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 pathologies. Traditional and nontraditional cardiovascular risk factors increase the prevalence of atherosclerotic cardiovascular disease(ASCVD) in this population. The aims of the study were: (a)to identify FH patients in the North-Eastern part of Romania and to analyze demographic, clinical and paraclinical data (b)to evaluate the risk of new cardiovascular events at follow-up in FH patients stratified by lipid-lowering agents.

Methods. This first prospective study in the North-Eastern part of Romania was carried out between October 2017 and October 2019; out of 980 patients with dyslipidemia evaluated with the Dutch Lipid Network(DLCN) and Simon Broome(SM) scores, 61 patients with DLCN score above 3 and possible/probable FH (SM score) were included.

Results. The 61 subjects with clinical diagnosis of FH (6.2% of all patients examined) recorded a mean age of 48.5±12.5 years, with more female patients than male patients. Hypertension was the main cardiovascular risk factor for both genders, followed by physical inactivity and obesity for the female group and active smoking for the male group. The measured DLCN score recorded: “possible” FH identified in 39.4%, “probable” FH in 45.9% and “definite” FH in 14.7%. As far as treatment was concerned, the effective lipid-lowering drugs were statin alone and statin in association with fenofibrate, which improved both the lipid profile values and the subclinical atherosclerosis markers (ankle-brachial index, carotid intima-media thickness and high-sensitivity C-reactive protein). New ASCVDs that emerged during the study were most commonly represented by coronary heart disease and stroke. At the same time, the new CV events were delayed in patients receiving the lipid-lowering drugs, without significant differences between them.

Conclusions. In patients with suspected FH, the lipid-lowering agents during the follow-up delayed the new cardiovascular events, yet failed to reach the goals proposed by the guidelines.

Background

Atherosclerosis is a complex multifactorial disorder, consisting in 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, and high blood pressure (1) (2).

The genetic cornerstone of atherosclerotic cardiovascular disease (ASCVD) includes both common deoxyribonucleic acid (DNA) variants (leading to polygenic susceptibility) and rare DNA versions that cause monogenic disorders, the typical monogenic disease being familial hypercholesterolemia (FH) (3). 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, representing a cause of premature coronary atherosclerotic

disease (4) (5). In Romania, the prevalence of FH records 1/213 for the heterozygous form (6) compared to other Caucasian populations with 1/500 individuals and to 1/1,000,000 for the homozygous form (7). There are three available criteria for the clinical diagnosis of FH: the Simon Broome criteria, the Dutch Lipid Clinic Network Criteria (DLCNC) and the MedPed criteria (8) (9).

Familial and personal history of hypertension, coronary heart disease (CHD), stroke, chronic kidney disease, smoking, eating habits, alcohol consumption, a sedentary lifestyle and nontraditional risk factors represented by the ankle-brachial index (ABI) and high-sensitivity C-reactive protein (hsCRP) were significantly associated with cardiovascular morbidity and mortality in FH patients (10) (11). Atherosclerotic plaques and the increase of mean cIMT were associated with a significantly expanded CHD risk (12) (13).

The management of cardiovascular risk factors is important for FH patients who associate cardiovascular atherosclerotic pathology (14), while statin therapy combined with ezetimibe and PCSK9 inhibitors contribute to an increased survival rate (7) (15) (16). Our prospective study was motivated by the current state of poor diagnosis, lack of a multidisciplinary approach and defective management of FH patients in Romania. The study included patients with suspected FH with the following objectives: (a) to identify FH patients in the North-Eastern part of Romania and to analyze the demographic, clinical and paraclinical data (b) to evaluate the risk of new cardiovascular events at follow-up in these patients stratified by lipid-lowering agents (at baseline, after 12 and 24 months of follow-up, respectively).

Methods

Inclusion and exclusion criteria for patients suspected with suspected FH

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

Study population: Out of 980 patients with dyslipidemia identified between September 2016 and October 2017, 61 patients met the following ***inclusion criteria:*** subjects with full mental capacity who signed the informed consent form; men and women aged over 18 years. Furthermore, the DLCN score above 3 and the Simon Broome criteria (probable or possible FH) were two important selection tools for the patients with clinical diagnosis of FH. These include the following elements: 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 combination with ezetimibe, as required) (Figure S1 flowchart for these patients).

Exclusion criteria: subjects lacking discernment or those who refused to sign the informed consent, patients under the age of 18, pregnant and breast-feeding women; subjects with severe physical disabilities, dementia, neoplasms and other causes of secondary hypercholesterolemia (uncontrolled diabetes, nephrotic syndrome, hypothyroidism, drug-induced dyslipidemia) (17) (Figure S1 flowchart for these 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 for the FH population (9). The reference values of the DLCN score were: 3-5 points highlighted possible FH, 6-7 points indicated probable FH, while over 8 points indicated definite FH (9). The other score, namely the Simon Broome score advertised between possible, probable or definitive FH (9). 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 the patients' enrollment (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). The medical history revealed that certain patients had received antihypertensive medication (those with BP>140/90 mmHg), or oral antidiabetic medication (those diagnosed with type 2 diabetes), which they continued to take throughout the study, according to the specialist doctors' prescriptions.

The lipid profile pointed out the total cholesterol (TC) 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 (hsCRP) mg / dL quantified by immunoturbidimetry. The spectrophotometric method (UV VIS Spectrophotometer - METTLER TOLEDO) is based on the selective micellar solubilization of LDL-cholesterol using a non-ionic detergent and the interaction between a saccharide compound and lipoproteins (VLDL and chylomicrons). In the presence of magnesium ions, the saccharide compound causes a marked reduction in the enzymatic reaction for measuring cholesterol in VLDL and chylomicrons and selectively determines LDL-c. The other technique, immunoturbidimetry, is based on the formation of antigen-antibody complexes in a solution. The formation of antigen-antibody complexes can be followed in a spectrophotometer as flocculation occurs, and absorbance increases.

Further explorations for cardiovascular evaluation included:

- an electrocardiogram (ECG) for ischemic changes assessment;
- a ABI measurement with a sphygmomanometer and a portable ultrasonography device, which determines sounds that detect systolic blood pressure in the lower limbs; the reference ABI values

were between 0.9 and 1.3.

- echocardiography (Siemens Acuson CV70 Cardiac Vascular Ultrasound Machine) highlighting left ventricular (LV) wall motion abnormalities and ejection fraction values, which are 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). The average of the cIMT (the average of the six quantified segments) was also recorded. The reference cIMT values were under 0.9 mm (13) (18).

Evaluation of cardiovascular risk factors and the new cardiovascular events

Treatment goals were defined by the 2019 Guidelines on Dyslipidaemias (9). ***The cardiovascular risk factors*** were defined according to the European Society of Cardiology (ESC): age and gender (> 50 years for men and > 60 years for women), genetic factors, race and ethnicity, diabetes mellitus (FPG ≥126 mg/dL: fasting was defined as no caloric intake for at least 8 h; 2-h plasma glucose≥200 mg/dL during oral glucose tolerance test (OGTT). The test should be performed using a glucose load containing the equivalent of 75-g anhydrous glucose dissolved in water; A1C ≥6.5% using a standardized assay, classic symptoms of hyperglycemia or hyperglycemic crisis with a random plasma glucose ≥200 mg/dL), obesity (BMI exceeding 30 kg/m², waist circumference in Caucasian females >88 cm and in males > 102 cm), physical inactivity (under 30-60 minutes in most days), smoking (active or passive or without exposure to tobacco in any form) and high blood pressure (BP>140/90 mmHg) (9) (16) (19) (20).

