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CLINICAL STUDY

Effect of warfarin anticoagulation on thrombin generation in patients with idiopathic pulmonary arterial hypertension

Jansa P[1], Hrachovinova I[2], Ambroz D[1], Maresova J[1], Polacek P[1], Simkova I[3], Linhart A[1], Aschermann M[1]

2nd Medical Department - Clinical Department of Cardiology and Angiology of the First Faculty of Medicine and General Teaching Hospital, Prague, Czech Republic. **jansapavel@yahoo.com**

Abstract: *Background:* Oral anticoagulant therapy is recommended for patients with pulmonary arterial hypertension (PAH). The rationale for the use of anticoagulant treatment is based on thrombophylic predisposition in PAH and improvement of survival in patients treated with anticoagulation. However, the target INR value has not been evaluated. The aim of this study was to analyze thrombin generation in patients with PAH treated with warfarin anticoagulation.
Methods: The study was performed in 58 patients with idiopathic PAH treated with warfarin at stable doses. Thrombin generation assay was performed in all subjects and three parameters were derived from the thrombin generation curves: lag time, maximal concentration of formed thrombin (peak thrombin) and area under the curve (AUC). Thrombin generation parameters were correlated with INR and compared between the patient groups with different intensity of anticoagulant therapy.
Results: Significant correlation between the lag time and INR was observed ($r = 0.495$, $p < 0.001$). Significant negative correlation between the maximal concentration of formed thrombin and INR and between the area under the curve of thrombin generation and INR was observed ($r = -0.709$, $p < 0.001$ and $r = -0.784$, $p < 0.001$, respectively). Thrombin generation was significantly reduced in patients with INR between 1.5 and 2.5.
Conclusions: Low-intensity warfarin anticoagulation with target INR between 1.5 and 2.5 could be effective and sufficient to suppress thrombin generation in patients with idiopathic PAH *(Fig. 3, Tab. 4, Ref. 12).* Full Text in free PDF *www.bmj.sk.*
Key words: pulmonary arterial hypertension, anticoagulant therapy, thrombin generation.

Pulmonary arterial hypertension (PAH) is a group of diseases affecting small pulmonary arteries defined by the mean pulmonary arterial pressure higher than 25 mmHg with a concomitant pulmonary capillary wedge pressure lower than 15 mmHg and pulmonary vascular resistance higher than 3 WU (Wood units). Idiopathic PAH is relatively rare, the estimated incidence is 1–2 per million in the population.

Oral anticoagulant therapy is widely recommended for patients with PAH. It has been shown to improve survival in patients with idiopathic PAH and PAH associated with anorexigens (1). The rationale for the use of anticoagulant treatment is based on the demonstration of thrombophilic predisposition in patients with pulmonary hypertension. The anticoagulant therapy in PAH may be associated with an increased risk of bleeding (hemoptysis, gastrointestinal). Therefore, the low-intensity warfarin anticoagulation with the target INR (international normalized ratio) between 1.5 and 2.5 is recommended (2, 3). It is the consensus of experts and lacks sufficient evidence. The efficacy and safety of low-intensity anticoagulation in preventing thromboembolism have been reported in patients with various cardiac disorders (4, 5, 6).

Recently, it has been shown that thrombin generation tests could be used in monitoring of anticoagulant treatment and that they could also be more sensitive than the traditional tests (7). Moreover, the assays for determination of thrombin generation over time can be used to calculate INR values for patients and to determinate states of bleeding disorders or thrombophilia as well as the activity of circulating microparticles. This broad range of applications is possible by providing different tissue factor concentrations and by monitoring thrombin generation during initiation, amplification and downregulation of thrombin formation.

Patients and methods

The study group consisted of 58 patients with idiopathic PAH evaluated at the Centre for Pulmonary Hypertension, Department of Cardiology and Angiology of Charles University in Prague.

[1]2nd Medical Department – Clinical Department of Cardiology and Angiology of the First Faculty of Medicine and General Teaching Hospital, Prague, Czech Republic, [2]Institute of Hematology and Blood Transfusion, Prague, Czech Republic, and [3]Cardiology Clinic, National Institute of Cardiovascular Diseases, Department of Cardiology, Slovak Medical University, Bratislava, Slovak Republic

Address for correspondence: P. Jansa, MD, 2nd Medical Dept – Clinical Dept of Cardiology and Angiology of the First Faculty of Medicine and General Teaching Hospital, U nemocnice 2, CZ-128 02 Praha 2, Czech Republic.
Phone: +420.728717041, Fax: +420.224912154

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Tab. 1. Demographic, clinical and hemodynamic parameters of the study population.

