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ORIGINAL ARTICLE

Secondary Prevention and Extreme Cardiovascular Risk Evaluation (SEVERE-1), Focus on Prevalence and Associated Risk Factors: The Study Protocol

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Abstract

Introduction Despite significant improvement in secondary CardioVascular (CV) preventive strategies, some acute and chronic coronary syndrome (ACS and CCS) patients will suffer recurrent events (also called "extreme CV risk"). Recently new biochemical markers, such as uric acid (UA), lipoprotein A [Lp(a)] and several markers of inflammation, have been described to be associated with CV events recurrence. The SEcondary preVention and Extreme cardiovascular Risk Evaluation (SEVERE-1) study will accurately characterize extreme CV risk patients enrolled in cardiac rehabilitation (CR) programs. Aim. Our aims will be to describe the prevalence of extreme CV risk and its association with newly described biochemical CV risk factors.

Aim Our aims will be to describe the prevalence of extreme CV risk and its association with newly described biochemical CV risk factors.

Methods We will prospectively enrol 730 ACS/CCS patients at the beginning of a CR program. Extreme CV risk will be retrospectively defined as the presence of a previous (within 2 years) CV events in the patients' clinical history. UA, Lp(a) and inflammatory markers (interleukin-6 and -18, tumor necrosis factor alpha, C-reactive protein, calprotectin and osteoprotegerin) will be assessed in ACS/CCS patients with extreme CV risk and compared with those without extreme CV risk but also with two control groups: 1180 hypertensives and 765 healthy subjects. The association between these biomarkers and extreme CV risk will be assessed with a multivariable model and two scoring systems will be created for an accurate identification of extreme CV risk patients. The first one will use only clinical variables while the second one will introduce the biochemical markers. Finally, by exome sequencing we will both evaluate polygenic risk score ability to predict recurrent events and perform mendellian randomization analysis on CV biomarkers.

Conclusions Our study proposal was granted by the European Union PNRR M6/C2 call. With this study we will give definitive data on extreme CV risk prevalence rising attention on this condition and leading cardiologist to do a better diagnosis and to carry out a more intensive treatment optimization that will finally leads to a reduction of future ACS recurrence. This will be even more important for cardiologists working in CR that is a very important place for CV risk definition and therapies refinement.

Keywords Extreme CV risk · Uric acid · Lipoprotein(a) · Inflammatory markers

1 Introduction: The Extreme Cardiovascular Risk

CV diseases are the leading cause of death worldwide with Acute Coronary Syndrome (ACS) representing the most frequent one [1]. In the last years the number of myocardial infarctions and its related morbidity and mortality have

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progressively reduced [2]. This is determined by a significant improvement in secondary prevention strategies including better blood pressure (BP) control, novel dyslipidemia and diabetes mellitus (DM) targets, new specific drugs (for dyslipidemia and DM) and longer period of dual anti-platelet therapy (DAPT) with stronger drugs.

Now the more urgent problem is turned on prevention of further CV events. All the patients that experienced an ACS or underwent a coronary revascularization (chronic coronary syndrome—CCS) have a very high risk of CV events recurrence. However, among them, most of the subjects will respond very well to secondary prevention therapies while some others will develop further CV events. The one with the higher risk for subsequent CV events has been called "extreme CV risk". This is a really new and interesting research topic with important publication in recent years [3–5]. The more simple inclusion criteria in this group of patients is the presence of a second CV events within two years from the previous one and those are estimated to be 10% of the total ACS subjects [4]. However, this criteria determine a tardive identification that doesn't permit to take advantages from the use of the most intensive and innovative treatments in order to reduce the risk of recurrence till the ideal goal of the absence of ACS recurrence. A prompt identification, ideally directly at the first ACS, will give the opportunity to really prevent future events and save lives. A step forward in this direction has been achieved by a recent consensus paper from the European Society of Cardiology (ESC) [5]. In this paper at the classic definition of 2 events within 2 years, also other definition has been added allowing a more early diagnosis (Table 1). This new definition will help identifying these subjects at the moment of the first ACS allowing them to be directly treated more intensively and with newer approach such as Lipoprotein(a)—Lp(a)—treatment, triglycerides reduction, further LDL reduction (target < 40 mg/dL), new anti-diabetic agents or new antiaggregant approach [6–9]. Furthermore, a better and rapid identification will give the opportunity to concentrate research effort on the subjects in this category for a newer further therapeutic approach in the next future.

