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A PCSK9 inhibitor induces a transient decrease in the neutrophil-lymphocyte ratio and monocyte-lymphocyte ratio in homozygous familial hypercholesterolemia patients

Fangyuan Li [a, 1], Pucong Ye [b, 1], Yu Hao [c], Juan Du [c], Hang Zhang [d], Zengtao Wang [c], Xumin Wang [e], Hui Zeng [a, *], Yaluan Ma [f, **], Jie Lin [d, ***]

[a] Beijing Key Laboratory of Emerging Infectious Diseases, Institute of Infectious Diseases, Peking University Ditan Teaching Hospital, Beijing, China
[b] Department of Critical Care Medicine, Beijing Shijitan Hospital, Capital Medical University, Beijing, China
[c] Beijing Key Laboratory of Emerging Infectious Diseases, Institute of Infectious Diseases, Beijing Ditan Hospital, Capital Medical University, Beijing, China
[d] Department of Atherosclerosis, Beijing Anzhen Hospital, Capital Medical University, Beijing Institute of Heart, Lung and Blood Vessel Diseases, Beijing, China
[e] College of Life Sciences, Yantai University, Yantai, China
[f] The Institute of Basic Medical Theory of Chinese Medicine, China Academy of Chinese Medical Sciences, Beijing, China

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ABSTRACT

Background and aims: Extremely elevated levels of low-density lipoprotein-cholesterol (LDL-C) contribute to long-term chronic systemic inflammation in homozygous familial hypercholesterolemia (HoFH) patients. The aims of this study is to describe the inflammatory profile of HoFH patients and explore the effect of a PCSK9 inhibitor (PCSK9i) on a series of inflammatory biomarkers, including the neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR), monocyte-HDL ratio (MHR), monocyte-lymphocyte ratio (MLR) and mean platelet volume-lymphocyte ratio (MPVLR).
Methods: In this prospective cohort study, 25 definitive HoFH patients on high-intensity statins plus ezetimibe were administered subcutaneous injections of 420 mg PCSK9i every 4 weeks (Q4W). The biochemical parameters and inflammatory profile were analyzed on the day before PCSK9i therapy and 3 months and 6 months after PCSK9i therapy.
Results: HoFH on the maximum tolerated statin dose plus ezetimibe displayed elevated lipid and disturbed blood biomarker profiles. After 3 months of add-on PCSK9i therapy, a significant reduction in LDL-C was observed. Moreover, the percentage and count of neutrophils, monocyte counts, MPV, and two inflammatory biomarkers, the NLR and MLR, were reduced. However, at 6 months of PCSK9i treatment, the NLR and MLR returned to pre-PCSK9i treatment levels.
Conclusions: PCSK9i induces a transient decrease in the NLR and MLR in HoFH patients in a lipid-lowering independent manner.

Introduction

Familial hypercholesterolemia (FH) is an autosomal dominant hereditary disease that is characterized by elevated levels of total cholesterol (TC) and low density lipoprotein cholesterol (LDL-C) [1], premature atherosclerosis (AS) and cardiovascular diseases (CVDs). The most frequently reported gene mutated is the LDL-receptor (LDLR), accounting for 85–90% of reported FH cases. In addition, mutations in apolipoprotein B (APOB), proprotein convertase subtilisin/kexin-9 (PCSK9), and low-density lipoprotein receptor adaptor protein 1 (LDLRAP1) were also reported to cause FH [2]. In the general population, the prevalence of heterozygous FH (HeFH)

* Corresponding author.. Beijing Ditan Hospital, Jingshundongjie 8, Beijing, 100015, China.
** Corresponding author.
*** Corresponding author.. Department of Atherosclerosis, Beijing Anzhen Hospital, Capital Medical University, Beijing Institute of Heart, Lung and Blood Vessel Diseases, Beijing, 100029, China.
E-mail addresses: zenghui@ccmu.edu.cn (H. Zeng), yaluanma@yahoo.com (Y. Ma), linjie1998@126.com (J. Lin).
[1] Fangyuan Li and Pucong Ye contribute equally to this work.

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Abbreviations

HoFH	homozygous familial hypercholesterolemia
HeFH	heterozygous familial hypercholesterolemia
cHeFH	compound heterozygous familial hypercholesterolemia
DH	double heterozygous familial hypercholesterolemia
TC	total cholesterol
TG	triglycerides
HDL-C:	high-density lipoprotein cholesterol
LDL-C:	low-density lipoprotein-cholesterol
FPG	fasting plasma glucose
ALT	alanine transaminase
AST	aspartate transaminase
CREA	creatinine
MPV	mean platelet volume
hsCRP	high sensitivity C-reactive protein
AS	atherosclerosis
CVD	cardiovascular diseases
CAD	coronary artery disease
LDLR	LDL-receptor
APOB	apolipoprotein B
PCSK9	proprotein convertase subtilisin/kexin-9
LDLRAP1	low-density lipoprotein receptor adaptor protein 1
PCSK9i	PCSK9 inhibitor
NLR	neutrophil-lymphocyte ratio
PLR	platelet-lymphocyte ratio
MHR	monocyte-HDL ratio
MLR	monocyte-lymphocyte ratio
MPVLR	mean platelet volume-lymphocyte ratio
ATT	Achilles tendon thickness
ROS	reactive oxygen species
MPO	myeloperoxidase

patients who carry a mutation in one of the alleles is approximately 1 in 300 [3,4], while homozygous FH(HoFH), in which both alleles harbor mutations, affects 1 in 160,000−300,000 individuals [5]. Compared to HeFH patients, HoFH patients usually have higher LDL-C levels and poorer clinical prognosis [5].

For a long time, lipid lowering has been the principal target in FH treatment. As the first-line pharmacological treatment for dyslipidaemia, statins can significantly reduce plasma level of LDL-C and the risk of cardiovascular events [6]. However, the majority of FH patients cannot achieve optimal LDL-C reduction even with the maximum tolerated doses of statins [7,8]. Recently, several large randomized clinical trials demonstrated that the addition of PCSK9 inhibitors (PCSK9i) to statins may lead to a further reduction in LDL-C and cardiovascular risk [9,10], even in HoFH patients [11,12].

Of note, hypercholesterolemia causes chronic systemic and vascular inflammation [13−16]. A number of immunocytes and blood components, such as monocytes [17], macrophages [18], dendritic cells [19], lymphocytes [20], neutrophils [21], platelets [22] and the complement system [23] contribute to the proinflammatory environment in AS, and promote the development of atherogenesis, plaque destabilization and plaque erosion. In the peripheral blood, a series of blood cellular component-related parameters, such as the neutrophil-lymphocyte ratio (NLR) [24,25], platelet-lymphocyte ratio (PLR) [26,27], monocyte-HDL ratio (MHR) [28,29], monocyte-lymphocyte ratio (MLR) [30,31] and mean platelet volume-lymphocyte ratio (MPVLR) [32], are able to illustrate systemic inflammation status and evaluate the risk of future CVD events. For example, the NLR and MLR reflect the balance between the innate (neutrophils and monocytes) and adaptive (lymphocytes) immune responses in the body [33]. Platelets directly contribute to the progression of thrombosis and CVD [34,35], and their activity becomes significantly enhanced in hyperlipidaemia [36]. Platelet counts and MPV are the two main parameters in evaluating platelet activity [37].