Female patiets (n/%)	39 / 67.2
Age, yrs	49 ± 17
WHO functional class, I/II/III/IV (n)	0 / 26 / 32 / 0
(%)	0 /44.8 /55.2/ 0
Hemodynamic data at the time of diagnosis	
RAP, mmHg	12 ± 6
mPAP, mmHg	60 ± 15
PCWP, mmHg	11 ± 4
CI, $L.min^{-1}.m^{-2}$	2.22 ± 0.52
PVR, Wood units	12.83 ± 5.46

RAP = right atrial pressure, mPAP = mean pulmonary artery pressure, PCWP = pulmonary capillary wedge pressure, CI = cardiac index, PVR = pulmonary vascular resistance

All patients met the European guidelines on diagnosis and treatment of pulmonary arterial hypertension. None had a history of pulmonary embolism and hereditary thrombophilia. All patients were treated with warfarin (Warfarin Orion, Orion Corporation, Finland). No changes in warfarin dosages were made 1 month prior the study. All patients were treated with PAH specific therapy. Twenty patients were treated with bosentan monotherapy, twelve patients with sildenafil monotherapy, five patients with subcutaneous treprostinil monotherapy and one patient with inhaled iloprost monotherapy. Eleven patients received a combination of intravenous epoprostenol and sildenafil, three patients a combination of subcutaneous treprostinil and sildenafil, two patients a combination of subcutaneous treprostinil and bosentan, two patients a combination of bosentan and sildenafil, one patient a combination of intravenous epoprostenol and bosentan and one patient was treated with a combination of inhaled iloprost and sildenafil.

The reference laboratory values were derived from a group of 22 healthy individuals.

After obtaining an informed consent, 4 ml of blood were collected in 3.2 % buffered sodium citrate and centrifuged at 1,700 g for 30 minutes within one hour after collection. Platelet-poor plasma was divided and than stored at –70 °C until tested. Prothrombin time was measured by Neoplastin R, recombinant human thromboplastin (Stago, France) with an international sensitivity index of 1.03 and was expressed in terms of INR. Thrombin generation over time was determined by thrombin generation assay (Technothrombin R TGA f. Ceveron R, Technoclone GmbH, Vienna, Austria). Three parameters were derived from the thrombin generation curves: lag time (moment that the signal deviates by more than 2 standard deviations from the horizontal baseline), maximal concentration of formed thrombin (peak thrombin) and area under the curve (AUC) – endogenous thrombin potential.

Data were obtained from all subjects regarding the demographic, clinical and hemodynamic variables, including age, sex, functional capacity according to the New York Heart Association (NYHA) classification, mean right atrial pressure, mean pulmonary artery pressure, pulmonary capillary wedge pressure, cardiac index and pulmonary vascular resistance.

Results were expressed as mean ± standard deviation. Differences between parameters were analyzed using the Student´s t-test. Correlations were expressed as Spearman coefficients.

Results

Fifty eight patients with idiopathic PAH participated in the study. Demographic, clinical and hemodynamic parameters of the patients are listed in Table 1. Thirty nine patients were women. Twenty six patients were in NYHA class II, and thirty two patients were in NYHA class III. The mean pulmonary artery pressure was 60±15 mmHg. Mean cardiac index was 2.22±0.52 $L.min^{-1}.m^{-2}$. The mean pulmonary vascular resistance was 12.83±5.46 Wood units.

Because our patients received low-intensity warfarin anticoagulation, the value of INR in all patients was 1.8±0.7. The cor-

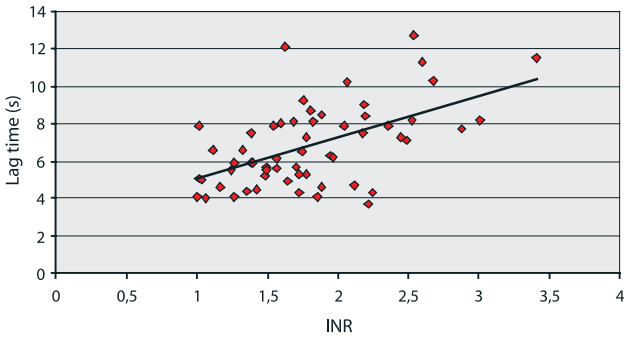

Fig. 1. Correlation between lag time (s) and INR (r=0.495, p<0.001).

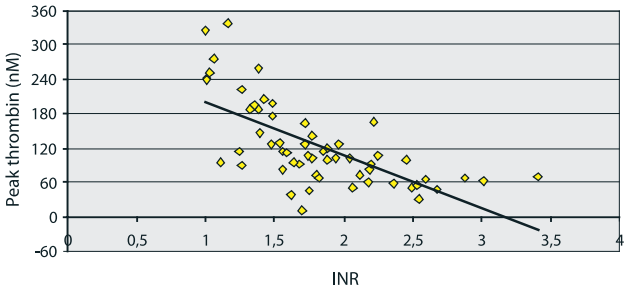

Fig. 2. Negative correlation between peak thrombin (nM) and INR (r=-0.709, p<0.001).

