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Original Article

Evaluations of metabolic and innate immunity profiles in subjects with familial hypercholesterolemia with or without subclinical atherosclerosis

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ABSTRACT

Background: Familial hypercholesterolemia (FH) is a genetic condition characterized by high low-density lipoprotein cholesterol (LDL-C). The presence of risk modifiers could promote the atherosclerotic injury beyond LDL-C. Our aim was to evaluate metabolic and innate immunity profiles in FH subjects with or without subclinical atherosclerosis.

Methods: In this cross-sectional observational study, we evaluated 211 genetically confirmed FH subjects on LDL-C target and without cardiovascular diseases. Biochemical analyses, LDL-C burden (LCB) calculation and vascular profile evaluation were obtained from all subjects. Study population was divided into two groups according to subclinical atherosclerosis: the subclinical atherosclerosis (SA) group and non-subclinical atherosclerosis (NSA) group.

Results: SA group had higher LDL-C at diagnosis (288.35 ± 24.52 vs 267.92 ± 23.86, $p < 0.05$) and LCB (13,465.84 ± 3617.46 vs 10,872.63 ± 3594.7, $p < 0.001$) than NSA group. SA group had higher white blood cell count (WBCC, 6.9 ± 1.66 vs 6.1 ± 1.16), neutrophil count (NC, 4.2 ± 1.3 vs 3.6 ± 1.11), monocyte count (MC, 0.8 ± 0.2 vs 0.4 ± 0.1), triglyceride to high-density lipoprotein ratio (TG/HDL, 1.73 ± 0.72 vs 1.45 ± 0.69), triglyceride-glucose index (TyG, 8.29 ± 0.35 vs 8.01 ± 0.33) than NSA group (p value for all < 0.01). Multivariate logistic regression analysis showed that LCB ($p < 0.01$), WBCC ($p < 0.01$), NC ($p < 0.05$), MC ($p < 0.05$) were associated with subclinical atherosclerosis. Simple linear regression analyses showed that LCB was associated with WBCC, NC, MC (p value for all < 0.01).

Conclusion: An increased LCB and an impaired innate immunity profile were found in FH subjects with subclinical atherosclerosis and they were independently associated with atherosclerotic injury. LCB could modulate the innate immunity profile.

1. Introduction

Atherosclerosis is a chronic and progressive process promoted by several environmental and genetic factors [1,2]; among these, low-density lipoprotein cholesterol (LDL-C) has been recognized to have a causal role in the pathogenesis of atherosclerotic cardiovascular disease (ASCVD) [3]. Familial hypercholesterolemia (FH) is a genetic condition characterized by lifelong exposure to high levels of LDL-C, which favors atherosclerosis progression and increases ASCVD risk especially in early life [4]. Therefore, an important effort is required to detect and treat FH subjects in clinical practice [5]. However, not all FH subjects have the same cardiovascular risk and thus the presence of risk modifiers could promote the atherosclerotic injury beyond LDL-C levels [6].

Several studies have demonstrated the crucial role of inflammation in the pathophysiology of ASCVD and it appears to be the final expression of the systemic interplay between hypercholesterolemia and the immune system during atherosclerosis progression [7]. The

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accumulation of LDL-C in the arterial wall disrupts endothelial function, leading to increased secretion of pro-inflammatory cytokines and chemokines as well as abnormal mobilization of innate immune cells with specific pro-atherosclerotic activities, ranging from initial leukocyte recruitment to plaque rupture [1,8,9]. It has been shown that in FH subjects neutrophils infiltrated the arterial wall in an early atherosclerotic stage and the activated monocytes exhibited an important pro-inflammatory profile by increasing the atherosclerosis-related gene [10,11]; thus, the assessments of these immune parameters could be useful to identify FH subjects with a higher risk of atherosclerotic damage.

Impairment of glucose homeostasis is recognized as an important risk factor that exacerbates the atherosclerotic injury by several mechanisms [12]; in particular, insulin resistance increases liver uptake of free fatty acids derived by adipose-tissue triglyceride (TG) hydrolysis and thus the production of TG-rich lipoproteins (TRLs) that promote the atherosclerotic damage [13]. In this context, previous studies showed that in the general population the triglyceride to high-density lipoprotein ratio (TG/HDL) and the triglyceride-glucose index (TyG) were useful metabolic parameters associated with insulin sensitivity as well as with the amount of TRLs and thus they could be able to reflect the glucose-lipid balance in clinical practice [14,15]

To better define the clinical parameters that may favor atherosclerotic injury, this study aimed to investigate the metabolic and innate immunity profiles in FH subjects with or without subclinical atherosclerosis.

