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ACTIVATED PARTIAL THROMBOPLASTIN TIME AS INDICATOR OF DABIGATRAN EFFICIENCY IN PATIENTS WITH NON-VALVULAR ATRIAL FIBRILATION

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Dabigatran, a new generation anticoagulant, is a direct thrombin inhibitor, which has a rapid onset of action and relatively wide therapeutic scope, with no need for monitoring of efficacy, as is the case with vitamin K antagonists. However, there are certain emergency situations that require immediate assessment of the effectiveness of dabigatran. The aim of this study was to determine whether the aPTT as a screening coagulation test, can be reliably used to assess the anticoagulant effect of dabigatran.

The study included 32 patients with non-valvular atrial fibrillation who received dabigatran (Pradaxa, Boehringer Inhelheim) in a single dose of 110 mg or 150 mg twice a day. In all patients screening coagulation (PT, aPTT, INR) was done before the treatment. aPTT was performed 4 hours, 8 hours and 12 hours after taking the drug.

There was a statistically significant prolongation of aPTT after 4 hours and 8 hours of taking the drug in patients who were treated with 150 mg of dabigatran compared to 110 mg, while after 12 hours there was no statistically significant difference in aPTT between these two groups. There was a strong correlation between the control values of aPTT and the total increase in aPTT after dabigatran administration (r = 0.96 for a dose of 150 mg IR = 0.83 for a dose of 110 mg).

aPTT is a useful test for assessing the effect of dabigatran and can be used as a screening test in patients who urgently need to determine the efficacy of the drug.

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Key words: *dabigatran, aPTT, anticoagulant, bleeding*

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Introduction

For more than 50 years, anticoagulant therapy has been effectively used in the treatment of venous thromboembolism and prevention of stroke and systemic embolism (1). In addition, anticoagulants are used in the prevention of ischemic stroke in patients with atrial fibrillation, in the prevention of early and late recurrence of thromboembolism, in patients with acute coronary syndrome (ACS), deep venous thrombosis (DVT) or pulmonary embolism (PE) (2). In practice, anticoagulant therapy involves the use of warfarin or acenocoumarol taken per os, non-fractionated heparin (NH), low molecular weight heparins (LMWH) and parenteral inhibitors of the activated factor Xa (e.g., fondaparinux). Despite the proven clinical efficacy of these drugs, there are some limitations to their use, such as warfarin interactions with other drugs and some foods, prolonged onset of action, narrow therapeutic range and mandatory laboratory control, while the use of NH or LMWH carries the risk of heparin-induced thrombocytopenia (HIT), it requires laboratory control and dose adjustments. That is the reason why clinicians are introducing new oral anticoagulant drugs in routine practice, which are structurally direct inhibitors of thrombin (dabigatran) and activated factor Xa (rivoroxaban, apixaban, edoxaban), they are applied in a fixed dose and do not require laboratory control, have rapid onset of action and a relatively wide therapeutic range (1, 3). These drugs are more comfortable both for patients and for doctors, they have shown good clinical results so far and high profitability in the "cost-benefit" analysis (4). It is certain that the new oral anticoagulants bring significant changes in the management of anticoagulant therapy, both in prevention and treatment of thrombosis.

Dabigatran etexilate (Pradaxa, Boehringer In-

gelheim) is an oral anticoagulant that is hydrolyzed in the liver to dabigatran, which is a direct thrombin inhibitor. Recommended doses for clinical use are 110 mg and 150 mg twice a day, whereby the maximum drug concentration in plasma (100-400 ng/ml) is reached after 2-3 hours of ingestion. Dabigatran is mostly eliminated in the kidney (80%), in the glomerular filtration rate (GFR) > 80 ml/min half-life is about 13 hours, while at the GF of 30-50 ml/min half-life of dabigatran is about 18 hours (1,5). Dabigatran affects coagulation screening tests, in terms of their prolongation, which depends on the dose and the time that has elapsed since the last dose of the drug is taken (6).