Since hypercholesterolemia and inflammation are now considered as “two sides of the same coin”, anti-inflammation becomes a target for therapeutic strategies [38]. In addition to lipid-lowering effects, PCSK9i showed anti-inflammatory and immunomodulatory effects in FH patients. Scicali et al. [39] found that six month of add-on PCSK9i in HeFH patients significantly reduced LDL-C levels, neutrophil counts and the inflammatory marker MHR, while the NLR was not altered. However, few studies have investigated the effects of PCSK9i on systemic inflammation in HoFH patients.

Patients and methods

Study design and participants

This study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki. This study was reviewed and approved by the ethics committees of Beijing Anzhen Hospital, Capital Medical University. All subjects voluntarily participated in the study, signed informed consent forms, and cooperated with the medical staff to complete the follow-up. Forty-seven healthy donors (HD) with LDL-C < 3.5 mmol/L were recruited. The exclusion criteria for HD included a history of established cardiovascular disease, lipid-lowering drugs, clinical signs of acute infection, and anti-inflammatory medication.

Eligible HoFH participants were diagnosed by genetic testing (two alleles both carrying mutations in the regions of LDLR, APOB, PCSK9 or LDLRAP1), whether they were true HoFH (the same mutation in both alleles of the same gene), compound heterozygous FH (cHeFH-different mutations in the two alleles of the same gene) or double heterozygotus FH (DH-two different alleles of two separate genes). The pathogenic genes were detected by the second-generation sequencing technique. In addition, their Achilles tendon thickness (ATT), a sensitive index in diagnosing FH [40], was measured before starting the genetic test. The eligibility criteria were age between 12 and 75 years old, bodyweight ≥40 kg, fasting triglyceride (TG) ≤ 4.5 mmol/L, and fasting LDL-C ≥ 3.4 mmol/L after at least four weeks of stable high-intensity statins plus ezetimibe therapies. Participants were willing to maintain a regular healthy diet and comply with clinic visits during the study period. The exclusion criteria included uncontrolled cardiac arrhythmias, myocardial infarction, unstable angina, percutaneous coronary intervention, coronary artery bypass grafting, stroke, deep vein thrombosis, pulmonary embolism (<3 months before study start), systolic blood pressure >180 mmHg and/or diastolic blood pressure >110 mmHg, having received other PCSK9i and cholesteryl ester transfer protein inhibitors (>6 months before study start). Patients did not have any clinically significant endocrine disease that influenced serum lipids. Women who were pregnant or breast-feeding were excluded. The inclusion of patients was consecutive.

From May 2019 to March 2022, 67 probable HoFH patients were enrolled from Beijing Anzhen Hospital, Capital Medical University. The participants had to have received stable maximum statin therapy with ezetimibe for at least 4 weeks (that is, atorvastatin 40 mg/d or rosuvastatin 20 mg/d, ezetimibe 10 mg/d) before they

added the PCSK9i evolocumab 420 mg administered subcutaneously every 4 weeks (Q4w). Biochemical analyzes were performed on the day before PCSK9i administration (T0) and 1 month (T1), 2 months (T2), 3 months (T3) and 6 months (T6) after the start of PCSK9i administration. At T3, the lipid-lowering effects of PCSK9i were evaluated. If patients did not attain their LDL targets, the follow-up was terminated at T3. The LDL target was defined by a reduction in the mean level of LDL-C at T1, T2 and T3 > 5% compared to T0.

Biochemical analysis

The counts and percentages of neutrophils, lymphocytes and monocytes, serum TC, TG, LDL-C, high-density lipoprotein cholesterol (HDL-C), fasting plasma glucose (FPG), alanine transaminase (ALT), aspartate transaminase (AST), creatinine (CREA), platelet count, mean platelet volume (MPV) and high-sensitivity C-reactive protein (hsCRP) were assessed using a Roche COBAS 701 analyser. The levels of LDL-C were measured with a direct assay (LDL-Cholesterol Gen.3 (LDLC3), Roche Diagnostics). The NLR, PLR, MHR, MLR and MPVLR were calculated by the aforementioned values.

Statistical analysis

Continuous data are expressed as the mean ± standard deviation (SD), and categorical data are expressed as the frequency (percentage). SPSS software version 25.0 (SPSS, Inc., Chicago, IL) and R software (R Studio, Version February 1, 5001; Boston, MA, USA). were used for statistical analyzes and graphs. Normality of the distribution was assessed by the Kolmogorov-Smirnov test. For continuous variables that satisfied a normal distribution, independent two-sample t tests or paired t tests were used; otherwise, the Mann-Whitney test was used. Categorical data were compared by the Chi-square test. For all analyzes, P values < 0.05 were considered indicative of statistical significance.

Results

In this study, 67 probable HoFH patients were evaluated. Of these, 25 definitive patients had received maximum stable statins plus ezetimibe therapy for at least one month. Then these patients started to add 420 mg PCSK9i Q4W and were followed up once a month. Twelve patients withdrew (2 at T1, 2 at T2, 5 at T3 and 3 at T4; Fig. 1). Ultimately, 13 patients completed the 6-month follow-up. Meanwhile, 47 HD were recruited as controls.

Baseline characteristics of the participants before PCSK9i therapy

Among the 25 patients enrolled in this study, six patients had homozygous LDLR mutations, 15 patients had compound heterozygous LDLR mutations, one patient had LDLR and PCSK9 double heterozygous mutations, one patient had homozygous LDLRAP1 mutations, and two patients had double LDLR mutations and APOB heterozygous mutations (Fig. 2; Supplemental Table 1). Twenty-four patients performed the ATT test and 22 displayed a prominent thicker Achilles tendon (normal value is approximately 4–7 mm [41]) (Supplemental Table 1). The baseline characteristics of the participants before PCSK9i therapy are summarized in Table 1. Compared to HD, HoFH patients had markedly higher levels of TC and LDL-C (4.17 ± 0.81 *vs.* 10.80 ± 4.07 mmol/L, P < 0.0001, 1.36 ± 0.30 *vs.* 9.00 ± 3.76 mmol/L, P < 0.0001, respectively). Of note, HoFH patients displayed a disturbed blood biomarker profile. Among eight platelet and white blood cell (WBC) parameters, seven were significantly different between HD and HoFH patients, including increased MPV (10.02 ± 0.99 *vs.* 10.61 ± 1.22 fL, P = 0.03),