2. Methods

2.1. Study design and population

This was a cross-sectional observational study involving genetically confirmed FH subjects [16] enrolled from the University Hospital of Catania, Italy, from October 2022 to June 2024. This is a tertiary center for the screening, diagnosis and management of familial dyslipidemias. All subjects were aged between 18 and 70 years and were on LDL-C target at the time of enrollment; moreover, all subjects were free of ASCVD, hematopoietic disorders, malignancies and/or treatment with chemotherapy, acute infections, chronic inflammatory status and glucocorticoid therapy within the past three months. After a 12-hour fast, all participants underwent a physical examination and review of their clinical history as well as biochemical analyses and vascular profile evaluation by assessments of coronary artery calcium (CAC) score and carotid and femoral plaques. Based on the recommendations of the 2019 ESC/EAS guidelines for the management of dyslipidemias, LDL-C target was defined as a lipid value < 70 mg/dL [17]. Subclinical atherosclerosis was defined as a CAC score > 0 and/or presence of carotid and/or femoral plaques [18,19]. ASCVD was defined as documented previous myocardial infarction (MI), acute coronary syndrome (ACS), coronary revascularization (percutaneous coronary intervention (PCI) or coronary artery bypass graft surgery (CABG)) or other arterial revascularization procedures, stroke or transient ischemic attack (TIA), or peripheral arterial disease [20]. Arterial hypertension was defined as brachial blood pressure (BP) $\geq$ 140 mm Hg (systolic) and/or 90 mm Hg (diastolic) on at least two different occasions, or if the subject was on antihypertensive therapy [21]. Lipid lowering therapy was defined as a daily intake of one of the following drugs: statins, ezetimibe, or proprotein convertase subtilisin/kexin type 9 inhibitor (PCSK9i, alirocumab or evolocumab or inclisiran) [22]. Body weight and height were measured, and body mass index (BMI) was calculated as weight divided by the squared value of height (kg/m^2). Diabetes mellitus was defined as fasting plasma glucose $\geq$ 7.0 mmol/L on two consecutive readings and/or glycated hemoglobin (HbA1c) $\geq$ 48 mmol/mol or the use of anti-diabetic medications [23]. Smoking habits were divided into either current smoking (defined as any cigarette in the last month) or not.

Participants were stratified into two groups according to the presence of subclinical atherosclerosis (subclinical atherosclerosis [SA] group, 129 FH subjects) or not (non-subclinical atherosclerosis [NSA] group, 82 FH subjects). In a secondary analysis, participants were stratified into two groups according to LDL-C burden (LCB) median value of 12,148 mg-years/dL: high LCB group (HLCB group, 106 subjects) and low LCB group (LLCB group, 105 subjects).

The study was approved by the local ethics committee in accordance with the ethical standards of the institutional and national research committees and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. Informed consent was obtained from each subject enrolled in the study.

2.2. Biochemical analyses

Fasting plasma glucose (FPG) was measured with the glucose oxidase method. Serum total cholesterol (TC), TG, high-density lipoprotein cholesterol (HDL-C), high sensitivity protein C reactive (hs-CRP) were assessed by currently available enzymatic methods. LDL-C was calculated using the Friedewald formula. Non-HDL cholesterol (Non-HDL-C) and TG/HDL were derived from baseline values. TyG was calculated according the following formula: Ln [(TGxFPG)/2)] [24]. Apolipoprotein B (ApoB) and Apolipoprotein A1 (ApoA1) were evaluated with a nephelometer assay (Siemens AG Healthcare Sector, Erlangen, Germany). Levels of lipoprotein(a) [Lp(a)] were measured with the latex agglutination immunoassay [25]. Glycated hemoglobin A1c (HbA1c) was measured with high-performance liquid chromatography using a National Glycohemoglobin Standardization Program and standardized to the Diabetes Control and Complications Trial assay reference. Chromatography was performed using a certified automated analyzer (HPLC; HLC-723G7 hemoglobin HPLC analyzer; Tosoh Corp.; normal range 4.25–5.9 % [23–41 mmol/mol]). White blood cell count (WBCC) as well as neutrophil count (NC) and monocyte count (MC) were performed by a blood cell analyzer (UniCel DxH-900, Beckman Coulter, Milan, Italy) [26].

2.3. LCB calculation

Lifelong LDL-C exposure was calculated as LCB (mg-years/dL) [27–29]. LCB is the addition of LDL-C exposure at diagnosis (by multiplying the initial serum LDL-C value by the age of the patient at diagnosis) and post-diagnosis (by adding the LDL-C values annually measured during follow-up using patients' medical records). Missing LDL-C values during follow-up were replaced by the mean of all available LDL-C values.

2.4. CAC assessment

Each patient underwent a multi-detector computed tomography (CT) scan (Definition Flash, Siemens, Erlangen, Germany) for a total radiation exposure of 1 to 3 mSv. CAC was quantified using the previously described Agatston scoring method [30]. Briefly, coronary visualization was achieved without contrast by using the high-resolution volume mode of the ultrafast computed tomographic scanner in conjunction with a 100 ms scan time, a 3 mm slice thickness, ECG triggering and breath holding. Twenty contiguous slices (60 mm) were acquired with the most cephalad at the lower margin of the bifurcation of the main pulmonary artery. To determine the presence and quantity of coronary calcium, each of the 20 levels was evaluated sequentially. The threshold for a calcific lesion was set at a computed tomographic density of 130 Hounsfield units having an area $\geq$1 mm^2. CAC was determined by the product of the calcified plaque area and maximal calcium lesion density (from 1 to

4 based on Hounsfield units). All CT scans were quantified in an expert central reading center and supervised by a senior cardiovascular radiologist who was blinded to patients' recent and past medical history. CAC presence was defined as a CAC > 0.

2.5. Carotid and femoral plaque assessments

Ultrasonography measurements of the carotid and femoral arteries were performed using an ACUSON Sequoia ultrasound machine with an 8 MHz transducer as previously described [31]. Carotid measurements were performed over a 1 cm segment in the distal common carotid artery (1 cm proximal to dilation of the carotid bulb) and over a 1 cm segment of the carotid artery bifurcation (1 cm proximal to the flow divider). Femoral measurements were performed on the common femoral artery (1 cm proximal to common femoral artery bifurcation). Three longitudinal sections of both right and left carotid and femoral arteries were acquired. Both carotid and femoral plaques were defined as an intima-media thickness (IMT) greater than 1.5 mm. All the exams were performed by a single blinded operator.

