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Apo-calypse now? Apolipoprotein profiling to reduce residual cardiovascular disease

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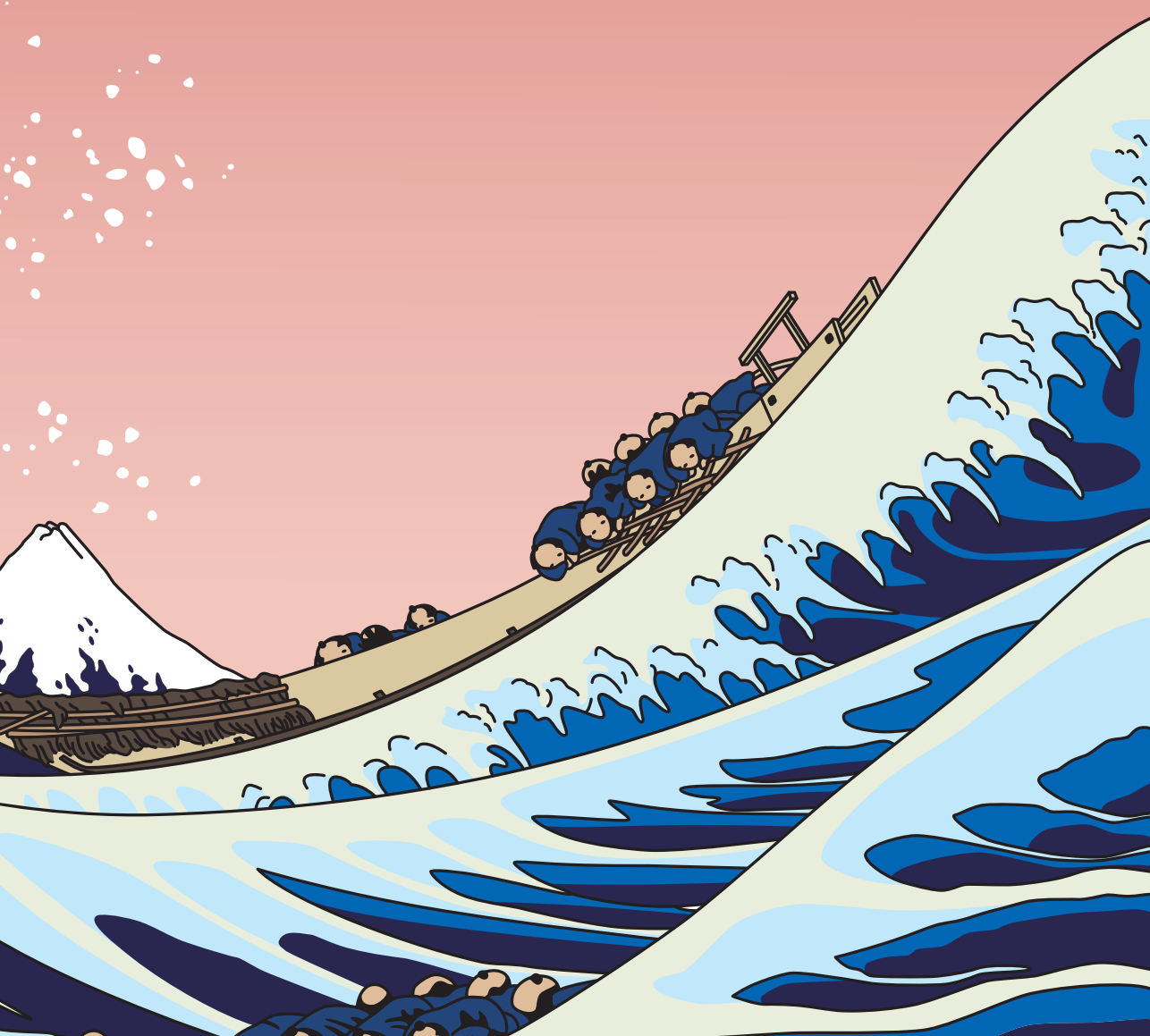
CHAPTER 6



Multiplex Apolipoprotein Panel Improves Cardiovascular Event Prediction and Cardiovascular Outcome by Identifying Patients who Benefit from Targeted PCSK9i Therapy

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ABSTRACT

Background: Considerable residual cardiovascular risk persists despite optimized statin therapy, which may not be fully quantified by risk prediction scores that only incorporate conventional lipid measures. Apolipoproteins offer the potential to complement residual risk evaluation and inform patient management. We assessed the prognostic performance of a 9-plex serum apolipoprotein (apo) panel in patients with recent ACS and the panel's utility to predict treatment benefit of alirocumab, a PCSK9 inhibitor.

Methods: Baseline serum samples from 11,843 participants in the ODYSSEY OUTCOMES trial were analyzed for levels of Apo(a), ApoA-I, ApoA-II, ApoA-IV, ApoB, ApoC-I, ApoC-II, ApoC-III, and ApoE, and ApoE phenotyping using mass spectrometry. We used logistic regression modeling with restricted cubic splines to estimate the probability of first major adverse cardiovascular events (MACE) and all-cause death based on baseline apolipoprotein and lipid concentrations in the placebo group. Clinical performance was assessed by comparing the area under the receiver operating characteristic curve (AUC) of models based on (1) the baseline apo panel, (2) the baseline conventional lipid panel, and (3) a combination of the two panels. Additionally, models estimating the treatment benefit of alirocumab by the apo panel were developed.

Results: The prognostic performance of the apolipoprotein panel for MACE showed an AUC (95% confidence interval) of 0.648 (0.626, 0.670), compared to 0.579 (0.557, 0.602) for the lipid panel. For all-cause death, the apolipoprotein panel had an AUC of 0.699 (0.664, 0.733), while the lipid panel had an AUC of 0.599 (0.564, 0.635). Adding the apolipoprotein panel significantly improved performance of the conventional lipid panel ($p<0.0001$): AUC 0.659 (0.637, 0.681) for MACE and 0.724 (0.691, 0.756) for all-cause death. Higher risk for MACE based on the baseline apo panel was found to predict greater treatment benefit with alirocumab.

Conclusions: A multiplex apo panel led to better prediction of MACE and all-cause death, beyond the conventional lipid panel, in patients with recent ACS on optimized statin therapy. The panel could also predict treatment benefit of alirocumab. This comprehensive apo panel is a promising asset for precision diagnostics and personalized disease management, with potential to guide targeted treatments.

REGISTRATION: URL: https://www.clinicaltrials.gov; Unique identifier: NCT01663402.

CLINICAL PERSPECTIVE

What is new?

- Models to predict major adverse cardiovascular event (MACE) and all-cause death in patients with recent ACS based on an apolipoprotein panel were developed and compared to models based on the conventional lipid panel.
- Apolipoprotein profiling led to better prognostic performance for MACE and all-cause death than the conventional lipid panel.
- Models using the baseline apolipoprotein panel to estimate risk of MACE and all-cause death also predict treatment benefit of alirocumab on those outcomes, thus allowing treatment benefit to be estimated in individual patients, enabling personalized medicine.

What are the clinical implications?

- In patients on optimized statin treatment, an integrated serum apolipoprotein panel significantly improved the estimation of residual cardiovascular risk compared with the conventional lipid panel.
- The apolipoprotein panel has potential to improve health outcomes in patients with ACS by guiding the identification and treatment of those most likely to benefit from additional lipid-lowering drugs such as alirocumab, thus potentially reducing healthcare costs.

INTRODUCTION

In individuals with dyslipidemia and coronary artery disease, considerable residual risk of cardiovascular events persists despite optimized statin treatment.[1, 2] Some of this residual risk is attributable to persistent lipoprotein abnormalities. However, estimation of that risk from a conventional lipid panel, including TC, high-density lipoprotein cholesterol (HDL-C), LDL-C, and triglyceride levels, may not be fully quantified.[1, 3-5] There is a clear unmet clinical need for more refined phenotyping of dyslipidemia to improve risk prediction and guide treatment decisions in the management of dyslipidemia.[6]

Apolipoproteins, the functional proteins of lipid metabolism, might be a missing link to fulfill this unmet clinical need.[6, 7] Over recent years, apolipoproteins have gained recognition as significant biomarkers for cardiovascular disease.[6, 8] Notably, apolipoprotein B (ApoB), found on all atherogenic lipoprotein particles, was shown to clinically and metrologically outperform traditional markers such as LDL-C.[9-11] In addition, ApoB is reported to be equivalent or even better of a predictor of cardiovascular events than non-HDL-C.[9-14] Apo(a), associated with the genetically determined risk factor lipoprotein(a), and ApoC-III, an inhibitor of triglyceride-rich lipoprotein clearance, have also become important markers in cardiovascular research.[15-17] Therapies targeting ApoB, Apo(a), and ApoC-III have been, or are currently being developed,[18-23] and therapies targeting other apolipoproteins may lie ahead. Consequently, measuring a panel of apolipoproteins may be valuable for both diagnostic purposes and for selecting patients for advanced lipid-lowering therapies.

Pechlaner *et al.* and Clarke *et al.* have reported that a panel of apolipoproteins measured by mass spectrometry could help predict cardiovascular events.[24, 25] To date, however, the clinical effectiveness of an apolipoprotein multimarker panel has not yet been demonstrated.