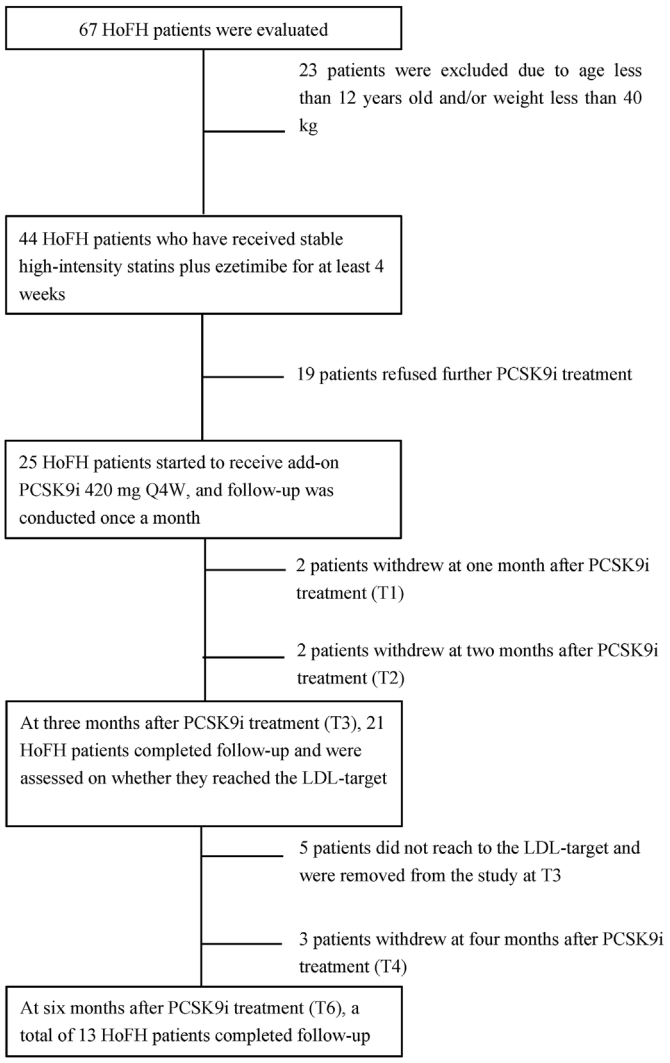

Fig. 1. Study population flowchart.

neutrophil counts and percentages (4.05 ± 13.56 *vs.* 6.23 ± 3.65 *10^9/L, P < 0.001, 56.08 ± 10.33 *vs.* 66.80 ± 7.25, P < 0.0001, respectively), decreased lymphocyte counts and percentages (2.59 ± 0.79 *vs.* 2.29 ± 1.22 × 10^9/L, P = 0.03, 35.86 ± 9.32 *vs.* 26.11 ± 6.41, P < 0.0001, respectively), monocyte percentages (8.06 ± 2.44 *vs.* 7.09 ± 1.75 × 10^9/L, P = 0.04), and platelet counts (242.60 ± 59.37 *vs.* 210.24 ± 61.06 × 10^9/L, P = 0.007) in HoFH patients. The monocyte counts were comparable between HoFH patients and HD. The systemic inflammatory biomarkers NLR, MHR, MLR and MPVLR that derived from the above parameters were significantly higher in HoFH patients (68.90%, 106.67%, 27.27%, and 29.50% higher than in HD, respectively). The PLR and hsCRP showed no significant difference between the two groups. In addition, no correlation between LDL and systemic inflammatory biomarker was observed (Supplemental Fig. 2).

Effects of PCSK9i therapy on inflammatory biomarkers in HoFH patients

After 3 months of add-on PCSK9i therapy, the levels of TC and LDL-C were significantly reduced by 13.56% and 20.28% (from 11.06 ± 4.21 mmol/L to 9.56 ± 4.80 mmol/L, P < 0.01; from 9.37 ± 3.84 mmol/L to 7.47 ± 4.17 mmol/L, P < 0.001, respectively), which were still obviously higher than HD. Moreover, three of seven

