

Research Article

Confluence of Mendelian Randomization with Clinical Trials as To Very Low Levels of LDL Cholesterol

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Received: 20 December, 2023

Accepted: 23 January, 2024

Published: 29 January 2024

Abstract:

Atherosclerotic disease and its clinical manifestations, including acute myocardial infarction and ischemic stroke, are the leading cause of morbidity and mortality worldwide. Among the atherogenic risk factors, the most well-documented and the one that determines a causal relationship with atherosclerotic disease is low-density lipoprotein cholesterol (LDL-C) values. It is essential to identify LDL-C as a therapeutic target to reduce cardiovascular risk, especially after the emergence of new drugs that further reduce LDL-C levels, with additional risk reduction. Our objective is to demonstrate that LDL-C is an important atherogenic risk factor and that any mechanism of reduction of plasma LDL-C concentrations reduces the risk of events proportional to the absolute reduction of LDL-C and the cumulative time of exposure to it. Mendelian randomization studies: even though the association between LDL-C and cardiovascular risk is well demonstrated and reproducible in meta-analyses of prospective cohort studies, such studies are not randomized and therefore susceptible to biases such as reverse causality and confounders. Mendelian randomization is used especially when randomized controlled trials to examine causality are not feasible. This method will assess the causal relationship between a modifiable exposure, or risk factor, and a clinically relevant outcome. The confluence of the results of both Mendelian randomization and clinical trials leads to the same proposition: the lower the LDL-C, the better for the prevention of atherosclerotic lesions.

Key words: LDL cholesterol, Mendelian randomization, Clinical trials, Cardiovascular diseases, Statins, PCSK9 inhibitors.

Introduction:

Even though the association between low-density lipoprotein cholesterol (LDL-C) and cardiovascular risk is well demonstrated and reproducible in meta-analyses of prospective cohort studies, such studies are not randomized and therefore susceptible to biases such as reverse causality and confounding factors. Mendelian randomization is used especially when randomized controlled trials to examine causality are not feasible. This method will assess the causal relationship between a modifiable exposure, or risk factor, and a clinically relevant outcome. Using genetic variants as instrumental variables for the exposure tested, the alleles that determine these variants are randomly allocated and are not subject to reverse causality. This, together with the wide availability of genetic associations for screening for appropriate instrumental variables, makes Mendelian randomization an efficient approach for the shortest time and reduced cost, compared to a randomized trial. An observed association between the genetic instrumental variable and the outcome supports the hypothesis that the exposure in question is causally related to the outcome.

To increase statistical power, several variants associated with the same exposure can be combined into a genetic score. If only independently inherited (unlinked) variants are included, the genetic score of the instrumental variable can be used to assess the dose-dependent effect of exposure on an outcome, thereby strengthening inferences about the causal relationship between exposure and outcome [1].

By the process of Mendelian randomization, numerous variants in multiple genes associated with low LDL-C levels are randomly inherited [2]. Inheriting an allele that causes LDL-C lowering is analogous to randomly allocating that allele to the cholesterol-lowering drug treatment "arm" and the other allele to the control "arm." Comparing the risk of cardiovascular events in the groups with and without these variants establishes the causal effect of risk reduction with lower LDL-C levels, in a manner analogous to randomized trials [3]. In this context, individuals who inherit the 46 L allele of the PCSK9 (proprotein convertase subtilisin/kexin type 9) gene with loss of function of this protein, have lifelong exposure to lower LDL-C values, resulting in a reduced risk of coronary heart disease,

even higher than expected [4]. Subsequent studies of this and other polymorphisms report similar, although less impactful results [5].

