Evaluation of Cardiovascular Risk Factors In Patients With Familial Hypercholesterolemia From The North-Eastern Area of Romania

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Abstract

Background. Familial hypercholesterolemia (FH) is one of the most frequent and important monogenic cholesterol pathology. Traditional and nontraditional cardiovascular risk factors increase the prevalence of atherosclerotic cardiovascular disease (ASCVD) in this population.

Objective. To establish the prevalence and the cardiovascular risk factors of FH population, to identify the ASCVD through the clinico-biological and imaging modifications during the 24-months follow-up.

Methods. This first prospective study in the north-eastern part of Romania, carried out between October 2017-October 2019, out of 980 patients with dyslipidemia evaluated with the Dutch Lipid Network (DLCN) and Simone Broome (SM) scores, only 61 patients with DLCN score above 3 and FH possible/probably (SM score) were included.

Results. The 61 FH subjects recorded a mean age of 48.46±12.53 years, with more women compared to men. Regarding the traditional cardiovascular risk factors, we identified that high blood pressure was the main factor in all patients, followed by sedentary lifestyle, obesity, smoker status, personal cardiovascular history and type 2 diabetes. The measured DLCN score recorded: “possible” FH identified in 39.4%, the “probable”FH in 45.9% and the “definite” FH in 14.7%. After the administration of the lipid-lowering agents for 24 months, TC and LDL-C levels, carotid intima-media thickness (cIMT) and ankle-brachial index (ABI) decreased and HDL-C levels increased, but without reaching the guideline goals. In addition, the high-dose of statin alone, the high-dose of statin with fenofibrate, subjects with „possible” FH, the normal values of cIMT and ABI, had a reduced time of ASCVD occurrence. Also, the high cIMT values, physical inactivity, high TC, TG and high-sensitivity C-reactive protein (hsCRP) levels were associated with an increased risk of ASCVD.

Conclusions. To reduce cardiovascular risk, the FH patients need a cascade screening and a specific management. Even though it was the first observational study in the north-eastern part of Romania, further molecular genetics studies are needed to confirm the FH cases.

Background

Atherosclerosis is a complex multifactorial disorder, consisting of a chronic inflammatory response, which causes plaque formation in the intima and media of medium and large arteries (1). Atherosclerosis can affect the vessels throughout the life of an individual, several risk factors being significant contributors to the atherosclerotic plaques formation: dyslipidemia, diabetes mellitus, high blood pressure (1).

The genetic cornerstone of atherosclerotic cardiovascular disease (ASCVD) includes both common DNA variants (leading to polygenic susceptibility) and rare DNA versions that cause monogenic disorders, typical monogenic disease being familial hypercholesterolemia (FH) (2). FH is an autosomal-dominant pathology, identified in all races and ethnic groups and determined by mutations of the *low density*

lipoprotein receptor (*LDLR), apolipoprotein B (APOB), proprotein convertase subtilisin/kexin type 9 (PCSK9)* genes, being a cause of premature coronary atherosclerotic disease (3) (4). In Romania, the prevalence of FH records 1/213 for the heterozygous form (5) compared to other caucasian population with 1/500 individuals and to 1/1,000,000 for the homozygous form (6).

At present, there are three criteria available for the clinical diagnosis of FH: the Simon Broome criteria in the UK, the Dutch Lipid Clinic Network Criteria (DLCNC) in the Netherlands and the MedPed criteria in the US (7) (8). There is no international consensus for the diagnostic foundation to be applied preferentially; instead, in both lipidology clinics and clinical trials, both Simon Broome and DLCN criteria are used (9).

Familial and personal history of hypertension, coronary heart disease (CHD), stroke, chronic kidney disease, smoking, diet, alcohol consumption, sedentary lifestyle and nontraditional risk factors represented by the ankle-brachial index (ABI), high-sensitivity C-reactive protein (hsCRP) were significantly associated with cardiovascular morbidity and mortality in FH patients (10) (11).

Peripheral arterial disease (PAD) is common in FH patients, and for the PAD diagnosis ABI is useful, being therefore a non-invasive, reliable and low-cost method (12). In addition, low ABI is a marker of increased CHD risk in the general population and even asymptomatic patient (12).

Coronary atherosclerosis can be identified by using different type of methods: carotid ultrasound (with measurement of mean of carotid intima-media thickness cIMT), CT/MRI angiography, or cardiac stress scintigraphy (13). Subclinical atherosclerosis, as the first manifestation of CHD, is essential for an adequate prevention program, an idea supported by the ESC/EAS guidelines (13). Atherosclerotic plaques and the increases of mean cIMT were associated with a significantly expanded CHD risk, being a sensitive method for identification of subclinical atherosclerosis and the efficacy of therapeutic conduct (14) (15).

Management of cardiovascular risk factors is important for FH patients who associate cardiovascular atherosclerotic pathology (16). Statin therapy and the opportunity for fast access to health care for FH patients have improved survival, the screening of this high-risk coronary population as part of a multifactorial approach being a must (6). In the general population, statins reduced LDL-C, ASCVD and all-cause mortality (17), while in FH patients it's recommended to take maximum tolerated dose of a statin, in association with ezetimibe for achieving cardiovascular goals (18).

Methods

Objectives

Due to poor diagnosis, lack of multidisciplinary approach and management of FH patients in Romania, we conducted a prospective study that included possible FH patients with the following objectives:

- - the prevalence of FH population in the north-eastern part of Romania

- - description of cardiovascular risk factors and their recognition in predicting ASCVD
- - description of ASCVD throughout the follow-up
- - clinico-biological and imaging follow-up for FH patients based on lipid lowering drugs (after 12 and 24 months respectively of treatment).

Inclusion and exclusion criteria of FH patients

Study design: observational, prospective, carried out over 2 years (between October 2017 and October 2019) in the Clinical Hospital "Dr. CI Parhon", Iași, the Institute of Cardiovascular Diseases, Iași and the Emergency Hospital "Sfântul Spiridon", Iași.

Study population: Out of 980 patients with dyslipidemia identified between september 2016 and october 2017, only 61 patients met the following inclusion criteria: subjects with judgment and who signed the informed consent form; men and women > 18 years-old; identification of a family history of hypercholesterolemia or cholesterol deposits in vascular and extravascular tissues; setup of a personal history of early onset of coronary, cerebrovascular and peripheral vascular diseases; clinical observations regarding the presence of either xanthomas, xanthelasma and / or arcus cornealis; biological identification of total cholesterol (TC) > 300 mg / dL, low density lipoprotein cholesterol (LDL-C) > 190 mg/dL without treatment or > 100 mg/dL following treatment with maximum doses of statins (40 mg rosuvastatin, 80 mg atorvastatin, in association with ezetimibe or not) (Figure S1 flowchart for FH patients).

