A New Risk Chart for Acute Myocardial Infarction by a Innovative Algoritm

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Abstract: Acute myocardial infarction (AMI) is complex disease; its pathogenesis is not completely understood and several variables are involved in the disease.. The aim of this paper was to assess: 1) the predictive capacity of Artificial Neural Networks (ANNs) in consistently distinguishing the two different conditions (AMI or control). 2) the identification of those variables with the maximal relevance for AMI. Genetic variances in inflammatory genes and clinical and classical risk factors in 149 AMI patients and 72 controls were investigated. From the data base of this case/control study 36 variables were selected. TWIST system, an evolutionary algorithm able to remove redundant and noisy information from complex data sets, selected 18 variables. Fitness, sensitivity, specificity, overall accuracy of the association of these variables with AMI risk were investigated. Our findings showed that ANNs are useful in distinguishing risk factors selectively associated with the disease. Finally, the new variable cluster, including classical and genetic risk factors, generated a new risk chart able to discriminate AMI from controls with an accuracy of 90%. This approach may be used to assess individual AMI risk in unaffected subjects with increased risk of the disease such as first relative with positive parental history of AMI.

1 INTRODUCTION

Morbidity and mortality of coronary heart disease (CHD) are high and acute myocardial infarction (AMI) is the major clinical complication of CHD (Yusuf S , 2001, Levi F, 2002).

AMI is a multi-factorial disease with a complex and incompletely defined pathogenesis. In fact, genetic, clinical and phenotypic factors are involved in the clinical history of the disease.

Knowledge regarding risk factors predisposing to AMI is still incomplete. It is known that many CHD events occur in individuals with at most one risk factor among those included in the Framingham risk assessment.

Different investigations confirmed that biomarkers of inflammation, such as increased blood homocysteine (Cummings DM, 2006), C-reactive protein (Ridker PM, 2000) and cytokine levels (Packard RR, 2008; Zhang C, 2008; Andersson J, 2010; Pamukcu B, 2010 and Biasillo G, 2010) could be considered new risk factors for CHD and AMI.

Genetic variations, represented by single nucleotide polymorphisms (SNPs) in the promoter region of several genes regulating metabolic and immune functions from case/control studies were found to be associated with an increased risk of AMI (Licastro F, 2010 and Ianni M, 2012).

Moreover, recent genome-wide association (GWA) studies have contributed to the discovery of new SNPs associated with CHD and AMI (Patel RS, 2011).

However, a single gene variant has a limited contribution to the total genetic load of AMI.. This

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situation may partially explain the contradictory results of genetic association studies using the candidate gene SNP approach in AMI (Kullo IJ, 2007; Hamsten A, 2008; Chiappelli M, 2005).

Therefore it is important to introduce new statistical methods to approach relationship of risk factors with AMI.

The Auto Contractive Map algorithm (AutoCM) has already been used to explore the concomitant presence and the associations among several genetic and phenotypic variables with AMI in a multi factor network (Licastro F, 2010). Individual phenotype biomarkers can vary widely as a function of time and may be different among subjects as result of gender, concomitant diseases, metabolic disorders, dietary intake and other environmental variables; therefore, to understand natural processes and recreate those processes may be useful to employ machine learning systems able to manage highly non linear interactions (Penco S, 2005; Lisboa PJC, 2002; Grossi E, 2007).

Artificial neural networks (ANNs) function are able to reconstruct the imprecise rules which may be underlying a complex set of data (testing) (Coppedè F, 2013). In recent years ANNs have been used successfully in medicine, for example they have been used to investigate the predictive values of risk factors related to the conversion from amnestic mild cognitive impairment to AD (Tabaton M, 2010), to differentiate fronto-temporal dementia from AD (Franceschi M, 2011) to identify genetic variants essential to differentiate sporadic amyotrophic lateral sclerosis cases from controls (Penco S, 2008; Buscema M, 2012), or maternal risk for Down syndrome child (Coppedè F, 2010).

