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ORIGINAL RESEARCH ARTICLE

Relating Lipoprotein(a) Concentrations to Cardiovascular Event Risk After Acute Coronary Syndrome: A Comparison of 3 Tests

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BACKGROUND: Lipoprotein(a) is a risk factor for cardiovascular events and modifies the benefit of PCSK9 (proprotein convertase subtilisin/kexin type 9) inhibitors. Lipoprotein(a) concentration can be measured with immunoassays reporting mass or molar concentration or a reference measurement system using mass spectrometry. Whether the relationships between lipoprotein(a) concentrations and cardiovascular events in a high-risk cohort differ across lipoprotein(a) methods is unknown. We compared the prognostic and predictive value of these types of lipoprotein(a) tests for major adverse cardiovascular events (MACE).

METHODS: The ODYSSEY OUTCOMES trial (Evaluation of Cardiovascular Outcomes After an Acute Coronary Syndrome During Treatment With Alirocumab) compared the PCSK9 inhibitor alirocumab with placebo in patients with recent acute coronary syndrome. We compared risk of a MACE in the placebo group and MACE risk reduction with alirocumab according to baseline lipoprotein(a) concentration measured by Siemens N-latex nephelometric immunoassay (IA-mass; mg/dL), Roche Tina-Quant turbidimetric immunoassay (IA-molar; nmol/L), and a noncommercial mass spectrometry–based test (MS; nmol/L). Lipoprotein(a) values were transformed into percentiles for comparative modeling. Natural cubic splines estimated continuous relationships between baseline lipoprotein(a) and outcomes in each treatment group. Event rates were also determined across baseline lipoprotein(a) quartiles defined by each assay.

RESULTS: Among 11 970 trial participants with results from all 3 tests, baseline median (Q1, Q3) lipoprotein(a) concentrations were 21.8 (6.9, 60.0) mg/dL, 45.0 (13.2, 153.8) nmol/L, and 42.2 (14.3, 143.1) nmol/L for IA-mass, IA-molar, and MS, respectively. The strongest correlation was between IA-molar and MS (r=0.990), with nominally weaker correlations between IA-mass and MS (r=0.967) and IA-mass and IA-molar (r=0.972). Relationships of lipoprotein(a) with MACE risk in the placebo group were nearly identical with each test, with estimated cumulative incidences differing by ≤0.4% across lipoprotein(a) percentiles, and all were incrementally prognostic after accounting for low-density lipoprotein cholesterol levels (all spline $P \leq 0.0003$). Predicted alirocumab treatment effects were also nearly identical for each of the 3 tests, with estimated treatment hazard ratios differing by ≤0.07 between tests across percentiles and nominally less relative risk reduction by alirocumab at lower percentiles for all 3 tests. Absolute risk reduction with alirocumab increased with increasing lipoprotein(a) measured by each test, with significant linear trends across quartiles.

CONCLUSIONS: In patients with recent acute coronary syndrome, 3 lipoprotein(a) tests were similarly prognostic for MACE in the placebo group and predictive of MACE reductions with alirocumab at the cohort level.

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ORIGINAL RESEARCH ARTICLE

Clinical Perspective

What Is New?

- In patients with recent acute coronary syndrome, we compared 2 immunoassay-based tests that report mass and molar lipoprotein(a) concentration with a mass spectrometry–based test that reports molar apolipoprotein(a) concentration in relation to major adverse cardiovascular event (MACE) risk and reduction of this risk with the PCSK9 (proprotein convertase subtilisin/kexin type 9) inhibitor alirocumab.
- Estimated cumulative incidence of MACE within the placebo group increased with higher lipoprotein(a) percentiles and differed by ≤0.4% among tests.
- Across percentiles of lipoprotein(a) as determined by the 3 tests, the, estimated treatment hazard ratios for MACE (alirocumab:placebo) differed by ≤0.07 among tests, with nominally less relative risk reduction by alirocumab at lower percentiles.

What Are the Clinical Implications?

- At a cohort level, the evaluated mass and molar lipoprotein(a) immunoassays were similarly prognostic for risk of MACE and predictive of MACE reduction with alirocumab.
- In terms of choosing a commercially available lipoprotein(a) immunoassay for individual prognosis, both of the evaluated tests provide comparable risk assessment.

Nonstandard Abbreviations and Acronyms

ACS	acute coronary syndrome
apo(a)	apolipoprotein(a)
HR	hazard ratio
IA-mass	immunoassay-based mass test
IA-molar	immunoassay-based molar test
LDL-C	low-density lipoprotein cholesterol
MACE	major adverse cardiovascular event(s)
MS	mass spectrometry
PAD	peripheral artery disease
PCSK9	proprotein convertase subtilisin/kexin type 9
VTE	venous thromboembolism

A relationship between the circulating concentration of lipoprotein(a), a genetically determined low-density lipoprotein particle, and the risk of cardiovascular events has been established in epidemiological studies,[1] in Mendelian randomization analyses,[2,3] and in the setting of cardiovascular outcomes trials.[4–6] Furthermore, a recent European Atherosclerosis Society consensus statement summarized the cumulative evidence supporting a causal continuous association between lipoprotein(a) concentration and cardiovascular events, substantiating clinical guideline recommendations and the inclusion of lipoprotein(a) in estimates of global cardiovascular risk.[7]

Initial reports relating lipoprotein(a) to cardiovascular events typically used immunoassay-based measurements of mass (ie, in units of milligrams per deciliter). More recently, commercial lipoprotein(a) tests have been developed with standardization against the internationally endorsed ELISA-based Reference Measurement Procedure and World Health Organization/International Federation of Clinical Chemistry and Laboratory Medicine SRM2B reference material, expressing lipoprotein(a) concentration in molar units (nanomoles per liter).[8] Previous clinical studies and guidelines have variably defined risk in terms of immunoassay-based mass[4] or molar[5] concentration of lipoprotein(a). In addition, eligibility criteria for ongoing trials of therapeutics that substantially decrease lipoprotein(a) levels have variably set minimum qualifying concentrations in mass[9] or molar[10] units. The potential transition to molar concentration being the preferred unit of measurement is further complicated by the fact that there is no consistent conversion factor between mass and molar scales because of differing lipoprotein(a) isoform dependency of each immunoassay-based analytic method.[7,11] Although commercially available immunoassays that measure lipoprotein(a) in mass or molar units have been compared in patients unselected for cardiovascular risk,[12] they have not been compared in terms of prognosis for cardiovascular events in a cohort of high-risk patients. A third, investigational analytic approach to lipoprotein(a) concentration utilizing mass spectrometry (MS) has the potential to improve measurement because it is unaffected by apolipoprotein(a) (apo(a)) isoform (number of kringle IV type 2 repeats) and has an extended measurement range.[13] However, no information exists on the prognostic information provided by this assay method.

