Lipidomic profile in patients with a very high risk of atherosclerotic cardiovascular disease on PCSK9 inhibitor therapy

Kui Huang[1,†], Xiao-Qin Wen[2,†], Ning Ren[1], Li Yang[1], Bo Gao[2,*]

[1] Department of Cardiology, Tianjin Chest Hospital, 300222 Tianjin, China

[2] Department of Cardiology, Tianjin Hospital, 300211 Tianjin, China

*Correspondence: dtgb8559@126.com (Bo Gao)
† These authors contributed equally.

We evaluated the lipidomic profile of patients with very high-risk atherosclerotic cardiovascular disease (ASCVD) by ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UPLC-MS). A total of 64 patients with a very high risk of ASCVD were recruited and randomLy divided into the atorvastatin group (20 mg, every night, 4 weeks) or the combined group (evolocumab, 140 mg, once every 2 weeks on top of atorvastatin (20 mg per day)). The level of serum lipids was detected before and after treatment for 4 weeks. The lipid classes of triacylglycerols, cholesteryl esters, and sphingomyelins were analyzed using an ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry system. There were 32 patients in each group. After 4 weeks of treatment, the levels of total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) in both groups and the level of lipoprotein-a (Lp-a) in the combined group were lower. In the combined treatment group, the levels of TC, LDL-C, and Lp-a decreased significantly ($P < 0.05$) after 4 weeks of treatment. Most of the lipid classes in plasma decreased in the combined group at 4 weeks, especially sphingolipids. Only 1 patient had an adverse event (a rash) in the combined group, which improved after anti-allergic treatment. PCSK9 inhibitors can rapidly and effectively reduce most lipid classes in patients with very-high-risk ASCVD.

Keywords

1. Introduction

Atherosclerotic cardiovascular disease (ASCVD) is still the leading cause of death worldwide [1]. Low-density lipoprotein cholesterol (LDL-C) is not only the main component of atherosclerotic plaque formation but also a crucial risk factor for ASCVD. The management of cardiovascular events in very-high-risk patients with ASCVD has always attracted much attention [2, 3]. There is no doubt that intensive lipid-lowering therapy can further reduce the incidence of cardiovascular events in patients with very-high-risk ASCVD.

Statins are considered to be the cornerstone of lipid-lowering therapy. Statins are not only widely used but also have anti-inflammatory properties [4]. However, the TNT study of 100,001 patients with stable coronary disease and LDL-C <3.4 mmol/L showed that patients with ASCVD still had residual cardiovascular risk even if they were treated with high-intensity statins (atorvastatin 10 mg/d and 80 mg/d) [5]. In addition, remnant cholesterol has been proven to be a possible risk factor for ASCVD [6]. Therefore, how to reduce these residual risks still requires further efforts.

Proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors have been a significant breakthrough in the field of cardiovascular lipid-lowering therapy in recent years [7, 8]. The results of the FOURIER study confirmed the role of PCSK9 inhibitors in the prevention of cardiovascular events on a background of statin therapy [9]. The FOURIER study, which involved 27,564 patients, showed that the use of a PCSK9 inhibitor reduced the level of LDL-C, the risk of cardiovascular events, the risk of myocardial infarction, and the risk of stroke within 2 years [9].

To date, the attainment rate of LDL-C goals in very-high-risk ASCVD populations remains elusive in many countries and needs to be improved [10]. However, the therapeutic effect of PCSK9 inhibitors on the blood lipid profile and tolerance in very-high-risk ASCVD populations remain unclear. In this study, a randomized controlled trial was conducted to investigate the effects and adverse reactions of PCSK9 inhibitors on the blood lipid profile and adverse reactions in ASCVD patients at very high risk.

2. Methods

2.1 Study design and patients

This two-center study was conducted from April 2019 to June 2020. ASCVD patients at very high risk in the Department of Cardiology, Tianjin Chest Hospital and the Department of Cardiology, Tianjin Hospital were recruited. Very-high-risk ASCVD patients who met the criteria of the guidelines on the management of blood cholesterol of

the American Heart Association/American Heart Association (AHA/ACC) [2] were enrolled. Very high risk was defined by a history of multiple (≥2) major ASCVD events (recent occurrence of acute coronary syndrome (within 12 months), myocardial infarction (MI) ≥12 months, ischemic stroke, and symptomatic peripheral arterial disease) or a history of 1 major ASCVD event along with ≥2 high-risk conditions [2]. Patients with severe hepatic or renal insufficiency, heart failure, or type 1 diabetes mellitus were excluded. This study was performed in accordance with the Helsinki Declaration and was approved by the Hospital Ethics Committee of Tianjin Chest Hospital. All treatments and assessments were performed after the informed consent of patients or their families was obtained.