In this pilot study we applied ANNs to investigate genetic, clinic and phenotypic markers in 149 patients with AMI and 72 healthy subjects selected from a previously described investigation (Licastro F, 2010). The present study was performed by using supervised ANNs to develop a predictive model able to distinguish AMI patients from healthy controls on the basis of classical risk factors and gene variants involved in the disease.

Moreover, TWIST algorithm (Buscema M, 2004) was applied to the genetic, clinical and phenotypic dataset in order to select relevant variables associated with AMI risk. Sensitivity and specificity of this selected group of variables in relation with AMI risk was then tested. No previous study has investigated the relationship among clinic, phenotypic and genetic polymorphisms by ANNs and TWIST in AMI.

The aim of this study was to investigate whether these innovative mathematical approaches might identify key new variables to better assess AMI risk and describe a new risk chart for the disease.

2 MATERIALS AND METHODS

2.1 Subjects

149 consecutive patients with clinical diagnosis of AMI (mean age = 71 ± 12; 70% male and 29% female) from the Cardiology Unit of Ferrara University Hospital were enrolled. Each patient met diagnostic criteria for AMI based on electrocardiography changes and standard laboratory findings confirmed by echocardiography and coronary angiography.

Controls consisted of 72 healthy subjects (mean age = 75 ± 5; 49% male and 51% female) belonging to a longitudinal population study, called "Conselice study of brain aging" (Ravaglia G, 2001) All controls did not show cardiovascular or inflammatory diseases at the beginning of the follow up (1999-2000) and were still free of these pathological conditions at the end of the follow up (2004-2005).

The research protocol was approved by relevant institutional review boards, all participants gave written and informed consent and the investigation is consistent with the principles outlined in the Declaration of Helsinki.

2.2 SNP Detection

Genomic DNA from peripheral blood leukocytes of AMI and healthy controls was obtained by a method described elsewhere (Grimaldi LM, 2000). Genetic determination of polymorphism in promoter regions of IL-1β-511 C/T (rs16944), IL-6 -174 C/G (rs=1800795), IL-10 -1082 G/A (rs1800896), TNF-α -308 G/A (rs1800629), ACT -51 G/T (rs1884082), VEGF -2578 C/A (rs699947) and HMGCR -911 C/A (rs3761740) genes was performed by Real Time-PCR method. SNP-specific primers and probes were designed according to the TaqMan genotyping assay by ABI (Foster City, CA, USA) and the assays were performed in 25 µl total volume on Stratagene MX3000P following manufacturer's instructions (Licastro F, 2010; Chiappelli M, 2005). IFNγ (rs2430561) and IL-6 (rs1800795) genotypes were assayed by Real Time-PCR using the following allele specific modified LNA primers (Latorra D, 2003).

IFN(+874)(rs2430561):
Primers F:
5'TTTATTCTTACAACACAAAATCAAATC+T-3',
5'-TTTATTCTTACAACACAAAATCAAATC+A-3',
Primer R
5'-TGTGCCTTCCTGTAGGGTATTATTA-3'
Il-6(-174)(rs1800795):
Primer F
5'-TCCCCCTAGTTGTGTCTTGC+C-3',
5'-TCCCCCTAGTTGTGTCTTGC+G-3',
Primer R
5'-AATCCCACATTTGATATAAATCTTTGT-3'

RT-PCR was performed in 96 well plates using Stratagene MX3000P platform. Reaction volume included a SYBR Green PCR Master Mix with the enzyme, Mg^{2+} and dNTPs (ABI, Fosteer City, CA, USA) PCR primers and genomic DNA (0.5ng/µl) was of 25µl . A start of 10 min at 95°C was followed by 40 cycles at 95°C for 15 s and 60°C for 60 s.

2.3 Plasma Lipid Profile Detection

Plasma levels of total cholesterol, triglycerides and HDL were measured by commercial clinical laboratory assay.

2.4 Artificial Neural Networks (ANNs)

ANNs are adaptive models for the analysis of data; these algorithms are inspired to the functioning processes of the human brain (Grossi E, 2007) and they are able to modify their internal structure in relation to a function objective. The adaptive feature is fundamental in case of complex data set in which non linearity prevails.