The ODYSSEY OUTCOMES trial (Evaluation of Cardiovascular Outcomes After an Acute Coronary Syndrome During Treatment With Alirocumab) compared the

convertase subtilisin/kexin type 9 inhibitor alirocumab with placebo in patients with recent acute coronary syndrome (ACS). In previous reports from the trial, we found that lipoprotein(a) concentration measured with a commercial immunoassay-based mass test (IA-mass) was prognostic for future major adverse cardiovascular events (MACE) and total cardiovascular events and predictive of the effect of alirocumab to reduce the risk of these events.[4,14] In light of the methodologic issues pertaining to lipoprotein(a) assessment, we measured its concentration in trial participants using 3 methods: the aforementioned IA-mass test, a commercial immunoassay-based molar test (IA-molar), and a semiautomated laboratory-developed multiplex MS-based test. The aims of this study were to evaluate the degree of correspondence among the 3 measurement methods in a well-characterized clinical trial population and compare the prognostic and predictive information for cardiovascular events provided by each test.

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 Population

The design,[15] primary results,[16] total events results,[17] and lipoprotein(a) mass concentration findings[4,14,18] from the ODYSSEY OUTCOMES trial have been published. A total of 18 924 patients from 1315 sites in 57 countries were randomized in a 1:1 ratio to receive 75 mg of alirocumab (increased to 150 mg for those who did not achieve a low-density lipoprotein cholesterol [LDL-C] level of <50 mg/dL [1.29 mmol/L]) or matching placebo subcutaneously every 2 weeks. Key inclusion criteria included age ≥40 years, hospitalization with ACS (myocardial infarction or unstable angina) 1 to 12 months before randomization, an LDL-C level ≥70 mg/dL (1.81 mmol/L), a non–high-density lipoprotein cholesterol level ≥100 mg/dL (2.59 mmol/L), or apolipoprotein B ≥80 mg/dL during stable treatment with 40 to 80 mg of atorvastatin daily, 20 to 40 mg of rosuvastatin daily, or the maximum tolerated dose of either statin. The trial was approved by the institutional review board of each site, and all patients provided informed consent.

Participants without available baseline results from all 3 lipoprotein(a) tests were excluded from the current analyses, including those recruited from countries or sites where additional exploratory laboratory testing was not permitted or not possible. In addition, results from month-4 samples from this cohort were included in the analyses if they were available.

Outcomes

The primary efficacy outcome of the study and of the current analysis was time to first occurrence of a MACE, consisting of death from coronary heart disease, nonfatal myocardial infarction, fatal or nonfatal ischemic stroke, or unstable angina requiring hospitalization. We also analyzed all cardiovascular outcomes observed during the trial, including cardiovascular death and total (first and subsequent) nonfatal cardiovascular events. The latter category included nonfatal primary outcome events, hemorrhagic stroke, hospitalization for heart failure, ischemia-driven coronary revascularization, major peripheral artery disease (PAD) events (critical limb ischemia, lower extremity revascularization procedures, or amputation for ischemia), venous thromboembolism (VTE), deep vein thrombosis and pulmonary embolism. Noncardiovascular deaths were also recorded during follow-up but were not included as events in any analyses. All events included in the analyses except PAD events and VTE were adjudicated by an independent committee blinded to treatment assignment; PAD events and VTE were reported by investigators blinded to treatment assignment on a specific case report form.

Measurement of Lipoprotein(a)

Lipoprotein(a) measurement by IA-mass was performed as part of the lipid central laboratory assessments for all randomized patients. Samples frozen at −20 °C were shipped from study sites to Covance Laboratories, where they were stored at −70 °C until measurement on a Siemens BNII analyzer using an immunonephelometric assay (Siemens N Latex) with rabbit polyclonal anti-lipoprotein(a) detection antibody with interassay coefficient of variation of 3.1% to 4.8%, depending on lipoprotein(a) levels (Siemens, Healthcare Diagnostics).

Molar concentrations of lipoprotein(a) were assessed by an immunoassay (IA-molar) and by MS in serum samples received on dry ice and stored at −80 °C at Leiden University Medical Center, performed in a single laboratory as part of a prespecified biomarker substudy. Samples were thawed and divided into 2 aliquots in batches, of which the immunoassay aliquot was stored at 4 °C overnight and analyses were performed daily on freshly thawed samples. The MS aliquot was stored at −80 °C for up to 1 year before analysis on freshly thawed samples.

IA-molar measurements of lipoprotein(a) were assessed by a Roche Cobas C 502 analyzer using an immunoturbidimetric assay (Roche Tina-Quant Lipoprotein(a) Gen.2) with rabbit polyclonal anti-lipoprotein(a) detection (Roche Diagnostics). Bilevel internal quality control samples were measured at the start and end of every analysis day. Three high-lipoprotein(a) internal quality control sample lots resulted in mean values (SD) of 106.6 nmol/L (2.8), 112.5 nmol/L (3.6), and 111.2 nmol/L (3.0), with an overall interassay coefficient of variation of 2.9%.

Apo(a) in lipoprotein(a) was measured with a higher-order[19] semiautomated laboratory-developed multiplex MS test (1290 Infinity II ultra-high-performance liquid chromatography instrument coupled to 6495 triple quadrupole-MS by Agilent Technologies).[20] Sample preanalysis was performed on a 96-channel Agilent BRAVO automated liquid handling platform. Proteins in serum samples were denatured, reduced, alkylated, and, after tryptic digestion, measured by MS as published previously, for 6 apolipoproteins.[20] For apo(a) quantification, proteotypic peptides LFLEPTQADIALLK (in the peptidase domain) and GISSTTVTGR (in the kringle 4 type 9 domain) were measured. Apo(a) was quantified with 5 value-assigned native serum calibrators, guaranteeing metrologic traceability to SRM2B and the World Health Organization/International Federation of Clinical Chemistry and Laboratory Medicine

reference measurement system.[8,21] Bilevel native serum internal quality control samples were measured in triplicate and evaluated by Levey-Jennings plots with adjusted Westgard rules to fit a multiplex test to assess assay performance.[22,23] One high-lipoprotein(a) internal quality control sample lot resulted in a mean value (SD) of 89.6 nmol/L (2.7) and an interassay coefficient of variation of 3.0%. This test is validated according to clinical and laboratory standards institute protocols.

Additional technical details of the tests are provided in Table S1. IA-mass relies on serial dilution of a single calibrator; IA-molar and MS use 5 independent calibrators. The immunoassay tests use polyclonal apo(a)-directed antibodies that are reported to detect the repeating KIV2 of apo(a), making their results apo(a) isoform dependent.[24] This effect is more pronounced when combined with a single serially diluted calibrator, as in the case of the IA-mass test.[25,26] The MS test is apo(a) isoform independent by design as the selected peptides are KIV2 independent. Both immunoassay tests require dilution steps (by design to calibrators with nonmatching apo(a) isoforms) if measurements exceed the upper limit of the measuring range, whereas this is less frequently required for the MS test because of its relatively extended measurement range.

