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THROMBOEMBOLIC STROKE PREDICTORS IN PAROXYSMAL ATRIAL FIBRILLATION: COX MODELING BASED ON STUDIED HEMOSTASIS INDICATORS

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Abstract: Thromboembolic stroke in paroxysmal atrial fibrillation (PAF) is associated with high disability and mortality, therefore its prediction is extremely important for clinical practice. The coagulation and fibrinolytic systems are the key factors, responsible for thrombus formation in atrial fibrillation. In this sense, it is reasonable to search for thromboembolic stroke predictors among coagulation and fibrinolytic indicators in PAF patients. This determined the aim of our study, namely to study the predictive value for thromboembolic stroke of twenty coagulation and fibrinolytic indicators in PAF patients. In this article, overall survival until stroke onset was modeled using the Kaplan-Meier curve. Multivariate Cox regression analysis was used to investigate the relationship between survival time and twenty coagulation and fibrinolytic parameters measured in 51 patients. Significant predictive value ($p < 0.05$) was found for plasma TF levels (HR 1.022 [95% CI, (1.004-1.040)], FVIII levels (HR 1.084 [95% CI, (1.005-1.170)]) and vitronectin. (HR 0.946 [95% CI, (0.895-0.999)]). From the tested clinical parameters, only age and CHA2DS2-VASC score showed a predictive value for ischemic stroke occurrence (HR 1.237 [95% CI, (1.036-1.478]); HR 1.324 [95% CI, (1.054-1.662)] respectively).

Statistical analysis of the data was performed using the specialized software product STATISTICA 13.3.0, StatSoft Inc., USA.

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1. Introduction

Atrial fibrillation (AF) affects about 3% of the entire population over 20 years of age [see 1]. Thromboembolic events are a fundamental clinical problem in AF [2]. Worldwide, 20% to 30% of all strokes occur in AF conditions [3]. Short episodes and frequently mild PAF clinical manifestation have led several authors to allow for a low thromboembolic risk, which has not been confirmed in clinical observations [4]. Incidence of thromboembolic complications is not only significant, but also decisive for AF mortality and disability as a whole [5, 6]. Every third ischemic stroke is associated with the disease [7]. Presented epidemiological data not only do not allow its underestimation, but also emphasize its social and clinical significance and require optimization of the possibilities for reliable thromboembolic events prediction in AF.

Thrombogenesis is the link between rhythm disturbance and thromboembolic stroke [8, 9]. The key factor for its manifestation is the development of coagulation imbalance, leading to increased fibrin formation [9]. Histological studies give us objective evidence for that process, showing fibrin abundance in thrombi structure [10]. These data gave us a serious prerequisite for coagulation and fibrinolytic indicators to be analyzed in terms of their predictive value for manifestation of thromboembolic complications, including stroke.

2. Study design and target population

In our previous studies, we found significant deviations occurring even in short PAF episodes (episode duration $\leq$ 24 hours), namely, significantly increased plasma activity of FII, FV, FVII, FVIII, FIX, FX, FXI, FXII, vWF, plasminogen and decreased activity of PAI-1; increased plasma levels of TF, FVIII, vWF, FPA, F1+2, t-PA and D-dimer and decreased α2-antiplasmin and vitronectin levels [11, 12, 13]. The study was conducted on 51 non-anticoagulated patients with a first AF episode (26 men, 25 women; 59.84 ± 1.60 years) and in 52 controls (26 men, 25 women; 59.50 ± 1.46 years) without previous AF data, selected in the period from October 2010 to May 2012, after approval of the Ethics Committee at St. Marina University Hospital – Varna (No. 35/29.10.2010)

