

# ROLE OF INNATE AND ADAPTIVE IMMUNITY ON ATHEROSCLEROSIS IN SUBJECTS WITH FAMILIAL HYPERCHOLESTEROLEMIA

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## Background and aims

Familial hypercholesterolemia (FH) is characterized by exposure to high LDL and premature atherosclerotic cardiovascular disease (ASCVD). However, ASCVD severity can be heterogeneous among FH subjects with similar LDL levels. To evaluate the role of immune-inflammatory system on atherosclerosis development in FH individuals under high-intensity lipid-lowering therapy (HI-LLT).

## Methods

One-hundred patients of both sexes with definite/probable diagnosis of FH, under HI-LLT with statin/ezetimibe were submitted to Coronary Computed Tomography Angiography (CCTA), for coronary calcium score (CCS) and Coronary Artery Disease - Reporting and Data System (CAD-RADS) evaluation. Lipids, apolipoproteins A1, B and Lp(a), sequencing of FH causing genes, anti-oxLDL and anti-ApoB-D autoantibodies (IgG and IgM), lymphocytes phenotype, platelet, monocyte and endothelial microparticles, IgM production by B1 cells (ELISPOT), and cytokines (TNF- $\alpha$ , IFN-gamma, IL-10, IL-6, IL-4, and IL-2) in the supernatant of cultured cells were determined. Results were compared among CCS and CAD-RADS categories.

## Results

Mean treated LDL was  $154 \pm 52$  mg/dL; mean and maximal CAC were 173 and 2139 UA, respectively, with median and P75th of 75 and 512 UA. Lipid parameters were similar among participants with CAC=0 vs. CAC>0. There were no differences in the distribution of immune cells, cytokines, autoantibodies, or MPs among CCS (0 vs. >0; 0, 1-99, 100-399, 400-999, and > 1000; P $\geq$ 75th vs. P<75th) or CAD-RADS (0-5) categories. CCS was not correlated with LDL-c levels.

## Conclusions

The immune-inflammatory system does not influence CCS in subjects with FH under HI-LLT. Atherosclerosis in FH subjects may result from the balance between exposure to LDL levels and to lipid-lowering therapy.

**Table 1. Demographic and clinical characteristics according to CCS**

Variable	CCS=0	CCS > 0	P-value
Males (%)	8 (17)	20 (39)	0.015
Age (y, median, IQ)	53 (15)	58 (10)	0.059
Hipertension (%)	21 (45)	30 (58)	0.196
Smokers/ex-smokers (%)	6 (13)	21 (40)	0.016
Diabetes (%)	7 (15)	8 (13)	0.625
CHD (%)	4 (9)	12 (23)	0.049
Stroke (%)	3 (6)	3 (6)	0.898
PAD (%)	0 (0)	3 (3)	0.094
Family history of premature CHD	16 (34)	16 (31)	0.728
CCS (U Agatston)	0 (NA)	100 (40-512)	<0.0001
CAD-RADS score			
0	42 (88)	1 (2)	<0.0001
1	4 (8)	10 (19)	
2	1 (2)	20 (38)	
3	0 (0)	5 (9)	
4	1 (2)	8 (15)	
5	0 (0)	9 (17)	
Lipid-lowering drugs (%)			
None	9 (19)	6 (11)	0.380
Moderate intensity	0(0)	1 (2)	
High-intensity	39 (81)	46 (87)	

Categorical variables are N (%); numerical variables are mean  $\pm$  SD, or median, IQ. Categorical variables compared by Pearson's Chi-square test; numerical variables compared by Student's t-test Mann-Whitney test.

**Table 2. Laboratory parameters, according to CCS**

Variable	CCS = 0	CCS > 0	P-value
Total Cholesterol (mg/dL)	236 (65)	240 (66)	0.787
HDL-C (mg/dL)	56 (15)	53 (15)	0.517
LDL-C (mg/dL)	149 (61)	158 (63)	0.471
Non-HDL-C (mg/dL)	180 (65)	186 (65)	0.646
Triglycerides (mg/dL)	183 (91-169)	143 (95-181)	0.505
Apo A (mg/dL)	154 (28)	146 (27)	0.255
Apo B (mg/dL)	118 (37)	130 (62)	0.442
Lp (a) (mg/dL)	35 (13-67)	27 (11-53)	0.668
AST (U/L)	21 (17-27)	20 (15-24)	0.251
ALT (U/L)	21 (15-29)	21 (16-25)	0.911
CK (U/L)	131 (108-176)	135 (110-252)	0.445
TSH ( $\mu$ UI/L)	1.93 (1.63-3.37)	2.53 (1.64-4.19)	0.145
Creatinine (mg/dL)	0.83 (0.71-1.08)	1.01 (0.75-1.16)	0.084
Glycemia (mg/dL)	94 (86-112)	93 (87-107)	0.850
HbA1c (%)	5.8 (5.4-6.1)	5.7 (5.5-6.0)	0.814

Numerical variables are mean  $\pm$  SD, or median, IQ, and were compared by Student's t-test Mann-Whitney test.

**Table 3. Circulating microparticles according to CCS**

Variable	CCS = 0	CCS > 0	P-value
Endotelial MP (%)	0.14 (0.11)	0.11 (0.13)	0.250
Platelet MP (%)	78 (24)	82 (15)	0.290
Monocyte MP (%)	3.31 (1.91-5.20)	2.33 (0.39-4.15)	0.053

Numerical variables are mean  $\pm$  SD, or median, IQ, and were compared by Student's t-test Mann-Whitney test.

