METHODS

Study Participants

We included all individuals free of clinically recognized cardiovascular and cancer disease, recruited in the *MOLI-SANI* study (24-30). Briefly, the *MOLI-SANI* project is a cohort study, started in March 2005. Participants, men and women aged ≥35, living in Molise, a Southern Italian region, were randomly recruited from city-hall registries. Up to April 2010 24,325 subjects were recruited at baseline. Exclusion criteria were pregnancy at the time of recruitment, disturbances in understanding or willingness, current poly-traumas or coma, or refusal to sign the informed consent. We excluded participants with a previous personal history of cardiovascular disease (heart attack, angina, stroke, transient ischemic attack, coronary bypass, angioplasty, or other cardiovascular surgery, 6%) or cancer (3%), those with missing value for D-dimer or other relevant variables (14%) or lost at follow-up (5%). The final study sample consisted of 17,639 individuals. The mean age of subjects was 55±11 years and 47% were men. The cohort was followed-up for mortality for any cause for a median of 4.2 years (max 6.5 y). Follow-up based on the mortality regional registry was from the baseline examination until death or 31 December 2011 for persons staying alive.

The ascertainment of vital status was carried out through linkage with demographic rosters, to identify the date of death. For deceased subjects, families were asked to confirm and about cause of death. Moreover, death certificates were retrieved; these report the initial and underlying causes of death and were coded according to the International Classification of Diseases, ninth Revision (ICD-9). In-hospital death were also checked through regional hospital discharge databases by record linkage to the MOLI-SANI database.

On 31/12/2011, in the whole MOLI-SANI cohort (n=24,325 subjects) a total of n=596 deaths were recorded. The number of deaths in the sample (n=17,639) included in the present study was 280.

The *MOLI-SANI* study complies with the Declaration of Helsinki and was approved by the Catholic University ethical committee. All participants enrolled provided written informed consent.

Risk Factor Ascertainment

Cigarette smoking was defined by self-report of currently smoking every day or some days and having smoked >100 cigarettes in their lifetime. Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared. Socio-economic status was defined by a score based on 6 variables (education, housing, ratio between the number of live-in partners and the number of rooms - both current and in the childhood - and availability of hot water at home in the childhood); the highest the score, the highest the level of socio-economic status. Physical activity was assessed by a structured questionnaire and expressed as

daily energy expenditure in metabolic equivalent task-hours (MET-h). Pre-hypertensive status was defined if diastolic or systolic pressure ranged in 90-95/140-160 mmHg; hypertensive status was defined if diastolic or systolic pressure was ≥95 or ≥160 mmHg or by the use of anti-hypertensive medications. Pre-diabetic status was defined if glucose level ranged in 110-126 mg/dL; diabetic status was defined if glucose level was ≥126 mg/dL or by the use of relevant medications. Pre-hypercolesterolemia status was defined if total cholesterol level ranged in 200-240 mg/dL; hypercolesterolemia status was defined if total cholesterol level was ≥240 or by the use of relevant medications. Blood pressure was measured by an automatic device (OMRON-HEM-705CP) three times on the non-dominant arm, with the patient lying down for about 5 minutes. Serum lipids and blood glucose were assayed by enzymatic reaction methods using an automatic analyzer (ILab-350). LDL-cholesterol was calculated according to the Friedewald formula. White blood cells (WBC) were counted by a Coulter LH Hematology analyzer (Becker-Coulter, Brea, CA). High sensitivity (hs) CRP was measured in fresh serum samples within 3 hrs from collection. A latex particle enhanced immunoturbidimetric assay (IL-Coagulation-Systems ACL9000, IL, Milan, Italy) was used. D-dimer was measured on fresh citrated plasma by an automated latex-enhanced immunoassay (HemosIL-IL, Milan). Quality control was maintained using internal laboratory standard in-house plasma pool. Inter and intra-day variability coefficients were 5.4% and 7.6%, respectively.

Statistical Methods

Baseline characteristics were presented as numbers and percentage, or mean values and standard deviation or geometric means and 95% confidence interval (for triglycerides, glucose, C-reactive protein and white blood cell). The baseline associations between quartiles of D-dimer and baseline covariates (means or percents) were assessed and adjusted for age and sex using linear models. Hazard ratios of death for D-dimer, modelled as quartiles or continuous variables, were calculated using the following Cox proportional hazard models: (1) crude, (2) adjusted for baseline age and sex and (3) additionally adjusted for other risk factors: baseline body mass index, hypertensive, diabetics and dyslipidemic status (no, pre, yes; modelled by dummies variables with no as referent), usual alcohol intake (gr/day), smoking status (current, ex, never), exercise (MET-h/wk, continuous), and score of social status (continuous). Another model added CRP and WBC. In supplemental analyses, we also modelled D-dimer as a continuous variable on its natural scale and as an ordinal variable with values of 0, 1, 2 and 3 representing successive quartile groups. The data analysis was generated using SAS/STAT software, Version 9.1.3 of the SAS System for Windows©2009. SAS Institute Inc. and SAS are registered trademarks of SAS Institute Inc., Cary, NC, USA.

APPENDIX

Moli-sani project Investigators

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