Although it is not necessary to test the efficacy of dabigatran routinely, there are some emergencies that require measurement and assessment of the anticoagulant effect of this drug. These are, primarily, the state before surgical or invasive procedure when there is an indication that the patient took the drug in the last 24 hours (or longer if the creatinine clearance is less than 50 ml/min), if the patient is bleeding, if the patient had taken a dose greater than prescribed, in the developing renal failure or if there is a development of thrombosis while the patient is on therapy (3, 7). The literature describes the cases of serious gastrointestinal and intracranial bleeding associated with taking dabigatran, so in these cases it is very important to assess whether dabigatran is achieving supratherapeutic, therapeutic or subtherapeutic anticoagulant effect (5, 8). For this purpose, it is necessary to employ rapid laboratory testings, sensitive to dabigatran, which will give results within 30-60 minutes. These are: activated partial thromboplastin time (aPTT), thrombin time (TT) and ecarin clotting time (ECT)

Aim

The aim of this study was to determine:

- the effect of dabigatran on aPTT as a screening coagulation test,
- whether this test can be reliably used to assess the effect of dabigatran, especially in patients who are preparing for surgical intervention or those with bleeding.

Patients and methods

The study included 32 patients with non-valvular atrial fibrillation taking dabigatran (Pradaxa, Boehringer Inhelheim) in a single dose of 110 mg or 150 mg twice a day. A total of 14 patients were previously treated with anticoagulant therapy, vitamin K antagonists (warfarin (Farin), acenocoumarol (Sinkum, Sintrom), but because of the poor therapeutic efficacy of these drugs dabigatran was used instead, while 18 patients were on oral anticoagulant therapy for the first time.

Before the introduction of dabigatran, in all patients coagulation screening tests were done in the Department for monitoring of coagulation disorders in the Blood Transfusion Institute (BTI) of Niš, by determining the prothrombin time (PT/INR) and aPTT-on ACL Elite Pro (Instrumentation Laboratoty, USA). After two weeks of starting the therapy, patients came again to the BTI Niš when we took three blood samples in order to determine their, aPTT on the same day but in different time interval from taking the drug: 4 hours, 8 and 12 hours after the last taken dose of dabigatran. Blood samples for determining the aPTT were taken into 3.8 mL tubes with sodium citrate, aPTT was determined using the aPTT reagent HemosIL SP within 30 min of sampling.

Statistical analysis was performed using the Statistical Package for Social Science (SPSS Software GmbH, Germany), version 18.0. The results were presented in tables and graphs, using the mean values and standard deviations (SD). The relationship between aPTT before dabigatran therapy and aPTT after dabigatran administration was determined using the Pearson's correlation analysis.

Results

From the total of 32 patients in this study, there were 20 men (20/32 or 62.50%) and 12 women (12/32 or 37.50%), which represented a statistically significant difference ($p > 0.05$). The average age of patients in the study was 52.36 ± 10.14 years (the youngest patient was 42 and the oldest one was 70 years old). A total of 20 patients were treated with dabigatran in the dose of 150 mg twice a day, while 12 patients received 110 mg of dabigatran twice a day.

The average values of aPTT in the examined patients are presented in Table 1.

Table 1. aPTT (sec) in patients who are treated with dabigatran ($\overline{X}$ ± SD)

