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The Prediction of Coronary Atherosclerosis Employing Artificial Neural Networks

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Summary

Background: Atherosclerosis is a complex histopathologic process that is analogous to chronic inflammatory conditions. Several factors have been shown to correlate with the extent of atherosclerosis. Whereas hypertension, obesity, hyperlipidemia, diabetes, smoking, and family history are all well documented, recent literature points to additional associated factors. Thus, antibodies to oxidized low-density lipoprotein (oxLDL), cytomegalovirus (CMV), *Chlamydia pneumonia*, *Helicobacter pylori*, as well as homocysteine and C-reactive protein (CRP) levels have all been implicated as independent markers of accelerated atherosclerosis.

Hypothesis: In the current study we attempted to formulate a system by which to predict the extent of coronary atherosclerosis as assessed by angiographic vessel occlusion.

Methods: The 81 patients were categorized as having single-, double-, triple-, or no vessel involvement. The clinical data concerning the "classic" risk factors were obtained from clinical records, and sera were drawn from the patients for determination of the various parameters that are thought to be associated with atherosclerosis.

Results: Using four artificial neural networks, we have found the most effective parameters predictive of coronary vessel involvement were (in decreasing order of importance) antibodies to oxLDL, to cardiolipin, to CMV, to *Chlamydia pneumonia*, and to β2-glycoprotein I (β2GPI). Although important in the prediction of vessel occlusion, hyperlipidemia, hypertension, CRP levels, and diabetes were less accurate.

Conclusion: The results of the current study, if reproduced in a larger population, may establish an integrated system based on the creation of artificial neural networks by which to predict the extent of atherosclerosis in a given subject fairly and noninvasively.

Key words: atherosclerosis, autoimmunity, infectious agents, oxidized low-density lipoprotein, anticardiolipin

Introduction

The initiation and progression of atherosclerosis results from an interplay of multiple factors.[1] Traditionally, risk factors such as hyperlipidemia, diabetes, hypertension, and family history have been assessed to define the combined risk. However, in recent years it has become apparent that the "risk-factor based approach" suffers from lack of sensitivity and specificity, neglecting additional potentially important factors.

Among the factors involved in the pathogenesis of atherosclerosis, the role of the immune system has recently been highlighted due to the finding of activated lymphocytes and immunoglobulins within the atherosclerotic lesions.[2, 3] Furthermore, it has been postulated that autoantigenic materials in the vicinity of the lesions elicit the immune-mediated inflammation resulting in accelerated atherogenesis. Possible antigenic candidates include heat shock protein-65[2] and oxidized forms of low-density lipoprotein (oxLDL).[4] Accordingly, titers of antibodies toward these antigens correlated with enhanced atherosclerosis in humans.[2, 5] The view of atherosclerosis as a chronic inflammatory condition[1] can serve to explain the correlation between elevated levels of acute phase

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reactants [C-reactive protein (CRP)] and the extent of the atherosclerotic process.[6–7]

There is growing interest concerning the role of infectious agents in atherosclerosis progression.[8–10] Circumstantial data exist on the involvement of *Clamydia pneumoniae*, *Helicobacter pylori*, and herpes viruses. The mechanisms by which the infectious agent enhances atherosclerosis is not clear, yet it appears to be multifactorial. Indeed, these findings support the paradigm of atherosclerosis as an inflammatory condition, and since infectious agents have also been incriminated in the initiation of autoimmunity, they may serve as the common denominator of these two conditions.

In view of the multiple factors that have been suggested to influence the progression of atherosclerosis, it is justified to assess comparatively the relative contribution of each of these factors. Such comparison may help in establishing an integrated approach for the assessment of risk for atherosclerotic disease.

We evaluated the relative contribution of each of a number of serologic and biochemical markers to the extent of atherosclerosis as defined by involvement of the coronary arteries in patients referred for coronary angiography.

Materials and Methods

Patients

We studied 81 consecutive patients referred for coronary angiography because of chest pains. Sera were obtained from routine blood sampling that is made automatically prior to the procedure, centrifuged, and stored in −70°C until testing.

Coronary Angiography

Cardiac catheterization was performed by the Judkins or Sone technique. Angiographic films were interpreted by an angiographer blinded to the condition of patients studied. For the purpose of the study, significant coronary disease was defined as either ≥ 50% reduction of the internal diameter of the left main coronary artery or ≥ 70% of the internal diameter of the left anterior descending artery, and the right coronary or left circumflex artery distribution.[11] The designation of single-, double-, or triple-vessel disease was based on criteria of the Coronary Artery Surgery Study (CASS).[12]

Serologic Studies

Enzyme-linked immunosorbent assay was applied to assess antibody levels. Oxidized LDL (10 μg/ml), native LDL (10 μg/ml), β2-glycoprotein I (β2GPI; 10 μg/ml), cardiolipin (CL; 50 μg/ml), phosphatidylserine (PS; 50 μg/ml), phosphatidylcholine (PC; 50 (g/ml), phosphatidylethanolamine (PEA; 50 (g/ml) were used for coating 96-well enzyme-linked immunosorbent assay (ELISA) plates.