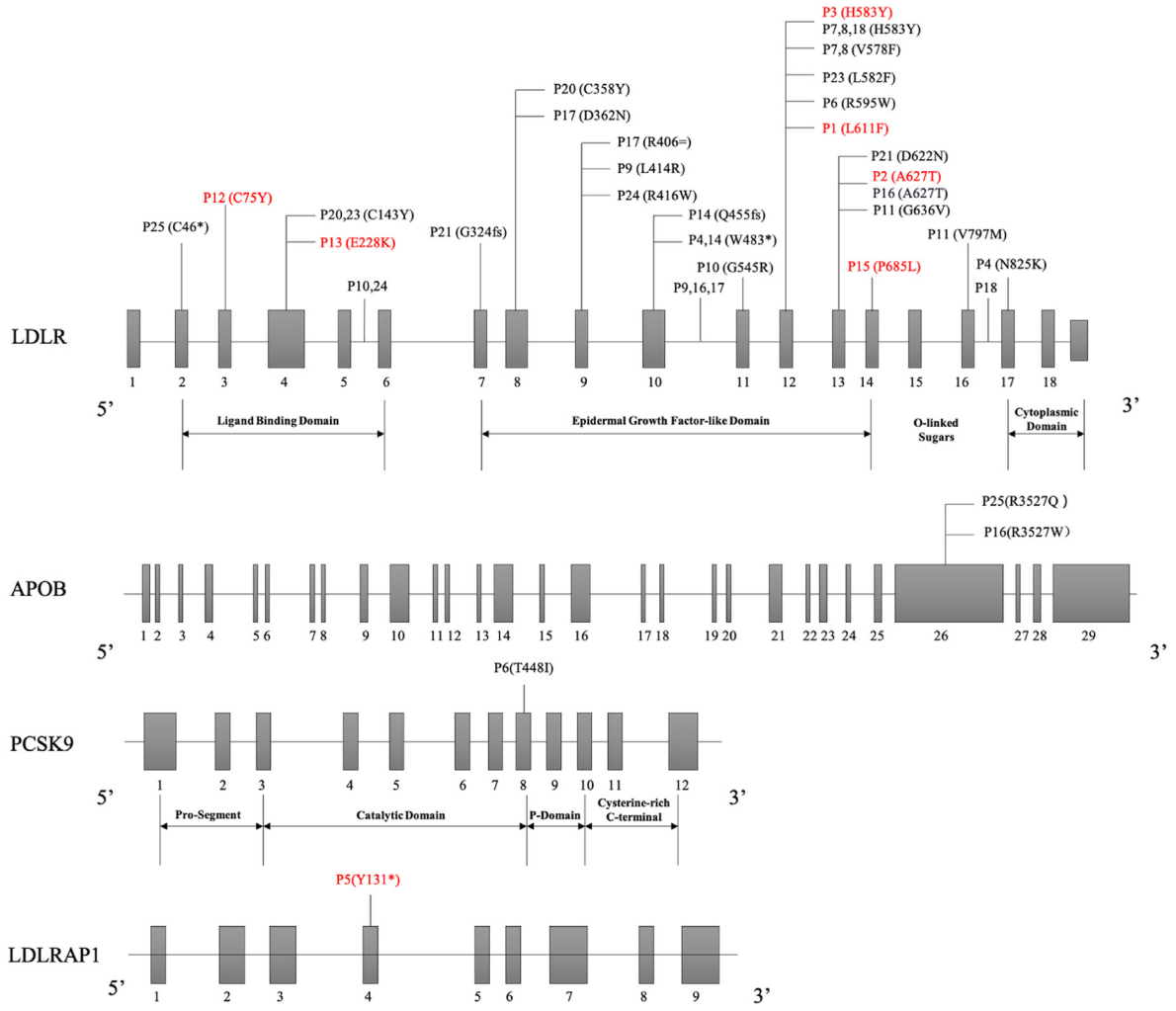

Fig. 2. The location of mutations in LDLR, APOB, PCSK9 and LDLRAP1 gene.

of the above disturbed platelet and WBC parameters statistically recovered, including percentage and count of neutrophils (from 68.06 ± 6.47 to 63.47 ± 5.24, $P < 0.01$; from 6.80 ± 3.72 to 4.38 ± 1.31 mmol/L, $P < 0.001$, respectively), and percentage of lymphocytes (from 24.98 ± 5.66 to 29.72 ± 5.13 mmol/L, $P < 0.001$). The monocyte counts significantly decreased after 3 months of PCSK9i therapy (from 0.70 ± 0.48 to 0.45 ± 0.13 mmol/L, $P < 0.001$). Furthermore, PCSK9i therapy reduced two inflammatory biomarkers, the NLR and MLR (−22.68% and −17.24%, respectively) (Table 2). And MLR returned to a level that was not statistically different from HD.

The NLR and MLR were further analyzed in 13 patients who completed 6 months of therapy. The LDL levels at T6 were lower than the levels before PCSK9i treatment ($P < 0.01$). However, the NLR and MLR at T6 returned to the levels of T0 ($P = 0.07$ and $P = 0.32$, respectively). Furthermore, the cell counts of lymphocytes at T6 were statistically comparable to the levels before PCSK9i treatment ($P = 0.59$) (Fig. 2). Each color represented an individual (Fig. 3). Furthermore, we analyzed the above changes according to their genetics type (true HoFH vs. cHeFH and DH). No noticeable differences in the index between the two groups was observed (Supplemental Fig. 1).

Discussion

Since hypercholesterolemia-induced inflammation contributes to the pathogenesis of AS and CVD [42], it is tempting to propose that the anti-inflammation effects of lipid-lowering drugs could be a consequence of reduced levels of LDL cholesterol. However, the lipid-lowering effect of statins was limited in HeFH and HoFH patients [43]. Even with the highest doses of most efficacious statins, only approximately 20% of HeFH patients can meet LDL targets [44], and LDL levels were still approximately 10 mmol/L in most HoFH patients [5]. Consistent with the previous studies, our HoFH cohort with maximum tolerated statin dose plus ezetimibe displayed high LDL-C levels (9.00 ± 3.76 mmol/L) and a hyperinflammatory state (an increase in MPV, neutrophil count and percentage; a decrease in monocyte percentage, platelet counts, lymphocyte count and percentage). Although LDL-C levels were reduced by 20.28% (from 9.37 ± 3.84 mmol/L to 7.47 ± 4.17 mmol/L) after adding on three months of PCSK9i treatment, the absolute LDL-C levels were still dramatically higher than the recommended levels in current guidelines [6] (<1.8 mmol/L for patients without CVD, and <1.4 mmol/L for patients with CVD). Strikingly, we found partial recovery of several systemic inflammation parameters in the presence of high cholesterol levels. In addition, we did not observe a close correlation between NLR and LDL-C levels, and noticeable differences in the tested parameters between HoFH and cHeFH. Therefore, PCSK9i might exert anti-inflammation effects independent its lipid-lowering effect.

Our results are consistent with several previous studies showing a relationship between PCSK9 and inflammation. It have been reported that PCSK9 directly promotes pro-inflammatory responses

Table 1
Characteristics of HD and HoFH patients on maximum tolerated statin dose plus ezetimibe.

	HD (n = 47)	HoFH (n = 25)	P value
Demographic profile			
Age, yr	29.34 ± 8.03	29.08 ± 11.32	
Men, n (%)	22 (47)	11(44)	
Genetic status, n			
Homozygous LDLR		6	
Compound heterozygous LDLR		15	
Double heterozygous (LDLR + PCSK9)		1	
Homozygous LDLRAP1		1	
Double LDLR mutation + APOB heterozygous		2	
Lipid profile			
TC, mmol/L	4.17 ± 0.81	10.80 ± 4.07	< 0.0001
TG, mmol/L	0.97 ± 0.39	0.88 ± 0.44	0.55
LDL-C, mmol/L	1.36 ± 0.30	9.00 ± 3.76	< 0.0001
HDL-C, mmol/L	2.42 ± 0.64	0.83 ± 0.31	< 0.0001
Glucose profile			
FPG, mmol/L	5.07 ± 0.44	4.79 ± 0.33	< 0.001
Liver and kidney index			
ALT, U/L	18.67 ± 15.20	25.24 ± 17.56	0.16
AST, U/L	17.58 ± 5.59	24.92 ± 9.58	< 0.01
CREA, umol/L	64.66 ± 13.70	60.49 ± 10.17	0.13
Platelet profile			
Platelets, 10^9/L	242.60 ± 59.37	210.24 ± 61.06	0.007
MPV, fL	10.02 ± 0.99	10.61 ± 1.22	0.03
White blood cell count and percentage			
Neutrophil, %	56.08 ± 10.33	66.80 ± 7.25	< 0.0001
Neutrophil count, 10^9/L	4.05 ± 13.56	6.23 ± 3.65	< 0.001
Lymphocyte, %	35.86 ± 9.32	26.11 ± 6.41	< 0.0001
Lymphocyte count, 10^9/L	2.59 ± 0.79	2.29 ± 1.22	0.03
Monocyte, %	8.06 ± 2.44	7.09 ± 1.75	0.04
Monocyte count, 10^9/L	0.57 ± 0.20	0.66 ± 0.45	0.68
Inflammatory profile			
NLR	1.64 ± 0.59	2.77 ± 0.92	< 0.0001
PLR	99.07 ± 29.56	102.41 ± 3.59	0.94
MHR	0.45 ± 0.25	0.93 ± 0.75	< 0.01
MLR	0.22 ± 0.06	0.28 ± 0.07	< 0.01
MPVLR	4.17 ± 1.20	5.40 ± 2.29	< 0.01
hsCRP	1.49 ± 0.90	2.87 ± 4.44	0.78

