



Vierde Nationale Lipidendag
Utrecht, May 17, 2018



Role of Proteomic Analysis in Cardiovascular Risk Categorisation?



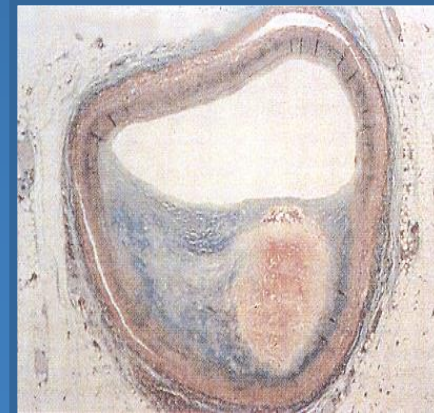
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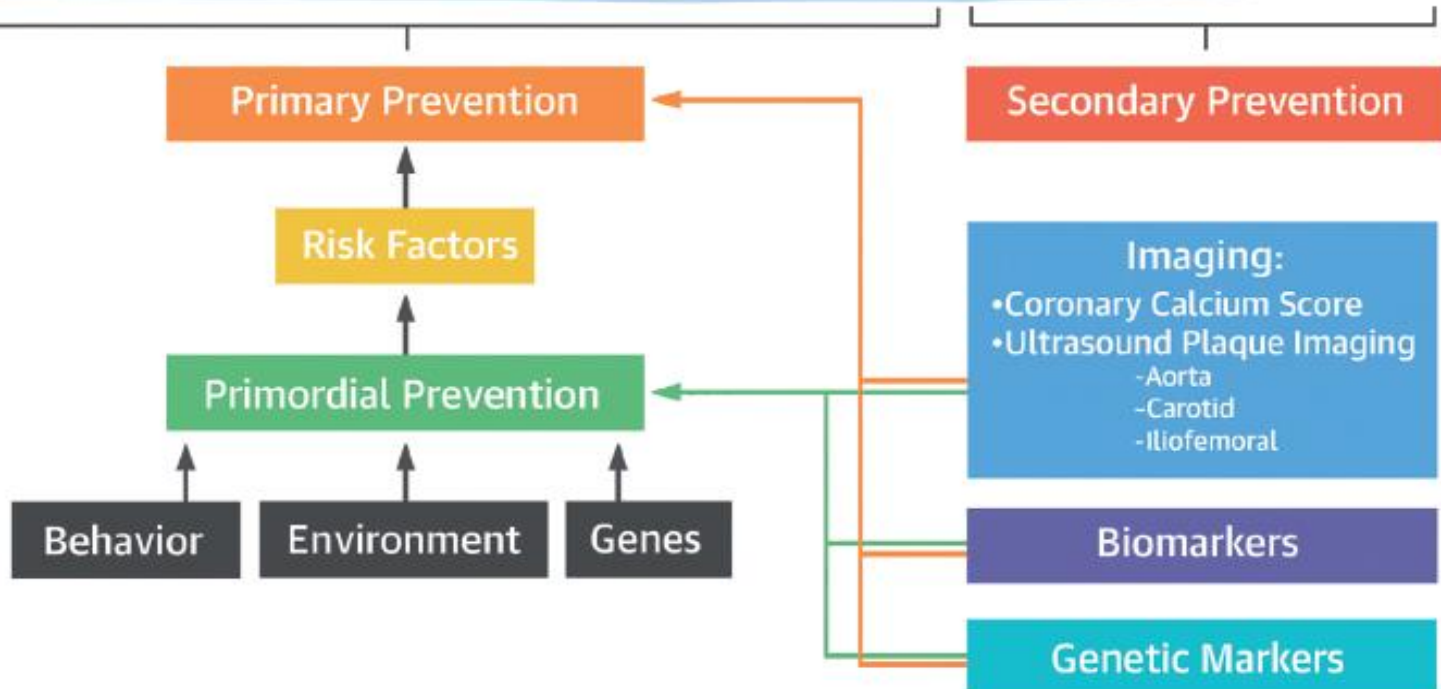
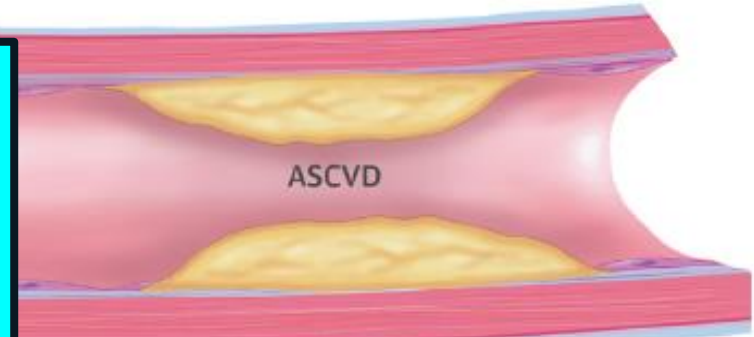


Conflict of Interest (COI) - Disclosure

- *Honorarium for Lectures:* AstraZeneca, Novartis, MSD, Amgen, Sanofi, Actavis, Berlin-Chemie
- *Consulting:* Novartis, Pfizer, The Medicines Company, Amgen, AstraZeneca, MSD, Kowa
- *Participation in Clinical Trials:* LEADER (Novo Nordisk), CANTOS (Novartis), FOURIER, GLAGOV (Amgen), OPTIONS I und II (Sanofi/Regeneron), SPIRE (Pfizer), CAIN III (MHICC), PROMINENT (Kowa), DalGene (DalCor), COLCOT (MHICC)
- *Research Contracts:* Abbott, Roche Diagnostics, Beckmann, Singulex
- *Stockholder of a Healthcare Company:* none

Prevention of Atherosclerotic Cardiovascular Disease (ASCVD)

50% of 1,779 subjects in PESA with no conventional risk factor had subclinical atherosclerosis as detected by US or CAC imaging
JACC 2017;70:2979-91



Systematic Coronary Risk Evaluation (SCORE)

Andorra, Austria, Belgium, Cyprus, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Israel, Italy, Luxembourg, Malta, Monaco, The Netherlands, Norway, Portugal, San Marino, Slovenia, Spain, Sweden, Switzerland and the UK

The rest of Europe

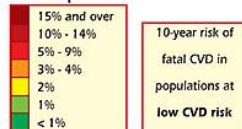
CLASS I

1. Global risk scores (such as the Framingham Risk Score [FRS]) that use multiple traditional cardiovascular risk factors should be obtained for risk assessment in all asymptomatic adults without a clinical history of CHD. These scores are useful for combining individual risk factor measurements into a single quantitative estimate of risk that can be used to target preventive interventions (25).
(*Level of Evidence: B*)

- Ger
- Smo
- Age
- Sys
- pres
- Total

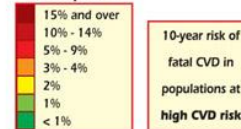
10 year risk of
fatal CVD

SCORE

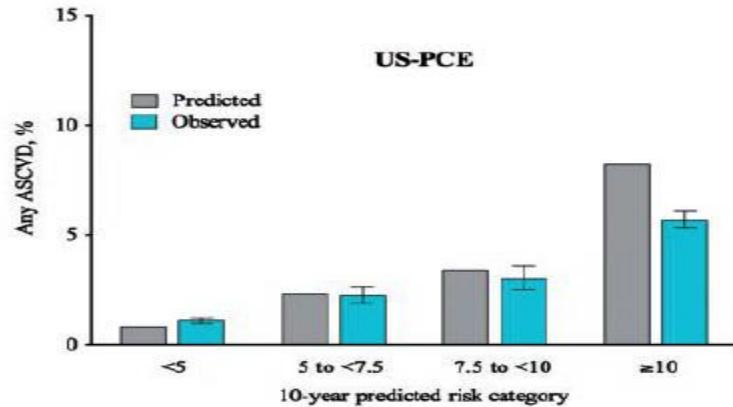


Based on Conroy
Society of Cardiology

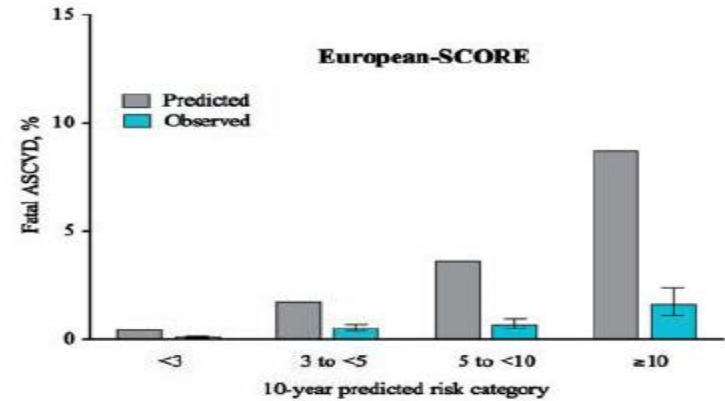
SCORE



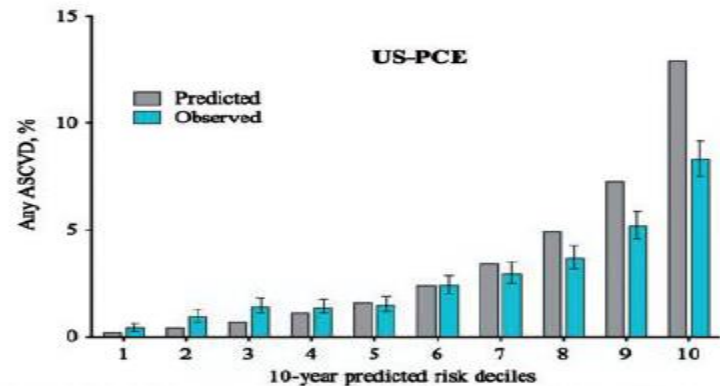
Calibration Comparing Observed and Predicted Events in 40- to 75-Year-Old Individuals in the CGPS



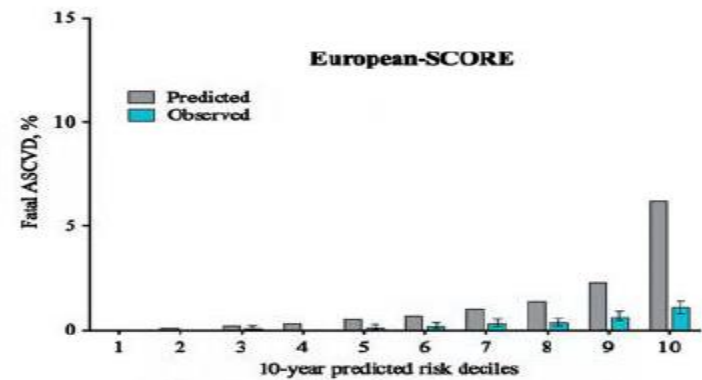
Mean predicted 10-year risk	2.1	6.2	8.7	19.2	8.5
No. of individuals	21746	5332	3839	13972	44 889
No. of observed events	235	123	115	792	1265
No. of predicted events	164	125	130	1144	1562
Predicted / observed	0.7	1.0	1.1	1.4	1.2



	1.0	3.9	6.8	14.3	2.6
No. of individuals	31040	6762	5534	1553	44 889
No. of observed events	28	32	37	25	122
No. of predicted events	116	115	200	134	565
Predicted / observed	4.1	3.6	5.4	5.4	5.0