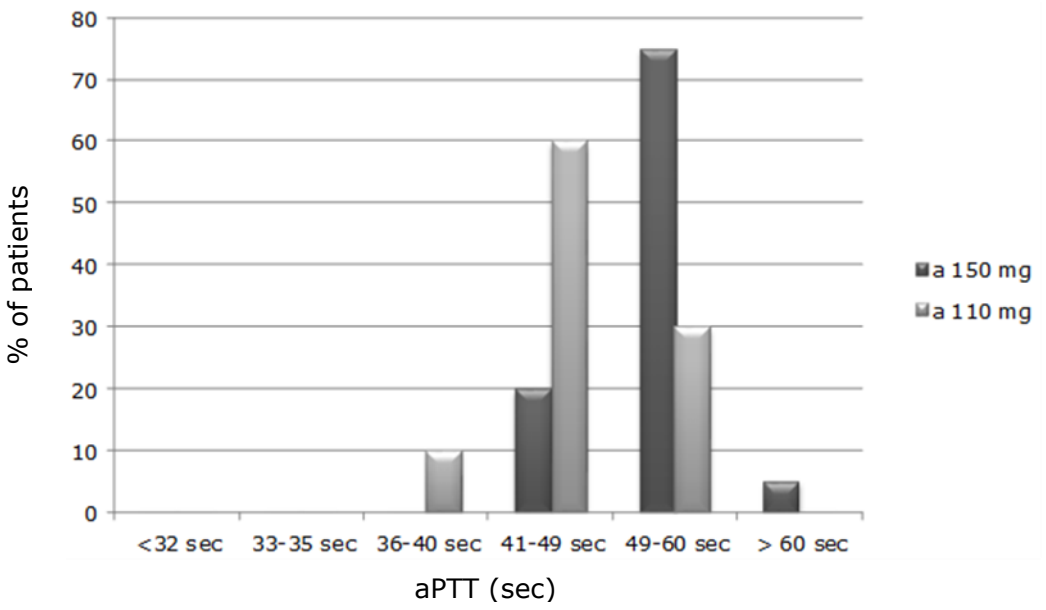
	a 150 mg	a 110 mg	p
Control*	31,23 ± 3,17	30,46 ± 2,79	> 0,05
After 4 hrs	52,95 ± 7,27	45,00 ± 2,64	< 0,001
After 8 hrs	44,15 ± 7,51	38,10 ± 2,05	< 0,001
After 12 hrs	36,70 ± 6,48	34,12 ± 1,12	> 0,05

* aPTT before dabigatran therapy

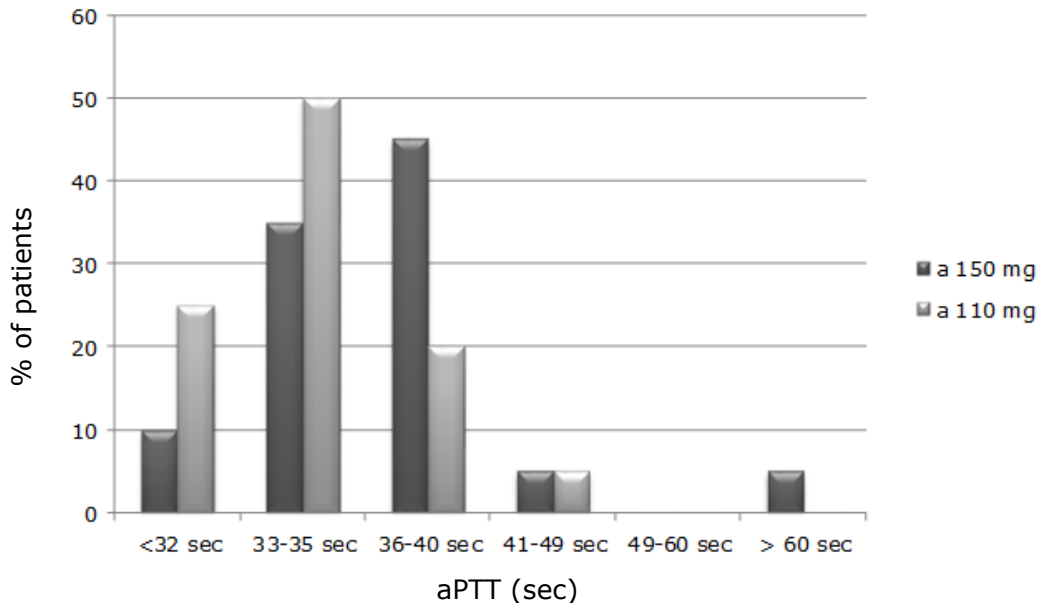
All patients had aPTT within normal ranges (25-35 sec) before the introduction of dabigatran. There was a statistically greater prolongation of aPTT values after 4 hours and 8 hours of taking the drug in patients who were treated with 150 mg of dabigatran as compared to patients taking 110 mg of dabigatran ($p < 0,001$), while after 12 hours of taking dabigatran there was no statistically significant difference in aPTT values between these two groups.