Atherosclerotic cardiovascular disease (ASCVD) was defined as a history of one of the following diseases identified in the medical records: coronary heart disease (CHD) with the following particularities: acute coronary syndrome, myocardial infarction (MI), stable angina, coronary revascularization, ischemic stroke, or transient ischemic attack and peripheral artery disease (PAD) (21).

Statistical analysis

The data of the patients with clinical diagnosis of FH 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, with the data being calculated as: mean and standard deviation (SD) for normal distribution variables, percent for categorical variables by using a 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 analyze the associations between ordinary and / or nominal variables with specific variables, specific association coefficients were used (Cramer's, Phi, contingency coefficient). Comparative analyses between the pathological, family history, clinical and paraclinical history according to gender were performed for the values that did not meet the criteria of normal homogeneity, whereas normal distribution was performed for nonparametric tests: Mann-Withney U sample, Wilcoxon Signed-rank, Kruskall-Wallis H test, Friedman

test. They were also employed for the categorical variables Chi Square (χ^2) and Fisher Exact Test. Survival free of ASCVD, defined as 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 inclusion in the study to the date of the cardiovascular events. Multiple logistic regression analysis was applied to identify the independent factors for cardiovascular events. The *P* value <0.05 was considered statistically significant.

Results

Baseline data of patients with suspected FH in the North-Eastern part of Romania

The study group included 61 patients (6.2% of all patients examined), with a mean age of 48.5 ±12.5 years, all subjects being Caucasian (Figure 1a. and Figure 1b.), with a higher number of women compared to men (63.9% versus 36%). The laboratory results 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), whereas the lipid profile did not differ according to gender (Table 1). Moreover, 36.1% of the patients had ASCVD history.

Table 1 presents the demographic and clinical data of the patients with clinical diagnosis of FH, as well as the main cardiovascular risk factors for male and female patients. Furthermore, uric acid and smoker status (active or passive) displayed different values according to gender.

At baseline, all the patients in the study had lipid-lowering therapies (about 1 year of treatment until inclusion in the study) the most frequent being the treatment with statin monotherapy 36.1%, followed by the associations between statin and ezetimibe, statin and fenofibrate, and the triple combination between them respectively. Also, a small number of patients (3%) reported adverse effects to statin such as myalgia and headache (Table 1).

The FH status of the enrolled subjects was assessed by calculating the DLCN score, with reference values between 4 and 19. ***Possible FH*** was identified with a score of 4 in 31.2% (n = 19 patients), a score of 5 in 8.2% (n = 5 patients). ***Probable FH*** was identified with a score of 6 in 32.8% (n=20 patients), a score of 7 in 4.9% (n=3 patients), a score of 8 in 8.2% (n=5 patients). ***Definite FH*** was identified with a score of 10 in 6.6% (n=4 patients), a score of 11 in 1.6% (n=1 patient), a score of 13 in 4.9% (n = 3 patients) and a score of 19 in 1.6% (n=1 patient) (Figure 2a. and Figure 2b.). Moreover, the DLCN score showed no variations between patients, either according to gender (U = 432.5, z = -0.05, *P* = 0.95) or the age (U = 494, z = -0.45, *P* = 0.65). According to the Simon Broome score, patients were classified as possible FH (n = 47, 77%) and probable FH (n = 14, 23%).

Both ischemic changes on the ECG and LV wall motion abnormalities following echocardiography were identified in the 24 patients suspected of having FH, , while in the other patients (n=37) these pathological aspects were not observed ($\chi^2(1) = 61$, *P* = 0.001).

TC values were correlated with increased cIMT values (r = +0.37, *P* = 0.03), with low values of ejection fraction (EF) (r = -0.43, P = 0.001) and with low ABI levels (r = - 0.64, *P* = 0.001). The significantly increased LDL-C values were positively correlated with high values of cIMT (r = +0.39, *P* = 0.002) and negatively correlated with low EF values (r = -0.42, *P* = 0.001), and low ABI values (*P* = 0.001). The significantly increased TG concentrations were positively correlated with high values of cIMT (r = +3.30, *P* = 0.02), while low HDL-C did not correlate with any of the parameters.

In addition, nontraditional cardiovascular risk factors represented by hsCRP and uric acid were correlated as follows: high values of hsCRP were positively 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 positively correlated with the higher TG (r = +0.29, *P* = 0.02) values.

ASCVD inpatients with **suspected FH follow-up based on lipid lowering drugs**

Intensive lipid-lowering therapy administered for 12 months, respectively 24 months from the enrollment in the study, compared to baseline, statin alone and statin in association with fenofibrate, were found to decrease the TC levels (Figure 3a). In addition, a significant reduction of LDL-C concentrations was observed at 12 months after the enrollment in the study, for the patients with maximum treatment dose (statin in association with fenofibrate) compared to baseline, but with a minimum decrease later, after 24 months (Table 2 and Figure 3b). Furthermore, at both 12 months and 24 months follow-ups, the most efficient treatment to improve HDL-C values and to decrease TG and hsCRP levels was statin alone (Figure 3c-e and Table 2). At the same time, both ABI and cIMT levels recorded significant differences between the groups of patients receiving lipid-lowering agents after 24 months of follow-up (Table 2). On the other hand, lipid-lowering therapy affected significantly neither the blood glucose levels, nor transaminases levels (P>0.05) (Table 2).

In this study, 26.2% (n=16 patients) of the population with clinical diagnosis of FH displayed new cardiovascular events during the follow-up, as follows: CHD in 13.1% of 61 enrolled patients (n=8 patients), stroke in 4.9% of 61 enrolled patients (n=3 patients) and PAD in 8.2 % of 61 enrolled patients (n=5 patients) (Figure 4a). Moreover, more women had new cardiovascular events represented by PAD (4.9% of 61 enrolled patients, n=3 patients) and stroke (6.6% of 61 enrolled patients, n=4 patients), as compared to men, in which CHD occured more frequently (8.2% of 61 enrolled patients, n= 5 patients) (Figure 4b).

For the subjects who received statin associated with fenofibrate (23 months), respectively high-dose of statin alone (22 months), the time-interval for ASCVD (composite endpoint) occurrence was not significantly postponed, as compared to patients receiving the 3 lipid-lowering drugs association (20 months) or statin associated with ezetimibe (18 months) (log rank χ^2 = 1.7, P = 0.6) (Figure 5).

Furthermore, following the multiple logistic regression, only LDL-C over 190mg/dL, and hsCRP>5 mg/L were predictors of cardiovascular events in patients with clinical diagnosis of FH (Table 3).

Discussion

This is the first epidemiological study aiming at identifying the population with FH in Romania's NE region, which includes a population of approximately 3,979,978 inhabitants (including eight counties). Besides, this study is useful for identifying cases with familial dyslipidemia to make the differential diagnosis between FH and polygenic dyslipidemia. In Romania, there is no active FH registry. However, it is necessary to create a database: for the implementation of a national policy for early screening through genetic tests, in order to find out the approximate number, the phenotype and genotype of FH patients in different regions of Romania, for a targeted management with monoclonal antibodies resulting in the reduction of new cardiovascular events; and finally for various international collaborations (in clinical trials) for a better understanding of this pathology.

In the aforementioned study, 980 patients with dyslipidemia from the North-Eastern part of Romania were evaluated, measuring the DLCN and Simon Broome (SM) scores, a number of 61 patients with DLCN score above 3 and possible/probable FH at SM score being included. The demographic data identified more female patients than male patients, indicating that women address the primary health care units earlier. The laboratory tests acknowledged significantly higher values of lipid profile by gender stratification, except for TG levels (the female patients had greater TG compared to male patients: 180.5 ± 94 vs 163.7 ± 90 mg dL). In contrast to this 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 the subjects included in our study (22). Hypertension was the main cardiovascular risk factor for both sexes, followed by sedentary lifestyle and obesity for the female group and active or passive smoking for the male group.

