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Atherosclerosis

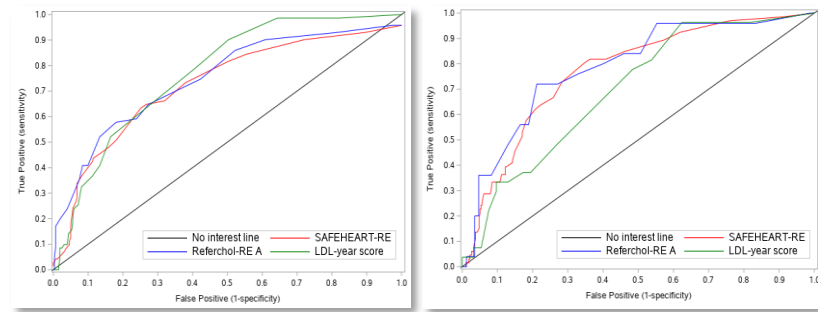
SAFEHEART risk-equation and cholesterol-year-score are powerful predictors of cardiovascular events in French patients with Familial Hypercholesterolemia --Manuscript Draft--

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Abstract:	<p>Background and aims: Patients with heterozygous Familial Hypercholesterolemia (HeFH) present elevated cardiovascular (CV) risk. Current CV risk stratification algorithms developed for the general population are not adapted for HeFH patients. It is therefore of singular importance to develop and validate CV prediction tools which are dedicated to the HeFH population. Methods: Our first objective was to validate the Spanish SAFEHEART-risk equation (RE) in the French HeFH cohort (REFERCHOL), and the second to compare SAFEHEART-RE with the low-density-lipoprotein-cholesterol (LDL-C)-year-score for the prediction of CV events in the HeFH French population. Results: We included HeFH (n=1473) patients with a genetic or clinical diagnosis (DLCN score ≥ 8). Among them, 512 patients with a 5-year follow-up were included in order to validate the 5 year-CV-RE. A total of 152 events (10.3%) occurred in the entire population of 1473 patients during a mean follow-up of 3.9 years. Over the five-year follow-up, non-fatal CV events occurred in 103 patients (20.2%). Almost all the parameters used in the SAFEHEART-RE were confirmed as strong predictors of CV events in the REFERCHOL cohort. The C-statistic revealed a satisfactory performance of both the SAFEHEART-RE and LDL-C-year-scores in predicting CV events for all the patients (primary and secondary prevention) (C-index 0.77 and 0.70, respectively) as well as for those in primary prevention at the inclusion (C-index 0.78 and 0.77, respectively). Conclusion: This analysis represents the first external validation of the SAFEHEART-RE and demonstrated that both SAFEHEART-RE and the LDL-C-year-score are good predictors of CV events in primary prevention HeFH patients. Abstract word count: 249 words Registry REFERCHOL, was declared to the ANSM (the French National Agency for Medicines safety) and received an ID-RCB declarant number: 2014-A01549-38 Key words: familial hypercholesterolemia, cardiovascular risk, cardiovascular risk equation, cholesterol-year-score, primary</p>

SAFEHEART-risk equation can now be used in other European population of HeFH patients to predict CV events

The cholesterol-year-score is a robust predictor of CV events in HeFH patients

SAFEHEART-RE and the cholesterol-year-score are valid in primary prevention heFH patients



SAFEHEART-RE and LDL-C-year-score are good predictors of CV events in patients with **heterozygous Familial Hypercholesterolemia** in primary prevention (left ROC curves) and in primary and secondary prevention (right ROC curves)

SAFEHEART risk-equation and cholesterol-year-score are powerful predictors of cardiovascular events in French patients with Familial Hypercholesterolemia

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Abstract:

Background and aims: Patients with heterozygous Familial Hypercholesterolemia (HeFH) present elevated cardiovascular (CV) risk. Current CV risk stratification algorithms developed for the general population are not adapted for heFH patients. It is therefore of singular importance to develop and validate CV prediction tools which are dedicated to the HeFH population.

Methods: Our first objective was to validate the Spanish SAFEHEART-risk equation (RE) in the French HeFH cohort (REFERCHOL), and the second to compare SAFEHEART-RE with the low-density-lipoprotein-cholesterol (LDL-C)-year-score for the prediction of CV events in the HeFH French population.

Results: We included HeFH (n=1473) patients with a genetic or clinical diagnosis (DLCN score ≥ 8). Among them, 512 patients with a 5-year follow-up were included in order to validate the 5 year-CV-RE. A total of 152 events (10.3%) occurred in the entire population of 1473 patients during a mean follow-up of 3.9 years. Over the five-year follow-up, non-fatal CV events occurred in 103 patients (20.2%). Almost all the parameters used in the SAFEHEART-RE were confirmed as strong predictors of CV events in the REFERCHOL cohort. The C-statistic revealed a satisfactory performance of both the SAFEHEART-RE and LDL-C-year-scores in predicting CV events for all the patients (primary and secondary prevention) (C-index 0.77 and 0.70, respectively) as well as for those in primary prevention at the inclusion (C-index 0.78 and 0.77, respectively).

Conclusion: This analysis represents the first external validation of the SAFEHEART-RE and demonstrated that both SAFEHEART-RE and the LDL-C-year-score are good predictors of CV events in primary prevention HeFH patients.

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Registry REFERCHOL, was declared to the ANSM (the French National Agency for Medicines safety) and received an ID-RCB declarant number: 2014-A01549-38

Key words: familial hypercholesterolemia, cardiovascular risk, cardiovascular risk equation, cholesterol-year-score, primary cardiovascular prevention

Introduction

Heterozygous Familial Hypercholesterolemia (HeFH) is one of the most prevalent autosomal dominant genetic diseases, and is characterized by mutations in genes encoding proteins involved in low-density-lipoprotein-cholesterol (LDL-C) catabolism. As a consequence, HeFH patients feature elevated circulating levels of LDL-C from birth. This condition remains underdiagnosed and undertreated, (1) (2) (3) and is characterized by premature cardiovascular (CV) events driven by lifelong exposure of the arterial wall to hypercholesterolemia (4).

Traditional risk factors such as smoking and hypertension may further increase CV risk in HeFH patients. Indeed, cumulative traditional CV risk factors are currently used to classify HeFH patients into categories of lesser or greater severity (5, 6). Cardiovascular risk is however expressed heterogeneously among HeFH patients (7). It is therefore essential to identify the more severe patients early in life, and to initiate treatment to the guideline-recommended goal of LDL-C commensurate with their level of risk (8, 9). Moreover, it is of particular importance to precisely evaluate CV risk in primary prevention HeFH patients, in order to guide the choice of the type and the frequency of CV explorations, the initiation of aspirin or PCSK9 inhibitors.

Classical CV risk equations (RE), such as the European SCORE equation or the US Framingham RE were formulated with data from the general population and are not therefore recommended for application to FH patients, as they underestimate their CV risk (1). Moreover, the SCORE RE is only valid for persons older than 40 years-old.

Recently, Pérez de Isla et al. developed the first specific CV-RE dedicated to genetically-confirmed HeFH patients, in primary or in secondary prevention: the SAFEHEART-RE (10) (11). This algorithm was constituted of traditional risk factors: age, gender, smoking, hypertension, body mass index, a history of CV events, and levels of LDL-C and lipoprotein (a) (Lp(a)). To date, the SAFEHEART RE has not been validated in another HeFH cohort and has not been tested in patients free of CV events at the inclusion. An external validation is critical for the extension of this CV-RE to other HeFH populations.