Statistical Analysis

For statistical modeling purposes, an apo(a) concentration below the corresponding lower limit of quantification (4.0 mg/dL for IA-mass, 7.0 nmol/L for IA-molar, and 3.8 nmol/L for MS) was set to the midpoint between 0 and the respective lower limit of quantification (ie, 2.0 mg/dL for IA-mass, 3.5 nmol/L for IA-molar, and 1.9 nmol/L for MS-molar). Continuous variables are described by median (quartile 1 and quartile 3); categorical variables are presented as counts and percentages. Comparisons of demographic and baseline characteristics between patients included in or excluded from the analysis cohort were determined by Wilcoxon or χ^2 tests. Relationships between baseline lipoprotein(a) and LDL-C were estimated by Spearman correlations. Distributions of lipoprotein(a) are described by treatment group at baseline, along with the absolute and percentage change from baseline to month 4 (122±28 days) after randomization. The first value was analyzed if a participant had multiple values within the time window.

To facilitate comparisons among measurements of lipoprotein(a) using different units of concentration, baseline values from each analysis method were converted into percentiles. With the MS test selected as the comparison method to which the other 2 assessments were compared, the difference between baseline IA-mass or IA-molar percentile and MS percentile was plotted as a function of MS percentile. In this fashion, positive values indicate overestimation with the comparator method versus MS and negative values indicate underestimation. Because IA-molar and MS share units of measurement, a Bland-Altman plot of lipoprotein(a) concentrations from these tests was also generated, and equivalence was tested by Deming regression using jackknife estimates for standard errors to calculate 95% CIs for the slope and *y* intercept.[27]

Within the placebo group, cumulative incidence of a first MACE and total cardiovascular events per 100 patients through 4 years by continuous baseline IA-mass, IA-molar, and MS concentration percentiles were estimated by natural cubic splines and associated 95% CIs from Poisson regression models. The models included log follow-up time as an offset and baseline LDL-C as a covariate; sensitivity analyses included additional covariates that are associated with risk or lipoprotein(a) concentrations (ie, age, sex, race, body mass index, history of diabetes, and baseline triglycerides). Knots were specified at the 25th, 50th, and 75th percentiles, and Wald tests were used to assess significance of the spline effects. To quantify the incremental value of each test for a MACE prognosis after accounting for LDL-C, absolute and relative integrated discrimination improvement was calculated for IA-mass, IA-molar, and MS natural cubic splines, both for percentiles and in their original units.[28]

Treatment hazard ratios (HRs) by percentiles of the baseline lipoprotein(a) concentrations were assessed by natural cubic splines and associated 95% CIs from proportional hazards models for a first MACE and, for total cardiovascular events, marginal proportional hazards models that allow for a given patient to have multiple events. A robust sandwich variance estimate for the estimated standard error of the log HR was applied to account for the dependence of event times within individual patients. Model covariates, locations of knots, and testing for significance of spline effects followed the specifications for the Poisson regression models described previously.

To further illustrate the relationships between baseline lipoprotein(a) and absolute risk of a first MACE and total cardiovascular events, rates per 100 patient-years of follow-up with corresponding 95% CIs within each treatment group and alirocumab treatment absolute rate reductions with 95% CIs were estimated by baseline lipoprotein(a) quartile of each analytical method by Poisson regression models. Log follow-up time was included in the models as an offset. To assess patterns of risk within the placebo group and heterogeneity in the treatment effects, *P* values were computed for linear trend in the estimated placebo rates and absolute rate reductions across quartiles.

P values <0.05 from 2-sided tests were considered statistically significant. All analyses were conducted according to intention-to-treat, including all patients and events from randomization to the common study end date (November 11, 2017). Analyses were conducted using SAS version 9.4 (SAS Institute).

RESULTS

Participant Characteristics and Relationships Between Baseline Lipoprotein(a) Assessments

A total of 11 970 participants had baseline IA-mass, IA-molar, and MS lipoprotein(a) assessments; of these patients, 11 167 (93%) also had month-4 assessments. Patients from Canada or the United States were overrepresented among the participants included in the analysis cohort, whereas participants from Central and Eastern Europe were overrepresented among the 6954 excluded participants (Table S2). Relative to the excluded participants, included participants had higher lipoprotein(a) IA-mass and apolipoprotein B concentrations and were less likely to have a history of heart failure. Of note,

nearly 90% of participants had been receiving high-intensity statin treatment with 40—80 mg of atorvastatin or 20—40 mg of rosuvastatin at the time of randomization. Characteristics of those included in the analysis cohort are summarized by treatment assignment in Table 1.

Baseline lipoprotein(a) IA-mass, IA-molar, and MS concentrations were highly correlated, with the strongest correlation between IA-molar and MS (r=0.990) and nominally weaker correlations between IA-mass and MS (r=0.967) and IA-mass and IA-molar (r=0.972; Table S3). All 3 measures had modest correlations with LDL-C. As shown in Figure S1, the distributions of lipoprotein(a) IA-mass, IA-molar, and MS concentrations at baseline in the overall analysis cohort were right-skewed. The proportions of patients with values below the lower limit of quantification at baseline were 15.7%, 13.6%, and 5.6% for IA-mass, IA-molar, and MS concentrations, respectively.

The ratios of baseline IA-molar to IA-mass and MS to IA-mass were not constant across the range of lipoprotein(a) concentration (Figure S2). Examination of the baseline molar:mass ratios across quartiles also indicated variability across the distributions (Table 2), ranging from ≈1.8 nmol/10 mg in the first quartile to ≈2.5 nmol/10 mg in the fourth quartile. Thus, a fixed molar/mass concentration ratio was deemed inappropriate for comparison of tests with mass versus molar concentration readouts. Instead, this finding supported the use of ordinal ranking in percentiles for comparative modeling. Plots of baseline MS percentile versus the difference in percentile between IA-mass and MS or IA-molar and MS revealed generally greater differences between the IA-mass and MS percentiles (Figure 1A) than between the IA-molar and MS percentiles (Figure 1B). Overall, IA-mass overestimated or underestimated concentration by ≥20 percentiles on the lipoprotein(a) distributions compared with MS in 1.5% of patients (overestimation 0.4% and underestimation 1.1%), whereas IA-molar concentration overestimated or underestimated concentration by ≥20 percentiles on the lipoprotein(a) distributions relative to MS in 0.5% of patients (overestimation 0.1% and underestimation 0.4%). Using a threshold of ±10 percentiles, IA-mass overestimated or underestimated lipoprotein(a) concentration by MS in 11.9% of patients, while IA-molar overestimated or underestimated concentration by MS in 2.2% of patients.

