e-08
CX3CL1	0.45	3393	1.31 [1.18,1.45]	1.44e-07
SLAMF1	0.54	3393	1.31 [1.20,1.43]	1.04e-09
PTX3	0.55	3393	1.30 [1.18,1.44]	2.03e-07
CDCP1	0.63	3393	1.30 [1.18,1.42]	2.08e-08
TGF-alpha	0.44	3393	1.29 [1.20,1.38]	1.29e-12
Lp-PLA2 (lab log2)	0.43	3393	1.29 [1.16,1.43]	1.97e-06

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Validated biomarkers significantly associated with CV-death at Random Survival Forest-Boruta analyses and at Cox regression adjusted for clinical factors and renal function (Cystatin-C) in both STABILITY and LURIC

	Random Forest Order		Cox full adjustment LURIC			Cox full adjustment STABILITY		
	LURIC	STABILITY	HR	CI	P	HR	CI	P
NT-ProBNP	1	1	1.779	(1.495-2.117)	8.60E-11	2.348	(2.051-2.689)	4.30E-35
cTnT-hs	2	2	1.266	(1.065-1.505)	7.63E-03	1.483	(1.323-1.663)	1.43E-11
GDF-15*	3	9	1.397	(1.180-1.654)	1.05E-04	1.264	(1.1-1.452)	9.50E-04
OPG	5	13	1.256	(1.087-1.452)	2.04E-03	1.29	(1.139-1.46)	5.76E-05
TIM	6	28	1.294	(1.123-1.491)	3.66E-04	1.162	(1.049-1.287)	4.11E-03
REN	7	36	1.523	(1.329-1.745)	1.50E-09	1.209	(1.089-1.343)	3.97E-04
sST2	10	29	1.250	(1.100-1.422)	6.36E-04	1.199	(1.079-1.332)	7.32E-04
HGF	17	12	1.157	(1.031-1.299)	1.32E-02	1.263	(1.137-1.404)	1.44E-05
IL-6*	18	10	1.261	(1.110-1.432)	3.76E-04	1.133	(1.021-1.257)	1.84E-02

* Measured with conventional method in STABILITY and PEA in Luric

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LIPID: planned biomarker studies

- Cystatin C: Malcolm West
- BNP: Ralph Stewart
- CRP; Andrew Tonkin

LIPID: planned biomarker studies

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- BNP: Ralph Stewart
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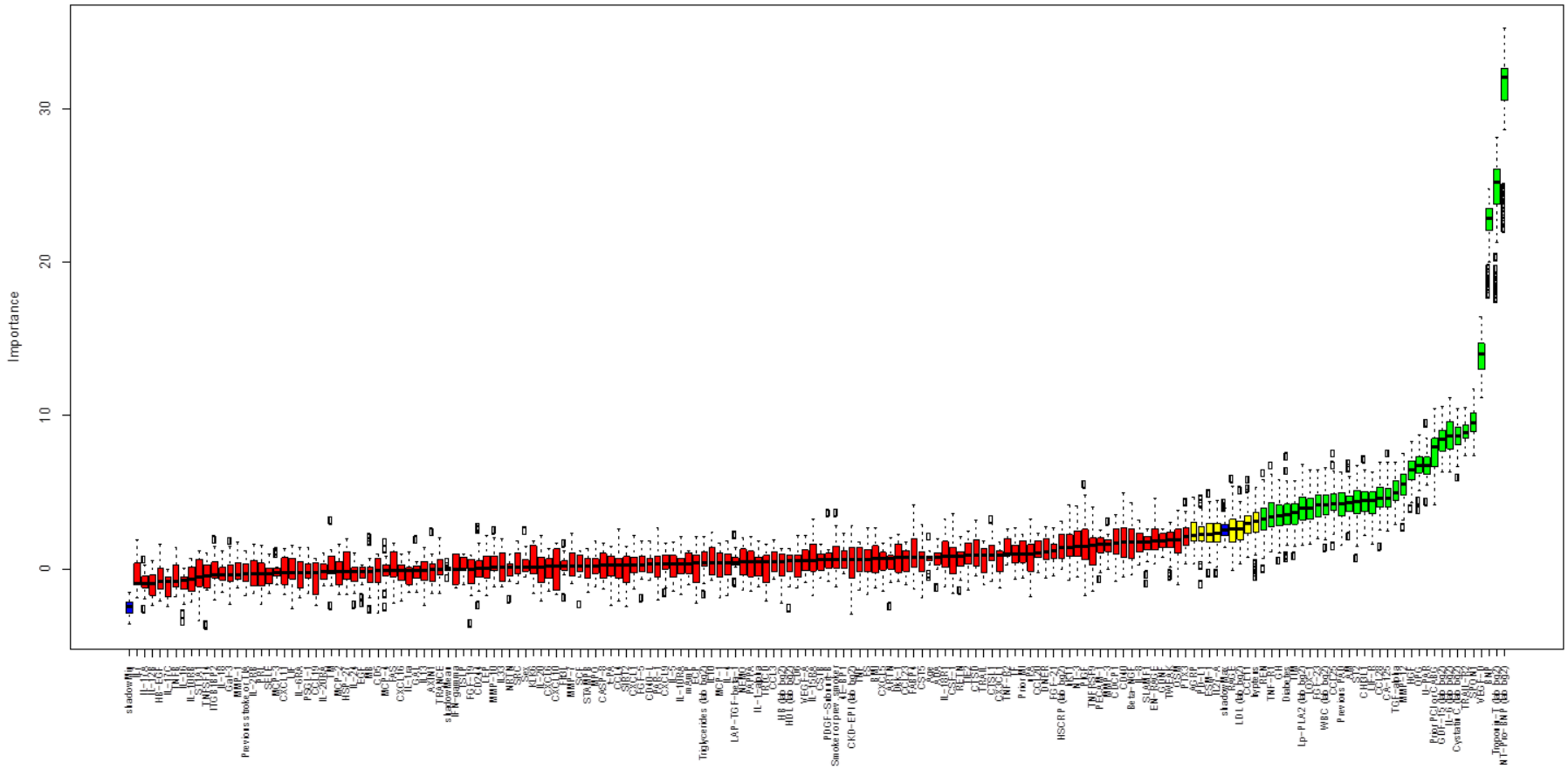
At the Executive teleconferences they each say "we will have a draft next week"

LIPID Study

With especial thanks to the many LIPID investigators and especially the patients

Boruta analysis in the STABILITY cohort of the significance of variable importance for cardiovascular death in the Random Survival Forest analysis, including clinical variables as well as established and PEA biomarkers



(Values are NPX values for the PEA biomarkers and log2 for the ng/L levels of the other biomarkers NTproBNP, cTnT-hs, IL-6, GDF-15, Cystatin-C, CRP-hs, Lp-PLA2 and WBC measured by conventional quantitative assays.)

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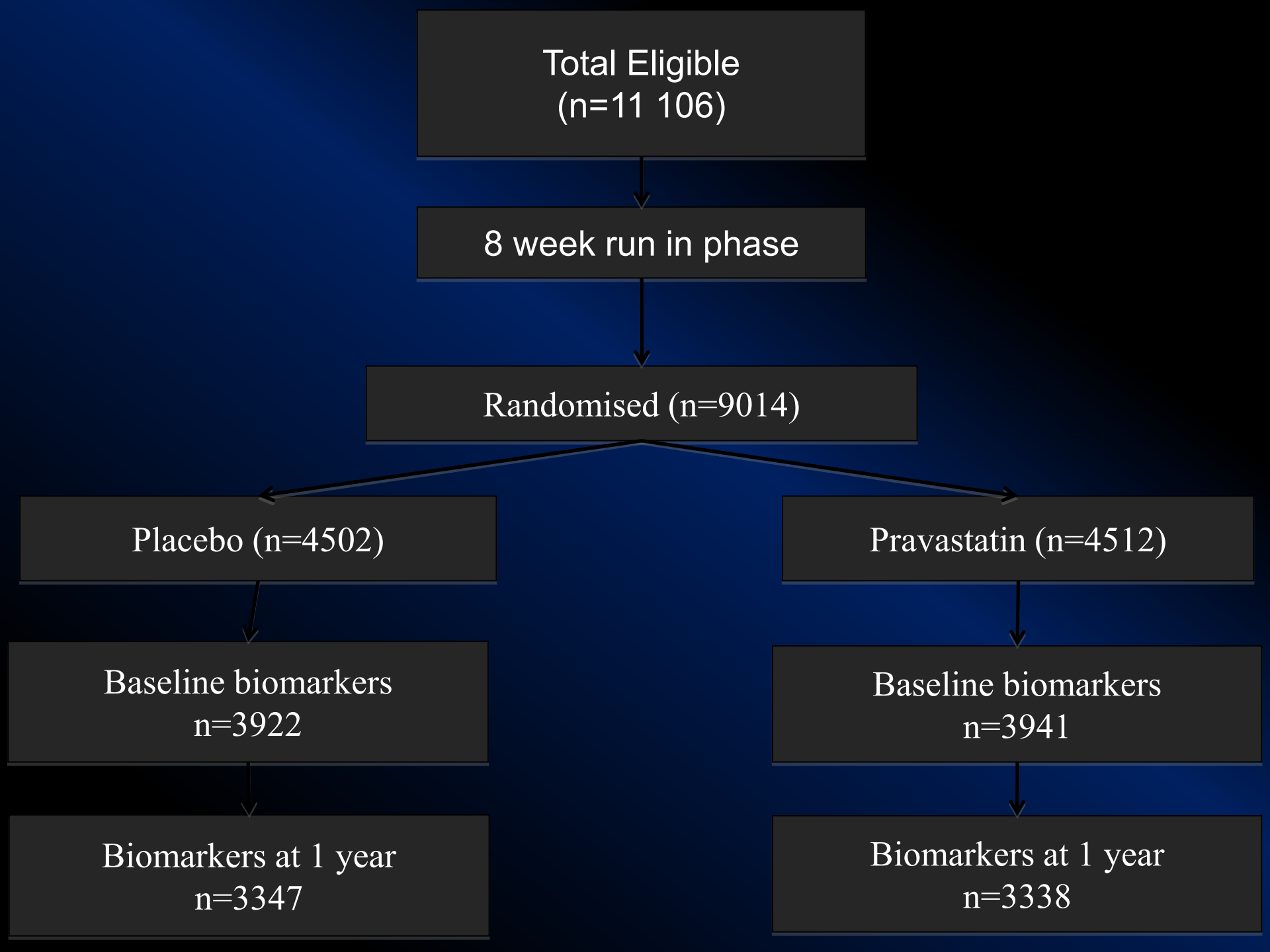
Background