Table 1 Expert recommendations on the definition of extremely high cardiovascular disease risk

1. SCORE > 20% in primary prevention
2. ACS and another CV events within the last 2 years
3. ACS with peripheral vascular disease or polivascular disease
4. ASC with multivessel coronary artery disease
5. ACS in patients with familiar hypercholesterolaemia
6. ACS with diabetes and one additional risk factor (Lp(a) > 50 mg/dL, CRP > 3 mg/dL, GFR < 60 mL/min

ACS acute coronary syndrome, *CV* cardiovascular, *Lp(a)* lipoprotein(a), *CRP* C-reactive protein, *GFR* glomerular filtration rate

However, the epidemiology of the extreme CV risk and their associated biochemical markers remain largely unknown. Due to this, diagnosis is actually based on clinical characteristics (such as number of vessels involved, already occurred second CV events, atherosclerotic disease in non-coronary vessels).

Recently some new biochemical markers associated with a higher risk of subsequent CV events have been described. The most important ones are Lp(a) [10, 11], Uric Acid (UA) [12, 13] and inflammatory markers [14, 15].

2 Aims of the Research Study

Aim of our study will be to accurately evaluate the prevalence of extreme CV risk as well as its associated biomarkers (Lp(a), UA and inflammatory markers) in ACS and CCS patients. The values of CV biomarkers will be compared with two control groups: one of 1180 hypertensives and another of 765 healthy subjects (Fig. 1).

We will create two scoring systems: the first one will use only clinical variables while the second one will introduce the biochemical markers evaluated. Finally, by exome sequencing we will both evaluate polygenic risk score ability to predict recurrent events and perform Mendellian randomization (MR) analysis on CV biomarkers. In fact, genetic predisposition of the new CV biomarkers has been poorly evaluated.

3 The Study Setting: Cardiac Rehabilitation

Patients will be enrolled on the first day of their cardiac rehabilitation (CR) program after an ACS or a CCS hospitalization. Current international guidelines strongly recommended (class I-A) CR for all the subjects with a recent hospitalization for an ACS or a CCS since it demonstrated to reduce CV mortality and morbidity [16, 17].

CR programs are based on monitored exercise both with cycloergometer and physiokinesis but are not limited to this. The program is completed with diagnostic test (echocardiography, carotid doppler ultrasound, ankle brachial index, 24 h ECG monitoring, ECG stress test and biochemical evaluation) that help the physician to optimize medical therapies for secondary prevention, a nutritional and psychological interview as well as a cardiological tutorial on heart physiology and pathology and cardio-active therapies (Fig. 2).

The last point is particularly important in order to increase patients' engagements and long-term patients' compliance to pharmacological and non-pharmacological therapies. In fact, since the hospital period progressively reduced, the attention of the clinicians is focused on the resolution of the acute problem. This gives further importance to CR that needs to