The cholesterol-year-score is a simple tool which evaluates the duration and the intensity of vascular exposure to elevated cholesterol levels (12), and in a similar manner to that in which pack-year is used to measure lifelong exposure to tobacco. An increased prevalence of early

1 atherosclerotic burden has been observed in young HeFH subjects who present a high vascular
2 cholesterol burden (13). **The cholesterol-year-score was first used in homozygous FH subjects (12)**
3 **but its validation in HeFH patients has not been reported to date.** To our knowledge, the
4 predictive power of the cholesterol-year-score in such subjects with respect to CV events has not
5 yet been evaluated.
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10 Our first objective was the validation of the Spanish SAFEHEART-RE in our French cohort of HeFH
11 patients (REFERCHOL cohort), and the second, a comparison of the predictive power of
12 SAFEHEART-RE with that of the LDL-C-year-score for CV events, in the whole cohort and in
13 primary prevention HeFH patients.
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19 Patients and Methods

20 21 22 *Study Design and Population*

23 The national French Register of FH (REFERCHOL) was established in 2015 by the *Nouvelle Société*
24 *Françophone d'Athérosclérose* (NSFA) as previously described (14). For the present study, the
25 population was prospectively enrolled between November 2015 and December 2018 and
26 retrospective data were recorded from 2005 to 2015. A total of 2311 adults (≥ 18 years old) with
27 either a genetic or a clinical diagnostic (DLCN score ≥ 8) of HeFH were enrolled. Among them,
28 1473 patients with at least two visits in the participating lipid clinics were retained for the analysis
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39). For the validation of the 5 year CV-RE, we included 512 patients with either 5-years of follow-
40 up, or a CV event within the 5 years following the enrollment visit.
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44 *Collection of Patient data*

45 Demographic and clinical characteristics at the enrollment visit (baseline) were used in this study.
46 For most patients, DNA was isolated and the genetic diagnosis of FH was evaluated. Maximum
47 statin dose was defined as previously described (atorvastatin 40 or 80 mg/day, rosuvastatin 20 or
48 40 mg/day, or simvastatin 80 mg/day) (15). Maximum combined lipid-lowering therapy was
49 defined as maximum statin dose plus ezetimibe 10 mg/day. Assessment of biological parameters,
50 including a plasma lipid profile, were performed in the clinical laboratory attached to each lipid
51 clinic, using assay methods subject to quality control. **The majority of Lp(a) measurements were**
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1 made using an immunonephelometric method (Siemens-France). However, some Lp(a)
2 measurements were made with alternative methods in laboratories of other University Medical
3 Centres, the latter corresponding to the location of heFH patients. The LDL-C-year-score was
4 calculated as previously described by multiplying the highest LDL-C value by the patient's age at
5 clinical diagnosis or at the initiation of statin therapy, to which was added the LDL-C value at the
6 inclusion visit in the registry multiplied by the time spent until the inclusion visit, as follows: LDL-
7 C-year-score = $LDL-C_{max} * [age\ at\ diagnosis/initiation\ of\ statin] + LDL-C\ at\ inclusion * [age\ at$
8 $inclusion - age\ at\ diagnosis/initiation\ of\ statin\ therapy]$

16 *Definition of outcomes*

19 Cardiovascular events included coronary heart disease (acute coronary syndromes, myocardial
20 infarction and unstable angina, percutaneous coronary intervention or coronary artery bypass
21 graft), stroke or transient ischemic attack, peripheral artery disease (defined as carotid
22 endarterectomy, carotid angioplasty, peripheral artery angioplasty or bypass), resuscitated
23 sudden death and cardiovascular death (16). A family history of early CV events was defined as
24 the occurrence of the first event before 55 years of age in men and before 65 years of age in
25 women in first-degree relatives. The duration of follow-up was defined as the time from the
26 enrollment visit to the last available visit at the Lipid Clinic.

36 *Statistical analysis*

38 These analyses were conducted in accordance with the TRIPOD (Transparent Reporting of a
39 Multivariable Prediction Model for Individual Prognosis or Diagnosis) requirements (17). Univariate
40 effects were analyzed with Cox models by hazard ratios and their 95% confidence intervals. For
41 multivariate analysis, several Cox models were adjusted. REFERCHOL equation A was estimated
42 by forcing variables derived from SAFEHEART-RE in order to compare effect size regardless of
43 statistical significance. REFERCHOL equation B included only independent significant factors with
44 a p value <0.05 from the analysis population. We modified some parameters as compared to the
45 SAFEHEART cohort. First, maximal LDL-cholesterol (ie LDL-C before treatment) was not
46 extrapolated from LDL-C under lipid lowering therapy but obtain from patient's medical record.
47 Secondly, the segmentation for ranks according to LDL-C levels was modified in view of
48 differences in sample sizes: there were only 91 patients with LDL < 100 mg/dL and 427 between
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100 and 160 mg/dL. New cut-points for LDL-C were updated from <100 mg/dl in SAFEHEART-RE to ≤ 160 mg/dL as the reference level, to 160-200 mg/dL and to >200 mg/dL. LDL-C-year-score and the variables included in its calculation (age at initiation of statin treatment, maximum LDL-C before therapy) were also tested in the univariate analysis and LDL-C-year-score was included in the REFERCHOL Equation B.

As in SAFEHEART-RE, the Kaplan-Meier estimator from the analysis population to obtain the 5-year risk was used according to the method described by D'Agostino (18). To compare ROC curves and C-indexes, the Kendall's Tau-b was computed to measures the ranking association between risk equations, and evaluated the concordance between pairs of estimated risks from two RE for every given risk threshold, ranging from -1 (negative association) to +1 (positive association). For additional validation of the Safeheart-RE and the REFERCHOL equation A, the degree of over-optimism (standard deviation from the standard error of risk estimations) on the REFERCHOL analysis population was estimated with bootstrap resampling of 100 randomized samples as recommended by TRIPOD (17).

Results

Characteristics of the HeFH REFERCHOL population at the enrollment visit

The mean characteristics of the analysis population are presented in Table 1. The mean age of the patients was 49.6 ± 16.7 years. Genetic causal mutations were found in 70.1% (n=1032) of the REFERCHOL cohort, with 88.9% in the *LDLR* gene, 7.8% in the *APOB* gene and 3.4% in the *PCSK9* gene. Among patients with a clinical diagnosis (n=441), more than half (n=236, 53.5%) did not have a genetic test and a small number of them lacked detectable causal mutations (n=33, 7.5%). For other patients (n=172, 39%), genetic tests are in process. At the enrollment visit, a third of patients were not receiving lipid lowering therapy (n=491, 33.3%). Almost a quarter of patients were receiving maximally tolerated statin therapy and 40% were treated with maximum combined therapy (ie maximum tolerated statin therapy plus ezetimibe). The mean age at statin initiation was 32 ± 14.3 years. The characteristics of the SAFEHEART population used for the development of the SAFEHEART risk equation (10) are also presented in Supplemental data 1.