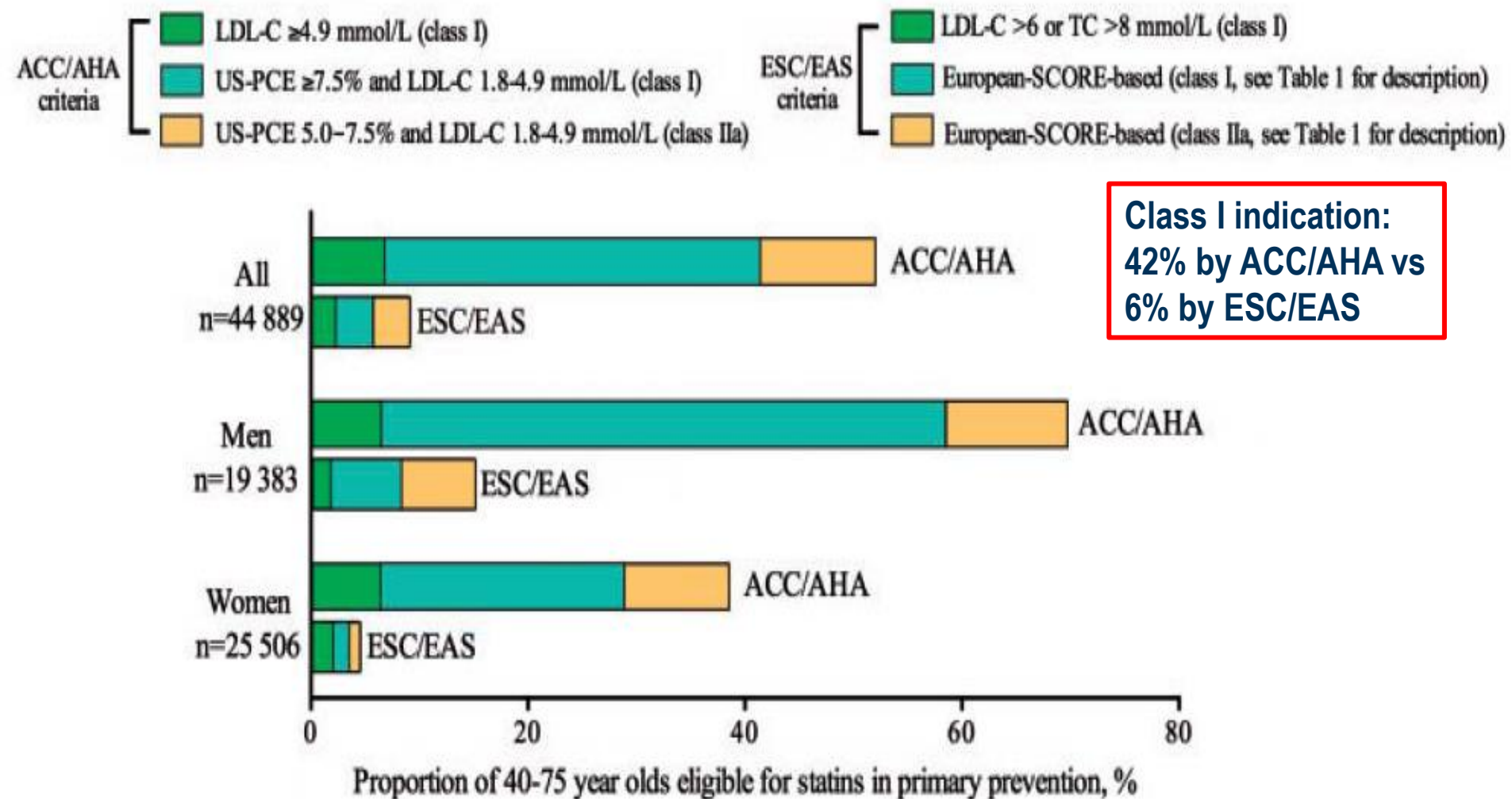


Mean predicted 10-year risk	0.5	1.1	1.9	3.0	4.5	6.4	8.9	12.4	17.6	28.3
No. of individuals	4488	4489	4489	4489	4489	4489	4489	4489	4489	4489
No. of observed events	18	42	63	61	66	107	132	166	234	374
No. of predicted events	7	16	30	49	73	106	153	221	327	580
Predicted / observed	0.4	0.4	0.5	0.8	1.1	1.0	1.2	1.3	1.4	1.6



	0.1	0.2	0.5	0.8	1.3	1.9	2.7	3.8	5.6	9.6
No. of individuals	4488	4489	4489	4489	4489	4489	4489	4489	4489	4489
No. of observed events	0	0	3	0	5	8	14	15	28	49
No. of predicted events	1	3	7	12	20	31	44	64	104	278
Predicted / observed	-	-	2.3	-	4.0	3.9	3.1	4.3	3.7	5.8

ACC/AHA guidelines superior to ESC/EAS guidelines for primary prevention with statins in non-diabetic Europeans: the Copenhagen General Population Study



Identification of vascular patients at very high risk for recurrent cardiovascular events: validation of the current ACC/AHA very high risk criteria

SMART and REACH registries:

- VHR criteria: CVD, +/-DM, SMK, HLP, recent CHD event
- C-statistics: 0.53 in REACH and 0.54 in SMART
- Clinical criteria like eGFR, PVD, or age > 70 performed as well
- 2/3 of patients meeting the ACC/AHA VHR criteria had a 10 year risk of recurrent MACE < 30%

2.9/100PY) and in REACH this was 64% (5.9/100PY, 95% CI 5.7–6.1/100PY). The C-statistic for the ACC/AHA VHR criteria was 0.53 in REACH and 0.54 in SMART. Very high risk factors with comparable or slightly better performance were eGFR < 45, polyvascular disease and age > 70 years. Around two third of the patients meeting the ACC/AHA VHR criteria had a predicted 10-year risk of recurrent MACE < 30%.

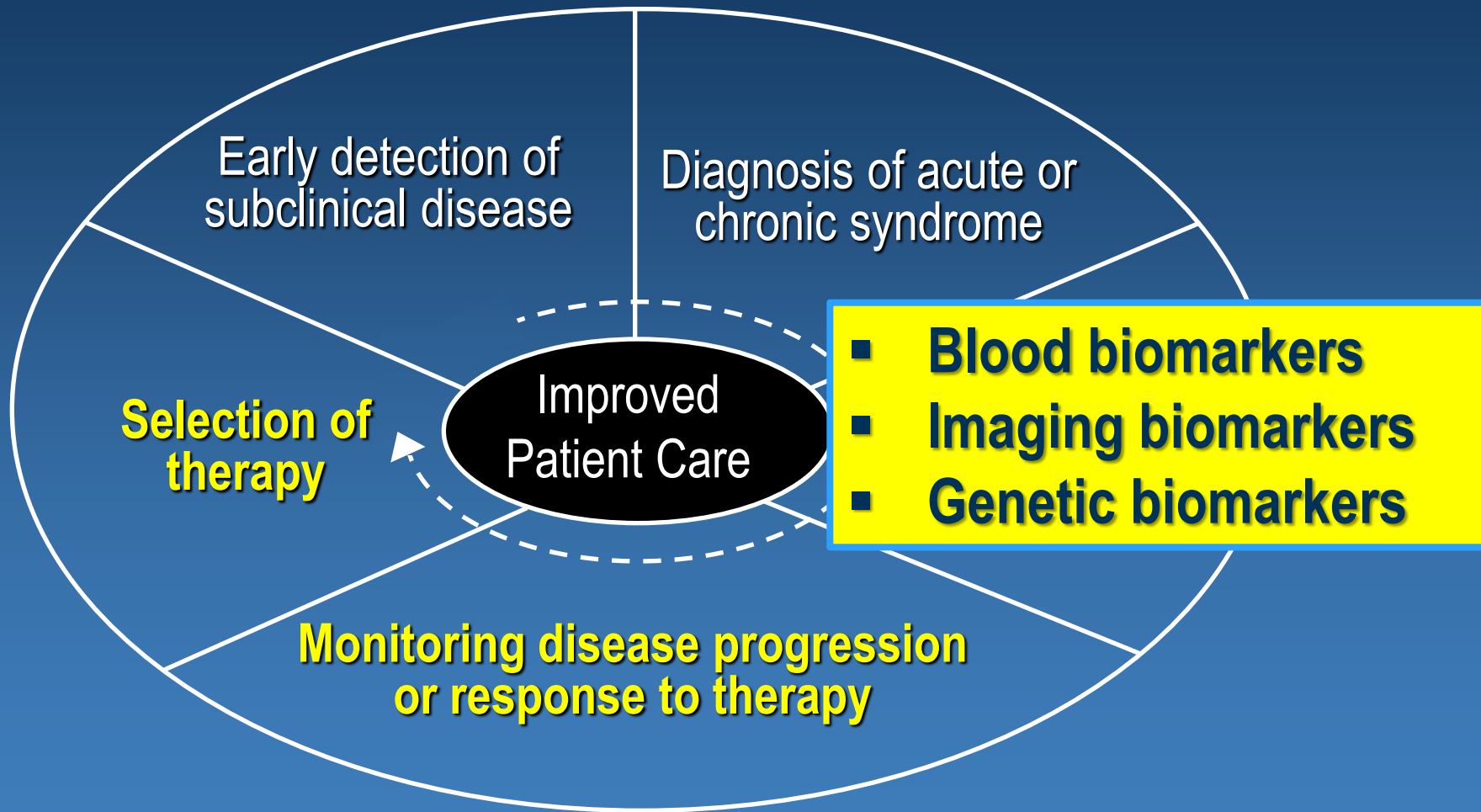
Conclusion

The ACC/AHA VHR criteria have limited discriminative power. Identifying patients with clinically manifest arterial disease at VHR for recurrent vascular events using eGFR < 45, polyvascular disease, or age > 70 years performs as well as the ACC/AHA VHR criteria.

Keywords

Cardiovascular events • Risk prediction • Secondary prevention • Very high risk

Clinical Applications of Cardiovascular Biomarkers



The MORGAM Biomarker Study: CHD

METHODS

- Prospective evaluation of 30 biomarkers reflecting inflammation, oxidative stress, lipid metabolism, renal function, hemodynamic stress, metabolic processes, coagulation, vitamins, and markers of myocardial necrosis
- 7,915 men and women from FINRISK_97 study w/o Hx of MI or stroke at baseline (derivation) and 2551 men in PRIME (validation)
- Follow-up 10 years; 538 incident CV events in FINRISK and 260 in PRIME.
- Fully adjusted Cox Proportional Hazards models
- Endpoint: CVD (MI, UA, CABG/PCI, ischemic stroke)

Biomarkers in the MORGAM-Cohorts

Lipid related markers

Apolipoprotein A1
Apolipoprotein B100
Lipoprotein - associated phospholipase A2
- activity
- mass
Paraoxonase-1