We developed a mass spectrometry-based multiplex apolipoprotein panel test, which is currently comprised of Apo(a), ApoA-I, ApoA-II, ApoA-IV, ApoB, ApoC-I, ApoC-II, ApoC-III and ApoE quantification as well as ApoE phenotyping.[26-28] To implement the apolipoprotein panel test as a new medical test, we relied on the test evaluation framework, developed by the European Federation of Clinical Chemistry and Laboratory Medicine Working Group Test Evaluation[29]. Since analytical performance validation of the apolipoprotein panel has been completed[26, 30], showing accuracy and robustness over time[28], we undertook an evaluation of its clinical performance and clinical effectiveness in a large, post-ACS cohort of patients on high-intensity statin therapy.

Accordingly, the goal of the current post hoc analysis of the ODYSSEY OUTCOMES trial was to compare performance of the apolipoprotein panel versus the conventional lipid panel in predicting major adverse cardiovascular events (MACE) and all-cause death, as well the treatment benefit of alirocumab, a PCSK9 inhibitor, on MACE.

METHODS

Requests from qualified investigators for data from the ODYSSEY OUTCOMES Trial will be considered by its Executive Steering Committee and the sponsor and should be submitted to odysseyoutcomesesc@gmail.com.

Study design

The design[31], primary results[32], and total events analyses[33] from the ODYSSEY OUTCOMES Trial (NCT01663402) have been published. In brief, 18,924 patients from 1,315 sites across 57 countries were randomized in a 1:1 ratio to receive either alirocumab (75 mg, increased to 150 mg for those who did not achieve an LDL-C level <1.29 mmol/L (50 mg/dL)) or matching placebo, administered subcutaneously every 2 weeks. Key inclusion criteria were recent hospitalization (1 to 12 months prior to randomization) due to ACS (myocardial infarction or unstable angina), and an LDL-C level ≥1.81 mmol/L (70 mg/dL), a non-HDL-C level ≥2.59 mmol/L (100 mg/dL), and/or ApoB ≥0.80 g/L on treatment with atorvastatin (40 to 80 mg daily), rosuvastatin (20 to 40 mg daily), or the maximum tolerated dose of either statin. A triglyceride level ≥4.52 mmol/L (400 mg/dL) at the screening visit was exclusionary. The trial was approved by the responsible institutional review board at each participating site. Informed consent was obtained from all participants.

Patient Outcomes

The primary efficacy outcome of the trial and of the current analysis was first occurrence of MACE, comprising death from coronary heart disease, nonfatal myocardial infarction, fatal or nonfatal ischemic stroke, or unstable angina requiring hospitalization.[32] Additionally, we analyzed the incidence of all-cause death.[32] All events included in the analyses were adjudicated by an independent committee blinded to treatment assignment.

Measurement of the conventional lipid panel

The conventional lipid panel, including total, LDL, and HDL cholesterol and triglycerides, was measured centrally at Covance Laboratories. LDL-C levels were calculated using the Friedewald formula[34], except when triglyceride levels exceeded 4.52 mmol/L (400 mg/dL) or when the calculated LDL-C level was <0.39 mmol/L (15 mg/dL). In these cases, LDL-C was assessed by preparative ultracentrifugation and β quantification.

Measurement of serum apolipoproteins

Serum apolipoprotein Apo(a), ApoA-I, ApoA-II, ApoA-IV, ApoB (reflecting total ApoB including ApoB48 and ApoB100), ApoC-I, ApoC-II, ApoC-III, ApoE levels including ApoE phenotype were

measured with a semi-automated lab-developed multiplex test by liquid chromatography tandem mass spectrometry (LC-MS/MS) (1290 Infinity II UHPLC coupled to 6495 QQQ-MS Agilent Technologies, Santa Clara, CA, USA). The analytical method has been published for seven apolipoproteins.[26, 30] In brief, sample pre-analysis was performed semi-automatically on a 96-channel Agilent BRAVO automated liquid handling platform. Thereafter, samples were either stored overnight at -80 °C with solid-phase extraction the next day or immediately subjected to solid-phase extraction after quenching. Subsequently, the samples were measured on the mass spectrometer. More specific details on the process, the measurements, and the quality of the data have been published.[35]

Statistical analysis

Patients without available baseline samples for apolipoprotein levels were excluded from the current analyses, including those recruited from countries or sites where additional exploratory laboratory testing was not permitted or not possible. Apolipoproteins were also measured in available month 4 samples on assigned treatment.

Distributions of apolipoproteins at baseline are described by treatment group, along with the absolute and percentage change from baseline to month 4 (122±28 days) after randomization. The first measurement result was analyzed if a patient had multiple measurements within the time frame. For statistical analyses, Apo(a) concentrations below the lower limit of quantification (3.8 nmol/L) were set to the midpoint between 0 and the lower limit of quantification (1.9 nmol/L). Median apolipoprotein levels for baseline and month 4 serum samples were calculated for each treatment arm, including both the absolute and percentage changes from baseline to month 4.

To evaluate prognostic performance, logistic regression modeling with restricted cubic splines was used to estimate the probabilities of a MACE and all-cause death based on continuous baseline biomarker concentrations within the placebo group. Three models were constructed, based on, respectively, (1) baseline apolipoprotein concentrations including the ApoE phenotype, (2) baseline conventional lipid concentrations including TC, HDL-C, and triglycerides, and (3) both baseline apolipoprotein concentrations, the ApoE phenotype and conventional lipid concentrations. LDL-C was not included in the models, as it is calculated using the Friedewald formula based on TC, HDL-C, and triglycerides, making its inclusion redundant. Direct and calculated LDL-C values also tend to be inaccurate, especially at lower levels under conditions of intensive lipid-lowering treatment.[6, 11, 36] The ApoE phenotype, was added as a categorical variable with six possible phenotypes (E2/E2, E2/E3, E2/E4, E3/E3, E3/E4, and E4/E4). The significance of non-linear effects, necessitating the use of splines, was assessed using Wald tests. Four knots were used for all biomarkers, in accordance with standard practice.[37] For Apo(a), three knots were applied due to its highly skewed

distribution (Supplemental Figure S1). We used partial Wald tests for testing the partial effect of each of the apolipoproteins, adjusted for the other variables in the model.

We calculated the area under the empirical receiver operating characteristic curve (AUC) with 95% CI for each model as an expression of the prognostic performance. We compared the performance of the combined model relative to the model based on conventional lipids only using the generalized likelihood ratio test. Additionally, we compared the ROC curves of the apolipoprotein panel with those of the lipid panel using the DeLong test.

Additionally, two models were built to quantify the incremental value of apolipoproteins after accounting for demographic and clinical information. The first model included age, sex, ethnicity, body mass index, smoking status, and history of diabetes, while the second model added the apolipoprotein panel to these parameters. The models were compared using the generalized likelihood ratio test.

To evaluate predictive performance, the same prognostic modeling strategy was applied for the alirocumab treatment arm, to estimate the probabilities of a MACE and all-cause death with alirocumab. This resulted in three pairs of models, one for each treatment arm, based on baseline apolipoprotein concentrations, baseline conventional lipids, and the combination of the two.

Placebo and alirocumab models were then run on the full study group to generate two estimated probabilities of an event per patient, based on that patient's profile of baseline values: one for the likelihood of the event if in the placebo group and another for the likelihood of that event if in the alirocumab group. The treatment effect was calculated as the difference between both probabilities. The threshold for a treatment benefit was set based on an absolute risk reduction of MACE of 2.1% and for all-cause death of 0.7%.

P values <0.05 from 2-sided tests were considered statistically significant. Analyses were conducted using R version 4.3.2.

RESULTS

Baseline characteristics

A total of 11,843 participants had baseline conventional lipid and apolipoprotein measurements and formed the analysis cohort, which is a subcohort from the original ODYSSEY OUTCOMES cohort. Baseline characteristics are shown in Table 1. Median age was 58 years, 24% were female, and 81% were White. Over 95% of patients received statin therapy at randomization; 89% received high-intensity statin therapy. Median ApoB at baseline was 0.80 g/L (interquartile range 0.69-0.96 g/L) median LDL-C at baseline was 2.24 mmol/L (86.49 mg/dL) (interquartile range 1.89-2.70 mmol/L (73.00-104.40 mg/dL)). Distribution plots of baseline apolipoprotein levels of the ODYSSEY OUTCOMES study group are depicted in Supplemental Figure S1.