Predictive factors for incident CV events in the REFERCHOL cohort

A total of 152 events occurred in the entire population of 1473 patients included for the analysis (10.3%) during a mean follow-up of 3.9 years (SD: 3.4 years; Med: 2.6 years). A large proportion of events occurred in patients with a history of CV events (n=79/326, 24.2%); a small proportion of events occurred in patients free of CV events at the enrollment visit (n=73/1147, 6.3%). Kaplan-Meier curves showing freedom from recurrent cardiovascular events in all patients, and patients with history of CV events or patients without history of CV events, are shown in Figure 2. Table 2 presents univariate analysis of predictors of incident CV events. With the exception of a family history of premature CV event, ApoA1 and Lp(a) levels, the predictors observed in the SAFEHEART cohort were statistically significant in the REFERCHOL cohort and the effect size of HRs varied globally over a similar range. Among the additional variables in REFERCHOL, LDL-C-year score was the strongest predictor of CV events. Table 3 shows multivariate analysis for REFERCHOL equations A and B. Data on the HRs for patients from the SAFEHEART study are reported in the first column, and are compared to those of the REFERCHOL cohort. REFERCHOL equations A and B were tested in the entire REFERCHOL population of 1473 patients. The REFERCHOL equation-A was applied by forcing the inclusion of all the significant variables of the SAFEHEART study in the

1 model. Significant variables in the multivariate analysis in the equation-A model were age, the
2 history of a CV event, active smoking and LDL-C levels. The REFERCHOL equation-B was
3 calculated with the inclusion of only significant variables from the univariate analysis in the
4 REFERCHOL cohort: gender, history of CV events, LDL-C levels and LDL-C-year-score. As maximal
5 LDL-C levels and the age of statin initiation were used for the calculation of the cholesterol-year-
6 score, they were removed from the multivariate analysis. Gender, history of CV events, LDL-C
7 levels and LDL-C-year-score were independent CV risk predictors in the REFERCHOL cohort. LDL-
8 C-year score was a strong predictor for CV events, as HeFH patients with a LDL-C-year-score
9 between 6000 and 16000 mg/dL had a 5.7 fold increased in CV risk (95%CI, p=0.09) and those
10 with a cholesterol-year-score above 16000 mg/dL had a CV risk multiplied by 17.4 fold (95%CI,
11 p=0.006).

22 *Validation of the SAFEHEART risk prediction model and comparison to REFERCHOL-RE and*
23 *cholesterol-year-score for predicting CV events in the REFERCHOL cohort*

26 The analysis of the validation population (512 patients with five-year follow-up or a CV event
27 within the five-year following enrollment visit) showed non-fatal CV events occurring in 103
28 patients (20.1%; 34 nonfatal myocardial infarctions, 28 coronary artery revascularization
29 procedures or bypass, 22 unstable anginas, 18 nonfatal strokes or transient ischemic attacks).
30 Among the 512 patients, 393 were in primary prevention at inclusion and 46 of them had a CV
31 event within the five 5 years of follow-up (11.7%). Among the 119 patients in secondary prevention
32 at inclusion, 57 of them had a recurrent CV event (47.9%) event. Only the first event during follow-
33 up was considered for analysis.

42 Considering the 512 patients cohort (primary and secondary prevention patients at the inclusion),
43 the C-index of SAFEHEART-RE for predicting five-year CV events in the REFERCHOL cohort was
44 0.77, and the C-index of REFERCHOL-RE A and the cholesterol-year-score were respectively 0.78
45 and 0.70. Figure 3A shows the ROC curves for the 3 models. The ability of SAFEHART-RE,
46 REFERCHOL-RE and the LDL-C-year-score to predict five-year CV events was quite similar
47 between the 3 models, although a little less effective for the LDL-C-year-score (supplemental data
48 2).

56 Considering the 393 patients cohort (primary prevention patients), the sensitivity analysis showed
57 that the all three risk prediction models were good predictors of CV events in this patients free of
58 CV event at the inclusion with the following C-indexes: 0.78 for SAFEHEART-RE, 0.79 for the
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REFERCHOL-RE A and 0.77 for the cholesterol-year-score. (Figure 3B and supplemental data 2).

The population of the 119 patients in secondary prevention at the enrollment was too small to be analyzed separately with unstable and uninterpretable results (supplemental data 2).

To compare ROC curves and C-indexes, the Kendall's Tau-b was computed to measure the ranking association between risk equations. The results were as following: Safeheart RE vs Referchol RE: 0.92, Safeheart RE vs LDL-year score: 0.78, Referchol RE vs LDL-year score: 0.83 (Kendall's Tau-b ranging from -1 (negative association) to +1 (positive association)). Additional validation assessed with bootstrap resampling (100 randomized sample of the original dataset) showed a degree of over-optimism of 0.01 and 0.007 for SAFEHART-RE and REFERCHOL-RE respectively.

Discussion:

The estimation of CV risk in HeFH has led to the development of dedicated CV-RE derived from the earliest longitudinal, prospective studies of national HeFH cohorts. SAFEHEART-RE was the first European HeFH cohort to develop a prospective model of prediction of CV events in this pathology (10). However, an external validation has not been performed to date, mainly as a result of the quality of data collection (namely, the lack of a molecular diagnosis of FH), of a relatively shorter follow-up for the comparison of CV outcomes, and of incompatibility between the databases. Indeed, HeFH is characterized by marked phenotypic variability, which often occurs independently of a molecular diagnosis, and which makes lifelong-cholesterol exposure (the so-called cholesterol burden) potentially a major surrogate marker for future CV disease. Moreover, SAFEHEART-RE was developed in a mixt population of primary and secondary prevention patients.

In the present study, we validated two different tools for the prediction of CV events in HeFH patients, SAFEHEART-RE and the LDL-C-year-score. Both SAFEHEART-RE and LDL-C-year-score performed in a highly satisfactory manner as concerns the prediction of CV events in the whole REFERCHOL cohort and specifically in patients in primary prevention. To our knowledge, this is the first external validation of the Spanish CV risk equation SAFEHEART dedicated to familial hypercholesterolemia, and the first validation of this CV risk equation in the specific population of HeFH patients free of CV events at the inclusion. .

Differences between the Spanish and the French heFH cohorts:

Our REFERCHOL cohort exhibited some differences as compared to that from Spain. First, patients included in the REFERCHOL cohort had either a genetic diagnosis with a heterozygous causal gene mutation, or a clinical diagnosis based on the DLCN score, whereas all patients belonging to the SAFEHEART cohort had a genetic diagnosis of HeFH. In order to be included in the study, a DLCN ≥ 8 was needed, resulting in a more severe CV risk profile in the REFERCHOL cohort, the consequence of the absence of a molecular diagnosis (which itself accounts for a DLCN score =8). Indeed, 20.1% of the REFERCHOL cohort was in secondary prevention as compared to 10% in the Spanish cohort and a higher prevalence of personal and/or family history of CV events, as well as higher LDL-C levels and/or xanthoma, were observed in the REFERCHOL cohort as compared to that of SAFEHEART. Xanthoma are a well-known CV risk factor in FH as they are correlated with the cholesterol burden (19) (20). Moreover, patients in the REFERCHOL cohort were older (49.6 years vs 45.5 years) and cumulated more CV risk factors (hypertension prevalence 27.3% vs 14%; LDL-C levels at the enrollment visit: 200 mg/dL vs 177 mg/dL and Lp(a) levels 44.5 mg/dL vs 38 mg/dL) than those in the SAFEHEART cohort. Together, these differences between the two cohorts may explain the finding that French patients were more clinically severe, but they equally reinforce the fact that the SAFEHEART-RE is valid in different populations of HeFH patients. We observed that one third of HeFH patients were not treated at the enrolment visit, which corresponds to the first visit to a specialist Lipid Clinic. This observation implies that a selection bias may be operative as more severe patients are probably referred to Lipid clinics, while less severe patients are probably followed by their general practitioner. Lipid lowering therapies are typically initiated and / or intensified in specialist lipid clinics. Furthermore, some patients exhibit statin intolerance and are therefore addressed to a specialist lipidologist.