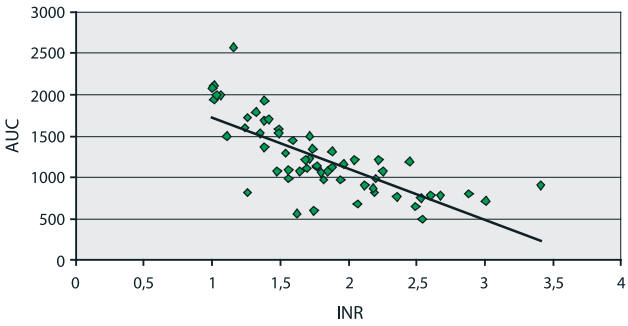

Fig. 3. Negative correlation between AUC (area under the curve) of thrombin generation and INR (r=-0.784, p<0.001).

Tab. 2. Thrombin generation measurements in patients with INR value below 1,5 and in controls.

Variables	Patients (n=20)	Controls (n=22)	p value
Lag time	5.47±1.08	4.1±0.86	< 0.01
Peak thrombin	204±69	169±44	NS
AUC	1713±382	1902±196	NS

AUC–area under the curve

Tab. 3. Thrombin generation measurements in patients with low-intensity warfarin and in controls.

Variables	Patients (n=32)	Controls (n=22)	p value
Lag time	6,9±1,93	4,1±0,86	<0.001
Peak thrombin	96±32	169±44	<0.001
AUC	1055±230	1902±196	<0.001

AUC–area under the curve

Tab. 4. Comparison of thrombin generation measurements in patients with low-intensity warfarin and in patients with INR value below 1.5.

Variables	INR 1.5–2.5 (n=32)	INR<1.5 (n=20)	p value
INR	1.9±0.27	1.25±0.17	<0.001
Lag time	6.9±1.93	5.47±1.09	<0.01
Peak thrombin	94±35	204±69	<0.001
AUC	1056±230	1713±381	<0.001

INR – internationalized normalized ratio, AUC–area under the curve

relation between lag time and INR is expressed in Figure 1 ($r = 0.495$, $p < 0.001$). The relation between the maximal concentration of formed thrombin (nM) and INR is shown in Figure 2. There was a significant negative relation between maximal concentration of formed thrombin and INR ($r = –0.709$, $p < 0.001$). The relation between the area under the curve of thrombin generation is shown in Figure 3. It also shows a significant inverse correlation with INR ($r = –0.784$, $p < 0.001$).

In patients with the INR value below 1.5, the maximal concentration of formed thrombin and the area under the curve did not differ significantly from the controls (Tab. 2). In patients with the INR between 1.5 and 2.5 (target value in PAH patients), the lag time, the value of maximal concentration of formed thrombin and the area under the curve differed significantly from controls (Tab. 3). Similarly, parameters derived from the thrombin generation curves in patients with the INR value between 1.5 and 2.5 differed significantly from those in patients with the INR below 1.5 (Tab. 4).

Discussion

Current evidence indicates that chronic pulmonary vascular thrombosis is present in patients with PAH. Thrombotic arteriopathy may alter the progression and prognosis of the disease. Systemic anticoagulation therapy appears to predict a better prognosis, especially for patients with disease not responsive to vasodilators. One case series, three retrospective cohort studies and one prospective cohort study have demonstrated a survival benefit of anticoagulation therapy (1, 8, 9, 10, 11). The degree of warfarin exposure is not consistently specified in the studies.

In recent randomized controlled trials, oral anticoagulants were administered in 51–86 % of subjects. The highest prevalence of oral anticoagulant treatment was seen in the trials involving mainly idiopathic PAH patients in NYHA classes III and IV.

No conclusive evidence for optimal INR can be derived from published studies. The target INR in patients with idiopathic PAH varies between 1.5 and 2.5 in American centers and 2.0–3.0 in European centers. The American College of Chest Physicians Clinical Practice Guidelines recommend a target INR of 1.5–2.5. The validity of the recommended therapeutic range of INR has not been evaluated. Updated American College of Chest Physicians Evidence-Based Clinical Practice Guidelines does not recommend any dosage of warfarin (12).

Traditional coagulation tests, such as the prothrombin time, do not assess the whole coagulation system. These tests use clot formation as their endpoint, which occurs when only around 5 % of all physiologically relevant thrombin is formed. Measurement of an individual´s capacity to generate thrombin, however, captures the end result of the interaction between proteases and their inhibitors and is therefore potentially more useful compared to conventional coagulation tests.

We observed a significant correlation between the lag time and INR, and significant negative correlation between the maximal concentration of formed thrombin and INR and between the area under the curve of thrombin generation and INR in our group of patients.

Our data shows that thrombin generation is significantly reduced in patients with INR between 1.5 and 2.5 compared to thrombin generation in patients with INR below 1.5 or in a reference population of healthy individuals.

In conclusion, our findings show a clear difference in thrombin generation already in PAH patients with low-intensity warfarin antiocoagulation (INR value between 1.5 and 2.5) as compared to the group with INR value below 1.5. This leads to the conclusion that already low-intensity warfarin antiocoagulation could be effective and sufficient in patients with idiopathic PAH. This therapeutic strategy may lead to a reduction of bleeding complications during anticoagulant treatment or may be used in patients with a higher risk of bleeding.

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