A Bland-Altman analysis of baseline IA-molar versus MS concentrations in their original molar units is presented in Figure S3, with positive differences indicating patients with higher IA-molar concentrations and negative differences indicating patients with higher MS concentrations. The overall bias was small (3.8 nmol/L), but 95% limits of agreement were relatively wide (—50.7 to 58.3 nmol/L). Among 5993 patients in the placebo group, there were 108 (1.8%) above, 5734 (95.7%) within, and 151 (2.5%) below the 95% limits of agreement. In a Deming regression analysis, the 95% CI for the

Table 1. Demographic and Baseline Clinical Characteristics of the Analysis Cohort by Treatment Assignment

Characteristics	Alirocumab (n=5977)	Placebo (n=5993)
Age, y	58 (51, 65)	58 (52, 65)
Female sex	1421 (23.8)	1438 (24.0)
Body mass index, kg/m²	28.0 (25.4, 31.2)	28.0 (25.2, 31.2)
Systolic blood pressure, mm Hg	127 (118, 138)	126 (116, 138)
Revascularization for index acute coronary syndrome	4342 (72.6)	4423 (73.8)
Race and ethnicity		
White	4786 (80.1)	4836 (80.7)
Asian	721 (12.1)	721 (12.0)
Black	168 (2.8)	157 (2.6)
Other	302 (5.1)	279 (4.7)
Region of enrollment		
Central and Eastern Europe	1159 (19.4)	1159 (19.3)
Western Europe	1406 (23.5)	1414 (23.6)
Canada or United States	1405 (23.5)	1409 (23.5)
Latin America	799 (13.4)	795 (13.3)
Asia	690 (11.5)	691 (11.5)
Rest of world	518 (8.7)	525 (8.8)
Baseline laboratory data		
Lipoprotein(a) immunoassay-based mass concentration, mg/dL	21.1 (6.9, 58.7)	22.4 (6.8, 61.1)
Lipoprotein(a) immunoassay-based molar concentration, nmol/L	44.4 (13.3, 150.6)	45.4 (13.1, 157.3)
Lipoprotein(a) mass spectrometry–based concentration, nmol/L	41.1 (14.4, 139.9)	43.0 (14.3, 146.3)
Low-density lipoprotein cholesterol, mg/dL	86.5 (72.6, 103.9)	86.5 (73.0, 105.0)
Apolipoprotein B, mg/dL	80.0 (69.0, 93.0)	80.0 (69.0, 94.0)
Non—high-density lipoprotein cholesterol, mg/dL	115.0 (98.8, 137.0)	115.8 (99.2, 138.0)
High-density lipoprotein cholesterol, mg/dL	42.1 (36.0, 50.0)	42.1 (36.0, 49.8)
Triglycerides, mg/dL	130.1 (94.7, 182.0)	131.9 (95.1, 185.4)
High-sensitivity C-reactive protein, mg/dL	0.17 (0.08, 0.37)	0.17 (0.08, 0.40)
Estimated glomerular filtration rate, mL/min/1.73 m²	77.9 (67.2, 90.1)	78.2 (67.4, 90.5)
Hemoglobin A1c, %	5.8 (5.5, 6.3)	5.8 (5.5, 6.4)
Medical history before index acute coronary syndrome		
Hypertension	3807 (63.7)	3739 (62.4)
Diabetes	1746 (29.2)	1790 (29.9)
Current tobacco smoker	1436 (24.0)	1457 (24.3)
Myocardial infarction	1136 (19.0)	1185 (19.8)
Percutaneous coronary intervention	1126 (18.8)	1128 (18.8)
Coronary artery bypass grafting	374 (6.3)	356 (5.9)

(*Continued*)

Table 1. Continued

Characteristics	Alirocumab (n=5977)	Placebo (n=5993)
Stroke	187 (3.1)	188 (3.1)
Peripheral artery disease	240 (4.0)	251 (4.2)
Congestive heart failure	544 (9.1)	567 (9.5)
Chronic obstructive pulmonary disease	263 (4.4)	272 (4.5)
Malignancy	207 (3.5)	206 (3.4)
Time from index acute coronary syndrome to randomization, mo	2.7 (1.7, 4.5)	2.7 (1.7, 4.5)
Background lipid lowering therapy at randomization		
High-dose atorvastatin/rosuvastatin	5287 (88.5)	5360 (89.4)
Low- or moderate-dose atorvastatin/rosuvastatin	475 (7.9)	425 (7.1)
No statin or other lipid-lowering therapy	75 (1.3)	78 (1.3)
Only lipid-lowering therapy other than statin	127 (2.1)	107 (1.8)
Other statin	13 (0.2)	23 (0.4)

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

estimated slope excluded 1 and the 95% CI for *y* intercept excluded 0, indicating that the 2 testing methods are not equivalent (Figure S4). Results were essentially identical from sensitivity analyses excluding observations below the lower limit of quantification on the MS test or on either test.

Baseline Lipoprotein(a), Risk of First MACE and Total Cardiovascular Events in the Placebo Group, and Effects of Alirocumab

Patients in the analysis cohort were followed for cardiovascular events for a median of 2.9 years (interquartile range, 2.4–3.5). A first MACE event was experienced by 725 patients in the placebo group and 604 patients in the alirocumab group; the types and counts of total cardiovascular events and noncardiovascular deaths are presented by treatment group in Table S4. The first MACE alirocumab:placebo treatment HR (95% CI) for patients in the analysis cohort was 0.83 (0.74, 0.92), compared with 0.85 (0.78, 0.93) for the entire study population. Likewise, the corresponding results for total cardiovascular events were 0.83 (0.75, 0.92) for included patients and 0.85 (0.78, 0.93) for the entire study population.

In the placebo group, baseline IA-mass, IA-molar, and MS lipoprotein(a) concentrations had significant and nearly identical relationships with cumulative incidence of a first MACE through 4 years; spline P=0.0001, 0.0002, and 0.0003 for IA-mass, IA-molar, and MS, respectively, adjusted for LDL-C, with significance essentially unchanged in sensitivity analyses featuring adjustment by additional baseline characteristics (Figure 2A). Differences in the cumulative incidence rate point estimates (adjusted for LDL-C) from the 10th to the 100th percentiles were small, differing by ≤0.4% among the 3 tests (Table S5). In addition, the confidence boundaries around the splines were nearly superimposable, indicating similar population precision of the estimated lipoprotein(a)-associated risk with each of the 3 measurement techniques. These relationships were not modified by baseline levels of LDL-C (spline × LDL-C interaction P>0.10 for all 3 measurements). Furthermore, the alirocumab relative treatment effect was nearly identical for each of the 3 lipoprotein(a) assessments, with point estimates of the HRs differing by ≤0.07 among the tests (Table S6). There was evidence of effect modification across lipoprotein(a) IA-mass percentiles, but not IA-molar or MS percentiles (spline interaction P=0.047, 0.21, and 0.16 for IA-mass, IA-molar, and MS, respectively, when adjusted for LDL-C; Figure 2B). With each test, there was nominally less risk reduction by alirocumab on a relative scale at lower lipoprotein(a) percentiles than at higher lipoprotein(a) percentiles. Corresponding findings were similar for total cardiovascular events and are presented in Figure S5, Table S5, and Table S6.