Exclusion criteria: subjects without discernment or who refuse to sign the informed consent, subject lost to follow-up, young patients (< 18 years of age), women during pregnancy and lactation; subjects with severe physical disabilities, dementia, neoplasms and other causes of secondary hypercholesterolemia (uncontrolled diabetes, nephrotic syndrome, hypothyroidism, drug-induced dyslipidemia) (9) or other missing data (Figure S1 flowchart for FH patients).

Clinical and biological evaluation in FH patients

The follow-up package comprised 3 components: clinical examination data, laboratory investigations and ultrasound parameters.

The study included patients with Dutch Lipid Clinic Network score > 3 especially for the FH population (8). The reference values of the DLCN score were: 3–5 points highlighted possible FH, 6–7 points a probable FH, over 8 a definite FH (8). The other score, Simone Broome score advertised between possible, probable or definitive FH (8). The patients included in the study were coded with the letter H and the corresponding ID number.

Laboratory analysis included values at baseline, 12- and 24 months after lipid-lowering drugs administration (statin alone - atorvastatin 80 mg maximum, rosuvastatin 40 mg, simvastatin 80 mg; combinations between high dose statin and ezetimibe 10 mg and / or Fenofibrate 160 mg). Lipid profile

pointed out the total cholesterol (CT mg/dL), low density cholesterol lipoprotein (LDL-C mg/dL), high density cholesterol lipoprotein (HDL-C mg/dL), triglycerides (TG mg/dL) measured by spectrophotometric method; blood glucose (mg/dL), aspartate transaminase (AST mg/dL) and alanine aminotransferase (ALT mg/dL), uric acid (UA mg / dL) measured by spectrophotometric method; high-sensitivity C-reactive Protein (hsPCR mg / dL) quantified by immunoturbidimetry.

Further explorations for cardiovascular evaluation included:

- • EKG for ischemic changes assessment;
- • ABI measurement with a sphygmomanometer and portable ultrasonography device, which determines sounds that detect systolic blood pressure in the lower limbs;
- • echocardiography (Siemens Acuson CV70 Cardiac Vascular Ultrasound Machine) highlighting left ventricular (LV) wall motion abnormalities and ejection fraction values, being important predictors of left ventricular systolic dysfunction;
- • measurement of cIMT (at the levels of carotid bifurcation, internal, external, right and left carotid arteries) by using Siemens Acuson CV70 Cardiac Vascular Ultrasound Machine, B-mode and color Doppler ultrasound (5–10 MHz). Also the average of the cIMT (the average of the six quantified segments) was recorded (15) (19).

Evaluation of cardiovascular risk factors and the prediction of ASCVD

Treatment goals were defined by the 2019 Guidelines on Dyslipidaemias (8). The cardiovascular risk factors defined according to the European Society of Cardiology (ESC) are: the age and gender (> 50 years for male and > 60 years for female), genetic factors, race and ethnicity, dyslipidemia, diabetes mellitus, obesity, physical inactivity, smoking and high blood pressure (8).

ASCVD in the study was defined as the occurrence after enrollment of one of the following: coronary heart disease (CHD), ischemic stroke (stroke) and peripheral artery disease (PAD) (20).

Statistical analysis

The data of the FH patients were introduced into a database and processed through the statistical functions of the SPSS version 20.0 system. One-sample Kolmogorov-Smirnov for normal distribution tests were performed, and the data were calculated as: mean and standard deviation (SD) for normal distribution variables, percent for categorical variables by using frequency test, median and interquartile range (IQR) for continuous variables with asymmetrical distribution. Bivariate correlation analysis was achieved between the scale variables, using the Pearson correlation coefficient. To analyse the associations between ordinary and / or nominal variables with specific variables, we used specific association coefficients (Cramer's, Phi, contingency coefficient). Comparative analysis between the pathological, family history, clinical and paraclinical history according to gender were performed by means of parametric tests (paired sample t-test, One-Way ANOVA), while for the values that did not met

the criteria of normal homogeneity and normal distribution, we used nonparametric tests: Mann-Withney U sample, Wilcoxon Signed-rank, Kruskall-Wallis H test, Friedman test. They were also utilized for the categorical variables Chi Square and Fisher Exact Test. Post-hoc Tukey's HSD was adopted to accurately assess group differences (to compare all combinations between treatment groups). Survival free of ASCVD, defined as an cardiovascular events (CHD, stroke, PAD) during follow-up, was estimated using the Kaplan-Meier method. The duration of follow-up was calculated from the date of study inclusion to the date of cardiovascular events. Univariate and multivariable associations with cardiovascular events were evaluated using Cox proportional hazards regression models and summarized with hazard ratios (HRs) and 95% confidence intervals (CIs). The p value < 0.05 was considered statistically significant.

Results

Identification of cardiovascular risk factors and the prevalence of FH population in the north-eastern part of Romania:

The study group included 61 patients, with a mean age of 48.46 ± 12.53 years, and a range of variation between 19 and 65 years, all subjects being Caucasian (Fig. 1a. and Fig. 1b.). The study group comprised more women (n = 39, 63.93%) compared to men (n = 22, 36.07%). The laboratory results for FH population were: TC 315 ± 56 mg/dL, LDL-C 254.2 ± 53 mg/dL, HDL-C 45.8 ± 18 mg/dL, TG 174.4 ± 92 mg/dL (for all patients); the same values of lipid profile by gender (Table 1). Also, the prevalence of FH population (with DLCN score above 3) in the north-eastern part of Romania was 6.2%.