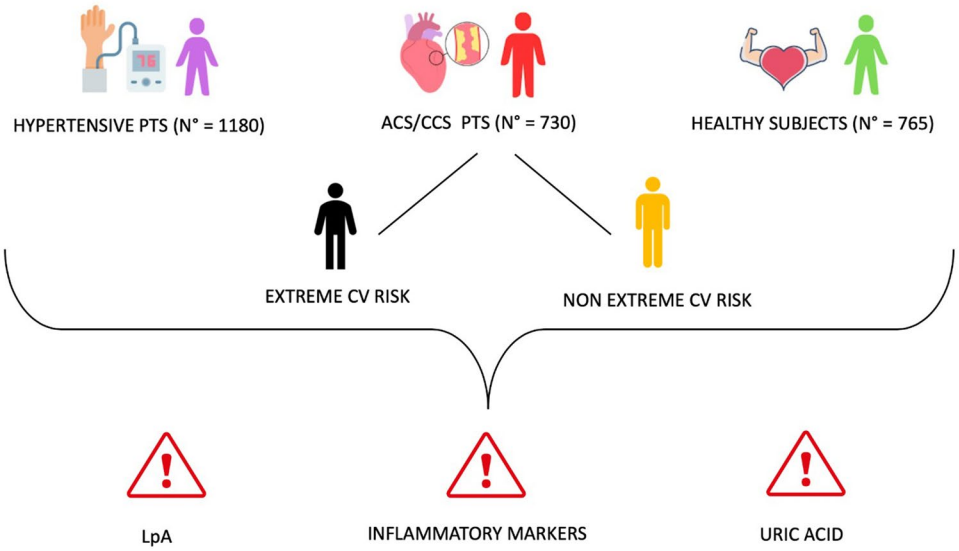

Fig. 1 Summary of study populations and evaluated biomarkers. *ACS* acute coronary syndrome, *CCS* chronic coronary syndrome, *CV* CardioVascular, *Lp(a)* lipoprotein(a)

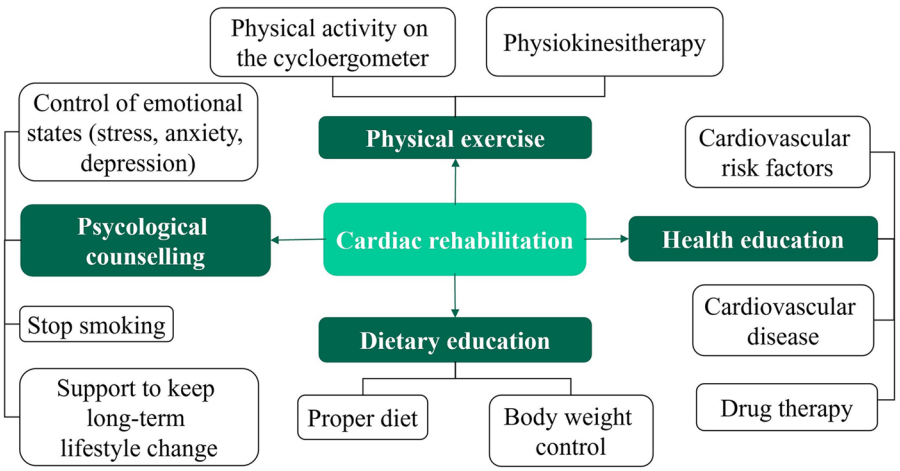

Fig. 2 Summary of a cardiac rehabilitation programs item. Obtained with permission from: Maloberti et al. [97]

be focused not only on the acute problem but on the patients as a whole. The staff is composed of figures who complement their skills: physicians, nurses, physiotherapists, dieticians and psychologists.

The CV events reduction is surely determined by the physical exercise itself but also because, through all the features previously identified, CR represents the context within which preventive strategies are introduced, tailored and refined. During CR clinicians can dedicate to a better stratification or residual CV risk in order to identify those subjects that are at extreme risk.

A patient informed on his/her risk factor and on how drugs he/she takes works and why they are important, is a patient with a higher compliance. As well known, compliance to therapies, as well to lifestyle habits, is something that progressively decreased after a CV events with significant decline already after 6 months [18, 19]. In fact, it was observed that individuals not referred to CR were less likely to receive guidelines suggested therapies (aspirin, beta-blockers, angiotensin-converting enzyme inhibitors or angiotensin receptor blockers, lipid-lowering agents and smoking cessation counseling) [20].