Table 1: Demographic and Baseline Clinical Characteristics of the Study Group by Treatment Assignment

Characteristics	Alirocumab (n = 5,917)	Placebo (n = 5,926)
Demographics		
Age, y	58 (51, 65)	58 (52, 65)
Male sex	4500 (76.1)	4492 (75.8)
Ethnicity		
White	4775 (80.1)	4823 (80.7)
Asian	719 (12.1)	721 (12.1)
Black or African American	167 (2.8)	157 (2.6)
Unknown	5 (0.1)	2 (0)
Medical history		
Hypertension	3796 (63.7)	3731 (62.4)
Diabetes	1741 (29.2)	1788 (29.9)
Current tobacco smoker	1434 (24.1)	1450 (24.3)
Time from index ACS to randomization, months	2.6 (1.7, 4.5)	2.7 (1.7, 4.5)
Background lipid-lowering therapy		
High-intensity statin	5275 (88.5)	5347 (89.5)
Low- or moderate intensity statin	485 (8.1)	446 (7.5)
No statin or other lipid-lowering therapy	75 (1.3)	78 (1.3)
Only non-statin lipid-lowering therapy	127 (2.1)	106 (1.8)
Baseline biometric and laboratory data		
Body mass index, kg/m2	28.1 (25.4, 31.3)	28 (25.2, 31.2)
Systolic blood pressure, mm Hg	127 (118, 138)	126 (116, 138)
Hemoglobin A1c, %	5.8 (5.5, 6.3)	5.8 (5.5, 6.4)
High-sensitivity C-reactive protein, mg/dL	0.2 (0.1, 0.4)	0.2 (0.1, 0.4)
Estimated glomerular filtration rate, mL/min/1.73 m2	77.9 (67.2, 90.1)	78.2 (67.4, 90.5)
Conventional lipid panel		
Total cholesterol (mmol/L)	4.12 (3.67, 4.72)	4.14 (3.67, 4.73)
High-density lipoprotein cholesterol, mmol/L	1.09 (0.93, 1.29)	1.09 (0.93, 1.29)
Low-density lipoprotein cholesterol, mmol/L	2.24 (1.89, 2.69)	2.24 (1.89, 2.72)
Triglycerides, mmol/L	1.46 (1.07, 2.06)	1.48 (1.07, 2.1)
Non−high-density lipoprotein cholesterol, mmol/L	2.97 (2.56, 3.54)	3 (2.57, 3.57)
Apolipoprotein panel		
Apolipoprotein(a), nmol/L	41.1 (14.4, 140.1)	43.0 (14.3, 146.2)
Apolipoprotein B, g/L	0.80 (0.69, 0.95)	0.80 (0.69, 0.96)
Apolipoprotein A-I, g/L	1.20 (1.06, 1.37)	1.20 (1.06, 1.36)
Apolipoprotein A-II, mg/L	246 (217, 280)	244 (216, 279)
Apolipoprotein A-IV, mg/L	182 (145, 224)	181 (144, 223)
Apolipoprotein C-I, mg/L	16.0 (13.0, 19.0)	16.0 (13.0, 19.0)
Apolipoprotein C-II, mg/L	34.0 (26.0, 44.0)	34.0 (26.0, 45.0)
Apolipoprotein C-III, mg/L	85.0 (65.0, 112.0)	86.0 (65.0, 114.0)
Apolipoprotein E, mg/L	23.0 (19.0, 28.0)	23.0 (19.0, 28.0)
ApoE2/E2	17 (0.3)	12 (0.2)
ApoE2/E3	310 (5.2)	298 (5.0)
ApoE2/E4	72 (1.2)	69 (1.2)
ApoE3/E3	3826 (64.7)	3917 (66.1)
ApoE3/E4	1551 (26.2)	1506 (25.4)
ApoE4/E4	141 (2.4)	124 (2.1)

Values are medians (interquartile range) for continuous variables and n (%) for categorical variables.

The Effect of Alirocumab on Apolipoprotein Levels

The effect of alirocumab on apolipoprotein levels was evaluated by examining changes from baseline to four months after randomization (Table 2). Alirocumab treatment led to a reduction in apolipoproteins associated with ApoB-containing lipoprotein particles including decreased levels of Apo(a), ApoB, ApoC-I, ApoC-II, ApoC-III, and ApoE.

Prognostic Performance

Participants were followed for cardiovascular events for a median of 2.9 years (interquartile range 2.4-3.5). A MACE event was experienced by 721 (12.2%) in the placebo group and 596 (10.1%) in the alirocumab group ($p<0.0005$). All-cause death was experienced by 250 (4.2%) patients in the placebo group and 208 (3.5%) in the alirocumab group.

Spline analysis revealed associations between baseline apolipoproteins and the incidence of MACE events and all-cause death (Supplemental Figure S2 and S3). Apo(a), ApoB, and ApoC-III exhibited a generally linear relationship where higher levels of these apolipoproteins were associated with higher odds of experiencing a MACE event or all-cause death. In contrast, ApoA-II and ApoC-II showed an inverse linear relationship with the odds of these events. ApoA-IV demonstrated a J-shape pattern with all-cause death, indicating that both low and high levels were associated with increased odds, whereas its relationship with MACE appeared more linear. ApoC-I showed a linear relationship with MACE and an inverse J-shape relationship with all-cause death. For ApoE, an inverse J-shape pattern was observed with both MACE and all-cause death, suggesting that both low and high levels were associated with decreased odds of these events.

The apolipoprotein panel model was prognostic for MACE, with an AUC of 0.648 (95% CI 0.626, 0.670). The apolipoprotein panel was prognostic for all-cause death as well, with an AUC of 0.699 (95% CI 0.664, 0.733). In comparison, the prognostic model based on the conventional lipid panel had a lower AUC for MACE (0.579; 95%CI 0.557, 0.602) and for all-cause death (0.599; 95%CI 0.564, 0.635). The apolipoprotein panel had significantly better prognostic performance than the lipid panel for both MACE and all-cause death ($p<0.0001$). Adding the apolipoprotein panel significantly improved performance of the conventional lipid panel ($p<0.0001$ for both MACE and all-cause death). With both lipids and apolipoproteins in a single model the AUC for MACE increased to 0.659 (95% CI 0.637, 0.681) and to 0.732 (95% CI 0.691, 0.756) for all-cause death.

Table 2: Baseline, month 4 and absolute and percentile change from baseline to month 4 in apolipoprotein concentrations by treatment group.

Protein	Baseline		Month 4		Absolute change baseline to month 4		Percentage change baseline to month 4	
	Alirocumab	Placebo	Alirocumab	Placebo	Alirocumab	Placebo	Alirocumab	Placebo
Apo(a), nmol/L	41.1 (14.4, 140.1)	43.0 (14.3, 146.2)	23.7 (7.6, 107.6)	37.1 (12.0, 134.1)	-10.8 (-29.0, -2.8)	-1.5 (-10.4, 3.5)	-28.1 (-48.1, -9.8)	-4.2 (-20.1, 9.9)
ApoA-I, g/L	1.20 (1.06, 1.37)	1.20 (1.06, 1.36)	1.27 (1.13, 1.45)	1.23 (1.08, 1.39)	0.07 (-0.04, 0.18)	0.02 (-0.08, 0.13)	6.1 (-3.1, 15.9)	1.8 (-6.7, 11.3)
ApoA-II, mg/L	246 (217, 280)	244 (216, 279)	253 (225, 287)	250 (221, 284)	7 (-15, 29)	5 (-17, 27)	2.7 (-5.8, 12.4)	2.0 (-6.6, 11.3)
ApoA-IV, mg/L	182 (145, 224)	181 (144, 223)	190 (152, 234)	188 (152, 233)	8 (-22, 41)	8 (-23, 39)	4.7 (-11.4, 24.5)	4.7 (-11.6, 23.5)
ApoB, g/L	0.80 (0.69, 0.95)	0.80 (0.69, 0.96)	0.38 (0.30, 0.52)	0.81 (0.69, 0.98)	-0.41 (-0.53, -0.28)	0.01 (-0.09, 0.11)	-52.5 (-62.0, -38.5)	1.1 (-10.4, 15.0)
ApoC-I, mg/L	16 (13, 19)	16 (13, 19)	14 (11, 17)	16 (13, 19)	-2 (-4, 0)	0 (-2.0, 2)	-13.3 (-25.0, 0.0)	0.0 (-11.1, 16.7)
ApoC-II, mg/L	34 (26, 44)	34 (26, 45)	29 (22, 38)	34 (26, 45)	-4 (-10, 1)	0 (-6, 6)	-13.9 (-28.2, 3.7)	0.0 (-16.1, 19.4)
ApoC-III, mg/L	85 (65, 112)	86 (65, 114)	74 (56, 97)	87 (66, 117)	-10 (-27, 4)	2 (-14, 18)	-13.2 (-28.7, 6.5)	2.2 (-15.7, 24.4)
ApoE, mg/L	23 (19, 28)	23 (19, 28)	16 (12, 20)	23 (19, 29)	-7 (-11, -3)	0 (-3, 4)	-31.6 (-43.5, -17.4)	0.0 (-13.0, 16.7)

Values are medians (interquartile range) for continuous variables.