Table 1
Baseline characteristics of FH patients

Characteristics	FH Patients		
	Total	Male	Female
N	61	22	39
Age - yo (mean ± SD)	48.46 ± 12.53	46.14 ± 13	49.77 ±11
Smoking status n (%)	18 (29.5%)	12 (54.5%)	6 (15.4%)
High blood pressure n (%)	31 (50.8%)	13 (59.1%)	18 (46.2%)
CHD history n (%)	28 (21.3%)	6 (27.3%)	22 (17.9%)
PAD history n (%)	10 (14.8%)	3 (13.6%)	7 (15.4%)
CHD + PAD history n (%)	11 (23%)	5 (22.7%)	6 (23.1%)
Obesity n (%)	36.1%	8 (36.4%)	14 (35.9%)
Type 2 diabetes n (%)	8 (13.1%)	4 (18.2%)	4 (10.3%)
Physical inactivity n (%)	30 (49.2%)	14 (63.6%)	16 (41%)
TC mg/dL (median ± IQR)	315 ± 56	313.5 ± 41	315 ± 60
LDL-C mg/dL (mean ± SD)	254.2 ± 53	257 ± 63	252.5 ± 47
HDL-C mg/dL (median ± IQR)	45.8 ± 18	44 ± 19	46 ± 18
TG mg/dL (mean ± SD)	174.4 ± 92	163.7 ± 90	180.5 ± 94
Uric acid mg/dL (mean ± SD)	5.79 ± 1.22	6.2 ± 1.1	5.5 ± 1.2
hsCRP mg/L (mean ± SD)	5.85 ± 2.29	6.3 ± 2.5	5.9 ± 2
EKG changes n (%)	25 (41%)	11 (50)	14 (35.9)
LV wall motion abnormalities n (%)	25 (41%)	11 (50)	14 (35.9)
ABI (mean ± SD)	0.96 ± 0.93	0.84 ± 0.09	1.01 ± 1.4
cIMT mm (mean ± SD)	0.94 ± 0.33	1.02 ± 0.34	0.9 ± 0.32

Legend: CHD- coronary heart disease, PAD- Peripheral arterial disease, TC-Total cholesterol, LDL-C - low density cholesterol lipoprotein, HDL-C- high density cholesterol lipoprotein, TG- triglycerides, hsCRP- high-sensitivity C-reactive protein, EKG- electrocardiogram, LV-left ventricular, ABI- ankle-brachial index, cIMT- carotid intima-media thickness

a)

b.

Characteristics	FH Patients		
	Total	Male	Female
Lipid-Lowering Agents	22 (36.1%)	7(39.8%)	15(38.5%)
Statin n (%)	18 (29.5%)	11 (50%)	7 (17.9%)
Statin + ezetimibe n (%)	8 (13.1%)	3 (13.6%)	5 (12.8%)
Statin + fenofibrate n (%)	13 (21.3%)	1 (4.5%)	12 (30.8%)
Statin + fenofibrate + ezetimibe n (%)	3%	1%	2%
Adverse effects n (%)			
Legend: CHD- coronary heart disease, PAD- Peripheral arterial disease, TC-Total cholesterol, LDL-C - low density cholesterol lipoprotein, HDL-C- high density cholesterol lipoprotein, TG- triglycerides, hsCRP- high-sensitivity C-reactive protein, EKG- electrocardiogram, LV-left ventricular, ABI- ankle-brachial index, cIMT- carotid intima-media thickness			
a)			
b.			

The cardiovascular risk factors in the enrolled patients group recorded: high blood pressure (50.8%), followed by sedentary lifestyle (49.2%), obesity (36.1%), smoking (29.5%), cardiovascular history (ischemic heart disease and peripheral arterial disease) (23%) and type 2 diabetes (13.1%). Table 1 presents the demographic and clinical data of patients with FH.

The main cardiovascular risk factors for men and for female were presented in Table 1.

The FH status of the enrolled subjects has been assessed by calculating the DLCN score, the values between 4 and 19 being the reference. Possible FH was identified with score of 4 at 31.2% (n = 19 patients), the score 5 at 8.2% (n = 5 patients), probable FH with score 6 at 32.8% (n = 20 patients), score 7 at 4.9% (n = 3 patients), score 8 at 8.2% (n = 5 patients) and definite FH with score 10 at 6.6% (n = 4 patients), score 11 at 1.6% (n = 1 patient), score 13 at 4.9% (n = 3 patients) and score 19 at 1.6% (n = 1 patient) (Fig. 2a. and Fig. 2b.). Also, the DLCN score did not vary between patients according to the gender ($U = 432.5$, $z = -0.05$, $p = 0.95$) and neither the age ($U = 494$, $z = -0.45$, $p = 0.65$). By the Simone Broome score, patients were classified as possible FH (n = 47, 77%) and probable FH (n = 14, 23%).

In 24 FH patients, both ischemic changes on the EKG and LV wall motion abnormalities following echocardiography were identified, while in the other patients (n = 37) these pathological aspects were not achieved ($\chi^2 = 61$, $df = 1$, $p = 0.001$). At the same time, our data revealed a relationship between the two diagnostic methods used in the examination of the cardiovascular system for patients included in the study by gender ($p < 0.05$) (Supplemental Table 1).

TC values were correlated with increased mean cIMT values in 37% patients (r = + 0.37, p = 0.03), with low values of ejection fraction (EF) in 43% patients (r = -0.43, p = 0.001) and low ABI levels at 64% patients (r = − 0.64, p = 0.001) (Supplemental Table 1).

The significantly increased of LDL-C values were positive correlated with high values of cIMT (r = + 0.39, p = 0.002) and negative correlated with the low EF values (r = -0.42, p = 0.001), and low ABI values (p = 0.001) (Supplemental Table 2) .

The significantly increased of TG concentrations were positive correlated with high values of cIMT (r = + 3.30, p = 0.02), while low HDL-C did not correlate with any of the parameters (Supplemental Table 2) .

In addition, nontraditional cardiovascular risk factors represented by hsCRP and uric acid were correlated as follows: high values of hsCRP were positive and significantly correlated with high concentrations of TC (r = + 0.45, p = 0.001) and LDL-C (r = + 0.47, p = 0.001), and the increased values of uric acid were positive correlated with the elevated TG (r = + 0.29, p = 0.02) (Supplemental Table 3).

Clinical, biological and imaging FH patients follow-up based on lipid lowering drugs

All the patients in the study had lipid-lower therapies, the highest frequency being the treatment with statin monotherapy 36.1%, followed by the associations between statin and ezetimibe, statin and fenofibrate, and the triple association between them respectively. Also, a small number of patients (3%) reported adverse effects to statin manifested by myalgia and headache (Table 1).