2.6. Statistical analysis

The distributional characteristics of each variable, including normality, were assessed by the Kolmogorov-Smirnov test. Data are reported as mean ± standard deviation (SD) for continuous parametric and median (interquartile range-IQR) for continuous non-parametric variables and as frequency (percentage) for categorical variables. When necessary, continuous non-parametric variables (TG, Lp(a), hs-CRP) were logarithmically transformed for statistical analysis to reduce skewness. The Chi square ($\chi 2$) test was used for categorical variables. To test differences in clinical and biochemical characteristics between the groups Student's *t*-test was used. Moreover, to test differences in WBCC, NC and MC between HLCB and LLCB groups Student's *t*-test was used. In order to evaluate the role of LCB as well as WBCC, NC, MC on subclinical atherosclerosis, we performed a stepwise logistic regression analysis adjusted for age, gender, TG/HDL, HbA1c and BMI. Model 1 was adjusted for LCB, age, gender; model 2 was adjusted for LCB, age, gender, WBCC, NC, MC; model 3 was adjusted for LCB, age, gender, WBCC, NC, MC, TG/HDL, HbA1c, BMI. Prior to multivariate analyses, variance inflation due to covariates was verified by estimating a variance inflation factor <2; for this reason, age at diagnosis, FPG, LDL-C at diagnosis, TG and TyG were excluded. Finally, simple linear regression analyses were performed to relate WBCC, NC and MC with LCB.

All statistical analyses were performed using IBM SPSS Statistics for Windows version 23. For all tests, a $p < 0.05$ was considered significant.

3. Results

In total, 432 FH subjects were evaluated; of these, 211 subjects satisfied the inclusion criteria and participated in this study (Fig. 1).

The characteristics of the study population including age, sex, lipid as well as genetic and vascular profiles and treatments are presented in Table 1. All subjects were heterozygous FH and the most frequent genetic variant was the LDL receptor (LDLR) mutation. The prevalence of subclinical atherosclerosis was 61.1 %; of these, CAC and presence of peripheral plaques were 40.3 % and 96.1 %, respectively. Moreover, a CAC score > 0 as well as the presence of peripheral plaques were observed in more than a third of these subjects.

Table 2 shows the characteristics of the study population stratified according to subclinical atherosclerosis. The SA group was older than the NSA group (59.39 ± 7.63 vs 51.76 ± 8.05, $p < 0.01$) and the age at FH diagnosis was higher in the SA group compared with the NSA group (38.63 ± 8.06 vs 31.11 ± 8.59, $p < 0.01$). Furthermore, the percentage of men in the SA group was significantly higher than in the NSA group (51.9% vs 39 %, $p < 0.05$); finally, BMI was higher in the SA group than the NSA group (26.13 ± 3.27 vs 25.01 ± 3.22, $p < 0.05$). While the prevalence of type 2 diabetes was similar between the two groups, FPG as well as HbA1c were higher in the SA group compared with the NSA group (for FPG 92.43 ± 5.21 vs 86.33 ± 5.16, $p < 0.05$; for HbA1c 5.63 ± 0.29 vs 5.44 ± 0.31 $p < 0.05$). The prevalence of LDLR null subjects was higher in the SA group compared to NSA group (45.8% vs 27.5 %, $p < 0.05$) while the opposite was observed in the LDLR defective distribution (54.8% vs 72.5 %, $p < 0.05$) [32]. LDL-C at diagnosis as well as LCB were higher in the SA group than the NSA group (for LDL-C at