To explore the therapeutic effect of PCSK9 inhibitors on the lipidomic profile of those patients, we compared the alterations in blood lipid composition between patients treated with atorvastatin alone and patients treated with PCSK9 inhibitors on top of statins. Patients were randomly divided into the combined group and the atorvastatin group according to the envelope method. A random grouping scheme within a sealed opaque envelope was opened by the researchers according to the sequence of patients, and the patients were divided into groups according to the scheme within the envelope.

Atorvastatin was given 20 mg orally once a night for 4 weeks in the atorvastatin group. Evolocumab injection (Amgen Manufacturing Limited, USA) was used (140 mg, every 2 weeks) in the combined group for 4 weeks on top of atorvastatin (20 mg per day). All patients received lipid-lowering therapy for the first time. Standard secondary prevention of ASCVD, such as antiplatelet therapy, was provided equally in both groups [2, 3]. Fig. 1 shows the flowchart of this study.

2.2 Primary outcomes: serum lipid profile

The serum of all patients was collected before treatment and 4 weeks after treatment in each group. Participants were required to avoid consuming high-fat, high-protein food and alcohol at the last meal before examination. After 12 hours of fasting, forearm venous blood was drawn for assessment. The laboratory results of triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), LDL-C, and lipoprotein-a (Lp-a) were recorded after each assessment. Adverse reactions during drug treatment were carefully observed in all patients.

2.3 Secondary outcomes: changes in lipid profiles

Serum was pretreated with a methyl tert butyl ether (MTBE)-methanol extraction system. To evaluate the stability of the determination process, quality control samples were inserted into the analysis sequence. The lipid classes of triacylglycerols (TAG), cholesteryl esters (CE), and sphingomyelins (SM) were analyzed using ultra-performance liquid chromatography (UPLC) (Waters Corp., Milford, MA, USA)-quadrupole time-of-flight mass spectrometry (MS) (TripleTOF 5600, AB Sciex, Framingham, MA, USA) system
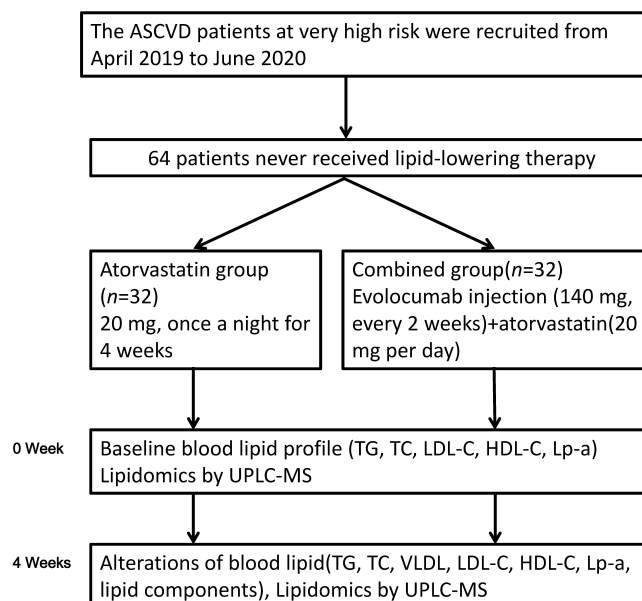
Fig. 1. Flowchart of this study. HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Lp-a, lipoprotein-a; TC, total cholesterol; TG, triglycerides; UPLC-MS, ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry.

[11].

The prepared samples were chromatographed on an Acquity UPLC HSS C18 (2.1 mm × 100 mm × 1.7 μm), column temperature, 55 °C; mobile phase A, acetonitrile: water (3: 2, v/v); mobile phase B, isopropanol: acetonitrile (9: 1, v/v); and mobile phases A and B containing 10 mM ammonium acetate, flow rate, 0.26 mL/min. High-resolution electrospray ionization (ESI) positive ions and negative ions were scanned separately. Mass spectrometry parameters: ion source voltage, 4500 V (–) and 5500 V (+); declustering voltage, 100 V (–) and 100 V (+); and interface heater temperature, 600 °C (–) and 500 °C (+). Analyst TF version 1.6.2, Lipidview, Peakview, and Multiquant software (AB Sciex, Framingham, MA, USA) was used to collect and qualitatively and quantitatively analyze the data.

