Improved accuracy of anticoagulant dose prediction using a pharmacogenetic and artificial neural network-based method

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Abstract

Background The unpredictability of acenocoumarol dose needed to achieve target blood thinning level remains a challenge. We aimed to apply and compare a pharmacogenetic least-squares model (LSM) and artificial neural network (ANN) models for predictions of acenocoumarol dosing.

Methods LSM and ANN models were used to analyze previously collected data on 174 participants (mean age: 67.45 SD 13.49 years) on acenocoumarol maintenance therapy. The models were based on demographics, lifestyle habits, concomitant diseases, medication intake, target INR, and genotyping results for CYP2C9 and VKORC1. LSM versus ANN performance comparisons were done by two methods: by randomly splitting the data as 50 % derivation and 50 % validation cohort followed by a bootstrap of 200 iterations, and by a 10-fold leave-one-out cross-validation technique.

Results The ANN-based pharmacogenetic model provided higher accuracy and larger R value than all other LSM-based models. The accuracy percentage improvement ranged between 5 % and 24 % for the derivation cohort and between 12 % and 25 % for the validation cohort. The increase in R value ranged between 6 % and 31 % for the derivation cohort and between 2 % and 31 % for the validation cohort. ANN increased the percentage of accurately dosed subjects (mean absolute error ≤1 mg/week) by 14.1 %, reduced the percentage of mis-dosed subjects (mean absolute error 2-3 mg/week) by 7.04 %, and reduced the percentage of grossly mis-dosed subjects (mean absolute error ≥4 mg/week) by 24 %.

Conclusions ANN-based pharmacogenetic guidance of acenocoumarol dosing reduces the error in dosing to achieve target INR. These results need to be ascertained in a prospective study.

Keywords Artificial neural network · Least-squares modeling · Anticoagulation · Pharmacogenetics · INR

Introduction

The use of “classic” oral anticoagulants such as warfarin (Coumadin®) and acenocoumarol (Sintrom®) is constantly increasing worldwide [1, 2]. This is paralleling the increased need for prophylactic anticoagulation in atrial fibrillation [3], deep venous thrombosis [4], after heart valve replacement [5], and other conditions [6]. Yet, the decreased risk of embolic and thrombotic events with this form of anticoagulation carries a significant competing risk of bleeding [7, 8]. The
unpredictability of the dose needed to achieve target blood thinning level remains a major challenge to healthcare providers [9] and, given its magnitude, has led to the introduction of the “Coumadin Clinic” concept [10]. Multiple randomized trials and observational studies continue to show that a significant number of patients are either under or over treated [11].

Recently, studies have supported a genetic-basis for inappropriate dosing of oral anticoagulation [2]. These provided strong evidence correlating efficacy and toxicity of acenocoumarol and warfarin with genetic variants including vitamin K epoxide reductase complex 1 (VKORC1) and cytochrome P450 2C9 (CYP2C9) [12–15]. In 2009, The International Warfarin Pharmacogenetics Consortium showed that a pharmacogenetic algorithm provided an estimated suitable starting dose of warfarin that was significantly closer to the needed stable therapeutic dose than that derived from a clinical algorithm or a fixed-dose approach [16, 17]. Other studies validated this conclusion and showed encouraging results, however, with some population variations [14, 15, 18–20]. One group also demonstrated that genetic testing and consequent dosing of anticoagulation decreased hospitalization for bleeding and thrombosis [21].

Dosing algorithms proposed thus far have been predominantly based on least-squares modeling (LSM) methods applied to observed dosing efficacy/toxicity data [22]. Known limitations of LSM will affect the wide prospective applicability and accuracy. In particular, as the complexity of the relationship between the dependent and independent variable increases and is non-linear, the LSM method becomes not the best fit to predict the outcome correctly [23]. The artificial neural network (ANN) system is a method that is gaining increasing application in the prediction of outcome in clinical research. It aims to “uncover the hidden causal relationships between single or multiple responses and a large set of properties” [24]. It has already been compared to conventional modeling techniques in prediction of outcome in cardiovascular research, with encouraging results [25]. In a recent study, ANN has shown superiority over established computational models (including linear regression) in early prediction of early risk detection of cardiovascular events [26]. Earlier attempts to use neural networks for prediction of warfarin dose needed and patient outcome with or without genetics have hypothesized improved accuracy by neural network based algorithms [27–29].