Renal function markers

Creatinine
Cystatin-C

Metabolic markers

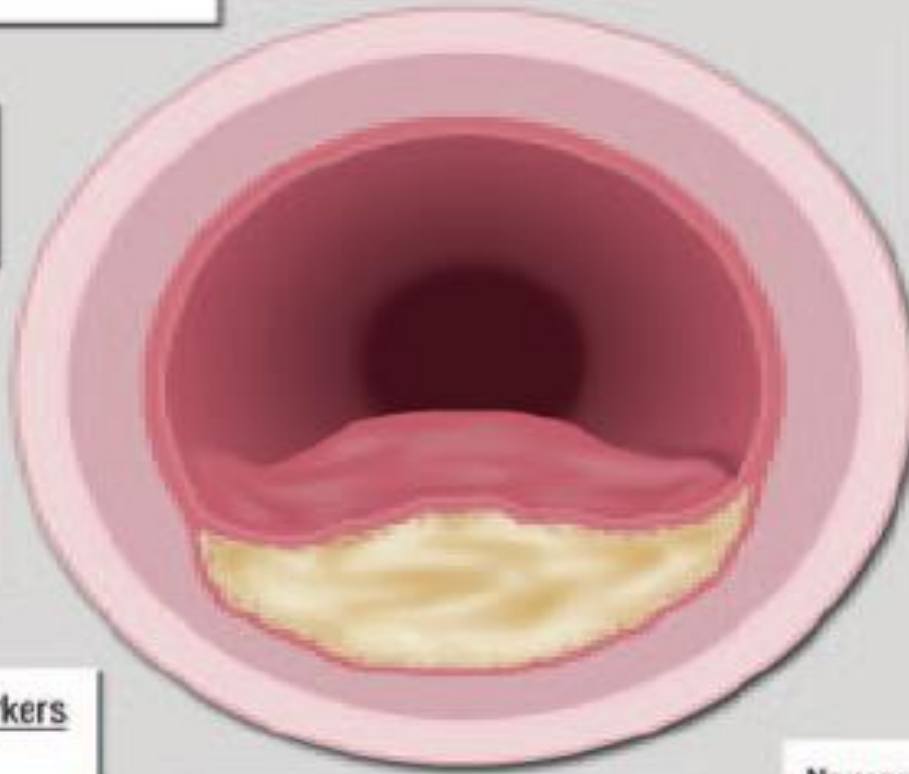
Adiponectin
Leptin
Insulin
Ferritin
Glucose

Coagulation markers

D-Dimer

Markers of vascular function and neurohumoral activity

(N-terminal pro) B-type natriuretic peptide
C-terminal pro-vasopressin
C-terminal pro-endothelin-1
Mid-regional pro-adrenomedullin
Mid-regional pro-atrial natriuretic peptide
Tissue inhibitor of metalloproteinase-1



Inflammatory markers

C-reactive protein
Interleukin-18
Interleukin-1 receptor antagonist
Neopterin

Markers of oxidative stress and antioxidants

Homocysteine
Myeloperoxidase
Vitamin B₂
Active vitamin B₁₂

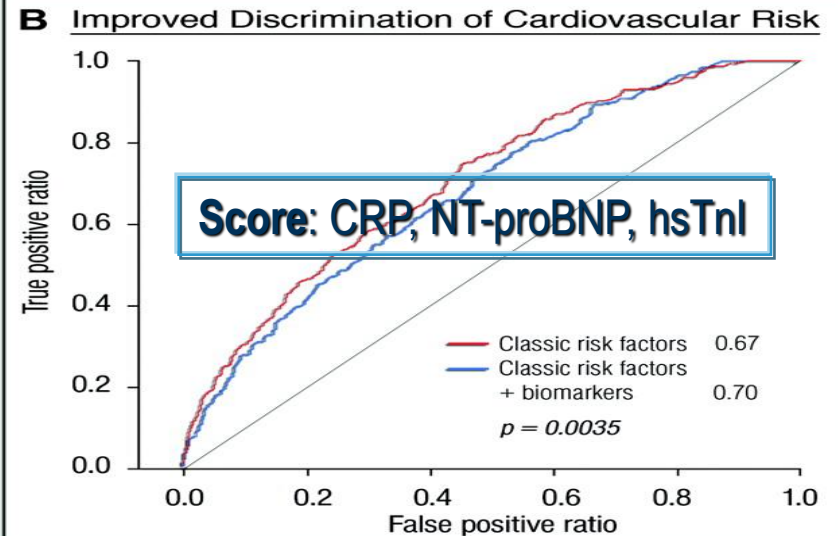
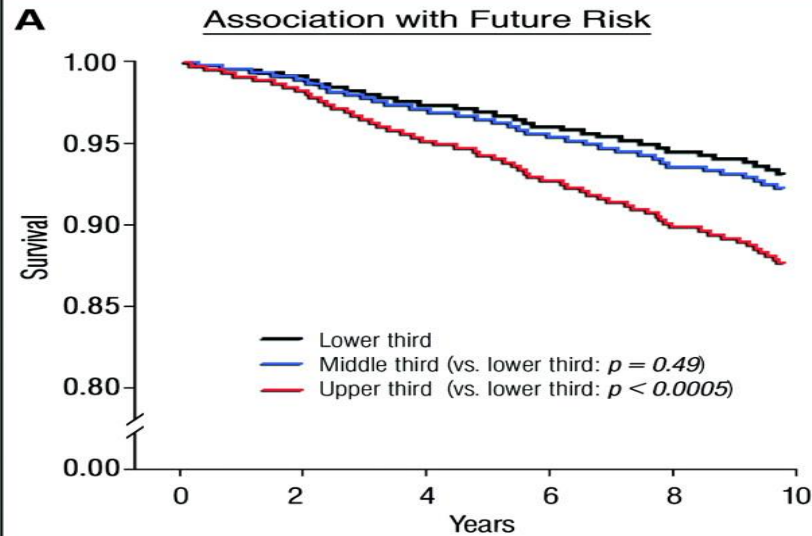
Angiogenesis markers

Cardiac placental growth factor

Necrosis markers

Creatine kinase-MB
Troponin I

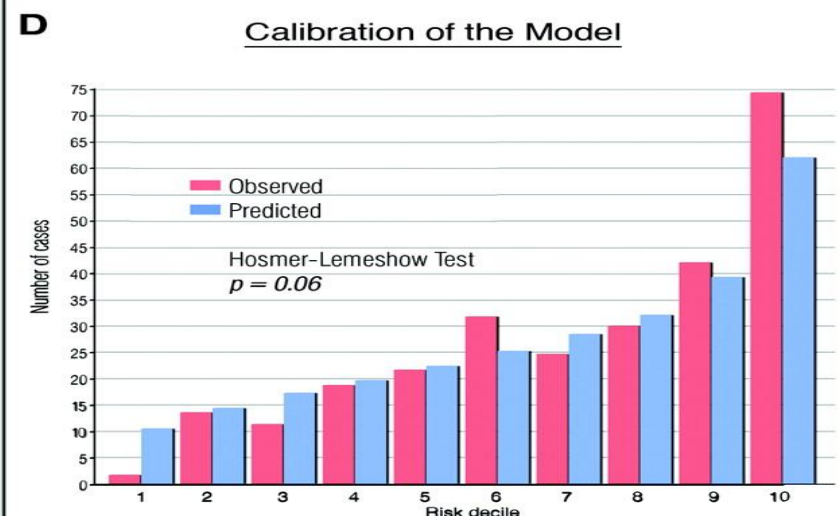
Contribution of 30 Biomarkers to 10-Year CV Risk in 2 Population Cohorts: The MORGAM Biomarker Project



C Reclassification of Risk

Predicted Risk Without Biomarker Score	Predicted Risk With Biomarker Score					
	<5%	5-10%	10-20%	>20%	Up	Down
Participants developing cardiovascular events during 10 year follow up						
<5%	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		
5-10%	2 (2.3%)	66 (73.3%)	20 (21.9%)	2 (2.6%)	43 (15.8%)	27 (9.9%)
10-20%	0 (0.0%)	20 (14.6%)	99 (70.6%)	21 (14.9%)		
>20%	0 (0.0%)	0 (0.0%)	4 (10.0%)	37 (90.0%)		
Participants not developing cardiovascular events during 10 year follow up						
<5%	152 (81.7%)	33 (17.7%)	1 (0.5%)	0 (0.0%)		
5-10%	80 (7.6%)	869 (82.3%)	101 (9.6%)	6 (0.5%)	190 (8.3%)	300 (13.2%)
10-20%	0 (0.0%)	197 (2.7%)	702 (74.1%)	49 (5.2%)		
>20%	0 (0.0%)	0 (0.0%)	24 (26.5%)	66 (73.5%)		

Net reclassification 0.11 (SE 0.03); $p = 0.0008$



Predictive Value of Inflammatory Biomarkers Assessed by AUC for Incident Coronary Events (1): KORA CCS

Biomarker	AUC1 ^a	Δ AUC1 (95% CI) ^a	AUC2 ^b	Δ AUC2 (95% CI) ^b
None	0.807	-	0.845	-
hsCRP	0.809	0.002 (0.001–0.008)	0.845	0.000 (-0.001–0.003)
IL-6	0.810	0.003 (0.001–0.008)	0.846	0.001 (-0.000–0.004)
IL-18	0.808	0.001 (0.001–0.005)	0.845	0.000 (-0.001–0.003)
TGF- β 1	0.808	0.001 (0.001–0.005)	0.845	0.000 (-0.001–0.003)
MIF	0.809	0.002 (0.001–0.008)	0.845	0.000 (-0.001–0.003)
MCP-1	0.808	0.001 (0.001–0.005)	0.845	0.000 (-0.001–0.002)
IL-8	0.808	0.001 (0.001–0.003)	0.845	0.000 (-0.000–0.002)
IP-10	0.808	0.001 (0.000–0.005)	0.845	0.000 (-0.001–0.005)
RANTES	0.808	0.001 (0.001–0.006)	0.845	0.000 (-0.000–0.003)
Adiponectin	0.808	0.001 (0.000–0.007)	0.845	0.000 (-0.000–0.005)

*CPHM

Predictive Value of Inflammatory Biomarkers Assessed by AUC for Incident Coronary Events (2): KORA CCS

Biomarker	AUC1 ^a	Δ AUC1 (95% CI) ^a	AUC2 ^b	Δ AUC2 (95% CI) ^b
Leptin	0.811	0.004 (0.001–0.010)	0.845	0.000 (-0.000–0.004)
sE-selectin	0.817	0.010 (0.004–0.018)	0.847	0.002 (-0.000–0.009)
sICAM-1	0.817	0.010 (0.005–0.021)	0.848	0.003 (0.000–0.011)
With all 13 biomarkers	0.825	0.018 (0.013–0.038)	0.851	0.006 (0.003–0.021)
With IL-18, adiponectin, sE-selectin, sICAM-1 ^c	0.819	0.012 (0.006–0.024)	0.849	0.004 (0.001–0.012)

Bold print denotes statistical significance for DAUC (P,0.05). “20.000” denotes values between 20.0005 and 0.0000.

