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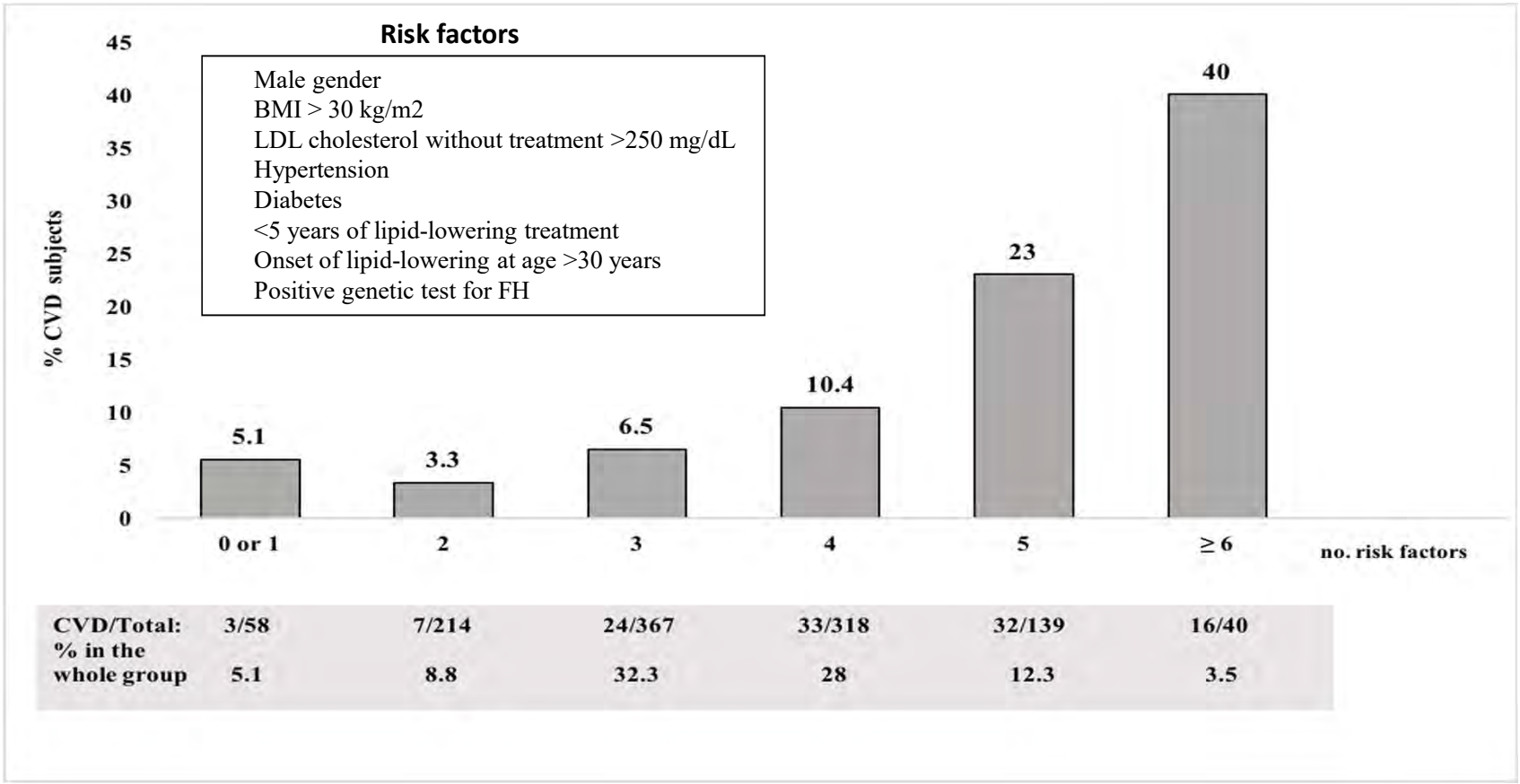
Prevalence of a first cardiovascular disease event among HeFH after 9.7 years in high-intensity lipid-lowering treatment stratified by the count of cardiovascular risk factors



Effect of lipid-lowering treatment in cardiovascular disease prevalence in familial hypercholesterolemia

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Abstract

Background and aims: The impact on heterozygous familial hypercholesterolemia (HeFH) health led by high-intensity lipid-lowering therapy (HILLT) is unknown, and the question remains if there is still an unacceptably high residual risk to justify treatment with new lipid-lowering drugs.

Methods: This observational, retrospective, multicenter, national study in Spain, whose information was obtained from a national dyslipemia registry was designed to establish the current prevalence of cardiovascular disease (CVD) in HeFH and to define the impact of HILLT on CVD in this population. Odds were estimated using several logistic regression models with progressive adjustment.

Results: 1958 HeFH, mean age 49.3±14.3 years, were included in the analysis. At inclusion in the registry, 295 patients (15.1%) had suffered CVD and 164 (55.6%) had suffered the first event before the onset lipid-lowering treatment. Exposition to treatment associated more than ten times lower odds for CVD than those subjects naïve to treatment (OR 0.085, 95% CI 0.063-0.114, $p<0.001$). A first CVD event after a mean treatment period of 9.1±7.2 years occurred in 131 out of 1615 (8.1%) HeFH subjects and 115 (87.8%) of them were on HILLT.

Conclusions: Current prevalence of CVD among HeFH is one third of that reported before the statins era. Early initiation and prolonged lipid-lowering treatment was associated with this reduction in CVD. New cases of CVD, in spite of HILLT appeared, mostly, among patients accumulating risk factors and probably they may be considered for further lipid-lowering drugs.