Intensive lipid-lowering therapy in the 12 months and 24 months, respectively, compared to baseline, statin alone or statin in association with ezetimibe and / or fenofibrate, was found to decrease TC, LDL-C levels and to augment plasma HDL-C levels. In addition, a significant reduction of TC and LDL-C concentrations were observed at 12 months for the patients with maximum treatment dose compared to baseline, but with a minimum decreased later, at 24 months. The most effective treatment was intensive dose of statin monotherapy in association with fenofibrate. As for the HDL-C values improvement, the most efficient treatment was statin alone, while association between high-dose of statin, ezetimibe 10 mg and fenofibrate 160 mg proved to be the best for lowering TG (Fig. 3a.-d.).

Also, hsCRP values were decreased after administration of all combinations of lipid-lowering treatment at both 12 months and 24 months, high-dose statin monotherapy being the most powerfull (Fig. 4).

At the same time, cIMT levels recorded significant differences ($p < 0.05$) between the groups of patients receiving lipid lowering agents ($F (22, 61) = 3.97$; $p = 0.01$) (Supplemental Table 4). After stratifying the group by gender, cIMT did not differ according to the treatment for male patients, whereas for female subjects, cIMT decreased significantly between treatment groups ($p = 0.007$). Following the posthoc tests, patients administered statin alone recorded significant cIMT reduction, the association between the 3 lipid lowering drugs contributing to a minimum reduction on it (Supplemental Table 5).

The ABI values were significantly affected by the administration of lipid lowering treatment after 24 months of follow-up ($p < 0.05$) ($\chi^2 = 18.31$, $p = 0.001$, Fig. 5a.-b. and Supplemental Table 6).

Lipid-lowering therapy did not significantly affect blood glucose levels χ^2 (2) = 5.93, p = 0.051, nor of transaminases χ^2 (2) = 5.31, p = 0.07 for AST, χ^2 (2) = 2.98, p = 0.22 for ALT (Figure S2).

ASCV in FH patients

In our study, FH population had 68.9% (n = 42 patients) ASCVD represented by: 37.7% (n = 23 patients) with CHD, 22.9% (n = 14 patients) with stroke and 8.1% (n = 5 patients) with PAD (Fig. 6a). Moreover, women often had CHD (n = 12 FH patients) and stroke (n = 8 FHpatients) compared to men that had PAD more frequently (n = 3 FH patients) (Fig. 6b).

In FH subjects that were given high-dose of statin alone (23 months), respectively statin associated with fenofibrate (22 months), the time-interval for ASCVD (composite endpoint) occurrence was somehow postponed, compared to patients receiving the 3 lipid-lowering drugs association (8 months) or statin associated with ezetimibe (3 months) (log rank $\chi^2 = 17.13$, $p = 0.001$) (Fig. 7a. and Supplemental Table 7). Moreover, in FH population selected by DCLN score, for subjects with possible FH, the time-interval for ASCVD occurrence was higher compared with „probable" FH or „definite" FH respectively (log rank $\chi^2 = 43.73$, $p = 0.001$) (Fig. 7b. and Supplemental Table 8). Likewise, FH subjects with normal values of cIMT and ABI had a time-interval for ASCVD occurrence higher than FH patients with atherosclerosis (log rank $\chi^2 = 20.75$, $p = 0.001$, log rank $\chi^2 = 4.5$, $p = 0.03$ respectively) (Fig. 7c.-d. and Supplemental Table 9 and Table 10).

FH population with physical inactivity had a 5-fold increased risk of ASCVD compared to patients with physical activity (HR = 5.34, 95% CI: 1.86–15.34, p = 0.002). FH subjects with high values of cIMT had 11-fold enhanced risk of ASCVD compared to FH patients with normal cIMT values (HR = 11.17, 95% CI: 1.21-102.91, p = 0.03). FH subjects with high TC and TG levels had 1% increased risk of ASCVD (HR = 1.1, 95%CI: 1.0-1.2, p = 0.01), while hsCRP had 113% increased risk of ASCVD (HR = 2.14, 95% CI: 1.58–2.89, p = 0.001). Also, FH patients with statin therapy and the association between statin and ezetimibe were associated with 76% and 79% reduced risk of ASCVD (HR = 0.27, 95% CI: 0.08–0.88, p = 0.05, HR = 0.21. 95% CI: 0.06–0.70, p = 0.02 respectively), but they lost their statistical significance when they adjusted for all confounders (Table 2).

Table 2
Univariate and multivariable associations with cardiovascular events in FH patients

Variable	Crude	Model 1	Model 2	Model 3	Model 4
Age		0.99 (0.97–1.01)	0.98 (0.95–1.02)	0.99 (0.95–1.03)	1.01 (0.94–1.08)
Gender					
Female	0.27 (0.09–0.85)*	0.99 (0.48–2.06)	1.09 (0.50–2.34)	0.57 (0.20–1.59)	0.35 (0.09–1.23)
Male	1.0 (reference)				
DLCN score					
Possible FH (3–5)	0.45 (0.01–1.96)	0.12(0.03–0.51)*	0.09 (0.02–0.47)*	0.06 (0.01–0.35)*	0.28 (0.01–5.78)
Probable FH(6–8)	0.39 (0.03–4.52)	0.54 (0.16–1.84)	0.45 (0.12–1.72)	0.34 (0.09–1.35)	0.82 (0.09–7.59)
Definite FH (> 8)	1.0 (reference)				
CV personal history					
CHD	2.48 (0.49–12.35)		1.28 (0.46–3.54)	1.75 (0.57–5.41)	2.22 (0.48–10.30)
PAD	0.93 (0.21–4.22)		0.69 (0.19–2.49)	0.70 (0.19–2.49)	0.79 (0.12–5.22)
CHD + PAD	0.336 (0.050–2.276)		0.509 (0.112–2.312)	0.397 (0.084–1.877)	0.160 (0.022–1.169)
Without CHD or PAD	1.0 (reference)				
Phisical activity	3.96 (1.48–10.61)*			1.79 (0.73–4.40)	5.34 (1.86–15.34)*
Lipid-lowering therapy					

Model 1: adjusted for age, gender, DLCN score and Simon Broome score. Model 2, Model 1 + adjusted for CV personal history. Model 3, Model 2 + adjusted for physical activity, smoking status, lipid-lowering therapy. Model 4, model 3 + adjusted for laboratory finding (TC, LDL-C, HDL-C, TG, uric acid, hsCRP) and imagistic findings (ischemic changes EKG, EF, ABI, cIMT). * $p < 0.05$