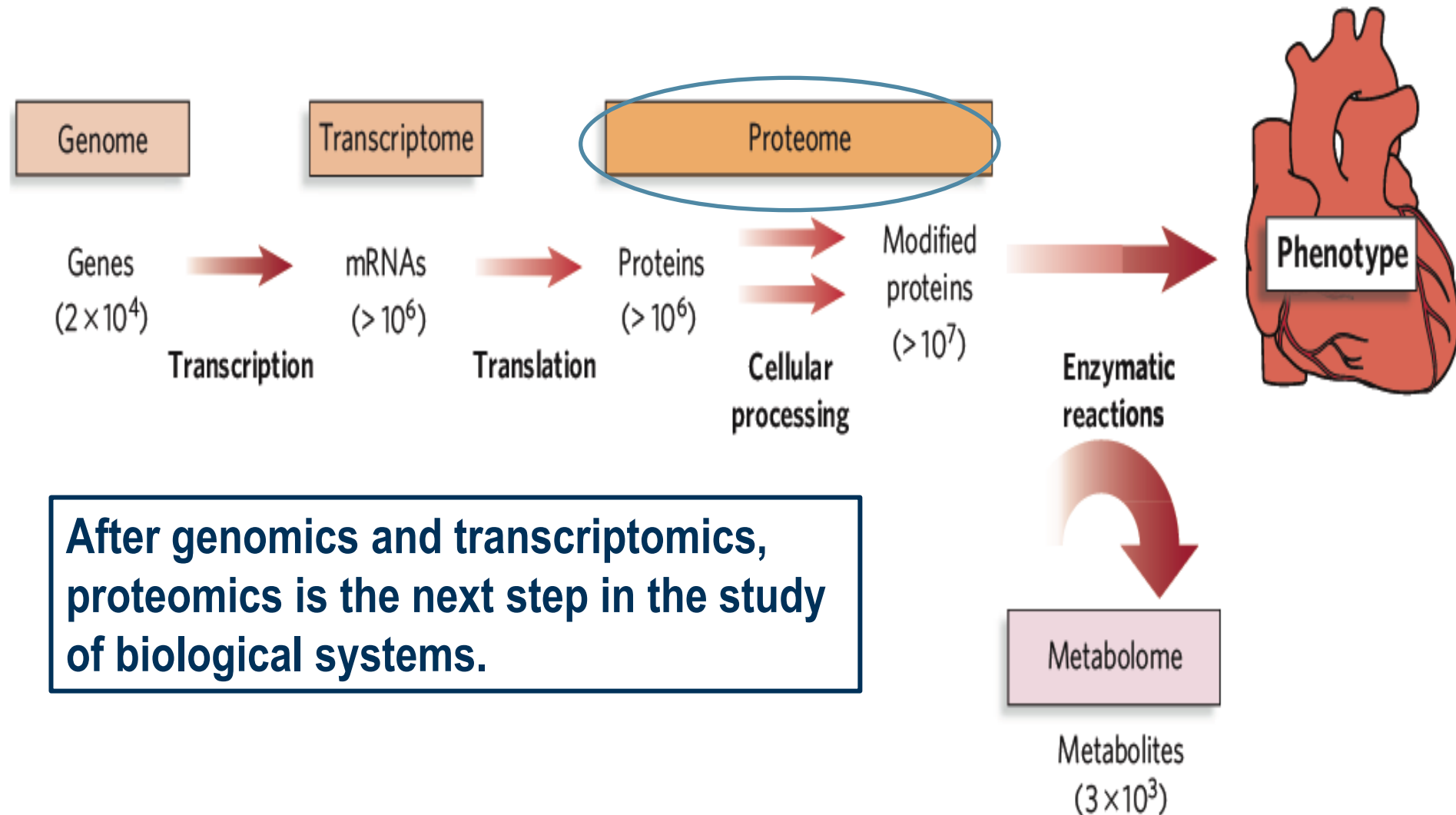
^aAdjusted for age, sex and survey (model 1).

^bAdjusted for age, sex, survey, BMI, systolic blood pressure, ratio of total cholesterol/HDL cholesterol, smoking, alcohol, physical activity, parental myocardial Infarction and prevalent diabetes (model 2).

^cWith biomarkers that were significantly associated with incident coronary events in multivariable-adjusted models (IL-6, sICAM-1).

DAUC denotes the differences between the model with the respective inflammation-related biomarker and the model without any inflammation-related biomarker. DAUC for the difference between the model adjusted for age, sex, survey and cardiometabolic risk factors (model c) and the basic model adjusted for age, sex And survey (model a) was 0.038 [95% CI 0.026–0.055].

The Conceptual Relationship of the Genome, Transcriptome, Proteome and Metabolome



The background of the slide shows a blurred image of laboratory equipment, likely a mass spectrometer or a similar analytical instrument, with various metal components and a scale visible at the bottom.

Definition:

Proteomics is the large scale study of proteins. The proteome („portmanteau of protein and genome“) represents the entire set of proteins that are produced by an organism or system, varying with time and from cell to cell.

Methods:

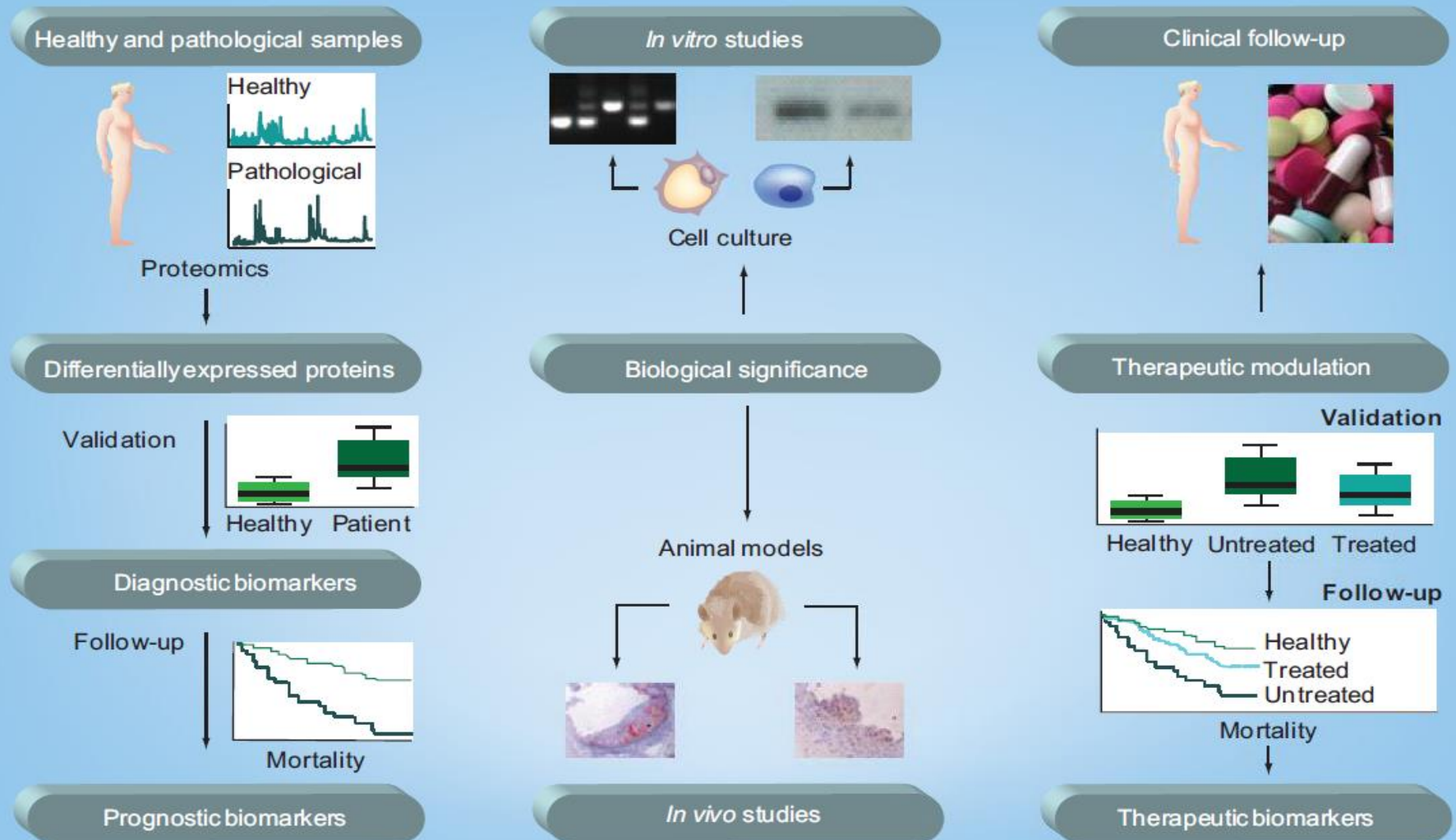
- 1. Immunoassays (e.g. ELISA), Western blot**
- 2. 2-D gel electrophoresis**
- 3. High-throughput proteomic technologies (MALDI-TOF/mass spectrometry)**
- 4. Protein microarrays (protein chips)**

Diagnostic biomarkers in cardiovascular disease: the proteomics approach

Applicable for
QSOX1⁵

- | | |
|--|---------------|
| 1 Proof of concept—do novel marker levels differ between subjects with and without disease? | Yes |
| 2 Prospective validation—does the novel marker predict development of disease in a prospective cohort or nested case-cohort/case-cohort study? | Yes |
| 3 Incremental value—does the novel marker add predictive information to the established risk marker? | Yes |
| 4 Clinical utility—does the novel marker change predicted risk sufficiently to change recommended therapy? | To be studied |
| 5 Clinical outcome—does the use of the novel marker improve clinical outcomes, especially when tested in randomized clinical trial? | To be studied |
| 6 Cost-effectiveness—does the novel marker improve clinical outcomes sufficiently to justify the additional costs of testing and treatment? | To be studied |

Vascular Proteomics, a Translational Approach: From Traditional to Novel Proteomic Techniques



Protein Biomarkers of New-Onset Cardiovascular Disease

Prospective Study From the Systems Approach to Biomarker Research in

- **Discovery MS (LC-MS/MS)** to determine 861 proteins in a nested case-control study from the Framingham Heart Study (135 MI/135 non-MI)
- **Validation MS (MRM)** with 59 most promising markers by targeted MS in 336 ASCVD case-control pairs
- Single and multi-marker analyses adj. for established CVRF
- 12 single markers from discovery MS were associated with MI incidence
- 7 proteins in aggregate were highly associated with MI and improved prediction compared to a clinical RF base model (AUC 0.71 vs 0.84)
- Through targeted MS 12 single proteins were predictors of ASCVD
- In multi-marker analyses 4 proteins in combination predicted ASCVD and moderately improved C-statistics (AUC 0.69-0.73)

Conclusions—Proteomics profiling identified single- and multiple-marker protein panels that are associated with new-onset ASCVD and may lead to a better understanding of underlying disease mechanisms. Our findings include many novel protein biomarkers that, if externally validated, may improve risk assessment for MI and ASCVD. (*Arterioscler Thromb Vasc Biol.* 2014;34:939-945.)

Yin et al. ATVB 2014;34:939-945

Key Words: biological markers ■ cardiovascular diseases ■ epidemiology ■ myocardial infarction ■ proteomics

Protein Biomarkers of ACVD and MI: Multiple-Marker Analyses From MRM and Depletion MRM MS

Gene Symbol	Protein Name	Odds Ratio (95% CI)	<i>P</i> Value
ASCVD (n=336 pairs)			
ORM1	Alpha-1–acid glycoprotein 1	1.45 (1.17, 1.80)	0.0007
PON1	Paraoxonase 1	0.75 (0.60, 0.94)	0.014
CLEC3B	Tetranectin	0.76 (0.61, 0.95)	0.017
CD5L	CD5 antigen-like	0.81 (0.67, 0.98)	0.031
MI (n=135 pairs)			
SERPINA10	Protein Z–dependent protease inhibitor	1.70 (1.16, 2.50)	0.0070
CRP	C-reactive protein	1.51 (1.03, 2.23)	0.037
CD5L	CD5 antigen-like	0.70 (0.50, 0.98)	0.039

Results are all from stepwise selection in conditional logistic regression models, adjusting for age, sex, current smoking status, statin use, systolic blood pressure, hypertension treatment status, total cholesterol, high-density lipoprotein–cholesterol, diabetes mellitus status and body mass index. Candidate markers include all 59 proteins measured by MRM and depletion MRM. *P* value of 0.05 was used as both enter and stay criteria. For each biomarker, data were rank normalized and have mean 0, SD 1. Odds ratios are in unit of 1 SD. ASCVD indicates atherosclerotic cardiovascular disease; CI, confidence intervals; MI, myocardial infarction; and MRM, multiple reaction monitoring.

Original Investigation | INNOVATIONS IN HEALTH CARE DELIVERY

Development and Validation of a Protein-Based Risk Score for Cardiovascular Outcomes Among Patients With Stable Coronary Heart Disease

Peter Ganz, MD; Bettina Heidecker, MD; Kristian Hveem, MD, PhD; Christian Jonasson, PhD; Shintaro Kato, MS; Mark R. Segal, PhD; David G. Sterling, PhD; Stephen A. Williams, MD, PhD

938 patients with stable CAD followed for 5.6 years

IMPORTANCE Precise stratification of cardiovascular risk in patients with coronary heart disease (CHD) is needed to inform treatment decisions.