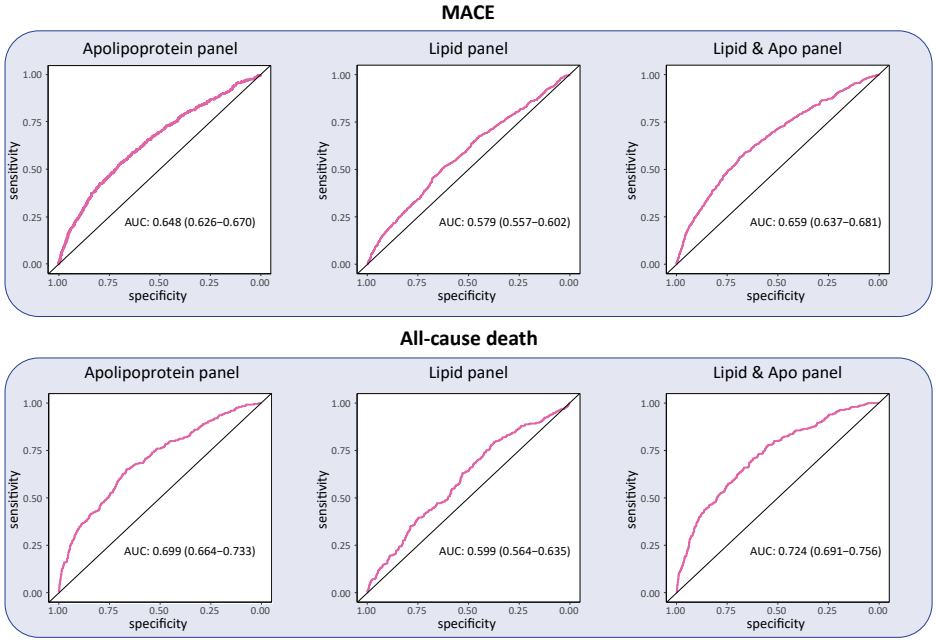


Figure 1: Clinical performance of prediction models on MACE and all-cause death. ROC curves of clinical performance for MACE (top) and all-cause death (bottom) based on the prognostic models based on: the apolipoprotein panel (left) consisting of baseline apolipoproteins and ApoE phenotype, the lipid panel (center) consisting of baseline TC, HDL-C, and triglycerides, and the combination of the two panels (right).

The apolipoproteins that significantly contributed to the prognostic full apolipoprotein panel model for MACE, as indicated by the partial Wald tests, are: Apo(a) (linear relationship, $p<0.0001$), ApoB (linear relationship, $p<0.001$), ApoC-I (linear relationship, $p<0.05$), ApoA-II (inverse relationship, $p<0.0001$), and ApoA-IV (linear relationship, $p<0.0001$). For all-cause death, ApoA-II (inverse relationship, $p<0.0001$), ApoA-IV (J-shape relationship, $p<0.0005$), ApoC-II (inverse relationship, $p<0.005$) and ApoE (inverse J-shape, $p<0.05$) significantly contributed to the full apolipoprotein panel model. Models created based on individual apolipoproteins showed only marginal discrimination for predicting MACE or all-cause death, as indicated by their AUCs (Supplemental Figure S4 and S5). Splines of the individual apolipoproteins are shown in Supplemental Figure S6 and S7. A model incorporating the apolipoprotein panel alongside age, sex, ethnicity, body mass index, smoking status and history of diabetes alongside had a significantly better goodness-of-fit than a model using only these readily available variables ($p<0.0001$).

Predictive Performance

Baseline Apolipoprotein Panel Predicts Treatment Benefit of Alirocumab on MACE and All-cause Death

Clinical effectiveness for MACE and all-cause death was evaluated based on baseline apolipoprotein levels of the placebo group (5,926 patients) and the alirocumab group (5,917 patients). The results of the estimated probabilities during a median follow-up of 2.9 years by the apolipoprotein panel per patient for the full study group are shown in Figure 2A. For comparison, the probabilities estimated with the lipid panel and with the lipid and apolipoprotein panels combined are presented in Supplemental Figure S8 and S9.

With higher baseline risk for MACE as estimated by the apolipoprotein panel, a larger number of patients had a lower calculated probability of an event with alirocumab than with placebo, indicating that the apolipoprotein panel can predict a treatment benefit of alirocumab on MACE (Figure 2A). The same trend is observed for prediction models based on the conventional lipid panel and the combination of the two panels (Supplemental Figure S8). For all-cause death a comparable pattern emerged, with individuals at higher estimated baseline risk having a lower estimated probability of an event when allocated to alirocumab (Figure 2C).

Using the observed overall 2.1% absolute reduction in risk of MACE with alirocumab as a minimum criterion for treatment benefit, two subgroups were defined based on the baseline apolipoprotein panel: those with a benefit from alirocumab treatment (n = 5,045) and those without (n = 6,798) (Figure 2B). A similar dichotomization was performed for all-cause death, using the observed overall 0.7% absolute reduction in risk of all-cause death with alirocumab as the threshold. A total of 4,384 patients had a risk reduction of 0.7% or more, while 7,459 patients fell below this threshold.

Figure 2B and 2D show the classification of benefit versus no benefit, using the predefined thresholds, for different baseline risks, as calculated with the apolipoprotein panels. In a relative sense, more were found to benefit from alirocumab treatment in those at a higher calculated baseline risk of a cardiovascular event. Analogous distributions of benefit versus no benefit based on the lipid panel and the combination of the lipid and apolipoprotein panels are shown in Supplemental Figure S8.

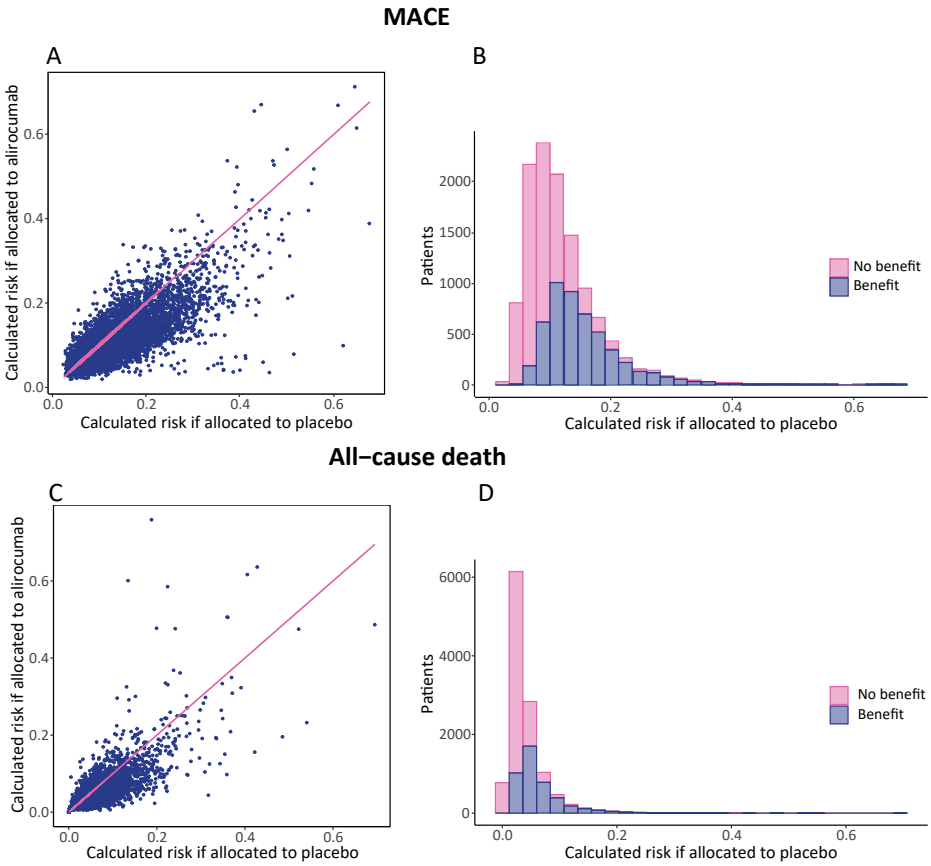
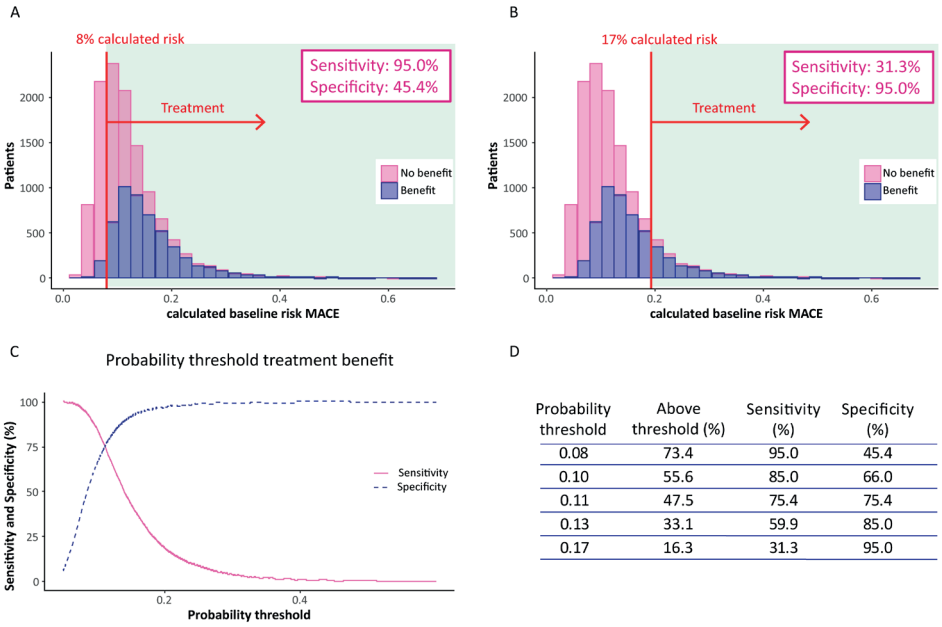


Figure 2: Model-based calculated risk fraction in the placebo respectively targeted treatment group of the apolipoprotein panel for MACE and all-cause death. Panel A for MACE and C for all-cause death display scatter plots of model-based calculated risk if allocated to placebo or allocated to alirocumab. Panel B and D present the stacked distribution of risk in the subgroups defined to achieve meaningful benefit or not for MACE and all-cause death (absolute risk reduction with alirocumab of 2.1% or higher for MACE and 0.7% or higher for all-cause death). Risk is calculated based on the apolipoprotein panel.