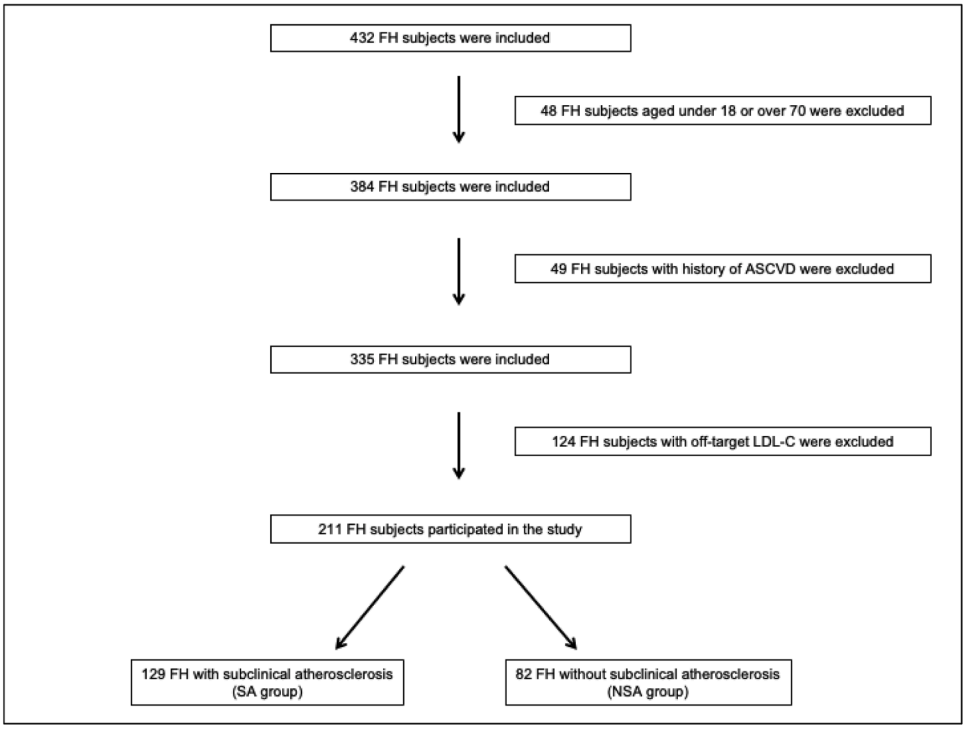

Fig. 1. Enrollment flowchart of the study population.
FH = familial hypercholesterolemia, ASCVD = atherosclerotic cardiovascular disease, LDL-C = low-density lipoprotein cholesterol, SA = subclinical atherosclerosis, NSA = non-subclinical atherosclerosis.

Table 1
Characteristics of the study population.

Demographic	
N	211
Age, years	54.73 ± 7.93
Men, n (%)	99 (46.9)
Age at diagnosis, years	35.04 ± 8.32
Lipid Profile	
LDL-C at diagnosis, mg/dL	274.76 ± 23.62
LDL-C burden, mg-years/dL	12,049.63 ± 3602.52
LDL-C, mg/dL	50.17 ± 6.39
FH Genotype	
LDLR, n (%)	204 (96.7)
- *LDLR defective, n (%)*	126 (61.8)
- *LDLR null, n (%)*	78 (38.2)
ApoB, n (%)	4 (1.9)
PCSK9, n (%)	2 (0.9)
ApoE, n (%)	1 (0.5)
FH Phenotype	
Heterozygous FH, n (%)	211 (100.0)
Vascular Profile	
Subclinical Atherosclerosis, n (%)	129 (61.1)
CAC > 0, n (%)	52 (40.3)
Peripheral plaque, n (%)	124 (96.1)
- Carotid plaque, n (%)	72 (58.1)
- Femoral plaque, n (%)	79 (61.2)
CAC + Peripheral plaque presence, n (%)	46 (35.7)
Treatment	
Antihypertensive therapy, n (%)	73 (34.6)
Glucose lowering therapy, n (%)	7 (3.32)
Lipid lowering therapy, n (%)	211 (100.0)

Data are presented as mean ± standard deviation or percentages. LDL-C = low-density lipoprotein cholesterol, FH = familial hypercholesterolemia, LDLR = low-density lipoprotein receptor, ApoB = apolipoprotein B; PCSK9 = proprotein convertase subtilisin/kexin type 9; ApoE = apolipoprotein E; CAC = coronary artery calcium score.

diagnosis 288.35 ± 24.52 vs 267.92 ± 23.86, $p < 0.05$; for LDL-C burden 13,465.84 ± 3617.46 vs 10,872.63 ± 3594.7, $p < 0.001$); moreover, TG were increased in the SA group than the NSA group (88 [56–140] vs 67 [43–120]). TG/HDL as well as TyG were higher in the SA group compared with the NSA group (for TG/HDL 1.73 ± 0.72 vs 1.45 ± 0.69, $p < 0.01$; for TyG 8.29 ± 0.35 vs 8.01 ± 0.33, $p < 0.01$). While more than a third of subjects were on antihypertensive therapy in both groups, all FH subjects were on high-intensity statin plus ezetimibe plus PCSK9i.

Fig. 2 illustrates the innate immunity profile distribution of the study population stratified according to subclinical atherosclerosis. Increased amounts of WBCC as well as NC and MC were found in the SA group compared with the NSA group (for WBCC 6.9 ± 1.66 vs 6.1 ± 1.16; for NC 4.2 ± 1.3 vs 3.6 ± 1.11; for MC 0.8 ± 0.2 vs 0.4 ± 0.1, p value for all < 0.01). In a secondary analysis, participants were stratified into two groups according to LCB median value of 12,148 mg-years/dL: high LCB group (HLCB group, 106 subjects) and low LCB group (LLCB group, 105 subjects). Fig. 3 illustrates the innate immunity profile distribution of the study population stratified according to LCB. Increased amount of WBCC as well as NC and MC were found in the HLCB group compared with LLCB group (for WBCC 7.2 ± 1.58 vs 6.3 ± 1.41; for NC 4.3 ± 1.2 vs 3.5 ± 1.13; for MC 0.9 ± 0.18 vs 0.5 ± 0.11).

Multivariate logistic regression analysis showed that LCB ($p < 0.01$), WBCC ($p < 0.01$), NC ($p < 0.05$) and MC ($p < 0.05$) were significantly associated with subclinical atherosclerosis and that these associations were independent after adjustment for age, gender, TG/HDL, HbA1c, BMI (Table 3). Moreover, simple linear regression analyses showed that LCB was significantly associated with WBCC, NC and MC (p value for all < 0.01) (Table 4).

4. Discussion

In this study, we investigated the impact of metabolic and innate immunity profiles on atherosclerotic injury in FH subjects on LDL-C target; to the best of our knowledge, this is the first study to explore the interactions of metabolic profile and the innate immunity system with the atherosclerotic damage in this population with cumulative exposure to high LDL-C. We found that LCB was higher in the SA group than in the NSA group; moreover, increased amounts of WBCC as well as NC and MC were found in the SA group compared with the NSA group.

