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Combination of biomarkers to predict mortality in elderly patients with myocardial infarction

Fabiola Olivieri [a,b,1,*], Liana Spazzafumo [a,1], Roberto Antonicelli [a], Francesca Marchegiani [a], Maurizio Cardelli [a], Cristina Sirolla [a], Roberta Galeazzi [a], Simona Giovagnetti [a], Eugenio Mocchegiani [a], Claudio Franceschi [a,c,d]

[a] *Center of Genetic and Molecular Biology, Cardiology-CCU Unit, Center of Statistics, Center of Immunology and Nutrigenomics, Center of Citology, Italian National Research Centers on Aging (I.N.R.C.A.), Ancona, Italy*
[b] *Department of Cellular Pathology and Innovative Therapy, Faculty of Medicine, Polytechnic University of Marche, Ancona, Italy*
[c] *CIG, Interdipartimental Centre "L. Galvani", University of Bologna, Bologna, Italy*
[d] *Department of Experimental Pathology, Bologna University, Bologna, Italy*

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Abstract

Objective: The elderly subjects affected by Acute Myocardial Infarction (AMI) have the highest risk of mortality. Our study was designed to improve the capability of mortality risk stratification in elderly AMI patients through the concurrent evaluations of different biomarkers, including genetic markers.

Methods and results: One-year follow-up study was performed in 250 elderly AMI patients. The combination of high total Homocysteine (tHcy), low folate and vitamin B12 plasma levels (18.0 ± 9.0 μmol/l; 4.4 ± 1.2 ng/ml; 404.2 ± 287.5 pg/ml, respectively) and elevated CRP plasma levels (≥6 mg/dl) identify the highest-risk pathway of heart mortality (RR = 4.20, IC 95% 1.62–10.89, $P < 0.002$) with respect to the combination of low total tHcy, high folate and vitamin B12 plasma levels (12.4 ± 5.2 μmol/l; 8.9 ± 2.5 ng/ml; 546.9 ± 379.8 pg/ml, respectively) and low CRP plasma levels (<6 mg/dl).

Conclusion: In elderly AMI patients the concomitant elevation of CRP and tHcy, associated with folate and vitamin B12 low levels, could be considered a significant predictive heart mortality risk factor.

Keywords: Decision-tree analysis; Homocysteine; CRP; Acute myocardial infarction; Elderly cardiovascular patients

1. Introduction

With the increase of the human life-span and the growth of the elderly as population, subjects aged more than 65 years, represent one of the largest part of patients affected by acute myocardial infarction (AMI) with an high risk of mortality (Stork et al., 2006). As a consequence, the need to more precisely stratify mortality within the group of the elderly has grown as well (Pearte et al., 2006). It is well known that in very elderly patients some tests commonly performed for the cardiovascular mortality risk assessment, like exercise stress test or miocardioscintigraphy, are not always feasible and reliable (Laupacis et al., 1990; Fletcher et al., 2006). Moreover in very elderly patients, more than in other age classes, the improvement of risk stratification is necessary to prescribe a more or less aggressive treatment for individual patients and to reduce and rationalize the costs of each patient's management (Stork et al., 2006; Pearson et al., 2003). Nevertheless, ways to predict the risk of cardiovascular events or all-cause mortality have largely been derived from populations in which very old subjects were usually underrepresented (Stork et al., 2006). Although >80% of annual coronary heart disease (CHD) deaths occur in adults aged >65 years and the population is aging rapidly, CHD event fatality and its predictors in the elderly have not been well described (Pearte et al., 2006). As a

* Corresponding author at: Department of Research, I.N.R.C.A. National Institute, Via Birarelli, 8, 60121 Ancona, Italy. Tel.: +39 071 8004322; fax: +39 071 206791.
E-mail address: b.molecolare@inrca.it (F. Olivieri).
[1] The authors contributed equally to the manuscript.

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consequence, the conventional risk scores for the mortality risk prediction seem to determine a misclassification of middle-aged and older subjects (Pearte et al., 2006; Pearson et al., 2003).

Moreover, if there are multiple markers affected by the cardiovascular disease which are not strongly correlated with one other, a composite index combining these markers may provide a much more robust indication of the disease prognosis (Anderson, 2005). Some scientific evidences demonstrate the possibility of improving the stratification of cardiovascular mortality risk in elderly patients through the concurrent evaluation of multiple biomarkers, even if only preliminary data were reported (Beckman et al., 2005; Lee et al., 2006; Cummings et al., 2006). The most promising biomarkers include C Reactive Protein (CRP), total Homocysteine (tHcy), folic acid, B12 vitamin, IL-6, TNF-alpha, brain natriuretic peptide and fibrinogen serum levels (Gori et al., 2005; Weikert et al., 2005; Tuomisto et al., 2006; Foussas et al., 2005; Bonaa et al., 2006; Girelli et al., 2006; Giacconi et al., 2004, 2006; Rossi et al., 2006; Palazzuoli et al., 2006). Also the genetic markers located in the IL-6, TNF-alpha, MTHFR and CRP genes, fulfil most of the requirements needed to serve as a new cardiovascular risk factor (CRF), but still several issues await further confirmation and clarification before these markers can ultimately be included in the routine risk profile (Roest et al., 2001; Antonicelli et al., 2005a,b; Lange et al., 2006; Zee et al., 2007).