The cholesterol-year-score, a simple tool to predict CV events in HeFH:

Cholesterol-year-score is an easy-to-use tool for the estimation of lifelong vascular exposure to elevated levels of lipoprotein cholesterol. The cholesterol-year-score was first used in the nineties as a tool for evaluating the duration and the severity of hypercholesterolemia in homozygous FH (12) (21). Twenty years later, the concept of cholesterol burden is at the forefront of care recommendations in FH, and emphasizes the fact that lipid lowering treatment must be initiated early (1). An elevated cholesterol burden is associated with an increased atherosclerotic

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burden. In an earlier study, we showed that up to 40% of young HeFH subjects aged 20-45 years exhibited coronary artery calcification, which was itself frequently associated with a high cholesterol burden (13). Interestingly, late initiation of lipid-lowering therapy, indirectly resulting in a higher pre-treatment cholesterol burden, was in turn associated with a higher atherosclerotic burden. This relationship has been extensively observed in other FH registries: the risk of CV death related to FH diagnosis was increased despite treatment in subjects in whom the age of initiation of lipid lowering therapy was late. A recent long-term follow-up study of young FH patients and their unaffected siblings has shown that lipid-lowering treatment that is initiated early in life results in a lower subclinical atherosclerotic burden and a significantly lower CV morbidity and mortality as compared to those FH subjects in whom treatment was initiated later (22). In the present study, the LDL-C-year-score was a strong predictor of CV events in HeFH patients especially in those in primary prevention. The CV risk is multiplied by 5.7 fold for patients with a cholesterol-year-score between 6000 and 16000 mg/dL and by 17.4 fold for those with a cholesterol year score above 16000 mg/dL. More interestingly, we observed that a LDL-C-year-score above 6000 mg/dL provided a similar CV risk as that in patients who had already presented clinically with a CV event, suggesting that earlier initiation of lipid lowering therapy might contribute to reduction in the incidence of CV events.

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In the REFERCHOL cohort, the predictive power of cholesterol-year-score for CV events was as robust as SAFEHEART-RE for patients in primary prevention but quite less robust when considering all patients (primary and secondary prevention). Nonetheless, these results demonstrated that arterial exposure to cholesterol as evaluated by the LDL-C-year-score is a satisfactory predictor of CV events, especially in patients free of CV events and that it summarized the effect of all other variables. These results reinforce the well-known major role of cholesterol burden in the development of CV disease in FH, as the duration and the severity of the hypercholesterolemia performs as well as the combination of other traditional risk factors in prediction of CV events.

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Other markers or predictors of CV risk in HeFH patients:

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We did not evaluate the burden of subclinical atherosclerosis in this study: carotid IMT is a valid marker for the assessment of early atherosclerosis, namely in FH children (23), although

1 carotid atherosclerosis (evaluated by non-invasive ultrasound or the presence of plaque) has not
2 proven its efficacy in further stratification of CV risk (24). Assessment of coronary artery calcium
3 represents a more promising approach. Apart from showing the highest reclassification power in
4 the general population, CAC score was found to be superior in improving CV event prediction
5 when added to the Framingham risk score in HeFH subjects (25). It has also recently been shown
6 that SAFEHEART-RE correlates with the CAC Score (26) and with other biological parameters such
7 as circulating microvesicles that may represent surrogate biomarkers of coronary calcification in
8 FH patients (27). Confirmation of the contribution of CAC in the context of FH-specific CV risk
9 algorithms is awaited in large populations.
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11 Classical cardiovascular risk factors were used to construct the SAFEHEART risk equation: age,
12 gender, smoking, high blood pressure, BMI, history of CV events, LDL-C levels and Lp(a) levels. A
13 recent analysis from the SAFEHEART study has confirmed the independent predictive role of Lp(a)
14 in determining CVD risk in subjects with or without HeFH (28). High levels of Lp(a) were not
15 confirmed as predictors in the REFERCHOL cohort, although this result might be due to variability
16 in the methods used for its assessment and to the potentially higher genotypic variability, which
17 differentially impacts CV risk (29) (30). Further efforts are needed for the optimal personalized
18 stratification of CV risk in HeFH: novel approaches such as Genome Wide Association Studies
19 have already strengthened the evidence of the role of cholesterol burden on CV risk (31), and
20 have equally integrated the possibility to predict therapeutic response to lipid lowering treatment.
21 Such approaches may be invaluable in the future in further defining the latent CV risk in HeFH
22 (32).
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44 *Differences between the analysis population (n= 1473) and the validation population (n=512)*

45 HeFH patients with a 5-year follow-up presented a more severe clinical profile than those
46 included for the complete analysis (20.1% of CV events as compared to 10% respectively). This
47 difference may be due to a recruitment bias as more severe patients tend to benefit more
48 frequently from follow-up in specialist Lipid clinics, thereby explaining the longer follow-up.
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Strengths and limitations

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This analysis represents the first study to validate a CV risk equation in a population of HeFH patients with either genetic or clinical diagnosis of FH. Furthermore, the cholesterol-year-score, a very simple tool, predicted risk as well as a CV risk equation integrating multiple CV risk factors, especially in primary prevention. Data employed to estimate the LDL-C-year-score were robust, as the score was calculated with the value for the maximal LDL-cholesterol level recorded in the medical dossiers of almost all patients, and was not extrapolated from values under treatment. In addition, the number of CV events in the French population with a five-year-follow-up was similar to that in the SAFEHEART cohort, ie. 103 and 122 CV events respectively. Only national and international FH registries with lifelong patient follow-up can of course provide robust data on CV risk in this specific population (30). Finally, we could only validate the SAFEHEART-RE for the 5 years follow-up. However, a longer follow-up would provide greater numbers of both patients and CV events, and would permit a 10-year CV – RE to be constructed for both the in SAFEHEART and REFERCHOL cohorts.

In conclusion, a pragmatic assessment of intermediate and long-term CV risk in HeFH is provided by the SAFEHEART- RE and the Cholesterol-year score evaluation, highlighting the importance of early CV risk assessment in guiding more efficient therapeutic strategies. **Both SAFEHEART and cholesterol-year-score were powerful predictors of CV events in primary prevention. However in the entire cohort, (ie. Including both primary and secondary prevention patients), SAFEHEART was superior to the cholesterol-year-score. This first external validation of the SAFEHEART-RE is promising as it validates the wider applicability of SAFEHEART-RE in other HeFH populations.**

Conflicts of interest statement: SB have received honoraria for board, conferences, clinical trial or congress from Aegerion, Akcea, Elivie, Sanofi or Amgen. AV has no conflict of interest to declare. E.B. reports grants from Sanofi and Amgen, and personal fees from Aegerion, Danone, Genfit, MSD, Sanofi/Regeneron Pharmaceuticals, Inc., AstraZeneca, Servier, AMGEN, AKCEA, Mylan.