Table 2
General characteristics of the Study Population stratified according to Subclinical Atherosclerosis.

	SA group	NSA group	*p* Value between two groups
Demographic Characteristics			
N	129	82	–
Age, years	59.39 ± 7.63	51.76 ± 8.05	< 0.01
Age at diagnosis, years	38.63 ± 8.06	31.11 ± 8.59	< 0.01
Men, n (%)	67 (51.9)	32 (39.0)	< 0.05
Body mass index, kg/m^2	26.13 ± 3.27	25.01 ± 3.22	< 0.05
Glucose Profile			
Type 2 diabetes, n (%)	5 (3.9)	2 (2.4)	–
FPG, mg/dL	92.43 ± 5.21	86.33 ± 5.16	< 0.05
HbA1c,%	5.63 ± 0.29	5.44 ± 0.31	< 0.05
FH Genotype			
LDLR, n (%)	124 (96.1)	80 (97.6)	0.82
- *LDLR defective, n (%)*	68 (54.8)	58 (72.5)	< 0.05
- *LDLR null, n (%)*	56 (45.8)	22 (27.5)	< 0.05
Lipid Profile			
LDL-C at diagnosis, mg/dL	288.35 ± 24.52	267.92 ± 23.86	< 0.05
LCB, mg-years/dL	13,465.84 ± 3617.46	10,872.63 ± 3594.7	< 0.001
TC, mg/dL	122.35 ± 10.88	116.33 ± 10.02	0.16
HDL-C, mg/dL	53.62 ± 8.14	54.37 ± 8.21	0.85
Triglycerides, mg/dL	88 (56–140)	67 (43–120)	< 0.05
LDL-C, mg/dL	51.68 ± 6.41	48.21 ± 6.36	0.71
Non-HDL-C, mg/dL	66.74 ± 9.73	61.67 ± 9.93	0.48
TG/HDL	1.73 ± 0.72	1.45 ± 0.69	< 0.01
TyG	8.29 ± 0.35	8.01 ± 0.33	< 0.01
ApoB, mg/dL	43.64 ± 5.44	40.42 ± 5.01	0.56
ApoAI, m g/dL	154.15 ± 10.63	156.18 ± 10.44	0.54
ApoB to ApoAI ratio	0.35 ± 0.13	0.32 ± 0.17	0.79
Lp(a), mg/dL	34.7 (10.4–55.3)	32.1 (10.4–52.5)	0.16
Risk Factors			
Systolic BP, mmHg	122.23 ± 9.38	119.42 ± 9.22	0.49
Diastolic BP, mmHg	72.34 ± 9.52	70.86 ± 9.71	0.48
Smoking, n (%)	43 (33.3)	24 (29.3)	0.62
hs-CRP, mg/dL	0.16 (0.05–0.25)	0.11 (0.05–0.23)	0.29
Treatment			
Antihypertensive therapy, n (%)	46 (35.7)	27 (32.9)	0.63
Glucose lowering therapy, n (%)	5 (3.9)	2 (2.4)	–
- *metformin (Glucophage)*, n (%)	5 (100.0)	2 (100.0)	–
Lipid lowering therapy, n (%)	129 (100.0)	82 (100.0)	–
- *High-intensity statin plus ezetimibe plus PCSK9i, n (%)*	129 (100.0)	82 (100.0)	–

Data are presented as mean ± standard deviation, percentages, or median (interquartile range).
SA = subclinical atherosclerosis, NSA = non-subclinical atherosclerosis, FPG = fasting plasma glucose, HbA1c = glycated hemoglobin, LDLR = low-density lipoprotein receptor, LDL-C = low-density lipoprotein cholesterol, LCB = low-density lipoprotein cholesterol burden, TC = total cholesterol, HDL = high-density lipoprotein, Non-HDL-C = non-HDL cholesterol, TG/HDL = triglyceride HDL ratio, TyG = triglyceride-glucose index, ApoB = apolipoprotein B, ApoAI = apolipoprotein AI, Lp(a) = lipoprotein (a), BP = blood pressure, hs-CRP = high sensitivity C-reactive protein, PCSK9i = Proprotein Convertase Subtilisin/Kexin type 9 inhibitor.