2.4 Statistical analysis

SIMCA-P version 13.0 (Umetrics, Umea, Sweden) was used for multivariate statistical analysis. An orthogonal partial least squares discriminant analysis (OPLS-DA) model was constructed, and then the data were summarized, classified and discriminantly analyzed. The reliability of the model was tested by 7 cross-validations. According to the OPLS-DA model, the variable importance in projection (VIP) was obtained, and the metabolites with significant changes in serum were preliminarily screened. A combined VIP value >1 and $P < 0.05$ were used to screen the differential lipid components. The measurement data with a normal distribution are expressed as the mean ± standard deviation, and the t-test was used for comparisons between groups. For nonnormally distributed data, medians with interquartile ranges were ex-

Table 1. Comparison of baseline data between two groups with different lipid-lowering regimens.

Characteristics	Atorvastatin group	Combination group	t/χ^2	P value
n	32	32	-	-
Male n (%)	14 (43.75)	16 (50.00)	0.251	0.616
Age (y)	57.38 ± 13.63	58.42 ± 14.61	0.294	0.769
BMI (kg/m^2)	28.44 ± 3.47	28.52 ± 3.61	0.088	0.931
Current smoking n (%)	3 (9.38)	4 (12.50)	0.160	0.689
Hypertension n (%)	16 (50.00)	17 (53.13)	0.063	0.802
Myocardial infarction n (%)	9 (28.13)	8 (25.00)	0.080	0.777
PCI n (%)	17 (53.13)	18 (56.25)	0.063	0.802

pressed and tested by the Mann-Whitney U-test. The count data were analyzed by the χ^2 test. $P < 0.05$ was considered significant. SPSS version 22.0 (SPSS Inc., Chicago, IL, USA) was used to process the data. GraphPad Prism 7 software (GraphPad Inc., San Diego, CA, USA) was used for graph production.

3. Results

3.1 Patient characteristics

A total of 64 patients were included. All patients in the atorvastatin group (n = 32) and the combined group (n = 32) completed a 4-week treatment without loss of follow-up. The baseline patient characteristics of the two groups are shown in Table 1. There was no significant difference in the baseline data of age, BMI, underlying diseases, or the proportion of percutaneous coronary intervention (PCI) between the two groups ($P > 0.05$).

3.2 Effects of PCSK9 inhibitors on blood lipidomic profile in patients with very-high-risk ASCVD

There was no significant difference in blood lipidomic profile between the two groups before treatment. After 4 weeks of treatment, the levels of LDL-C in both groups were significantly lower than before (all $P < 0.05$). The level of Lp-a in the combined group was significantly lower than that before treatment, but this result was not found in the atorvastatin group. It should be pointed out that the decrease in LDL-C and Lp-a levels in the combined treatment group was significantly greater than that in the atorvastatin group after treatment (Fig. 2, **Supplementary Tables 1,2**). Unfortunately, there was no significant change in the levels of TG and HDL-C after 4 weeks of treatment in either group. Differences in the blood lipidomic profile in the two groups are shown in Fig. 2 and **Supplementary Table 2**.

3.3 Effect of PCSK9 inhibitors on the LDL-C compliance rate

After 4 weeks of treatment, the level of LDL-C decreased to less than 1.4 mmol/L in 16 patients in the combined group [3]. The rate of reaching the LDL-C standard was 50.0%. However, only 7 patients treated with atorvastatin had an LDL-C level of 1.4 mmol/L or less after 4 weeks of treatment. The rate of reaching the standard was 21.8%, which was significantly lower than that in the combined group (χ^2 = 5.497, P = 0.019).

3.4 Change in all lipid classes after the administration of the PCSK9 inhibitor

Principal component analysis (PCA) was used to analyze the changes in all lipid classes in serum before and after treatment in each group. The principal component scores were analyzed by cluster analysis. The score chart of each sample data reflecting the dispersion degree between the two groups was obtained (Fig. 3A,B). Before treatment, the distance between the control group and the statin group was very small, and it was difficult to distinguish between the two groups (Fig. 3A). This finding means that there was no significant difference in the principal component score between the two groups before treatment. However, after 4 weeks of treatment, there was a significant difference in lipid metabolites between the combined group and the atorvastatin group. The cluster analysis of the principal component scores of the two groups was obviously separated into two parts (Fig. 3B).

Next, the changes in different lipid metabolism pathways were further analyzed. After 4 weeks of treatment with PCSK9 inhibitors, most of the lipid classes in plasma decreased. In the combined group, sphingolipids were the most reduced lipids, including dihydroceramide (Cer d18: 0), ceramides (Cer d18: 1), glucosyl/galactosylceramides (Glc/GalCer), globular trisaccharide ceramide (Gb3), sphingomyelins (SM), and lactosylceramides (LacCer). Significantly decreased levels of cholesterol esters (CE), free cholesterol (FC), phospholipids, triacylglyceride (TAG), and diacylglycerol (DAG) were also found, as shown in Fig. 3C and **Supplementary Table 3**.