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3 and coordinated by the CHU of Nantes.
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6 Author's contributions: SB and EB are the principal investigators of the REFERCHOL registry, they
7 contributed to the design and the setting of the registry. AG, SC, SB and EB designed the study
8 and wrote the manuscript. AV made the statistical analyses of the data. JC, DA, FB, BC, VC and AC
9 are co-investigators of the REFERCHOL registry and they made a critical revision of the
10 manuscript. SB and EB are the guarantors of this work and, as such, had full access to all the data
11 in the study and take responsibility for the integrity of the data.
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20 the statistical analysis.
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Figure (n=3) and tables (n=3):

Figure 1: Patient flowchart for the REFERCHOL cohort for the analysis study and the validation study. HeFH= Heterozygous familial hypercholesterolemia. DLCN= Dutch Lipid Clinic Network

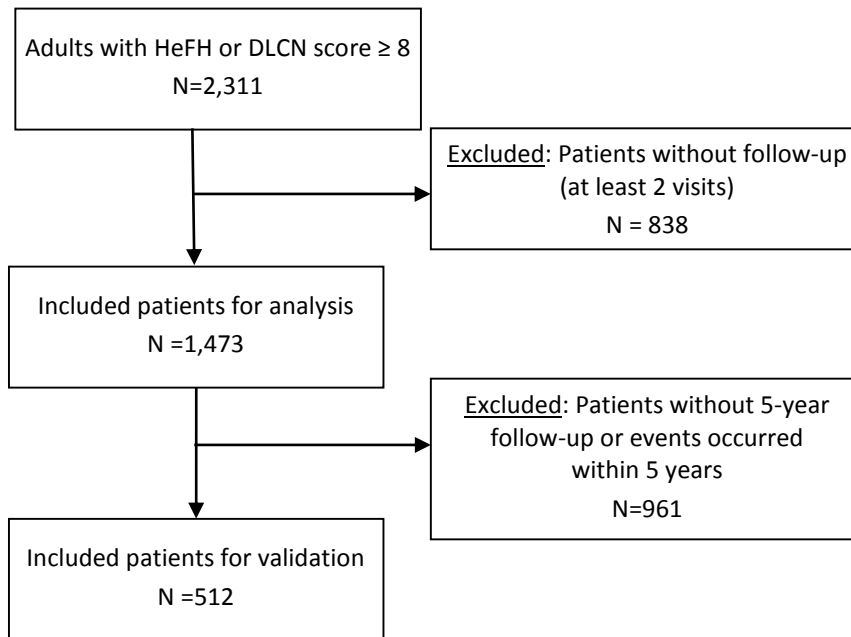
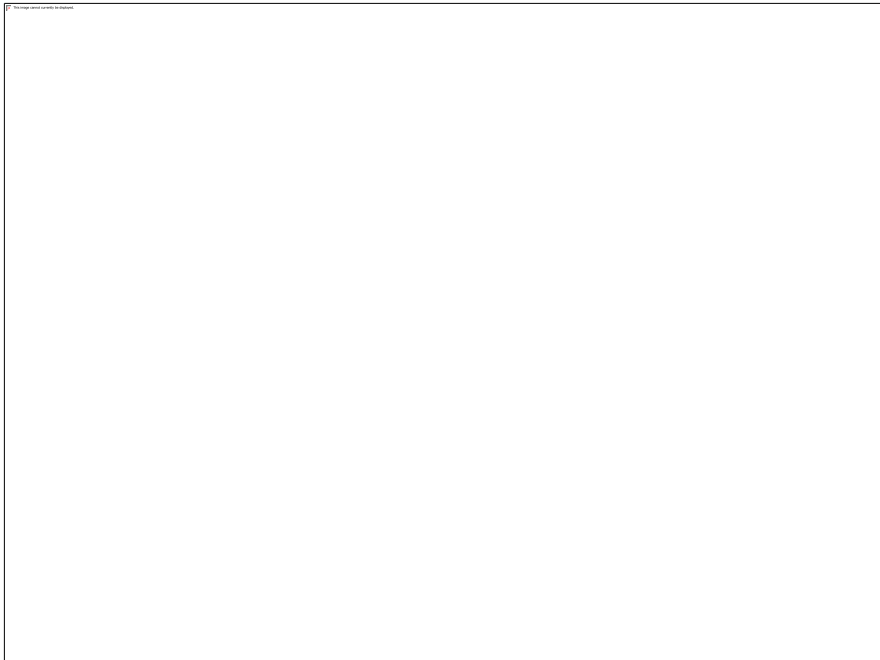


Table 1: Characteristics of the French REFERCHOL HeFH population at the enrolment visit

Variable	Value	REFERCHOL patients (N=1473)
Gender (n)	Male	712 (48.34%)
Age (years)	Mean (Sd)	49.61 (16.65)
Age (class)	<30	244 (16.56%)
	30-60	794 (53.90%)
	>60	435 (29.53%)
Mutation (n)	Yes	1032 (70.06%)
	LDL-R	917 (88.86%)
	APOB	80 (7.75%)
	PCSK9	35 (3.39%)
History of ASCVD (n)	Yes	326 (22.13%)
Premature history of familial ASCVD (n)	Yes	379 (32.39%)
Type II diabetes (n)	Yes	61 (4.39%)
HTA (n)	Yes	377 (27.26%)
Current smoker (n)	Yes	369 (27.05%)
BMI (Kg/m²)	Mean (Sd)	24.69 (4.76)
TC (mg/dl)	Mean (Sd)	277.12 (91.21)
	Median	261.0
Maximum TC (mg/dl)	Mean (Sd)	388.78 (102.20)
	Median	376.0
LDL-C (mg/dl)	Mean (Sd)	199.88 (83.33)
	Median	186.0
Maximum LDL-C(mg/dl)	Mean (Sd)	299.75 (95.12)
	Median	288.5
HDL-C (mg/dl)	Mean (Sd)	54.14 (16.43)
	Median	52.0
TG (mg/dl)	Mean (Sd)	111.72 (69.24)
	Median	94.0
ApoA1 (mg/dl)	Mean (Sd)	144.04 (32.39)
	Median	142.0
ApoB (mg/dl)	Mean (Sd)	140.90 (47.02)
	Median	130.0
Lp(a) (mg/dl)	Mean (Sd)	44.55 (55.28)
	Median	25.0
Lp(a) > 50 mg/dl(n)	-	265 (31.36%)
Treatment(n)	None	491 (33.33%)
	Statins only	464 (31.50%)
	Bitherapy	517 (35.10%)
Maximum statin dose(n)		364 (24.71%)
Ezetimibe(n)		454 (30.82%)
Maximum combined therapy (n)		226 (15.34%)
LDL-apheresis		40 (2.7%)
Time on statins (years)	Mean (Sd)	10.84 (10.73)
	Median	8.0
Age at initiation of statins (years)	Mean (Sd)	32.03 (14.28)
	Median	30.9
LDL-C-year-score (mg/dl)	Mean (Sd)	11.075 (5.863)
	Median	10.424
LDL-C-year-score mg/dl (Class)	<6.000	275 (21.62%)
	6.000-16.000	756 (59.20%)
	>16.000	244 (19.18%)

1 ASCVD= AtheroSclerotic cardiovascular Disease. Sd= Standard deviation. A premature family
2 history of ASCVD was defined as the occurrence of the first event before 55 years of age in men
3 and before 65 years of age in women in first-degree relatives. Maximum statin dose was defined
4 as atorvastatin 40 or 80 mg/day, rosuvastatin 20 or 40 mg/day, and simvastatin 80 mg/day) (15).
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10 Maximum combined lipid-lowering therapy was defined as maximum statin dose plus ezetimibe
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13 10 mg/day. LDL-C-year-score = LDL-C max * [age at diagnosis/initiation of statin] + LDL-C at
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15 inclusion * [age at inclusion - age at diagnosis/initiation of statin].
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Figure 2: Kaplan-Meier curves of freedom from cardiovascular event in all HeFH patients (black line), with history of CV event (blue line) or without history of CV event (green line).
CV=cardiovascular.