The data shown are the mean ± SD or number (%). TC = total cholesterol; TG = triglycerides; LDL-C = low-density lipoprotein cholesterol; HDL-C = high-density lipoprotein cholesterol; FPG = fasting plasma glucose; ALT = alanine transaminase; AST = aspartate transaminase; CREA = creatinine; MPV = mean platelet volume; NLR = Neutrophil-lymphocyte ratio; PLR = Platelet-lymphocyte ratio; MHR = Monocyte-HDL ratio; MLR = Monocyte-lymphocyte ratio; MPVLR = MPV-lymphocyte ratio; hsCRP = high-sensitivity C-reactive protein.

Table 2
Characteristics of HoFH patients before and after 3 months of PCSK9i therapy.

	HD (n = 47) (1)	HoFH subjects (n = 21) T0 (2)	HoFH subjects (n = 21) T3 (3)	P value (2) Vs (3)	P value (1) Vs (3)
Lipid profile					
TC, mmol/L	4.17 ± 0.81	11.06 ± 4.21	9.56 ± 4.80	< 0.01	< 0.001
TG, mmol/L	0.97 ± 0.39	0.82 ± 0.33	0.81 ± 0.36	0.60	0.12
LDL-C, mmol/L	1.36 ± 0.30	9.37 ± 3.84	7.47 ± 4.17	< 0.001	< 0.001
HDL-C, mmol/L	2.42 ± 0.64	0.83 ± 0.31	0.85 ± 0.34	0.67	< 0.001
Glucose profile					
FPG, mmol/L	5.07 ± 0.44	4.80 ± 0.35	4.96 ± 0.55	0.19	0.36
Liver and kidney index					
ALT, U/L	18.67 ± 15.20	22.48 ± 13.58	24.67 ± 14.76	0.85	0.03
AST, U/L	17.58 ± 5.59	24.14 ± 9.12	27.14 ± 10.34	0.17	< 0.001
CREA, umol/L	64.66 ± 13.70	61.2 ± 8.93	60.36 ± 11.73	0.58	0.22
Platelet profile					
Platelets, 10^9/L	242.60 ± 59.37	214.48 ± 65.60	214.33 ± 67.51	0.98	0.08
MPV, fL	10.02 ± 0.99	10.65 ± 1.25	10.42 ± 1.03	0.13	0.13
White blood cell count and percentage					
Neutrophil, %	56.08 ± 10.33	68.06 ± 6.47	63.47 ± 5.24	< 0.01	< 0.01
Neutrophil count, 10^9/L	4.05 ± 13.56	6.80 ± 3.72	4.38 ± 1.31	< 0.001	< 0.001
Lymphocyte, %	35.86 ± 9.32	24.98 ± 5.66	29.72 ± 5.13	< 0.001	< 0.01
Lymphocyte count, 10^9/L	2.59 ± 0.79	2.40 ± 1.30	2.05 ± 0.70	0.32	< 0.01
Monocyte, %	8.06 ± 2.44	6.97 ± 1.59	6.80 ± 1.75	0.70	0.05
Monocyte count, 10^9/L	0.57 ± 0.20	0.70 ± 0.48	0.45 ± 0.13	< 0.001	< 0.001
inflammatory profile					
NLR	1.64 ± 0.59	2.91 ± 0.90	2.25 ± 0.77	< 0.001	< 0.01
PLR	99.07 ± 29.56	100.27 ± 37.93	111.51 ± 37.97	0.12	0.15
MHR	0.45 ± 0.25	1.01 ± 0.79	0.75 ± 0.80	0.10	0.02
MLR	0.22 ± 0.06	0.29 ± 0.07	0.24 ± 0.08	< 0.01	0.57
MPVLR	4.17 ± 1.20	5.35 ± 2.43	5.76 ± 2.38	0.25	< 0.001
hsCRP	1.49 ± 0.90	2.75 ± 4.35	2.84 ± 4.73	0.99	0.67