Summarizing, CR aims are: achievement of clinical stability, assessment and reduction of CV risk factors, reduction of the risk of subsequent CV events, improvement of functional capacity, modification of lifestyle and, even more, its long-term maintenance, consolidation of the results achieved by patients and the promotion of long-term adherence and the recovery of autonomy and independence in order to promote social and working reintegration [16].

Despite the strong guidelines indication, only half of the patients with a recent ACS participate in a similar program [21]. The overall cardiologists' referral rate to cardiac rehabilitation (56%) was far lower than what is expected and indicated by cardiological guidelines, suggesting that physician awareness about the benefits of CR is still low [22]. The

most referred patients are the frailest one such as those with previous CV events, uncontrolled risk factors of the need for CV therapies optimization. For this reason, we expect to find higher prevalence of extreme CV risk than actually believed when systematically assessed in patients undergoing CR [5].

4 Populations

Three Italian Hospital will be involved in patients enrolment: Niguarda Hospital (Milan, Outpatients Cardiac Rehabilitation Unit, Cardiology 4), Federico II University Hospital (Naples, Inpatients Cardiac Rehabilitation Unit) and S. Anna e S. Sebastiano Hospital (Caserta, Outpatients Cardiac Rehabilitation Unit, Cardiology Unit).

In the three hospitals, nearly 1200 patients/year are hospitalized for an ACS and a similar number for a CCS. 600 patients/year begin a CR program every year in the three hospitals (200 patients/year for the Niguarda Hospital, 100 patients/year for the Federico II University Hospital and 300 patients/years for the S. Anna e S. Sebastiano Hospital). Considering a retention rate of 90% (10% drop-out from rehabilitation program) and an enrolling proportion of 90%, we expect to enrol about 486 patients/year. A total enrolment period of 18 months is expected with a total of about 730 recruited subjects.

Inclusion criteria will be: (1) recent (< 12 months) hospitalization for an ACS (ST elevated myocardial infarction, non-ST elevated myocardial infarction and unstable angina) or a CCS leading to coronary revascularization; (2) being recruited in a CR program (both inpatients or outpatients); (3) being enrolled at least two weeks after the ACS (in order to exclude changes in biochemical risk factors determined by the acute phase of the disease); (4) signed informed consent.

Exclusion criteria will be: (1) inability (mainly due to orthopedic condition) to participate in a cardiac rehabilitation program; (2) active tumor or any other condition (as defined by the enrolling physician) that is able to determine a reduced life expectancy of less than 2 years; (3) being enlisted for Heart Transplantation; (4) dementia or mild cognitive impairment; (4) inability to understand the study protocol or to sign the informed consent.

Despite the new definitions recently proposed, only the older but most accepted one (patients that experienced two CV events within a period of two years) will be used for the retrospective definition of extreme CV risk in this study [5].

This definition was chosen, among the different one listed in the 2022 ESC consensus on extreme CV risk [5], because it was already described in the 2019 ESC guidelines on lipids management [4] and it's the most accepted one in clinical practice. The other new definitions are mainly based on consensus between expert and need further evaluation in clinical studies. However, sensitivity analysis (see statistical analysis section) will be done with the other definitions.

The following CV events will be considered: (1) any previous ACS (ST elevated myocardial infarction, non-ST elevated myocardial infarction and unstable angina); (2) any previous coronary revascularization both as percutaneous intervention and as coronary artery by-pass grafting (both programmed hospitalization and access from the emergency department will be considered); (3) any previous stroke or transient ischemic attack (lacunar infarction or positive imaging for cerebrovascular events, such as identification of ischemic area on computer tomography or magnetic resonance, will not be considered as a CV event since its dating is impossible without the contemporary insurgence of clinical neurological symptoms); (4) Any previous peripheral artery disease revascularization (thromboendoarterectomy or stenting) both at lower extremity and/or at the carotid levels; (5) any previous acute lower limb events (ischemia, arterial occlusion and artery to artery embolization).

Data collection will be obtained from patients interviews and from discharge letters as well as Electronic health records (EHRs) of the hospitals involved.