Treatment Benefit of Alirocumab as Estimated by the Apolipoprotein Panel Prediction Model

The results of estimating treatment benefit of alirocumab for reducing the risk of MACE or all-cause death based on apolipoproteins can help identify the subgroup most likely to benefit from treatment. For instance, if one were willing to treat only those with an estimated absolute MACE risk above 8%, 73% of the cohort would be treated, which would be 95% of those with a calculated treatment benefit and 55% of those without. (Figure 3A). In contrast, if the clinical decision threshold to treat with alirocumab was set at a higher estimated risk, 17% (Figure 3B), only 16% would be treated: 31 of those with a calculated benefit but only 5% of those without.

Figure 3C shows these proportions for other probability thresholds. For clinical decision making an example of probability thresholds is shown in Figure 3D, where increasing the threshold means decreasing the sensitivity, but increasing the specificity of the apolipoprotein panel test.



Probability threshold	Above threshold (%)	Sensitivity (%)	Specificity (%)
0.08	73.4	95.0	45.4
0.10	55.6	85.0	66.0
0.11	47.5	75.4	75.4
0.13	33.1	59.9	85.0
0.17	16.3	31.3	95.0

Figure 3: Probability thresholds for treatment allocation based on estimated treatment benefit by the apolipoprotein panel. Panel A and B show the clinical decision limit at 8% and 17% estimated risk of MACE and the resulting sensitivity and specificity, respectively. Panel C shows the sensitivity or specificity at specific probability thresholds. Panel D shows a table with five examples of probability thresholds and the resulting sensitivity, and specificity.

DISCUSSION

In this study, we assessed the clinical performance and clinical effectiveness of a comprehensive 9-plex apolipoprotein panel in a subset of the ODYSSEY OUTCOMES trial participants, comprised of patients with recent ACS on optimized statin therapy. The baseline apolipoprotein panel led to better and incremental classification of MACE and all-cause death, compared with the conventional lipid panel. The apolipoprotein panel was also able to predict treatment benefit of alirocumab on MACE and all-cause death.

Our results further demonstrate that individual apolipoproteins were not prognostic for MACE or all-cause death in this population, suggesting that interdependent apolipoproteins should preferably be measured as a full panel to predict cardiovascular events. In the full apolipoprotein panel model, the partial effects of each apolipoprotein, adjusted for other variables, significantly

contributed to the prediction of MACE according to the partial Wald tests. Specifically, Apo(a), ApoB, ApoC-I, and ApoA-IV demonstrated a positive linear relationship with MACE, while ApoA-II exhibited an inverse relationship. For all-cause death, the apolipoproteins that significantly contributed to the full panel model were ApoA-II and ApoC-II, which demonstrated an inverse relationship, ApoA-IV, which showed a J-shaped relationship, and ApoE, which exhibited an inverse J-shaped relationship (all $p<0.05$).

Several of the apolipoproteins in our panel are well-established risk factors. Apo(a) is recognized as an important cardiovascular risk factor as supported by Mendelian randomization studies[38, 39], epidemiological evidence[40], and cardiovascular outcome trials.[17, 41, 42] Similarly, ApoB, another contributing predictor, is a well-established cardiovascular risk factor as well and its measurement is recommended for cardiovascular risk assessment.[9, 14, 43, 44] ApoC-I plays a dual role in lipid metabolism, acting as either atherogenic by inhibiting triglyceride-rich lipoprotein metabolism or atheroprotective by facilitating HDL synthesis and stabilization.[6]

ApoA-II and ApoA-IV made significant contributions to the prediction models for both outcomes according to the partial Wald tests. The role of ApoA-II in cardiovascular disease remains poorly understood, with conflicting findings in the literature.[25, 45-47] In the present study, spline analysis in this ACS cohort on optimized statin therapy revealed an inverse relationship between ApoA-II concentrations and the likelihood of MACE and all-cause death, indicating that lower ApoA-II levels may increase the risk of adverse cardiovascular outcomes (Supplemental Figure S2 and S3). Interestingly in the present study, ApoA-IV, which is generally considered a cardioprotective factor, was associated with MACE (linear relationship) and all-cause death (J-shape) (Supplemental Figure S2 and S3). This is in contrast with findings from the PROCARDIS study, where ApoA-IV, as part of an apolipoprotein panel, was inversely associated with coronary heart disease.[25] The reasons behind the conflicting results for ApoA-IV need further investigation to understand the underlying mechanisms.

ApoE plays a crucial role in lipid metabolism and cardiovascular disease risk, particularly through its involvement in the clearance of remnant lipoproteins. ApoC-II, on the other hand, is essential for activating lipoprotein lipase, promoting the metabolism of triglyceride-rich lipoproteins, and its inverse association with all-cause death in this study suggests a protective role. ApoC-I and ApoC-III are associated with the risk of cardiovascular events which is also demonstrated in the current study. For ApoC-III this can be explained by the fact that it acts as an inhibitor of lipolysis and impairs the clearance of triglyceride-rich lipoproteins, leading to the accumulation of these atherogenic particles in circulation.[6] ApoC-I plays a dual role and can act as an inhibitor of lipolysis, which explains the association with MACE demonstrated in this study with its linear relationship with MACE and all-cause death.[6]

Some potential limitations of our analysis should be acknowledged. It is important to note that this was a post hoc analysis in a subgroup from a randomized clinical trial. Nevertheless, the characteristics of the analysis cohort were generally representative of the full trial cohort. Ideally, the clinical effectiveness of a new medical test should be evaluated using direct outcome data in a comparative randomized controlled trial. This would involve a study comparing lipids and apolipoproteins in separate arms of the trial, assessing the impact on patient outcomes based on management guided by the test results. However, such a study design is challenging and very expensive. In the current study, we adopted an alternative approach in which we measured apolipoprotein levels and developed prognostic and predictive models based on these levels, comparing them with predictions made using the conventional lipid panel.

We developed and evaluated multivariable prediction models in a single study group and did not rely on a separate validation cohort for obtaining independent estimates of performance. While this does not jeopardize the validity of our comparison of models based on either lipids, apolipoproteins or their combination, it may mean that performance of each of the three models is overestimated, compared to what would have been obtained in an independent evaluation of performance.

There are numerous apolipoproteins, of which we carefully selected nine for measurement in the context of cardiovascular disease.[6, 8] Not measuring all apolipoproteins may present a limitation, as it leaves uncertainty regarding whether these selected apolipoproteins provide the greatest amount of prognostic or predictive information.

The ODYSSEY OUTCOMES Trial recruited from a population with established cardiovascular disease, which allowed us to assemble a large, multinational cohort for assessing clinical and predictive performance of apolipoprotein profiling. Performance in other populations and for other testing purposes is still unknown. In general, clinical performance will differ, according to the testing purpose, the target population, target condition or event, and with other comparator index tests.

The study group for our analyses was highly selected, as a results of the trial inclusion criteria, which focused on inadequate control of ApoB-containing lipoproteins, reflected in levels of LDL-C and non-HDL-C, while excluding patients with markedly elevated triglyceride levels. Consequently, patients with pronounced elevations of remnant lipoproteins, a condition often underdiagnosed in current clinical practice, were likely excluded. It is possible that the apolipoprotein panel has particular value in remnant disease.[4, 8] Nearly 90% of the current analysis cohort was treated with high-intensity statin therapy, which might have curbed the prognostic and predictive performance of the apolipoprotein panel.

The apolipoprotein panel was able to predict treatment benefit with alirocumab for MACE. These results suggest that the apolipoprotein panel can be used to identify patients more likely to benefit from addition of a PCSK9 inhibitor to statin. Based on the risk of MACE estimated with the apolipoprotein panel, physicians might select individuals who are at a sufficiently high estimated risk and more likely to benefit from treatment, while not treating patients at a lower estimated risk and lower expected benefit of treatment. This personalized approach might enhance the cost-effectiveness of treatment.

We speculate that the predictive performance of the apolipoprotein panel in estimating treatment benefit of therapies that directly target a single apolipoprotein may be even stronger than observed in the current analysis for alirocumab. For example, it is possible that apolipoprotein profiling could help guide the selection of patients for therapies in development that specifically target Apo(a)[23, 48] or ApoC-III[49, 50].

In addition to treatment decisions, apolipoprotein profiling could serve as a valuable tool for precision diagnostics and personalized medicine, enabling a more refined diagnosis of dyslipidemia beyond the traditional classification based on lipids and potentially uncover novel dyslipidemic phenotypes. Specifically, the comprehensive biomarker panel integrates the interdependencies between the apolipoproteins and by “thinking in wholes” this multiplex approach improves clinical performance and clinical effectiveness of testing.

CONCLUSION

In patients with recent ACS receiving optimized or maximum-tolerated statin treatment, a 9-plex comprehensive apolipoprotein panel including ApoE phenotype significantly improved classification for MACE and all-cause death beyond the conventional lipid panel. In addition, the apolipoprotein panel may help select patients most likely to benefit from treatment with PCSK9 inhibition therapy versus standard statin therapy. Hence, the 9-plex apolipoprotein panel may be a valuable asset for cardiovascular precision diagnostics and personalized cardiovascular disease.