As in the studies conducted by Averna et al and Yudi et al., this study revealed the same results regarding the DLCN score between 3-6 (possible FH or probable FH), but different results for the DLCN score above 8 (definite FH) - they have a higher number of patients with DLCN>8 (23) (24).

Patients with suspected FH were identified with ischemic changes on ECG and LV wall motion abnormalities (LV hypokinesia or akinesia) on echocardiography, as in the case of Asian FH patients included in the study conducted by Song et al. (25).

hsCRP is a nontraditional marker of cardiovascular risk, which occurs in all stages of atherogenesis and correlates with different cardiovascular pathologies (26). 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 (27), equivalent findings being also observed in the above-mentioned study.

The ankle-brachial index (ABI) had significantly lower values in men compared to women, with male patients frequently displaying subclinical atherosclerosis. Moreover, this specific group of patients needs screening for the early identification of PAD, even if they are asymptomatic, an approach also supported by Pereira et al. (28). In this 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 supported by Khan et al. (mean cIMT was significantly correlated with LDL-C) (13). In contrast to the results described above, in a meta-analysis that included 51 studies and 4057 FH patients, Masoura et al. showed that mean cIMT correlates neither with LDL-C levels, nor with TG values in the FH population (12). Also, in the ASAP study, Smilde et al. found the same trend regarding the baseline mean of cIMT grouped by gender (29).

Likewise, in an observational study, Elis et al. found the same frequency of lipid-lowering agents, with a higher administration of statin in monotherapy compared the combination of these drugs (30). Furthermore, in this study, the adverse effects (myalgia and headache) affected a small number of patients (3%), similar to the results reported by Elis et al. (none of the FH patients had severe adverse reactions such as hepatic impairment or rhabdomyolysis) (30). Even though this study indicates that the lipid-lowering therapy did not cause significant changes on glycemic levels or transaminases concentrations, the glycemic control and liver function tests should be performed for safety reasons (9). This research indicates that all lipid-lowering drugs have contributed to the postponement of cardiovascular events, without significant differences between them. The administration of the lipid-lowering agents during the follow-up resulted in decreased TC and LDL-C levels and elevated HDL-C levels, yet without reaching the goals established by the European Guide of Dyslipidemias. In order to achieve the target objectives, it is necessary to discuss the introduction of monoclonal antibodies in lipid-lowering medication combinations, a conclusion also supported by Cesaro et al (31). The PCSK9 inhibitors had a good adherence (a good safety profile and a good efficacity) (32) (2). The PCSK9 inhibitors represent a valuable therapy in patients with FH, with a significant reduction in LDL-C values, their bi-weekly or monthly administration contributing to an increased quality of life (31). Yet, because of the low economic level and moderate addressability of health care, this medication is difficult to implement as a national public health program.

Similarly, Khan et al. found that increased hsCRP and LDL-C levels (caused by prolonged exposure of vessels to high cholesterol levels) were associated with a significantly increased risk of CHD (13).

STUDY STRENGTH AND LIMITATIONS

This is the first epidemiological study conducted in order to identify the population with FH in Romania's NE region, as it is absolutely necessary to create a database, since currently no active FH registry is available. The region is homogenous as far as genetic elements, culture, eating habits, and socio-economic status are concerned, being served by several county hospitals and one referral university and research center.

Nevertheless, this study had significant limitations. First of all, the study was longitudinal and the patients were followed-up for only 2 years. Secondly, the small number of patients included in the study might suggests that FH is a relatively rare genetic pathology, their selection being made according to the scores provided by the 2019 Guidelines on Dyslipidemias (9). Thirdly, 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 Romanian population with ASCVD. At the same time, the diagnosis of FH should be considered by performing the DLCN and Simon Broome scores, especially among Romanian patients with a high risk for CVD and LDL-C>190 mg/dL without treatment, or above 100 mg/dL in patients with high-intensity lipid-lowering therapies. Moreover, for most enrolled patients there were no values for cIMT, ABI and lipid profile prior to their inclusion in the study.

Conclusions

The patients with clinical diagnosis of FH included in the study were young patients, mainly women, whose lipid profile did not differ according to gender. Hypertension was the main cardiovascular risk factor, followed by a sedentary lifestyle and obesity for female patients, and by an active smoking status for male patients. Moreover, the DLCN score did not differ according to gender or age, and a significant number (36.1%) of patients had a history of ASCVD. At the end of the study, it was found that lipid-lowering drugs decreased LDL-C, hsCRP and cIMT levels, and increased ABI and HDL-C values, yet without reaching the goals established by the European Guide of Dyslipidemias. The most common new ASCVD event was CHD, followed by stroke and PAD. All lipid-lowering drugs delayed the new cardiovascular events, without significant differences between them. Even though this 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 opportunity of introducing monoclonal antibodies (alirocumab, evolocumab) as a therapy option, despite the socio-economic barriers.

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; ECG- 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” of Iasi, of the "Dr. C. I Parhon" Clinical Hospital, the Institute of Cardiovascular Diseases and the "Sf. Spiridon" Emergency Hospital of Iasi, the consent was signed by all the patients prior to their enrollement in the study. 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 major contributors in writing the manuscript. All authors read and approved the final manuscript.

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Tables

Table 1. Baseline characteristics of patients suspected for FH

Characteristics	Patients suspected for FH			
	Total	Male	Female	P
N	61	22	39	0.001
Age - yo (mean±SD)	48.4 ± 12.5	46.1 ± 1	49.7 ±1	0.4
Smoker n (%)	18 (29.5 %)	12 (54.5 %)	6 (15.4 %)	0.001*
High blood pressure n (%)	31 (50.8 %)	13 (59.1 %)	18 (46.2 %)	0.3
CHD history n (%)	28 (21.3%)	6 (27.3 %)	22 (17.9 %)	0.5
PAD history n (%)	10 (14.8%)	3 (13.6 %)	7 (15.4 %)	0.5
CHD+PAD history n (%)	11 (23%)	5 (22.7 %)	6 (23.1 %)	0.5
Obesity n (%)	36.1 %	8 (36.4 %)	14 (35.9 %)	0.9
Type 2 diabetes n (%)	8 (13.1 %)	4 (18.2 %)	4 (10.3 %)	0.4
Physical inactivity n (%)	30 (49.2 %)	14 (63.6 %)	16 (41 %)	0.09
TC mg/dL (median± IQR)	315 ± 56	313.5 ± 41	315 ±60	0.7
LDL-C mg/dL (mean±SD)	254.2 ± 53	257 ±63	252.5± 47	0.8
HDL-C mg/dL (median± IQR)	45.8 ±18	44 ±19	46 ±18	0.6
TG mg/dL (mean±SD)	174.4 ± 92	163.7± 90	180.5± 94	0.6
Uric acid mg/dL (mean±SD)	5.79 ± 1.22	6.2 ±1.1	5.5 ±1.2	0.02*
hsCRP mg/L (mean±SD)	5.85 ± 2.29	6.3 ±2.5	5.9± 2	0.5
ECG changes n (%)	25 (41%)	11 (50)	14 (35.9)	0.3
LV wall motion abnormalities n (%)	25 (41%)	11 (50)	14 (35.9)	0.1
ABI (mean±SD)	0.96±0.93	0.84±0.09	1.01±1.4	0.8
cIMT mm (mean±SD)	0.95±0.33	1.02±0.34	0.9±0.32	0.2
Lipid-Lowering Agents				0.3
Statin n (%)	22 (36.1%)	7(39.8 %)	15(38.5 %)	
Statin + ezetimibe n (%)	18 (29.5%)	11 (50 %)	7 (17.9 %)	
Statin + fenofibrate n (%)	8 (13.1 %)	3 (13.6 %)	5 (12.8 %)	
Statin + fenofibrate + ezetimibe n (%)	13 (21.3%)	1 (4.5 %)	12 (30.8 %)	
Adverse effects n (%)	3 %	1%	2%	