The CV biomarkers values found in the ACS/CCS population will be compared with two historical cohort control groups: one of 1180 hypertensives and one of 765 healthy subjects (blood donor). This comparison is important in order to assess prevalence/values differences in progressively higher CV risk groups. We will expect to find very low values in blood donors and very high ones in the ACS patients (particularly in those with extreme CV risk) with intermediate values in hypertensives. These subjects have been evaluated between 2006 and 2012 and they are both deeply phenotyped with demographic, biometric, blood pressure, biochemistry (total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, glucose, creatinine and estimated filtration rate), classic CV risk factor (hypertension, dyslipidaemia, diabetes mellitus and smoking) and treatment data (lipid lowering therapies, anti-hypertensive therapies, beta blockers and anti-diabetic drugs). Their specific inclusions and exclusions criteria can be found in previous publication [23]. Their plasma samples are already available through the relative biobank.

5 Evaluated Biomarkers

New biochemical CV risk factors assessed will be Lp(a), UA and inflammation markers including interleukin-6 (IL-6) and -18 (IL18), Tumor-Necrosis Factor α (TNF-α), C-Reactive Protein (CRP), calprotectin and osteoprotegerin). They have been selected by literature review being strongly related to future recurrent CV events in patients that already experienced an ACS.

5.1 Lipoprotein(a)

Lp(a) is a strong risk factor for CV events consisting of a polymorphic glycoprotein apo(a) covalently linked to an apoB100-containing lipoprotein [10]. It is synthesized in the hepatocyte and secreted into the plasma while clearance is determined by liver and kidney [24]. The relationship between Lp(a) and CV events has been well established in epidemiological and GWAS studies [25, 26] and it is considered the strongest single genetic risk factor known for CV diseases [27]. Lp(a) have been linked to atherosclerosis in all the vessels district (coronary, cerebral and peripheral arterial disease—PAD). In fact, a recent MR study found that a 2-fold higher level of genetically determined Lp(a) is associated with a 22% increase of myocardial infarction risk [28]. Regarding cerebrovascular events, an elevated Lp(a) caused a 1.97-fold increase in the risk of stroke [29]. Finally, Lp(a) levels correlate with new peripheral lesion, repeated peripheral artery revascularization and major amputation in a population with PAD [30–32]. However, to the best of our knowledge, no study evaluates if elevated Lp(a) levels determined a higher probability of a multivessels atherosclerotic localization. Particularly of interest for our study Lp(a) have been associated with early CV events recurrence [33, 34].

Lp(a) can determine vascular damage through three mechanisms: pro-inflammatory, pro-atherogenic and pro-thrombotic. Lp(a) is more atherogenic than LDL because of apo(a) presence that, with its lysine-binding sites bind to the endothelium and infiltrate the vessel. It is pro-inflammatory since up-regulates adhesion molecules, stimulates the proliferation of smooth muscle cells [35] and, inside the plaque, oxidize creating a highly pro-inflammatory oxidized complex [36]. In addition, in presence of high Lp(a) levels, monocytes have a greater production of pro-inflammatory cytokines and present an enhanced penetration capacity through the arterial wall. This determines foam cells formation, cellular apoptosis, acceleration and enlargement of the necrotic core [37, 38]. Lp(a) pro-thrombotic effects are determined by the reduction of plasminogen activation and fibrine degradation, the increase in Plasminogen Activator Inhibitor-1 expression and the activation of the tissue factor pathway inhibitor, altogether resulting into a more intense platelet activation and thrombus formation [10].