OBJECTIVE To derive and validate a score to predict risk of cardiovascular outcomes among patients with CHD, using large-scale analysis of circulating proteins.

DESIGN, SETTING, AND PARTICIPANTS Prospective cohort study of participants with stable CHD. For the derivation cohort (Heart and Soul study), outpatients from San Francisco were enrolled from 2000 through 2002 and followed up through November 2011 (≤ 11.1 years). For the validation cohort (HUNT3, a Norwegian population-based study), participants were enrolled from 2006 through 2008 and followed up through April 2012 (5.6 years).

EXPOSURES Using modified aptamers, 1130 proteins were measured in plasma samples.

Ganz et al. JAMA 2016;
315:2532-2541

Sample and Statistical Process for Evaluation of the 9-Protein Model

Protein quantification by modified aptamer assay
1130 Proteins measured in a total of 2496 samples^a

1054 Proteins and 2423 samples passed quality control^b

Derivation cohort

938 Baseline samples from participants in the Heart and Soul study

16 Proteins selected by the least absolute shrinkage and selection operator (LASSO)

9-Protein model constructed through stepwise backward elimination

Validation cohort

971 Baseline samples from participants in the HUNT3 (Helseundersøkelsen i Nord-Trøndelag) study

Evaluation of performance of 9-Protein model

Analysis of longitudinal changes in proteins

514 Paired samples, 5 years apart, from participants in the Heart and Soul study derivation cohort

Evaluation of longitudinal changes in 9-Protein model

Aptamer-based proteomic profiling:
Single stranded DNA aptamers (Oligos of appr. 50 base pairs in length selected to bind proteins with high specificity and affinity) and measure > 1100 proteins in a single blood sample

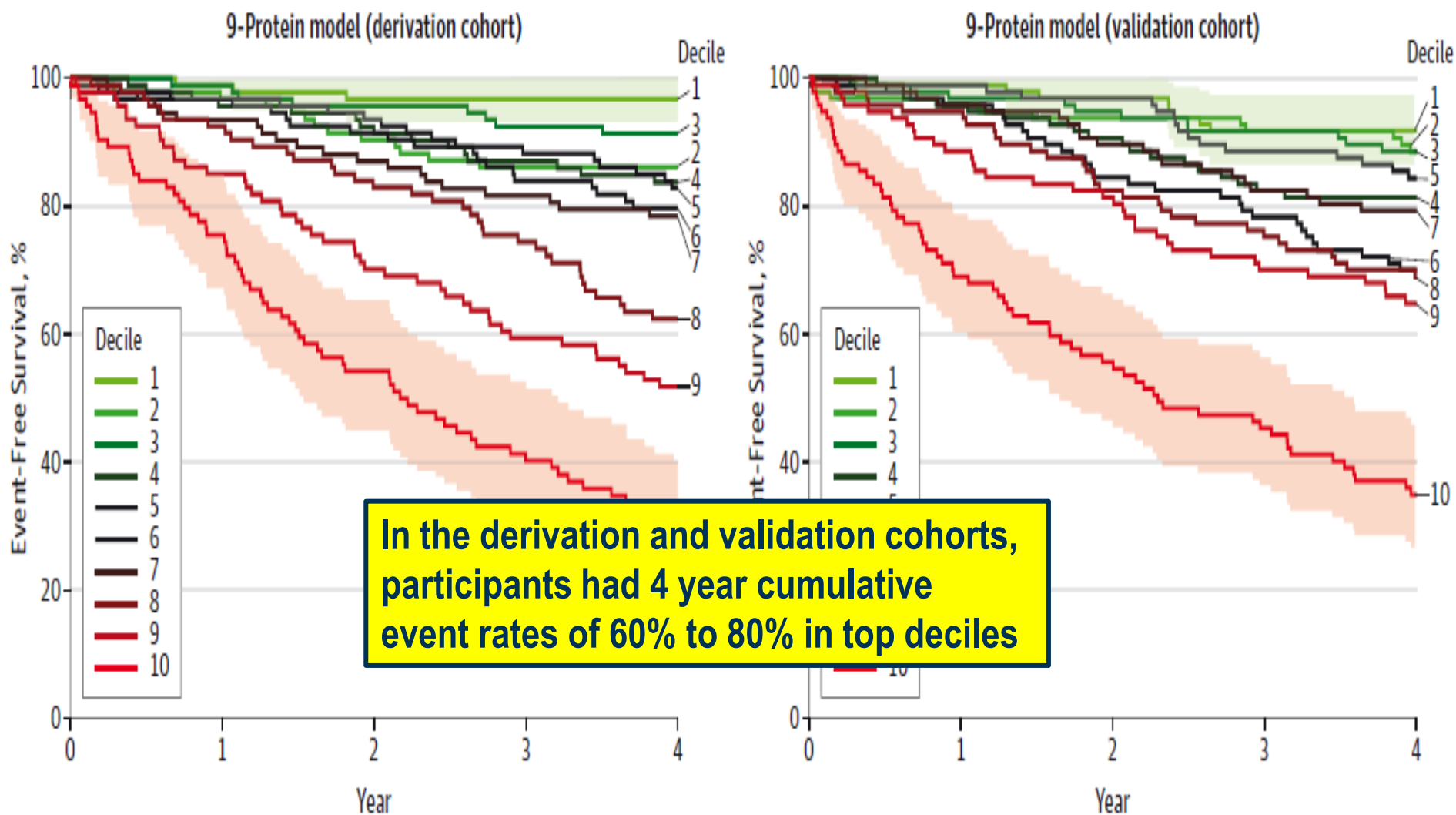
9 proteins identified ($p < 10^{-16}$):

- ANGPT2 (HR 1.67)
- MMP12 (HR 1.65)
- CCL18 (HR 1.47)
- Complement 7 (HR 1.47)
- SERPINA3 (HR 1.39)
- ANGPTL4 (HR 1.27)
- Troponin I (HR 1.27)
- GDF8/11 (HR 0.72)
- α_2 -antiplasmin (HR 0.64)

Risk Prediction Models for Primary End Point of Myocardial Infarction, Stroke, HF, and Death

	Framingham Variables Alone ^b			Framingham Variables ^b Plus 9-Protein Prognostic Index		
	HR (95% CI)	β	P Value	HR (95% CI)	β	P Value
Men	1.71 (1.26 to 2.32)	0.535	<.001	1.63 (1.20 to 2.20)	0.487	.002
Age, y	1.77 (1.58 to 1.99)	0.573	<.001	1.28 (1.13 to 1.44)	0.247	<.001
Total cholesterol, mg/dL	1.14 (1.03 to 1.26)	0.129	.01	1.20 (1.09 to 1.32)	0.178	<.001
HDL-C, mg/dL	0.88 (0.79 to 0.99)	-0.122	.03	0.95 (0.85 to 1.05)	-0.056	.28
Diabetes	1.84 (1.50 to 2.26)	0.611	<.001	1.44 (1.17 to 1.77)	0.363	<.001
Systolic blood pressure, mm Hg	1.03 (0.94 to 1.13)	0.029	.55	0.99 (0.90 to 1.08)	-0.014	.77
Current smoker	2.02 (1.58 to 2.58)	0.704	<.001	1.50 (1.16 to 1.94)	0.405	.002
9-Protein prognostic index	-	-	-	2.32 (2.08 to 2.58)	0.840	<.001

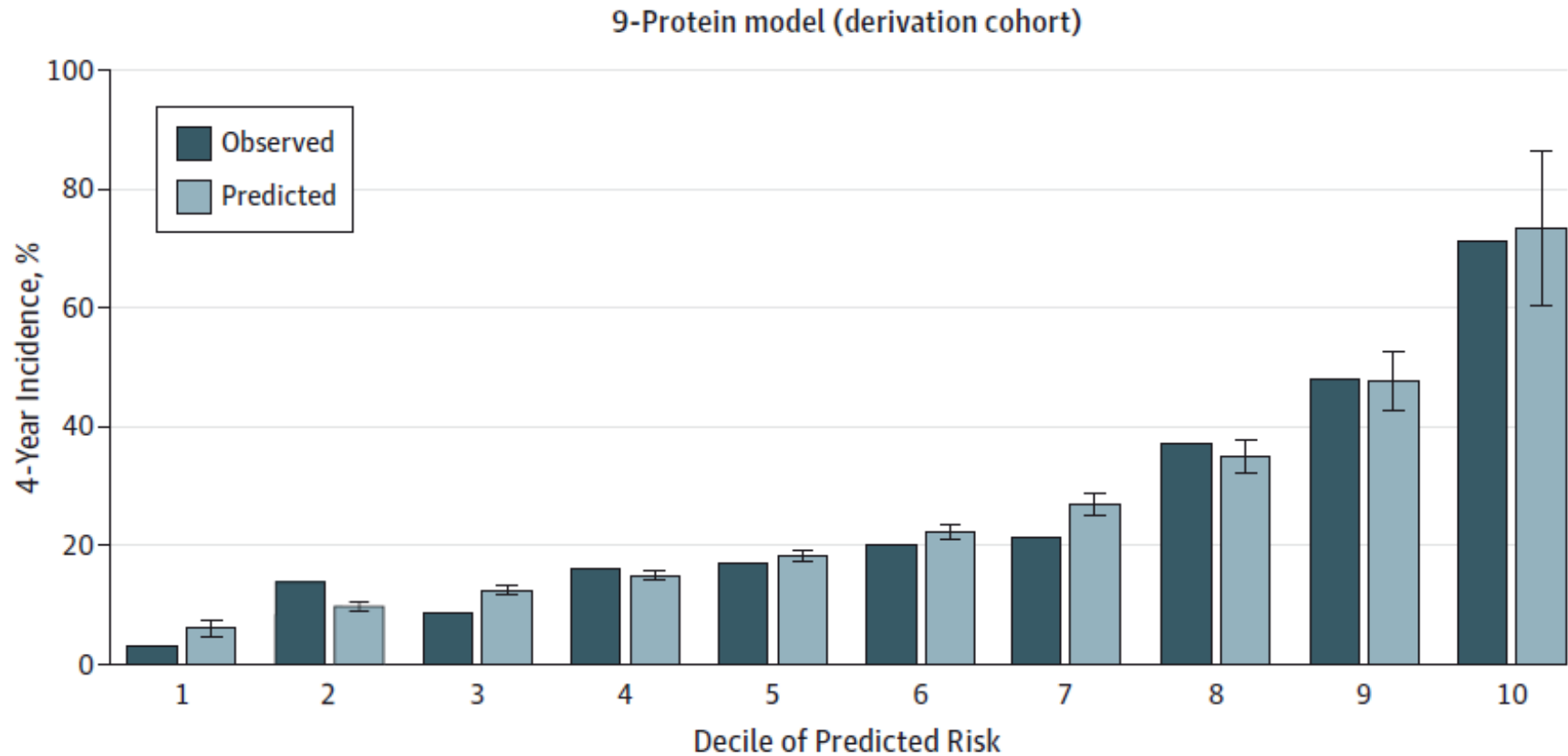
Event-Free Survival for MI, Stroke, HF, and Death, Stratified by Deciles of the 9-Protein 4-Year Risk Score



Metrics in Derivation/Validation Cohorts for Refit FRS, 9-Protein Model, and Their Comb. Predicting MI, Stroke, HF, and Death