Supplemental Table

Figure S1. Flowchart for FH patients

Variable	Crude	Model 1	Model 2	Model 3	Model 4
Statin	0.80 (0.15–4.41)			0.27 (0.08–0.88)*	0.84 (0.14–5.16)
Statin + Ezetimibe	0.54 (0.09–3.02)			0.20 (0.06–0.70)*	1.78 (0.26–12.11)
Statin + Fenofibrate	0.77 (0.15–3.81)			0.44 (0.12–1.59)	0.42 (0.08–2.30)
Statin + Fenofibrate + Ezetimibe	1.0 (reference)				
Ischemic changes on EKG	1.27 (0.39–4.05)				2.59 (0.51–13.15)
Ejection fraction	0.98 (0.90–1.06)				0.96 (0.88-1.00)
cIMT	0.72 (0.06–8.81)				11.17(1.21-102.91)*
ABI	0.03 (0.01-2258.43)				0.01 (0.01–5.33)
Total cholesterol	1.02 (1.01–1.03)*				1.01 (1.01–1.02)*
LDL-C	1.01 (0.99–1.02)				0.99 (0.98–1.01)
HDL-C	1.01 (0.98–1.05)				1.01 (0.97–1.04)
TG	1.00 (1.00-1.02)*				1.01 (1.00-1.02)*
Uric acid	0.87 (0.56–1.35)				1.04 (0.64–1.68)
hsCRP	2.17 (1.59–2.96)*				2.14(1.58–2.89)*

Model 1: adjusted for age, gender, DLCN score and Simon Broome score. Model 2, Model 1 + adjusted for CV personal history. Model 3, Model 2 + adjusted for physical activity, smoking status, lipid-lowering therapy. Model 4, model 3 + adjusted for laboratory finding (TC, LDL-C, HDL-C, TG, uric acid, hsCRP) and imagistic findings (ischemic changes EKG, EF, ABI, cIMT). * $p < 0.05$

Supplemental Table

Figure S1. Flowchart for FH patients

Discussions

In our study, 980 patients with dyslipidemia from the north-eastern part of Romania were evaluated, measuring the DLCN and Simone Broome (SM) scores, a number of 61 patients with DLCN score above 3 and FH possible/probably at SM score being included. The prevalence of possible FH was 6.2%. The study group recorded a mean age of 48.46 ± 12.53 years. Demographic data identified more female persons (n = 39; 63.93%) compared to the male (n = 23; 36.07%), revealing the early address of women to the primary health care units. The laboratory tests acknowledged significantly higher values of lipid profile by gender stratification (TC = 315 ± 56 mg/dL, LDL-C = 254.2 ± 53 mg/dL, HDL-C = 45.8 ± 18 mg/dL), except for TG levels (the female had greater TG compared to male 180.5 ± 94 *vs* 163.7 ± 90 mg dl). In contrast to our study, Casula et al., in a study of FH patients from Italy, identified no significant differences between genders, the means levels of lipid panel being close to those recorded by our subjects (21).

In our study, regarding the traditional cardiovascular risk factors, we identified that high blood pressure (50.8%) was the main factor in all patients, followed by sedentary lifestyle (49.2%), which results in obesity (36.1%), smoker (29.5%), personal cardiovascular history (23%) and last but not least type 2 diabetes balanced by diet or antidiabetic drugs (13%). In the LIPIGEN multicenter study from Italy the personal history for the premature coronary disease had a prevalence of 11.3%, and the peripheral vascular diseases a prevalence of 9.6% (22), while in our study the prevalence of the 2 pathologies was much higher (CHD were found in 21.3% of patients and PAD in 14.8%). Hypertension was the main cardiovascular risk factor, followed by sedentary lifestyle and obesity in women and smoking in men.

The measured DLCN score recorded values between 4 and 19, with the possible FH being identified in 39.4% (n = 24 patients), the probable FH in 45.9% (n = 28 patients) and the definite FH in 14.7% (n = 9 patients). The enrolled subjects were classified as possible FH (n = 47 patients; 77%) and probable FH (n = 14 patients; 23%) while using the Simone Broome score. Averna et al. found that 56.1% of FH patients had a DLCN score > 6, 23.6% had a score between 6 and 8 and 32.5% had a score above 8 (22). Also, Yudi et al. identified 3 patients (1%) with a DLCN score > 3 and 50 patients (24%) with a DLCN score > 6 (23).

In our group, 24 patients were identified with ischemic changes on EKG and LV wall motion abnormalities on echocardiography, while the other patients (n = 37) did not exhibited such pathological findigs. Song et al. reached at the same conclusions in an observational study performed on 28 homozygous FH patients, as little as 7 patients displayed reduction in segmental or diffuse ventricular contractility, while 21 patients did not show LV hypokinesia or akinesia (24).

hsCRP is a nontraditional marker of cardiovascular risk, which occurs in all stages of atherogenesis and correlates with different cardiovascular pathologies (25). In our study, the mean hsCRP value was above the upper normal limit of (hsCRP = 5.85 ± 2.29 mg /L), and male FH subjects recorded higher values compared to female. Also, the increased hsCRP levels were correlated with the elevated TC and LDL-C concentrantions, being an important factor for ASCVD in FH patients (113% increased risk of ASCVD). Likewise, in a cross-sectional study, Eltoft et al. pointed out that hsCRP was associated with the

identification of atherosclerotic plaques, but without influencing the formation of new plaque or its progression (26). Equivalent conclusions were observed in our study.

Uric acid is another marker of CVD risk as it can produce endothelial dysfunction, being a therapeutic target for endothelial dysfunction improvement (27). In our study, uric acid had levels at the upper limit of normal (5.85 ± 2.29 mg/dL), with elevated values in males (6.3 ± 2.5 mg / dl) and was significantly associated only with elevated plasma TG concentrations.

The ankle-brachial index (ABI), another nontraditional risk factor of CVD, had significantly lower values in men compared to women such that male gender had frequently subclinical atherosclerosis (they need a multidisciplinary approch for identification and for management). Moreover, the mean of ABI was 0.96 ± 0.93. Pereira et al. also found that PAD had a prevalence of 17.3% and should be routinely identified in these individuals, even if it was asymptomatic (12).