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SUPPLEMENTAL INFORMATION

Table S1: Incidence of MACE, cardiovascular death and all-cause death

	Alirocumab (5,917)	Placebo (5,926)	ARR (%)	Total
MACE	596 (10.1%)	721 (12.2%)	2.1	1317
All-cause Death	208 (3.5%)	250 (4.2%)	0.7	458

Values are n (%).

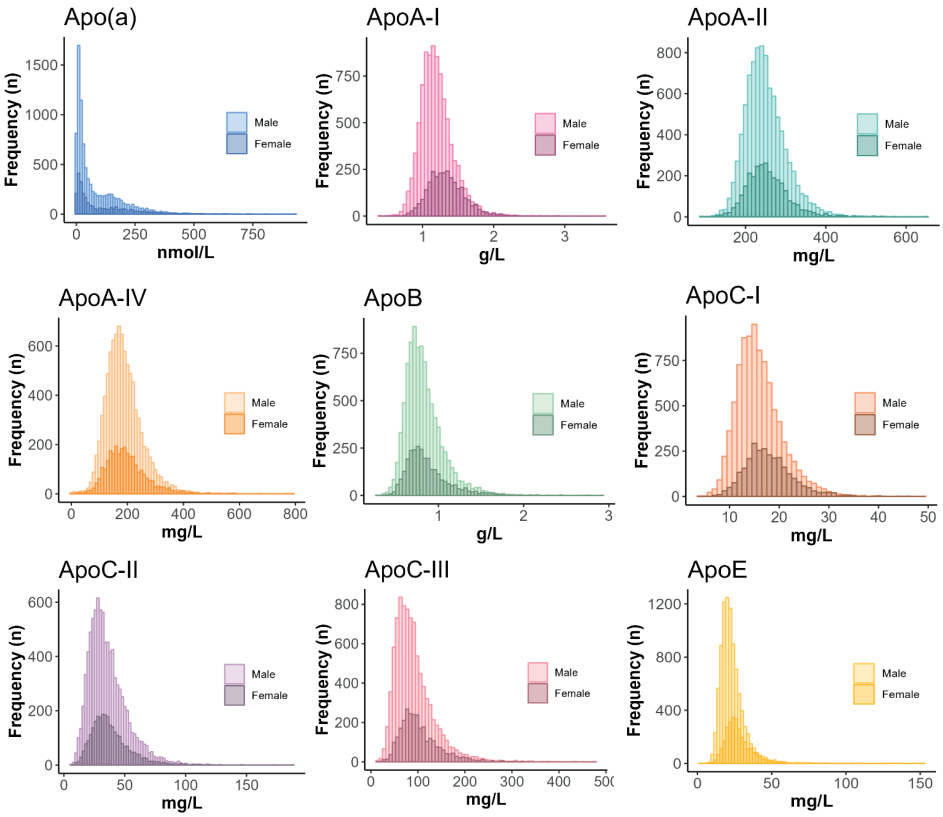

Figure S1: Baseline apolipoprotein levels. Distribution of baseline apolipoprotein concentrations as part of the apolipoprotein panel in the Odyssey Outcomes trial stratified by sex. Placebo and alirocumab are combined (n = 11,843)

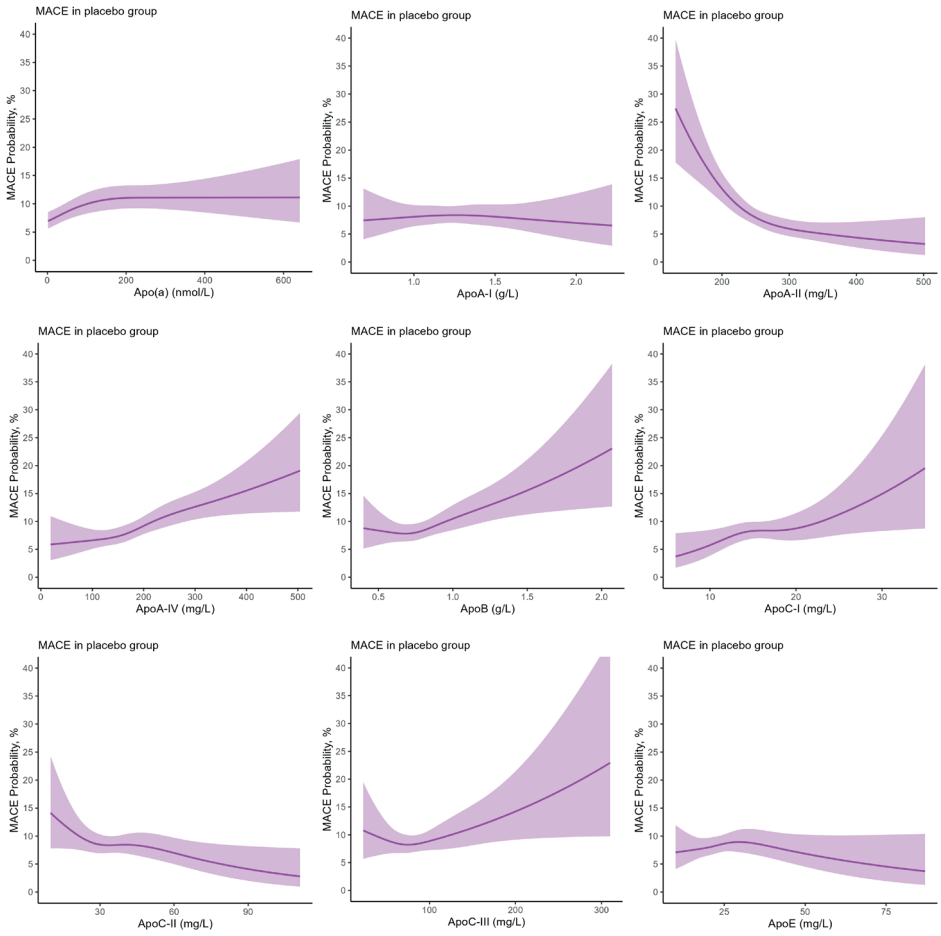

Figure S2: Spline analysis apolipoprotein profile for MACE. Results of spline analysis of baseline apolipoproteins within the apolipoprotein panel model, based on the placebo group (n = 5,926), adjusted for other apolipoproteins in the model, to predict MACE.

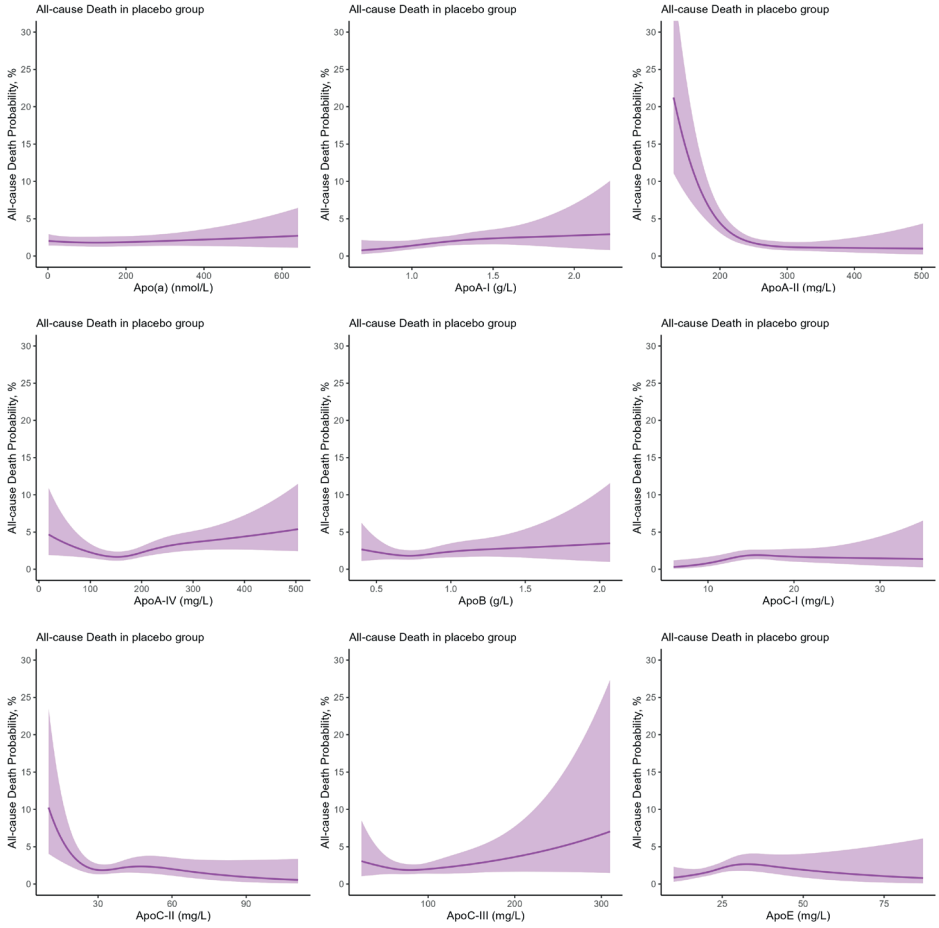

Figure S3: Spline analysis apolipoprotein profile for all-cause death. Results of spline analysis of baseline apolipoproteins within the apolipoprotein panel model, based on the placebo group (n = 5,926), adjusted for other apolipoproteins in the model, to predict all-cause death.