The role of higher sensitivity troponins in predicting long-term outcomes in patients with stable CHD is not clearly defined

Objective

- To assess the value of baseline and change in levels of a higher sensitivity troponin I (TnI) (Siemens Ultra) assay (99th % >0.04ng/ml) to predict outcomes in the LIPID trial with mean follow-up of 6.1 years
- To assess the effects of pravastatin on TnI levels and outcomes


Total Eligible
(n=11 106)
8 week run in phase
Randomised (n=9014)
Placebo (n=4502)
Pravastatin (n=4512)
Baseline biomarkers
n=3922
Baseline biomarkers
n=3941
Biomarkers at 1 year
n=3347
Biomarkers at 1 year
n=3338

Methods

- Baseline TnI levels (ng/ml) assessed in approximate tertiles:
  - Not detectable, below 0.006ng/ml (38%)
  - 0.006 to <0.018 ng/mL (31%)
  - >0.018 ng/mL (31%)
  - Change was defined as moving up or down one category and > or < 50%

Baseline characteristics according to levels of Troponin I

Baseline characteristic	Troponin level (ng/ml)		
	Not detectable	0.006 to < 0.018	≥ 0.018
n	2967	2436	2460
Troponin (ng/ml)		0.012 (0.003)	0.048 (0.097)
Age (years)	61.0 (54.0 – 67.0)	62.0 (56.0 – 68.0)	64.0 (57.0 – 68.0)
Female	18%	17%	16%
Current Smoker	10%	9%	9%
Diabetes	8%	8%	10%
Hypertension	39%	43%	44%
LDL – c (mmol/l)	3.9 (0.7)	3.9 (0.7)	3.9 (0.7)
HDL – c (mmol/l)	0.9 (0.2)	1.0 (0.2)	1.0 (0.2)
eGFR mLs/min/1.73m2	71 (61 - 82)	70 (61 - 80)	68 (58 - 78)

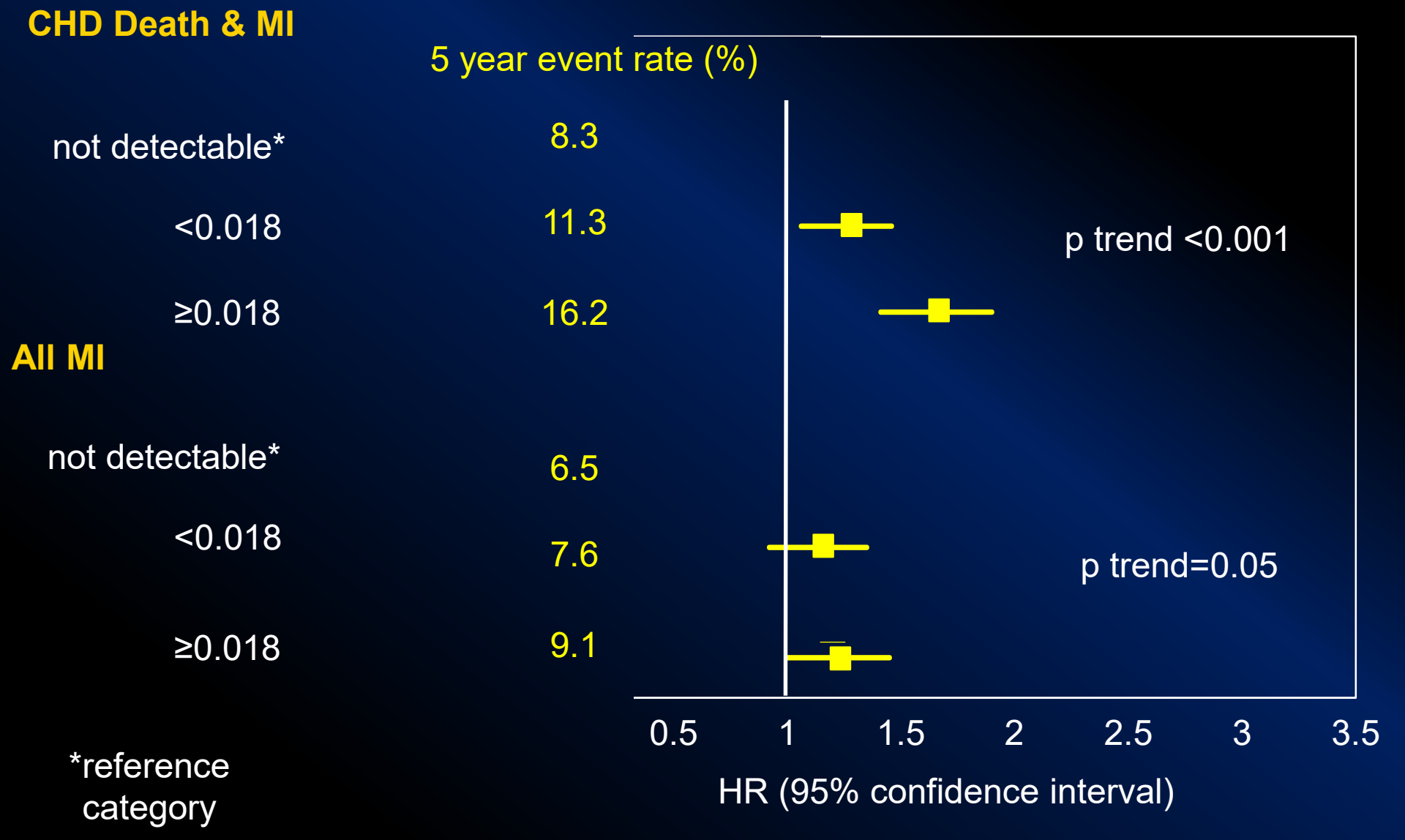
Statistics are %, mean (SD) or median (Q1-Q3)

Baseline characteristics according to levels of Troponin I

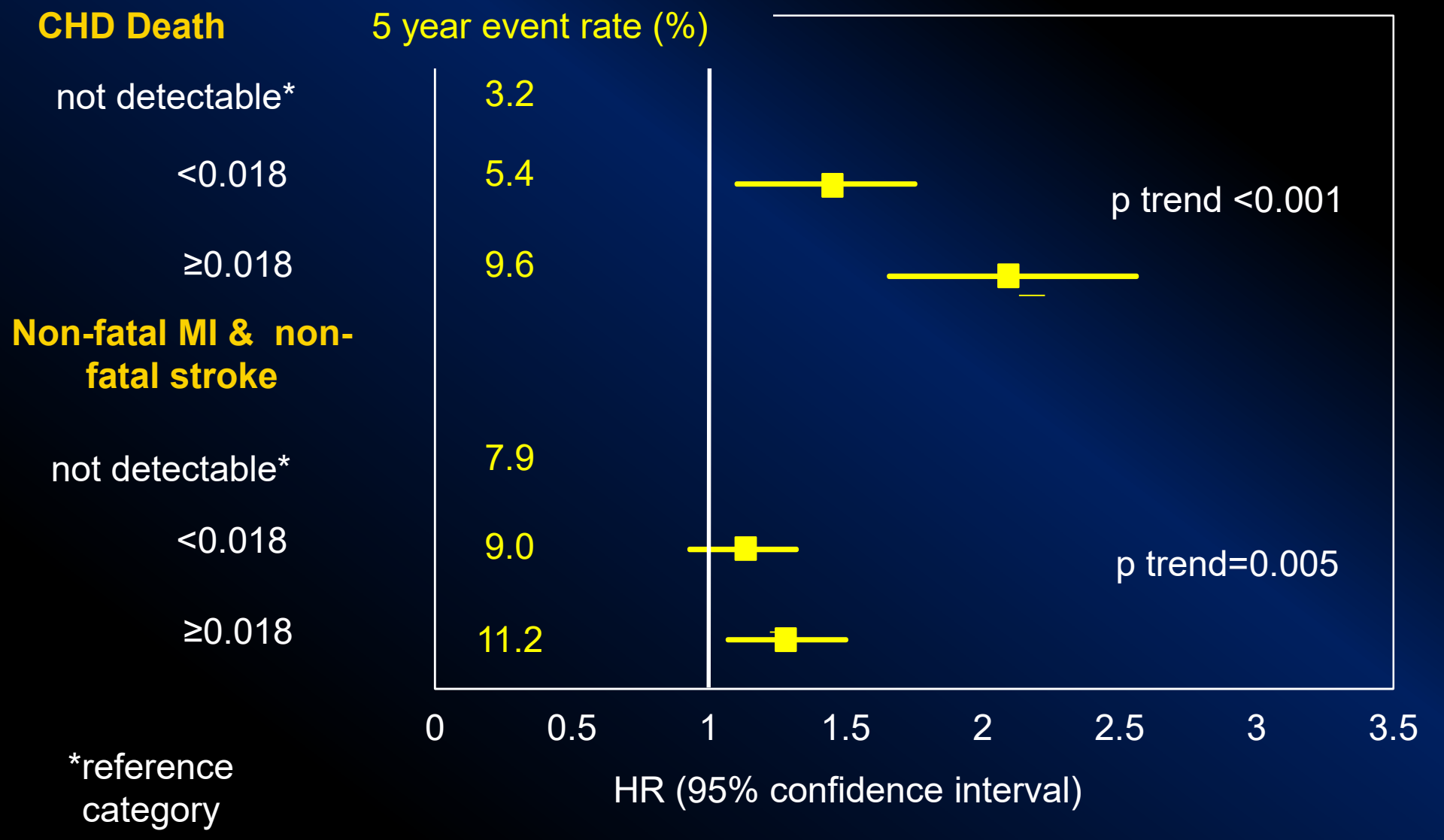
Baseline characteristic	Troponin level (ng/ml)		
	Not detectable	0.006 to < 0.018	≥ 0.018
n	2967	2436	2460
Qualifying event			
Unstable angina	42%	35%	30%
Single MI	49%	54%	55%
Multiple MI	8%	11%	15%
Medications			
Aspirin	83%	83%	82%
ACE inhibitors	11%	16%	22%
Beta blockers	48%	49%	44%
Calcium antagonist	35%	33%	34%

Statistics are %, mean (SD) or median (Q1-Q3)