By integrated discrimination improvement analyses within the placebo group, splines of baseline IA-mass, IA-molar, and MS, both for percentiles and in original units, were incrementally prognostic for a first MACE after accounting for LDL-C (Table S7). Participants who experienced an event during follow-up had an ≈1.0% higher expected risk of an event by 4 years compared with patients who did not have an event on the basis of baseline LDL-C concentration; this expected risk differential increased to ≈1.4% after additionally accounting for baseline lipoprotein(a). All 3 lipoprotein(a) tests were significant at P<0.05 for incremental integrated discrimination improvement after accounting for LDL-C.

Rates of a first MACE and total cardiovascular events and absolute rate reductions with alirocumab stratified by baseline lipoprotein(a) IA-mass, IA-molar, and MS concentration quartiles are shown in Figure 3 and Figure S6. Overall, the rate of first MACE rate (95% CI) was 3.6 (3.3, 3.9) events per 100 patient-years in the alirocumab group and 4.4 (4.1, 4.7) events per 100 patient-years in the placebo group, with an absolute rate reduction (95% CI) with alirocumab of 0.8 (0.3, 1.2) events per 100 patient-years. The corresponding results for total cardiovascular events were 10.6 (10.1, 11.0), 12.8 (12.2, 13.3), and 2.2 (1.5, 2.9). Within the placebo group, the event rates increased monotonically from the lowest to the highest quartiles, with P_{trend}<0.0001 for all 3 tests for a first MACE and total cardiovascular events. Absolute rate reductions with alirocumab also generally increased from the lowest to the highest quartiles, with significant linear trends in most cases.

Table 2. Baseline, Month 4, and Absolute Change From Baseline to Month 4 in Lipoprotein(a) Concentrations by Treatment Group Overall and by Biomarker-Specific Baseline Quartile

	Baseline		Month 4		Absolute change baseline to month 4		Percentage change baseline to month 4	
Characteristics	Alirocumab	Placebo	Alirocumab	Placebo	Alirocumab	Placebo	Alirocumab	Placebo
IA–mass concentration, mg/dL								
Overall	n=5977; 21.1 (6.9, 58.7)	n=5993; 22.4 (6.8, 61.1)	n=5587; 13.3 (2.0, 46.3)	n=5580; 20.7 (6.3, 59.5)	n=5587; −5.1, (−13.4, 0)	n=5580; 0 (−4.8, 2.8)	n=5587; −23.6 (−46.5, 0)	n=5580; 0 (−16.9, 11.8)
Quartile 1 (<6.9 mg/dL)	n=1486; 2.0 (2.0, 4.9)	n=1503; 2.0 (2.0, 4.9)	n=1393; 2.0 (2.0, 2.0)	n=1401; 2.0 (2.0, 5.1)	n=1393; 0 (−2.0, 0)	n=1401; 0 (0, 0.2)	n=1393; 0 (−30.8, 0)	n=1401; 0 (0, 4.1)
Quartile 2 (6.9 to <21.8 mg/dL)	n=1543; 12.6 (9.5, 16.4)	n=1446; 12.7 (9.6, 16.5)	n=1441; 6.7 (4.5, 10.8)	n=1360; 11.9 (8.0, 16.8)	n=1441; −5.2 (−8.0, −2.5)	n=1360; −0.5 (−3.0, 2.3)	n=1441; −41.7 (−65.9, −22.4)	n=1360; −4.1 (−25.3, 18.5)
Quartile 3 (21.8 to <60.0 mg/dL)	n=1491; 38.3 (29.3, 48.8)	n=1508; 38.6 (28.8, 48.5)	n=1390; 28.0 (18.6, 40.2)	n=1389; 37.8 (26.3, 50.0)	n=1390; −9.8 (−16.0, −3.3)	n=1389; −1.0 (−7.1, 5.5)	n=1390; −25.9 (−43.6, −8.7)	n=1389; −3.0 (−19.5, 14.8)
Quartile 4 (≥60.0 mg/dL)	n=1457; 90.1 (72.9, 117.0)	n=1536; 92.1 (73.6, 120.0)	n=1363; 71.7 (55.6, 96.1)	1430; 87.9 (69.0, 118.0)	n=1363; −20.0 (−33.7, −8.1)	n=1430; −5.0 (−17.2, 7.8)	n=1363; −22.0 (−33.8, −9.5)	n=1430; −5.2 (−17.7, 8.1)
IA–molar concentration, nmol/L								
Overall	n=5977; 44.4 (13.3, 150.6)	n=5993; 45.4 (13.1, 157.3)	n=5587; 24.4 (7.1, 119.4)	n=5580; 42.3 (11.9, 151.4)	n=5587; −11.9 (−32.0, −2.0)	n=5580; −0.1 (−9.4, 4.2)	n=5587; −26.8 (−49.0, −5.0)	n=5580; −0.1 (−6.0, 8.8)
Quartile 1 (<13.2 nmol/L)	n=1484; 3.5 (3.5, 9.6)	n=1505; 3.5 (3.5, 9.6)	n=1389; 3.5 (3.5, 3.5)	n=1408; 3.5 (3.5, 9.6)	n=1389; 0 (−4.3, 0)	n=1408; 0 (0, 0.2)	n=1389; 0 (−53.9, 0)	n=1408; 0 (0, 2.1)
Quartile 2 (13.2 to <45.0 nmol/L)	n=1520; 23.2 (17.4, 32.5)	n=1475; 25.0 (18.0, 33.1)	n=1421; 12.1 (8.5, 18.2)	n=1378; 23.4 (15.6, 32.6)	n=1421; −10.8 (−16.3, −5.8)	n=1378; −1.4 (−5.6, 3.3)	n=1421; −46.3 (−64.8, −28.0)	n=1378; −5.9 (−24.1, 14.0)
Quartile 3 (45.0 to <153.8 nmol/L)	n=1519; 85.5 (60.2, 123.1)	n=1471; 87.0 (61.1, 121.1)	n=1425; 60.3 (37.6, 94.8)	n=1356; 82.7 (56.5, 119.7)	n=1425; −24.1 (−36.7, −11.2)	n=1356; −3.5 (−15.5, 9.7)	n=1425; −29.0 (−45.5, −13.1)	n=1356; −4.4 (−18.4, 11.9)
Quartile 4 (≥153.8 nmol/L)	n=1454; 227.5 (186.0, 295.5)	n=1542; 225.3 (186.8, 301.5)	n=1352; 184.5 (146.1, 235.2)	n=1438; 217.5 (176.2, 286.3)	n=1352; −49.5 (−81.3, −22.2)	n=1438; −9.1 (−35.0, 14.3)	n=1352; −21.6 (−31.5, −10.1)	n=1438; −4.1 (−14.3, 5.6)
MS concentration, nmol/L								
Overall	n=5977; 41.1 (14.4, 139.9)	n=5993; 43.0 (4.3, 146.3)	n=5587; 25.8 (8.3, 110.3)	n=5580; 39.4 (13.1, 138.5)	n=5587; −10.4 (−28.2, −2.7)	n=5580; −1.2 (−9.4, 3.8)	n=5587; −27.4 (−46.5, −9.5)	n=5580; −3.4 (−18.5, 10.5)
Quartile 1 (<14.3 nmol/L)	n=1481; 7.0 (3.9, 10.5)	n=1495; 7.0 (3.9, 10.6)	n=1385; 4.3 (1.9, 7.0)	n=1400; 6.7 (2.9, 10.7)	n=1385; −2.0 (−4.4, 0)	n=1400; 0 (−1.9, 1.8)	n=1385; −25.9 (−56.2, 0)	n=1400; 0 (−20.4, 22.8)
Quartile 2 (14.3 to <42.2 nmol/L)	n=1534; 23.7 (18.5, 31.2)	n=1475; 24.5 (18.6, 31.7)	n=1434; 14.2 (10.0, 19.6)	n=1379; 23.1 (17.0, 31.2)	n=1434; −9.6 (−14.1, −5.0)	n=1379; −1.3 (−5.3, 3.4)	n=1434; −40.6 (−55.8, −22.3)	n=1379; −5.6 (−23.1, 12.7)
Quartile 3 (42.2 to <143.1 nmol/L)	n=1511; 80.6 (57.5, 112.8)	n=1481; 81.3 (57.3, 111.1)	n=1415; 57.2 (36.7, 89.5)	n=1370; 77.3 (52.5, 108.8)	n=1415; −21.1 (−32.5, −9.6)	n=1370; −4.3 (−14.2 7.4)	n=1415; −27.3 (−42.4, −11.9)	n=1370; −5.1 (−17.9, 8.7)
Quartile 4 (≥143.1 nmol/L)	n=1451; 219.1 (173.6, 284.4)	n=1542; 218.1 (175.8, 292.3)	n=1353; 176.6 (1381, 234.3)	n=1431; 211.0 (164.5, 279.2)	n=1353; −45.6 (−72.6, −18.0)	n=1431; −10.4 (−36.0, 13.0)	n=1353; −20.4 (−30.7, −8.9)	n=1431; −4.6 (−14.9, 5.6)