and Medical University of Varna (No. 9/14.10.2010). Indicators were studied once in peripheral venous blood. The two groups were balanced according to demographic and clinical indicators, comorbidity was reduced only to hypertension and diabetes mellitus [14, 15], ensuring maximum balance between the groups and objective comparison of the obtained results. The unidirectional and logically interrelated deviations in hemostatic markers gave us indisputable grounds to assume that significant systemic hypercoagulability occured during short PAF episodes (duration $\leq$ 24 hours) [11, 12, 13]. This determined the aim of the present study, namely to analyze the predictive value of the twenty examined coagulation and fibrinolytic parameters for ischemic stroke development after a short PAF episode ($\leq$ 24 hours), as well as clinical parameters with an already established prognostic value for ischemic stroke manifestation, namely: age, BMI (kg/m^2), eGFR (ml/min/1.73m^2) and thromboembolic risk characteristics defined as low-risk (CHA2DS2-VASc score = 0 in men and 1 in women) and increased risk (CHA2DS2-VASc score $\geq$ 1, regardless of gender) [16]. In this regard, the selected patients were monitored for manifestation of thromboembolic events until the end of December 2020, or earlier, in case of discontinuation of monitoring for reasons other than stroke, but before it has occurred (refusal to participate, death or cancer diagnosis).

3. Statistical analysis

Statistical analysis of the data was performed using the specialized software product STATISTICA 13.3.0, StatSoft Inc., USA. The level of significance for statistical testing of hypothesis was $p = 0.05$.

Survival analysis

The main purpose of survival analysis is to model and analyze data about the duration before a certain event, ie data for which the time endpoint is the occurrence of this event. In the present study, this analysis was used to model time data on ischemic stroke occurrence in PAF patients, ie for the purposes of the present study, survival was defined as the period (in years) without stroke, and the event ("failure") was the cerebrovascular incident itself. Patient follow-up was right-censored, i.e. until the end of the observation period or until the individual was removed for reasons other than ischemic stroke, but before it has occurred (described in Study design and target population).

Kaplan-Meier estimate for survival function

The Kaplan-Meier estimate, also called the product-limit estimate, is a popular approach which addresses this issue by re-estimating the survival probability each time an event occurs.

Appropriate use of the Kaplan-Meier estimate rests on the assumption that censoring is independent of the likelihood of developing the event of interest and that survival probabilities are comparable in participants who are recruited early and later into the study. For real data, the survival function $S(t)$ is unknown, but can be estimated from a sample. Estimation of the survival function can be realised nonparametrically by Kaplan-Meier estimate. If data were not censored, the obvious estimate would be the empirical survival function:

$$\hat{S}(t) = \frac{1}{t}\sum_{i=1}^{n} I(t_i > t),$$

where I is the indicator function that takes the value 1 if the condition in braces is true, and 0 otherwise. The estimator is simply the proportion alive at . Kaplan and Meier [18] extended the estimate to censored data. An observation is right-censored if the subject leaves the study or is alive when the study ends.

Let $t_1 < t < ... < t_m$ denote the distinct ordered times of death (not counting censoring times). Let d_i be the number of deaths at t_i, and let n_i be the number alive just before t_i. The intervals between each time typically will not be uniform. The Kaplan-Meier or product limit estimate of the survivor function is

$$S = \prod_{i:t_i>t} (1 - d_i/n).$$

In case of discrete covariates, survival curves for different factor levels can be calculated and compared by log-rank test which gives an indication of the relevance of the factor, i.e. whether survival in the groups differs significantly. If the two groups have the same survival function, the log rank statistic is approximately standard normal. In the present work, this method was used to estimate the mean annual stroke probability.

Cox proportional hazards model

The Cox proportional hazards model (Cox model) is a regression model and it is most often used with medical data to analyze the relationship between survival time and one or more risk variables (risk factors) [17]. The Cox model

allows for analysis of the influence of each of the variables by calculating the so-called Hazard ratio (HR) or Risk ratio (RR), which represents the value of the exponent in the risk function presented below ($h(t)$-hazard function). The function is interpreted as risk of death/incident at time t. Even if one factor is not statistically significant, the Cox model makes it possible to estimate its influence on survival prognosis of patients.

Let T represent survival time. T is regarded as a random variable with cumulative distribution function $P(t) = P(T \leq t)$ and probability density function $p(t) = dP(t)/dt$.

The survival function $S(t)$ is the compliment of the distribution function $S(t) = P(T > t) = 1 - P(t)$. A representation of the distribution of survival times is the hazard function $h(t)$, which assesses the instantaneous risk of death at time t, conditional on survival to that time.