5.2 Uric Acid

UA has been identified from latest guidelines as a biomarker able to reclassify high risk patients [39, 40] and its role as a prognostic marker in ACS patients has been clearly defined [32]. The relevance of UA in CV diseases is dramatically growing also outside gouty patients. In fact, the association of gout with CV and renal diseases is definitively established and the research moves on the topic of asymptomatic (not gouty) hyperuricemia. In these patients, UA levels lower than the conventional cut-off (6 mg/dL for females and 7 mg/dL for males) have been associated with death [41], ACS/CCS [42], heart failure [43] and stroke [44]. This suggests that the mechanisms through which UA determines CV events are not only related to crystal tissue deposition (as it is in gout) but also to inflammation, oxidative stress, association with other CV risk factors and with target organ damage. In fact, several epidemiological studies have reported a relationship between UA and traditional CV risk factors, including hypertension, dyslipidemia and diabetes mellitus, suggesting a possible pathophysiological link between these conditions [45–48] and also the possibility to include UA into the definition of metabolic syndrome.

Interestingly for our study, UA has been associated to CV events recurrence [49].

5.3 Inflammation Markers

Inflammatory processes play an important role in the pathogenesis of ACS/CCS and other CV events (stroke and PAD) [50–52], and the number of papers investigating inflammatory biomarkers in CV disease progressively increased in recent years.

The development of atherosclerosis is characterised by a chronic, low-grade inflammatory process.

Cytokine-mediated inflammation accompanies atherosclerosis from its initiation (plaque formation and progression as also seen in the Lp(a) paragraph) to the occurrence of clinical endpoints (plaque rupture). Acute phase response biomarkers (CRP first among all) have been significantly associated with CV events but they are only the latest part of the inflammatory process (downstream markers) [51, 53]. Researchers move upward in order to find (and eventually act on) which molecules really are involved into the inflammatory pathways of the atherosclerotic process (that can thereafter be evaluated with downstream markers) [54].

As already mentioned, among the hundreds of inflammatory biomarkers we have choose the one that has been already related to CV events recurrence.

IL-6, IL-18 and TNF-α are upstream regulators of inflammatory processes. In particular, IL-6 is produced by dendritic cell, fibroblast, macrophages, monocyte and type 2 T-Helper cell with the aim of induce the differentiation of B cells in plasma-cells, induce acute phase reaction biomarkers and promote T-cell proliferation. IL-18 is produced by monocytes, macrophages and dendritic cells in order to induce interferon-γ production and stimulate natural killer cells. Finally, TNF-α is produced by B-cells, dendritic cell, macrophages, mast cell, monocyte, natural killer cell and T-helper cell with the aim of inducing other cytokine production, stimulating E-selectin expression on endothelial cell and activate macrophages.

All of them have been found in human plaques and may play roles in plaque progression and rupture since they have been associated with ACS [52–58]. Furthermore, and more interestingly for our study, they have been associated with CV events recurrence [59–64].

Calprotectin is also known as S100A8/A9 complex or myeloid-related protein-8/14 and is an acute phase protein, mainly secreted by neutrophils [65]. Elevated levels of calprotectin have been reported in several chronic inflammatory conditions such as rheumatoid arthritis, systemic lupus erythematosus, cystic fibrosis, psoriasis, and inflammatory bowel diseases [66, 67]. In addition to its role in the modulation of inflammation, leukocyte trafficking, apoptosis, and immune response emerging evidence suggests that calprotectin may be implicated in the pathogenesis of CV disease. A number of studies have demonstrated elevated levels of calprotectin in patients with ACS, both in the systemic circulation and in atherosclerotic plaques [68–72] and it was found that adding it to the Framingham score determined a significantly better risk stratification for subsequent CV events [73]. Also in this case it is associated to recurrence in patients that already had a first CV events [74, 75].

Finally, osteoprotegerin is a member of the TNF family regulating the calcification process inhibiting osteoclastogenesis. For many years vascular calcification (an important phase of the atherosclerotic process) was thought to be an unregulated and degenerative process [76]. However, recently a role for osteoprotegerin in regulating this process have been found. In fact, it is secreted also in the heart and vessels and it has higher plasmatic values in ACS [77] and CCS patients [78] as well as a correlation with CV events recurrence [79, 80].