IA indicates immunoassay; and MS, mass spectrometry. Values are medians (interquartile range).

Effect of Alirocumab and Placebo on Lipoprotein(a) Concentrations

Baseline, month 4, and absolute and percentage change from baseline to month 4 in lipoprotein(a) concentration are summarized by treatment group in Table 2, overall, and by baseline quartiles. Overall, the median change in lipoprotein(a) within the alirocumab group was −5.1 mg/dL, −11.9 nmol/L, and −10.4 nmol/L for IA-mass, IA-molar, and MS concentrations, respectively; overall median changes in the placebo group were minimal. The magnitude of absolute change in the alirocumab group was dependent on baseline levels, in part because of fractions of patients in the lowest quartiles below or near the lower limits of quantification. In contrast, the magnitude of percent change in the alirocumab group was consistently greatest in the second quartile, with somewhat lower reductions in the upper quartiles. Histograms of absolute change from baseline to month 4 for the alirocumab group are presented in Figure S7.

DISCUSSION

It was previously reported from the ODYSSEY OUTCOMES trial that among patients with recent ACS who were receiving intensive or maximally tolerated statin treatment, baseline lipoprotein(a) mass measured by immunoassay was prognostic for a first MACE and total cardiovascular events. In a subset of the ODYSSEY OUTCOMES population, the current results demonstrate that in direct comparisons of mass and molar concentra-

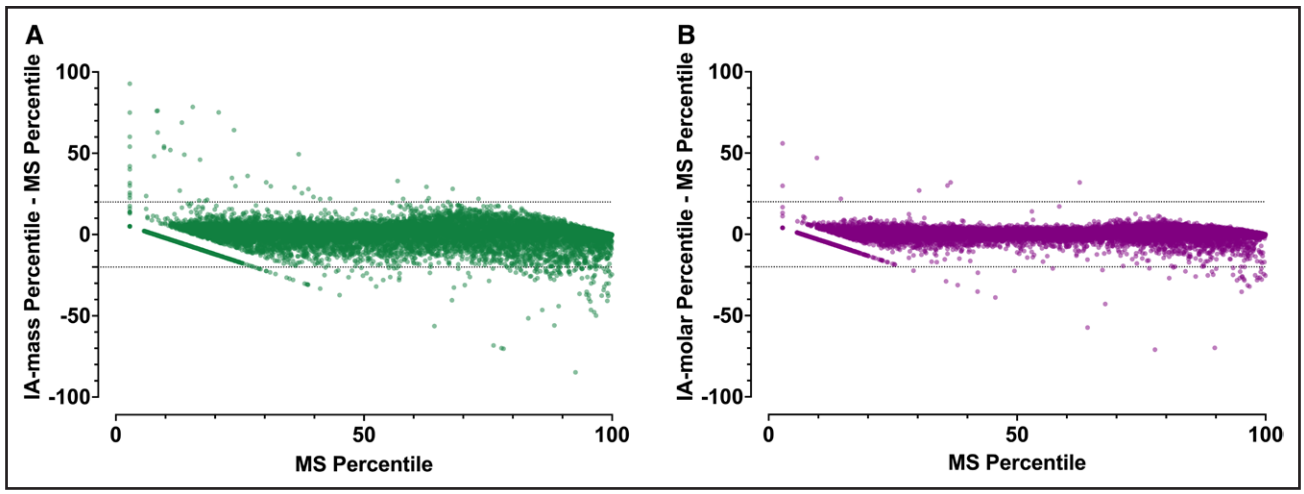

Figure 1. Baseline lipoprotein(a) MS concentration percentile vs difference in IA-mass and MS concentration percentiles and difference in IA-molar and MS concentration percentiles.
Scatterplots of baseline lipoprotein(a) mass spectrometry (MS) concentration percentile vs difference in immunoassay-based mass test (IA-mass; **A**) and MS concentration percentiles and MS concentration percentile vs difference in immunoassay-based molar test (IA-molar; **B**) and MS concentration percentiles. IA-mass overestimated and underestimated concentration by ≥20 (≥10) percentiles compared with MS in 0.4% (3.9%) and 1.1% (8.0%) of patients, respectively. IA-molar overestimated and underestimated concentration by ≥20 (≥10) percentiles relative to MS in 0.1% (0.2%) and 0.4% (2.0%) of patients, respectively.

tions by commercial immunoassay tests, as well as molar concentration from a noncommercial MS method, all 3 tests were similarly prognostic for cardiovascular events within the placebo group, with higher concentrations translating to higher risk. In addition, these relationships were not modified by LDL-C levels.