Baseline Troponin ng/mL and events HR are compared to Not Detectable TnI group



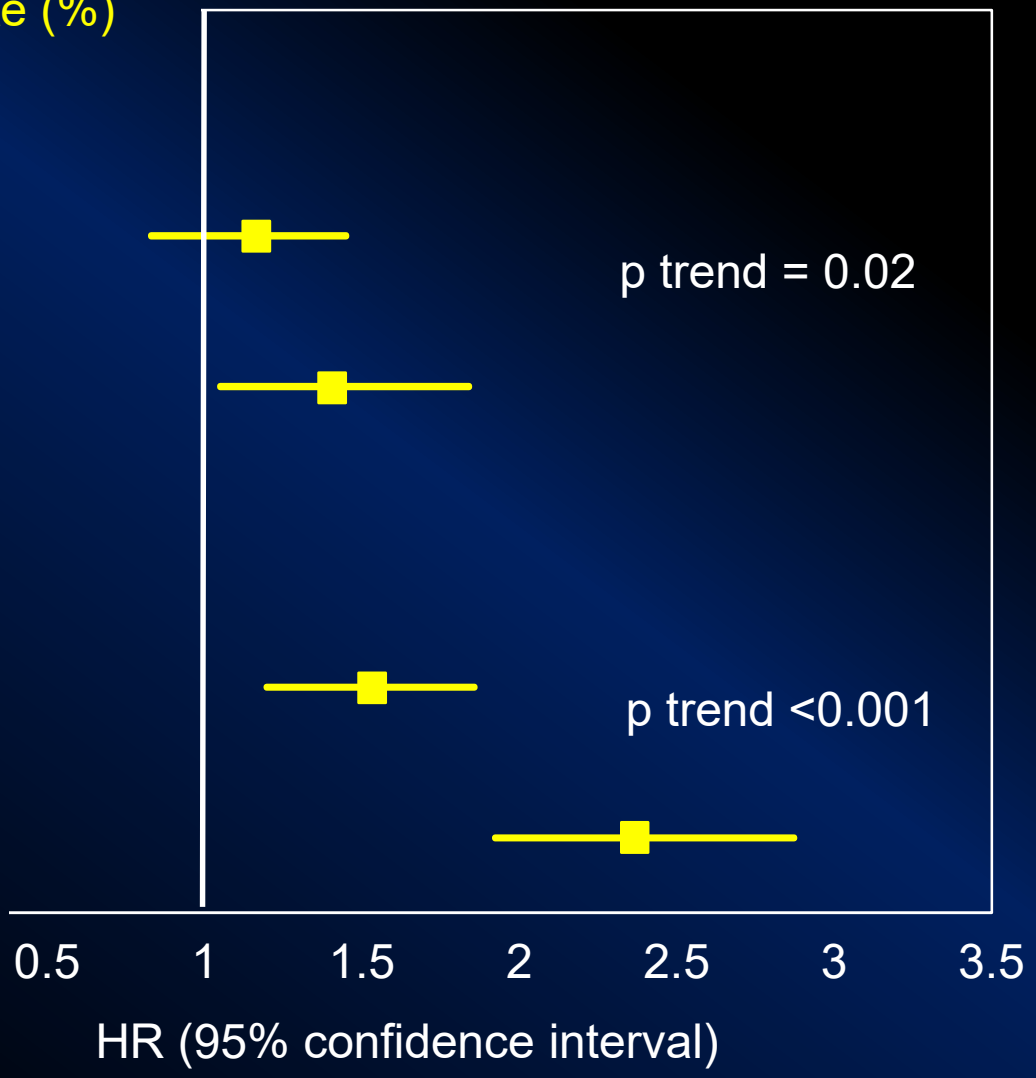
Baseline Troponin (ng/ml) and events



*reference category

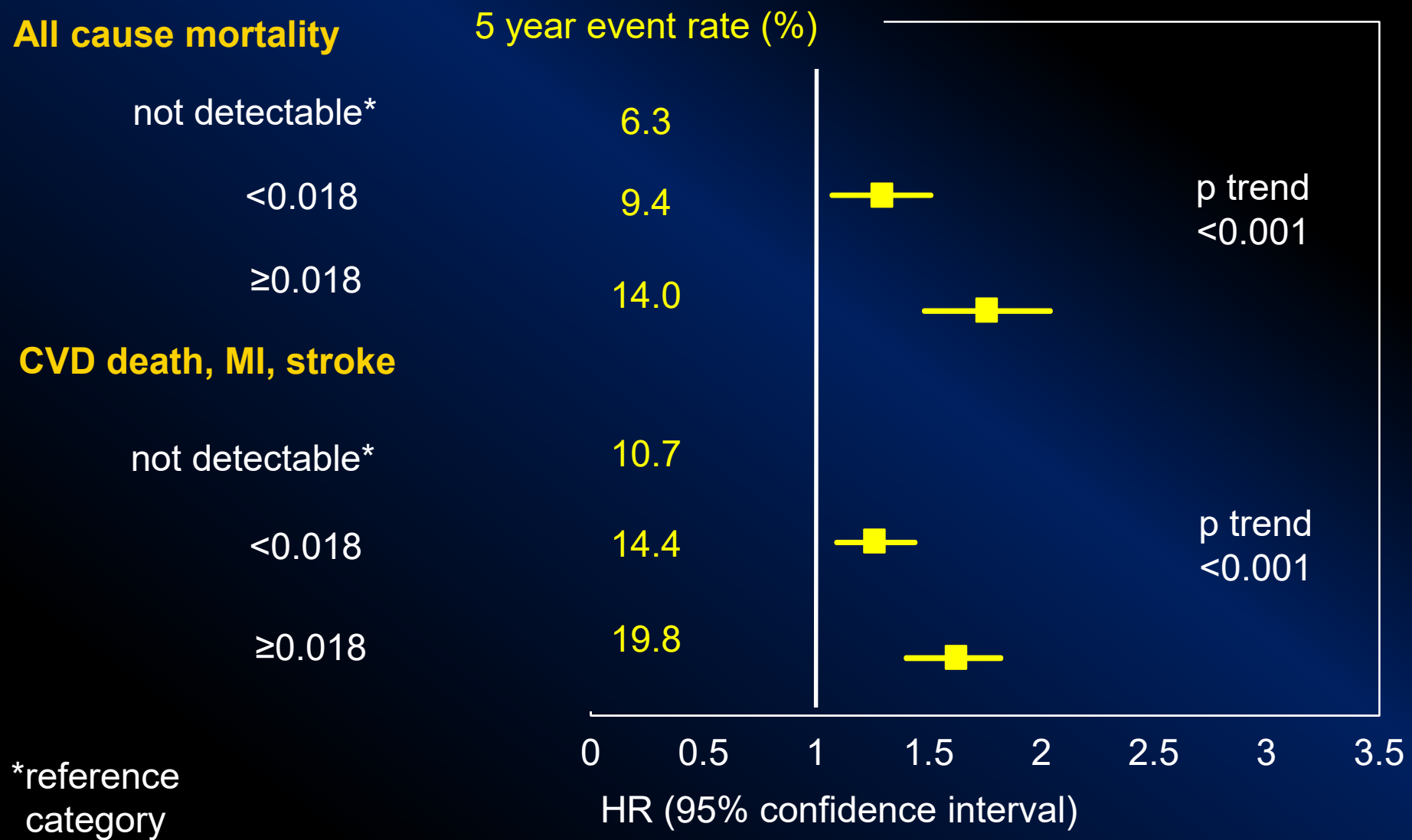
Baseline Troponin (ng/ml) and events

	5 year event rate (%)
Stroke	
not detectable*	2.5
<0.018	3.1
≥0.018	4.3
Heart Failure	
not detectable*	4.1
<0.018	7.2
≥0.018	12.4