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Table 2: Univariate analysis with Proportional Hazard Cox model for predicting CV events

	SAFEHEART patients (N=2404)				REFERCHOL patients (N=1473)			
	Hazard ratio	Lower 95% CI	Upper 95% CI	p-value	Hazard ratio	Lower 95% CI	Upper 95% CI	p-value
SAFEHEART variables								
Age <30	Ref.				Ref.			
30-59	5.88	2.22	15.59	<.001	6.479	2.37	17.714	0.0003
≥ 60	12.81	4.83	34.01	<.001	12.479	4.563	34.128	<.0001
Gender (Male)	2.76	1.89	4.02	<.001	1.935	1.391	2.692	<.0001
History of CV event	6.64	4.52	9.76	<.001	4.598	3.338	6.334	<.0001
Family History of premature CV event	2.66	1.76	4.05	<.001	1.150	0.805	1.644	0.4433
Diabetes mellitus	3.45	2.03	5.86	<.001	2.177	1.175	4.032	0.0134
High blood pressure	3.38	2.29	4.98	<.001	2.349	1.69	3.266	<.0001
Active smoker	1.77	1.20	2.60	0.004	1.834	1.308	2.571	0.0004
BMI Normal weight	Ref.				Ref.			
Overweight	4.69	2.71	8.12	<.001	2.08	1.405	3.079	0.0003
Obesity	6.12	3.51	10.70	<.001	2.371	1.42	3.958	0.001
TC (unit)	1.002	1.001	1.005	0.05	1.003	1.001	1.004	0.0007
LDL-c level < 100 mg/dl	Ref.				Ref.			
100-159 mg/dl	1.66	0.49	5.57	0.83	0.522	0.253	1.076	0.0781
≥ 160 mg/dl	2.06	0.63	6.72	0.23	0.96	0.501	1.84	0.9019
HDL-c (Unit)	0.97	0.96	0.98	<.001	0.975	0.964	0.987	<.0001
TG (Unit)	1.004	1.003	1.006	<.001	1.006	1.004	1.007	<.0001
APOA1 (Unit)	0.99	0.985	0.997	0.003	0.993	0.983	1.002	0.1358
APOB (Unit)	1.007	1.003	1.010	<.001	1.009	1.004	1.014	0.0004
Lipoprotein(a) >50 mg/dL	2.14	1.51	3.04	<.001	1.400	0.931	2.104	0.1057
Patients on treatment:								
Maximum statin dose	2.08	1.48	2.93	<.001	2.634	1.911	3.629	<.0001
Ezetimibe	3.42	2.38	4.92	<.001	2.272	1.643	3.142	<.0001
Maximum LLT	2.88	2.002	4.15	<.001	2.58	1.802	3.694	<.0001
Years on statins (Unit)	1.04	1.01	1.07	0.002	1.021	1.001	1.04	0.0349
REFERCHOL additional variables								
TC-c max ≤ 350 mg/dl	-				Ref.			
350-450 mg/dl					1.104	0.747	1.632	0.6186
> 450 mg/dl					2.162	1.480	3.159	<.0001
LDL-c max ≤ 250 mg/dl	-				Ref.			
250-350 mg/dl					1.149	0.790	1.671	0.4679
> 350 mg/dl					1.813	1.216	2.703	0.0035
LDL-c < 160mg/dl					Ref.			
160-200 mg/dl	-				1.491	0.942	2.359	0.0881
200 mg/dl	-				1.676	1.127	2.494	0.0108
Treatment class None					Ref.			
Statins	-				1.125	0.729	1.734	0.5947
Bitherapy	-				2.479	1.646	3.735	<.0001
Age at initiation of Statins (Unit)	-				1.051	1.037	1.065	<.0001
LDL-C-year-score* ≤ 6.000					Ref.			
6.000-16.000					5.462	2.371	12.580	<.0001
>6.000					14.095	6.031	32.938	<.0001

* Calculated by multiplying the highest LDL-C value by the patient's age at clinical diagnosis or initiation of statin therapy, to which was added the LDL-C value at the inclusion visit in the registry

1 multiplied by the time spent until the inclusion visit, as follows: LDL-C-year-score = LDL-C max *
2 [age at diagnosis/initiation of statin] + LDL-C at inclusion * [age at inclusion - age at
3 diagnosis/initiation of statin]. Sd= Standard deviation. A premature family history of ASCVD was
4 defined as the occurrence of the first event before 55 years of age in men and before 65 years of
5 age in women in patient's first-degree relatives. Maximum statin dose was defined as atorvastatin
6 40 or 80 mg/day, rosuvastatin 20 or 40 mg/day, and simvastatin 80 mg/day (15). Maximum LLT=
7 maximum lipid lowering therapy was defined as maximum statin dose plus ezetimibe 10 mg/day.
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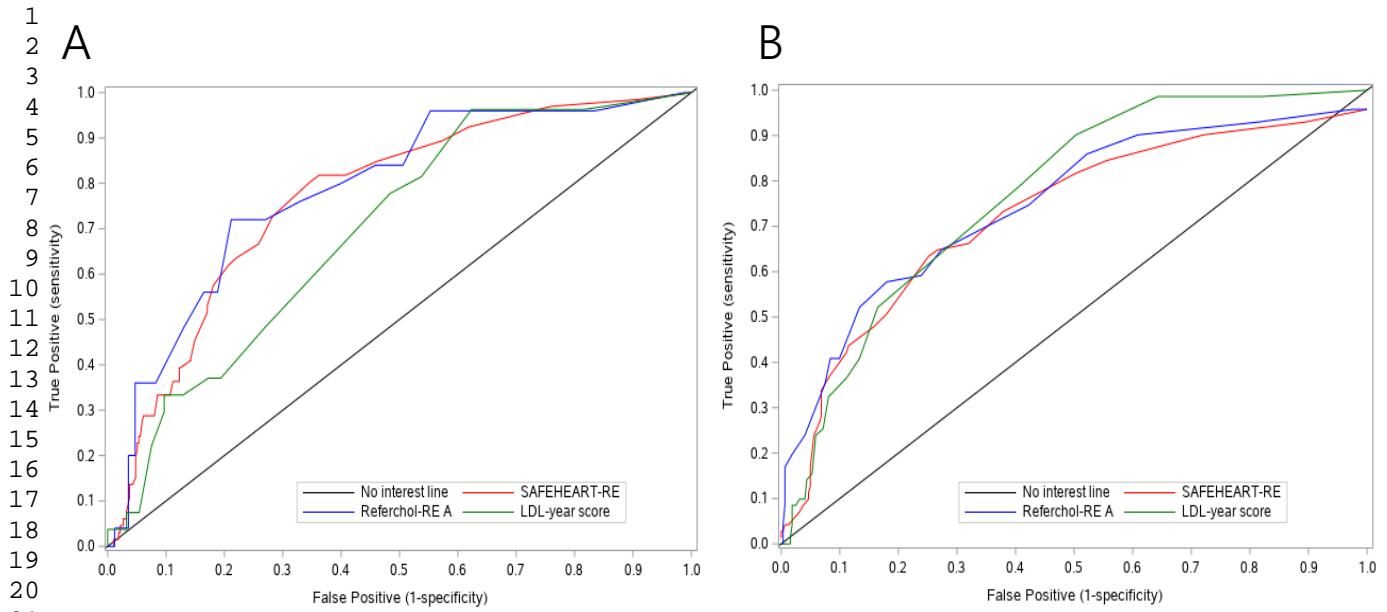
LDL-C-year-score = LDL-C max * [age at diagnosis/initiation of statin] + LDL-C at inclusion * [age
at inclusion - age at diagnosis/initiation of statin].