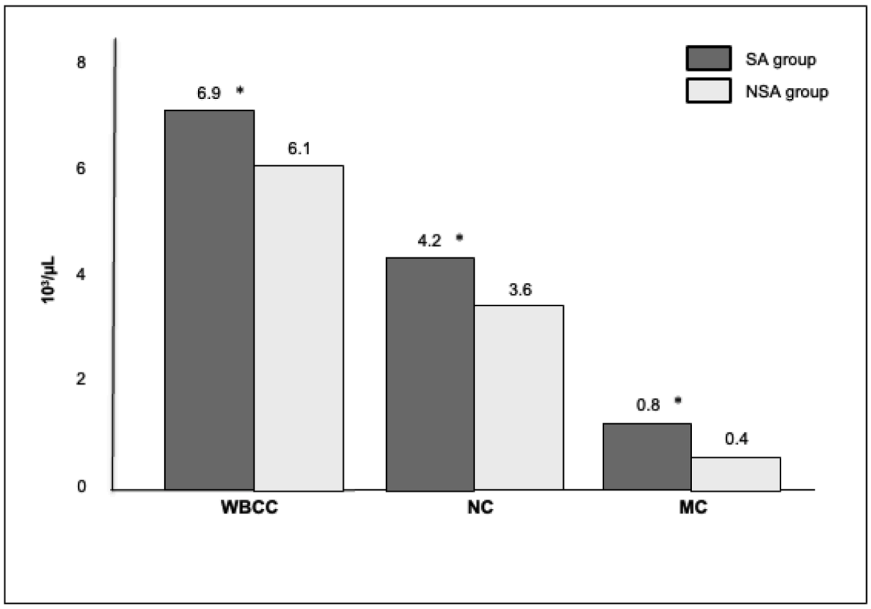

Fig. 2. Innate immunity profile distribution of the Study Population stratified according to subclinical atherosclerosis.
SA = subclinical atherosclerosis, NSA = non-subclinical atherosclerosis, WBCC = white blood cell count, NC = neutrophil count, MC = monocyte count, * = $p < 0.01$.

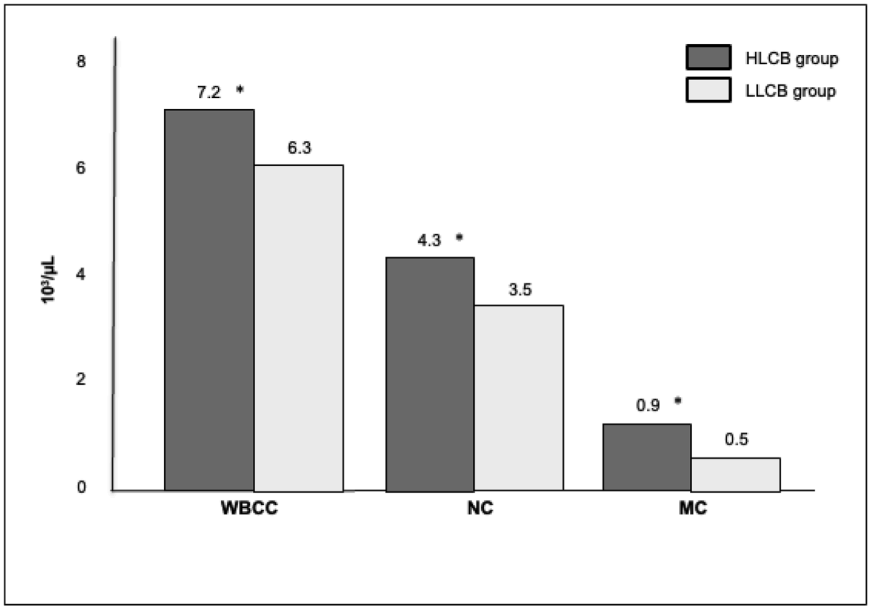

Fig. 3. Innate immunity profile distribution of the Study Population stratified according to LDL-C Burden.
HLCB = high LDL-C Burden, LLCB = low LDL-C Burden, WBCC = white blood cell count, NC =neutrophil count, MC = monocyte count, * = $p < 0.01$.

Furthermore, we found that LCB as well as WBCC, NC and MC were independent predictors of subclinical atherosclerosis in FH subjects. Our results are in line with previous studies that evaluated the atherogenic role of immune cells in FH subjects [8,33]. These studies showed that the accumulation of cholesterol crystals and oxidized LDL (oxLDL) in the vessels caused abnormal mobilization of immune cells, which have been suggested to display specific pro-atherosclerotic activities in FH patients. In this context, it is well established that once monocytes are recruited into plaques, they undergo extensive differentiation into macrophages, which are crucial in both the initiation and progression of atherosclerosis [34]. Thus, within the plaques, macrophages actively engulf modified LDL, particularly through the scavenger receptor A1 (SRA1), leading to the formation of foam cells, which can subsequently trigger the release of pro-inflammatory cytokines [35]. Moreover, Drechsler et al. demonstrated that hypercholesterolemia increased serum levels of the neutrophil chemoattractant CXCL1 that enhanced neutrophil mobilization. Additionally, the expression of CXCR2 (CXCL1 receptor) is also up-regulated in this condition, further promoting the release of neutrophils from the bone marrow [36]. Moreover, in FH subjects a high NC was associated with the release of a large amount of cytotoxic and destructive factors able to cause vascular tissue destruction [37]. Thus, the evaluation of leucocytes counts could be a useful parameter that link the systemic inflammation with the inflammatory process in the arterial wall. In fact, it was previously shown that a wide range of innate immune cells, including macrophages, dendritic cells, monocytes, mast cells and neutrophils are relevant to atherosclerosis progression [38]. Hypercholesterolemia promotes the proliferation of hematopoietic stem and progenitor cells, leading to systemic

Table 3
Logistic regression of Subclinical Atherosclerosis in the Study Population.