*reference category

Baseline Troponin (ng/ml) and events

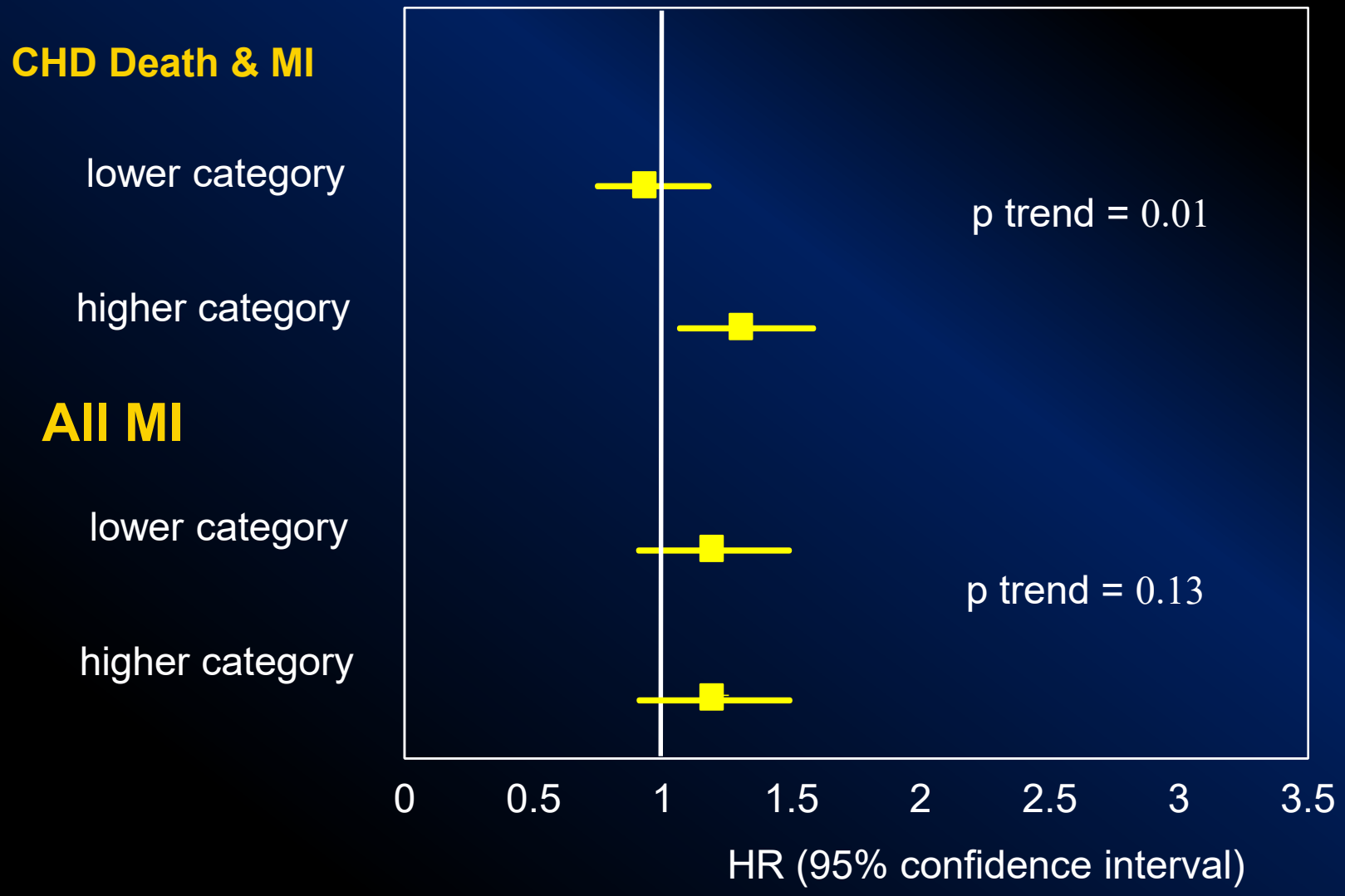


*reference category

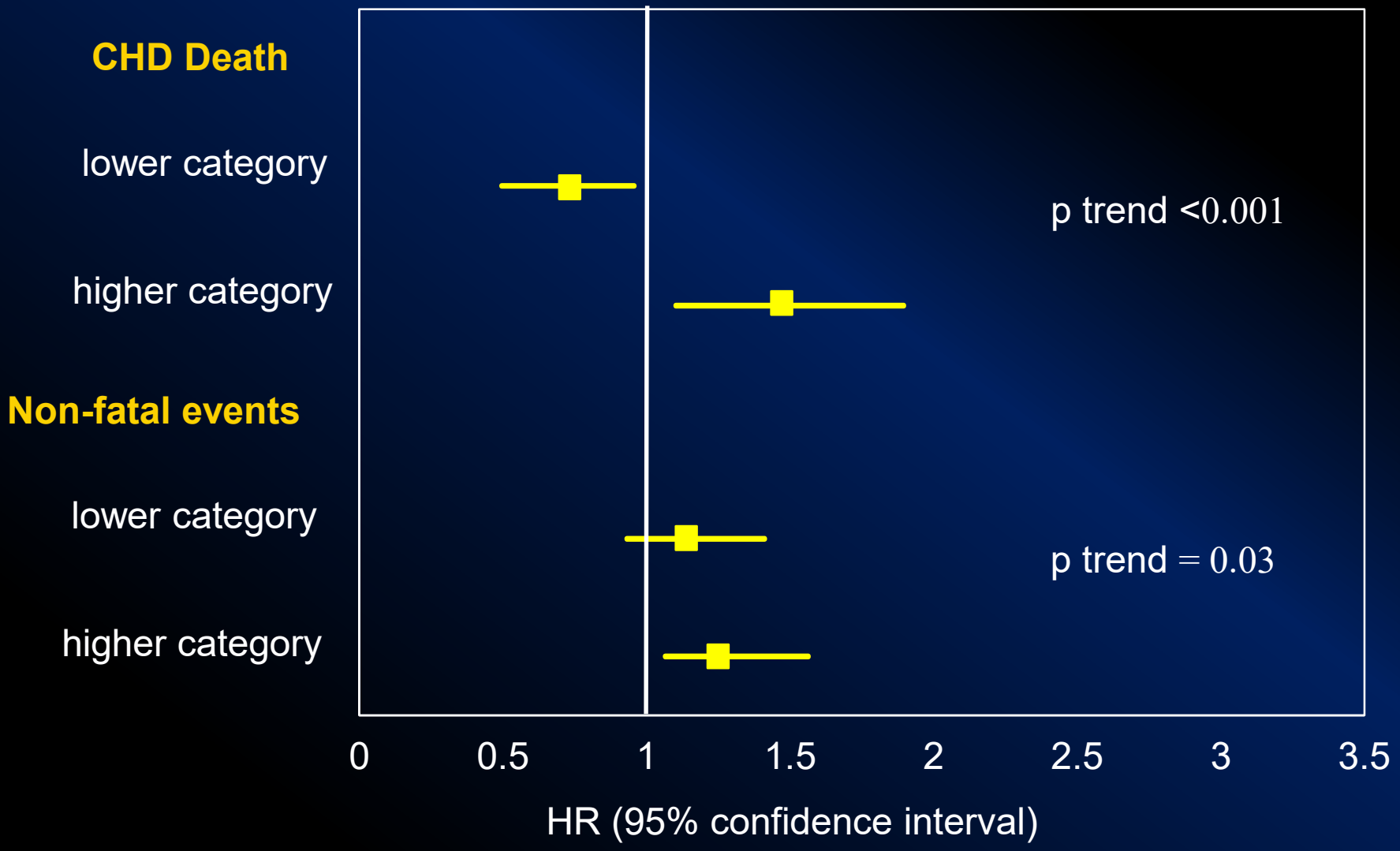
Results

- Levels of TnI at 1 year:
  - Decreased in 25% of patients
  - Unchanged in 51% of patients
  - Increased in 23% of patients

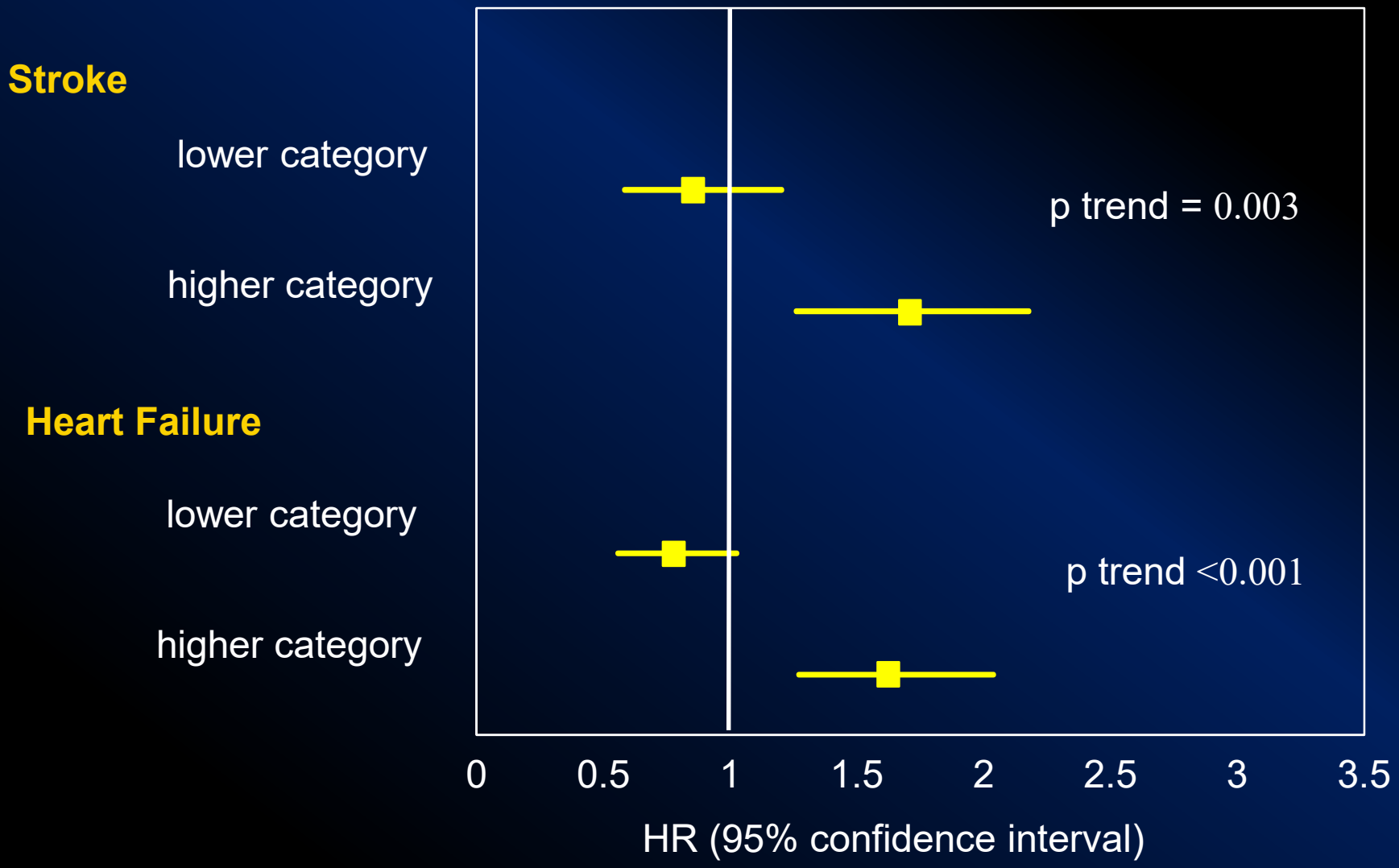
Events from Year One & change in Troponin (compared to no change)



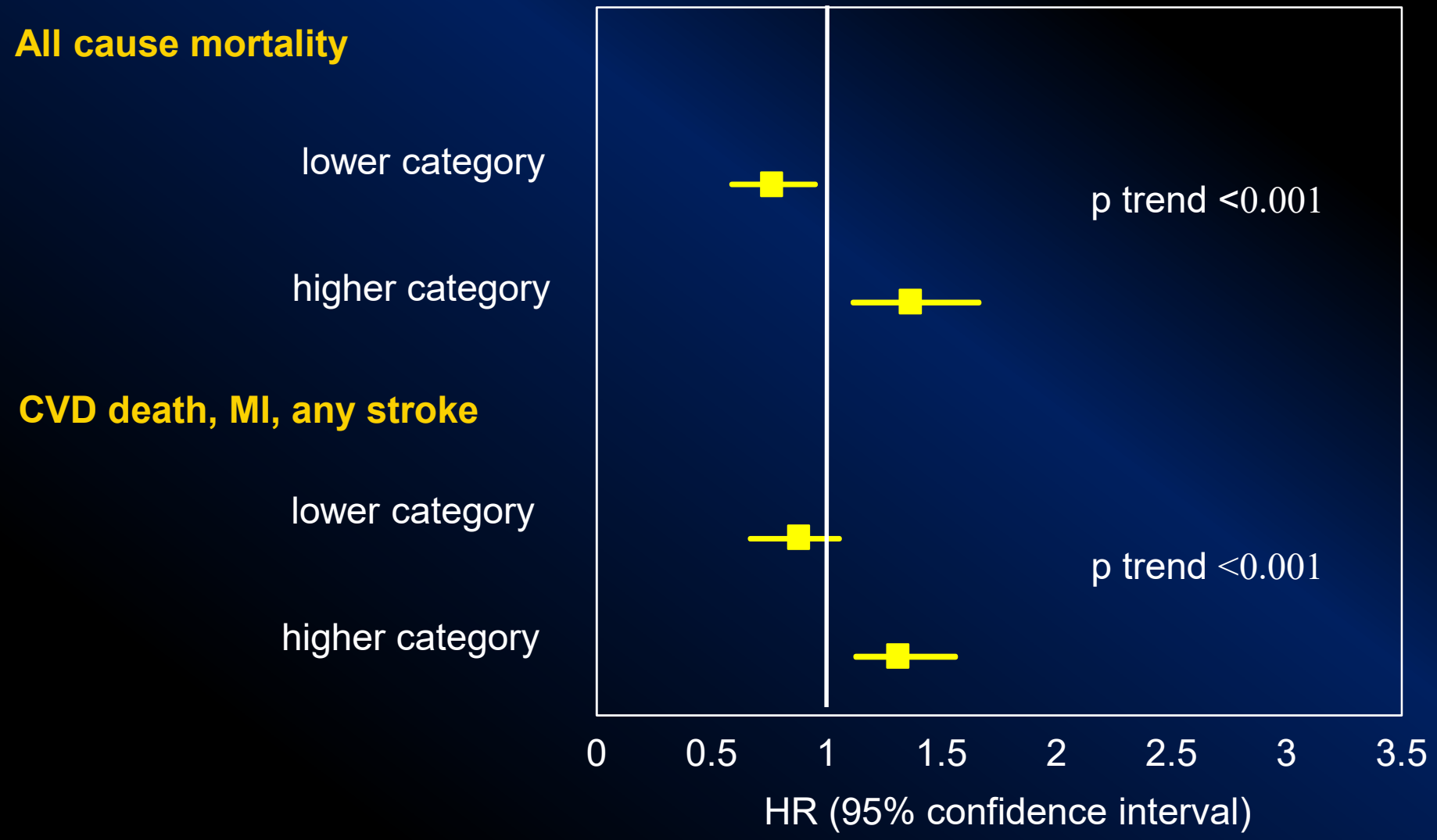
Events from Year One & change in Troponin (compared to no change)