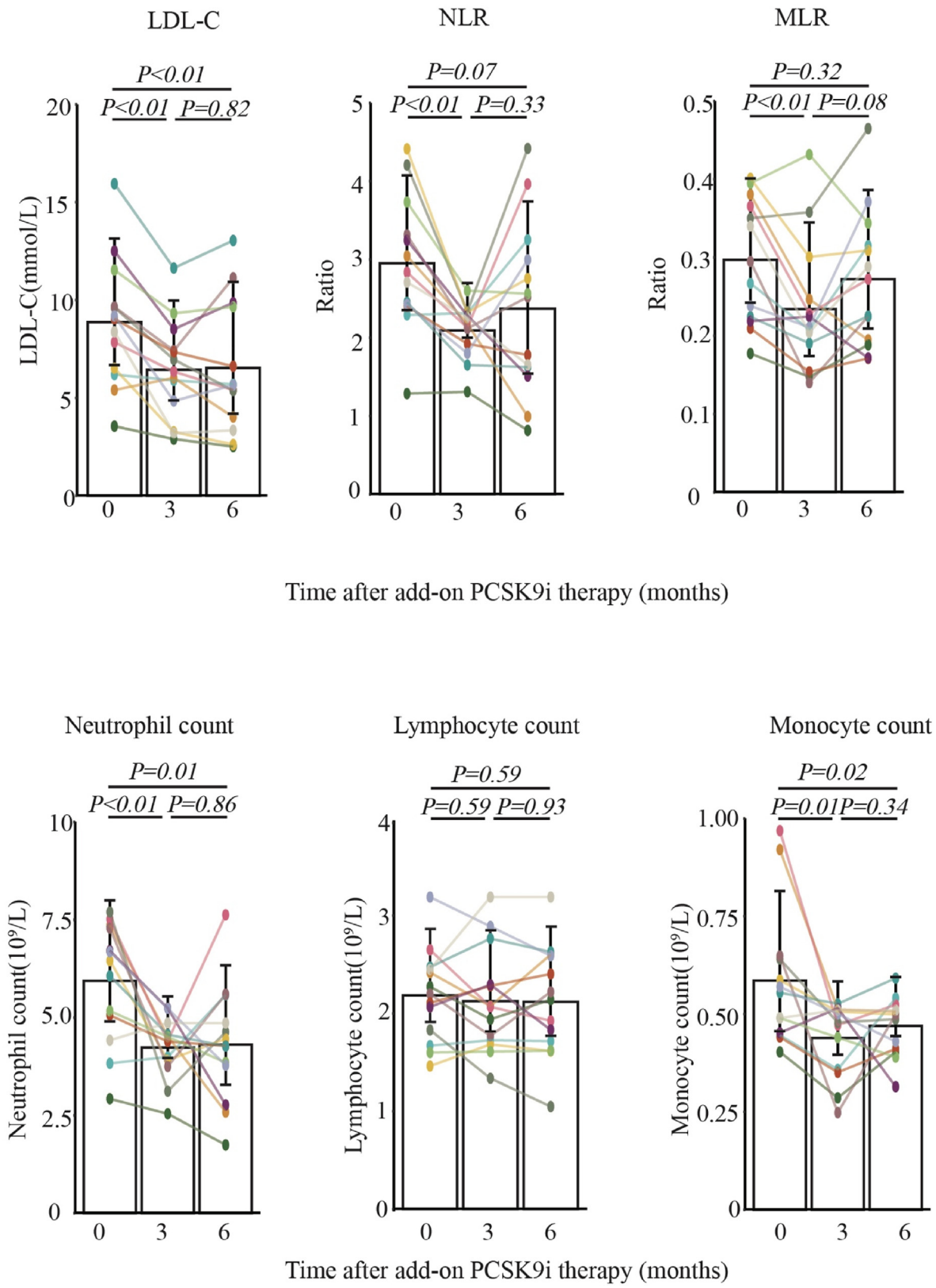

Fig. 3. The levels of LDL and the NLR, MLR, neutrophil count, lymphocyte count, and monocyte count before PCSK9i treatment and at 3 months and 6 months after PCSK9i treatment.

in macrophages [45], liver cells and a variety of tissues [46]. High serum PCSK9 levels in patients with CVD were associated with CRP and pro-inflammatory cytokines IL-6, IL-1β, TNF-α and M-CSF [46]. Giunzioni et al. [47] reported that PCSK9 could act directly on immune cells in a cholesterol-independently manner. Consistently, Liu et al. revealed a direct, LDL-C reduction independent, anti-inflammation effect of PCSK9i [48].

It should be emphasized that add-on PCSK9i therapy significantly reduced both NLR and MLR. Since lymphocyte counts were comparable during this period, the reduction in NLR and MLR should be attributed to a significant decrease in neutrophil counts and monocyte counts. Although further investigations are still needed, clinical trials with CAD patients revealed that serum PCSK9 concentration was positively correlated with neutrophil and lymphocyte numbers [49]. There is growing evidence suggesting that both monocytes and neutrophils contribute to cardiovascular inflammation and the development of atherosclerotic plaques [50,51]. For example, neutrophils stimulate the activation and dysregulation of the endothelial cells by secreting reactive oxygen species (ROS) [52] and myeloperoxidase (MPO) [53]. MPO also mediates the oxidation of LDL, promoting the formation of foam cells. Bernelot et al. [54] reported that PCSK9i decreased migration capacity of monocytes in FH patients. It should be noted that LDL-C also promotes pro-inflammatory status of monocytes and myelomonocytic skewing during bone marrow hematopoiesis [55]. Cholesterol-lowering treatment only reverts the myelomonocytic skewing in hematopoietic stem and progenitor cells. Further research is required to investigate whether a rebound of inflammatory parameters is related to the failure of recovery of the defects during early hematopoiesis.

There are some limitations of this study. First, this study had a single-center design and was limited to Chinese patients. However, the present study showed a similar prevalence of dyslipidaemia and other risk factors with large contemporary trials [56] and real-world registries [57] including those involving other ethnicities. Second, the small sample size of this study limited the generalizability of the results, and further analysis with large sample size is required to confirm these results. Third, since the reduction in LDL-C levels with PCSK9i treatment was more profound in HeFH patients than in HoFH patients, similar research should be carried out in HeFH patients to further illustrate the separate impact of LDL-C reductions and PCSK9i itself. Forth, several studies have shown that PCSK9 induced a pro-inflammatory phenotype in human macrophages, thus, additional analysis of more markers and the change of plasma PCSK9 levels should also be included.

Unfortunately, the NLR and MLR returned to pre-PCSK9i treatment levels at 6 months after PCSK9i treatment. It remains unclear why PCSK9i only induces a transient decrease in the NLR and MLR, whether HoFH patients may gain clinical benefit, and whether other therapies targeting PCSK9 such as inclisiran also show anti-inflammation effect. In-depth investigations should be conducted in further studies.

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Author contribution

F.L., P.Y.: Acquisition of data and writing; Y,H., J.D., H,Z., Z.W., X.W., Y.M.: Analysis, interpretation of data, and technical, material support; H.Z., J.L.: Study supervision and revision of the manuscript. All authors read and approved the final manuscript.

Declaration of competing interest

The authors declare that they have no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.athplu.2022.05.002.