For an estimate of the association between long-term exposure to lower LDL-C values and risk of coronary heart disease, meta-analyses evaluated the risk-reducing impact of 9 single nucleotide polymorphisms (SNPs) in 6 genes related to lower LDL-C values. The percentage reduction in LDL-C values according to each inherited variant and the impact on cardiovascular risk has been shown to be a continuum of risk reduction, dependent on the absolute reduction in LDL-C levels and the lifetime risk of ischemic events. In that same study, long-term exposure to lower LDL-C and the effect of lowering LDL-C during treatment with a statin started late were compared. The combination of 9 polymorphisms in 6 different genes related to LDL-C reductions, randomly allocated from birth, demonstrated a three-fold greater decrease in the risk of coronary artery disease (CAD), per unit of LDL-C reduction, than observed during treatment with a statin initiated in adulthood. Exposure from birth to reductions of 38.7 mg/dl in LDL-C was associated with a 55% reduction in the risk of CAD. Thus, treatment with a statin initiated late would require a three-fold reduction in LDL-C values (3 mmol/l or 116 mg/dl) to achieve the same magnitude of risk reduction [6].

Meta-analyses have confirmed that a dozen variants in more than 50 genes associated with lower LDL-C levels correspond to a lower risk of CAD, confirming the causal relationship between LDL-C and CAD [7].

In more than 300,000 participants, including 80,000 cases of chronic CAD, evidence that reduced LDL-C values are causally associated with reduced risk of cardiovascular events, and that this effect is independent of the mechanism by which LDL-C is reduced, has been observed in Mendelian randomization meta-analyses [8].

The clinical implications of randomization studies demonstrate that prolonged and early exposure to lower LDL-C levels is associated with substantially greater reduction in the risk of coronary heart disease. This explains, to a large extent, the residual risk of coronary events present even in individuals treated with statins or other lipid-lowering therapies. The reduction of LDL-C if started late, after the development of atherosclerosis, partially reduces the risk of cardiovascular events. Therefore, Mendelian randomization studies, in addition to implications for primary prevention, identify therapeutic targets that contribute to the development of new therapeutics.

Experimental, genetic, Mendelian randomization, and prospective studies have built a line of evidence that, over decades, has supported the lipid hypothesis. During this period, the need to reduce cholesterol became evident. Clinical trials of dietary intervention in the 1960s had limited efficacy and poor adherence. Consequently, interest in pharmacological approaches began as early as the 1950s and eventually led to the discovery of statins by Dr. Akira Endo in 1976 [9].

LDL-C reduction has been the cornerstone in managing atherosclerotic cardiovascular disease risk for more than three decades [8]. American, European and Brazilian [10,11]

guidelines have outlined algorithms for quantifying cardiovascular risk according to LDL-C values and their progressive reduction. In this context, statins are considered the drugs of choice for cholesterol reduction and their main effect is the lowering of LDL-C, through the inhibition of the limiting enzyme in cholesterol synthesis, 3-hydroxy-3-methylglutaryl coenzyme A (HMGCoA) reductase, which increases the expression of LDL-C receptors and removal of LDL-C particles from the circulation, reducing your serum levels.

The most robust clinical evidence that elevated plasma LDL-C values are considered causal predictors of coronary heart disease, and that plasma reduction of this lipoprotein reduces the risk of events, comes from randomized intervention studies with statins.

In a Cholesterol Treatment Trialists (CTT) meta-analysis of individual participant data from 26 statin studies, including nearly 170,000 subjects, a proportional reduction of 22% in the risk of major cardiovascular events per 40 mg/dL drop in LDL-C values was demonstrated over an average of five years of treatment. The effect was smaller in the first year, followed by a proportional reduction in events of 22-24% by a 40 mg decrease in LDL-C [12]. The magnitude of this effect was independent of LDL-C levels, similar in individuals with or without cardiovascular disease at baseline, and in all subgroups. The evidence of benefit from the use of statins in reducing the risk of major cardiovascular events is proportional to the absolute magnitude of the LDL-C reduction.

Meta-analyses have shown that LDL-C concentrations used for inclusion in studies have been increasingly lower, ranging from 155 mg/dl LDL-C in the initial studies with statins to LDL-C values below 130 mg/dl with more potent statins, to LDL-C concentrations below 70 mg/dl with combination therapy, and a substantial number of patients have been exposed to LDL-C levels below 50 mg/dL [8,13,14]. In terms of benefit, there was a gradual decrease in the risk of arterial cardiovascular disease, as LDL-C was reduced, and the 'rule of thumb' is that for every 40 mg of LDL-C reduction, there is a 22% reduction in relative risk of cardiovascular events [12]. As evidence of "lower is better," LDL-C targets have evolved, and studies that have influenced increasingly aggressive LDL-C target recommendations for risk reduction have included trials of statins versus placebo, more potent versus less potent statins, and statin monotherapy versus combination treatment. Although no specific LDL-C targets have been tested, statins were the drugs that inaugurated one of the pillars of lipid-lowering therapy: the lower the LDL-C values, the greater the reduction in cardiovascular risk, with no threshold below which a further reduction in LDL-C does not lead to incremental benefits [15].