The ANNs are particularly suited for solving problems of the non linear type, being able to reconstruct the approximate rules put into a certain set of data. In this study, we applied supervised ANNs to data network in which the result of the processing (the output desired) is already defined. Supervised ANNs calculate an error function that measures the distance between the desired fixed output (target) and their own output, and adjust the connection strengths during the training process to minimize the result of the error function. The learning constraint of the supervised ANNs tests its own output to overlap that of a determined target. The general form of these ANNs is: $y = f(x,w*)$, where we constitutes the set of parameters which best approximate the function. The ANNs used in the study are characterized by the law of learning and topology. The laws of learning identify equations which translate the ANNs inputs into outputs, and rules by which the weights are modified to minimize the error or the internal energy of the ANN. In this study, we have used as a standard model the Back Propagation standard (BP-FF) (Rumelhart DE, 1982), belonging to a very large family of ANNs defined by different interconnected layers of nodes. These are characterized by a non linear function, which can be differentiated and limited, and has a linear combination of the activations coming from the previous layer in the input. The function is generally a sigmoid type.

The fundamental equation that characterizes the activation of a single node and therefore, the transfer of the signal from one layer to another is showed in Fig 1.

Results obtained with these ANNs have also been compared with a model of linear statistic such as, the Linear Discriminant Analysis (LDA; Software SPSS) (Tabaton M, 2010).

$$x_j^{[s]} = f\left(\sum_{i=0}^{n} w_{ji}^{[s]} \cdot x_i^{[s-1]}\right)$$

Fig 1: Single node activated value equation xj = node activation value; f = sigmoidal function; W= vector of weights arriving at j-n node; s = weights and nodes layer; xi = Input nodes at J-n node.

2.5 Twist Algorithm

TWIST (Training With Input Selection and Testing) is a new evolutionary algorithm (Buscema M, 2004) able to generate two subsets of data with a very similar probability density of distribution and with the minimal number of effective variables for pattern recognition.

Consequently, in the TWIST algorithm every individual of the genetic population will be defined by two vectors of different lengths:

1) the first one, showing which records (N) have to be stored into the subset A and which ones have to be stored into the subset B;

2) the second one, showing which inputs (M) have to be used into the two subsets and which one have to be deleted.

TWIST has already been applied to medical data base with promising results (Penco S, 2005; Tabaton M, 2010; Coppedè F, 2010; Buscema M, 2005; Lahner E, 2008; Buri L, 2010; Street ME, 2008; Buscema M, 2010; Rotondano G, 2011; Pace F, 2010). TWIST consists of a population of Multilayer Perceptrons. The "reverse strategy" used

in this algorithm tends to generate two subsets with the same probability density function, and this is exactly the gold standard of every random distribution criterion (Buscema M, 2005).

In addition, when the "reverse strategy" is applied, two fitness indicators are generated: the accuracy on the subset B after the training on the subset A, and the accuracy on the subset A after the training on the subset B. But only the lower accuracy of the two is saved as the best fitness of each individual of the genetic population rather than an average of the two or the higher of the two. This criterion increases the statistical probability that the two sub-samples are equally balanced during the genetic evolution because of the quasi logarithmic increase of the optimization process. We have also demonstrated experimentally (Buscema M, 2013) that when there is no information in a dataset, the behaviors of the TWIST algorithm, the Training and Testing Random Splitting and the K-Fold Cross Validation are absolutely equivalent. Therefore, TWIST does not code noise to reach optimistic results (Buscema M, 2013).

Each ANN has to learn a subset of the global dataset and has to be tested with another subset of the dataset in a blind way. By this application the fitness function of TWIST is re-programmed: the population of Multilayer Perceptrons is exchanged with a population of simple K Nearest Neighbour (KNN), based on Euclidean metric. Basic kNN algorithm is able to find the Euclidean similarity between two samples that is training set and testing set. So kNN is a suitable cost function for Twist optimization. ANNs for classification are applied in a second step, when the two subsamples (Training and Test set) are already defined.