Table 3: Multivariate analysis with Proportional Hazard Cox model for predicting CV events

		SAFEHEART patients (N=2404)				REFERCHOL patients (N=1473)				REFERCHOL patients (N=1473)			
						<u>Forcing SAFEHEART variables</u>				<u>No Forcing (Selection of significant variables only)</u>			
		SAFEHEART equation				REFERCHOL Equation – A				REFERCHOL Equation - B			
		Hazard ratio	Lower 95% CI	Upper 95% CI	p- value	Hazard ratio	Lower 95% CI	Upper 95% CI	p- value	Hazard ratio	Lower 95% CI	Upper 95% CI	p- value
Age	<30					Ref							
	30-59	2.92	1.14	7.52	0.026	3.474	1.235	9.768	0.0182				
	≥ 60	4.27	1.60	11.48	0.004	5.452	1.883	15.787	0.0018				
Gender (Male)		2.01	1.33	3.04	0.001	1.335	0.889	2.005	0.1643				
History of CV event		4.15	2.55	6.75	<.001	4.192	2.783	6.314	<.0001	5.774	3.378	9.868	<.0001
High blood pressure		1.99	1.26	3.15	0.003	1.085	0.718	1.637	0.6992				
Active smoker		1.62	1.08	2.44	0.02	1.680	1.129	2.500	0.0106	2.146	1.283	3.587	0.0036
BMI	Normal weight	Ref				Ref							
	Overweight	2.40	1.36	4.23	0.002	1.052	0.694	1.594	0.8113				
	Obesity	2.67	1.47	4.85	0.001	1.585	0.914	2.750	0.1011				
LDL-c level*	< 100 mg/dl	Ref				Ref*				Ref*			
	100-159 mg/dl	2.50	0.60	10.53	0.21	2.261	1.359	3.759	0.0017	2.925	1.469	5.826	0.023
	≥ 160 mg/dl	4.80	1.15	20.01	0.032	3.348	2.094	5.353	<.0001	3.261	1.625	6.546	0.0009
Lipoprotein (a) > 50 mg/dl		1.52	1.05	2.21	0.028	1.413	0.929	2.149	0.1059				
LDL-C-year-score ≤ 6.000										Ref			
	6.000-16.000									5.685	0.761	42.491	0.0904
	>16.000									17.403	2.309	131.172	0.0056

* LDL-C level: < 160 mg/dl (Reference); 160-200 mg/dl; ≥200 mg/dl for REFERCHOL Equation A and B. LDL-C-year-score = LDL-C max * [age at diagnosis/initiation of statin] + LDL-C at inclusion * [age at inclusion - age at diagnosis/initiation of statin].

Figure 3: ROC curves for REFERCHOL-RE A, SAFEHEART-RE, and LDL-C-year-score.



A: HeFH patients in primary and secondary prevention. B: HeFH patients in primary prevention

$$\text{LDL-C-year-score} = \text{LDL-C max} * [\text{age at diagnosis/initiation of statin}] + \text{LDL-C at inclusion} * [\text{age at inclusion} - \text{age at diagnosis/initiation of statin}].$$

REBUTTAL LETTER TO THE EDITOR AND THE REVIEWERS:

Dear Dr. Arnold von Eckardstein, Dr. Gerald F Watts and Dr. Jan Borén,

Thank you for reviewing our manuscript entitled "**SAFEHEART risk-equation and cholesterol-year-score are powerful predictors of cardiovascular events in French patients with Familial Hypercholesterolemia**" (Ms. No. ATH-D-20-00496). We again thank the reviewers for their overall positive comments and for the pertinent points they have raised. Please find attached our responses to the comments of the reviewers in a point-by-point fashion. We have indicated clearly in each case the revisions to the manuscript that address his/her suggestions, in red in the text. We feel that the manuscript has benefited significantly from their critiques and we hope that you will now find it acceptable for publication in the journal "Atherosclerosis".

Yours sincerely,

Dr SOPHIE BELIARD, MD, PhD

Marseille, the 04 June 2020,

A handwritten signature in blue ink, consisting of a vertical line that loops back to the left, followed by a long, sweeping horizontal stroke that tapers to the right.

Responses to Reviewer#1:

We thank the reviewer for his/her very positive and encouraging assessment of this manuscript and for the pertinent points he/she has raised.

"Gallo et al validated SAFEHEART-risk-equation and compare with LDL-C year score in the French cohort. A valid CV risk equation is important for FH management. The manuscript is a well-written manuscript with detailed/appropriate statistical analysis. I have no major issue on the results and interpretation. Lp(a) was not a significant predictor of CV events".

1. Please provide kit details for Lp(a) measurement:

The majority of Lp(a) measurements were made by using an immunonephelometric method (Siemens- France). However, some Lp(a) measurements were made with alternatives methods outside in laboratories of other University Medical Centres, the latter corresponding to the location of heFH patients. We added this point in red in the Patients and Method section, page5.

2. Would Lp(a) be a predictor as a continuous variable or using other cut-cut off values were employed e.g 30mg/dL or 80mg/dL?

We thanks the reviewer 1 for this pertinent question. We decided to use the 50 mg/dL cut-off for two main raisons. First, as the subject of this study was to validate SAFEHEART-RE in the French cohort, we decided to use exactly the same variables as those used in the SAFEHEART-RE. Secondly, the 50 mg/dL cut-off is discriminant for the isolation of the 90th percentile of the general population.

As you required, we tested if Lp(a) as a **continuous variable** was predictive of the occurrence of a CV event in univariate analysis in the REFERCHOL cohort (table 1), age-adjusted or not. We did not found that Lp(a) as a continuous variable was statistically correlated to CV events, even though we found a clear tendency with a limit p value at 0.09. Our results probably lack power, as Lp(a) is a known CV risk factor.

	SAFEHEART patients (N=2404)				REFERCHOL patients (N=1473)			
	Hazard ratio	Lower 95% CI	Upper 95% CI	p-value	Hazard ratio	Lower 95% CI	Upper 95% CI	p-value
SAFEHEART variables								
Lipoprotein(a) (unit, mg/dL)	-	-	-	-	1.002	1.000	1.005	0.0984
Lipoprotein(a) (unit, mg/dL), age-adjusted	-	-	-	-	1.002	0.999	1.005	0.1936

Table: Univariate analysis with Proportional Hazard Cox model for predicting CV events for Lp(a) as a continuous variable

3. Had LDL-C been adjusted for Lp(a) cholesterol?

No, LDL-cholesterol has not been adjusted for the Lp(a) cholesterol. As the LDL-cholesterol was not directly dosed but was calculated by the Friedwald formula, LDL-cholesterol is the composite of the cholesterol in the LDL particles and the Lp(a) particles. That means, that the LDL-year-score also take into account the cholesterol contained in the Lp(a) particles.

4. It is of interest to explore Lp(a)- year score whether it predicts CV events.

You're right, it would be of interest to test a Lp(a)-year score. However, Lp(a) level is known to be lifelong stable, with no treatment for decreasing its level (except PCSK9 inhibitors which are few prescribed in France and since only two years). So a "Lp(a) year score" would be an age-adjusted Lp(a) level. As we showed in the question 2 (table 1), we tested in univariate analysis if age-adjusted-Lp(a) as a continuous variable was predictive of the occurrence of a CV event and results were negatives. As we already said in the previous answer, our results probably lack power, as Lp(a) is a known CV risk factor.

Responses to Reviewer#2:

We thank the reviewer for his/her very positive and encouraging assessment of this manuscript and for the pertinent points he/she has raised.

Gallo et al. Gallo A et al, analized the National French register of FH (REFERCHOL) with the main objective to validate the Spanish SAFEHEART-RE (SF-RE), and secondly to compare the predictive power of SF-RE with LDL-C year score.

They used 1473 FH patients from the registry with clinical and/or genetic diagnosis of FH with at least 2 visits in the follow-up. Specifically, for the validation of the SF-RE, they included 512 patients with a follow-up at least 5 years. Some variables were modified due to differences in samples size (mainly related to LDL-C).