Events from Year One & change in Troponin (compared to no change)



Events from Year One & change in Troponin (compared to no change)



Results

- Findings were similar using a 50% change criteria
- Levels of TnI >0.04 ng/mL (99th percentile) in 8.4%
- Levels of TnI >0.04 vs undetectable levels were associated with:
  - HR for CHD death or MI adjusted for baseline risk factors of 2.02 (95% CI 1.66 – 2.45), p for trend <0.001

Results

- Effect of pravastatin in reducing CHD events remained highly significant in each model ($p<0.02$) and was independent of TnI levels

Effect of pravastatin in each Troponin I category

Endpoint	Level	Placebo 5-yr events	Pravastatin 5-yr events	NNT	HR 95% CI	p trend
CHD death and MI	TnI not detectable	9.3%	7.3%	52	0.73 (0.58, 0.92)	0.3
	0.006–0.017 ng/mL	12.6%	10.0%	39	0.75 (0.60, 0.93)	
	>0.018 ng/mL	17.4%	15.1%	29	0.85 (0.71, 1.02)	
All cause mortality	TnI not detectable	6.9%	5.7%	63	0.74 (0.58, 0.94)	0.3
	0.006–0.017 ng/mL	10.6%	8.1%	42	0.68 (0.54, 0.86)	
	>0.018 ng/mL	14.7%	13.2%	31	0.84 (0.69, 1.01)	

Unadjusted and Adjusted models for CHD death & MI

Unadjusted model^	Level	HR (95% CI)	P value
Baseline Troponin	Not detectable	1	<0.001
	0.006 – 0.017 ng/mL	1.29 (1.11-1.51)	
	>0.018 ng/mL	1.89 (1.64-2.18)	

^Adjusted treatment and gender only

Adjusted model*	Level	HR (95% CI)	P value
Baseline Troponin	Not detectable	1	<0.001
	0.006 – 0.017 ng/mL	1.19 (1.02-1.40)	
	>0.018 ng/mL	1.50 (1.29-1.74)	

*Adjusted for BNP, Cystatin C, D Dimer, sex, treatment, nature of prior ACS, coronary revascularization, stroke, diabetes, smoking, angina grade, dyspnoea class, WBC, Apo B

Conclusions

- Baseline and change to 1 year in TnI levels were independent predictors of CHD death and MI
- Pravastatin had no effect on TnI levels
- The benefits of pravastatin were independent of TnI levels

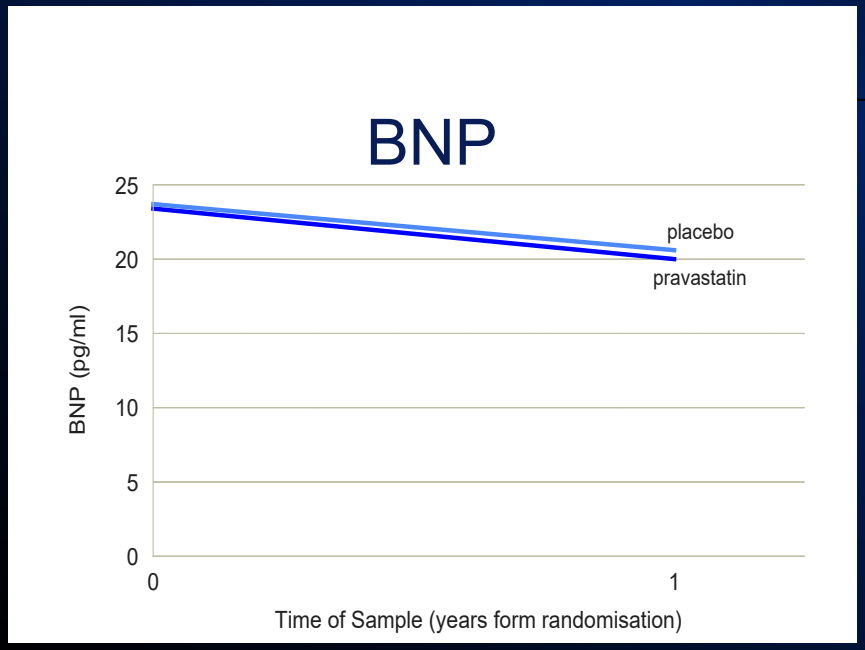
Baseline risk factors

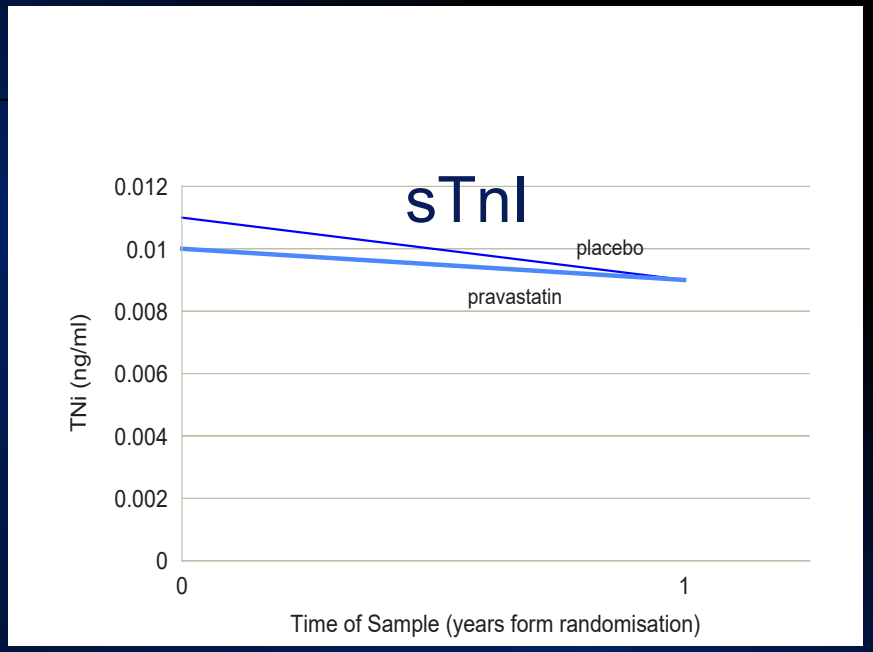
- gender
- age
- stroke
- diabetes
- smoking
- hypertension
- total cholesterol
- Apo B
- Apo A1
- HDL-c
- nature of prior ACS
- SBP
- timing of coronary revasc
- atrial fibrillation
- eGFR
- BMI
- dyspnoea class
- angina grade
- WBC
- peripheral vascular disease
- triglycerides
- fasting glucose
- aspirin

Conclusion

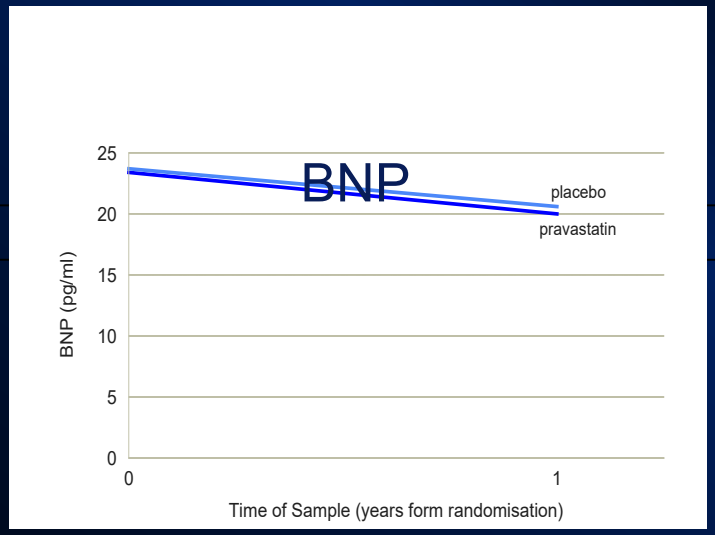
- Repeated measures of BNP and sensitive Troponin I might guide outcome in secondary prevention
- Prospective studies need to address treatment regimes by sensitive troponin and BNP testing in secondary and primary prevention