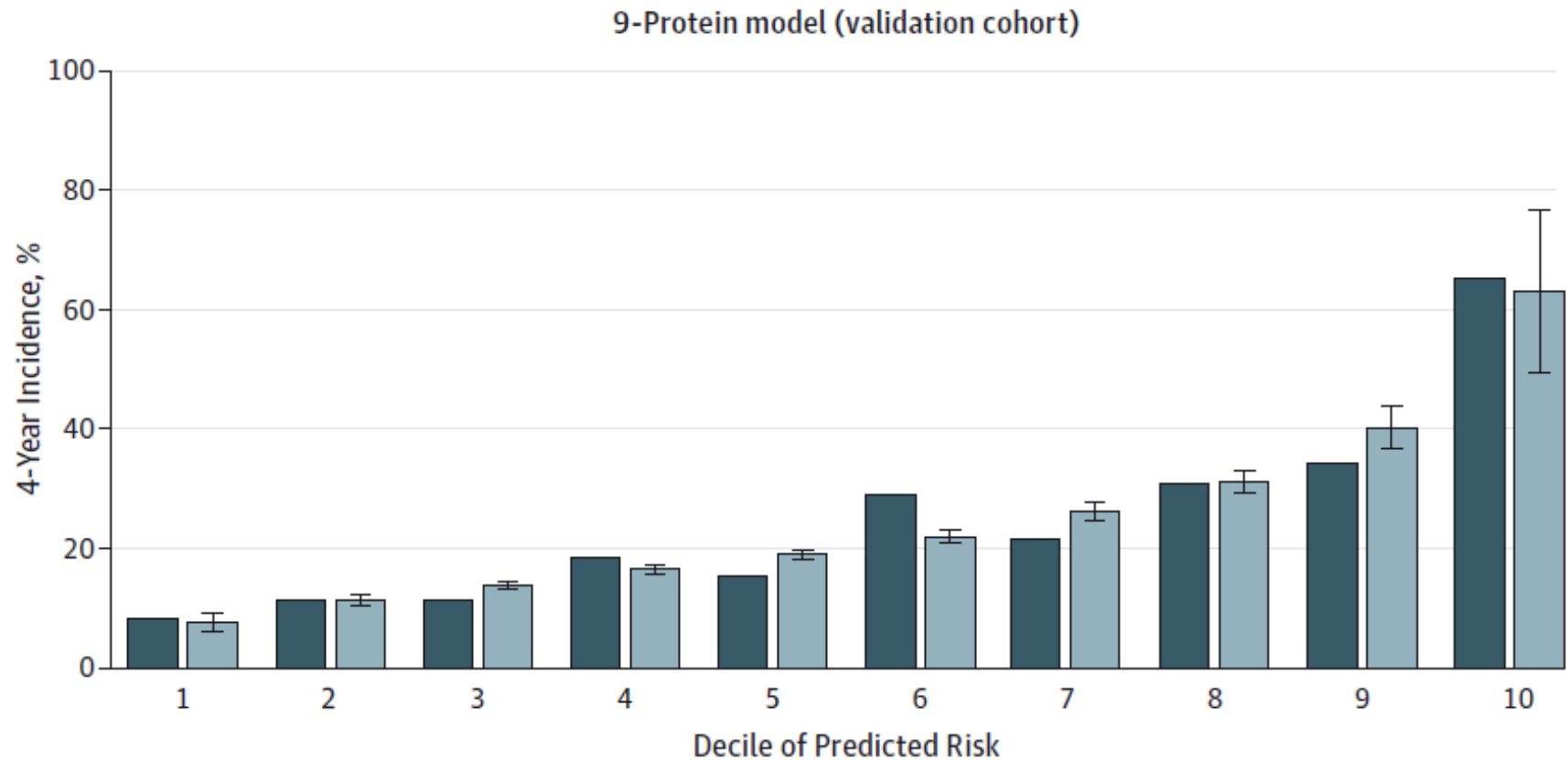
	Cohort	Refit Framingham Model	9-Protein Model	Refit Framingham Model Plus the 9-Protein Model
C statistic	Derivation	0.66 (0.63 to 0.68)	0.74 (0.72 to 0.77)	0.75 (0.73 to 0.78)
	Validation	0.64 (0.61 to 0.67)	0.70 (0.67 to 0.72)	0.71 (0.69 to 0.74)
Δ C statistic (derivation and validation) ^a	Both	0.01 (−0.01 to 0.04)	0.05 (0.03 to 0.07)	0.04 (0.02 to 0.07)
Discrimination slope	Derivation	0.09 (0.07 to 0.11)	0.21 (0.17 to 0.24)	0.23 (0.19 to 0.26)
	Validation	0.07 (0.05 to 0.08)	0.14 (0.12 to 0.17)	0.17 (0.14 to 0.20)
Δ Discrimination slope (derivation and validation) ^a	Both	0.02 (0 to 0.05)	0.07 (0.01 to 0.11)	0.06 (0.01 to 0.11)
Hazard Ratio (95% CI)				
Quintile ^b	Derivation	5.0 (3.60 to 6.94)	11.7 (8.08 to 16.86)	16.3 (10.69 to 24.93)
	Validation	6.6 (3.74 to 11.54)	7.6 (4.53 to 12.85)	9.8 (4.53 to 20.99)
Per standard deviation	Derivation	1.9 (1.72 to 2.15)	2.5 (2.27 to 2.73)	2.8 (2.49 to 3.05)
	Validation	1.7 (1.53 to 1.97)	2.1 (1.86 to 2.33)	2.2 (1.97 to 2.52)
Hosmer-Lemeshow ^c	Derivation	6.8 (5.57×10^{-1})	5.3 (7.25×10^{-1})	3.5 (9.02×10^{-1})
	Validation	23.5 (2.81×10^{-3})	6.8 (5.62×10^{-1})	9.7 (2.89×10^{-1})
Δ C statistic (refit Framingham model)	Derivation	1 [Reference]	0.09 (0.06 to 0.12)	0.10 (0.08 to 0.12)
	Validation		0.05 (0.02 to 0.09)	0.07 (0.04 to 0.09)
Integrated discrimination index ^d	Derivation	1 [Reference]	0.12 (0.08 to 0.16)	0.14 (0.10 to 0.17)
	Validation		0.08 (0.05 to 0.10)	0.10 (0.08 to 0.13)
NRI(>0) ^d	Derivation	1 [Reference]	0.52 (0.40 to 0.65)	0.72 (0.60 to 0.84)
	Validation		0.43 (0.26 to 0.57)	0.48 (0.33 to 0.62)
Event NRI ^d	Derivation	1 [Reference]	0.22 (0.11 to 0.36)	0.29 (0.19 to 0.42)
	Validation		0.08 (−0.06 to 0.22)	0.30 (0.16 to 0.44)
No-event NRI ^d	Derivation	1 [Reference]	0.30 (0.22 to 0.36)	0.43 (0.36 to 0.48)
	Validation		0.35 (0.28 to 0.41)	0.18 (0.11 to 0.24)

Observed vs Predicted 4-Year Incidence of MI, Stroke, HF, and Death With the 9-Protein Model (Derivation)



Median predicted risk, % (range)	6.2 (2.3 to <8.4)	9.7 (8.4 to <11.1)	12.4 (11.1 to <13.7)	15.0 (13.7 to <16.4)	18.2 (16.4 to <20.2)	22.2 (20.2 to <24.1)	26.7 (24.1 to <30.5)	34.8 (30.5 to <39.9)	48.3 (39.9 to <56.8)	69.1 (56.8 to 99.9)
No. of events	3	13	8	15	16	19	20	35	45	67
No. of participants	94	94	94	93	94	94	93	94	94	94

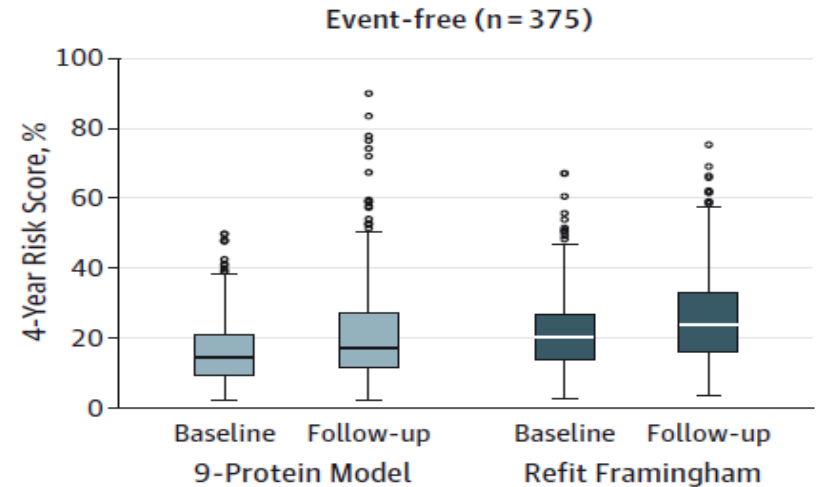
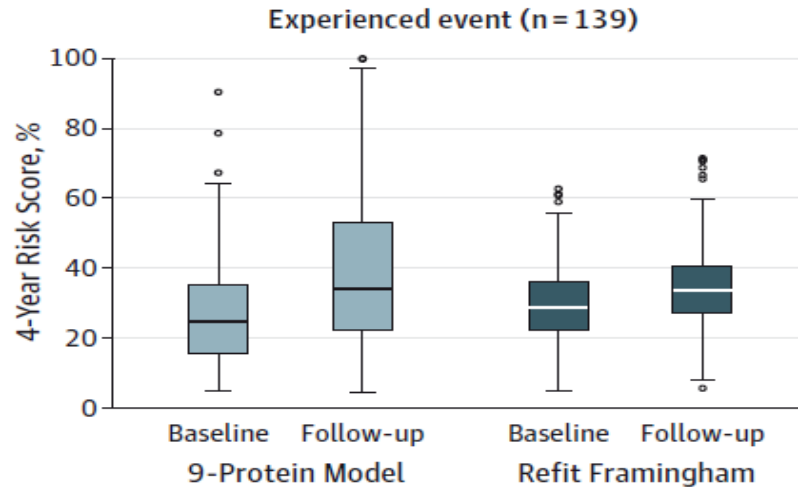
Observed vs Predicted 4-Year Incidence of MI, Stroke, HF, and Death With the 9-Protein Model (Validation)



Median predicted risk, % (range)	7.8 (2.7 to <9.7)	11.4 (9.7 to <12.6)	13.8 (12.6 to <15.0)	16.5 (15.0 to <17.9)	19 (17.9 to <20.5)	21.9 (23.6 to <23.6)	26.0 (24.1 to <28.6)	30.7 (28.6 to <34.7)	39.5 (34.7 to <46.6)	59.2 (46.6 to 99.2)
No. of events	8	11	11	18	15	28	21	30	33	64
No. of participants	98	97	97	97	97	97	97	97	97	97