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, ECG- electrocardiogram, LV-left ventricular, ABI- ankle-brachial index, cIMT- carotid intima-media thickness

*P<0.05

Table 2. Paraclinical characteristics of patients with clinical diagnosis of FH on the follow-up

Characteristics	Patients with clinical diagnosis of FH			
	Baseline	12 months	24 months	P
TC mg/dL (median ± IQR)	315 ± 56	258 ± 37	237 ± 33	0.001*
LDL-C mg/dL (mean ±SD)	254.2 ± 53	185± 46	156 ± 40	0.001*
HDL-C mg/dL (median± IQR)	45.8 ±18	59 ± 15	68 ± 11	0.001*
TG mg/dL (mean ± SD)	174.4 ± 92	130± 49	119 ± 32	0.001*
Uric acid mg/dL (mean ± SD)	5.8 ± 1.22	5.3 ± 0.9	5.1 ± 1	0.001*
hsCRP mg/L (mean ± SD)	5.9 ± 2.29	5 ± 1.9	4.2 ± 1.7	0.001*
Glucose (median ± IQR)	97 ± 18	100 ± 15	97 ± 23	0.06
AST (median ± IQR)	25 ± 14	28 ± 15	28 ± 31	0.07
ALT (median ± IQR)	28 ± 20	33 ± 15	32 ± 15	0.2
ABI (mean ± SD)	0.96 ± 0.9	0.92 ± 0.6	0.91 ± 0.08	0.001*
cIMT mm (mean ± SD)	0.95 ± 0.3	0.91 ± 0.3	0.85 ± 0.3	0.001*

Legend: TC-Total cholesterol, LDL-C - low density cholesterol lipoprotein, HDL-C- high density cholesterol lipoprotein, TG- triglycerides, hsCRP- high-sensitivity C-reactive protein, AST- aspartate transaminase, ALT- alanine aminotransferase, ABI- ankle-brachial index, cIMT- carotid intima-media thickness

*P<0.05

Table 3. Independent factors for cardiovascular events in patients suspected for FH

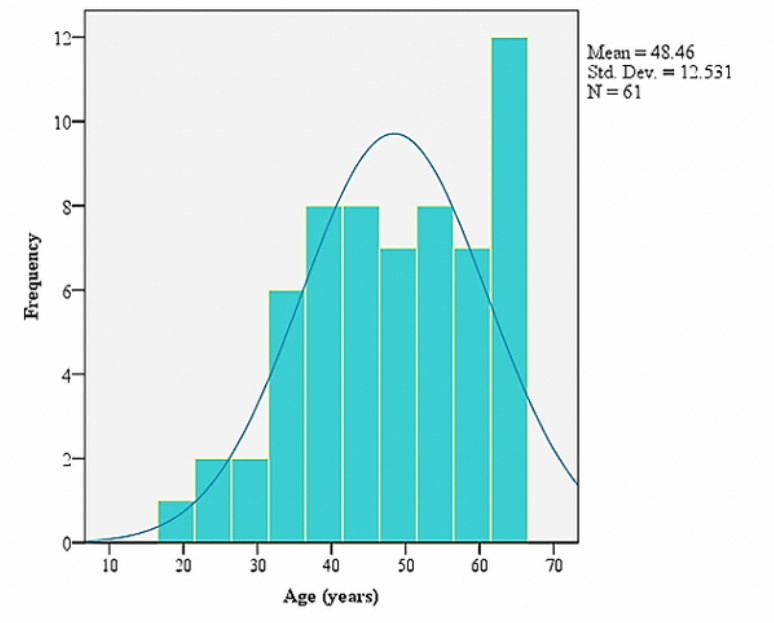
Variables	OR	95% C.I. for OR		P
		Lower	Upper	
cIMT baseline	4.2	0.1	178	0.5
ABI baseline	0.2	0.1	87	0.4
LDL-C at baseline	1.1	1.01	1.2	0.007*
hsCRP at baseline	1.7	1.03	2.81	0.04*
Lipid-lowering drugs				
Statin+Ezetimibe+Fenofibrate	ref	ref	ref	0.1
Statin	206	2.7	1583	0.02*
Statin+Ezetimibe	17.5	0.4	693	0.1
Statine+Fenofibrate	992	2.2	4523	0.03*

Legend: LDL-C - low density cholesterol lipoprotein, hsCRP- high-sensitivity C-reactive protein, cIMT- carotid intima-media thickness, OR odd ratio

*P<0.05

Figures

a)



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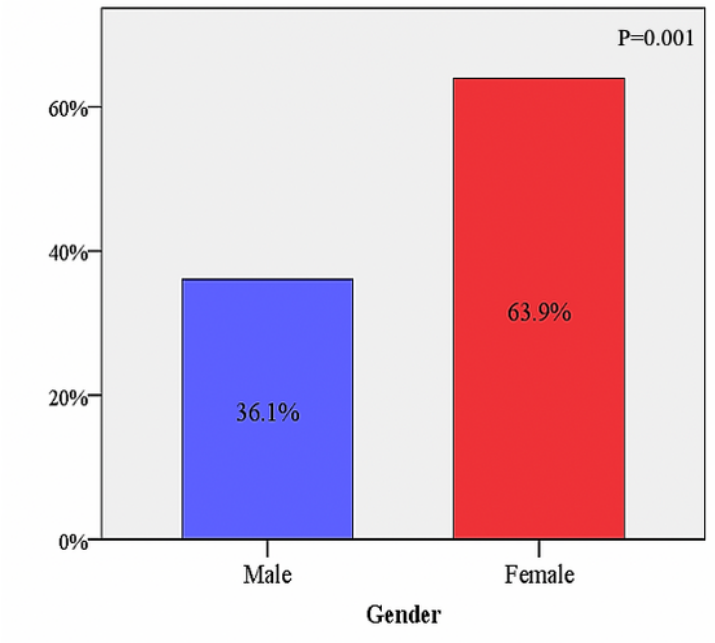
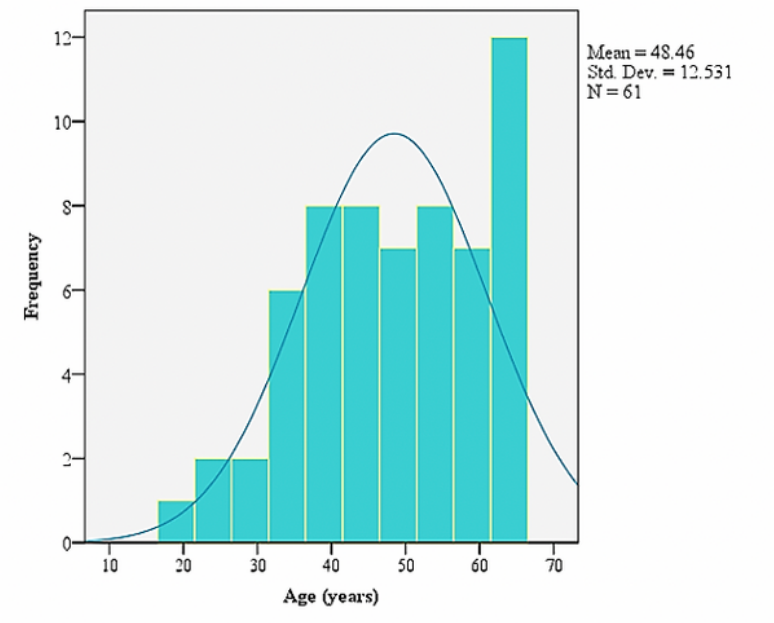