Keywords

Familial hypercholesterolemia, cardiovascular disease, lipid-lowering, statins

1. Introduction

Familial hypercholesterolemia (FH) is one of the most common genetic diseases in the world [1]. The estimated prevalence of heterozygotes FH (HeFH) is one in every 200-250 persons [2,3] and it is even higher in areas with some genetic isolation [4]. FH subjects are characterized by very high plasma concentration of low-density lipoprotein (LDL) cholesterol with autosomal co-dominant pattern of transmission, tendon xanthomas and high risk of premature coronary heart disease (CHD) [5]. Most cases of FH are caused by loss-of-function mutations in the genes encoding the LDL particle receptor (*LDLR*) [6], or apolipoprotein B (*APOB*) [7], but also by gain-of-function mutations in the genes encoding for proprotein convertase subtilisin/kexin type 9 (*PCSK9*) [8] or apolipoprotein E (*APOE*) [9].

Untreated affected subjects have a markedly elevated long-term CHD risk, with hazard ratios up to 5.0 with respect to the general population [10], and early mortality with up to 100-fold increase from CHD in young adults. This high CHD risk reduces life expectancy in 20 years for men and 12 years for women [10]. Consequently, international clinical guidelines classified HeFH as a high-risk condition which deserves early diagnosis and treatment [1,5,12].

The advent of potent lipid-lowering drugs, specially 3-hydroxy-3-methyl-glutaryl-CoA reductase inhibitors or statins, has been a landmark for people suffering from FH. Since the late 1980s this pharmacological therapy, sometimes in association with ezetimibe, has substantially reduced or even normalized LDL cholesterol concentrations in HeFH, and the natural history of the disease has been importantly modified [5]. Different reports from United Kingdom [13], Norway [14], Denmark [15] and The Netherlands [16] indicate that, although cardiovascular disease (CVD) remains significantly higher in treated heterozygous FH than in subjects from the general

population, CVD has substantially improved in HeFH in recent years. However, the impact on HeFH health led by high-intensity lipid lowering therapy is basically unknown, as well as the question whether it is sufficient with this treatment or still the residual risk is unacceptably high to justify treatment with new lipid-lowering drugs, such as PCSK9 inhibitors.

Most diagnosed cases of HeFH in Spain, especially those with genetic diagnosis, are controlled in specialized lipid units distributed throughout the country that are organized in a network within the Spanish Atherosclerosis Society (SEA). SEA created in 2013 a National Registry that includes primary dyslipidemias using homogeneous clinical diagnostic criteria [17,18]. We hypothesized that lipid-lowering therapy has improved HeFH cardiovascular prognosis in recent years. Thus, the objective of this analysis was to establish the current prevalence of CVD in HeFH adults, and to assess the impact of high intensity lipid lowering treatment on CVD in this population.

2. Material and methods

2.1 Study characteristics

This observational, retrospective, multicenter, national study in Spain was designed to determine current prevalence of CVD in patients with HeFH in the era of statin treatment. The impact of lipid lowering treatment was studied with a case-control approach. The information was obtained from the Dyslipidemia Registry of the SEA. This is an active online registry, where 50 certified lipid clinics distributed throughout all regions of Spain report cases of various types of primary hyperlipidemias [17]. The anonymous clinical data collection in this registry was approved by a central ethical committee (Comité Ético de Investigación Clínica de Aragón, CEICA). Inclusion criteria were standardized in 5 training sessions before case recruitment before. For

HeFH, the registry includes personal and family health history, anthropometry, physical examination, laboratory data, presence of CVD, age at which CVD events occurred, age at which statin treatment began, history of lipid-lowering treatment, and genetic data regarding mutations in *LDLR, APOB* or *PCSK9* (positive, negative or unknown). Patients were eligible for inclusion in this study if they were 18 years of age or older with clinical or genetic diagnosis of HeFH. Clinical diagnosis was based on the diagnostic criteria proposed by the Dutch Lipid Clinics Network (DLCN): 6-8 points (probable), and >8 points (definite) [1]. Genetic diagnosis was based on tested carrier status of a known pathogenic mutation for FH. Pathogenicity definition of mutations followed the American College of Medical Genetics ACMG recommendations [18]. Only pathogenic and likely pathogenic mutations were considered as causal in this analysis. Additional written informed consent was required for genetic analysis. Homozygous FH were not included in this study. CVD is defined as: coronary (myocardial infarction, coronary revascularization procedure, sudden death); cerebral (stroke with >24h neurological deficit without evidence of bleeding in brain imaging tests); peripheral vascular disease (intermittent claudication with ankle arm index <0.9, or arterial revascularization of lower limbs); or, symptomatic or asymptomatic abdominal aortic aneurysm. Arterial hypertension was defined as systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg, or self-reported use of antihypertensive medication. Diabetes was defined as fasting plasma glucose ≥126 mg/dl, HbA1c ≥6.5% or self-reported treatment with antidiabetic medication. Current smoking was defined as smoking in the present or having smoked in the last year. Former smoker was defined as a subject having smoked at least 50 cigarettes in his lifetime, but not having smoked in the last year. Severe high LDL cholesterol was considered when >250 mg/dl in absence on lipid-lowering drugs [12].

Lipid-lowering treatment was classified into three categories according to the type of drug and the daily dose: low intensity treatment (ezetimibe 5-10 mg, simvastatin 5-10 mg, lovastatin 20 mg, pravastatin 10-20 mg, fluvastatin 20-40 mg or pitavastatin 1 mg), moderate intensity treatment (atorvastatin 10-20 mg, rosuvastatin 5-10 mg, simvastatin 20-40 mg, fluvastatin 80 mg, lovastatin 40 mg, pravastatin 40 mg or pitavastatin 2-4 mg) and high intensity treatment (rosuvastatin 20-40 mg, atorvastatin 40-80 mg, or any daily statin doses plus ezetimibe) [19]. Only extended lipid-lowering (>6 months) at entry was considered.

We conducted this study in accordance with the Declaration of Helsinki for the protection of the rights and welfare of people participating in biomedical research.