Dependent Variable	Subclinical atherosclerosis					
Independent Variable	Multivariate ORs (95 % CIs)		Multivariate ORs (95 % CIs)		Multivariate ORs (95 % CIs)	
	Model 1	*P* value	Model 2	*P* value	Model 3	*P* value
LCB, mg-years/dL	1.012 (1.007–1.017)	< 0.001	1.349 (1.324–1.445)	< 0.01	1.948 (1.041–3.127)	< 0.01
Age, years	1.107 (1.078–1.137)	< 0.001	1.124 (1.090–1.158)	< 0.001	1.141 (1.084–1.204)	< 0.001
Gender, men = 1	1.018 (1.011–1.026)	< 0.001	1.115 (1.084–1.149)	< 0.001	1.138 (1.075–1.198)	< 0.001
WBCC, 10^3/μL			1.519 (1.059–1.980)	< 0.01	1.909 (1.180–3.090)	< 0.01
NC, 10^3/μL			1.418 (1.041–1.853)	< 0.01	1.776 (1.119–2.819)	< 0.05
MC, 10^3/μL			1.124 (1.071- 1.682)	< 0.05	1.287 (1.091–1.520)	< 0.05
TG/HDL, value					1.107 (0.856–1.432)	0.18
HbA1c,%					1.041 (0.229–4.738)	0.42
BMI, kg/m^2					1.005 (0.787–3.534)	0.57

Stepwise logistic regression model was used to estimate ORs and 95 % CIs.
Model 1 was adjusted for, LCB, age, gender; Model 2 was adjusted for LCB, age, gender, WBCC, NC, MC; Model 3 was adjusted for LCB, age, gender, WBCC, NC, MC, TG/HDL, HbA1c, BMI.
LCB = low-density lipoprotein cholesterol burden, WBCC = White blood cell count, NC = Neutrophil count, MC = Monocyte count, TG/HDL = triglyceride to high-density lipoprotein ratio, HbA1c = glycated hemoglobin, BMI = body mass index.

Table 4
Simple Linear Regression of Innate Immunity and LDL-C Burden.

Dependent Variables	WBCC		NC		MC	
Independent Variable	β	*p* Value	β	*p* Value	β	*p* Value
LCB, mg-years/dL	0.296 ± 0.041	< 0.01	0.274 ± 0.035	< 0.01	0.261 ± 0.028	< 0.01

WBCC = white blood cell count, NC = neutrophil count, MC = monocyte count, LCB = low-density-lipoprotein cholesterol burden.

monocytosis; consequently, the subendothelial accumulation of lipoproteins and chemokines could promote the influx of monocytes into the vessel wall [39]. Moreover, it was demonstrated that a vulnerable plaque can entrap and may form neutrophil extracellular traps (NETS); these cells release very high amounts of reactive oxygen species (ROS) and the pro-oxidant enzyme myeloperoxidase. Thus, the arrival of neutrophils in the vulnerable plaque could aggravate oxidative stress and amplify plaque progression [40]. In line with these findings, in our study the SA group exhibited a significant difference of innate immunity profile compared with the NSA group; thus, in FH subjects the evaluation of innate immunity profile could be useful to identify subjects with more pronounced atherosclerotic damage and thus at higher risk of ASCVD in clinical practice. However, further prospective observational studies are needed to confirm these findings. hs-CRP plasma levels were higher in the SA group than NSA group but the difference was not significant. This could be due to the effect of statin therapy on hs-CRP [41]. In fact, it was note that statins reduced hs-CRP levels and in our study all FH subjects were on high intensity statin therapy. Conversely, according to our data, it is possible to hypothesize that other inflammatory parameters such as WBCC, NC and MC are not modulated by statins. Moreover, recent findings by Tajani et al. did not find a relationship between the concentration of hs-CRP and the incidence and progression of subclinical atherosclerosis assessed by CAC levels [42]. A possible explanation may be that hs-CRP is better correlated with other parameters than biomarkers of subclinical atherosclerosis. Despite hs-CRP is an inflammatory biomarker largely utilized in clinical practice, there is still conflicting opinion on the usefulness of adding hsCRP among traditional risk factors for relevant prognostic information to the prediction of cardiovascular risk [43].

In our study we found that the SA group were older at diagnosis as well as having a higher LCB compared with the NSA group and a significant association of cumulative exposure to high LDL-C and subclinical atherosclerosis was observed. In line with these findings, previous studies documented that FH was often diagnosed too late and thus the cumulative exposure to high LDL-C levels promoted atherosclerotic injury [44]. The concept of cumulative LDL-C burden and its association with ASCVD has been clearly recognized in the FH population. Indeed, high LDL-C levels during childhood and young adulthood exacerbated the severity of atherosclerotic plaque development and it has been demonstrated that young FH adults who started treatment between the ages of 8 and 10 years experienced only 1 % of cardiovascular events during adulthood compared with 26 % in their parents who started LLT later in life [45]. Moreover, we demonstrated that LDL-C burden was associated with WBCC as well as NC and MC and thus the prolonged exposure to elevated LDL-C levels may reflect a more pronounced inflammatory profile in FH individuals despite LDL-C target achievement. Early diagnosis and treatment of FH individuals are essential to reduce the incidence of early cardiovascular events [46]. Unfortunately, despite substantial reductions in LDL-C levels, a residual cardiovascular risk remains, contributing to the incidence of MACE. Interventions targeting inflammatory processes could positively affect cardiovascular outcomes and provide clinical benefit independent of lowering lipoproteins. In this context, the Colchicine Cardiovascular Outcomes Trial (COLCOT) has revealed that daily dose of 0.5 mg of colchicine resulted in 23 % reduction in the relative risk of MACE among patients who had recently experienced an acute coronary syndrome [47]. Moreover, the Low-Dose-Colchicine-2 (LoDoCo2) trial has showed a 31 % decrease in MACE when comparing colchicine treatment to placebo in patients on secondary prevention [48]. It is well established that inflammation plays a significant role in residual cardiovascular risk [49]; thus, in our study we demonstrated that in clinical practice the evaluation of LCB beyond the last-visit LDL-C could be useful to detect FH subjects with a higher inflammatory profile and thus more vulnerable to atherosclerotic injury.