Therefore, although current US guidelines recommend consideration of some of these new markers measurement as part of routine individual risk factor screening and management in cardiovascular prevention, several international commentators have not yet approved these recommendations suggesting that such markers do not add significance to the established predictive value of current clinical scores, such as the Framingham score (Lowe, 2005, 2006). Moreover, the finding of a significant association of some cardiovascular biomarkers (such as CRP and IL-6) with all cause death provided some evidences that elevated levels of such markers may reflect the cumulative effects of an adverse environment on the non specific inflammatory response (Lowe, 2005).

Taking into account that there are currently conflicting evidences to support the measurement of new biomarkers as part of routine cardiovascular risk assessment in clinical practice and that the conventional mortality risk scores could induce a misclassification of old subjects, we was aimed to verify, in a sample of elderly AMI patients, the predictive power of different biomarkers, including some genetics markers, on cardiac mortality. Multivariate model, such as the decision-tree analysis, was chosen to identify important interaction between predictors and quantify their relative contribution to mortality risk.

2. Methods

2.1. Sample

250 AMI patients (150 males and 100 females, age range: 65–99 years, mean age 80 ± 8 years) consecutively admitted (during 1 year) to the Hospital Coronary Care Unit (CCU) of Italian National Research Center on Aging (I.N.R.C.A.) were enrolled in our study and were followed for 1-year period. 62 patients were affected by a STEMI and 188 by a NSTEMI. The patients were considered eligible if they fulfilled the diagnostic criteria for AMI according to European Society of Cardiology (ESC) guidelines (Bertrand et al., 2002). The diagnosis of AMI was confirmed in all patients, with conventional instrumental examinations. All subjects gave their informed consent to the study, which was approved by the Ethics Committee of the I.N.R.C.A. Institute.

Exclusion criteria were: renal failure, severe anemia, cancer, or life expectancy less than 12 months for other severe diseases, such as end stage respiratory failure, cachexia or neurological diseases. 25 of consecutively AMI patients admitted at the I.N.R.C.A. Coronary Care Unit of Ancona were excluded by the present study because they presented some of the exclusion criteria. Three of the excluded subjects were affected by renal failure. Moreover, on admission, a detailed history was elicited with regard to previous cancer without recurrence in the last 10 years (e.g., skin cancer or breast cancer), drugs used and presence of classical CRF, such as hypercholesterolemia, arterial hypertension, type 2 diabetes, presence of CHD history and smoking habit.

Hypercholesterolemia was defined by cholesterol levels >200 mg/dl and low level of HDL cholesterol was defined <30 mg/dl, according to WHO guideline.

The diagnosis of arterial hypertension was made according to the recent 2003 European Society of Hypertension/European Society of Cardiology Guidelines for the management of arterial hypertension, if blood pressure was repeatedly ≥140/90 mmHg, or if patients were under antihypertensive treatment (European Society of Hypertension, 2003).

Diagnosis of type 2 diabetes mellitus was based on revised American Diabetes Association diagnostic criteria (Report of the Expert Committee, 1997).

Moreover, the patients were stratified according to the presence of CHD history, if the presence of CHD was previously documented, or patients without a history of CHD, if the current AMI episode was the first CHD manifestation. Any smoking habit was assessed through a questionnaire, and each patient was coded as current or non-current smoker (non- and ex-smoker).

Moreover, at the time of the hospital admission, the following laboratory parameters were recorded: CRP, tHcy, vitamin B12, folic acid, creatine kinase, mass assay (CK-MB), Troponin I (TnI) and Mioglobin levels.

tHcy, folate and vitamin B12 values were detected by AxSYM systems (ABBOTT Diagnostic Division, USA).

The sensitivity of the AxSYM Homocysteine assay is <0.8 μmol/l. The normal range was 3.36–20.44 μmol/l.

The sensitivity of the AxSYM Folate assay is <0.9 ng/ml. The normal range was 7.2–15.4 ng/ml.

The sensitivity of the AxSYM vitamin B12 assay is <60 pg/ml. The normal range was 98.5–1.200 pg/ml.

CRP values were detected by CardioPhase hsCRP (Dade Behring Inc., USA). The sensitivity of the CRP assay is 0.01 mg/dl. The normal range was 0.03–10 mg/dl.

The pharmacological treatment of AMI patients followed the ESC Guidelines.

No patient has received folic acid or vitamin B12 supplementation.

We included in the 1-year follow-up survival analysis all the AMI patients fulfilled the inclusion criteria including patients who died during hospitalization. Cardiac causes of death were validated by an independent team of physicians.

Since one patient died of non-cardiovascular causes and in one case the clinical data were incomplete, 248 patients were included in the final survival analysis.

2.2. Genotyping MTHFR 677 C>T

An established procedure was used to genotype the MTHFR 677C>T SNP. Briefly, exon 4 was amplified using the following primers: 5′-CAA AGG CCA CCC CGA AGC and 5′-AGG ACG GTG CGG TGA GAG TG. Samples were amplified for 30 cycles, each of which consisted of denaturing at 94 °C for 30 s, annealing at 58 °C for 30 s, and extension at 72 °C for 30 s. After HinfI (New England Biolabs, Mississauga, Ontario, Canada) digestion of the resulting 245 bp fragment, the C allele yielded only a single 245 bp fragment, and the T allele yielded two fragments with sizes 176 and 69 bp. Electrophoresis in a