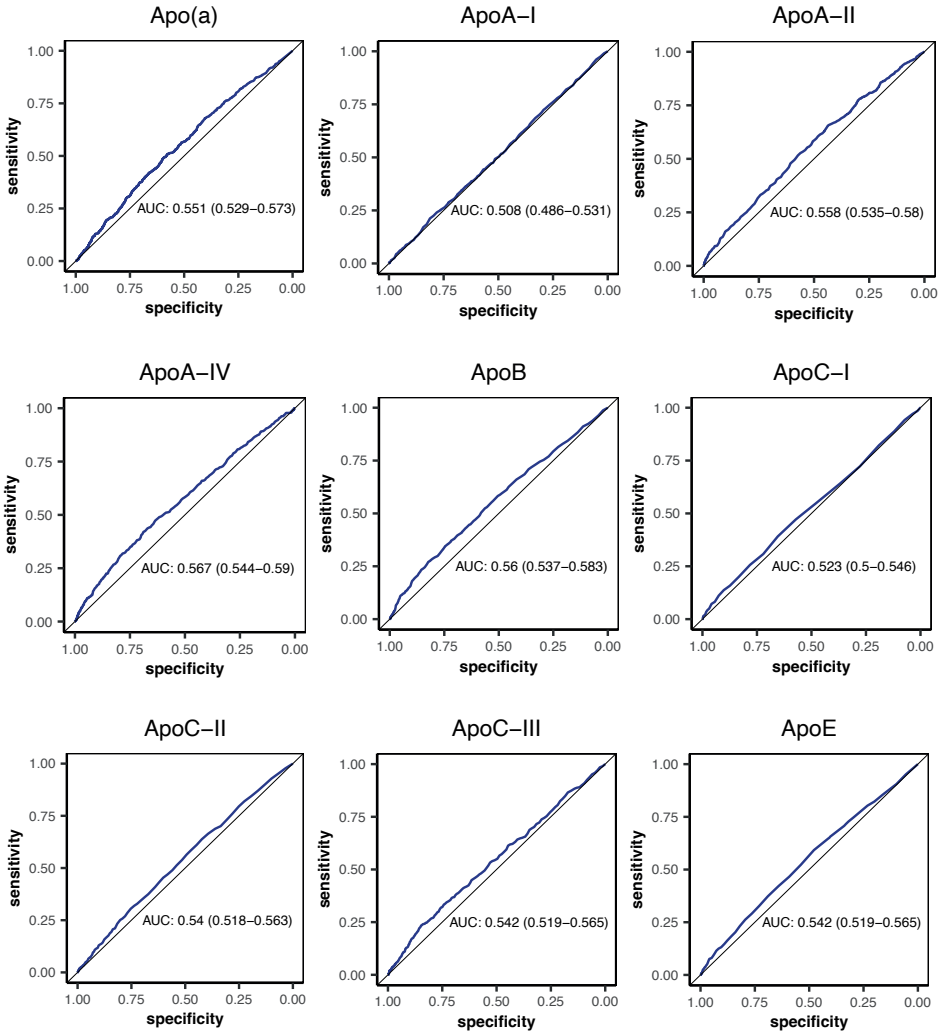

Figure S4: Individual apolipoproteins prognosis of MACE. ROC curves of nine prediction models based on individual baseline apolipoproteins to of the placebo group predict MACE.

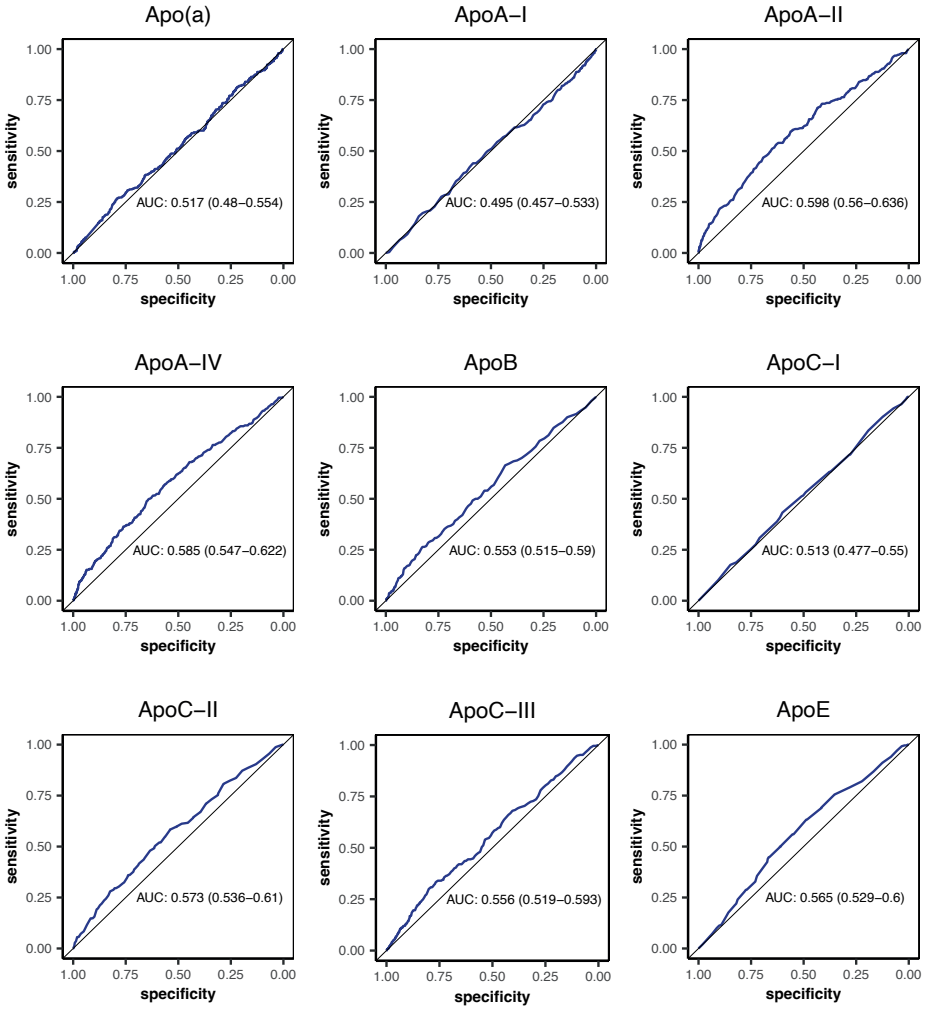

Figure S5: Individual apolipoproteins prognosis of all-cause death. ROC curves of nine prediction models based on individual baseline apolipoproteins of the placebo group to predict all-cause death.

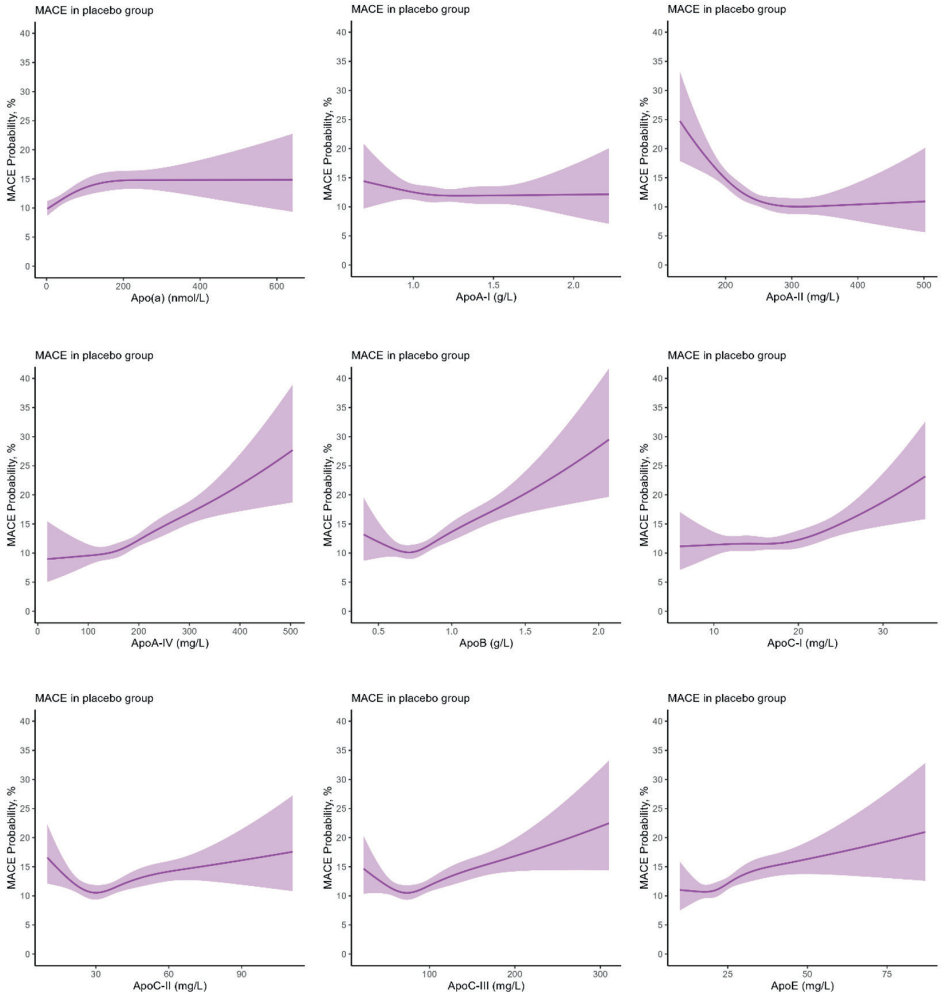

Figure S6: Spline analysis individual apolipoproteins for MACE. Results of spline analysis of individual baseline apolipoproteins, based on the placebo group (n = 5,926) to predict MACE.