3.5 Adverse drug reactions

During the course of 4 weeks of treatment, only one patient had an adverse drug reaction (a rash) in the combined group, which improved after anti-allergic treatment. There were no other adverse reactions, such as abnormal liver function or increased muscle enzymes, in either group.

4. Discussion

In the present study, the lipid-lowering effect of PCSK9 inhibitors on very-high-risk patients with ASCVD was investigated. Not only were the short-term effects of PCSK9 inhibitors on lipidomic profile analyzed, but specific changes in the lipidomic profile were also identified by UPLC-MS. We found that PCSK9 inhibitors on top of atorvastatin can
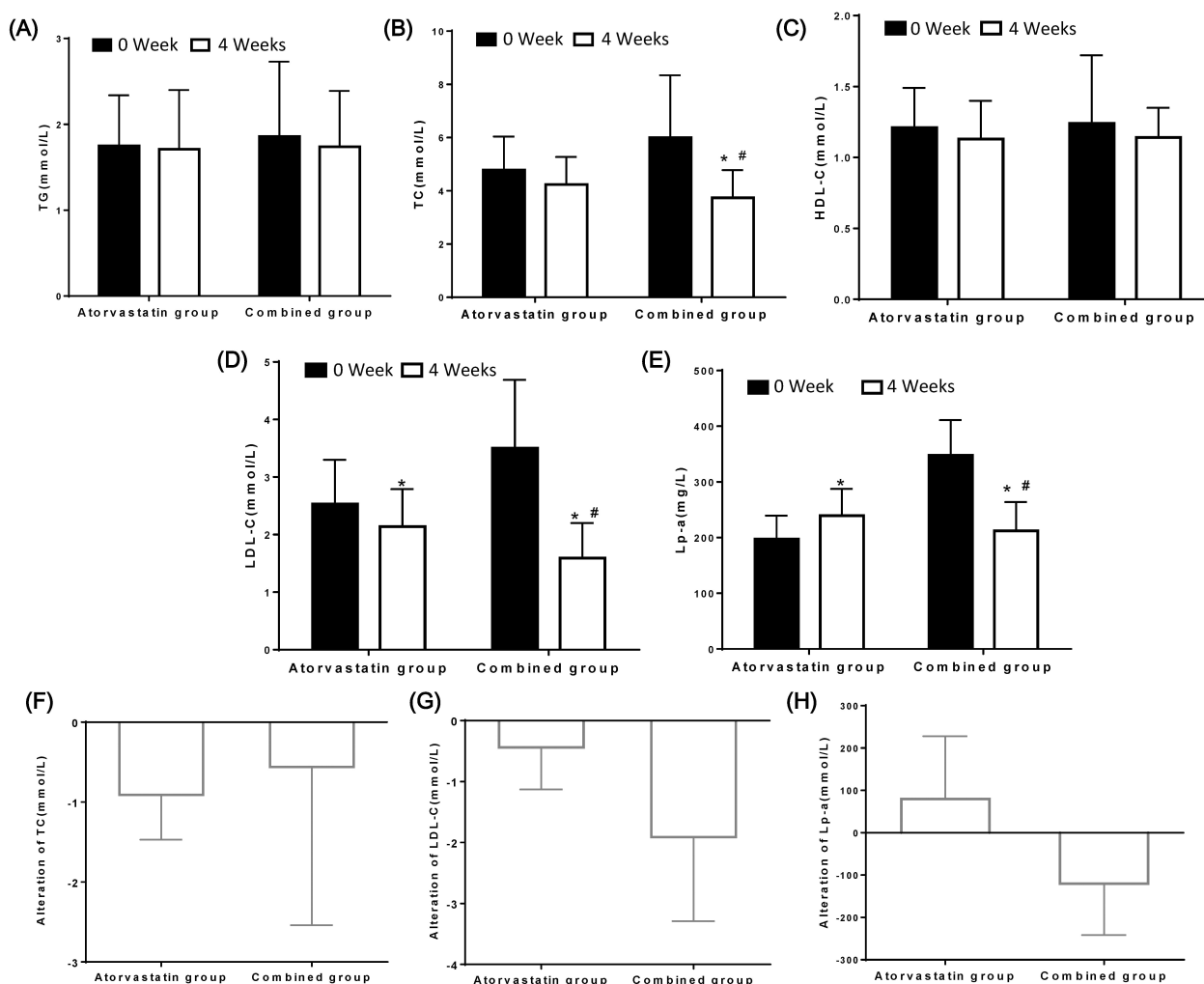
Fig. 2. The changes of lipidomic profile in ASCVD patients with a very high-risk before and after treatment in the two groups. The levels of TG (A), TC (B), HDL-C (C), LDL-C (D), Lp-a (E) were compared. The alteration of TC (F), LDL-C (G), Lp-a (H) in two group. *: $P < 0.05$, compared with the same group before treatment, #: $P < 0.05$ between the two groups. HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Lp-a, lipoprotein-a; TC, total cholesterol; TG, triglycerides.