The ANN is modeled after our biological neural network. Similar to the biological network, it is an interconnection of nodes, similar to neurons. Each node has a “character” which determines how signals are processed by the node, i.e. the number of inputs and outputs associated with the node, the weight of each input/output, as well as the activation function. This is much like what happens in a neuron, where there is a summation of the inputs, and if the summation reaches threshold, it would lead to an action potential, called the “firing” of the neuron. The nodes are organized into linear arrays, also known as layers. There are input layers, hidden layers and output layers. The initial design of the ANN is first by intuition and later improved following many cycles of model experimentation [24]. The relationships between the nodes can be excitatory, inhibitory, of modulating effect, or of potentiating impact. The summation effect of all these inputs together is complex and follows multilayered matrix decision model which in turn leads to the final outcome [30].

In theory, ANN modeling approaches to dose prediction may minimize or avoid some of the limitations of the LSM and may result in more accurate dosing with obvious clinical benefits [31]. Yet, to date, ANN modeling has not been applied using the pharmacogenetic parameters to this specific clinical problem. Accordingly, we aimed to apply and compare LSM and ANN model predictions of weekly acenocoumarol dosing on a previously collected cardiology patient series with known relevant genotyping.

Methods

This study is a secondary analysis approved by the American University of Beirut (AUB) institutional research board of previously collected data. Briefly, patients older than 18 years who were treated with an oral anticoagulant for different medical indications for at least 2 months, and maintained on the same weekly dose over the past 3 international normalized ratio (INR) (i.e. at steady state) were recruited. The previously published cohort included 237 patients who received either acenocoumarol (>75 % of the sample) or warfarin, and 50 were excluded because of INR being outside of the target range. In this study we included all patients who received acenocoumarol (N=174) even if the measured INR value was outside the target range; this is because our objective is to build a model that predicts the needed dosage of acenocoumarol for the desired target INR [12].

Baseline data such as gender, height, weight, body surface area (BSA), body mass index (BMI) age at recruitment, smoking and alcohol intake, concomitant diseases and medications were collected. Whole blood was drawn and genotyping for CYP2C9 (CYP2C9*2 allele: 430C>T and CYP2C9*3 allele: 1075A>C) and VKORC1 (I173C>T and -1639G>A known in complete linkage disequilibrium) was performed as previously described [12]. The role of CYP4F2 polymorphisms with oral anticoagulants was not known at the time of study inception, and it was hence not included [32]. Very few patients had renal or liver disease; hence these variables were not included. Data for clopidogrel and aspirin intake were incomplete and hence were not included in the analysis, and no patient was taking an azole antifungal. Target INR for patients with atrial fibrillation, aortic valve replacement, DVT±PE, or those categorized as other causes was
considered to be 2.0 to 3.0; patients with mitral valve replacement or thrombophilias were considered to be in the 2.5 to 3.5 INR target group.

**Statistical methods**

Data analysis

*Linear Regression (LR)* Using the LSM is the starting point for devising any best-fitting model. It is essential to implement LSM because it gives an indicator about the relevance (p-value) of each of the predictors used for risk prediction. A linear LSM is given in general by:

\[ y = \beta x \]

where \( x \) is a column vector of all predictors, \( \beta \) a column vector of the coefficients associated with every predictor and \( y \) represents the predicted risk. The LSM algorithm finds the best vector \( \beta \) that fits this model.