In FH patients in our study, the mean cIMT was 0.94 ± 0.33 mm, while cIMT changes were more frequent and more important in male FH subjects compared to female FH. In 2 control case studies, in patients with FH, cIMT had values between 0.77 ± 0.18 mm (15) (19). Also, in the ASAP study, Smilde et al. found the same trend regarding the baseline mean of cIMT grouped by gender (it was higher in the male 0.98 ± 0.22 mm compared to the female 0.93 ± 0.25) (28). In our study, the marker of subclinical atherosclerosis represented by cIMT correlated poorly with dyslipidemia (with increased TC, LDL-C and TG levels, but not with decreased HDL-C concentration), an idea reiterated by Khan et al. (mean cIMT was significantly correlated with LDL-C), cIMT being a predictor for CVD (15). In contrast to our study, in a meta-analysis that included 51 studies and 4057 FH patients, Masoura et al. showed that mean cIMT did not correlate with LDL-C levels ($p = 0.65$) nor with TG values ($p = 0.50$) in the FH population (14).

All the patients included received lipid-lowering therapies before enrollment in our study, the highest frequency being the treatment with high-dose statin monotherapy (36.1%), followed by the other associations between lipid-lowering drugs. Likewise, the same data were found in an observational study conducted by Elis et al., where 24% of all subjects with FH were treated with statin alone, 55% with association between statin plus another agent (44% received a combination of statin with ezetimibe) and 21% association between 3 drugs (29).

Furthermore, in our study, the adverse effects (myalgia and headache) were developed in a small number of patients (3%), similar to the reported results by Elis et al. (none FH patients had severe adverse reactions such as hepatic impairment or rhabdomyolysis) (29). Even though in our study lipid-lowering therapy did not cause significant changes on glycemic levels, or transaminases concentrations, for safety reasons, the glycemic control and liver function tests need to be performed (8).

After the administration of the lipid-lowering agents for 24 months, it was found a decreased of the TC, LDL-C levels and elevated HDL-C levels, but without reaching the goals according to the European Guide of Dyslipidaemias 2019, the most effective treatment being the double association between high-dose statin and fenofibrate. At the same time, in a case-control study (43 patients with FH and 26 controls)

with a 20 years follow up of lipid-lowering therapy (statin with niacin), patients exhibited improved lipid profile compared to patients treated with statin in monotherapy (30). The same results following the administration of lipid-lowering drugs were also revealed by Brunham et al., in a longitudinal observational study that enrolled 339 subjects based on clinical diagnosis of FH (≥ 50% LDL-C reduction in 34.5% FH patients) (31).

In addition, in our research we identified that the high-dose of statin alone, the high-dose of statin with fenofibrate respectively, reduced the time of ASCVD occurrence. However, we observed that FH patients that received statin in monotherapy had a significant reduction in subclinical atherosclerosis, identified through cIMT and ABI, while the 3 lipid-lowering drugs association manifested a mild effect. In 2 observational studies, the authors revealed that after 2 years double-dose of statin monotheraphy, cIMT dropped significantly, lowering the risk for CVD (19) (32). In FH patients, statins improved endothelial function in a manner related to the intensity of the therapy (14), results also identified in our study.

In our FH subjects, high cIMT values, physical inactivity, high TC, TG and hsCRP levels were associated with an increased risk of ASCVD. Similar to our study, Khan et al. found that increased cIMT and LDL-C levels (due to prolonged exposure of vessels to high cholesterol levels) were associated with a significantly increased risk of CHD (15). The prevalence of risk factors, their management and their impact, differ between the FH population and the non-FH population, including the calculation of the absolute CV risk due to FH (33).

Moreover, in our FH population, DLCN score that identified „possible” FH, normal values of cIMT and ABI, had a reduced time of ASCVD occurrence. In contrast to our findings, Brunham et al identified that the risk of CV recurrent event was significantly higher in men than in women, but it was not significantly different among people with DLCN score "definite" compared to "probably" or "possible" (31).

Research Limitations

There were significant limitations of this study. First of all, the study was longitudinal and the FH patients were followed-up for only 2 years. Second, the small number of patients included suggests that FH is a relatively rare genetic pathology, their selection being made according to scores of 2019 Guidelines on Dyslipidaemias (8). Third, the current study enrolled subjects from the north-eastern area of Romania, so that the current group of subjects did not significantly mirror the entire Romania population with ASCVD. At the same time, the diagnosis of FH should be considered by performing the DLCN and Simone Broome scores, especially among romanian patients with high risk for CVD and LDL-C > 190 mg/dL without treatment, or above 100 mg/dL at patients with high-intensity lipid-lowering therapies. Fourth, in all most enrolled patients there were no values for cIMT, ABI and lipid profile prior to inclusion in the study.

Conclusions

Dyslipidemia is a strong predictor for arterial wall thickness, and FH patients in our study had chronic inflammation status due to dyslipidemia, which contributed atheromatous plaques development.

However, to reduce cardiovascular risk, the FH patients need the cascade screening and a specific management. In addition, it is useful to setup clear protocols for straightforward identification of cases with familial dyslipidemia in order to make the differential diagnosis between FH and polygenic dyslipidemia. High doses of the lipid-lowering agents and administration for a long time resulted in a significant decreased decline of lipid profile (TC, LDL-C), of subclinical atherosclerosis markers (cIMT and ABI values), without significant adverse effects of the choiced therapy. High cIMT values, physical inactivity, high TC, TG and hsCRP levels were associated with an increased risk of ASCVD. DLCN score that identified „possible" FH, normal values of cIMT and ABI, had a reduced time of ASCVD occurrence. Even though it was the first observational study in the north-eastern part of Romania, further molecular genetics studies are needed to confirm the FH cases and to evaluate the oportunity of monoclonal antibodies (alirocumab, evolocumab) introduction into their therapies.

Abbreviations

FH- familial hypercholesterolemia; DLCN- Dutch Lipid Clinic Network score ASCVD- atherosclerotic cardiovascular disease; CVD
cardiovascular diseases; CHD -coronary heart disease; PAD- Peripheral arterial disease, cIMT - carotid intima-media thickness; ABI- ankle-brachial index; EKG- electrocardiogram, LV-left ventricular; EF ejection fraction TC- total cholesterol; LDL-C- low density cholesterol lipoprotein; HDL-C- high density cholesterol lipoprotein; TG- triglycerides; hsCRP- high-sensitivity C-reactive protein; CI:confidence interval; HR hazard ratio; IQR- interquartile range; SD-standard deviation.

Declarations

Ethical considerations

After the approval of the Informed Consent by the Ethics Commission of "Grigore T. Popa University of Medicine and Pharmacy Iasi", of the "Dr CI Parhon" Clinical Hospital, the Institute of Cardiovascular Diseases and the "Sf. Spiridon" Emergency Hospital in Iasi, the consent has been signed by all patients prior to study enrollement. The research could not state any physical or mental discomfort for the patients included in the study, nor any physical or mental risks, or obligation to participate in the study. The confidentiality of the personal and medical data of the subjects enrolled in the study was preserved.