effectively reduce the levels of TC, TG, LDL-C, and Lp-a in patients with very-high-risk ASCVD. PCSK9 inhibitors can further reduce residual blood lipids (ultimately be <1.4 mmol/L), especially sphingolipids, after using statins, which is a new method of lipid management in patients with a very high risk of ASCVD in the future based on oral statin treatment.

Very-high-risk ASCVD was defined for the first time in the 2018 AHA/ACC Cholesterol Management Guide [2]. LDL-C and Lp-a are independent risk factors for ASCVD [12]. At present, intensive lipid regulation is the key treatment to improve the prognosis of ASCVD. The importance of LDL-C level control in the secondary prevention of AS-CVD patients has been increasingly emphasized. The 2018 AHA/ACC Cholesterol Management Guide states that the LDL-C level of very-high-risk ASCVD patients should be below 1.8 mmol/L (70 mg/dL) [2]. Accoding to 2019 ESC/EAS Guidelines for the management of dyslipidaemias, for pa-

tients with very high risk, regardless of primary prevention or secondary prevention, the LDL-C goal should be less than 1.4 mmol/L [3].

At present, a number of studies have proven that PCSK9 inhibitors can effectively reduce the level of LDL-C [9, 13–15] and have been recommended by diverse guidelines [2, 3]. The new intensive lipid regulation regimen based on PCSK9 inhibitors is an important means and direction to improve the prognosis of very-high-risk ASCVD patients. However, in China, the application of PCSK9 inhibitors is still in the early stage of exploration. To the best of our knowledge, this is the first study on the effect of PCSK9 inhibitors on the blood lipidomic profile of patients with very-high-risk AS-CVD in China.

After 4 weeks of treatment with the PCSK9 inhibitor evolocumab supplementary to oral atorvastatin, the levels of TC, LDL-C, and Lp-a were significantly lower than those before treatment. Although the levels of TG and HDL-C
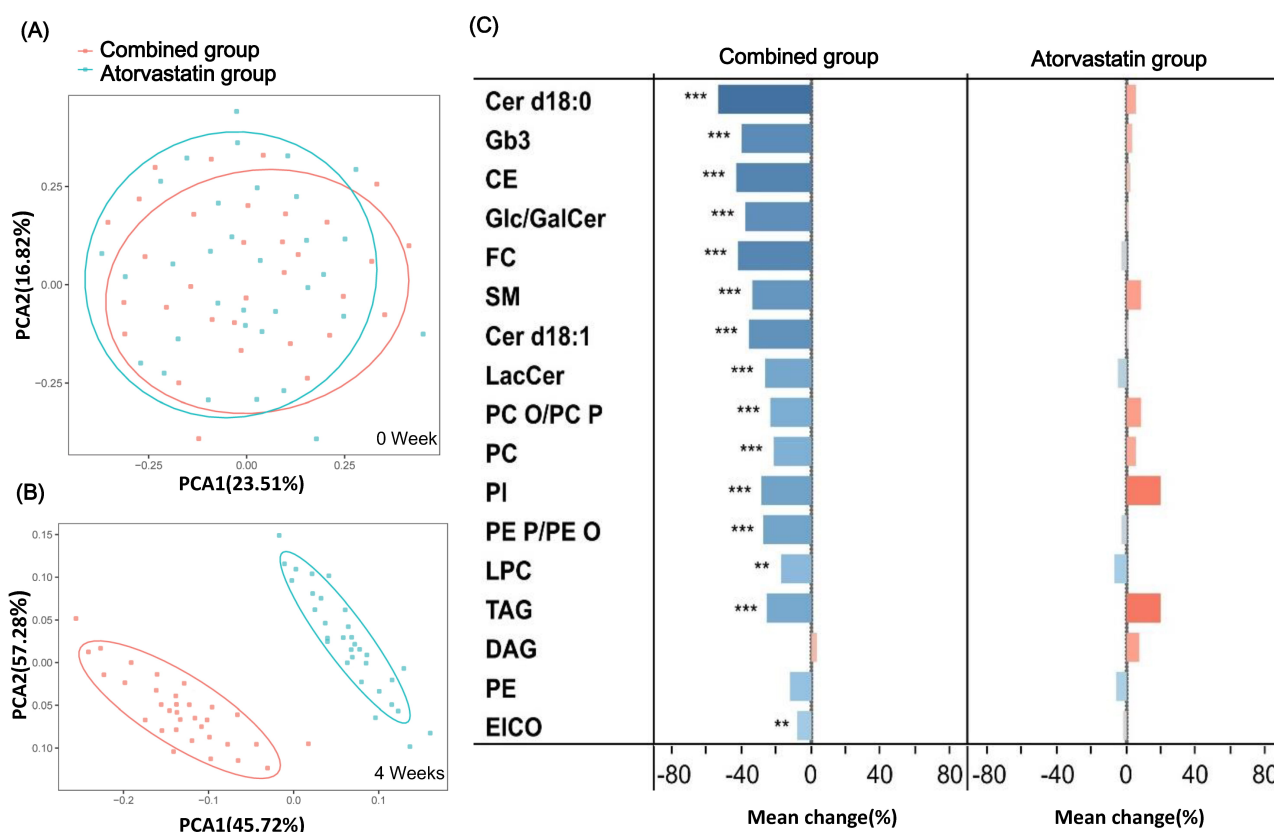
Fig. 3. Lipid classes were identified in plasma samples by ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry before and after the two groups. (A) Principal component analysis of metabolites at baseline in two groups. There was no significant difference in clustering. (B) Principal component analysis of metabolites after 4 weeks of treatment and the clustering separation was obviously. (C) The change trend of lipid classification in the two groups. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$. CE, cholesteryl ester; Cer, ceramide; DAG, diacylglycerol; EICO, eicosanoids; FC, free cholesterol; Gb3, globotriasoylceramide; PC, phosphatidylethanolamines; PCA, principal component analysis; PI, phosphatidylinositol; LPC, lysophosphatidylcholine; PE, phosphatidylethanolamines; SM, sphingomyelin; TAG, triacylglycerol.