The hazard is sometimes referred to as the "force of mortality" or "conditional failure rate." Survival analysis typically estimates the relationship of the survival distributions to covariates (explanatory variables). Most commonly, this estimation entails the specification of a linear-like model for the log hazard.

A parametric model based on the exponential distribution may be written as $\log h_i = \lambda + \beta_1 x_{i1} + \ldots + \beta_p x_{ip}$, where i is subscript for observations, x's are the covariates and $\beta = (\beta_1, \beta_2, \ldots, \beta_p)^{\mathrm{T}}$ is a vector of model parameters.

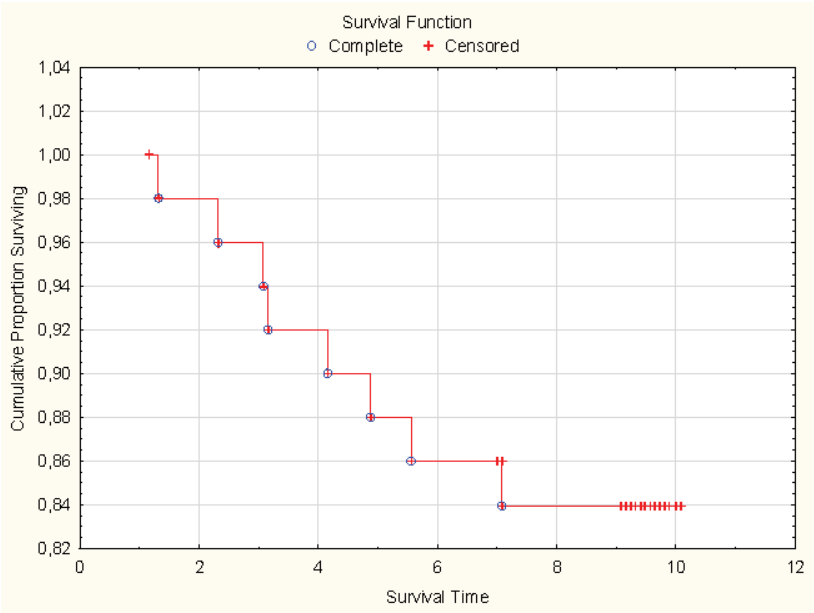
The Cox model leaves the baseline hazard function $\lambda(t) = \log h_0(t)$ unspecified. This model relates the hazard function of an individual at time t, with a vector $X = (X_1, X_2, \ldots, X_p)$ of p covariates, to a baseline hazard function $h_0(t)$ via a log-linear function $h(t; X) = h_0(t) \exp\left(\sum_{j=1}^{p} \beta_j X_j\right)$.

An important consequence of this formulation, and the reason for the name "Proportional Hazards Model," is that the hazard ratio-HR (also called relative risk – RR) for two individuals with covariates X and X^* does not depend on time:

$$HR = \frac{h(t; X)}{h(t, X^*)} = \exp\left(\sum_{j=1}^{p} \beta_j (x_j - x_j^*)\right).$$

The Cox model was used to evaluate the significance of the studied coagulation and fibrinolytic parameters as predictors of ischemic stroke.

Figure 1: Estimated event-free survival function in years for all 51 patients.



4. Results and discussion

Event-free survival (EFS) was calculated from the date of the stroke. Patients were followed-up from October 2010 until stroke or end-point of the study (December 2020), whichever occurred first. Survival curves were plotted using the Kaplan-Meier estimate.

Survival function assessment using the Kaplan-Meier estimate showed a mean follow-up period of 8.44 years (1.16-10.08), during which 9 ischemic strokes were recorded. Thus, 17.65% (9 patients) of the studied cases for an event occurrence were not censored, and 82.35% (42 patients) were censored. Mean annual incidence of thromboembolic events was 2%. Information was collected based on outpatient visits and available medical records in the hospital database.

Estimated event-free survival (EFS) function for all 51 patients is plotted in Figure 1.

The function estimates (predicts) the following EFS annual stroke-free survival: One-year: 98%; Two-year: 96%; Five-year: 86.2%; Ten-year: 82.3%. Using the Cox model, we checked to what extent deviations in hemostatic in-

Table 1: Parameter estimates for the Cox model with ischemic stroke occurrence data.