5.4 Biomarkers Summary

Although some papers have been already published with these biomarkers, their relationship with extreme CV risk as well as the prevalence of high values in these subjects and its predictive role for relapse of CV events it is still under evaluation.

Lp(a) will be considered high if > 70 mg/dL while for UA both the classic cut-off (6/7 mg/dL for females and males) and the newly described CV cut-off (5.1/5.6 mg/dL for females and males) will be used [39]. For the inflammatory markers, no validated cut-offs are present in literature and they will be analysed only as continuous variables, as recommended by the Clinical Laboratory Standards Institute (CLSI) [81].

The plasma sample will be drawn on the first day of the CR and, to minimize the analytical variability, all the biochemical analysis will be performed at the biochemical laboratory of the Niguarda Hospital (leading operating unit) using the same analyser. [82].

All the three operating units will collect blood locally and they will centrifuged all the samples at 3000 rpm, in order to obtain plasma that will be sent to the central laboratory for biochemical evaluation in a single analytical instrument.

For the determination of serum value of UA, LpA, CRP and IL-6, the samples will be analysed by a Roche Cobas 8000 system. In particular, UA will be evaluated through enzymatic colorimetric method (uricase/perossidasis) while for LpA and calprotectin we will use immunoturbidimetric method. IL-6 will be evaluated through sandwich immunologic assay and CRP through latex particle immunoturbidimetric method.

IL-18, TNF-α, serum calprotectin and osteoprotegerin will be evaluated with ELISA methods, reading sample assorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

As already mentioned, only patients that begin CR at least 2 weeks after the index ACS/CCS will be included in order to exclude markers changes related to the acute phase of the myocardial infarction or revascularization.

6 Statistical Analysis

With the planned sample size of 730 patients, expecting an observed proportion of patients with extreme CV risk around 10%, the variability of the estimated prevalence (semi-amplitude of the 95% confidence interval) would be small (around 2.2%). Even in the extreme case of an observed prevalence of 20%, this error would remain smaller than 3%.

Sample size is also adequate with reference to the biomarkers analysis. Considering that a two-sided T-test with 1% level of significance would reach a 90% power to detect a small difference (i.e. Cohen s d=0.2) in the mean of a possible marker in each pairwise comparison between groups. Finally, for the purpose of building a predictive model for extreme CV risk, considering that we expect at least 10% events among 730 study subjects, it will be possible to include an adequate number of covariates in the final model (up to 7 or 8), according to the ten-events-per-variable rule [83].

Descriptive statistics will be calculated as means (standard deviation) or median (first and third quartile) for continuous variables and as absolute frequencies and relative frequencies for categorical variables. The proportion of patients at extreme CV risk will be estimated and the 95% confidence interval according to Wilson score formula will be calculated.

The distribution of all candidate CV biomarkers will be described and compared among 3 groups (ACS patients vs hypertensives vs healthy subjects) and among 4 groups (ACS patients with extreme CV risk vs ACS patients without extreme CV risk vs the 2 controls groups). Markers

will be considered as continuous variables and univariate association with disease groups will be performed using one-way ANOVA or Kruskal–Wallis test for non-normally distributed markers. Post-hoc tests (i.e. Tukey's HSD test or Mann–Whitney test with Holm correction) will be performed for markers with a different distribution in ACS groups with respect to controls. For markers with established cut-off, the proportion of patients with high level will be compared using Chi-square test. The results of these analyses will guide the choice of candidate factors to be included as covariates in the final predictive model for extreme CV risk. Multivariable logistic regression will be used to create two models to predict the probability of being at extreme CV risk: a basic model, including as covariates only clinical variables, and an enhanced model, including new biochemical potential CV markers on top of clinical variables. Variable selection will be performed using LASSO or Elastic Net penalization methods but also considering results of univariable logistic models as well as existing clinical knowledge [84]. The association of each variable with the condition of extreme CV risk will be expressed as Odds ratios (OR) with 95% confidence intervals. The functional form of continuous variables will be examined using splines and relevant interactions between variables will be tested. Both the basic and the enhanced model will be used to build a predictive score for the condition of extreme CV risk. Calibration and discrimination ability of both scores will be assessed using calibration plots and ROC curves, respectively. Further, the area under the curve (AUC) index will be calculated on the original sample and after an internal validation procedure (10-fold cross-validation) to correct for the possible overfitting. Finally, the difference between the internally validated AUC of the two models will be used as a measure of the performance improvement provided by the CV biomarkers.