Figure 1

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

a)



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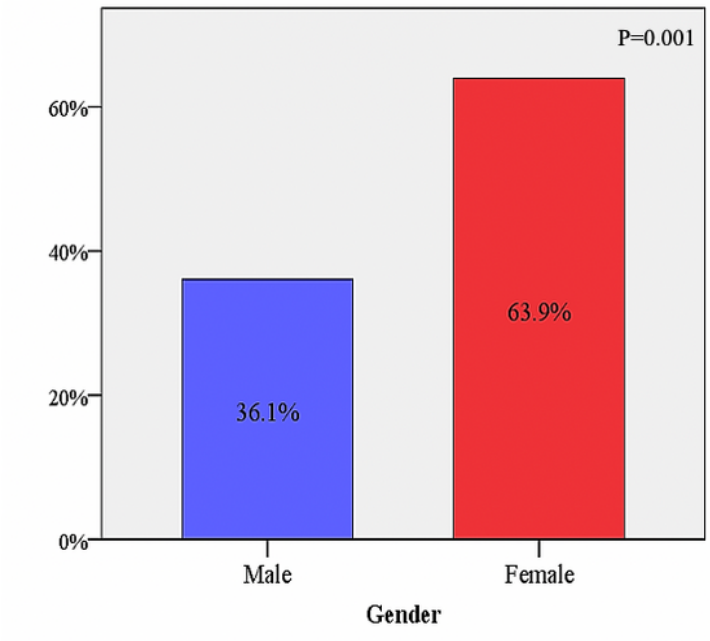

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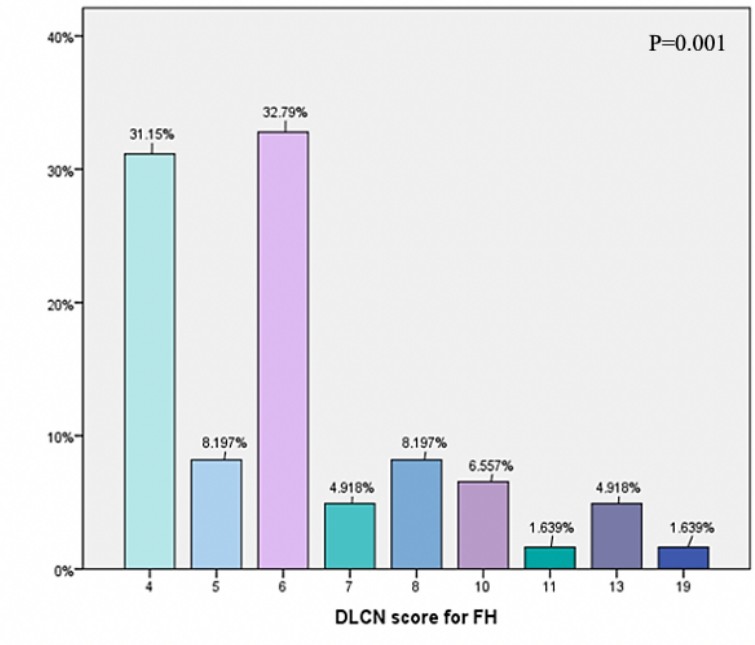

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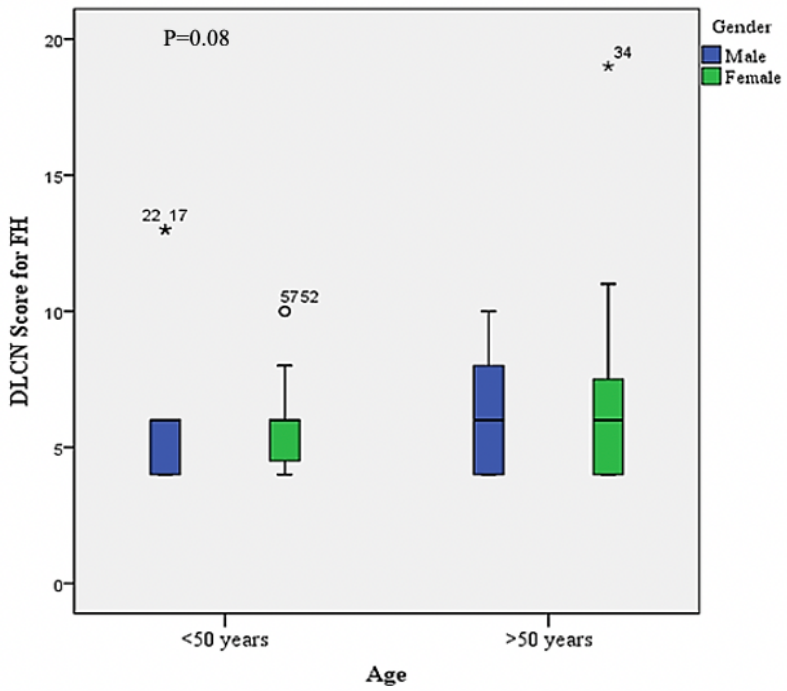
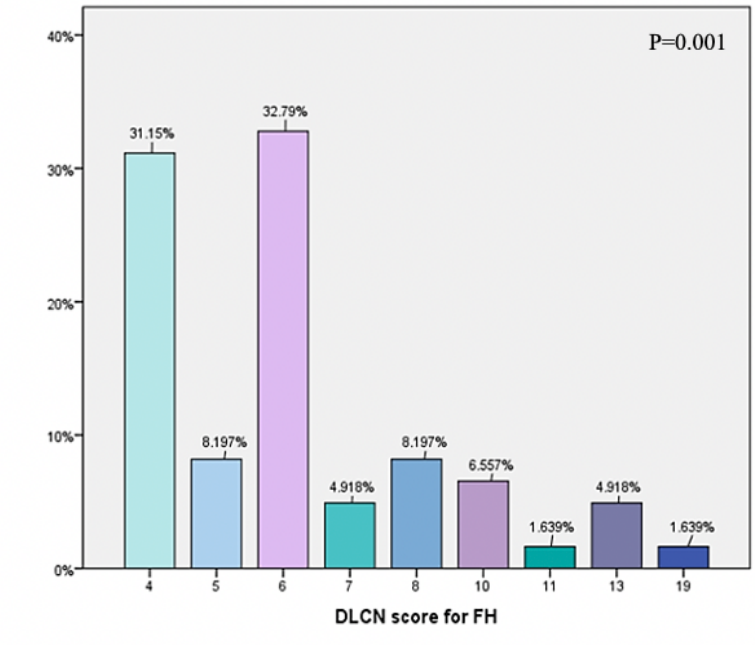


Figure 2

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

a.