2.2 Statistical analysis

Prevalence and HeFH description used the information at the time of data collection. Cases were participants who had suffered a first CVD event and controls were the remainder participants. Lipid-lowering treatment use defined the exposure variable. A participant was deemed exposed to lipid-lowering treatment if the recorded treatment start age predated the first CVD event in cases, and in all cases of treatment in controls. Duration of exposure was used for a dose-effect analysis. Years of lipid-lowering therapy were calculated by subtracting age of treatment to age of event among cases, and age of treatment to age at the time of data collection among controls. This variable was categorized in non-exposed, and tertiles of duration among those exposed (resulting in cutoff values at 5 and 12 years of treatment). Case-control age, for adjustment, was that of the first CVD event for cases and that at the time of data collection for controls. Other clinical variables used as potential determinants of CVD were values present at the time of data collection for both cases and controls.

Odds ratios (OR) were estimated using several logistic regression models with progressive adjustment: Model 1 was unadjusted, model 2 was adjusted for gender and age, model 3 was further adjusted for hypertension, diabetes, tobacco, HDL cholesterol (mg/dl), LDL cholesterol (mg/dl), and body mass index (BMI) (kg/m^2), and model 4 additionally for lipoprotein (a) [Lp(a) mg/dl].

A second logistic regression analysis with the same progressive adjustments was performed restricted to those exposed to high intensity treatment (as defined above) to study factors that influence CVD among those under such treatment.

3. Results

3.1 Study population

A total 1958 HeFH patients, 1016 women and 942 men, with a mean age of 49.3 years fulfilled inclusion criteria. Probable (6-8 DLCN points) and definite HeFH (>8 DLCN points) was diagnosed in 354 (18.12 %) and 1604 (81.98%) subjects, respectively. A positive genetic diagnosis was present in 1273 (65.0%) subjects. At inclusion in the registry, 295 patients (15.1%) had suffered CVD. Prevalence of CVD among HeFH increased with age and male gender (Fig. 1). Subjects with CVD were older, more frequently men, had higher BMI, tobacco consumption, and had more frequently hypertension and diabetes than those HeFH without CVD. In addition, HeFH patients with CVD had lower HDL cholesterol and higher LDL cholesterol, triglycerides and Lp(a) concentrations without lipid-lowering treatment (Table 1).

3.2 Effect of exposure to lipid-lowering treatment on CVD

Among HeFH patients with CVD, the first event occurred before the onset lipid-lowering treatment in 164 (55.6%) of the subjects. This percentage was slightly higher

for men (56.9%) than for women (52.7%) and had a tendency to decrease with age in both genders (Fig.2).

The mean exposure time to lipid-lowering treatment was 9.7 years, being lower in subjects with CVD, although without reaching statistical significance (Table 2).

To better establish the impact of lipid-lowering treatment on CVD we calculated the risk of presenting a first CVD event according to previous exposure to lipid-lowering treatment and its duration. Treatment exposure was associated with more than ten times, gender and age adjusted, lower odds of CVD than treatment naïve patients (OR 0.085, 95% CI 0.063 to 0.114, $p<0.001$) (Supplemental Figure). This CVD protection was also analyzed as the lipid-lowering treatment exposure increased. According to tertiles of years of exposure to statins, with respect to those not exposed to statins, the OR for those subjects with exposure <5 years, between 5 and 12 years and >12 years, was 0.095 (95% CI 0.065 to 0.139), 0.086 (95% CI 0.058 to 0.128) and 0.071 (95% CI 0.047 to 0.108), respectively, with significant differences versus non-exposed ($p<0.001$ in all cases) (Table 3). There was a trend for a greater protection among longer exposures but due to the small number of events among those exposed, the differences did not reach statistical significance. These ORs remained similar after further adjustment for sex, age, BMI, hypertension, diabetes, tobacco, HDL cholesterol and LDL cholesterol levels without treatment. Adjustment for Lp(a) concentration did not modify ORs in the regression (Table 3).

3.3 Risk factors for CVD in subjects with lipid-lowering treatment

A first CVD event occurred in 131 out of 1615 (8.1%) HeFH subjects already in extended lipid-lowering (>6 months) treatment. Among them, 115 (84.6%) were on high intensity therapy, including 75 (65.2%) combining ezetimibe, and 93 (71.0%) had

a positive genetic diagnosis. Clinical characteristics of these groups are shown in Table 2. There were more men and they were older, with higher BMI, more prevalence of hypertension and diabetes, more often confirmed genetically, and higher LDL cholesterol levels without lipid-lowering treatment among HeFH with CVD event than among HeFH without it. There were not differences in the presence of xanthomas, tobacco consumption, Lp(a) concentration and years of exposure to lipid-lowering treatment.

Two regression analysis calculating OR for CVD after lipid-lowering treatment are shown (Supplemental Table). The first analysis included all exposed HeFH subjects to treatment and in the second only those subjects treated with high intensity therapy. Independent risk factors were male gender, BMI, LDL cholesterol without treatment, history of hypertension or diabetes, less than 5 years of lipid-lowering treatment, onset of lipid-lowering at age >30 years, and a positive genetic test for FH. The intensity of association of these factors with CVD was similar in subjects with high intensity treatment to that in the whole group.

Then, restricted to those subjects treated with high intensity therapy. In this group the mean dose of atorvastatin or rosuvastatin was 41.8±0.61 or 24.8±0.44 mg/day, respectively, and 738 (64.9%) subjects were also taking ezetimibe. We calculated the proportion of subjects who developed CVD according to the count of independent risk factors found in the previous logistic regression analysis. CVD prevalence increases as the number of risk factors increases (Figure 3). Among our HeFH population with statin treatment previous to CVD, and treated with high intensity therapy, 56.2% showed ≤3 risk factors, but still 34 (5.3%) had developed CHD in spite of having undergone an average of 9.7 years of previous lipid lowering treatment.

4. Discussion

In the present study, we describe the prevalence of CVD in a registry of HeFH patients treated in specialized lipid units and the effect on CVD of prolonged treatment with lipid-lowering drugs. This is the first work where we can describe the characteristics of HeFH that suffered CVD in spite of lipid-lowering treatment, even with some of them on high intensity treatment, and provide information to assess the potential role of new drugs in the treatment of this disease.