Plates were blocked with 3% bovine serum albumin (BSA), and binding of the sera (diluted 1:50) was detected after addition of alkaline phosphatase conjugated mouse anti-human IgG and the appropriate substrate.

The serologic markers to different infectious agents were determined at Specialty Laboratories. Cytomegalovirus kits were from Trinity Biotech (Jamestown, N.Y., USA), herpes simplex virus (HSV)-1 and HSV-2 kits were from Wampole Laboratories (Cranbury, N.J., USA), *Helicobacter pylori* kits were from Micro Detect Inc. (Laguna Hills, Calif., USA) and the *Chlamydia pneumonia* kit was from Linmed Biologicals (Brea, Calif., USA).

Homocysteine and CRP levels were determined on the sera according to the previously described method.[13]

Application of the Artificial Neural Network

An artificial neural network has been constructed to predict coronary vessel involvement. The database used to develop the network consisted of the 81 patients undergoing coronary angiography and contained 20 input variables described in "Methods."

The database was randomly divided into two subsets. The first subset (85%) of the original database was used to train and test the neural networks. The second set was used to validate the learning of the networks and was not used in the development or testing of the networks. All statistics on the performance of the networks are based on the validation set. Four networks were trained; one each to recognize the presence or absence of one of the four angiographically defined conditions (0, single-, double-, or triple-vessel disease). Statistical results were obtained by adding the true positives, true negatives, false positives, and false negatives from all four networks for the validation set.

The neural network used in this study was a feed-forward supervised network. In a supervised network, the correct outcomes for the training patients are shown to the developing network. The learning process, or weight adjustment, lies at the heart of the network development and in this study was a modified form of backpropagation of errors. The network consisted of three layers of neurons or processing elements. Input values were weighted and passed to a second, or hidden, neuron layer. Neurons in the second layer "fire" or produce outputs that are based upon the sum of weighted values passed to them. In this study, the optimal number of hidden neurons was determined to be 83. The hidden layer passes values to the output layer consisting of two neurons, one neuron reserved for a negative and the other for a positive vessel occlusion. Typically, both neurons "fire" or produce a result, but typically one neuron dominates this process, indicating the outcome prediction of the network for a given patient.

This particular network utilizes a Bayes statistics approach during training to ensure overfitting will not occur. The training method used in this study is the turboprop variant. This is a backpropagation training algorithm that operates much faster than other methods. It has the significant advantage of not being sensitive to learning rate and momentum factors. Training proceeds through an entire epoch of patient cases before network weights are updated. It adds all of the weight and modi-

fies the weights at the end of the epoch. This method utilizes an independent weight update size for each different weight, rather than the usual method of having a single learning rate and momentum that applies to all weights. This combination of learning properties has been found to yield high accuracies for predicting prospective patient outcomes.

In the database, 15% of the patients were randomly chosen and withheld as a validation set; the remaining 85% were used to train the neural network. When training and architecture evolution (determination of the optimum number of hidden neurons) was complete, each of the four networks was presented with the validation set and asked to predict vessel occlusion outcome. The results were then compared with the actual occlusion extent and a set of statistics generated.

Results

Patient Characteristics

The mean age of the patients with no vessel involvement was 62 ± 14.5, with single-vessel disease 64 ± 9.5, with double-vessel disease 61 ± 12, and with triple-vessel disease 70 ± 9. All patients were males.

The networks were asked to determine the most important variables leading to identification of coronary vessel occlusion. Figure 1 indicates the 12 most important variables in order of importance. It is essential to realize that these variables taken individually may not correlate strongly with the vessel occlusion, but when taken in concert with other variables they may be quite important. For the validation set, the combined networks achieved a sensitivity of 70%, a specificity of 80%, a positive predictive value for vessel occlusion of 54%, a negative predictive value for vessel occlusion of 89%, and an overall accuracy of 78%.