Moreover, the current findings indicate numerically less relative treatment benefit of alirocumab on cardiovascular events at lower lipoprotein(a) concentrations, as measured by each of the 3 lipoprotein(a) tests. These findings are consistent with previous findings with lipoprotein(a) measurement by IA-mass in the full trial population.[4,14] Baseline lipoprotein(a) concentrations by each lipoprotein(a) test were strongly and similarly predictive of absolute treatment benefits, with numerically lower absolute benefit at lower lipoprotein(a) concentrations and greater absolute benefit at higher concentrations, as determined by each lipoprotein(a) test. In sum,

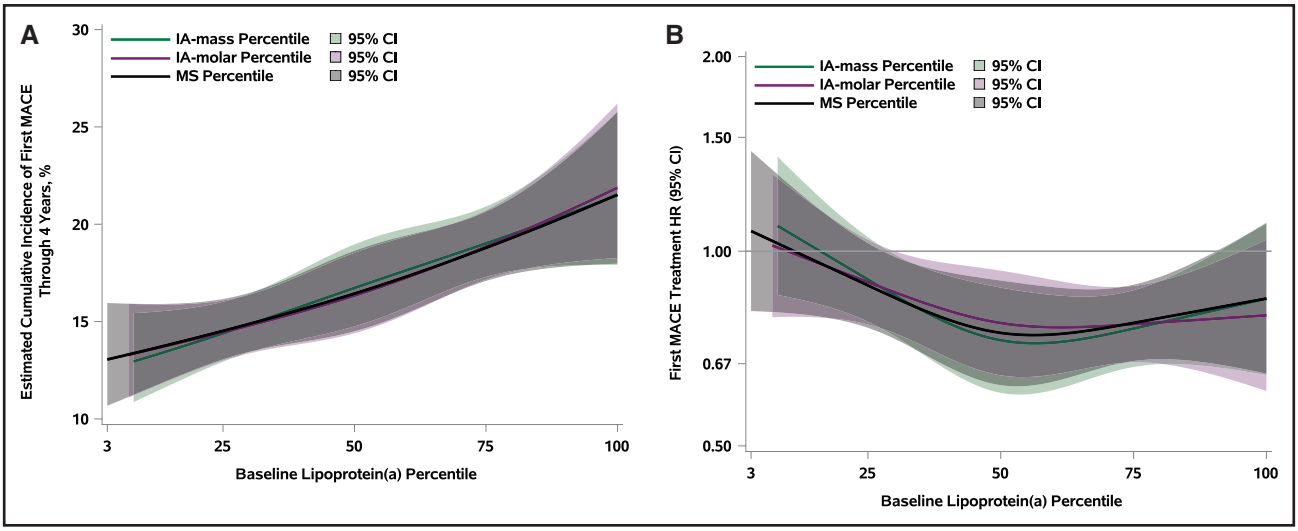

Figure 2. Spline analysis of risk of first MACE event by baseline lipoprotein(a) concentration percentiles.
A, Cumulative incidence of first major adverse cardiovascular event (MACE) through 4 years within the placebo group. **B**, First MACE alirocumab:placebo hazard ratio. Splines are natural cubic with knots specified at the 25th, 50th, and 75th percentiles and reflect adjustment for baseline low-density lipoprotein cholesterol (LDL-C). Within the placebo group, spline P=0.0001, 0.0002, and 0.0003 for immunoassay-based mass test (IA-mass), immunoassay-based molar test (IA-molar), and mass spectrometry (MS), respectively, adjusted for LDL-C; spline P<0.0001, P<0.0001, and P=0.0001 for IA-mass, IA-molar, and MS, respectively, adjusted for LDL-C, age, sex, race, body mass index, history of diabetes, and triglycerides; all spline × LDL-C interactions P>0.10. Treatment × spline interaction P=0.0474, 0.21, and 0.16 for IA-mass, IA-molar, and MS, respectively, adjusted for LDL-C. HR indicates hazard ratio.