**Table 4. Circulating immune cells and autoantibodies, according to CCS**

Variable	CCS = 0	CCS > 0	P-value
Total leukocytes (células/mL)	6767 (1910)	7832 (2123)	0.011
Lymphocytes (cells/mL)	2242 (561)	2286 (806)	0.759
T CD4+ lymphocytes (%)	44 (11)	45 (12)	0.650
T CD4+ lymphocytes (cells/mL)	7.26 (2.66)	7.63 (3.12)	0.533
T CD8+ lymphocytes (%)	17 (7)	17 (7)	0.858
T CD8+ lymphocytes (cells/mL)	3.35 (2.65)	3.50 (2.86)	0.789
B memory cells (%)	1.57 (1.08-2.39)	1.41 (0.84-2.19)	0.375
B memory cells (cells/mL)	0.0023 (0.001-0.0047)	0.0021 (0.0007-0.0043)	0.414
B1 cells (%)	0.15 (0.10-0.23)	0.13 (0.08-0.29)	0.534
B1 cells (cells/mL)	0.0002 (0.00009-0.0005)	0.0002 (0.00007-0.0009)	0.796
B naïve cells (%)	5.15 (3.15)	4.85 (3.18)	0.635
B naïve cells (cells/mL)	0.006 (0.003-0.014)	0.006 (0.0025-0.011)	0.488
B1 CD11b+ (%)	0.15 (0.10-0.23)	0.13 (0.08-0.29)	0.954
B1 CD11b+ (cells/mL)	0.00005 (0.00003-0.0002)	0.00006 (0.00002-0.0002)	0.954
B1 CD11b- (%)	0.11 (0.06-0.15)	0.08 (0.05-0.21)	0.378
B1 CD11b- (cells/mL)	0.00004 (0.00002-0.0001)	0.00004 (0.00001-0.0001)	0.663
B memory TLR4+ (%)	0.053 (0.035-0.127)	0.075 (0.044-0.189)	0.246
B memory TLR4+ (cells/mL)	0.000006 (0.000002-0.00004)	0.000009 (0.000003-0.00006)	0.380
B1 TLR4+ (%)	0.011 (0.005-0.036)	0.010 (0.006-0.044)	0.490
B1 TLR4+ (cells/mL)	0.000001 (0.0000002-0.00001)	0.000001 (0.0000003-0.00002)	0.490
B naïve TLR4+ (%)	0.054 (0.025-0.210)	0.080 (0.040-0.141)	0.155
B naïve TLR4+ (cells/mL)	0.000005 (0.000001-0.00007)	0.000008 (0.000002-0.00009)	0.289
IgM anti-Ox-LDL (UA)	0.51 (0.11)	0.49 (0.01)	0.317
IgG anti-LDLox (UA)	0.89 (0.05)	0.90 (0.04)	0.111
IgM anti-ApoB-D (UA)	3.43 (2.42)	3.18 (2.00)	0.567
IgG anti-ApoB-D (UA)	0.45 (0.29-0.58)	0.51 (0.27-0.89)	0.147

Numerical variables are mean  $\pm$  SD, or median, IQ, and were compared by Student's t-test Mann-Whitney test.

**Table 5. Cytokines produced by T and B cells, according to CCS (pg/mL)**

Variable	CCS=0	CCS > 0	P-value
T cells Interferon- $\gamma$	5.9 (0.00-159)	0.0 (0.0-84.0)	0.209
TNF- $\alpha$ (pg/mL)	7.38 (0.00-151.49)	0.19 (0.00-87.60)	0.452
IL-10 (pg/mL)	0.00 (0.00-3.50)	0.00 (0.00-2.17)	0.329
IL-6 (pg/mL)	0.00 (0.00-4.98)	0.00 (0.00-0.96)	0.710
IL-4 (pg/mL)	0.00 (0.00-2.66)	0.00 (0.00-1.69)	0.431
IL-2 (pg/mL)	5.58 (0.00-205.37)	1.83 (0.00-152.16)	0.672
B memory cells Interferon- $\gamma$	0.00 (0.00-2.62)	0.00 (0.00-0.00)	0.185
TNF- $\alpha$ (pg/mL)	0.00 (0.00-0.06)	0.00 (0.00-0.00)	0.585
IL-10 (pg/mL)	0.00 (0.00-0.00)	0.00 (0.00-0.00)	0.718
IL-6 (pg/mL)	0.32 (0.00-8.21)	0.00 (0.00-2.02)	0.345
IL-4 (pg/mL)	0.00 (0.00-0.00)	0.00 (0.00-0.00)	0.285
IL-2 (pg/mL)	0.00 (0.00-72.53)	0.00 (0.00-3.77)	0.234
B naïve cells Interferon- $\gamma$	0.00 (0.00-0.00)	0.00 (0.00-0.27)	0.221
TNF- $\alpha$ (pg/mL)	0.00 (0.00-4.62)	0.00 (0.00-0.00)	0.140
IL-10 (pg/mL)	0.00 (0.00-0.00)	0.00 (0.00-0.00)	0.693
IL-6 (pg/mL)	0.77 (0.00-12.54)	0.00 (0.00-3.65)	0.311
IL-4 (pg/mL)	0.00 (0.00-0.11)	0.00 (0.00-0.00)	0.087
IL-2 (pg/mL)	0.00 (0.00-12.37)	0.00 (0.00-0.00)	0.183

Numerical variables are mean  $\pm$  SD, or median, IQ, and were compared by Student's t-test Mann-Whitney test.

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