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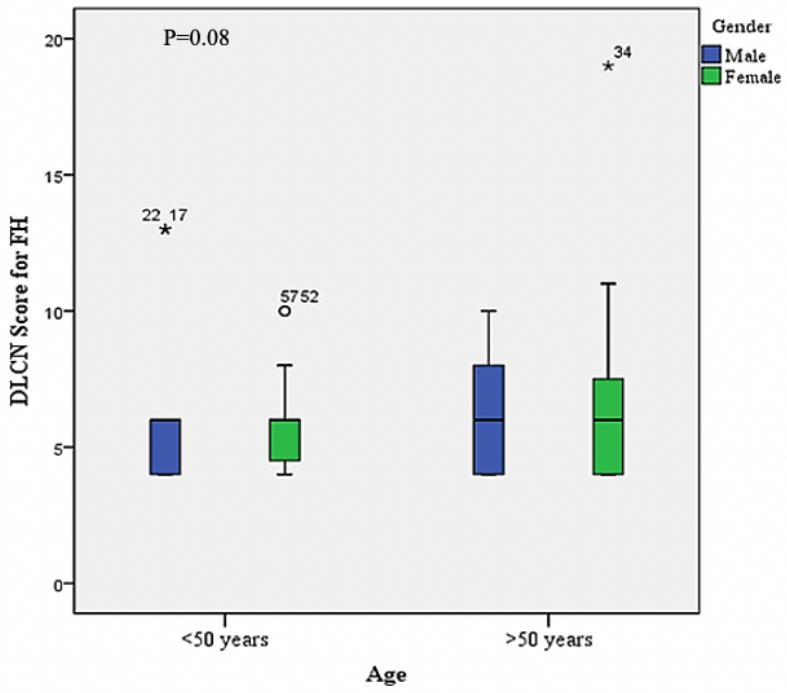

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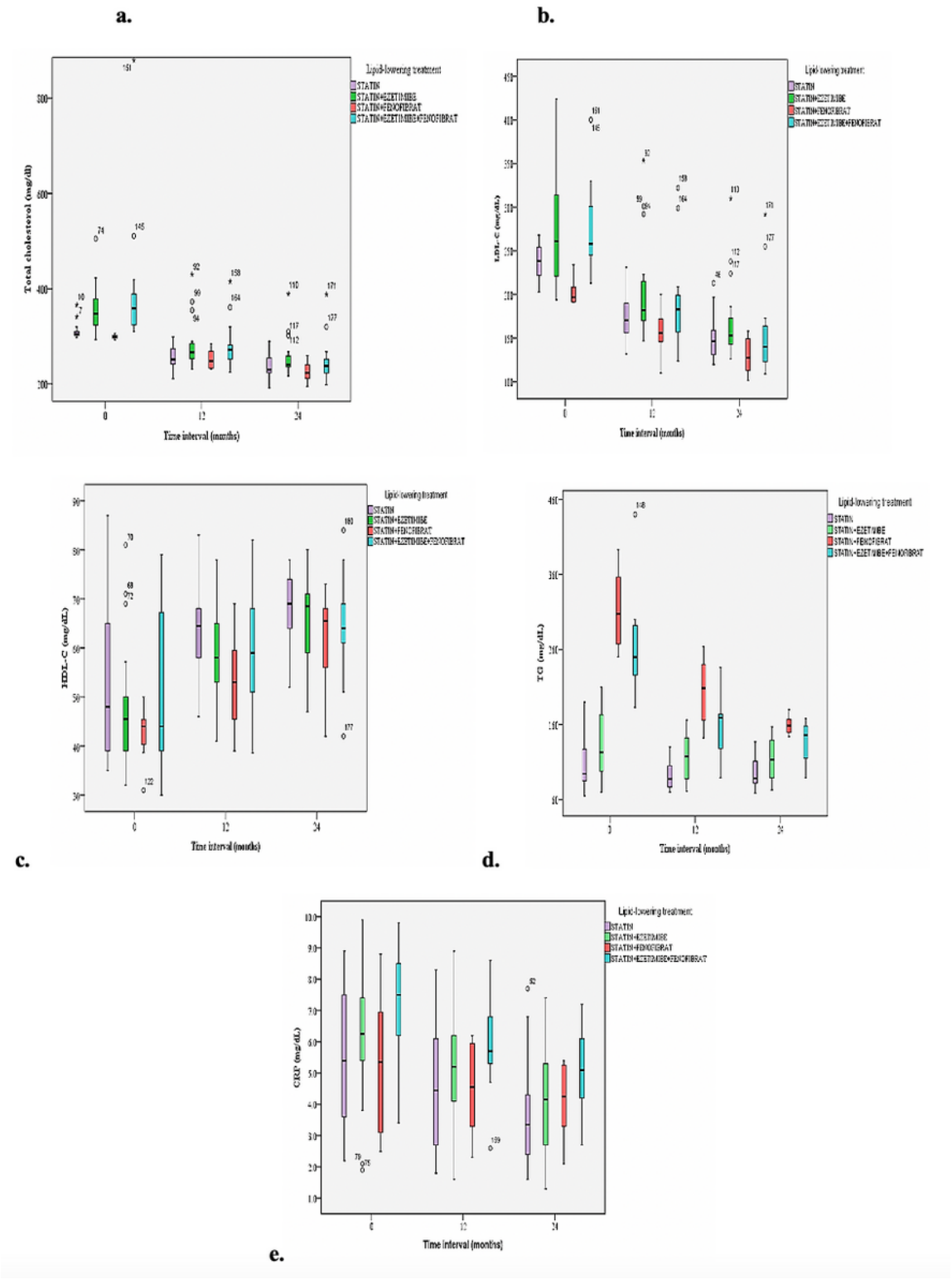
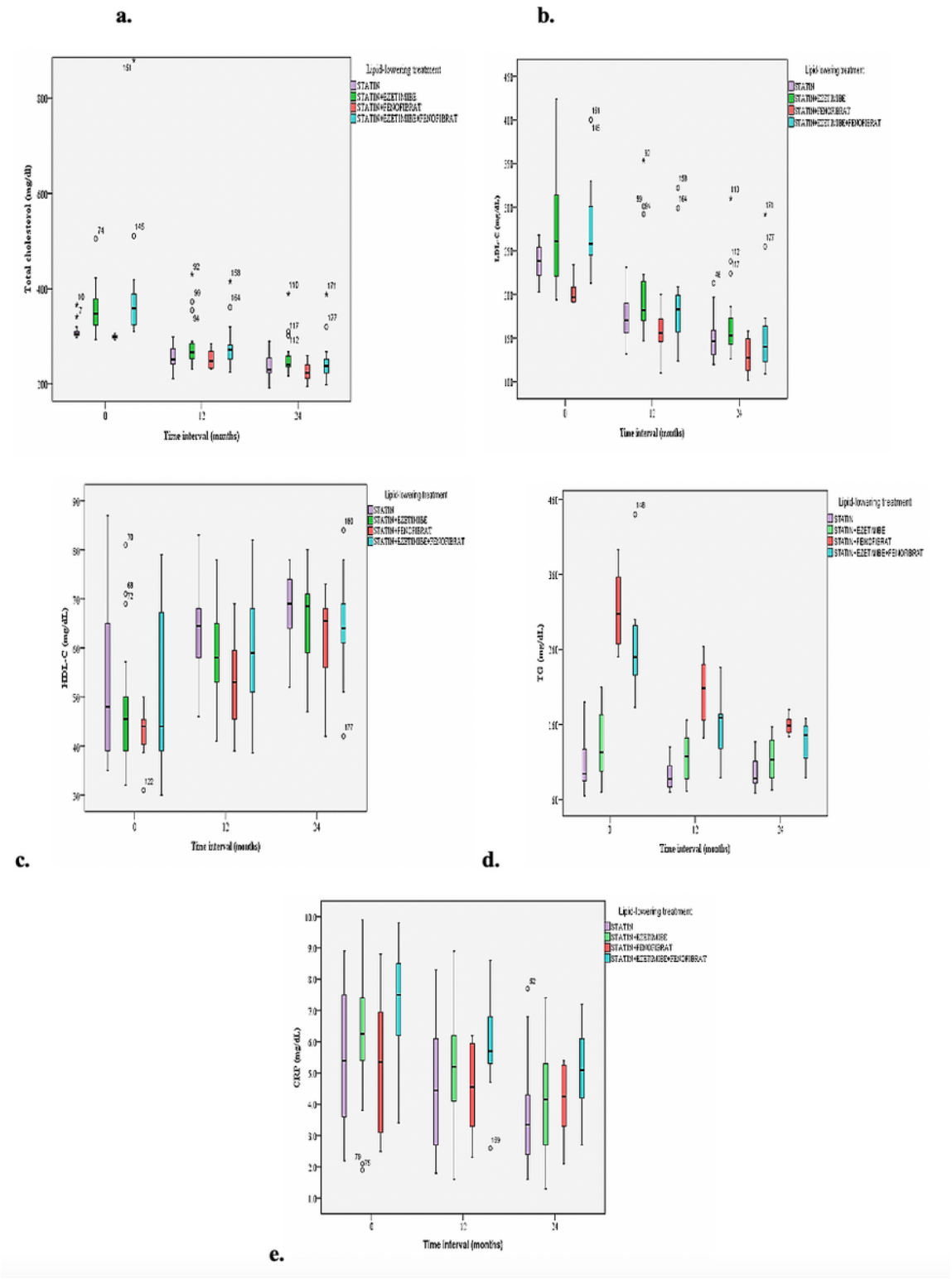