After the data collection phase, we place the predictors for every subject in a matrix \( X \), where every row corresponds to one subject, and the corresponding risks of all subjects are placed in a column vector \( y \). Then the coefficient vector is computed using:

\[ \beta = (X^TX)^{-1}X^Ty \]

This method yields the best linear (it does not have to be linear) model that fits the data. Referring to it as best indicates that this is the model that minimizes the square error.

*Artificial neural network* A detailed introduction to ANN has been described by Hagan and Demuth [33]. In this study a standard feed-forward multilayer network was used. It consisted of one input layer and one output layer. The input layer consists of a single neuron, to which is connected all the observed independent variables. The output layer consists also of 1 neuron since the output of the network has to be a single real number representing the predicted dosage. So in total we have two neurons: a hidden layer containing one neuron and an output layer containing another neuron. The transfer function in the hidden layer is the tangent sigmoid function defined by:

\[ f(n) = \frac{e^n - e^{-n}}{e^n + e^{-n}} \]

As for the output layer, the transfer function is the linear function defined by: \( f(n)=n \).

The training of the network was done using the Levenberg-Marquardt back-propagation algorithm. This algorithm finds the weights that minimize the error using a variation of Newton’s method for minimizing functions [34]. This algorithm was chosen because it is the fastest neural networks training algorithm for moderate size networks [33] as is the case in this study. During the training phase, the derivation cohort was randomly split into 80 % training and 20 % validation. The training was repeated 200 times and the model that yielded the lowest error was used on the validation set. The network architecture was chosen using a standard systematic method where the number of hidden neurons is changed incrementally, and the network that gives the highest overall accuracy (derivation/validation) is chosen.

**LR versus ANN performance comparisons**

To compare the performances of the LR and ANN in predicting the dose of acenocoumarol needed to achieve target INR, two methods were used. The first method was to split the data randomly as 50 % derivation cohort and 50 % validation cohort. Next, as some of the independent variables are not linearly correlated with the predicted dosage which induces error in the LR model, a bootstrap of 200 iterations was performed. A new LR model was run in every iteration and the p-value for every independent variable was computed. An independent variable was considered statistically significant for the prediction process if its p-value was <0.05. After performing the 200 iterations, if an independent variable was noted in>= 10 % of the iterations to have a p-value<0.05 then it was retained in the final model, referred to as the reduced model. For every iteration the optimal model was found, and at the end the optimal overall model is presented by giving every independent variable a weight equal to the average of its corresponding weights obtained during the 200 iterations. The validation cohort was used to test if the model derived by the derivation cohort is valid. It is important to note that the validation cohort was never used during the derivation phase.

The second method was to use a 10-fold leave-one-out cross-validation technique in which the original dataset was randomly split into 10 groups of equal size. Then 10 iterations are performed, where in every iteration, nine groups are used as the derivation cohort and one as the validation cohort (the results of this method are shown in the attached online resource 1).

The mean absolute error, that is, the absolute difference between the predicted dosage and the actual dosage that the patient was receiving, is used to evaluate the performance of the LSM model and the ANN model. In addition to the mean absolute error, the determination coefficient \( R^2 \) as a metric to evaluate the performance of both derivation and validation sets was included.

Finally, to show the impact of using genetic variables in dosage prediction, the same derivation/validation procedure
and analysis was repeated with and without genetic data (referred to as a model excluding genetics).

Results

Data from 174 patients (mean age in years: 67.45 SD 13.49) who were maintained on acenocoumarol was analyzed. The studied group included 94 (54 %) men; the mean BMI was 29.45 SD 5.15 kg/m² and the mean BSA was 1.85 SD 0.21 m². The mean acenocoumarol weekly maintenance dose was 17.32 SD 11.16 mg. The majority of the participants were non-smokers (79 %) and only 24 % drank alcohol. Only 26 (15 %) patients were receiving amiodarone. Of the 174 patients included 69 (39.6 %) had atrial fibrillation, 35 (20.1 %) had undergone aortic valve replacement, 14 (8.0 %) had history of deep venous thrombosis±pulmonary embolism, 19 (10.9 %) had undergone mitral valve replacement, 12 (6.9 %) had thrombophilias and 25 (14.3 %) had other causes.