Risk factor	**Estimator of the parameter Beta**	**P-value**	**Hazard Ratio (HR) (95% CI for HR)**
TF level (pg/mL)	0.022	0.015	1.022 (1.004-1.040)
FVIII level (%)	0.081	0.037	1.084 (1.005-1.170)
Vitronectin level (mcg/mL)	-0.055	0.049	0.946 (0.895-0.999)
Age (years)	0.213	0.019	1.237 (1.036-1.478)
CHA_2DS_2-VASc score*	0.281	0.016	1.324 (1.054-1.662)

** defined in both categories CHA2DS2-VASc score = 0 in men or 1 in women and CHA2DS2-VASc score ≥ 1 (regardless of gender)*

dicators, clinical indicators and thromboembolic risk characteristics of patients (low risk or increased risk) can be predictors of ischemic stroke occurrence. Five factors were identified as significant ($p < 0.05$) predictors: TF levels, FVIII levels and vitronectin, as well as clinical indicators: age and CHA2DS2-VASC score. The results are presented in Table 1.

As TF and FVIII values increased, probability of a cerebrovascular event (Beta> 0) increased. From constructed dichotomous logistic regression models for these two predictors with a variable indicating presence/absence of stroke in studied patients, it can be seen that at a value of TF=418.79 pg/mL the probability is 0.5 and increased to 0.75 at a value of 440 pg/mL. For FVIII, the probability increased from 0.5 at 46.95% to 0.9 at a value of 150% For the statistically significant predictor vitronectin, Beta coefficient is < 0 and the probability of stroke decreased from 0.9 at a value of 155 (mcg/mL) to 0.5 at a value of 180 (mcg/mL).

From the tested clinical parameters, only age and CHA2DS2-VASC score showed a predictive value for ischemic stroke occurrence (HR 1.237 [95% CI, (1.036-1.478); HR 1.324 [95% CI, (1.054-1.662)] respectively). Beta coefficient was > 0, so when age increased, stroke probability increased and CHA2DS2-VASc score ≥ 1 (regardless of gender) gives a poor stroke prognosis.

AF determines a significantly increased incidence of ischemic stroke development [19, 20, 21, 22]. At the same time, 30-day stroke mortality, associated

with rhythm disorder, is high, reaching up to 24% in some studies [23]. Therefore, precise stroke risk estimation has remained a challenge for more than 25 years. Its early and correct assessment is extremely important.

In the studied patient population, high plasma TF and FVIII levels, as well as low vitronectin levels, determined a high risk for ischemic stroke occurrence, and these indicators showed a predictive value, measured in the early hours (up to the 24th hour) of the rhythm disorder. The established results have a pathophysiological justification. Tissue factor is a key and irrevocable participant in the process of thrombus formation and more precisely in the initiation phase of coagulation. Circulating TF is even believed to be the one that carries TF/FVIIa activity to activated platelets [24, 25, 26, 27]. In this sense, it is precisely the freely circulating TF plasma levels that reflect very accurately the body's propensity for thrombus formation. The vitronectin molecule is a multifunctional complement regulator that can be found in almost all human cells. It is an important platelet adhesive glycoprotein and an indirect inhibitor of fibrinolysis, factors that determine its procoagulant role [26]. However, it inhibits at the same time the final pathway of the complement system and prevents formation of cell-lethal "pore-forming complexes." The exact mechanisms why low vitronectin levels predetermine a high stroke probability in the studied population are unestablished, but we speculatively assume its role in cellular protection by controlling complement system activity. The obtained CHA2DS2-VASc score results confirm its significance as a long term ischemic stroke risk stratifier in AF patients.

5. Conclusion

In our study, TF, FVIII and vitronectin plasma levels showed a significant predictive value for ischemic stroke occurrence. The simultaneous examination of twenty hemostasis indicators, as well as strict selection of participants, are factors of particular importance. In this sense, we consider that the obtained results are a serious prerequisite for larger clinical studies to confirm the strength of the conclusions drawn for clinical practice.

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