Figure 3

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



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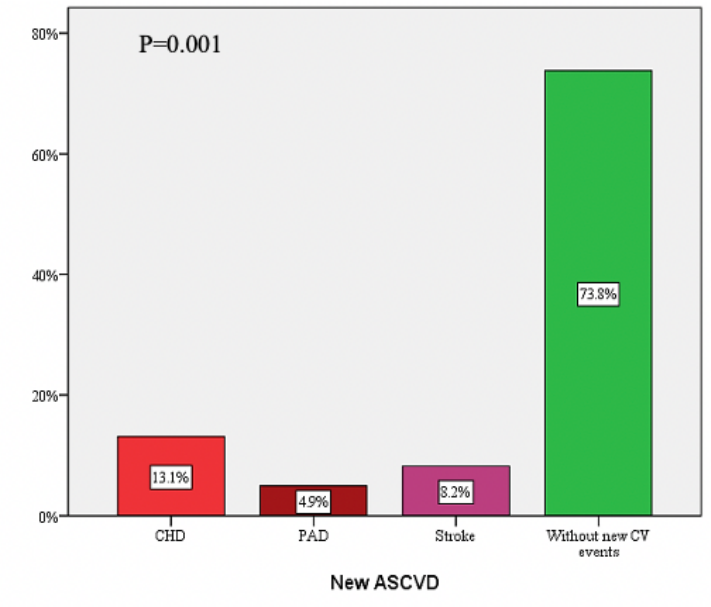


b.

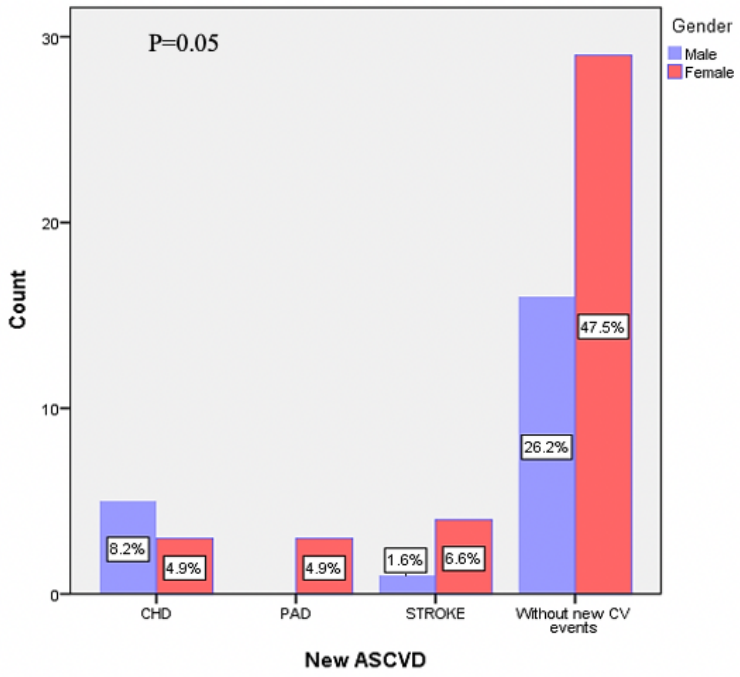
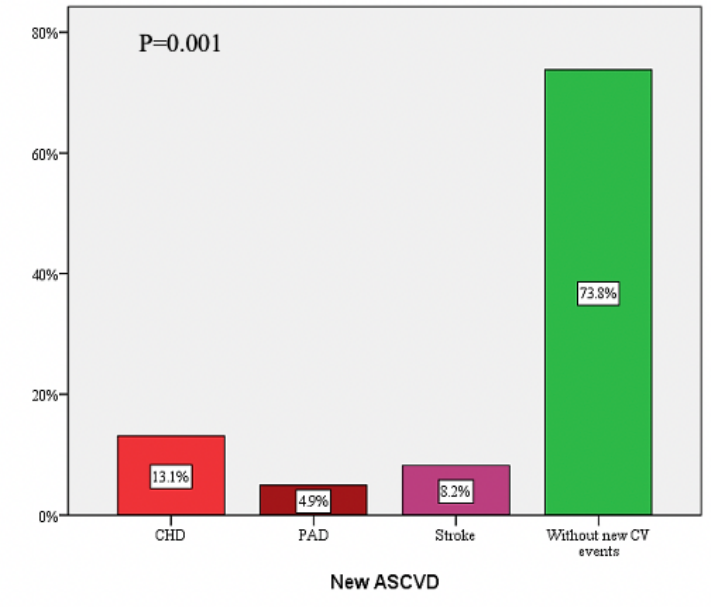


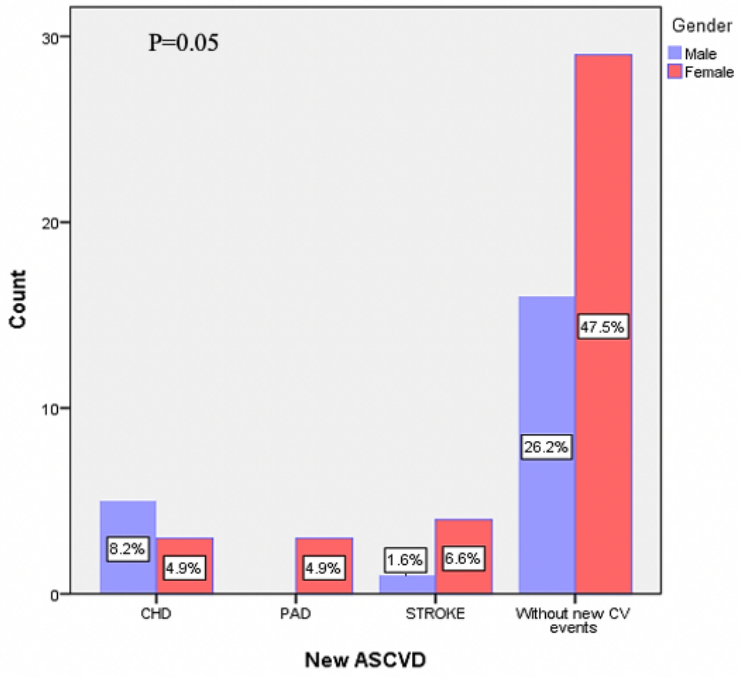
Figure 4

The frequency of the new ASCVD a. in population with clinical diagnosis of FH b. in this population by gender

a.



b.