In our opinion, three important conclusions can be drawn from our work. First, current CVD prevalence in HeFH is much lower in the statin era than was reported several decades ago; second, CVD in HeFH patients is highly dependent of the moment when lipid-lowering is started; and third, new cases of CVD under prolonged statin treatment are uncommon and concentrated in subjects with certain risk factors. Altogether, our results would indicate that with lipid-lowering treatment is started early in life, HeFH is no longer a high-risk CVD condition.

CVD prevalence estimated from this study is similar to that reported in other current registries from specialized lipid centers and, as expected, is highly dependent on the mean age of the cohort. The HeFH cohort from The Netherlands with a mean age of 38.3 years showed a CVD prevalence of 9.2% among 14,283 HeFH [20]; in a cohort from Canada with a mean age of 43.9 years it was 12.1% [21]; in our cohort with a mean age of 49.3 years it was 15.1%; and in Norway with a mean age of 58 years increased to 24% [22]. These prevalences are clearly much lower those reported years ago [23] and could probably be related to multiple factors including reduction of smoking habit that has been reduced near 50% in males in Spain in the last 20 years [24,25]; better medical cardiovascular risk factor control, specially hypertension [26]; and changes in the diagnosis of HeFH from clinical diagnosis where the weight of the

family and personal history of coronary disease is very strong and favors the selection of more serious cases versus the diagnosis based in the genetic diagnosis that eliminates these potential biases. In addition, early initiation of statin therapy seems to play major role [16]. In fact, in our sample over 50% of HeFH subjects who developed CVD it happened before initiating lipid-lowering treatment, while among all the patients that initiated treatment while free from CVD, only 8% had a subsequent CVD event.

We quantified the impact of lipid-lowering drugs, mainly statins, on CVD prevention in HeFH. In absence of randomized clinical trials with clinical events as main end-point, observational studies contribute to analyze this effect. Considering that the mean reduction of LDL cholesterol in our cohort was approximately 50%, which corresponds approximately to 135 mg/dl (3.5 mmol/L), the Cholesterol Treatment Trialists' (CTT) Collaboration [27] and epidemiological prospective studies [28] would predict a reduction of approximately 59% in CVD incidence in 5 years, because in mathematical terms, the decrease in CVD risk should be 0.78 to the power of the LDL cholesterol reduction in mmol/L [29]. Applying the formula *relative risk* ≈ *OR / [1 – absolute risk + (absolute risk · OR)]*, an OR= 0.10, that we find in our study, proyecting from, as an example, and an untreated absolute risk of 50% in HeFH, our OR would correspond to a relative risk of 19%, which corresponds to an 81% reduction. The impressing magnitude of the protection found in our work may be explained because the mean treatment duration in our study is almost 10 years. The estimation obtained in the present study overcomes that figure probably because the mean treatment duration in our study was almost 10 years, LDL cholesterol is the major, if not the only risk factors in many HeFH patients, and the treatment was started in most cases in primary prevention to avoid the development of atherosclerosis which is probably more effective than in subjects with advanced disease [29]. This result is in agreement with the large

CVD benefit observed of LDL cholesterol lowering effect of certain genetic variation that reduced LDL cholesterol early in life [28,30]. Our results emphasize the importance of an early in life diagnosis and intense treatment of HeFH [1,31].

Although CVD is drastically reduced with high intensity lipid-lowering treatment in our study, approximately 10% of HeFH patients that started treatment free of CVD events still had one event in spite of treatment, some of them even after more than 12 years of treatment. Probably, this group of patients are good candidates for more potent lipid-lowering treatments such inhibition of PCSK9 with monoclonal antibodies. The analysis of our cohort would indicate that HeFH subjects with 4 or more risk factors including: male gender, statin treatment duration less than 5 years, obesity, diabetes, hypertension, LDL cholesterol >250 mg/dl without treatment, presence of a causative mutation in candidate genes, or late-in-life initiation of statin treatment would be probably the best candidates for such approach. These conditions are well-recognized risk factors in general population and HeFH [32]. In contrast, CVD risk for HeFH subjects with early-in-life initiation, more than 5 years of treatment, and free from other risk factor is reasonably good.

Limitations. The design of our registry does not reliably allow calculating the cumulative LDL cholesterol of the subjects. This calculation has been related to the risk of cardiovascular disease [1]. However, the fact that CVD decreases so significantly with treatment suggests that cumulative LDL cholesterol above a certain threshold that many HeFH get with high-intensity lipid-lowering treatment is even more important than the total cumulative LDL cholesterol. During the period of registered treatment (approximately 10 years in average) it may not remain constant. We have information about the time of treatment onset but covariates are collected at the time of inclusion in the registry. However, these patients are usually treated with potent therapies from the

very beginning. Although all lipids clinics in the network follow homogeneous recommendations for the treatment of HeFH, some differences may be present. In addition, HeFH patients in our registry are followed at specialized lipid clinics, and perhaps, their phenotype or their management do not fully represent the whole spectrum of HeFH in the population. Finally, the retrospective study design implies that only HeFH who lived enough time to be registered in our sites are included, and thus most severe phenotypes leading to premature death, as well as mortal CVD episodes have not been considered, although cardiovascular death has been reported very low in HeFH under high intensity treatment [33].

In conclusion, current prevalence of CVD among treated HeFH in specialized lipid clinics for long period of time is one third of that reported before the statins were available. Early initiation and prolonged lipid-lowering treatment are associated with most of this benefit. However, new cases of CVD appear in spite of high-intensity statin but these episodes occur among high risk patients that should be considered for further lipid-lowering drugs such as PCSK9 inhibitors.

Conflicts of interest

J. Pedro-Botet received advisory and/or lecture fees from Astra-Zeneca, Esteve, Ferrer, Mylan, MSD and Sanofi. N. Plana received honoraria from Amgen, Merck, Ferrer, Alexion and Sanofi. A. J. Amor received lecture fees from Mylan and consulting fees from Sanofi-Aventis and Astra-Zeneca. F. Civeira receives grants, consulting fees, and/or honoraria from Amgen, Merck, Pfizer, and Sanofi-Aventis. All identified disclosures are modest. The other researcher did not receive any specific grant from agencies in the public, commercial, or not-for-profit sectors.