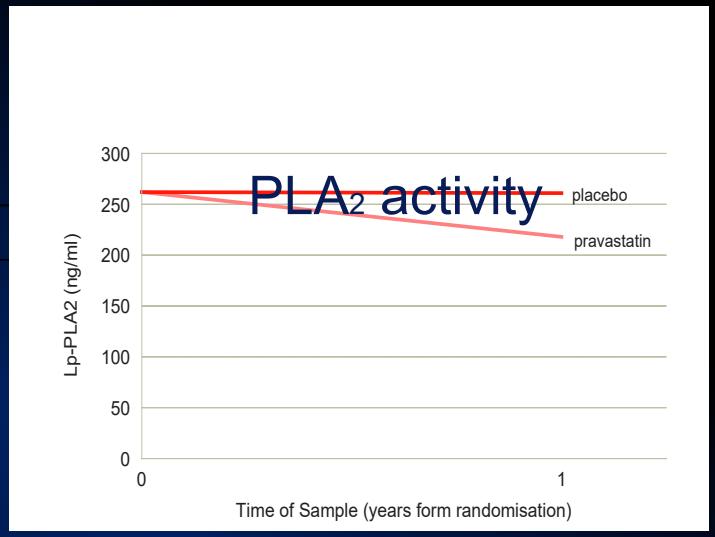
Change of Biomarkers

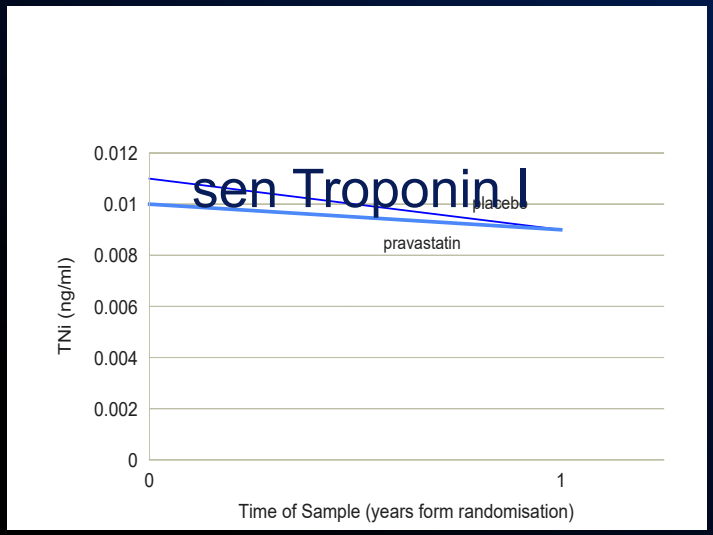


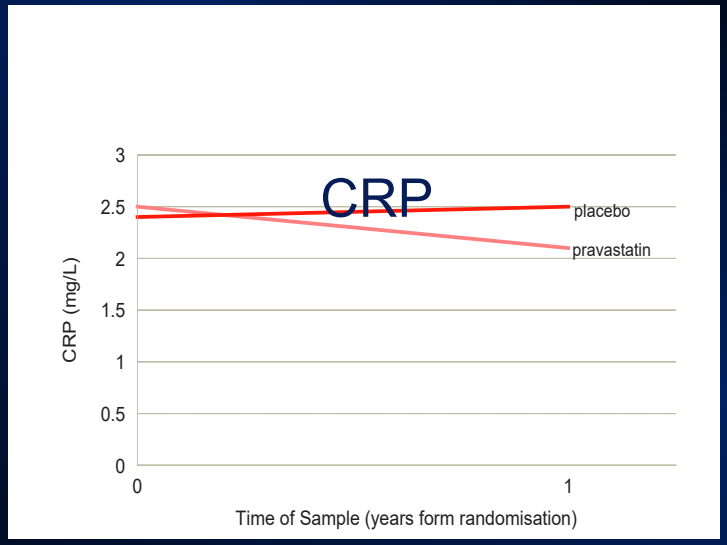


Change of Biomarkers









Baseline Variables

	Placebo		Pravastatin	
	Females	Males	Females	Males
N(%)	674	3248	659	3282
Age at randomisation; median(IQR)	64.0 (58.0 - 69.0)	62.0 (55.0 - 67.0)	64.0 (58.0 - 69.0)	62.0 (55.0 - 67.0)
Age >=65; N(%)	49%	38%	47%	37%
Baseline health				
Months from QE; median(IQR)	13.7 (7.4 - 24.6)	14.2 (8.2 - 25.3)	13.0 (7.7 - 24.0)	13.8 (7.8 - 25.1)
Current Smoker; N(%)	8%	10%	11%	9%
Hypertension; N(%)	57%	39%	55%	38%
Diabetes; N(%)	10%	8%	11%	8%
Obese; N(%)	23%	16%	26%	17%
Stroke; N(%)	4%	5%	24 (4%)	4%
Systolic BP; mean(SD)	138 (21)	134 (19)	138 (20)	133 (19)
Diastolic BP; mean(SD)	80 (11)	81 (11)	80 (11)	81 (11)
Dyspnoea NYHA Class>1; N(%)	13%	9%	14%	9%
Angina CCVS Grade>0; N(%)	45%	36%	49%	35%

Biomarkers at Baseline

	Placebo		Pravastatin	
	Females	Males	Females	Males
BNP [pg/mL]	29.5 (12.8 - 64.3)	22.6 (9.8 - 48.0)	26.2 (11.4 - 57.0)	22.6 (9.3 - 49.8)
C-reactive protein [mg/L]	2.9 (1.3 - 6.1)	2.3 (1.2 - 4.5)	3.3 (1.6 - 6.4)	2.4 (1.2 - 4.5)
Cystatin C [mg/L]	0.8 (0.7 - 1.0)	0.8 (0.7 - 0.9)	0.8 (0.7 - 1.0)	0.8 (0.7 - 0.9)
D Dimer [ng/mL]	194 (133 - 301)	170 (108 - 271)	191 (132 - 275)	167 (107 - 267)
Troponin [mg/L]	0.010 (0.006 - 0.020)	0.010 (0.006 - 0.020)	0.009 (0.006 - 0.019)	0.011 (0.006 - 0.021)
Lp(a) [nmol/L]	16.6 (7.5 - 52.4)	12.8 (6.3 - 42.2)	16.9 (8.3 - 57.3)	13.8 (6.5 - 43.5)
Mid Regional Pro-Adrenomedullin [nmol/L]	0.53 (0.42 - 0.65)	0.47 (0.38 - 0.57)	0.51 (0.40 - 0.64)	0.47 (0.38 - 0.56)
PLA Activity [nmol/min/mL]	230 (47)	269 (50)	231 (47)	268 (48)

*Median (Q1-Q3) is presented except for Lp-PLA_2 activity and LDL cholesterol where Mean(SD) is shown instead

Predictive value of baseline biomarkers on CHD events

Baseline model includes:

treatment with Pravastatin, prior stroke, diabetes mellitus, current smoker, history of hypertension, total cholesterol, HDL, age , gender, type of prior acute coronary syndrome, timing of coronary revascularisation, SBP, atrial fibrillation, eGFR, BMI, dyspnoea grade, angina grade, WBC, peripheral vascular disease, aspirin, fasting glucose, triglycerides, Apo B, Apo A1

Methods – Laboratory I

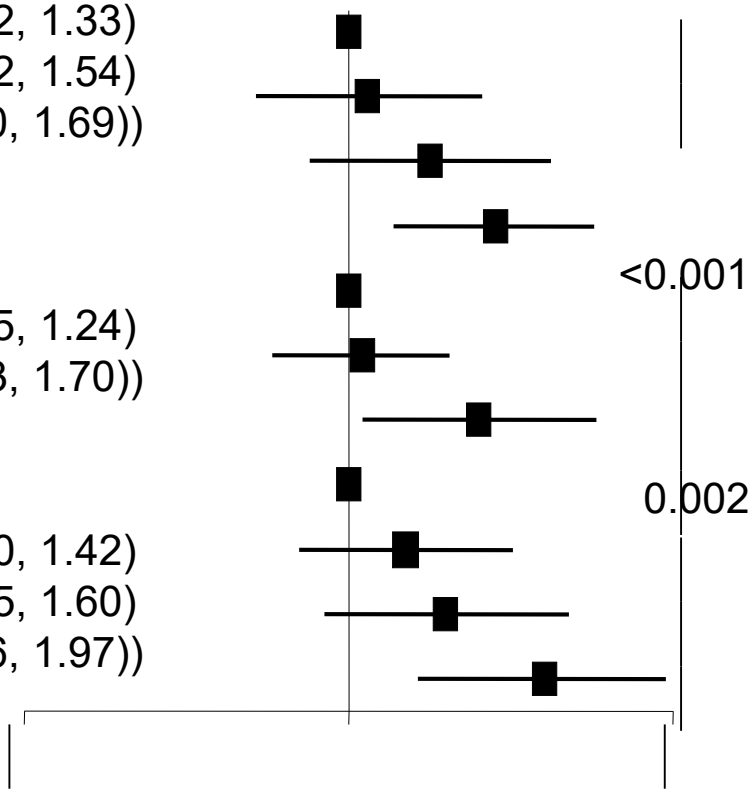
Analyte [unit]	Method	Abbreviation
B-type natriuretic peptide [pg/mL]	Chemiluminescent micro particleimmunoassay (SIEMENS)	BNP
C-reactive protein [mg/L]	Latex Immunoassay (Abbott Diagnostics)	CRP
Cystatin C [mg/L]	Latex Immunoassay (Abbott Diagnostics)	Cystatin C
D-Dimer [ng/mL]	Microparticle enzyme immunoassay (Abbott Diagnostics)	D-Dimer

Methods – Laboratory II

Analyte [unit]	Method	Abbreviation
Mid-regional pro adrenomedullin [nmol/L]	Immunoluminometric (BRAHMS)	MR-proADRM
Lipoprotein-associated phospholipase A_2 [nmol/min/mL]	Colorimetric activity assay	sPL A_2 (diaDEXUS)
Lipoprotein [a] [mg/dL]	Latex Immunoassay (Abbott Diagnostics)	Lp(a)
Sensitive Troponin I [mg/L]	Chemiluminescent particle immumoassay (SIEMENS)	sTnI

Multivariate model including all baseline and change in levels of multiple biomarkers on coronary events that remain significant

Significant Change Biomarker	Quartile levels	HR (95% CI)	P-value (trend)
BNP (pg/ml)	≤ -14.15	1	0.004
	-14.15 - -1.74	1.04 (0.82, 1.33)	
	-1.74 - 7.35	1.19 (0.92, 1.54)	
	> 7.35	1.37 (1.10, 1.69))	
sen TnI (ng/ml)	Lower category	1	<0.001
	Same category	1.03 (0.85, 1.24)	
	Higher category	1.32 (1.03, 1.70))	
Lp-PLA2 (mg/L)	≤ -46.6057	1	0.002
	-46.6057 - -19.8282	1.13 (0.90, 1.42)	
	-19.8282 - 2.8565	1.23 (0.95, 1.60)	
	> 2.8565	1.52 (1.16, 1.97))	