The frequencies of CYP2C9 genetic polymorphisms were as follows: 62.8 % CYP2C9*1/*1, 18.9 % CYP2C9*1/*2, 10.5 % CYP2C9*1/*3, 2.6 % CYP2C9*2/*2, 4.1 % CYP2C9*2/*3 and 1.1 % CYP2C9*3/*; and those for VKORC1 were: 26.2 % GG(CC), 45 % GA(CT), and 28.8 % AA(TT).

Bootstrap 50 % derivation-50 % validation simulation

Over the 200 iterations, age and the 2 genotypes VKORC1 (Mean P-Value=0.0008; CI=0.0003-0.0014) and CYP2C9 (Mean P-Value=0.0108; CI=0.0056-0.0159) had a significant p-value 100 % of the time; on the other hand, BMI (Mean P-Value=0.25; CI=0.2116-0.2887), BSA (Mean P-Value=0.458; CI=0.4101-0.4861) and the target INR (Mean P-Value=0.4599; CI=0.4195-0.5002) had a significant p-value in 20 %, 15 % and 15 % of the iterations, respectively. All the other independent variables achieved a significant p-value <10 % of the time and were, therefore, excluded from the reduced model (the reduced model included six independent variables: Age, BMI, BSA, VKORC1 and CYP2C9 genotype, and the target INR).

Models

Briefly, the dose prediction by LR is given using the following equation:

$$ D = 35.32 - 0.21 \text{Age} + 0.36 \text{BMI} - 0.48 \text{BSA} - 5.35 \text{VKORC1} - 2.7 \text{CYP2C9} + 0.76 \text{INR} $$

As for ANN, prediction is done using:

$$ D = \frac{D_n + 1.15}{0.044} $$

where Dn is given by:

$$ D_n = 3.29 n_1 + 2.45 $$

And n₁ is the output of the neural network. For more information on how n₁ is computed, refer to the online resource 1.

Table 1 shows that both ANN and LR methods lead to lower error and higher R with the models that include the genetic information (pharmacogenetic models), when compared to the models that exclude genetic information. It also shows that LR, using the reduced set of independent variables, outperforms its version where all independent variables were

Table 1  Bootstrap 50–50 simulation

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Derivation Cohort Mean Absolute Error (CI 95 %)** mg/week</th>
<th>R (%)</th>
<th>Validation Cohort Mean Absolute Error (CI 95 %)** mg/week</th>
<th>R (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LSM Reduced</td>
<td>4.9 (4.65-5.15)</td>
<td>67</td>
<td>7.1 (6.6-7.6)</td>
<td>55</td>
</tr>
<tr>
<td>LSM All variables</td>
<td>5 (4.7-5.3)</td>
<td>67</td>
<td>7.14 (6.6-7.6)</td>
<td>54</td>
</tr>
<tr>
<td>LSM Reduced excluding genes</td>
<td>6.07 (5.6-6.3)</td>
<td>42</td>
<td>7.25 (6.6-7.7)</td>
<td>42</td>
</tr>
<tr>
<td>LSM All variables excluding genes</td>
<td>5.92 (5.6-6.2)</td>
<td>52</td>
<td>7.45 (6.8-8)</td>
<td>34</td>
</tr>
<tr>
<td>ANN All variables</td>
<td>4.66 (4.4-4.8)</td>
<td>73</td>
<td>6.24 (5.8-6.7)</td>
<td>65</td>
</tr>
<tr>
<td>ANN Reduced</td>
<td>4.52 (4.35-4.7)</td>
<td>69</td>
<td>6.73 (6.2-7.1)</td>
<td>58</td>
</tr>
<tr>
<td>ANN All variables excluding genes</td>
<td>5.4 (5.1-5.6)</td>
<td>58</td>
<td>7.73 (7.2-8.2)</td>
<td>10</td>
</tr>
<tr>
<td>ANN Reduced excluding genes</td>
<td>6.23 (5.9-6.5)</td>
<td>38</td>
<td>7.13 (6.6-7.6)</td>
<td>43</td>
</tr>
</tbody>
</table>