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Author contributions

SP-C contributed to the data acquisition, analyzed the data and wrote the manuscript; ML analyzed the data and wrote the manuscript; VM-B, IL-M, JP-B, NP, RMS-H, AJA, FA, FF and MS-T contributed to the data acquisition and critically reviewed the manuscript; and FC designed the study, supervised the data analysis and drafted the manuscript.

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Figure legends

Figure 1. Prevalence of cardiovascular disease according to age at the moment of entry in the registry stratified by gender.

CVD, cardiovascular disease.

Figure 2. Distribution of a first cardiovascular event in relation to the onset of lipid-lowering treatment stratified by age and gender.

CVD, cardiovascular disease.

Figure 3. Prevalence of cardiovascular disease among HeFH in high-intensity lipid-lowering treatment stratified by the count of cardiovascular risk factors.

CVD, cardiovascular disease.

The risk factors considered were those that were statistically significant in Regression 1 described in Supplemental Table.

Supplemental Figure. Crude proportions of participants with previous lipid-lowering treatment exposure on the case (Cardiovascular Disease Present) and control (Cardiovascular Disease Absent) groups, and crude ORs for each age and sex stratum.

Table 1. Anthropometric, clinical and biochemical characteristic of HeFH subjects at registry inclusion.

Variables	**Total (n=1958)**	**Non-CVD (n= 1663)**	**CVD (n= 295)**	***p*[a]**
Age at registry, years	49.3 ± (14.3)	47.8 ± (14.3)	58.0 ± (10.2)	<0.001
Case-control age[b], years	47.7 ± (13.7)	47.8 ± (14.3)	47.6 ± (9.9)	0.267
Gender (Male)	48.1 (942)	44.4 (738)	69.2 (204)	<0.001
Body mass index, (Kg/m^2)	26.2 ± (4.4)	25.9 ± (4.4)	28.1 ± (4.3)	<0.001
Xanthomas, % (n)	32.0 (626)	31.9 (530)	32.5 (96)	0.545
Tobacco consumption				0.001
Never smoke, % (n)	53.9 (1056)	56.0 (932)	42.0 (124)	
Ever smoke, % (n)	46.1 (902)	44.0 (731)	58.0 (171)	
Hypertension, % (n)	19.6 (383)	15.5 (258)	42.4 (125)	<0.001
Diabetes, % (n)	6.5 (128)	4.4 (73)	18.6 (55)	<0.001
Lipids without treatment				
Total cholesterol, mg/dl	348 ± (76.2)	345 ± (72.0)	362 ± (95.0)	<0.001
HDL cholesterol, mg/dl	54.8 ± (15.7)	55.8 ± (15.5)	49.2 ± (15.8)	<0.001

LDL cholesterol, mg/dl	269 ± (74.7)	267 ± (69.9)	285 ± (96.3)	<0.001
Triglycerides, mg/dl	132 ± (118)	128 ± (120)	157 ± (98.2)	<0.001
Lipoprotein (a), mg/dl (n=1360)	49.4 ± (56.9)	47.8 ± (57.0)	59.0 ± (55.6)	0.004
Clinical HeFH diagnosis				0.101
Probable (6-8 DLCN points), % (n)	18.1 (354)	17.4 (290)	21.7 (64)	
Definite (>8 DLCN points), % (n)	81.9 (1604)	82.6 (1373)	78.3 (231)	
Genetic test				<0.001
Unknown, % (n)	24.4 (478)	22.9 (381)	32.9 (97)	
Negative, % (n)	10.6 (207)	11.3 (188)	6.4 (19)	
Positive, % (n)	65.0 (1273)	65.8 (1094)	60.7 (179)	

Values are numbers (%), mean ± (SD), as applicable. CVD denotes cardiovascular disease; HDL, high-density lipoprotein; HeFH, heterozygous familial hypercholesterolemia; LDL, low-density lipoprotein; DLCN, Dutch Lipid Clinic Network.

[a] *p* values refer to differences calculated after adjusting gender and case-control age, as appropriate

[b] Case-control age refers to the age of controls at their inclusion in the registry, and the age of the first CVD event in the group of cases.

Table 2. Anthropometric, clinical and biochemical characteristics of HeFH subjects with and without CVD after lipid-lowering treatment.

Variables	**No CVD n =1484**	**CVD n =131**	***p***
Control-cases age, years	48.3 ± (14.1)	50.0 ± (10.8)	0.022
Gender (Male), % (n)	45.3% (672)	67.2% (88)	<0.001
Body Mass Index, Kg/m^2	25.9 ± (4.3)	28.3 ± (4.5)	<0.001
Xanthomas, % (n)	33.7% (484)	37.3% (47)	0.660
Tobacco consumption			0.848
Never smoker, % (n)	55.3% (821)	51.1% (67)	
Ever smoker, % (n)	44.7% (663)	48.9% (64)	
Hypertension, % (n)	15.5% (230)	38.9% (51)	<0.001
Diabetes, % (n)	4.2% (63)	20.6% (27)	<0.001
Pre-treatment LDL-cholesterol, mg/dl	268 ± (69.0)	295 ± (102)	<0.001
Post-treatment LDL-cholesterol, mg/dL	140.1± (49.9)	126.7 ± (49.1)	0.016
Lp(a), mg/dl (n=1169)	47.7 ± (57.4)	55.8 ± (50.8)	0.152
Treatment intensity			0.014
Unknown, % (n)	8.5 (126)	7.6 (10)	
Low intensity, % (n)	1.9% (28)	0.8% (1)	
Moderate intensity, % (n)	20.8% (309)	3.8% (5)	

High intensity, % (n)	68.8% (1021)	87.8 (115)	
Time with lipid-lowering treatment, years	9.8 ± (7.4)	9.1 ± (7.2)	0.085
Probable FH, % (n)	17.0% (253)	16.0% (21)	0.540
Definite FH, % (n)	83.0% (1231)	84.0% (110)	
Genetic test			0.324
Positive test genetic, % (n)	67.0% (994)	71.0% (93)	
Negative test genetic, % (n)	11.8% (175)	7.6% (10)	
Unknown, % (n)	21.2% (315)	8.2% (28)	

Values are numbers (%), mean ± (SD), as applicable. *p* values refer to differences calculated after adjusting by gender and age, as appropriate.