The whole sample results will be repeated in pre-specified sub-population analysis: ACS only sub-group, CCS only sub-group and stratified by gender. Further sensitivity analysis will be conducted looking at differences in results based on the use of the different definitions of extreme CV risk category as listed in the 2022 ESC consensus [5].

All the analyses will be performed using R software. The performance of risk scores will be evaluated using "riskRegression" package [85].

7 Polygenic Risk Score

In order to assess the additional value of integrating genetic risk evaluation in extreme CV risk stratification, an established polygenic risk score (PRS) will be applied to the population with ACS and its incremental benefit in helping predict early recurrent ischaemic events will be evaluated.

First, multiple parallel sequencing will be used for genotyping of specific SNPs that will be used to calculate a coronary artery disease PRS, PGS000010, for all study participants. Details on the construction of this score have been previously published [86]. Further information and score weight files are available in PGSCatalog. The PRS will be calculated for all participants by taking the product of the risk allele counts and the risk allele weights at each of the loci included in the PRS, and then summing across all loci. This calculation will be implemented using the pgsc_calc [87–89] bioinformatic analysis pipeline.

In addition to the already described two primary models, the calculated PRS will be inserted into a third phenotypic and genetic model. The difference between the internally validated AUC of this further enhanced model will be used as a measure of the performance improvement provided by adding the PRS for clinical risk stratification.

8 Mendelian Randomization

The causal relevance of any novel biomarkers identified as associated with development of extreme CV risk will be evaluated using two-sample MR [90]. This will be carried out using existing, publicly available summary statistics of Genome Wide Association Studies (GWAS). Uncorrelated genome-wide significant markers for each of the exposures will be extracted from publicly available summary statistics. Corresponding information for all SNPs will then be extracted from the largest available GWAS on the outcome of coronary artery disease [91]. After harmonization and clumping of SNPs (at linkage disequilibrium threshold 0.001) inverse-variance weighted MR will be conducted. Sensitivity analyses with MR-egger and Weighted median MR will be conducted [92, 93] if sufficient instruments are available to carry out these analyses. This will be performed using the Mendelianrandomization and TwoSampleMR packages in R [94–96].

9 Conclusions

In conclusion, identification of the extreme CV risk patients is an important unmet clinical need in the field of secondary CV prevention. An early and more accurate diagnose of extreme CV risk will permit to carry out a more intensive treatment optimization in these patients probably leading to a reduction of future ACS recurrence.

With the present protocol we want to give definitive data on the prevalence of extreme CV risk in recently hospitalized ACS/CCS patients enrolled in CR programs and to evaluate the association of this condition with Lp(a), UA and inflammation comparing them with a group of hypertensive

subjects and one of blood donor (Fig. 1). Furthermore, in the ACS/CCS group, genetic SNP on loci found to be associated with coronary artery disease will be analysed in order to assess genetic predisposition to extreme CV risk.

From these data, we will derive three different scores system (phenotype only, biomarkers score and genetic score) that will help clinicians identifying patients at extreme CV risk. The first one will be of significance because it will not need any other assessment other than what is already done in clinical practice and will simplify the diagnosis of extreme CV risk in ACS/CCS patients. The second and the third score will give physician the opportunity to increase the diagnostic capability for identification of these subjects. The strength of our results will be further reinforced by the MR analysis.

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Data availability Data will be provided on resonable request to the corrisponding authors.

Declarations

Conflict of interest The authors declare they have no conflict of interest.

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