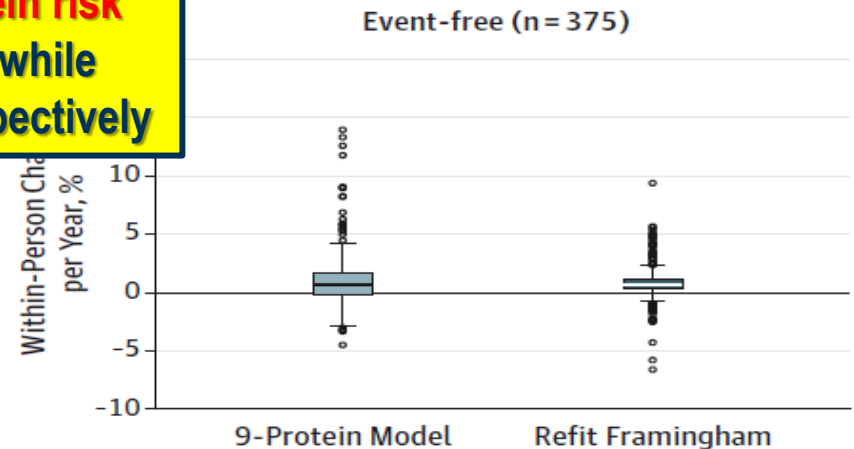
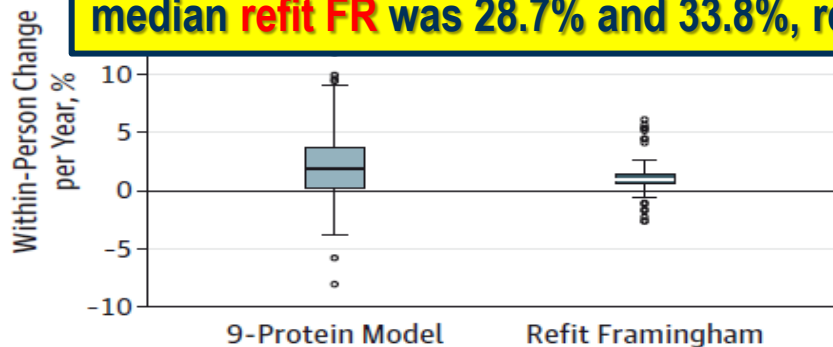
Changes in Risk Scores of MI, Stroke, HF, and Death in Paired Samples 4.8 Years Apart

A 4-Year risk prediction at baseline and follow-up



B

A 139 pts with an event had a median **9 protein** risk of 24.6% at baseline and 34% at 4.8 years, while median **refit FR** was 28.7% and 33.8%, respectively



Original Investigation | INNOVATIONS IN HEALTH CARE DELIVERY

Development and Validation of a Protein-Based Risk Score for Cardiovascular Outcomes Among Patients With Stable Coronary Heart Disease

Peter Ganz, MD; B
Mark R. Segal, PhD

Conclusions: Among patients with stable CHD, a risk score based on 9 proteins performed better than the refit FRS (for secondary events) in predicting CV events, but still provided only modest discriminative accuracy.

IMPORTANCE

disease (CHD) is needed to inform treatment decisions.

Other promising approaches:

96-Plex Proximity Extension (Immuno)assay (PEA) by OLINK®

with the main advantage: to relieve the shortcomings of antibodies and their inherent cross-reactivity in multiplex protein quantification applications.

Assarsson et al. PLoS ONE; 2014;9(4) e95192

EXPOSURES Using modified aptamers, 1130 proteins were measured in plasma samples. Ganz et al. JAMA 2016;315:2532

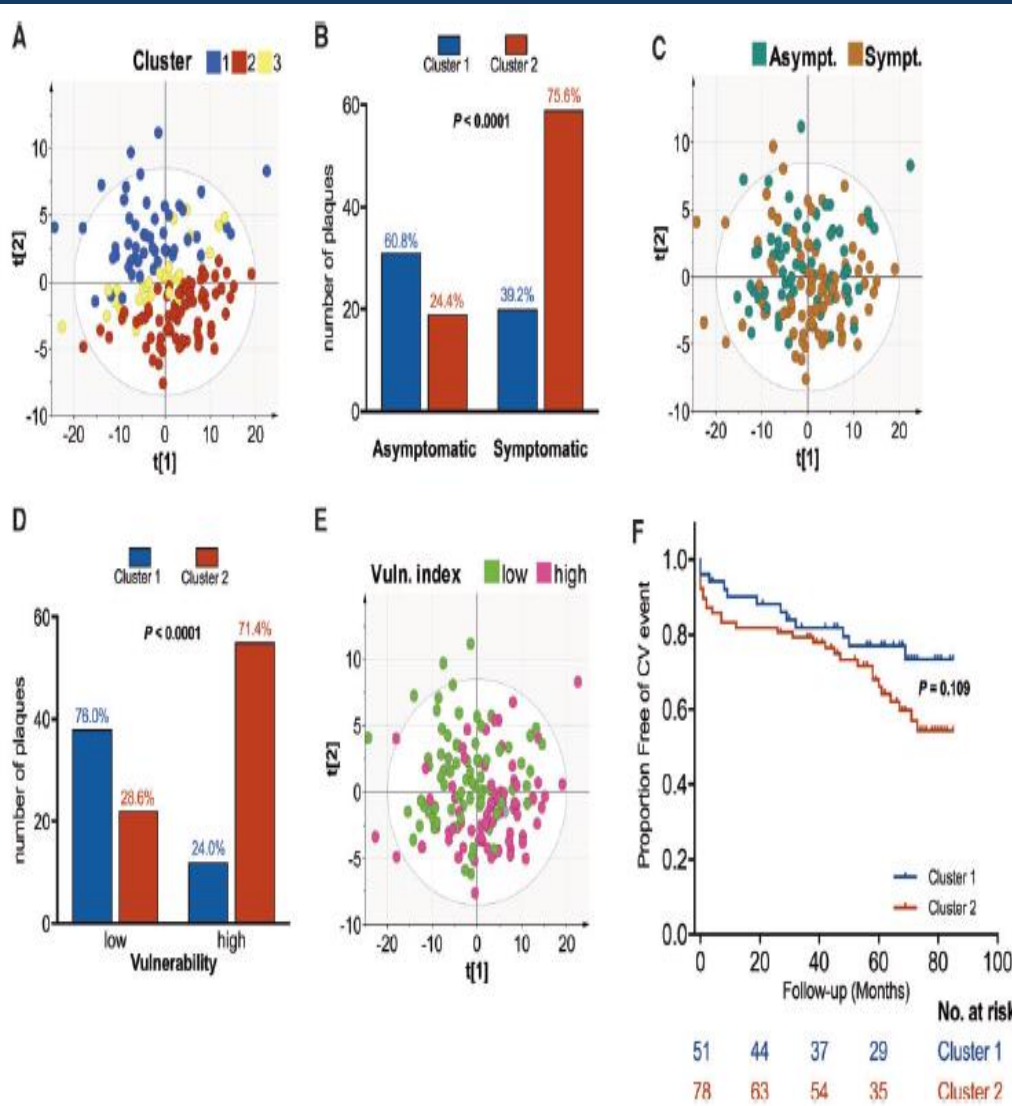
Altered metabolism distinguishes high-risk from stable carotid atherosclerotic plaques

- 159 carotid plaques from patients undergoing TEA
- Metabolites and cytokines by MS, enzymatic assays, PEA and Luminex assay as well as RNA sequencing
- PCA revealed a separation between cluster 1 and 2 according to 2 metabolite signatures (histologically evaluated vulnerability and inflammatory markers as well as symptoms; high risk vs stable plaque)
- Altered metabolic signature: increased glycolysis, elevated amino acids and decreased fatty acid oxidation

These results highlight a possible key role of cellular metabolism to support inflammation and a high-risk phenotype of atherosclerotic plaques. Targeting the metabolism of atherosclerotic plaques with novel metabolic radiotracers or inhibitors might therefore be valid future approaches to identify and treat the high-risk atherosclerotic plaque.

Atherosclerosis • Carotid plaque • High-risk plaque • Metabolism • Inflammation

A Distinct Metabolite Profile is Associated with Symptomatic and Vulnerable Plaques



A: PCA score plot of cluster 1, 2 and 3

B: Number of asymptomatic and symptomatic plaques assigned to cluster 1 and 2

C: PCA score plot with symptomatic and asymptomatic plaques

D: Number of plaques with a high and low vulnerability index assigned to cluster 1 and 2

E: PCA score plot showing plaques with a high or low vulnerability index

F: Kaplan Meier curves for CV event-free survival for cluster 1 and 2

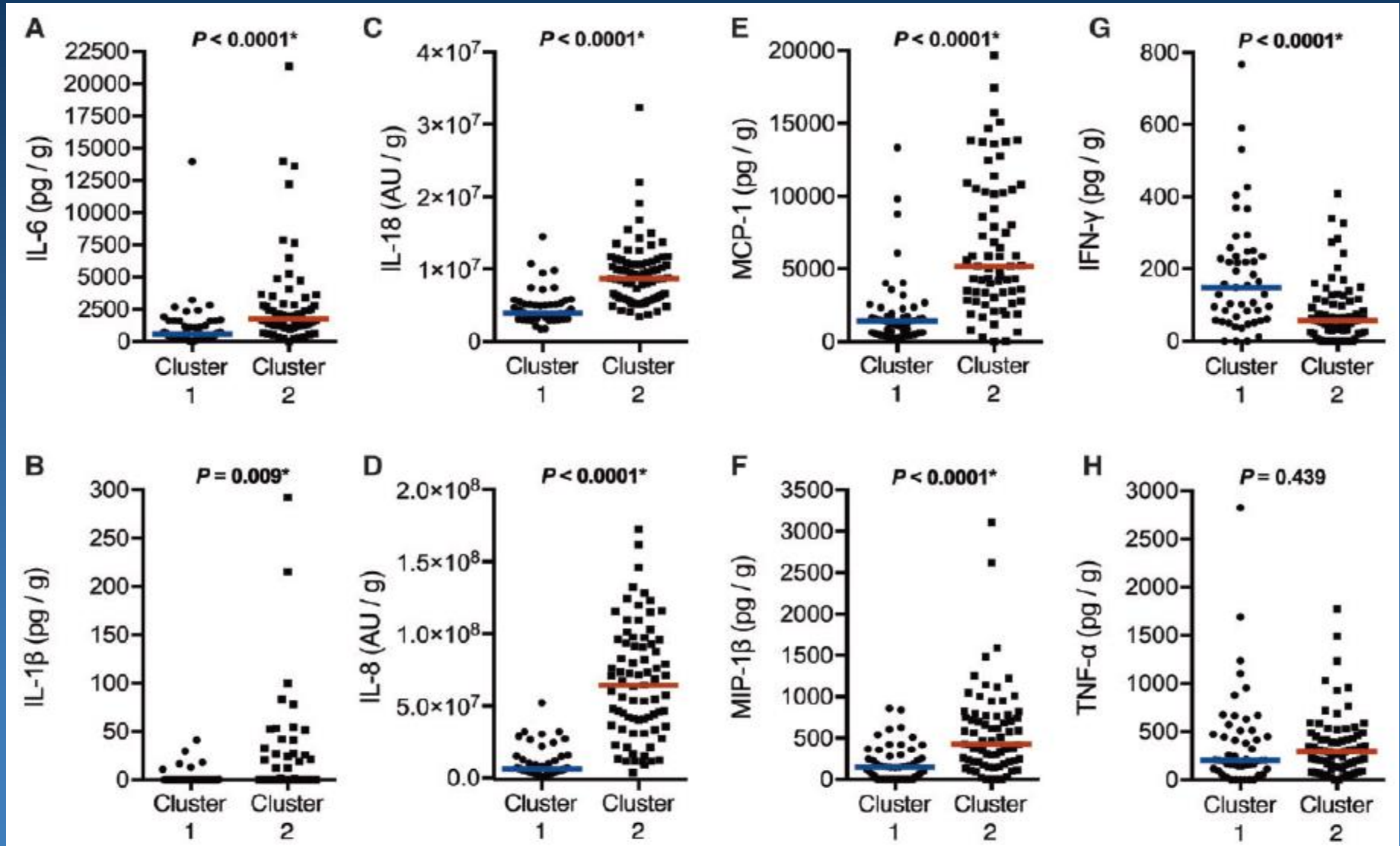
High Risk vs Stable Carotid Plaques: Patient Characteristics