Consent for publication

All authors consent to the publication of the data

Availability of data and material

The data and material of this study are available from the author (L.F.) on reasonable request.

Competing interests

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

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Authors' contributions

CEV, RP analyzed and interpreted the patients data regarding the FH criteria. CEV, AC, DRP, IIC performed the clinical and imagistic examination during the follow-up. FL, FL, AC were a major contributors in writing the manuscript. All authors read and approved the final manuscript.

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Figures

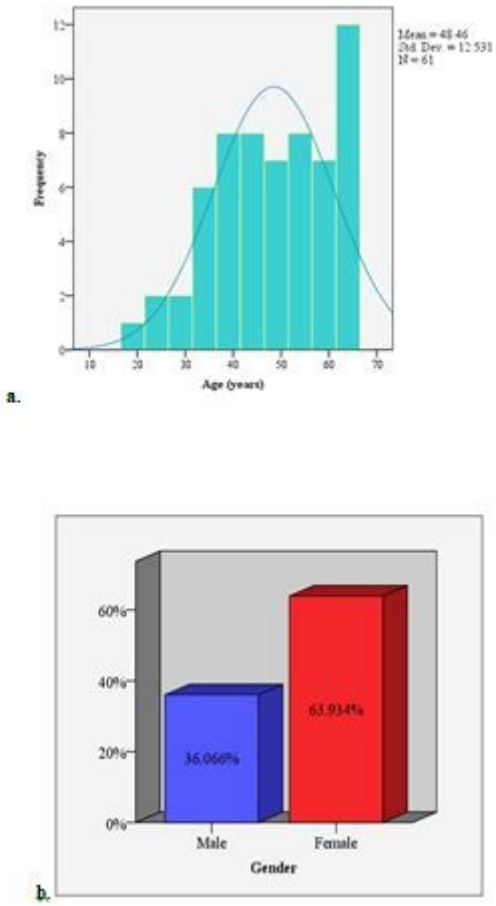

Figure 1

The frequency of FH patients according to a. age and b. gender

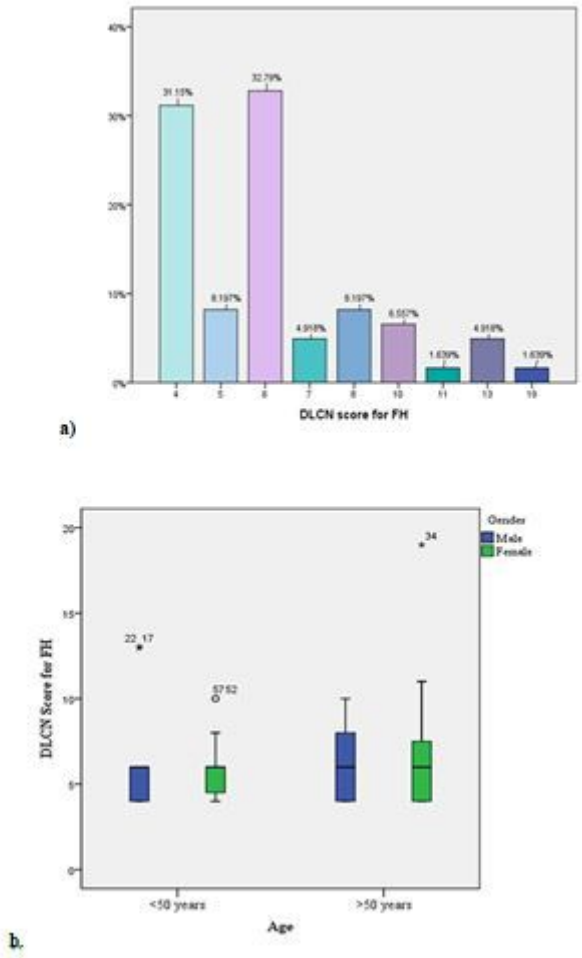

Figure 2

The frequency of the DLCN score and the distribution of the score by a. age and b. gender

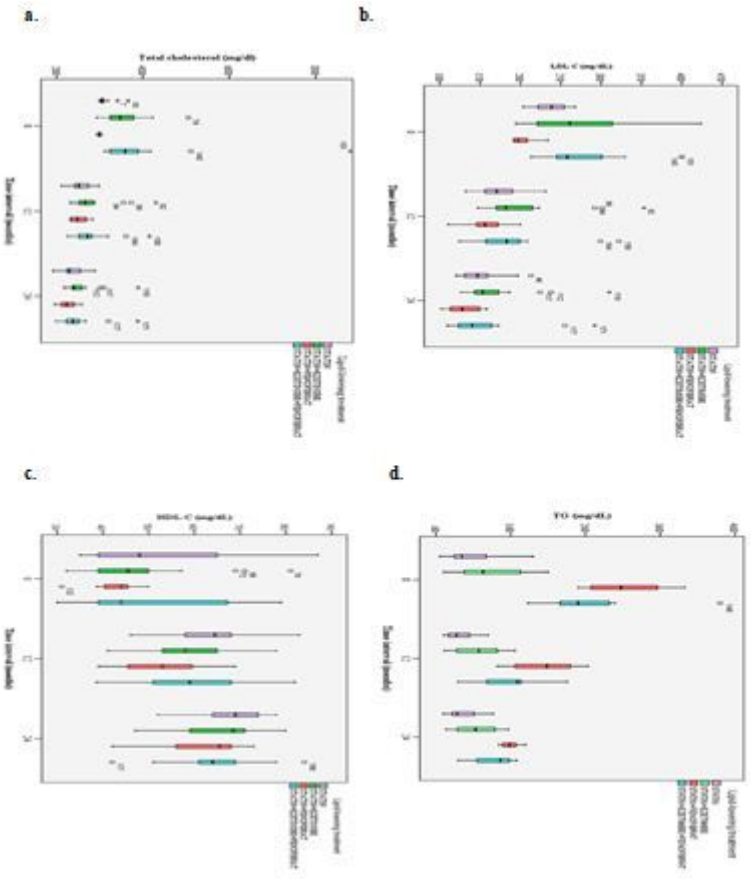

Figure 3

Lipid profile (a. total cholesterol; b. LDL-C; c.HDL-C; d.TG) based on the lipid-lowering treatment and the effect of time interval