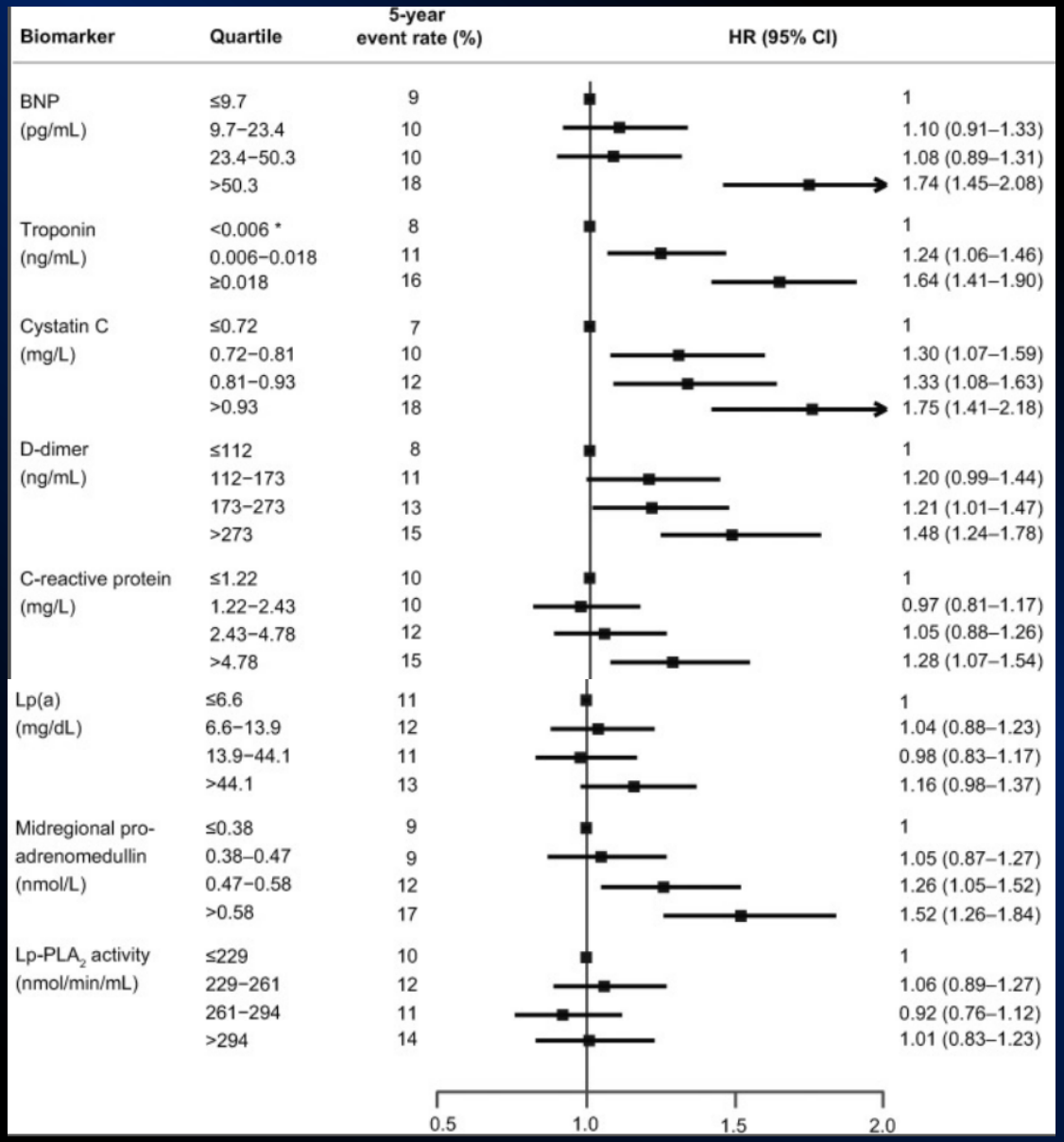


Change of Biomarkers

	Baseline*		Year 1*		change#		
	Placebo	Pravastatin	Placebo	Pravastatin	Placebo	Pravastatin	p-value
BNP	23.7 (10.2 - 50.3)	23.4 (9.5 - 50.9)	20.6 (7.6 - 45.4)	20.0 (7.2 - 44.1)	-1.64	-1.91	0.83
C-reactive protein	2.4 (1.2 - 4.8)	2.5 (1.2 - 4.8)	2.5 (1.3 - 5.2)	2.1 (1.1 - 4.2)	0.11	-0.2	<0.001
Cystatin C^	0.8 (0.7 - 0.9)	0.8 (0.7 - 0.9)	0.8 (0.7 - 0.9)	0.8 (0.7 - 0.9)	0	0	0.001
D Dimer^	173 (112 - 276)	172 (112 - 269)	178 (115 - 284)	166 (108 - 263)	5	-2	<0.001
Troponin	0.010 (0.006 - 0.020)	0.011 (0.006 - 0.021)	0.009 (0.006 - 0.020)	0.009 (0.006 - 0.019)	0	0	0.002
Lp(a)	13.4 (6.5 - 43.4)	14.3 (6.7 - 45.3)	12.9 (6.1 - 41.4)	13.4 (6.1 - 43.6)	-0.2	-0.3	0.11
Mid Regional Pro-adrenomedullin	0.47 (0.38 - 0.58)	0.48 (0.38 - 0.58)	0.47 (0.37 - 0.58)	0.46 (0.37 - 0.57)	-0.001	-0.005	0.03
PLA Activity	262 (51)	262 (50)	261 (50)	218 (47)	-1.02	-43.8	<0.001

*Median (Q1-Q3) is presented except for PLA activity where Mean(SD) is shown instead
^Apparent outliers are present for these variables
% change is the mean change in each treatment group/overall mean at baseline *100

Associations between biomarkers at baseline and coronary heart disease death and nonfatal myocardial infarction

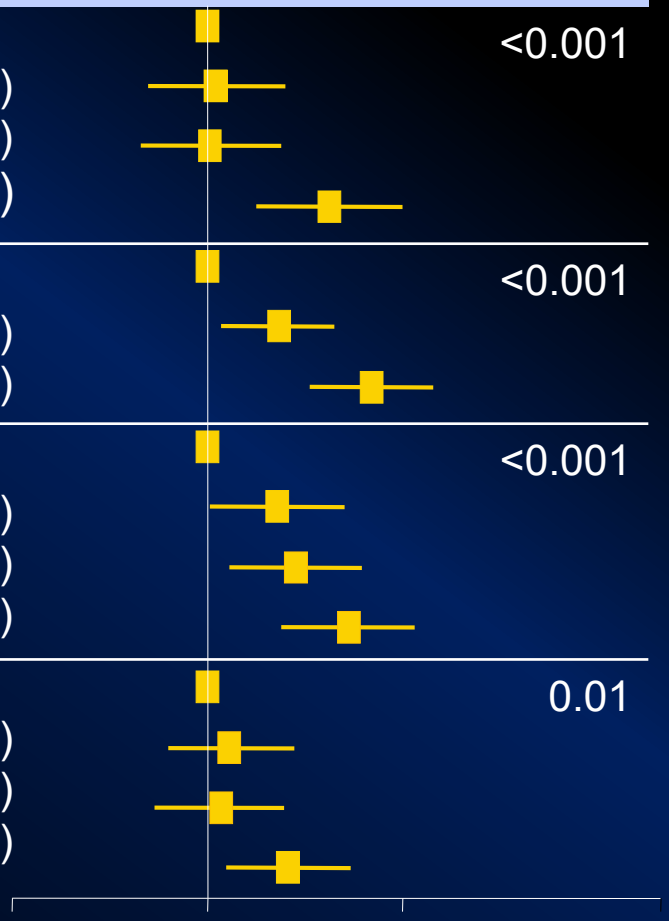


* Troponin not detected

Tonkin AM et al. Int J Cardiol. 2015;201:499-507

Multivariate model including all baseline and change in levels of multiple biomarkers on coronary events that remain significant

Significant Baseline Biomarker	Quartile levels	HR (95% CI)	P-value (trend)
BNP (pg/ml)	≤9.73	1	<0.001
	9.73-23.36	1.03 (0.81, 1.32)	
	23.36-50.29	1.01 (0.79, 1.30)	
	>50.29	1.54 (1.19, 2.00)	
sen TnI (ng/ml)	nd	1	<0.001
	<0.018	1.29 (1.05, 1.57)	
	≥0.018	1.79 (1.44, 2.23)	
Cystatin C (mg/L)	≤ 0.72	1	<0.001
	0.72-0.81	1.28 (1.01, 1.63)	
	0.81-0.93	1.37 (1.08, 1.73)	
	> 0.93	1.65 (1.30, 2.09)	
D dimer (ng/ml)	≤ 112	1	0.01
	112-173	1.08 (0.87, 1.36)	
	173-273	1.05 (0.83, 1.31)	
	> 273	1.33 (1.07, 1.66)	



Multivariate model including all baseline and change in levels of multiple biomarkers on coronary events that remain significant