in the combined group had a downward trend, the changes were not significant. These findings suggest that PCSK9 inhibitors can effectively reduce the level of cholesterol, except for HDL-C, in patients with very-high-risk ASCVD. Although the level of LDL-C decreased in the combined group, the decrease was smaller than that reported in other studies [16]. This difference may be related to the shorter treatment time in our study. In the postanalysis of the FOURIER phase III study [16], LDL-C decreased by approximately 59% at 48 weeks after treatment in the evolocumab injection group (95% CI: 58%–60%, $P < 0.001$). The average decrease was approximately 1.46 mmol/L (56 mg/dL). In the present study, only the lipidomic profile were observed after 4 weeks of PCSK9 inhibitor administration. Therefore, it is necessary to prolong the follow-up time and to increase the sample size, which is what we will do in the future.

At present, there are many kinds of drugs that can reduce LDL-C, and they are widely used in the clinic, but drugs that can effectively reduce Lp-a are still in the pipeline. Although this study confirmed that PCSK9 inhibitors can reduce the level of Lp-a, the mechanism is not completely clear [17–19].

After 4 weeks of treatment, the level of Lp-a decreased by approximately 21.5% in the combined group, which was similar to that reported in other studies [9, 13, 15]. Stiekema et al. [20] studied the effect of intensive lipid regulation, including PCSK9 inhibitors. The authors found that although LDL-C decreased significantly, the decrease in Lp-a was small. In patients with normal Lp-a levels, the level of LDL-C decreased by approximately 60%, and the level of Lp-a was reduced by approximately 20% to 30%. Interestingly, it is suggested that the reduction in Lp-a by PCSK9 inhibitors depends on the baseline level of Lp-a. The higher the baseline level is, the smaller the decrease is [3].

The attainment of the LDL-C target is the key to affecting the prognosis of patients with very-high-risk ASCVD. In this study, after 4 weeks of PCSK9 inhibitor administration, 50% of patients met the target LDL-C level in the combined group, which was higher than that in the atorvastatin group (23.3%). This finding also indicates that PCSK9 inhibitors can not only reduce the level of LDL-C but also decrease LDL-C faster. Earlier attainment of the target means an earlier benefit for patients.