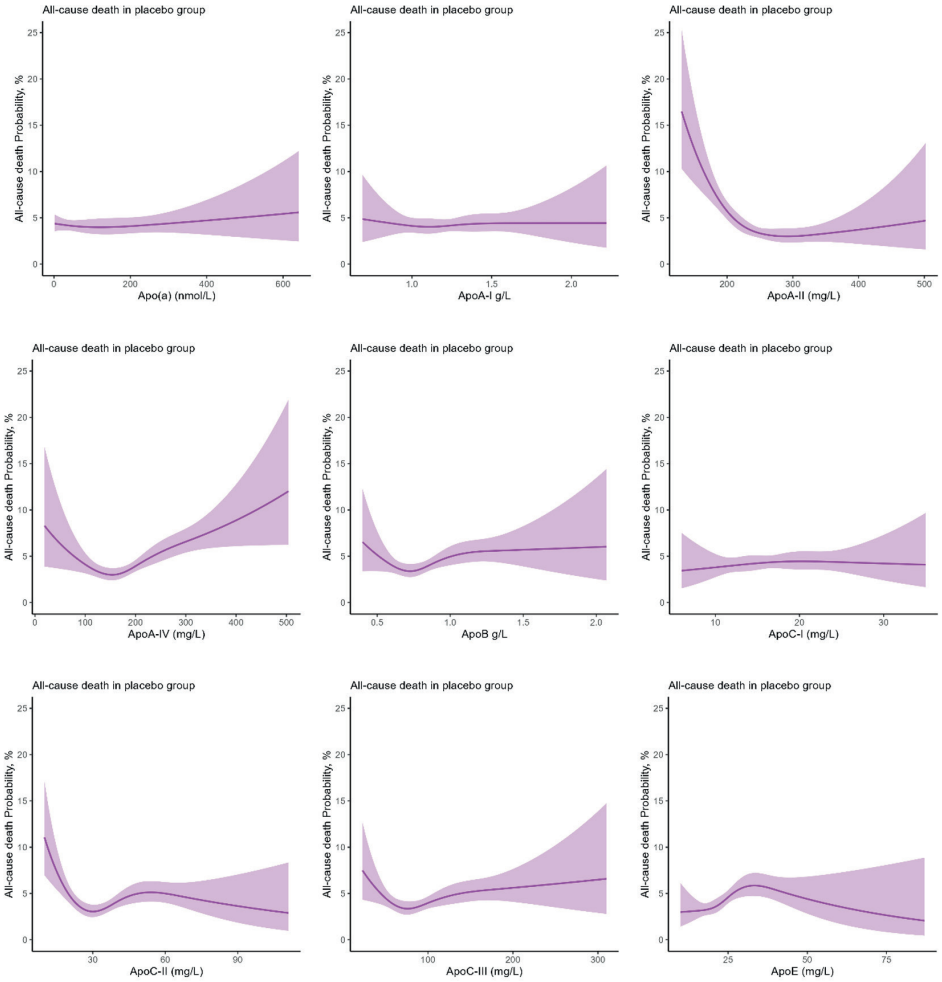

Figure S7: Spline analysis individual apolipoproteins for all-cause death. Results of spline analysis of individual baseline apolipoproteins, based on the placebo group (n = 5,926) to predict all-cause death.

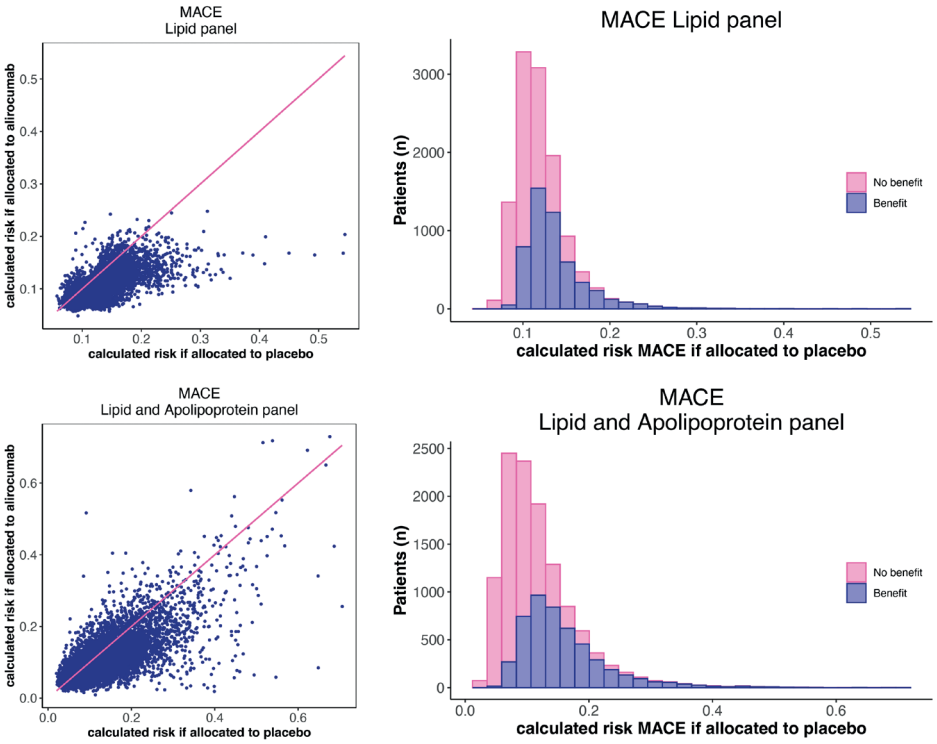

Figure S8: Estimated Treatment Effect and Treatment Benefit MACE by Lipid Profile and Lipid Profile in combination with Apolipoprotein Profile. On the left scatter plots of model-based calculated risk if allocated to placebo or allocated to alirocumab. The plots on the right show the stacked distribution of risk in the subgroups defined to achieve meaningful benefit or not for MACE (absolute risk reduction with alirocumab of 2.1% or higher). Risk is calculated based on the lipid panel and the combination of the lipid panel and the apolipoprotein panel.

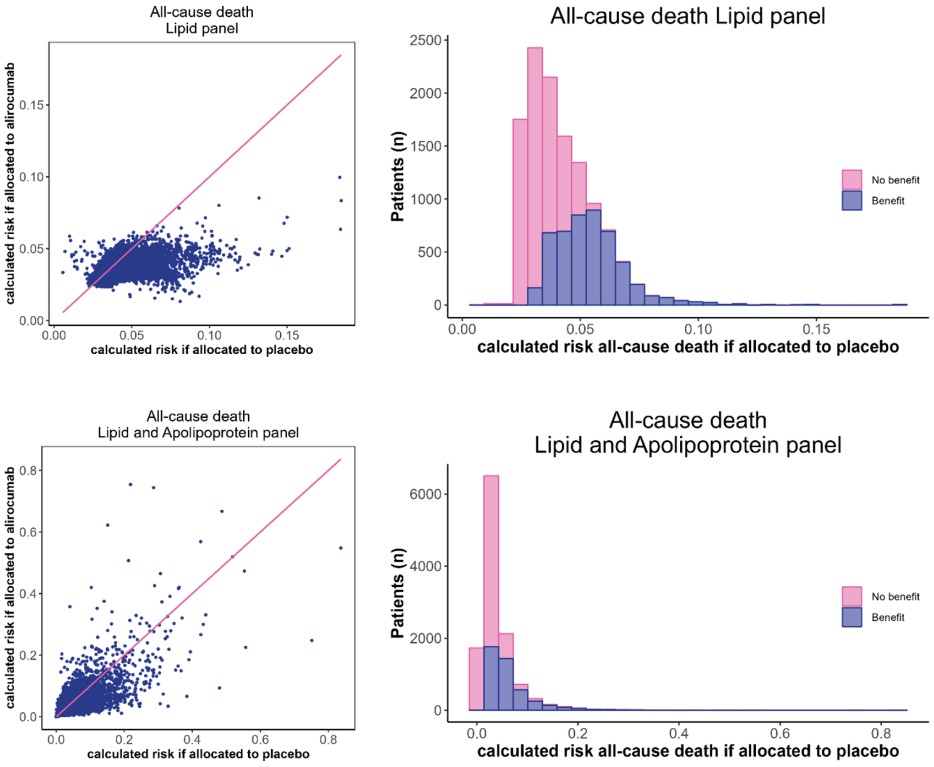

Figure S9: Estimated Treatment Effect and Treatment Benefit all-cause death by Lipid Profile and Lipid Profile in combination with Apolipoprotein Profile. On the left scatter plots of model-based calculated risk if allocated to placebo or allocated to alirocumab. The plots on the right show the stacked distribution of risk in the subgroups defined to achieve meaningful benefit or not for all-cause death (absolute risk reduction with alirocumab of 0.7% or higher for all-cause death). Risk is calculated based on the lipid panel and the combination of the lipid panel and the apolipoprotein panel.