For the LDL-C year score, they used the highest LDL-C at clinical diagnosis or initiation of statin therapy (obtained from the medical record) and then the LDL-C at the inclusion visit. Each value multiplied by the age in each moment, and then added.

Methods and Statistics are well explained

Main results:

In UV analysis, all parameters included in the SF-RE except family history of PCV and Lp(a) levels >50 were statistically significant with HR with similar range.

In the REFERCHOL cohort, LDL-C year score was a strong predictor in UV model.

In MV analysis, forcing the inclusion of all variables (A) from SAFEHEART, age, history of CV events, tobacco, and LDL-C levels were significant predictors. In option B including only significant variables in UV analysis, history of CV disease, tobacco, LDL-C and LDL-C years score were the independent predictors. The strongest predictor was LDL-C years score (17 times with a value > 16000).

For the validation of the SF-RE (the main objective of this study), the C-index for SF-RE, REFERCHOL A and LDL-C years score were: 0.77, 0.78 and 0.70 respectively.

LDL-C year score was less effective than the other 2 models.

In FH patients free of CV events at inclusion, all 3 models were good predictors (C-index 0.78, 0.79 and 0.77). No conclusion regarding patients with CV events at inclusion could be obtained by the sample size.

This is a novel and very interesting manuscript because it is the first external validation of the SF-RE, and probably also of the LDL-C year score in FH population.

The SF-RE was the first specific CV risk equation developed in a genetically confirmed heterozygous FH population from Spain, followed-up prospectively, including clinical and laboratory variables used in routine clinical practice in the evaluation of FH patients.

1) Although not described in the discussion the SF-RE correlates with the CAC Score (J Clin Lipidol. 2018;12(4):948-957) and with other biological parameters such as circulating microvesicles that may be surrogate biomarkers of coronary calcification in FH patients (ATVB 2019;39:945-955.DOI: 10.1161/ATVBAHA.118.312414). SF-RE shows strong clinical evidence and relates to as well as biological and image data.

We added this precise point on the correlation of SAFEHEART-RE with imaging data and circulating biomarkers of coronary calcifications in FH in the discussion, in red, page 13. We then added these two references (in red in the bibliography).

2) There are some differences among both populations like Age: 45 in SF vs 49, BMI 26.5 vs. 24.7, Lp(a) 38.2 vs. 44.55. Most patients in Safeheart were receiving LLT at inclusion. In the case of SAFEHEART Lp(a) was measured centralized. It is probably than in REFERCHOL; Lp(a) was measured in different labs with different methods and that's why the results may differ.

You are absolutely right, Spanish and French populations differ on many points as the age, the BMI, the number of treated patients. Also, Lp(a) measurements were not centralized as in SAFEHEART study. However, a large proportion of Lp(a) measurements were made by using an immunonephelometry method (Siemens- France). However, some Lp(a) measurements were made with various other methods outside from the university hospital. We added this point in red in the Patients and Method section, page 5.

3) Regarding LDL-C year score, this study also validates its utility. It is important to point out that this score was first used in homozygous FH patients (n=17), and its validation in heterozygous FH is scarce.

We added your pertinent remark in the introduction page 5 in red.

4) LDL-C years score or Cholesterol year score is supposed to be an easy and simple tool to determine the burden of disease (cholesterol burden associated to atherosclerotic burden). In this study, in the MV analysis, it is a strong predictor in values over 16.000 with a wide CI (2.39 to 131.7). It is important to remark in the discussion some practical issues, like to get the highest values of TC or LDL -C in patients can be difficult nowadays (except if they are recorded) because most of them are under long-term treatment and do not remember baseline values (practical issue). Also, values of LDL-C are susceptible of changes during time by modification in lipid-lowering treatments. In this sense, is the same as risk a patient that took medication during years and discontinued by other amount of years ??

You are right, LDL-C-year-score is an approximate data reflecting the lifelong cholesterol burden, but it is a good predictor of CV events in FH. It is approximate because the maximal cholesterol value, ie without treatment, is often hard to find. However many patients kept their old biological analyses with their lipid results before treatment initiation, and we could retrieved this data in the majority of the cases. Secondly, as you mentioned, some patients stop medications for some months or years for several reasons (pregnancy, statins intolerance etc.). We constructed the LDL-C year score with several LDL-C levels along the time (with or without lipid lowering therapy). Finally, the LDL-C year score is as approximate as the pack-year measuring lifelong exposure to tobacco, but it works to predict CV events in this FH cohort.

5) Considering the risk as a continuum, the SH-RE seems to be a good tool if all variables are available. Therefore, it can be used in both primary and secondary prevention. For the entire cohort (primary and secondary prevention) the SF-RE is better; and is close similar to LDL-C year score in primary prevention. In the conclusion authors need to point out that SF-RE is a better predictor in both primary and secondary prevention. In addition, this validation can strengthen the use of SF-RE as an easy and accurate tool in other countries.

As you required, we added this sentence in the conclusion, page 14 (in red in the text):

Both SAFEHEART and cholesterol-year-score were powerful predictors of CV events in primary prevention. However, in the entire cohort (ie. Including both primary and secondary prevention patients), SAFEHEART was superior to the cholesterol-year-score. This first external validation of the SAFEHEART-RE is promising as it validates the wider applicability of SAFEHEART-RE in other HeFH populations.

Marseille, April 15th 2020,

Dr A. von Eckardstein

Editor in Chief

Statement of originality:

Dear A. von Eckardstein,

I certify that the data of the present study are my own work and have not been published in this or any substantially similar form, nor accepted, nor is it under consideration for publication elsewhere.

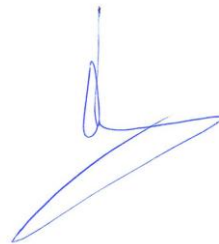
All authors have contributed to the preparation and development of this manuscript, and have approved the final draft prior to submission.

Thank you for considering the manuscript and I look forward to hearing from you.

Yours sincerely

Sophie BELIARD-LASSERRE, MD, PhD

APHM, Aix Marseille University, France

A handwritten signature in blue ink, consisting of a stylized 'S' followed by a horizontal line that curves upwards at the end.

Marseille, April 15th 2020,

Dr A. von Eckardstein

Editor in Chief

Dear A. von Eckardstein,

This is the conflict of interest form for the present work:

SB have received honoraria for board, conferences, clinical trial or congress from Aegerion, Akcea, Elivie, Sanofi or Amgen. AV and VC has no conflict of interest to declare. E.B. reports grants from Sanofi and Amgen, and personal fees from Aegerion, Danone, Genfit, MSD, Sanofi/Regeneron Pharmaceuticals, Inc., AstraZeneca, Servier, AMGEN, AKCEA, Mylan.

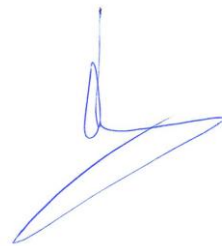
AG, FB, AC, BC, SC, DA have received honoraria for boards, and/or conferences, and/or clinical trial and/or congress from Sanofi or Amgen.

Thank you for considering the manuscript and I look forward to hearing from you.

Yours sincerely

Sophie BELIARD-LASSERRE, MD, PhD

APHM, Aix Marseille University, France

A handwritten signature in blue ink, consisting of a vertical line that curves into a horizontal stroke, followed by a large, sweeping loop that extends to the right and then curves back down.



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Checklist

All authors have contributed to the preparation and development of this manuscript, and have approved the final draft prior to submission.