	Cluster 1 (n = 51 patients)	Cluster 2 (n = 78 patients)	P-value
Sex (female)	15 (29%)	29 (37%)	0.363
Age (years)	68 (64–71)	73 (67–78)	0.001
BMI (kg/m ²)	26.8 ± 3.9	26.8 ± 3.7	0.938
Current smokers	20 (39%)	20 (26%)	0.103
Hypertension	37 (73%)	58 (74%)	0.820
Diabetes	22 (43%)	35 (45%)	0.846
HbA1c (mmol/mol)*	56.3 (50.0–65.7)	55.2 (45.8–67.5)	0.907
Total cholesterol (mmol/L)	4.2 (3.5–5.1)	4.4 (3.6–5.1)	0.531
LDL (mmol/L)	2.1 (1.6–3.1)	2.6 (2.1–3.4)	0.042
HDL (mmol/L)	1.2 (0.9–1.6)	1.1 (0.9–1.3)	0.186
Triglycerides (mmol/L)	1.2 (0.9–1.9)	1.3 (1.0–1.7)	0.892
hsCRP (mg/L)	4.3 (2.2–7.0)	4.0 (2.1–7.2)	0.975
WBC (10 ⁸ /L)	8.2 ± 2.0	7.8 ± 2.0	0.264
Creatinine (μmol/L)	82.0 (71.0–96.0)	90.5 (76.7–103.5)	0.104
Statins	45 (88%)	69 (88%)	0.969

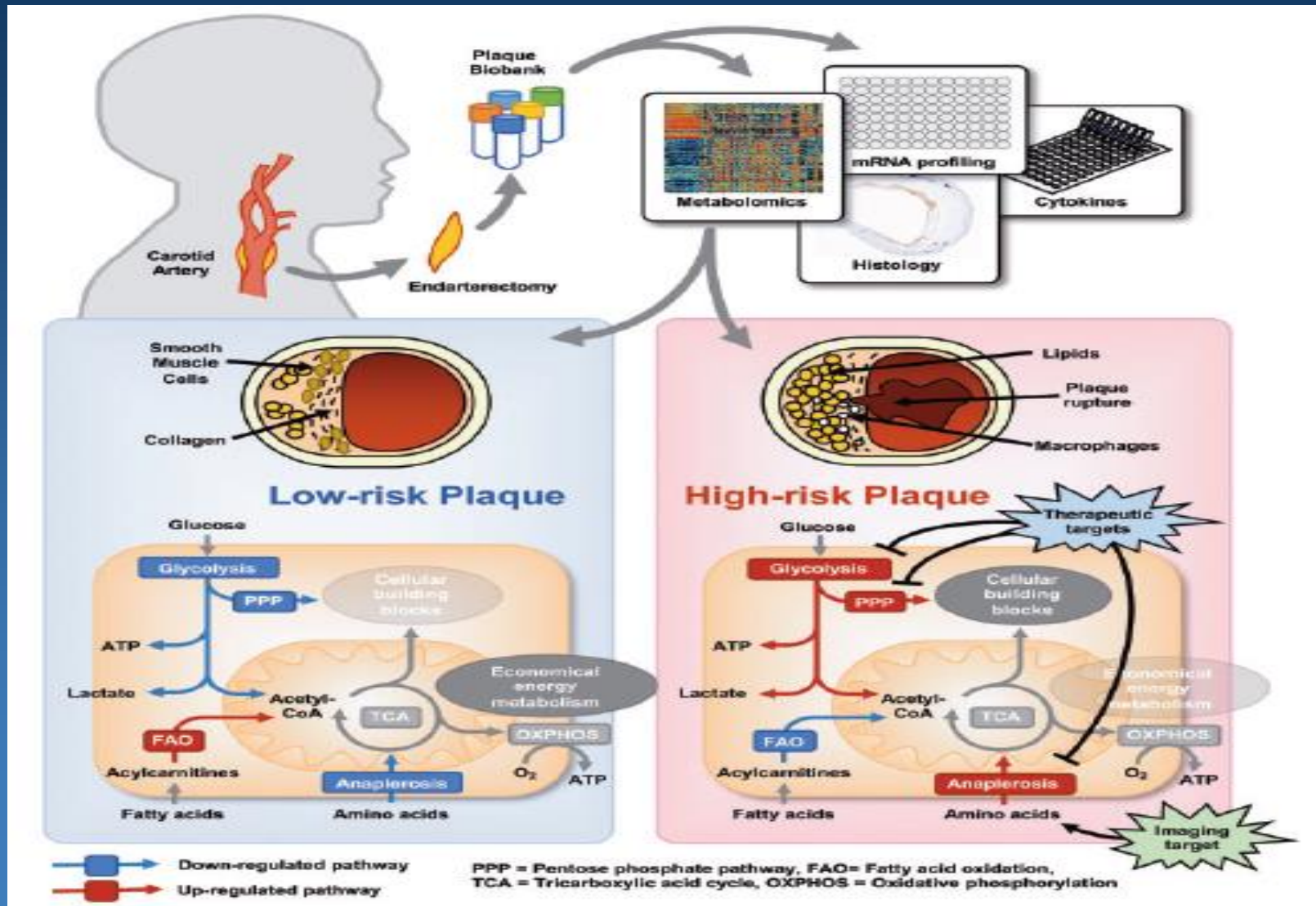
For all measurements $n \geq 50$ (cluster 1), $n \geq 65$ (cluster 2) except for * $n = 19$ (cluster 1), $n = 32$ (cluster 2). Data for cluster 3 can be found in Supplementary material online, Table S1.

WBC, white blood cell count; hsCRP, high-sensitive C-reactive protein.

Several Pro-Inflammatory Mediators are Elevated in Cluster 2 Carotid Plaques



Overview Highlighting the Differences in the Metabolic Profiles Between High-Risk and Stable Carotid Plaques



Potential and Caveats of Lipidomics (or Proteomics) for Cardiovascular Disease

Sources of variability

Population & clinical characteristics

demographic, genetic, lifestyle, comorbidities, medication, disease stage, etc.

Pre-analytical variability

blood sampling, storage and processing

Measurement

extraction method, separation, ionization, mode of MS, use of standards, etc.

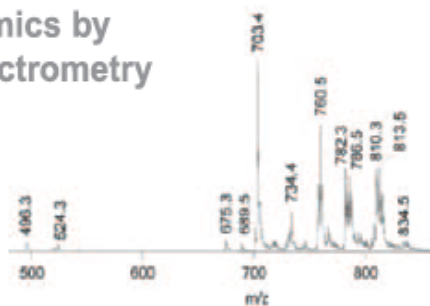
Statistical analysis

high data dimension, variable selection, multicollinearity and multiplicity

Random noise

True biological variation

Lipidomics by Mass Spectrometry



Myocardial infarction



Stroke



Diabetes & Metabolic syndrome

T2DM

Potential future clinical translation

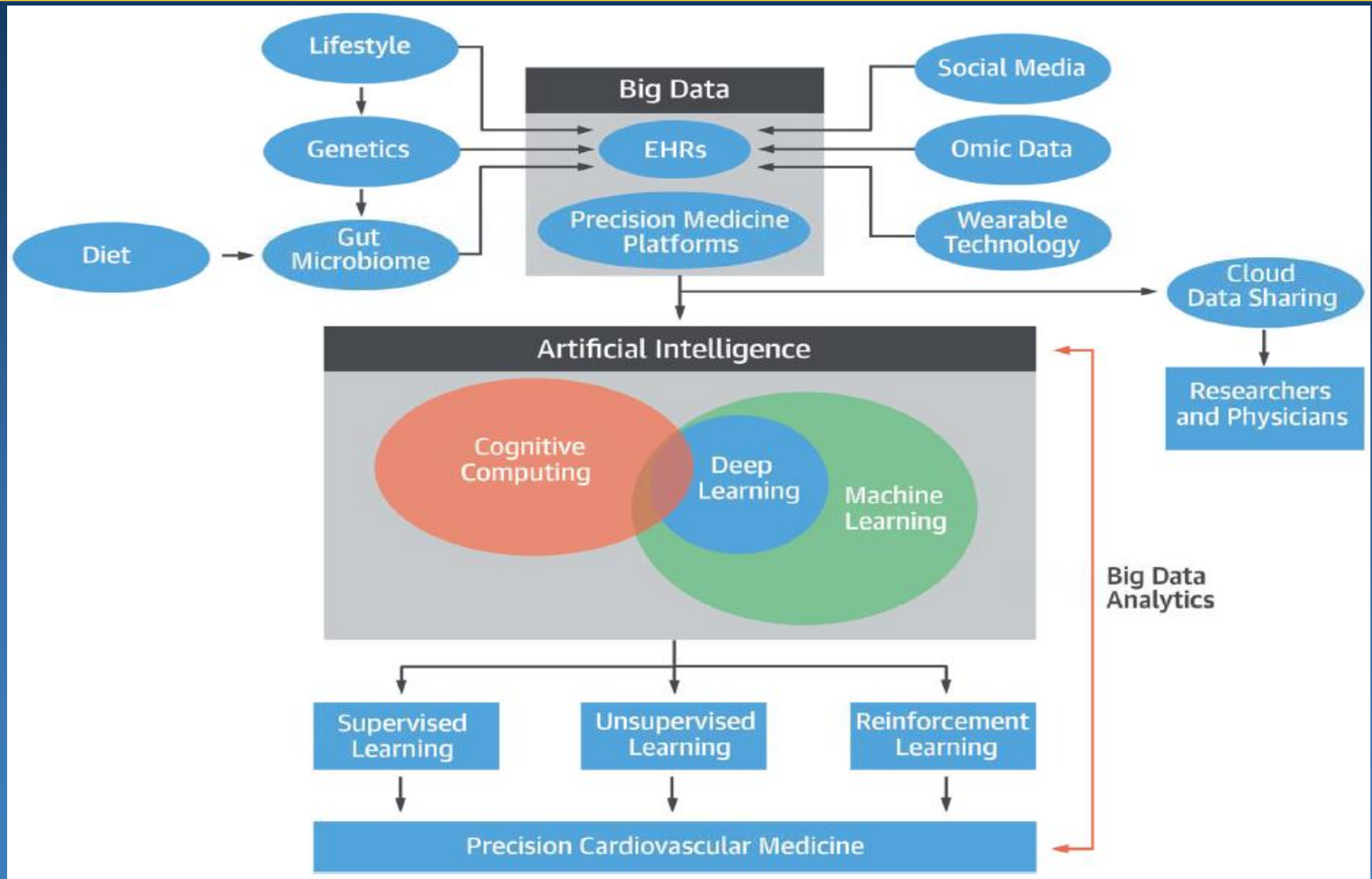
Improvement of CVD risk prediction and risk stratification of patients

Discovery of disease mechanisms and underlying molecular pathways

Diet and life-style-based modification of lipid quality

Novel therapeutic targets (e.g. change in lipid composition)

Artificial Intelligence in Precision Cardiovascular Medicine



Summary and Conclusions

- Proteomics offers the opportunity to assess in an unbiased way all proteins in the human body
- The proteome is highly dynamic
- Cross validation of results needed for various methods
- Large prospective, well-controlled studies in various populations are needed to determine the value of broad protein profiling to improve risk prediction above and beyond presently applied scores
- Proteomics may identify new targets for therapy

A photograph taken from the driver's perspective inside a car. The view is looking out through the windshield at a long, straight asphalt road that stretches towards the horizon. The road has a white dashed line down the center and solid white lines on the edges. The surrounding landscape is flat and arid, with low-lying green shrubs and patches of reddish-brown soil. The sky is filled with soft, white clouds. The car's dashboard and windshield wipers are visible at the bottom of the frame.

...still...true personalized
medicine is

A LONG WAY TO GO



Thank You for Your Attention!



Prof. Wolfgang Koenig
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