In patients who were taking 150 mg of dabigatran after 4 hours, aPTT was prolonged 1.5 to 1.8 times compared to controls; after 8 hours, 1.3 to 1.6 times; whereas after 12 hours it was 1.2 to 1.4 times greater then the initially measured aPTT values. In patients who received 110 mg of dabigatran after 4 hours, aPTT was prolonged 1.4 to 1.7 times, while after 12 hours of taking the drug aPTT was almost normalized (1.05 to 1.2 times greater). Patient distribution according to aPTT values after 4 hours and 12 hours of taking dabigatran are shown in Graph 1 and Graph 2.

Graph 1. Distribution of patients (%) according to aPTT values 4 hours after taking dabigatran



Graph 2. Distribution of patients (%) according to aPTT values 12 hours after taking dabigatran



Only one patient who was taking 150 mg of dabigatran had aPTT after 4 hours 2.8 times higher then normal, while after 12 h of dabigatran ingestion aPTT was still significantly prolonged (2.1 times). At the time of testing, the patient had bruises on the left arm and abdomen, a hematoma on the left forearm and complained of occasional epistaxis; the laboratory testings on the same day showed disturbed renal function (creatinine clearance about 35 ml/min).

Pearson's correlation analysis showed a strong positive correlation between initial aPTT (control) and total aPTT increase after taking dabigatran at a dose of 150 mg (r = 0,96, R2 = 0,93), as well as after 110 mg of dabigatran (r = 0,83, R2 = 0,89).

Discussion

When dabigatran was approved in October 2010 by the U.S. Food and Drug Administration (FDA) as an oral anticoagulant that can be used in the prevention of cerebrovascular events in patients with non-valvular atrial fibrillation, a major strength of this drug over vitamin-K antagonists, as indicated, was the drug pharmacokinetics that can be predicted, which eliminated the need for frequent laboratory testing to monitor its anticoagulant effect (9). The RE-LY study (Randomized Evaluation of Long-term anticoagulant therapy with dabigatran etexilate), conducted in order to investigate the efficacy and safety of dabigatran compared with warfarin in patients with non-valvular atrial fibrillation, showed that dabigatran at a dose of 150 mg twice a day had an efficacy equal to warfarin in achieving the anticoagulant effect, without any significant difference in the incidence of bleeding (10). The results in this study also showed that in patients older than 80 years and those with impaired renal function, bleeding occurred more frequently, but in these patients dabigatran is recommended in a single dose of 110 mg twice daily, while in those with significantly reduced renal function (creatinine clearance of 15-30 ml/min) a total daily dose of 150 mg (75 mg twice a day) is recommended. The factors that increase the risk of stroke, according to CHADS2 score (congestive heart failure, hypertension, age over 75 years, diabetes mellitus, previous stroke or transient ischemic attack), increase the risk of bleeding in patients with atrial fibrillation who are taking anticoagulant therapy (10). The later RELY-ABLE study (Long-term Multicenter Extension of Dabigatran Treatment in Patients with Atrial Fibrillation) showed no statistically significant difference in the incidence of stroke or mortality rate both in patients who received 150 mg of dabigatran and those taking 110 mg of the drug, but the higher dose of dabigatran was associated with a higher rate of bleeding (11). The literature describes 280 cases of fatal bleeding associated with dabigatran use in the last six years, especially in Japan and in the area of New Zealand (12), and the Australian Ministry of Health has confirmed that there are certain drugs that potentiate the effect of dabigatran and therefore increase the risk of bleeding, and those are primarily P-glycoprotein inhibitors such as ketoconazole, dronedarone and panrazol (13). Because of the risk of bleeding (primarily gastrointestinal and intracranial), as well as of overdosing dabigatran, it is necessary in certain situations to measure the anticoagulant effect of dabigatran and possibly introduce a reverse therapy.