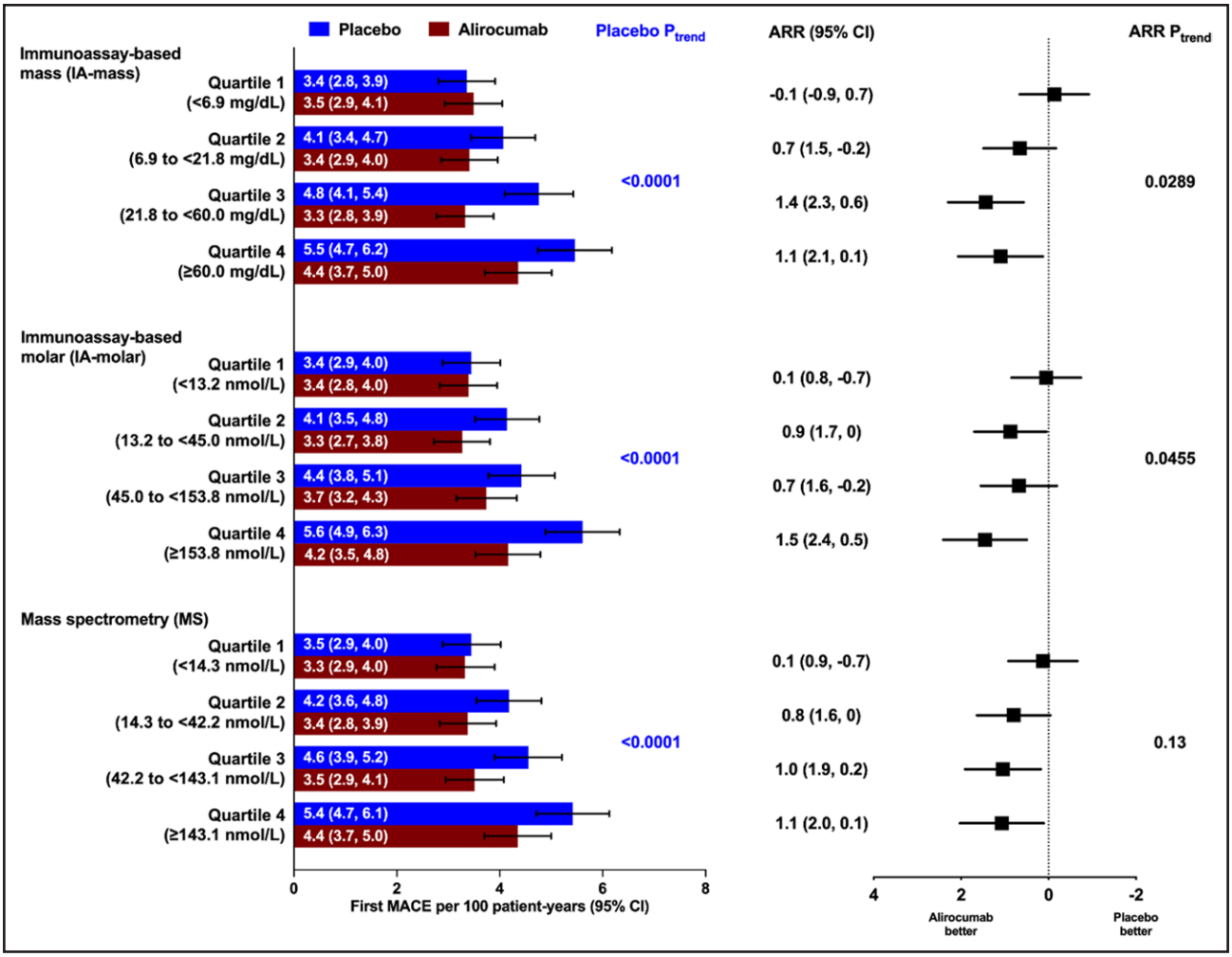

Figure 3. Rates of first MACE and absolute rate reductions with alirocumab stratified by baseline lipoprotein(a) IA-mass, IA-molar, and mass spectrometry concentration quartiles.

Absolute risk reductions (ARRs) reflect number of first major adverse cardiovascular events (MACEs) avoided with alirocumab treatment per 100 patient-years. IA-mass indicates immunoassay-based mass test; and IA-molar, immunoassay-based molar test.

the current findings indicate that at the cohort level, high lipoprotein(a) concentration by each of the 3 measurement methods identifies high risk and an expected large absolute benefit from treatment with a PCSK9 (proprotein convertase subtilisin/kexin type 9) inhibitor. In addition, relative reductions of lipoprotein(a) concentration by alirocumab were generally consistent across the 3 measurement techniques, both overall and by baseline quartile.

Although a previous study related different lipoprotein(a) measures with risk of death,[29] only a subset of the analysis cohort had lipoprotein(a) assessed by >1 method, and combined results of the tests were related to outcomes. In contrast, our study applied 3 lipoprotein(a) tests in all patients, comprising a large, high-risk cohort, allowing for a comparison of their individual relationships with cardiovascular events. The design of the ODYSSEY OUTCOMES trial was a suitable setting to explore this issue because patients were at high risk for events resulting from a recent ACS, but the duration between the index ACS and randomization (1–12 months) minimized any acute-phase effect on lipoprotein(a) or LDL-C concentrations, as previously demonstrated by stable measurements in the placebo group at multiple time points.[16] In addition, converting the units of measurements of each test to percentiles not only facilitated comparisons of 3 tests that comprised the current results but could provide an approach to compare some previous studies that reported on relationships between lipoprotein(a) and cardiovascular events using different assays or units of measurement.

At an individual patient level, all 3 measurement techniques were closely correlated. However, the relationship between baseline IA-molar and MS was nominally stronger than that for IA-mass and MS. Considering MS the gold standard method, 0.5% patients differed by at least 20 percentiles on the lipoprotein(a) distributions with IA-molar compared with 1.5% with IA-mass, implying slightly greater accuracy of IA-molar than IA-mass. In addition, Deming regression analyses indicated that

the IA-molar and MS tests are not equivalent, even after accounting for the different lower limits of quantification. Whether this evidence of laboratory nonequivalence indicates a difference in clinical usefulness of IA-molar and MS is uncertain. Moreover, none of these patient-level differences translated into heterogeneity in the cohort-level relationships of IA-mass, IA-molar, and MS with cardiovascular events.

There were a priori reasons to expect greater differences among the 3 evaluated lipoprotein(a) tests than were identified. First, the apo(a) component of lipoprotein(a) is heterogenous[19,25] because of size polymorphism from varying numbers of kringle IV type 2 repeats and varying degrees of N- and O-glycosylation among individuals. Because of an inverse association of number of kringle IV type 2 repeats with lipoprotein(a) particle concentration, polyclonal immunoassays recognizing epitopes on apo(a) may tend to underestimate high lipoprotein(a) concentrations and overestimate low lipoprotein(a) concentrations in the setting of small or large isoform composition, respectively.[7] This effect is mitigated by using calibrators with varying isoforms.[30] Quantification of lipoprotein(a) by MS through its proteotypic peptides offers a theoretical advantage over immunoassays by directly measuring apo(a) through its specific peptides, independent of kringle size.[19] Second, there is a strong rationale to measure lipoprotein(a) in molar rather than mass concentration if the atherogenicity of lipoprotein(a) is related to particle number, rather than size. If so, one would expect greater fidelity of lipoprotein(a) molar than mass concentration to the risk of a MACE. International consensus statements support expressing lipoprotein(a) concentration in molar units.[7,31] However, despite all these considerations, the IA-mass, IA-molar, and MS lipoprotein(a) tests used in this analysis performed almost indistinguishably in terms of their associations with MACE risk on a cohort level, with generally modest differences in percentile classification of individuals.

A limitation of the analyses is that the analysis cohort was a nonrandom subset of the study population, as indicated by differences in baseline characteristics between included and excluded patients. However, overall relative treatment benefits of alirocumab on a first MACE and total cardiovascular events were similar in the current analysis cohort and in the entire study population, and the relationship between lipoprotein(a) by IA-mass and cardiovascular events previously reported for the entire study population is similar to that for the current subset. Therefore, it is reasonable to expect that the current IA-molar and MS findings would extend to the full study population. In addition, comparisons of absolute differences in concentration between methods was possible for IA-molar and MS, which reported results in the same units. However, it was not possible to determine absolute differences in concentration with IA-mass versus the molar methods because of different units of measurement and because the ratio of mass and molar concentrations did not appear to be fixed, varying from 1.8 to 2.5 nmol/10 mg across. Previous studies have also compared lipoprotein(a) tests with different units of measurement using an ordinal (percentile) approach,[32] and this conversion facilitates comparisons across studies.

Conclusions

In patients with recent ACS receiving high-intensity or maximum-tolerated statin treatment, 2 commercially available immunoassay-based tests and 1 MS-based test for lipoprotein(a) were similarly prognostic for a first MACE and total cardiovascular events in patients assigned to placebo and similarly predictive of reductions of these outcomes with alirocumab at the cohort level. Values of the MS-based molar test were more closely correlated with the results from the commercial immunoassay-based molar test than those from the immunoassay-based mass test, although direct comparisons of values from the 2 molar tests indicated that the tests were not fully equivalent. Taken together, given their similar relationships with cardiovascular events, the 3 tests can provide comparable clinical use in terms of information for cardiovascular risk assessment.

ARTICLE INFORMATION

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Supplemental Material

ODYSSEY OUTCOMES Committees and Investigators
Tables S1—S7
Figures S1—S7

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