Evidence that the reduction in cardiovascular risk is progressive and proportional to the linear reduction in LDL-C values, with no clear threshold of what the reduction limit is [12], supported the way in which further decreases in LDL-C values could prevent cardiovascular events. In this context, Mendelian randomization studies with genetic variants that lower LDL-C from birth have identified important therapeutic targets for new lipid-lowering agents [16].

The development of PCSK9-targeted therapies has simultaneously provided intensive treatment options in lowering LDL-C, and has accelerated the debate about the potential benefits and safety of achieving very low LDL-C levels [16].

PCSK9 inhibitors belong to the class of novel lipid-lowering agents, which substantially decrease LDL-C levels with further reduction of cardiovascular risk.

PCSK9 promotes degradation of hepatic LDL receptors, which remove LDL-C from the circulation, raising serum levels. Inhibitors of this protein consist of monoclonal antibodies that bind to the PCSK9 protein in plasma, preventing the degradation of hepatic LDL receptors, which increases the plasma removal of the LDL-C particle from the circulation. Associated with statins, PCSK9 inhibitors promote additional reductions of up to 60% in LDL-C levels [16,17].

This new class of lipid-lowering agents has been tested in large clinical trials such as *The Further Cardiovascular Outcomes Research with PCSK9 Inhibition in subjects with Elevated Risk* or *FOURIER*, which included more than 27,000 patients with atherosclerotic cardiovascular disease and LDL levels ≥ 70 mg/dL. Participants, all on statin treatment, were randomly allocated to receive additional therapy with either evolucumab or placebo for a median duration of 2.2 years. In the evolucumab group (140 mg every 15 days or 420 mg once a month, injectable), the mean reduction in LDL-C levels was 59%, with values ranging from 92 mg/dl to 30 mg/d. There was a 15% risk reduction in the treatment group compared to placebo and an absolute risk reduction of 1.5% for both primary and secondary endpoints, with a number needed to treat (NNT) of approximately 67 [14]. Risk reductions persisted in the subgroup of patients with LDL-C below 25 mg/dl or even below up to 10 mg/dl. Exploratory analyses noted that safety events did not differ between the achieved LDL-C ranges, including comparisons between LDL-C < 10 mg/dL and LDL-C > 100 mg/dL. These analyses were limited to 2.2 years of median follow-up, the duration of the study [18].

Another important piece of evidence was the study "Alirocumab and Cardiovascular Outcomes after Acute Coronary Syndrome": ODISSEY Outcomes Committees and Investigators, which randomized 18,924 patients with previous acute coronary syndrome (1 to 12 months) and baseline LDL-C above 70 mg/dl, to the use of alirocumab plus statins at maximum tolerated doses (treatment group) versus statin and/or ezetimibe, (placebo) for 2 years and 8 months. The LDL-C target was 25-50 mg/dl. If LDL-C reached values < 15 mg/dL, the dose was reduced to 75 mg, and if it remained < 15 mg/dL, it was discontinued and replaced with placebo. The treatment group achieved mean LDL-C levels of 53.3 mg/dl and the group 101.4 mg/dl with a drop of 54.7mg/dl and reductions in combined outcomes including death from CAD of 15% [19].

The absolute benefit of alirocumab relative to the composite primary endpoint was more pronounced among patients with baseline LDL-C levels of 100 mg/dl than with lower values. The incidence of adverse events did not differ between the two treatment groups, including onset of diabetes, cognitive changes, hemorrhagic stroke, and liver changes. The safety and

efficacy of alirocumab may have been influenced by the study design itself, which used the blinded dose adjustment strategy to avoid extremely low LDL-C values [19].