** The 95 % confidence intervals (CIs) on the estimates of mean absolute error were computed by bootstrapping with 200 replications

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used. However, ANN outperforms LR in any model with reduced and all independent variables. The best overall model was obtained using ANN with all independent variables with a mean absolute error of 6.24 (5.8-6.7) mg/week and R 65 % in the validation cohort. The reduced model includes age, BMI, BSA, VKORC1 and CYP2C9 genotype, and the target INR, while the “All” variables model includes age, sex, weight, height, BMI, BSA, VKORC1 and CYP2C9 genotype, alcohol, smoking and the target INR.

Table 2 shows the percentage improvement of pharmacogenetic models compared to the clinical models. It is clear that whatever the model is, the pharmacogenetic version outperforms the clinical version for the derivation cohort and for the validation cohort. The improvement is substantial for all cases especially for ANN with all independent variables where the improvement in mean error is 14 % for the derivation cohort and 20 % for the validation cohort. The determination coefficient also improves by more than 550 % for the validation cohort.

Figures 1 and 2 show the error histograms for the two best models, LR Reduced and ANN with all variables, and one can infer the clinical relevance and relative performance of the two methods. Each histogram or group of histograms show the number of patients whose mean absolute error is: (a) Accurate (mean absolute error ≤1 mg/week); (b) Mis-dosed (mean absolute error 2-3 mg/week); (c) Grossly mis-dosed (mean absolute error ≥4 mg/week).

These histograms show the clinical relevance and relative performance of the two methods. The results show that the total number of patients who were highly accurate is 89 for ANN and 78 for LR; the number of patients who were mis-dosed is 66 for ANN and 71 for LR. Finally the number of patients who were grossly mis-dosed subjects by 14.1 %, reduces the percentage of mis-dosed subjects by 7.04 %, and reduces the percentage of grossly mis-dosed subjects by 24 %.

To further compare LR with ANN, in Table 3 we show the percentage improvement in mean error of an ANN model over its LR counterpart. Every row of Table 3 compares the corresponding LR model with all ANN models over both derivation and validation cohorts. The diagonal elements compare every LR model with its corresponding ANN model. It is noted that ANN outperforms LR in all models except for two. These two are the models where the two genotypes are excluded. In addition we here show the importance of the genetic independent variables as LR with the pharmacogenetic model outperforms ANN with clinical model; however, the ANN pharmacogenetic model outperforms every other model. Note that there is no LR model that outperforms every ANN method as we can always find an ANN model that outperforms LR.

Table 2 Percent improvement of pharmacogenetic models over clinical models

<table>
<thead>
<tr>
<th>Model</th>
<th>Derivation Cohort</th>
<th>Validation Cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td>LR Reduced</td>
<td>ME: +20 % R: +60 %</td>
<td>ME: +2 % R: +28 %</td>
</tr>
<tr>
<td>LR All variables</td>
<td>ME: +16 % R: +29 %</td>
<td>ME: +4 % R: +59 %</td>
</tr>
<tr>
<td>ANN Reduced</td>
<td>ME: +28 % R: +82 %</td>
<td>ME: +6 % R: +51 %</td>
</tr>
<tr>
<td>ANN All variables</td>
<td>ME: +14 % R: +26 %</td>
<td>ME: +20 % R: +550 %</td>
</tr>
</tbody>
</table>

Leave-one-out cross-validation method

All the results of this method were consistent with the Bootstrap 50 % derivation-50 % validation simulation method and are displayed in details in the online resource 1.
Discussion

The ANN-based pharmacogenetic algorithm provided superior accuracy for predicting target INR withacenocoumarol than the least-squares method-based pharmacogenetic model. This approach offered the ability to use genetic information and independent variables reported in the literature to be correlated with acenocoumarol dosing in a clinical decision making tool [16, 17]. Making the ANN-based model accessible for future large cohort validations is a necessary step. This can significantly impact the practice of anticoagulation, genetic testing, and clinical care of patients [1].

