A score of low-grade inflammation and risk of mortality: prospective findings from the Moli-sani study

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The Moli-sani study

The Moli-sani study is a prospective cohort study of men and women randomly recruited from the general population of a Southern Italian region with the purpose of investigating genetic and environmental risk factors in the onset of cardiovascular, cerebrovascular and tumour diseases[1]. The cohort of the Moli-sani study was recruited from about 200,000 persons, aged ×35 years, resident in the Molise region. Participants were recruited from city-hall registries by a multistage sampling. Exclusion criteria were pregnancy at the time of recruitment, disturbances in understanding or willingness, current poly-traumas or coma, refusal to sign the Informed Consent form. All subjects identified were sent a letter inviting them to participate in the project, followed a few days later by a phone call. From March 2005 to April 2010, 24,325 subjects were enrolled. The cohort was followed up with the main outcome of interest being all-cause mortality until May 2015. All-cause death was assessed by linkage with Offices of vital statistics of the Molise region. The MOLI-SANI study complies with the Declaration of Helsinki. All participants provided written informed consent.

Inflammatory biomarkers measurement

All blood samples were obtained from participants who had fasted overnight and had refrained from smoking for at least 6 h. Serum lipids and blood glucose were assayed by enzymatic reaction methods using an automatic analyzer (ILab 350, Instrumentation Laboratory (IL), Milan, Italy). LDL-cholesterol was calculated according to Friedewald [2].

High sensitivity CRP was measured in fresh serum samples by a particle-enhanced immunoturbidimetric assay (IL-Coagulation-Systems ACL9000, IL, Milan, Italy). Quality control for CRP was maintained using in-house serum pool and internal laboratory standard at 1.5 mg/L; inter-day coefficients of variability for CRP were 5.5% and 4.2% , respectively. Hemocromocytometric analysis was performed by cell count (Coulter HMX, Backman Coulter, IL Milan, Italy) within 3 h from blood collection. In our population, the correlation between granulocyte and neutrophil counts is extremely high as expected (Spearman correlation index r = 0.99; p <.0001) thus we used granulocyte as proxy of neutrophils for which we do not have complete data for the whole population and then calculated the ratio of granulocyte (%) to lymphocyte (%) as a proxy of the more used neutrophil to lymphocyte ratio (NLR).

Definition of risk factors

History of cardiovascular disease (including angina, myocardial infarction, revascularisation procedures and cerebrovascular events) and heart failure were self-reported and confirmed by

medical record and therapy. History of cancer, haematological diseases and hepatitis B or C were self-reported.

Hypertension was defined as systolic blood pressure >140 mm Hg or diastolic blood pressure >90 mm Hg or treatment for hypertension . Hypercholesterolemia was defined if total cholesterol >240 mg/dl or by use of specific medication. Diabetes was defined as blood glucose >126 mg/dl or by use of specific pharmacological treatment.

The ratio between waist and hip (WH ratio) circumferences was used to assess body weight distribution and expressed either as a continuous variable or as quartiles. For sensitivity analysis, WH ratio was dichotomized according to sex-specific cut-offs (>0.85 for women and >0.9 for men)[3]. Subjects were classified as never-smokers, current smokers or ex-smokers (quitting from at least 1 year). Leisure-time physical activity was assessed by a structured questionnaire [4] (questions on walking, gardening and sport participation) and expressed as daily energy expenditure in metabolic equivalent task-hours (MET/d). Education was based on the highest qualification attained and was categorized as low (up to lower secondary school; approximately Ö8 years of study) or high (upper secondary education or higher; approximately > 8 years of study).

Dietary information was collected by the validated Italian version of the EPIC food frequency questionnaire [5,6]; fruits and vegetables intake (gr/day) and energy intake (Kcal/day) were expressed both as continuous variables and quartiles.

Serum creatinine levels were used as a marker of renal function and determined in the frame of the BiomarCaRE project[7].

Data analyses

For each variable (continuous or categorical) with missing data, a dummy variable was created to indicate whether or not data are missing on that variable. All such dummy variables are included in the analyses. Subjects with missing data for continuous variables were coded as having the population mean value on that variable.

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.

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