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	-1.74 - 7.35	1.19 (0.92, 1.54)	
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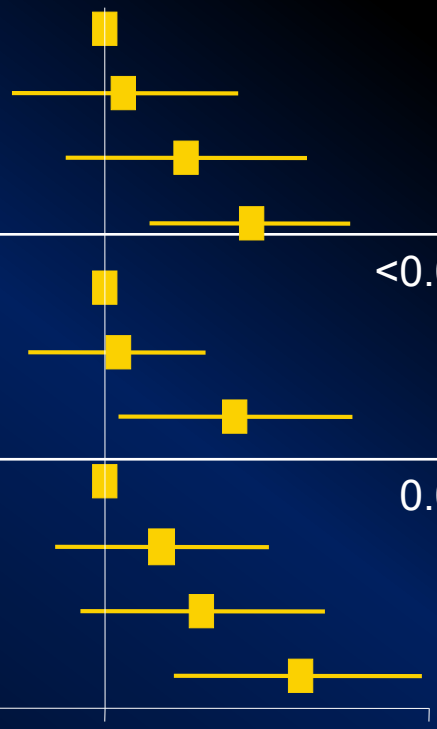
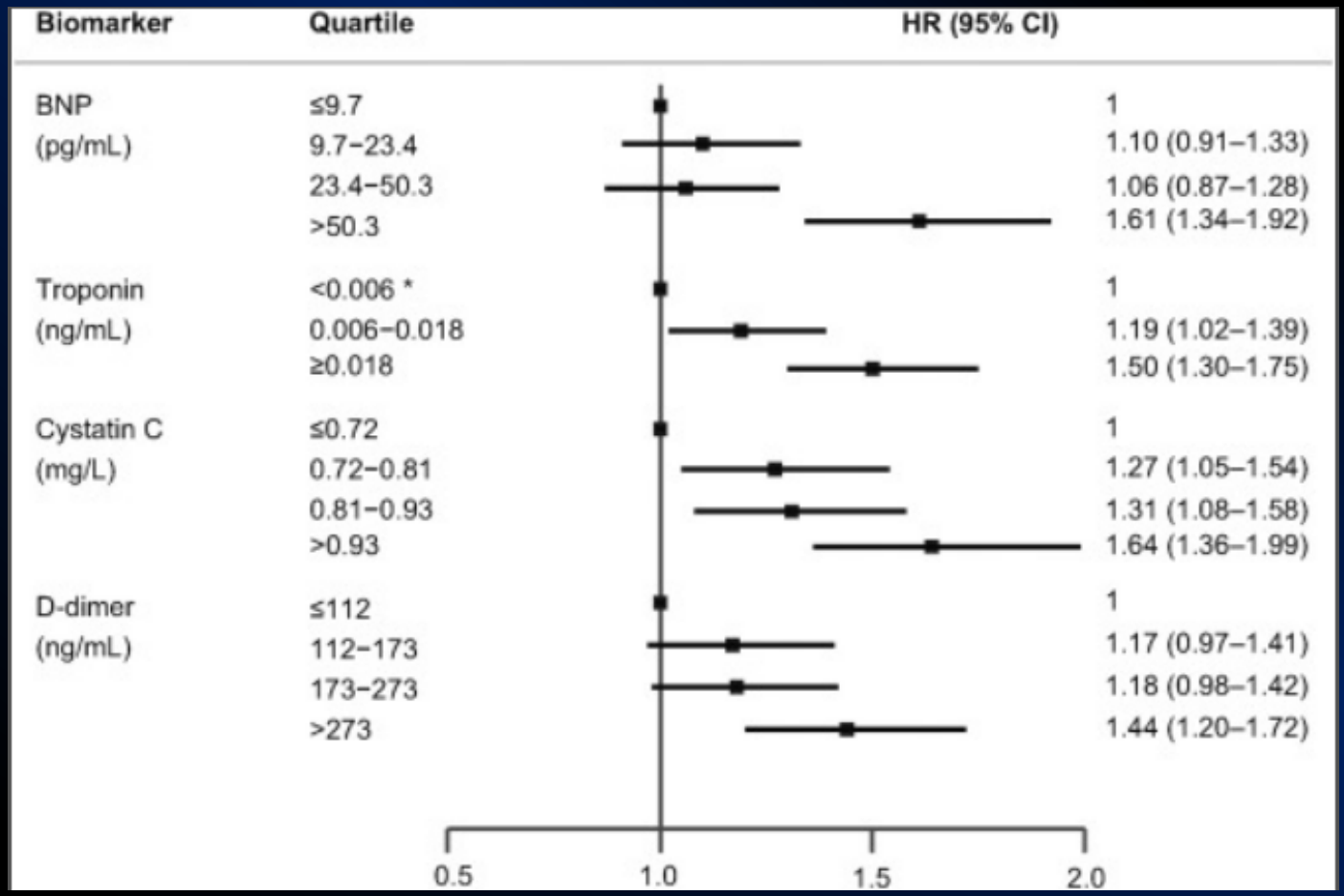
	-46.6057 - -19.8282	1.13 (0.90, 1.42)	
	-19.8282 - 2.8565	1.23 (0.95, 1.60)	
	> 2.8565	1.52 (1.16, 1.97))	



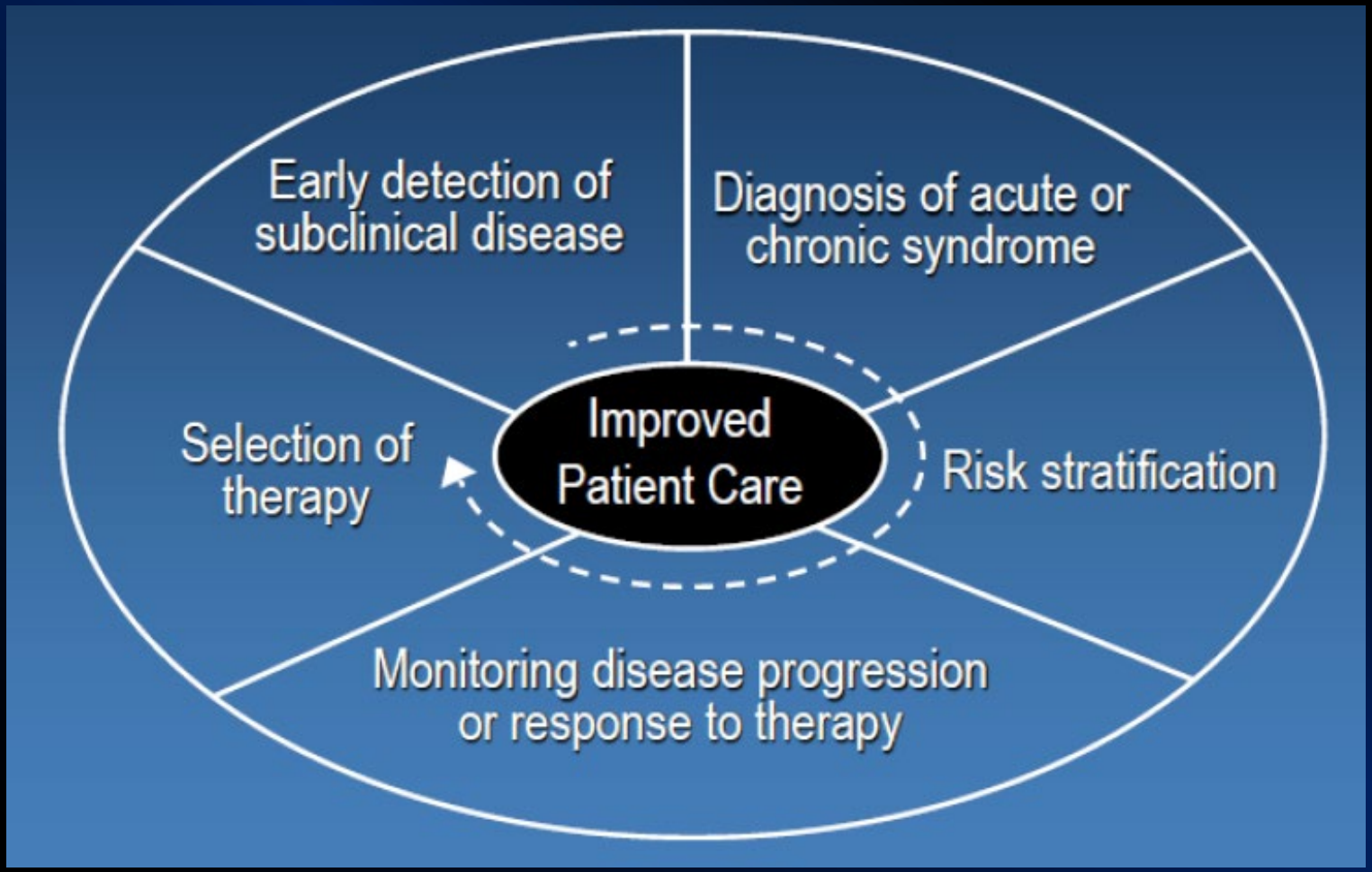
Multivariable associations between biomarkers and coronary heart disease death and nonfatal myocardial infarction



* Troponin not detected

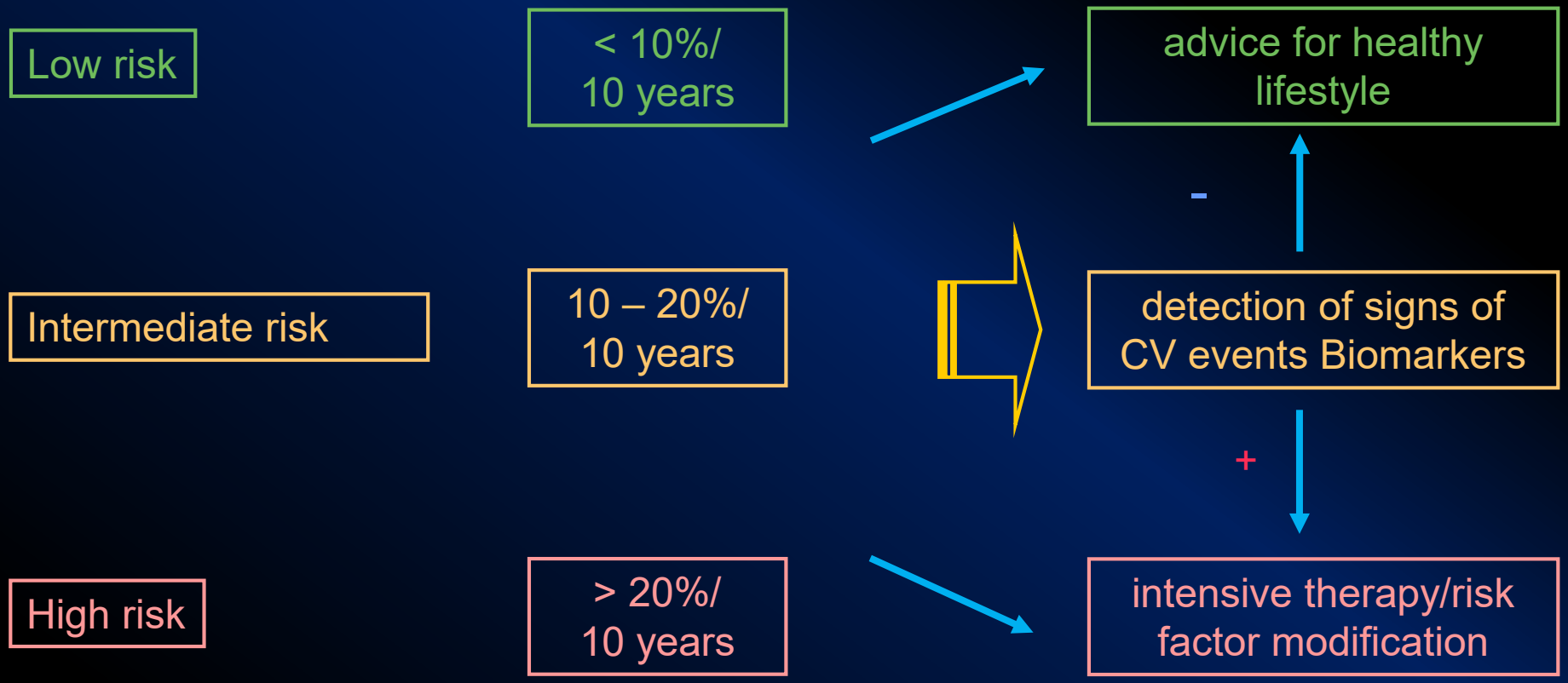
Tonkin AM et al. Int J Cardiol. 2015;201:499-507

Clinical Application of Biomarkers



Morrow et al, Circulation 2007; 1155; 949-52

Potential biomarkers for improving risk based categorization



Greenland et al. Circulation. 2000; 101: 111-116, Greenland et al. Circulation. 2001; 104: 1863-1867, NCEP / ATP III JAMA 2001; 285: 2486-97, Erbel et al. Atherosclerosis 2007; 197: 662-72