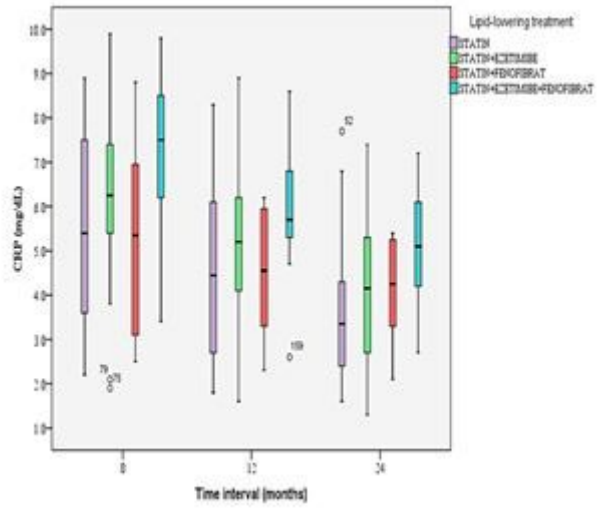

Figure 4

hsCRP based on the lipid-lowering treatment and the effect of time interval

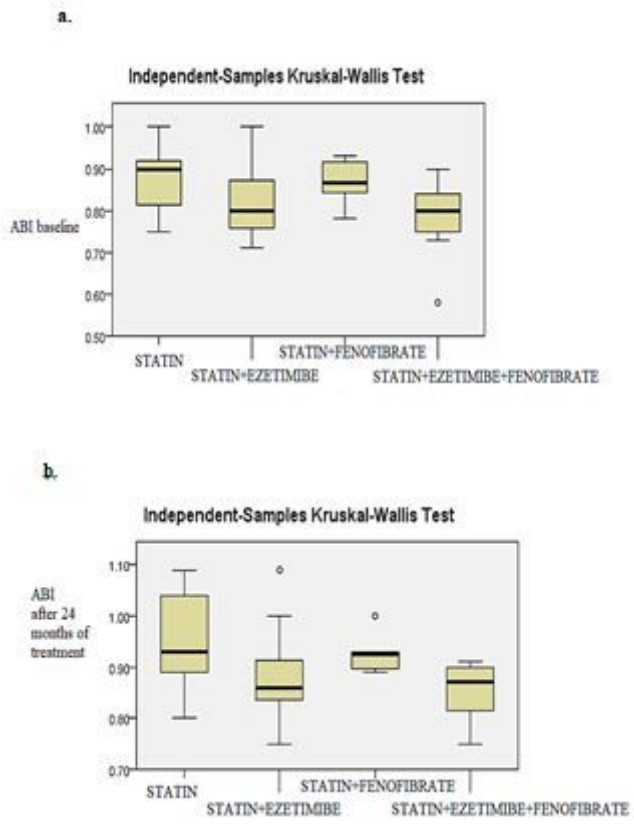

Figure 5

The relationship between lipid-lowering treatment and ABI values a) baseline b) after 24 months of treatment

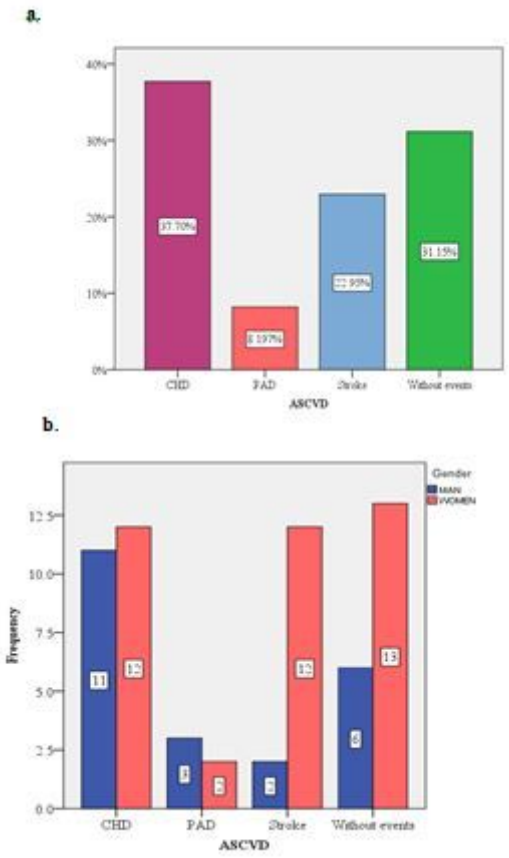

Figure 6

The frequency of the ACVD a. in FH population b. in FH population by gender

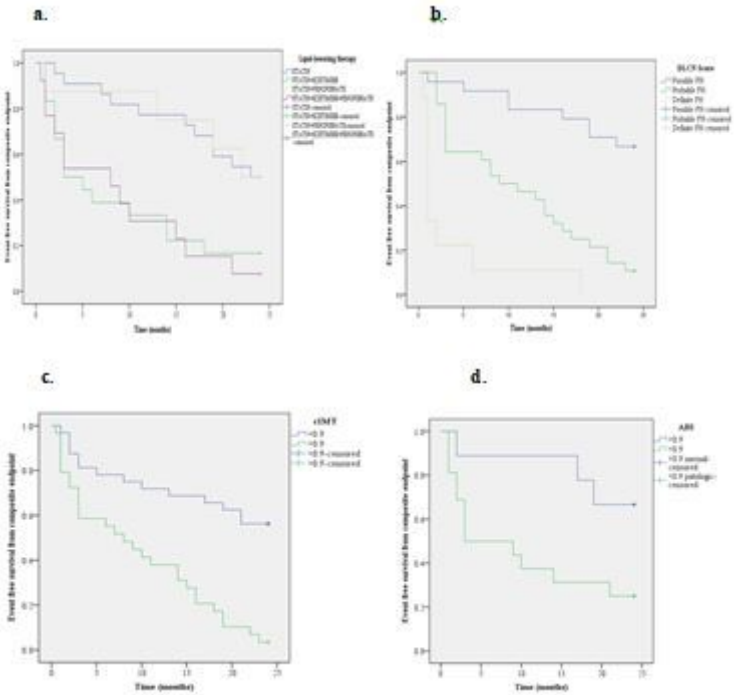

Figure 7

Kaplan Meier for ASCVD depending on a. the lipid-lowering therapy and time interval; b. the DLCN score and time interval; c. cIMT and time interval and d. ABI and time interval