CVD denotes cardiovascular disease; HeFH, heterozygous familial hypercholesterolemia; LDL, low-density lipoprotein; Lp(a), lipoprotein (a).

Table 3. Odds ratio (OR) for cardiovascular disease according to lipid-lowering treatment in subjects with heterozygous familial hypercholesterolemia.

	OR (CI 95 %)	***p***	**Lipid-lowering treatment**				***p***
	Exposure Yes vs No		Non-Exposure	< 5 years	5.12 years	>12 years	
n/N	131/1615 vs 164/343		164/343	48/575	42/499	41/541	
% subjects with CHD	8.1 % vs 47.8 %	<0.001	47.8 %	8.3 %	8.4 %	7.6 %	<0.001
Model 1 N=1958	0.096 (0.073-0.121)	<0.001	REF	0.099 (0.069-0.143)	0.100 (0.069-0.147)	0.090 (0.061-0.131)	<0.001
Model 2 N=1958	0.085 (0.063-0.114)	<0.001	REF	0.095 (0.065-0.139)	0.086 (0.058-0.128)	0.071 (0.047-0.108)	<0.001
Model 3 N=1958	0.092 (0.067-0.126)	<0.001	REF	0.115 (0.077-0.171)	0.089 (0.058-0.136)	0.070 (0.045-0.110)	<0.001
Model 4 n=1360	0.082 (0.054-0.123)	<0.001	REF	0.096 (0.058-0.160)	0.076 (0.045-0.130)	0.070 (0.040-0.121)	<0.001

n/N denotes number with CVD/ number in the exposure group.

Model 1: Univariate analysis

Model 2: After adjustment for gender and age.

Model 3: After adjustment for gender, age, hypertension, diabetes, tobacco consumption, HDL cholesterol (mg/dl), LDL cholesterol (mg/dl), and body mass index (Kg/m^2).

Model 4: After adjustment for gender, age, hypertension, diabetes, tobacco consumption, HDL cholesterol (mg/dl), LDL cholesterol (mg/dl), body mass index (Kg/m^2), and lipoprotein (a) (mg/dl).

F-test *p* are those for the lipid-lowering treatment variables.

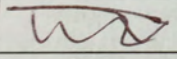
Name	Signature	Date	email
Sofía Perez-Calahorra			
Martín Laclaustra			
Victoria Marco-Benedí			
Itziar Lamiquiz-Moneo			
Juan Pedro-Botet			
Núria Plana			
Rosa M Sanchez-Hernandez			
Antonio J Amor			
Fátima Almagro		07 JAN 2019	MARIAFATIMA.ALMAGROMUGICA@osakidetza.eus
Francisco Fuentes			
Manuel Suarez-Tembra			