This change makes TWIST faster and more oriented to discover explicit similarities between input variables and classes (AMI and controls).

2.6 Training and Testing Protocol

Training and testing validation protocols consisted of the following steps:

1) Division of the data set in to two sub-samples: subset A and subset B. In the first run subset A was used as the Training Set and the subset B as the Testing Set.
2) Application of ANN trained on the Training Set. In this phase the ANN learns to associate the input variables with those that are indicated as targets.
3) At the end of training phase all the values adaptively created by the artificial neural networks and predefined as setting parameters are freezed and kept apart for the testing phase
4) The Testing Set, which has not be seen before, was then shown to the classificator that expressed an evaluation based on the previously training; this operation was performed for each input vector; each result (output vector) was not communicated to the classificator.
5) The ANN was evaluated only in reference to the generalization ability acquired during the Training phase.
6) In a second run an identical ANN is applied to subset B which was used as training subset and then to subset A which used as a testing subset.

2.7 The Receiver-Operating Characteristic Curves (ROCs) and Areas under Curves (AUC)

Sensitivity and specificity along with positive and negative predictive values with 95 % confidence intervals for each strategy were estimated by AUC. ROCs were calculated and compared for clinical and statistical rules with a nonparametric approach using a paired design (Delong ER, 1988). Odds ratio was used for computing the association of variables with the selected outcome. Chi-squared test was used to assess the statistical significance of differences among proportions. All p values involve hypothesis tests against a two-sided alternative. Differences were considered significant at a 0.05 probability level (Buri L, 2010).

3 RESULTS

The original data set included the following variables: *Male*, Female, *Age<50 years*, Age=50 years, *Age>50 years, High BMI, Diabetes, High cholesterol, Low HDL, High triglycerides*, AMI, Controls, CC genotype IL-1ß, CT genotype IL-1ß, *TT genotype IL-1ß*, GG genotype ACT, *GT genotype ACT, TT genotype ACT, GG genotype IL-6, GC genotype IL-6, CC genotype IL-6*, CC genotype HMGCR, CA genotype HMGCR, AA genotype HMGCR, GG genotype IL-10, GA genotype IL-10, *AA genotype IL-10*, *CC genotype VEGF*, CA genotype VEGF, AA genotype VEGF, TT genotype IFN-γ, *TA genotype IFN-γ*, AA genotype IFN-γ, GG genotype TNF-α, GA genotype TNF-α, *AA genotype TNF-α*.

The application of the TWIST algorithm allowed the selection of 18 variables (*italic variables*) from a previously established larger data set with 36

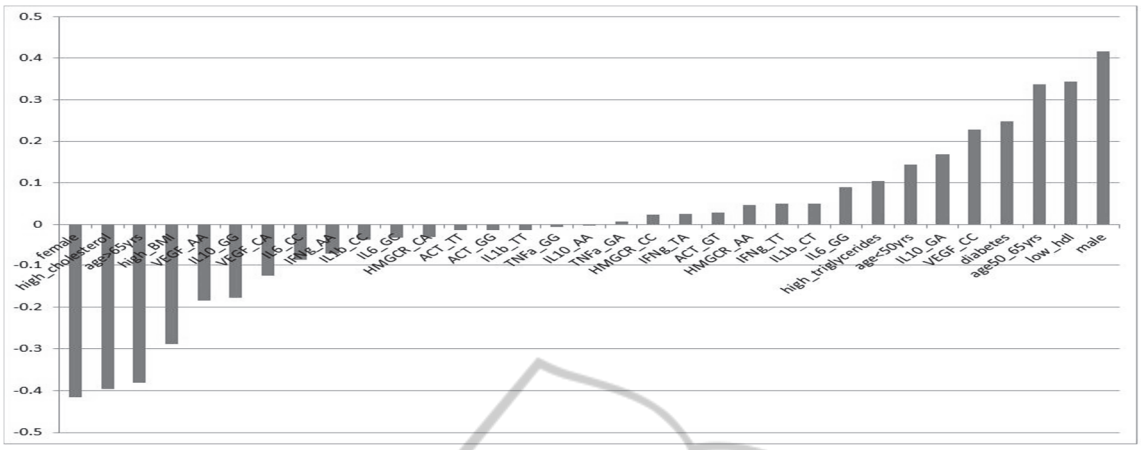

Figure 2: Linear correlation index in AMI.

variables (all variables).