We also found that evolocumab has little effect on TG while lowering cholesterol. In addition, there was no significant change in HDL-C levels before and after the administration of the PCSK9 inhibitor. There is still a large debate on the relationship between HDL-C and cardiovascular disease, which tends to be "U-shaped"; that is, high or low levels of HDL-C may increase the risk of cardiovascular disease [21, 22]. In this study, only one patient in the combined group had an allergic reaction, which manifested as a rash. After oral anti-allergic drugs were administered, the allergic reaction was mild. Thus, the PCSK9 inhibitor was safe. As reported, the main adverse drug reactions to PCSK9 inhibitors were nasopharyngitis, upper respiratory tract infection, influenza, and back pain, and the incidence rate was less than 2% [13]. However, there was no significant difference in the occurrence of adverse drug reactions between PCSK9 inhibitors and statins, which also shows that the use of PCSK9 inhibitors is safe [23].

5. Limitations

There are also some limitations in this study. First, this study is a double-center study with a small number of selected patients. Second, the impact of PCSK9 and atorvastatin on individual lipid species within each lipid class was not analyzed. Third, PCSK9-related genes, which may have some influence on the therapeutic effect of PCSK9 inhibitors, were not detected. Finally, we studied only the short-term changes in the blood lipidomic profile. There is a lack of long-term follow-up data for treatment effects and long-term impacts on cardiovascular events.

6. Conclusions

In conclusion, the PCSK9 inhibitor evolocumab is efficacious and safe in treating very-high-risk ASCVD patients. The universality of the efficacy needs to be further confirmed, and more large-scale, multicenter, long-term, randomized trials need to be carried out.

Abbreviations

ASCVD, Atherosclerotic cardiovascular disease; CE, cholesteryl esters; HDL-C, high-density lipoprotein cholesterol; LDL-C, Low-density lipoprotein cholesterol; Lp-a, lipoprotein-a; MS, mass spectrometry; OPLS-DA, orthogonal partial least squares discriminant analysis; PCSK9, Proprotein convertase subtilisin/kexin type 9; SM, sphingomyelins; TAG, triacylglycerols; TC, total cholesterol; TG, triglyceride; UPLC, ultra-performance liquid chromatography.

Author contributions

All authors contributed significantly to this study. KH, XQW, and BG designed the trial. KH, XQW, NR, LY, and BG were conducted the work and involved in data collection. KH, XQW and BG analyzed the data. NR, LY, and BG interpreted the data. KH and XQW wrote the manuscript. All authors revised the manuscript.

Ethics approval and consent to participate

The study was obtained permission from the Ethics Committee of the Tianjin Chest Hospital (Ethics approval number: 2019-00161). All treatments and assessments were performed after the informed consent of patients or their families was obtained.

Conflict of interest

The authors declare no conflict of interest.

Supplementary material

Supplementary material associated with this article can be found, in the online version, at https://rcm.imrpress.com/EN/10.31083/j.rcm2202052.

References

[1] Thirunavukkarasu S, Khader SA. Advances in cardiovascular disease lipid research can provide novel insights into mycobacterial pathogenesis. Frontiers in Cellular and Infection Microbiology. 2019; 9: 116.

[2] Grundy SM, Stone NJ, Bailey AL, Beam C, Birtcher KK, Blumenthal RS, et al. 2018 AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA guideline on the management of blood cholesterol: a report of the american college of cardiology/american heart association task force on clinical practice guidelines. Circulation. 2019; 139: e1082–e1143.

[3] Mach F, Baigent C, Catapano AL, Koskinas KC, Casula M, Badimon L, et al. 2019 ESC/EAS guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk. European Heart Journal. 2020; 41: 111–188.

[4] Ravnskov U, de Lorgeril M, Diamond DM, Hama R, Hamazaki T, Hammarskjöld B, et al. LDL-C does not cause cardiovascular disease: a comprehensive review of the current literature. Expert Review of Clinical Pharmacology. 2018; 11: 959–970.

[5] LaRosa JC, Grundy SM, Waters DD, Shear C, Barter P, Fruchart J, et al. Intensive lipid lowering with atorvastatin in patients with stable coronary disease. The New England Journal of Medicine. 2005; 352: 1425–1435.

[6] Sandesara PB, Virani SS, Fazio S, Shapiro MD. The forgotten lipids: triglycerides, remnant cholesterol, and atherosclerotic cardiovascular disease risk. Endocrine Reviews. 2019; 40: 537–557.

[7] Ali A, Costanzo P, Hoye A. Proprotein convertase subtilisin/kexin type 9 inhibition in cardiovascular prevention. Current Pharmaceutical Design. 2018; 24: 442–450.

[8] Spolitu S, Dai W, Zadroga JA, Ozcan L. Proprotein convertase subtilisin/kexin type 9 and lipid metabolism. Current Opinion in Lipidology. 2019; 30: 186–191.