Figure 1

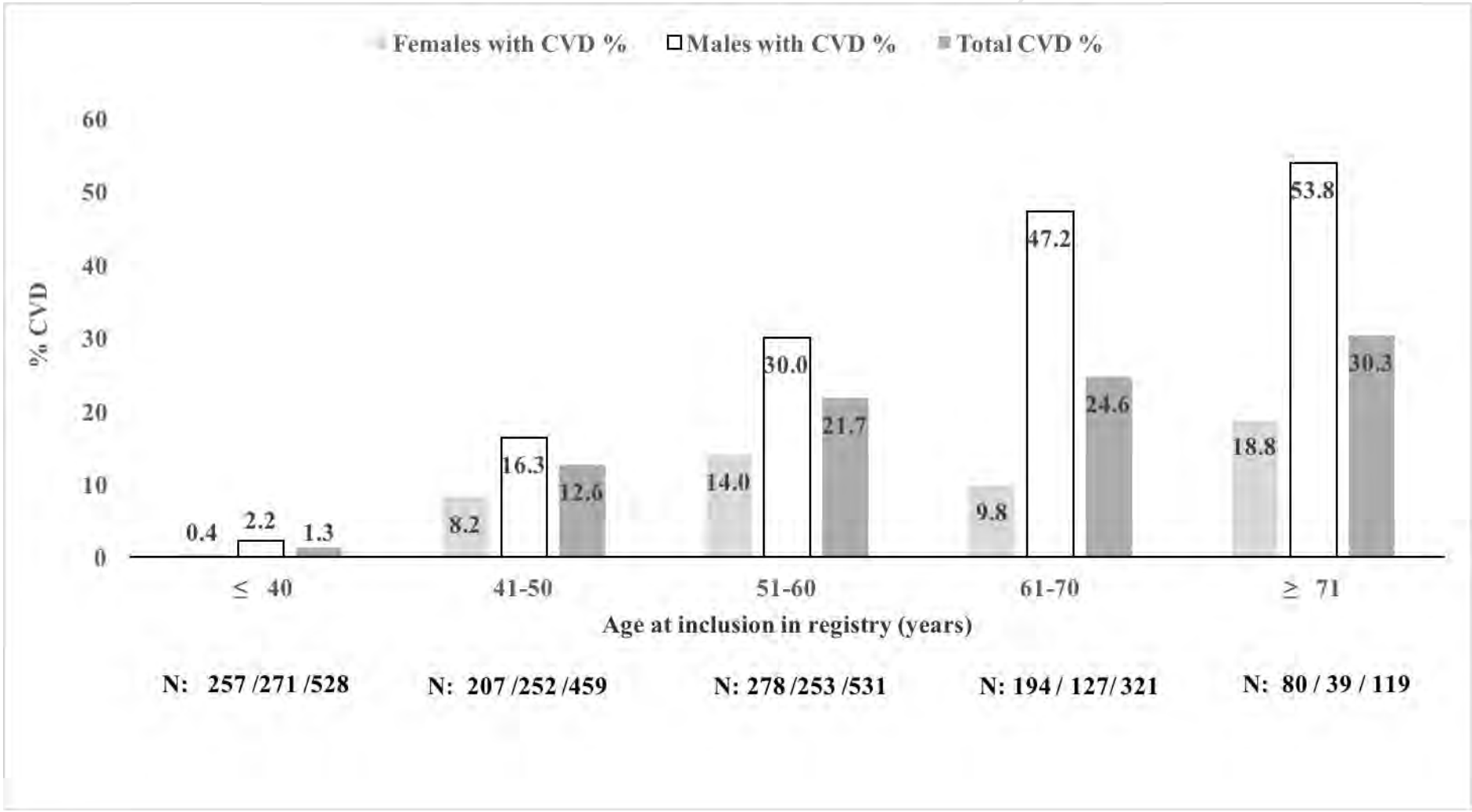

Females with CVD %
Males with CVD %
Total CVD %
% CVD
60
50
40
30
20
10
0
0.4
2.2
1.3
8.2
16.3
12.6
14.0
30.0
21.7
9.8
47.2
24.6
18.8
53.8
30.3
≤ 40
41-50
51-60
61-70
≥ 71
Age at inclusion in registry (years)
N: 257 /271 /528
N: 207 /252 /459
N: 278 /253 /531
N: 194 / 127/ 321
N: 80 / 39 / 119

Figure 2.

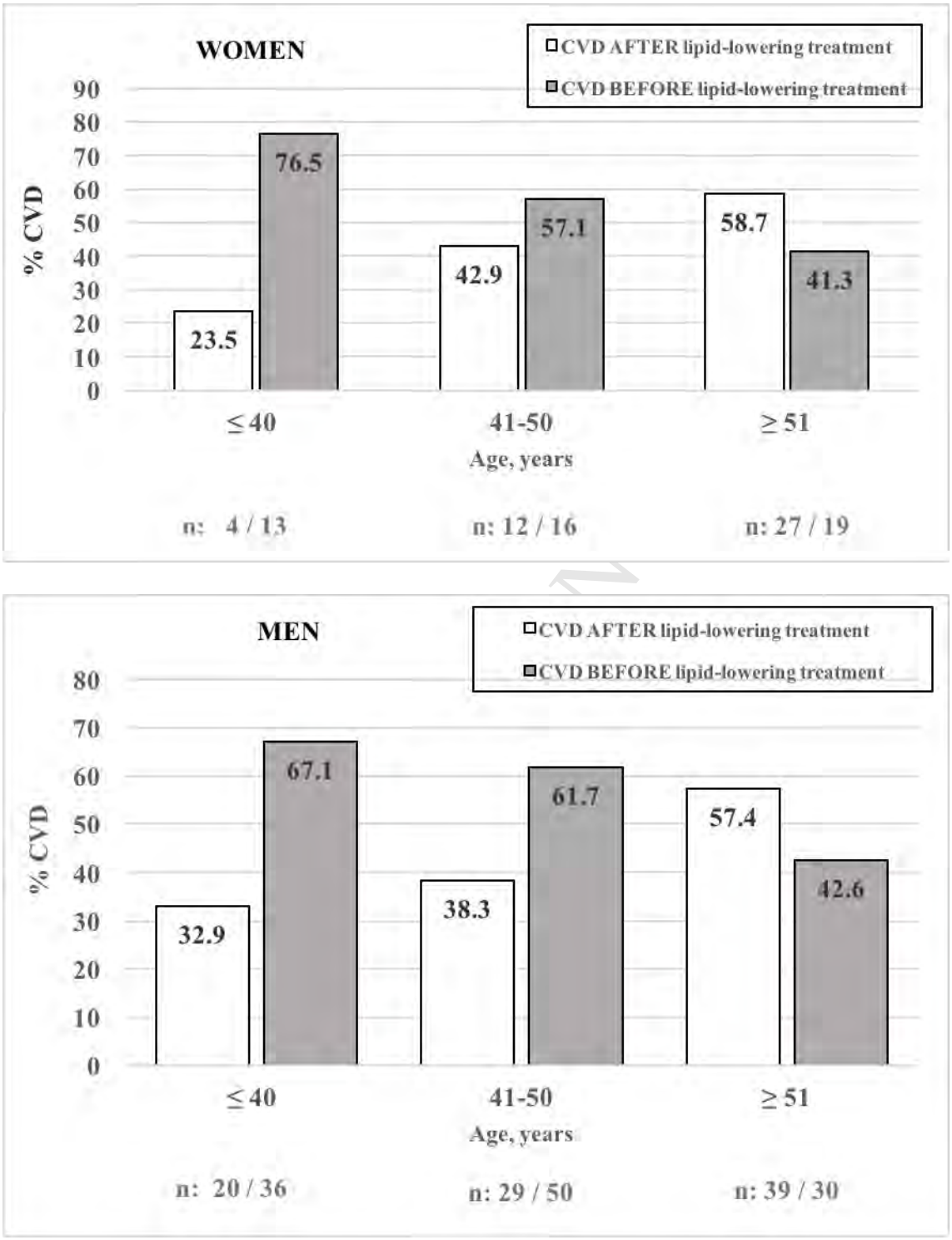
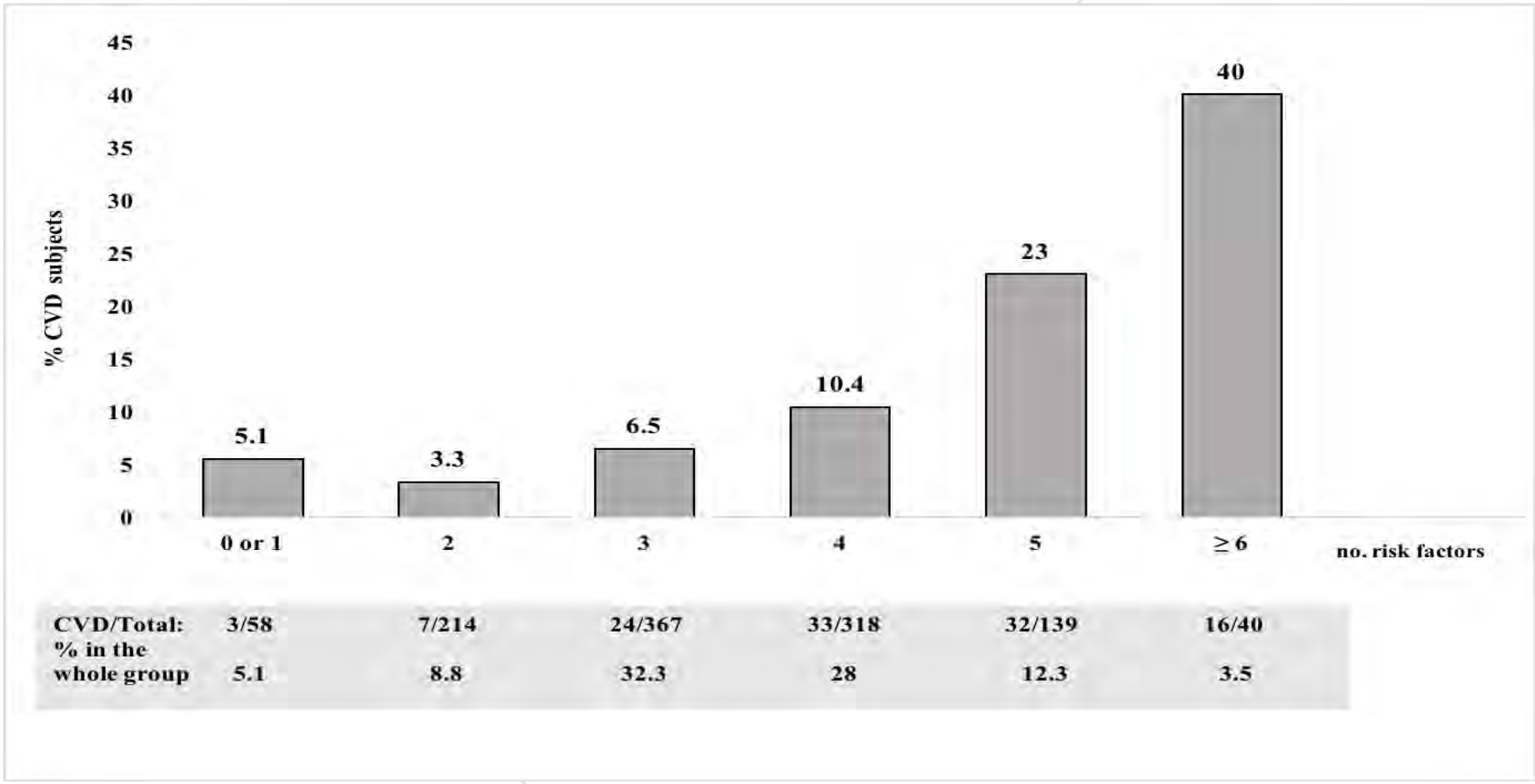