The linear correlation indexes of 36 variables in AMI patients has been reported in figure 2 where variables appears more associated (up the line) or less associated (down the line) with the AMI .

Tables 1 showed the results regarding sensitivity, specificity and overall accuracy according AUC analysis regarding association with AMI by using the variables data set after the TWIST selection. By 10 different applications, the mean sensitivity

resulted 89.9%, mean specificity 85.4%, mean overall accuracy 87.6.

The results obtained with independent application of Back Propagation feed forward artificial neural network using 8 hidden nodes in sequence a-b and b-a were stable and consistently reached an average overall accuracy near to 90%. Consequently, the tested algorithms were able to find a good correlation between some variables and diagnosis, after the removal of noisy attributes.

Data from this investigation suggest a new risk chart to be applied for the prevention of AMI in unaffected subjects.

Table 1.

N	ANN	Sens.	Spec	Acc.
1	*1 (ab)	83.51	90.48	86.99
2	*2 (ab)	86.6	85.71	86.16
3	*3 (ab)	84.54	83.33	83.93
4	*4 (ab)	85.57	88.1	86.83
5	*5 (ab)	96.15	83.33	89.74
6	*1 (ba)	92.31	83.33	87.82
7	*2 (ba)	94.23	83.33	88.78
8	*3 (ba)	94.23	86.67	90.45
9	*4 (ba)	94.23	86.67	90.45
10	*5 (ba)	87.63	83.33	85.48
	Mean	**89.9**	**85.43**	**87.66**

Table 1 Results obtained with the application of 10 independent supervised ANNs, five with the sequence a-b. and five with the sequence b-a (see text for explanation) in discriminating AMI from control status by using the 18 variables after selection operated by the TWIST algorithm.

4 DISCUSSION

A recent investigation by using a new CHD risk assessment model described that age, gender, diabetes and family history of AMI in combination with seven blood biomarkers yielded a 43% clinical net reclassification of patients previously considered of intermediate risk level by Framingham's criteria (Cross DS, 2012).

In a German population study, among 10,981 men followed up for 11 years, 378 subjects developed AMI; current smoking, excess body weight and physical inactivity were associated with the disease (Li K, 2014). A recent investigation confirmed that smoking (66%), hypertension (50%) and diabetes (43%) were the principal risk factors for AMI. However, the study conclusion was that none of these factors reached an association so solid to be used for AMI prediction in unaffected subjects (Juárez-Herrera Ú, 2013).

This notion was reinforced by results from an independent investigation on 605 consecutive patients hospitalized for a first AMI showing that the

preventive potential of a classical risk factor based health check was limited (Mortensen MB, 2013).

Moreover above quoted studies used conventional statistical models and therefore required a large number of patients and controls.

In the pilot study described here, ANNs and other new potent mathematical algorithms have been used and the need of large number of cases was not a limitation. By applying these new statistical algorithms we searched for a cluster of variables able to discriminate AMI cases form healthy controls.

TWIST algorithm operated a selection of 18 factors. and reached an average overall accuracy near to 90%. On the other hand, results obtained with the same validation protocol using all 36 variables were clearly inferior with an average overall accuracy of 74.99% (data not shown).

Present results partially confirm previously published findings showing that SNP in pro-inflammatory genes were associated with increased AMI risk in a different patients set by using a diverse non conventional statistical analysis (Licastro F, 2011) .

In conclusion the tested algorithms were able to find a set of variables highly associated with AMI diagnosis in men. This cluster is comprehensive of new SNPs which can be easily assessed in laboratory practice. Thereafter, the new cluster of variables might be used to better describe AMI risk in unaffected subjects with positive familiarity of the disease.

These findings need to be confirmed in larger case/control or longitudinal population studies.

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