[9] Sabatine MS, Giugliano RP, Keech AC, Honarpour N, Wiviott SD, Murphy SA, et al. Evolocumab and clinical outcomes in patients with cardiovascular disease. The New England Journal of Medicine. 2017; 376: 1713–1722.

[10] Volis I, Saliba W, Jaffe R, Eitan A, Zafrir B. Effect of cerebrovascular and/or peripheral artery disease with or without attainment of lipid goals on long-term outcomes in patients with coronary artery disease. The American Journal of Cardiology. 2020; 128: 28–34.

[11] Ståhlman M, Ejsing CS, Tarasov K, Perman J, Borén J, Ekroos K. High-throughput shotgun lipidomics by quadrupole time-of-flight mass spectrometry. Journal of Chromatography B. 2009; 877: 2664–2672.

[12] Yoshino S, Hamasaki S, Ishida S, Kataoka T, Yoshikawa A, Oketani N, et al. Characterization of the effect of serum bilirubin concentrations on coronary endothelial function via measurement of high-sensitivity C-reactive protein and high-density lipoprotein cholesterol. Heart and Vessels. 2013; 28: 157–165.

[13] Robinson JG, Nedergaard BS, Rogers WJ, Fialkow J, Neutel JM, Ramstad D, et al. Effect of evolocumab or ezetimibe added to moderate- or high-intensity statin therapy on LDL-C lowering in patients with hypercholesterolemia: the LAPLACE-2 randomized clinical trial. The Journal of the American Medical Association. 2014; 311: 1870–1872.

[14] Sabatine MS, Giugliano RP, Wiviott SD, Raal FJ, Blom DJ, Robinson J, et al. Efficacy and safety of evolocumab in reducing lipids and cardiovascular events. The New England Journal of Medicine. 2015; 372: 1500–1509.

[15] Nicholls SJ, Puri R, Anderson T, Ballantyne CM, Cho L, Kastelein JJP, et al. Effect of evolocumab on progression of coronary disease in statin-treated patients: the GLAGOV randomized clinical trial. The Journal of the American Medical Association. 2016; 316: 2373–2384.

[16] Bonaca MP, Nault P, Giugliano RP, Keech AC, Pineda AL, Kanevsky E, et al. Low-density lipoprotein cholesterol lowering with evolocumab and outcomes in patients with peripheral artery disease: insights from the FOURIER trial (further cardiovascular outcomes research with PCSK9 inhibition in subjects with elevated risk). Circulation. 2018; 137: 338–350.

[17] Rader DJ, Mann WA, Cain W, Kraft HG, Usher D, Zech LA, et al. The low density lipoprotein receptor is not required for normal catabolism of Lp(a) in humans. The Journal of Clinical Investigation. 1995; 95: 1403–1408.

[18] Reblin T, Niemeier A, Meyer N, Willnow TE, Kronenberg F, Dieplinger H, et al. Cellular uptake of lipoprotein[a] by mouse embryonic fibroblasts via the LDL receptor and the LDL receptor-related protein. Journal of Lipid Research. 1998; 38: 2103–2110.

[19] Romagnuolo R, Scipione CA, Boffa MB, Marcovina SM, Seidah NG, Koschinsky ML. Lipoprotein(a) catabolism is regulated by proprotein convertase subtilisin/kexin type 9 through the low density lipoprotein receptor. The Journal of Biological Chemistry. 2015; 290: 11649–11662.

[20] Stiekema LCA, Stroes ESG, Verweij SL, Kassahun H, Chen L, Wasserman SM, et al. Persistent arterial wall inflammation in patients with elevated lipoprotein(a) despite strong low-density lipoprotein cholesterol reduction by proprotein convertase subtilisin/kexin type 9 antibody treatment. European Heart Journal. 2019; 40: 2775–2781.

[21] Bowe B, Xie Y, Xian H, Balasubramanian S, Zayed MA, Al-Aly Z. High density lipoprotein cholesterol and the risk of all-cause mortality among U.S. Veterans. Clinical Journal of the American Society of Nephrology. 2016; 11: 1784–1793.

[22] Ko DT, Alter DA, Guo H, Koh M, Lau G, Austin PC, et al. High-density lipoprotein cholesterol and cause-specific mortality in individuals without previous cardiovascular conditions: the CANHEART study. Journal of the American College of Cardiology. 2016; 68: 2073–2083.

[23] Dixon DL, Pamulapati LG, Bucheit JD, Sisson EM, Smith SR, Kim CJ, et al. Recent updates on the use of PCSK9 inhibitors in patients with atherosclerotic cardiovascular disease. Current Atherosclerosis Reports. 2019; 21: 16.