WOMEN
CVD AFTER lipid-lowering treatment
CVD BEFORE lipid-lowering treatment
% CVD
90
80
70
60
50
40
30
20
10
0
23.5
76.5
42.9
57.1
58.7
41.3
≤ 40
41-50
≥ 51
Age, years
n: 4 / 13
n: 12 / 16
n: 27 / 19
MEN
CVD AFTER lipid-lowering treatment
CVD BEFORE lipid-lowering treatment
% CVD
80
70
60
50
40
30
20
10
0
32.9
67.1
38.3
61.7
57.4
42.6
≤ 40
41-50
≥ 51
Age, years
n: 20 / 36
n: 29 / 50
n: 39 / 30

Figure 3.


% CVD subjects
45
40
35
30
25
20
15
10
5
0
5.1
3.3
6.5
10.4
23
40
0 or 1
2
3
4
5
≥ 6
no. risk factors
CVD/Total: 3/58 7/214 24/367 33/318 32/139 16/40
% in the whole group 5.1 8.8 32.3 28 12.3 3.5

Highlights

1. Statins have changed the natural history of CVD in HeFH
2. We report the current CVD prevalence in HeFH after an average of 9.7 years of statins
3. 10% of HeFH suffered a CVD event after more than 12 years of statin treatment
4. HeFH at high risk with high-intensity statins are those with >3 risk factors
5. This study identifies HeFH patients susceptible for more intensive treatment

Oct 13rd, 2018

Prof. A. von Eckardstein,
Editor-in-Chief
ATHEROSCLEROSIS

Dear Professor von Eckardstein,

Concerning the manuscript entitle: "Effect of lipid-lowering treatment in cardiovascular disease prevalence in familial hypercholesterolemia" that we submit as "Original Research Paper (Clinical and Population Research Paper)" to be considered in *Atherosclerosis* journal.

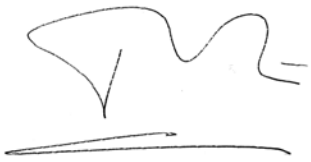
We wish to confirm that all potential conflicts of interest associated with this publication have been declared within the manuscript and there has been no significant financial support for this work that could have influenced its outcome.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

We further confirm that any aspect of the work covered in this manuscript that has involved either experimental animals or human patients has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript.

We understand that the Corresponding Author is the sole contact for the Editorial process (including Editorial Manager and direct communications with the office). He is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs. We confirm that we have provided a current, correct email address which is accessible by the Corresponding Author and which has been configured to accept email.



Fernando Civeira, MD, PhD, on behalf of all authors
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