To our knowledge, no previous study has shown a similar result using a pharmacogenetic model. However, earlier attempts to use neural networks for prediction of warfarin dose needed and patient outcome with or without genetics have hypothesized improved accuracy by neural network-based algorithms [27–29]. Narayanan et al. [28] suggested that genetic model with neural networks improves accuracy of prediction of the international normalized ratio (INR) to determine appropriate INR dosing. However, their finding was based on analysis of three cases only. The use of neural networks was further investigated by Byrne et al. [27] and employed in supporting the decision in prescription and outcome prediction in anticoagulation drug therapy. They showed that the prescribing practice of physicians may be improved through the use of ANN which can learn to predict the outcome and prescribe the dosage of anticoagulants with twice the accuracy than that of expert physicians. However, the combined effect of these neural networks didn’t improve in comparison to individual networks.

Advantages of using ANN in anticoagulation dosing decision making

In Table 1 we show that LR with all independent variables has a higher mean error in comparison to a reduced number of independent variables. This is the opposite of what happened with ANN where the model with all independent variables outperformed the one with reduced independent variables. The reasoning behind this is that not all independent variables are linearly correlated with the dosage, so including them while deriving the linear model becomes a source of error [35]. Conversely, for non-linear models such as ANN, this does not constitute a problem because the ANN algorithm has the potential to learn the non-linear relationship independent of its complexity [30, 35]. A literature review of independent variables listed to be correlated with dosing reveals significant discrepancy among studies [36]. Using ANN offers the ability to embrace this discrepancy making it part of the prediction model and enhancing the model’s accuracy contrary to what linear regression offers [37].

Moreover, the increased accuracy for predictive inference using ANN demonstrated in this study further supports ANN based decision making [31, 38–41]. As is noted in Table 1, the mean error for the weekly dose of acenocoumarol in the validation cohort was 6.24 mg/wk (5.8-6.7) with an R of 65 % using ANN with all independent variables, making this the lowest mean error and highest R. This was confirmed when using KF cross-validation simulation where ANN with all independent variables had the lowest mean error of 5.6 mg/wk and highest R of 62 % (Tables S1 and S2, in online resources 2 and 3, respectively). From a clinical standpoint, ANN increased the percentage of accurately dosed subjects (defined as mean absolute error ≥1 mg/week) by 14.1 %, reduces the percentage of mis-dosed subjects (defined as mean absolute error 2-3 mg/week) by 7.04 %, and reduces the percentage of grossly mis-dosed subjects (defined as mean absolute error ≥4 mg/week) subjects by 24 %. This reduction in error is expected to lead to a reduction in harm from anticoagulation and increased safety. However, this needs to be ascertained in a prospective trial.

ANN has been described as a useful tool in clinical decision making in particular in large data sets [31, 38–41]. In this
study, the dataset does not qualify as large; however, it is reasonable to hypothesize that a future study including larger datasets will lead to further improvement in the accuracy of prediction.

**Clinical need for similar studies**

Newer oral anticoagulants that are less cumbersome are the solution that was recently introduced into the arena. Similar to any other innovation, these anticoagulants’ benefits and risks are being uncovered and their utilization is under scrutiny [42]. For example, the increased myocardial infarction associated with dabigatran is a concern that is now being further investigated [43]. However, in our personal experience, causes of delay in adopting these newer anticoagulants include: 1) cost [44], 2) lack of randomized trials in indications other than atrial fibrillation, 3) concerns related to pharmacokinetics of these drugs in special populations (very old or those with chronic kidney disease), 4) lack of antidotes to reverse their effect and 5) physician learning curve. Therefore, newer models, similar to what is described in this study, that can help predict the dose of acenocoumarol remain a need in the foreseen future, particularly in countries were oral anticoagulants use will lag.