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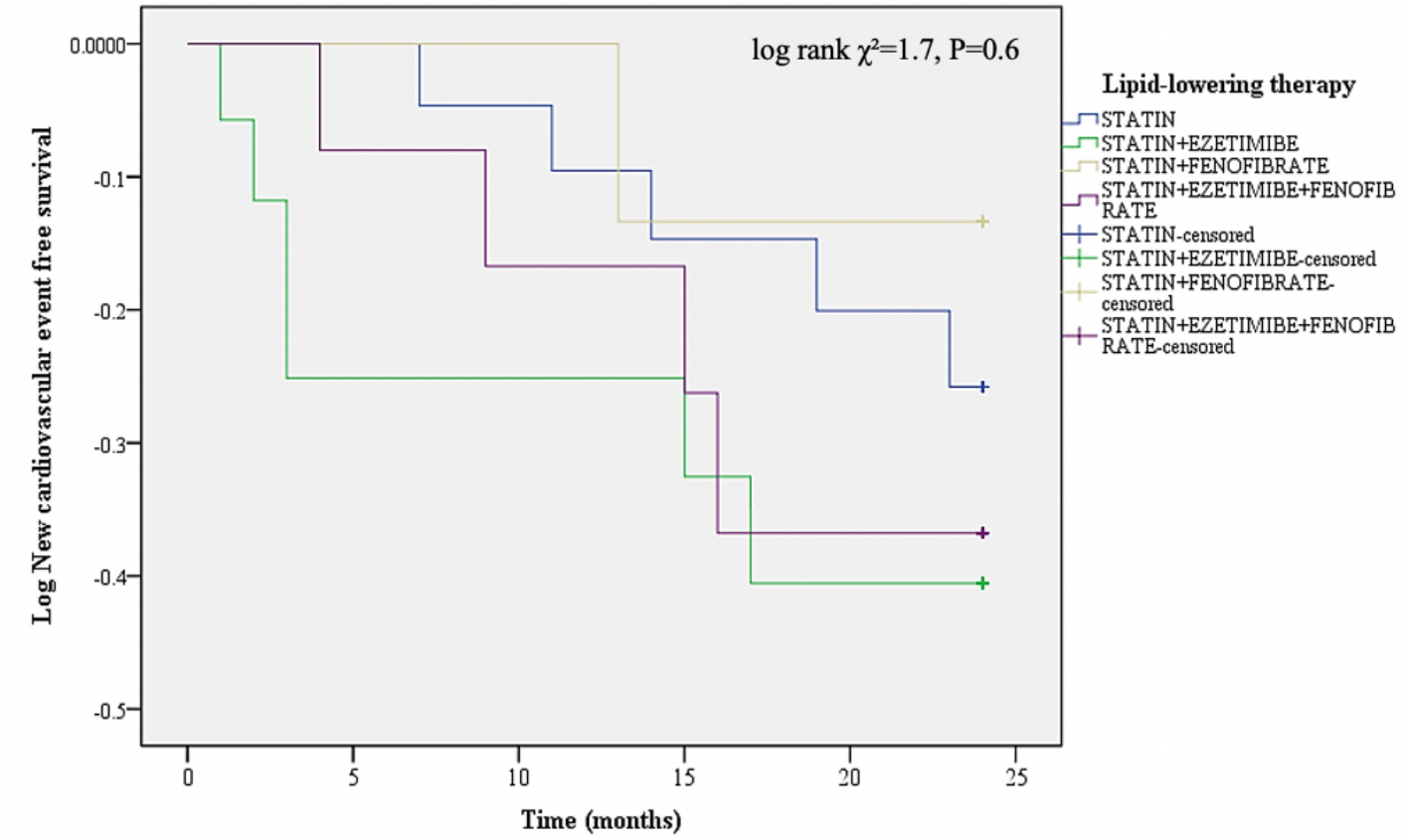
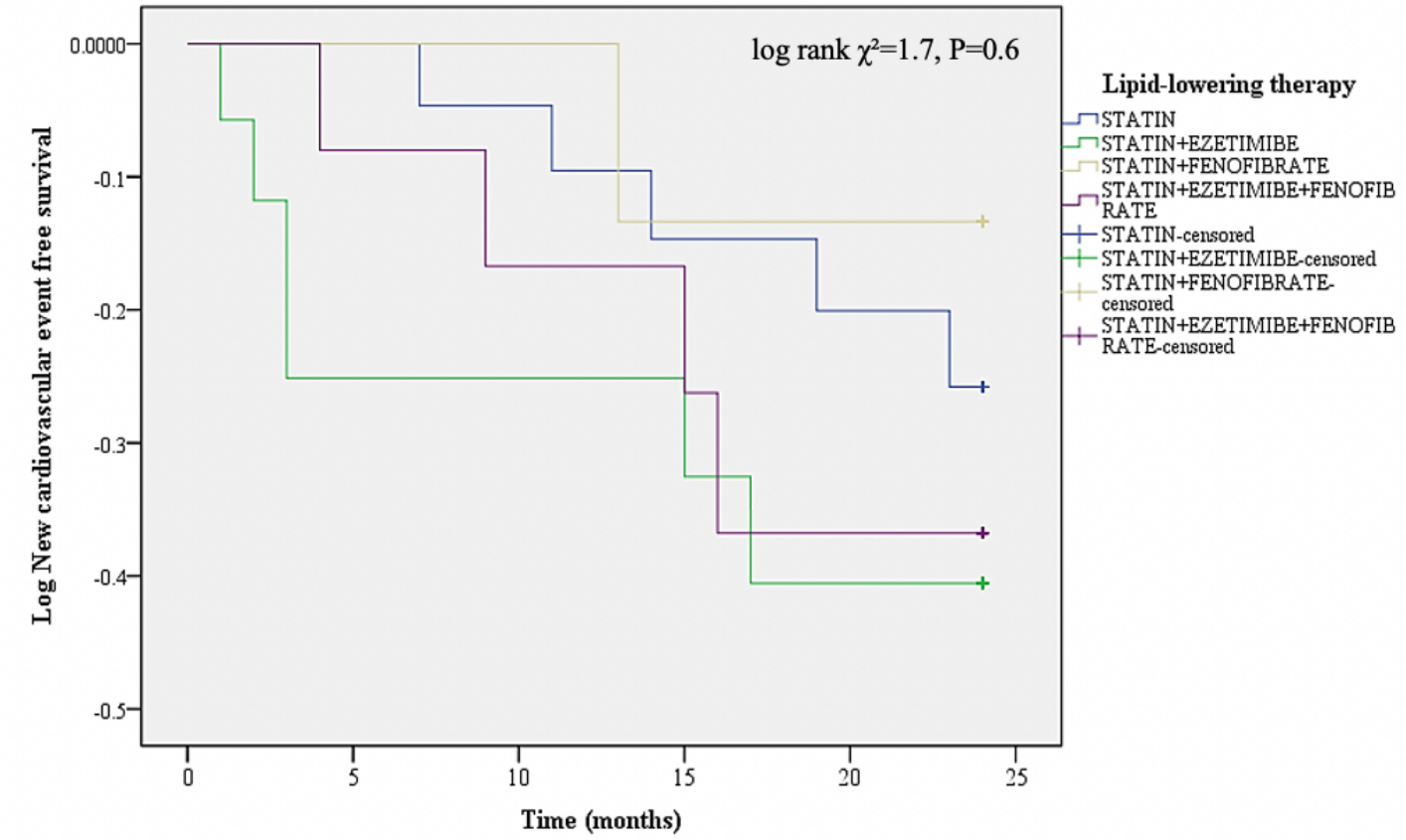


Figure 5

Kaplan Meier for ASCVD depending on the lipid-lowering therapy and time interval for the occurrence of new cardiovascular events



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