These large clinical trials have shown that evolucumab and alirocumab reduce the risk of cardiovascular disease when prescribed in addition to statins, in both high- and very high-risk patients. These drugs have also been evaluated in smaller studies in individuals who are intolerant to statins or have increased LDL-C levels, despite conventional therapy, and who did not reach the recommended targets, remaining at high residual risk.

Meta-analysis evaluated efficacy and safety of PCSK9 inhibitors in patients on primary and secondary prevention, in association and/or comparison with statins and ezetimibe. Data were collected from 60,997 participants, of which 26,538 were randomized to alirocumab (18 trials) and 34,435 to evolucumab (six trials). Most patients had a history of previous CAD and/or type 2 diabetes. Six studies with alirocumab used the active treatment comparison group (alirocumab versus statin or ezetimibe) and the remainder used placebo (alirocumab plus statins versus statin). Three trials with evolucumab versus active treatment and three trials with evolucumab versus placebo were included. Follow-up ranged from 6 to 36 months for placebo comparisons and from 6 to 12 months for active treatment comparisons [18].

Alirocumab compared to placebo significantly decreased the risk of cardiovascular events, all-cause mortality, acute myocardial infarction (AMI), and stroke. When compared to active treatment with ezetimibe or statins, there was no significant risk reduction [17].

Evolucumab compared to placebo decreased the risk of outcomes including AMI and stroke, with no differences in mortality. In comparison with active treatment, the risk reduction was modest [17].

There is strong evidence of benefit of PCSK9 monoclonal antibodies in individuals who are already on optimized treatment with statins and/or ezetimibe, still at high residual risk, and for those who do not achieve lipid targets with traditional therapy. When compared to active treatment, the evidence is of low quality and insufficient, and does not seem to indicate the benefit of monoclonal antibodies as replacement therapies [17].

In a recent meta-analysis of 83,660 subjects receiving maximal statin therapy, the addition of ezetimibe and/or PCSK9 inhibitors significantly reduced the risk of nonfatal AMI and nonfatal stroke in adults at high or very high cardiovascular risk, with no significant effect on cardiovascular or all-cause mortality. In statin-intolerant patients, the addition of ezetimibe and/or PCSK9 inhibitors reduced the risk of non-fatal AMI and stroke in the high- or very high-risk population [20].

In general, PCSK9 inhibitors have shown greater absolute reductions in AMI and stroke when added to baseline therapy or even in statin-intolerant patients, as they present a greater reduction in LDL-C values [20].

Having established the efficacy and safety of very low LDL-C values with PCSK9 inhibitors plus statins in the short and medium term, the long-term benefits were determined by the

open-label extension of the Fourier study (Fourier-Ole) [21]. This study covered a portion of the patients originally included in the FOURIER study, which originally included about 27,000 patients, of whom 6,635 participated in the extension. Regardless of the treatment in the original study, all patients converged to use evolocumab, at a dose of 140 mg subcutaneously every 2 weeks or 420 mg subcutaneously once a month. The maximum exposure to evolocumab in the original FOURIER plus FOURIER-OLE study was 8.4 years (median 7.1 years).

In the open-label extension, subjects remained at high residual risk even after an average of 7 years exposure to the most potent LDL-C lowering treatments available [22]. Current strategies are only partially effective in mitigating the risk of CAD, and late interventions to reduce LDL-C fail to eliminate much of the risk accumulated over years of exposure to elevated LDL-C values [22].

In randomized controlled trials of potent LDL-C lowering therapies (e.g., PCSK9 inhibitors), in patients with high atherosclerotic plaque burden, recurrent event rates and residual risk remain elevated.

The confluence of the results of both Mendelian randomization and clinical trials leads to the same proposition: the lower the LDL-C, the better for the prevention of atherosclerotic lesions.

Acknowledgments

None.

Conflict of interest

None.

Abbreviations

CAD: Coronary Artery Disease

HMGCoA: 3-Hydroxy-3-Methylglutaryl Coenzyme A

LDL-C: Low Density Lipoprotein Cholesterol

PCSK9: Proprotein Convertase Subtilisin/Kexin Type 9

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