Moreover, a secondary outcome of this study was to demonstrate the added value of genetic information on reducing weekly dose error of acenocoumarol. As is noted in Tables 2, 3 and Table S1 in online resource 2, all algorithms that included genetic information produced lower mean error and higher R, with the best model being ANN with all independent variables including pharmacogenetic information. Previous studies using genetic information for diagnostic purposes or prognostic purposes are well known [14, 29, 32]; however, developing a model for genetic testing to guide therapeutic dosing of medication and with accessibility to an electronic tool to perform is not widely available. The favored result of pharmacogenetic guided dosing is in-line with the studies published earlier by Gong et al. 2011 [45], Caraco et al. 2008 [18], and Anderson et al. 2007 [46]. In these studies time to achieving therapeutic INR and time within therapeutic INR were improved with pharmacogenetic guidance [18, 45, 46]. On the other hand, our study’s design differs from the others in that it included all comers regardless of their INR value at recruitment. Earlier studies have shown that the contribution of genetic guidance is mostly appreciated in dosing at initiation [47]. However, while time to achieving therapeutic INR is important, most of the embolic events in atrial fibrillation in particular were correlated with time within therapeutic range, i.e. in the chronic phase of treatment [48]. Hence, including in this study all individuals presenting regardless of their INR represents, in our opinion, a closer position to the reality in the clinic.

**Acenocoumarol dosage calculator**

To facilitate utilization of the ANN model, an application was developed in order to create a friendly interface to calculate acenocoumarol dosage. The application gives the ability for the user to input all the independent variables for a specific subject and obtain the predicted acenocoumarol dosage using both ANN and LR. The application also outputs a nomogram showing the variation of warfarin dosage with respect to the desired INR. It also gives a predictive nomogram for the next 8 years (assuming all other independent variables remain constant) and superimposes it on the nomogram obtained from LR. The software was developed using C# and will also be available as an online calculator. A screenshot of the described application is shown in the online resource 4.

**Limitations**

This study was a secondary analysis of a pre-existing data set, hence intrinsically carrying all the limitations of this design. In addition, the number of individuals recruited for this study affects the generalizations made, particularly when considering that the prevalence of some genetic polymorphisms was less than 5%. Furthermore, it is well known that diet (through vitamin K intake) affects the INR but this was not included in our analysis. The diet component, however, is challenging to control and assess, and has not been accounted for in other major studies either.

**Conclusion**

This study suggests that ANN-based pharmacogenetic guidance of acenocoumarol dosing reduces the error in comparison to LSM-based algorithms. Furthermore, contrary to the LSM-based model, increasing the number of independent variables to be included in an ANN-based algorithm is not a source of increased error, which explains some of the contradictions seen among other studies. The electronic tool provided facilitates future evaluation of this method in larger data sets and in a prospective design. A prospective study comparing the benefit and safety endpoints (time within therapeutic range, embolic and thrombotic events, and bleeding events) while using ANN-based pharmacogenetic guidance versus standard care would be the ideal trial to perform. However, as some studies addressing the relationship between pharmacogenetic testing and anticoagulation dosing are currently ongoing, our result may be an important drive for these colleagues to try ANN-based modeling.

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Dr. Robert H Habib: Analysis and interpretation of data, drafting and revising the article, and final approval of the manuscript.

Mr. Mohamad M Almedawar: Analysis and interpretation of data, critically revising the manuscript for important intellectual content, and final approval of the manuscript.

Dr. Nathalie K Zghieb: Acquisition of data, analysis and interpretation of data, critically revising the manuscript for important intellectual content, and final approval of the manuscript.

Dr. Imad H Elhajj: Conception and design, analysis and interpretation of data; drafting the article or revising it critically for important intellectual content; and final approval of the manuscript.

Conflict of interest  The authors declare that they have no conflict of interest.

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