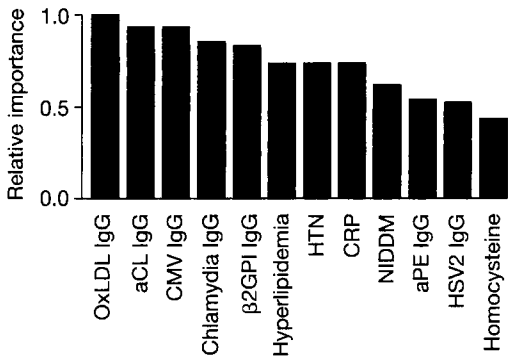

FIG. 1 The relative importance of the 12 leading parameters found to predict coronary vessel occlusion most accurately, as determined using artificial neural networks. HTN = hypertension, CRP = C-reative protein, aPE = antiphosphatidylethanolamine, CMV = cytomegalovirus, β2GPI = β2-glycoprotein I., aCL = anticardiolipin, oxDL = oxidized low-density lipoprotein, NIDDM = not insulin-dependent diabetes mellitus, HSV = herpes simplex virus.

Discussion

Traditional risk factors (i.e., hyperlipidemia, hypertension, cigarette smoking, diabetes, and family history of accelerated atherosclerosis) have long been recognized to be important for the progression of atherosclerosis.[1] However, the recent interest in inflammatory, infectious, and autoimmune factors has expanded the list of participants in the complex pathogenesis of this chronic process. These observations could result in therapeutic implications such as treatment with anti-inflammatory agents,[7] antibiotics,[14] or immunomodulatory drugs in atherosclerosis-related conditions.

The recent realization of the multiple factors involved in progression of atherosclerosis have raised the need to establish an integrated processing system that will help in assessing the severity of atherosclerosis as manifested by involvement of coronary arteries.

As depicted in Figure 1, we observed unexpectedly that anti-oxLDL and anticardiolipin (aCL) autoantibodies were the two most effective markers in predicting coronary vessel involvement. Oxidized LDL has been shown to play an important role in atherosclerosis.[4] The oxidative modification of LDL is thought to increase its atherogenicity by enhancing its accumulation in macrophages within the vessel wall. Antibodies to oxLDL are a fairly good marker of the oxLDL load, especially in view of the controversy regarding the presence of oxLDL in the plasma.

Anti-oxLDL have been shown to correlate with carotid atherosclerosis assessed by ultrasound.[5] Furthermore, these antibodies were found to be predictive of future myocardial infarction independent of LDL cholesterol levels.[15]

The second most effective parameter in predicting vessel atherosclerosis are antibodies to aCL. These antibodies, which are found in patients with systemic lupus erythematosus (SLE) and the antiphospholipid syndrome, have been shown to be associated with a procoagulant state.[16] Recently, it has been shown that high levels of aCL could predict myocardial infarction in a prospective follow-up of middle-aged dyslipidemic men.[17] In this study, a joint additive effect of aCL and anti-oxLDL was observed, emphasizing the independent importance of each in the risk for myocardial infarction.

β2-glycoprotein I (β2GPI) is a 50Kd protein that is a probable target of anticardiolipin antibodies.[18] Modern solid phase assays detect antibodies to this protein rather than to cardiolipin. We have shown that induction of anti-β2GPI antibodies by immunization with the respective protein accelerated atherosclerosis in transgenic mice.[19] These experimental findings may explain the observation that these antibodies were fifth in order of importance in predicting atherosclerotic vessel involvement.

Cytomegalovirus, a member of the herpes viruses, has recently attracted major attention in view of studies showing its presence in atherosclerotic plaques and the detection of antibodies in patients with accelerated atherosclerosis.[9, 10] Seropositivity for *Chlamydia pneumonia* has also been associated with coronary heart disease, and this intracellular pathogen has been found in macrophages within atherosclerotic plaques.[8, 9]

Antibodies to CMV and to *Chlamydia pneumonia* were indeed found to be among the most accurate factors (the third and fourth, respectively) in predicting vessel involvement, and thus the extent of coronary atherosclerosis.

It is interesting to note that the relative importance of traditional risk factors such as hyperlipidemia, hypertension, and diabetes in predicting vessel occlusion did not reach the values achieved by autoantibodies to oxLDL and cardiolipin or the antibodies to CMV and *Chlamydia*.

Recently, Lauerta *et al.* constructed an artificial neural network to predict coronary artery disease based on information from serum lipid profile.[20] This approach allowed for an effective prediction of outcome from a clinical trial (The Cholesterol Lowering Atherosclerosis Study) with variable follow-up times. However, the study was structured on the basis of a single risk factor rather than on a complex of variable parameters known to be associated with coronary artery disease.

The results of the current study represent a fairly strong predictive pattern and indicate the ability to identify a vessel state correctly in more than 50% of cases. The results of the neural networks are reasonably encouraging, indicating the networks have found a pattern in the patient data which is predictive of atherosclerotic vessel blockage. The main drawback of the study is, however, the relatively small number of patients which is insufficient to establish the neural network system as a useful diagnostic tool. If the network results can be repeated with a significantly larger patient population, this network may be found to be useful in predicting coronary vessel occlusion.

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