Concerning lipid values, we showed an increased amount of TG and TG/HDL in the SA group compared with the NSA group. This observation supports the growing evidence that triglyceride-rich lipoproteins (TRLs) may contribute to residual ASCVD risk, even after LDL-C reduction [50]. In this context, previous studies showed that TG/HDL was significantly associated with subclinical atherosclerosis in subjects with metabolic disorders [20,51]; however, further prospective studies are needed to evaluate the role of this lipid parameter in the atherosclerotic damage in large cohorts of FH subjects.

Moreover, we found that the SA group exhibited an abnormal glucose profile compared with the NSA group suggesting that it may significantly influence the atherosclerotic damage. In this context, it was previously shown that high glucose concentrations could damage the arterial wall and promote atherosclerosis by generating advanced glycation end products (AGEs) and reactive oxygen species (ROS) or by

leading to the activation of protein kinase C in vascular cells [52]. Additionally, our study revealed that TyG, a metabolic parameter associated with insulin resistance (IR), was elevated in the SA group compared with the NSA group, suggesting that insulin resistance could promote atherosclerotic injury. This finding is in line with previous studies showing that insulin-stimulated nitric oxide (NO) synthesis is selectively impaired by insulin resistance, leading to endothelial dysfunction [53,54]. Dyslipidemia interplays with metabolic conditions such as diabetes, obesity, as well as with kidney failure, concurring to an overall increased cardiovascular risk. In this contest, drugs for diabetes, such as sodium-glucose cotransporter 2 inhibitors (SGLT2i) and glucagon-like peptide 1 receptor agonist (GLP1-RA), not only influence metabolism, enhancing glycemic control, but also represent highly effective therapy agents in terms of cardiorenal outcomes. In the SMART-C collaborative meta-analysis, Patel et al. showed that SGLT2i consistently reduced the risk of MACE across a broad spectrum of patients, independent of baseline ASCVD status, diabetes, or kidney function [55]. Further data from a meta-analysis involving 78 607 subjects showed that, in addition to cardiovascular benefits, SGLT2i significantly reduced the risk of kidney disease progression independently of diabetes status [56]. As regards GLP1-RA, a meta-analysis involving 83,258 individuals showed that these drugs significantly reduced MACE in both males and females, irrespective of CVD history, and these benefits were observed across varying eGFR and BMI groups; moreover, it was demonstrated that GLP1-RA reduced the risk of all-cause and cardiovascular mortality, fatal and non-fatal stroke, coronary revascularization, and composite renal outcomes [57].

There are several limitations to our study. First, because of its cross-sectional design, the impact of LCB and impaired inflammatory profile on atherosclerosis progression cannot be established; further prospective studies are needed to evaluate the roles of these parameters on cardiovascular injury progression in FH subjects. Furthermore, data on the influence of diet and physical activity on the onset of subclinical atherosclerosis have not been included in this study. Moreover, total WBCC is not the main or only indicator of immune system response. Many other WBC parameters should be evaluated for a comprehensive picture of immune function. Data on specific surface proteins, such as CD4 and CD8 on WBCC, as well as cytokine levels like IL-6, which could provide valuable insights into immune activation status and response, are lacking [58,59] . These parameters should be considered for future further investigations. Moreover, our study population did not undergo CT angiography despite its advantages in providing more detailed information than other diagnostic methods, especially for plaque characterization and identifying vulnerable plaques. However, we chose less invasive and more accessible diagnostic methodologies, as previous studies have demonstrated that detecting subclinical atherosclerosis through noninvasive imaging techniques can significantly enhance risk stratification and clinical management in patients with FH [60]. Specifically, the Agatston CAC score has proven to be a well-established marker of subclinical atherosclerosis and a strong predictor of cardiovascular events and mortality risk in asymptomatic individuals, both with and without FH [61]. In this context, Miname et al. underscored the value of the CAC score for risk stratification in FH patients, showing a very low ASCVD event risk in asymptomatic individuals with a confirmed genetic diagnosis of FH and a CAC score of 0 after median follow-up periods of 3.7 and 2.7 years, respectively [62]. Finally, concerning the role of insulin resistance on the development of subclinical atherosclerosis, no data on insulin plasma levels as well as NO concentrations were available in our cohort of patients.

In conclusion, in our study an increased LCB as well as an impaired innate immunity profile were found in FH subjects with subclinical atherosclerosis and they were independently associated with atherosclerotic injury. Moreover, LCB appears to play a role in modulating the innate immunity profile; further prospective studies are needed to evaluate this possible modulation in a large cohort of FH subjects. While the risk of cardiovascular events is highly heterogeneous in the FH population, the evaluations of these parameters beyond the last-visit LDL-C could be useful to identify individuals at higher cardiovascular risk in clinical practice. However, further prospective observational studies are needed to confirm these findings in a large cohort of FH subjects.

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Declaration of competing interest

The authors have no conflicts of interest to disclose.

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S.P. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors approved the final version.

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Research involving human participants and/or animals

This study was approved by the local ethics committee in accordance with the ethical standards of the institutional and national research committees and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. This article does not contain any studies with animals performed by any of the authors.

Informed consent

Informed consent was obtained from each participant enrolled in the study.

Data availability statement

The data underlying this article will be shared on reasonable request to the corresponding author.

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