References

[1] Kastelein JP, Reeskamp LF, Hovingh GK. Familial hypercholesterolemia the most common monogenic disorder in humans. J Am Coll Cardiol 2020;75(20):2567–9. https://doi.org/10.1016/j.jacc.2020.03.058.
[2] Defesche JC, Gidding SS, Harada-shiba M, et al. Familial hypercholesterolaemia. Nat Rev Dis Prim 2017;3:1–20. https://doi.org/10.1038/nrdp.2017.93.
[3] Hu P, Dharmayat KI, Stevens CAT, et al. Prevalence of familial hypercholesterolemia among the general population and patients with atherosclerotic cardiovascular disease: a systematic review and meta-analysis. Circulation 2020;141(22):1742–59. https://doi.org/10.1161/CIRCULATIONAHA.119.044795.
[4] Defesche JC, Gidding SS, Harada-Shiba M, et al. Familial hypercholesterolaemia. Nat Rev Dis Prim 2017;3:17093. https://doi.org/10.1038/nrdp.2017.93.
[5] Cuchel M, Bruckert E, Ginsberg HN, et al. Homozygous familial hypercholesterolaemia: new insights and guidance for clinicians to improve detection and clinical management. A position paper from the Consensus Panel on Familial Hypercholesterolaemia of the European Atherosclerosis Society. Eur Heart J 2014;35(32):2146–57. https://doi.org/10.1093/eurheartj/ehu274.
[6] Mach F, Baigent C, Catapano AL, et al. ESC/EAS Guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk. Eur Heart J 2019;41(1):111–88. https://doi.org/10.1093/eurheartj/ehz455. 2020.
[7] Benn M, Watts GF, Tybjaerg-Hansen A, Nordestgaard BG. Familial hypercholesterolemia in the Danish general population: prevalence, coronary artery disease, and cholesterol-lowering medication. J Clin Endocrinol Metab 2012;97(11):3956–64. https://doi.org/10.1210/jc.2012-1563.
[8] Marais AD, Blom DJ, Firth JC. Statins in homozygous familial hypercholesterolemia. Curr Atherosclerosis Rep 2002;4(1):19–25. https://doi.org/10.1007/s11883-002-0058-7.
[9] Murphy SA, Pedersen TR, Gaciong ZA, et al. Effect of the PCSK9 inhibitor evolocumab on total cardiovascular events in patients with cardiovascular disease: a prespecified analysis from the fourier trial. JAMA Cardiol 2019;4(7):613–9. https://doi.org/10.1001/jamacardio.2019.0886.
[10] Preiss D, Tobert JA, Hovingh GK, Reith C. Lipid-modifying agents, from statins to PCSK9 inhibitors: JACC focus seminar. J Am Coll Cardiol 2020;75(16):1945–55. https://doi.org/10.1016/j.jacc.2019.11.072.
[11] Raal FJ, Honarpour N, Blom DJ, et al. Inhibition of PCSK9 with evolocumab in homozygous familial hypercholesterolaemia (TESLA Part B): a randomised, double-blind, placebo-controlled trial. Lancet 2015;385(9965):341–50. https://doi.org/10.1016/S0140-6736(14)61374-X.
[12] Blom DJ, Harada-Shiba M, Rubba P, et al. Efficacy and safety of alirocumab in adults with homozygous familial hypercholesterolemia: the ODYSSEY HoFH trial. J Am Coll Cardiol 2020;76(2):131–42. https://doi.org/10.1016/j.jacc.2020.05.027.
[13] Tall AR, Yvan-Charvet L. Cholesterol, inflammation and innate immunity. Nat Rev Immunol 2015;15(2):104–16. https://doi.org/10.1038/nri3793.
[14] Rahman T, Hamzan NS, Mokhsin A, et al. Enhanced status of inflammation and endothelial activation in subjects with familial hypercholesterolaemia and their related unaffected family members : a case control study. Lipids Health Dis 2017;16(81):1–12. https://doi.org/10.1186/s12944-017-0470-1.
[15] Stapleton PA, Goodwill AG, James ME, et al. Hypercholesterolemia and microvascular dysfunction: interventional strategies. J Inflamm 2010;7:1–10. https://doi.org/10.1186/1476-9255-7-54.
[16] Rao LN, Ponnusamy T, Philip S, et al. Hypercholesterolemia induced immune response and inflammation on progression of atherosclerosis in Apobtm2Sgy Ldlrtm1Her/J mice. Lipids 2015;50(8):785–97. https://doi.org/10.1007/s11745-015-4046-4.
[17] Groh L, Keating ST, Joosten LAB, et al. Monocyte and macrophage immunometabolism in atherosclerosis. Semin Immunopathol 2018;40(2):203–14. https://doi.org/10.1007/s00281-017-0656-7.
[18] Moore KJ, Sheedy FJ, Fisher EA. Macrophages in atherosclerosis: a dynamic balance. Nat Rev Immunol 2013;13(10):709–21. https://doi.org/10.1038/nri3520.

[19] Alberts-Grill N, Denning TL, Rezvan A, et al. The role of the vascular dendritic cell network in atherosclerosis. Am J Physiol Cell Physiol 2013;305(1). https://doi.org/10.1152/ajpcell.00017.2013.
[20] Saigusa R, Winkels H, Ley K. T cell subsets and functions in atherosclerosis. Nat Rev Cardiol 2020;17(7):387–401. https://doi.org/10.1038/s41569-020-0352-5.
[21] Silvestre-Roig C, Braster Q, Ortega-Gomez A, et al. Neutrophils as regulators of cardiovascular inflammation. Nat Rev Cardiol 2020;17(6):327–40. https://doi.org/10.1038/s41569-019-0326-7.
[22] Nording H, Baron L, Langer HF. Platelets as therapeutic targets to prevent atherosclerosis. Atherosclerosis 2020;307(January):97–108. https://doi.org/10.1016/j.atherosclerosis.2020.05.018.
[23] Martínez-López D, Roldan-Montero R, García-Marqués F, et al. Complement C5 protein as a marker of subclinical atherosclerosis. J Am Coll Cardiol 2020;75(16):1926–41. https://doi.org/10.1016/j.jacc.2020.02.058.
[24] Adamstein NH, MacFadyen JG, Rose LM, et al. The neutrophil-lymphocyte ratio and incident atherosclerotic events: analyses from five contemporary randomized trials. Eur Heart J 2021;42(9):896–903. https://doi.org/10.1093/eurheartj/ehaa1034.
[25] Corriere T, Di Marca S, Cataudella E, et al. Neutrophil-to-Lymphocyte Ratio is a strong predictor of atherosclerotic carotid plaques in older adults. Nutr Metabol Cardiovasc Dis 2018;28(1):23–7. https://doi.org/10.1016/j.numecd.2017.10.022.
[26] Temiz A, Gazi E, Güngör Ö, et al. Platelet/lymphocyte ratio and risk of in-hospital mortality in patients with ST-elevated myocardial infarction. Med Sci Mon Int Med J Exp Clin Res 2014;20:660–5. https://doi.org/10.12659/MSM.890152.
[27] Qiu Z, Jiang Y, Jiang X, et al. Relationship between platelet to lymphocyte ratio and stable coronary artery disease: meta-analysis of observational studies. Angiology 2020;71(10):909–15. https://doi.org/10.1177/0003319720943810.
[28] Cetin MS, Ozcan Cetin EH, Kalender E, et al. Monocyte to HDL cholesterol ratio predicts coronary artery disease severity and future major cardiovascular adverse events in acute coronary syndrome. Heart Lung Circ 2016;25(11):1077–86. https://doi.org/10.1016/j.hlc.2016.02.023.
[29] Canpolat U, Çetin EH, Cetin S, et al. Association of monocyte-to-HDL cholesterol ratio with slow coronary flow is linked to systemic inflammation. Clin Appl Thromb Hemost 2016;22(5):476–82. https://doi.org/10.1177/1076029615594002.
[30] Ji H, Li Y, Fan Z, et al. Monocyte/lymphocyte ratio predicts the severity of coronary artery disease: a syntax score assessment. BMC Cardiovasc Disord 2017;17(1):1–8. https://doi.org/10.1186/s12872-017-0507-4.
[31] Zuo B, Zhao D. Monocyte/lymphocyte ratio is associated with carotid stenosis in ischemic stroke : a retrospective analysis. Brain Behav 2019;9:1–8. https://doi.org/10.1002/brb3.1429.
[32] Besli F, Ilter A, Gungoren F. The link between mean platelet volume to lymphocyte ratio and complexity of coronary artery disease. Angiology 2018;69(4):358–9. https://doi.org/10.1177/0003319717743315.
[33] Fani L, Van Der Willik KD, Bos D, et al. The association of innate and adaptive immunity, subclinical atherosclerosis, and cardiovascular disease in the Rotterdam Study: a prospective cohort study. PLoS Med 2020;17(5):1–16. https://doi.org/10.1371/journal.pmed.1003115.
[34] Oikonomou E, Leopoulou M, Theofilis P, et al. A link between inflammation and thrombosis in atherosclerotic cardiovascular diseases: clinical and therapeutic implications. Atherosclerosis 2020;309(July):16–26. https://doi.org/10.1016/j.atherosclerosis.2020.07.027.
[35] Khodadi E. Platelet function in cardiovascular disease: activation of molecules and activation by molecules. Cardiovasc Toxicol 2020;20(1). https://doi.org/10.1007/s12012-019-09555-4.
[36] Wang N, Tall AR. Cholesterol in platelet biogenesis and activation. Blood 2016;127(16):1949–53. https://doi.org/10.1182/blood-2016-01-631259.
[37] Kamath S, Blann AD, Lip GYH. Platelet activation: assessment and quantification. Eur Heart J 2001;22(17):1561–71. https://doi.org/10.1053/euhj.2000.2515.
[38] Montecucco F, Carbone F, Liberale L, et al. Challenges in reducing atherosclerotic inflammation in patients with familial hypercholesterolemia. Eur J Prev Cardiol 2019. https://doi.org/10.1177/2047487319862907.
[39] Scicali R, Di A, Viviana P, et al. Effect of PCSK9 inhibitors on pulse wave velocity and monocyte - to - HDL - cholesterol ratio in familial hypercholesterolemia subjects : results from a single - lipid - unit real - life setting. Acta Diabetol 2021;58(7):949–57. https://doi.org/10.1007/s00592-021-01703-z.
[40] Baragetti A, Casula M, Scarinzi P, et al. Achilles tendon ultrasonography in familial hypercholesterolemia: a sub-study of the LIpid transPort disorders Italian GEnetic Network (LIPIGEN). J Intern Med 2021:1–3. https://doi.org/10.1111/joim.13421.
[41] Scott A, Zahradnik TM, Squier K, et al. Diagnostic accuracy of ultrasound and MRI for Achilles tendon xanthoma in people with familial hypercholesterolemia: a systematic review. J Clin Lipidol 2019;13(1):40–8. https://doi.org/10.1016/j.jacl.2018.10.013.
[42] Gungoren F, Besli F, Caliskan S, et al. Statin therapy may not effect NLR and MPV levels in patients with hypercholesterolemia: A Retrospective Study. Angiology 2015. https://doi.org/10.1177/0003319715604098.
[43] Raper A, Kolansky DM, Cuchel M. Treatment of familial hypercholesterolemia: is there a need beyond Statin therapy? Curr Atherosclerosis Rep 2012;14:11–6. https://doi.org/10.1007/s11883-011-0215-y.
[44] Korneva V, Kuznetsova T, Julius U. Efficiency and problems of statin therapy in patients with heterozygous familial hypercholesterolemia. Atherosclerosis Suppl 2019;40:79–87. https://doi.org/10.1016/j.atherosclerosissup.2019.08.029.
[45] Ricci C, Ruscica M, Camera M, et al. PCSK9 induces a pro-inflammatory response in macrophages. Nature 2018;8(1):1–10. https://doi.org/10.1038/s41598-018-20425-x.
[46] Ding Z, Pothineni NVK, Goel A, et al. PCSK9 and inflammation: role of shear stress, pro-inflammatory cytokines, and LOX-1. Cardiovasc Res 2020;116(5):908–15. https://doi.org/10.1093/cvr/cvz313.
[47] Giunzioni Ilaria, Tavori Hagai, Covarrubias Roman, et al. Local effects of human PCSK9 on the atherosclerotic lesion. Physiol Behav 2016;238(1):52–62. https://doi.org/10.1002/path.4630.
[48] Liu A, Frostegård J. PCSK9 plays a novel immunological role in oxidized LDL-induced dendritic cell maturation and activation of T cells from human blood and atherosclerotic plaque. J Intern Med 2018;284(2):193–210. https://doi.org/10.1111/joim.12758.
[49] Li S, Guo Y, Xu R, et al. Association of plasma PCSK9 levels with white blood cell count and its subsets in patients with stable coronary artery disease. Atherosclerosis 2014;234(2):441–5. https://doi.org/10.1016/j.atherosclerosis.2014.04.001.
[50] Gomez I, Ward B, Souilhol C, et al. Neutrophil microvesicles drive atherosclerosis by delivering miR-155 to atheroprone endothelium. Nat Commun 2020;11(1):1–18. https://doi.org/10.1038/s41467-019-14043-y.
[51] Warnatsch Annika, Ioannou Marianna, Qian Wang VP. Neutrophil extracellular traps license macrophages for cytokine production in atherosclerosis. Science 2015;349(6245):316–20. https://doi.org/10.1126/science.aaa8064.80-.
[52] Burtenshaw D, Kitching M, Redmond EM, et al. Reactive oxygen species (ROS), intimal thickening, and subclinical atherosclerotic disease. Front Cardiovasc Med 2019;6(August):1–18. https://doi.org/10.3389/fcvm.2019.00089.
[53] Cheng D, Talib J, Stanley CP, et al. Inhibition of MPO (myeloperoxidase) attenuates endothelial dysfunction in mouse models of vascular inflammation and atherosclerosis. Arterioscler Thromb Vasc Biol 2019;39(7):1448–57. https://doi.org/10.1161/ATVBAHA.119.312725.
[54] Bernelot Moens SJ, Neele AE, Kroon J, et al. PCSK9 monoclonal antibodies reverse the pro-inflammatory profile of monocytes in familial hypercholesterolaemia. Eur Heart J 2017;38:1584–93. https://doi.org/10.1093/eurheartj/ehx002.
[55] Stiekema LCA, Willemsen L, Kaiser Y, et al. Impact of cholesterol on proinflammatory monocyte production by the bone marrow. Eur Heart J 2021;42(42):4309–20. https://doi.org/10.1093/eurheartj/ehab465.
[56] Valgimigli M, Gragnano F, Branca M, et al. P2Y12 inhibitor monotherapy or dual antiplatelet therapy after coronary revascularisation: individual patient level meta-analysis of randomised controlled trials. BMJ 2021:373. https://doi.org/10.1136/bmj.n1332.
[57] Cesaro A, Gragnano F, Calabrò P, et al. Prevalence and clinical implications of eligibility criteria for prolonged dual antithrombotic therapy in patients with PEGASUS and COMPASS phenotypes: insights from the START-ANTIPLATELET registry. Int J Cardiol 2021;345(July):7–13. https://doi.org/10.1016/j.ijcard.2021.10.138.