Numerous studies have shown that aPTT as a screening coagulation test routinely used in almost all laboratories for testing hemostatic disorders is sensitive to the presence of dabigatran, and that there is a linear prolongation of aPTT with increasing concentrations of dabigatran up to 200 ng/mL (1, 14-16). In the presence of higher dabigatran concentrations there is a curvilinear prolongation of aPTT, which indicates that aPTT can not be used as a reliable test in the presence of supratherapeutic dabigatran concentration in the blood. On the other hand, TL Lindahl et al. in their study showed a linear response of aPTT even in the concentrations of dabigatran greater than 200 ng/mL (17). Our investigation confirmed that aPTT was a sensitive test for the presence of dabigatran, and it was most prolonged 4 hours after taking the drug (1.5-1.8 times when taking dabigatran in a single dose of 150 mg, and 1.4 to 1.7 times in the presence of 110 mg of dabigatran), but after 12 hours (immediately before taking the second dose of dabigatran) aPTT returned to the reference value or was barely prolonged. aPTT prolongation for more than twice, even 12 hours after taking the drug, was associated with bleeding and decreased renal function.

If bleeding occurs during the treatment with dabigatran, it should be immediately discontinued, and in case of bleedings that are life-threatening (especially intraocular, intracranial, gastrointestinal, retroperitoneal) it is recommended to apply prothrombin complex concentrate (PCC, activated and inactivated), activated factor VIIa or access hemodialysis (18). Today, a specific dabigatran antidote is available, idarucizumab (Praxbind), approved by the FDA and the European Medicines Agency (EMA), which reverses the effect of dabigatran in a few minutes, while the complete reversal of dabigatran is achieved in about 12 hours (19, 20).

Dabigatran therapy discontinuation because of surgery or other invasive procedures depends on the type and severity of the procedure and patient comorbidities, especially renal diseases. It is recommended that in the case of a minor intervention dabigatran has to be discontinued 2-3 days preoperatively and started again after 24 hours, while in the case of major surgical procedures or in those with impaired renal function (creatinine clearance less than 50 ml/min) dabigatran has to be discontinued 3-5 days before surgery and continued again after 48 hours (1). However, individual assessment of the anticoagulant effect of dabigatran in these cases is very important, and exactly here aPTT can be used as a semi-quantitative screening test that shows whether there is a pharmacologically significant anticoagulant effect of dabigatran at the time of testing. We must be particularly careful in the post-

operative period, because within 2-3 days after surgery false positive prolonged aPTT can occur due to heparin therapy in the perioperative period.

Conclusion

aPTT is a useful test for assessing the effect of dabigatran and can be used as a screening test in patients in whom the efficacy of the drug has to be urgently determined. Further studies should be directed towards determining the efficacy of aPTT in the assessment of the reverse effect of dabigatran in patients who are bleeding.

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AKTIVISANO PARCIJALNO TROMBOPLASTINSKO VREME KAO INDIKATOR PROCENE EFEKTA DABIGATRANA KOD BOLESNIKA SA NEVALVULARNOM ATRIJALNOM FIBRILACIJOM

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Dabigatran, antikoagulans nove generacije, po svojoj strukturi je direktni inhibitor trombina, ima brzi početak delovanja i relativno širok terapijski opseg, a njegova primena ne zahteva praćenje efikasnosti, kao što je to slučaj sa antagonistima vitamina K. Međutim, postoje određena hitna stanja koja zahtevaju procenu efikasnosti ovog leka. Cilj ovog rada bio je utvrditi da li se aPTT, kao skrining koagulacijski test, može pouzdano koristiti za procenu antikoagulantnog efekta dabigatrana.

Ispitivanje je obuhvatilo 32 bolesnika sa nevalvularnom atrijalnom fibrilacijom koji su uzimali dabigatran (Pradaxa, Boehringer Inhelheim) u pojedinačnoj dozi od 110 mg ili 150 mg dva puta dnevno. Svim bolesnicima je pre početka terapije urađen skrining koagualcije (PT, aPTT, INR). Merenje aPTT-a je vršeno 4 sata, 8 sati i 12 sati posle uzimanja leka.

Postoji statistički znajno veće produženje vrednosti aPTT-a posle 4 sata i 8 sati od uzimanja leka kod bolesnika koji su na terapiji dabigatranom od 150 mg u odnosu na bolesnike koji uzimaju 110 mg dabigatrana ($p < 0,001$), dok se posle 12 sati ne uočava statistički značajna razlika u vrednostima aPTT-a između bolesnika ove dve grupe. Postoji jaka korelacija između kontrolnih vrednosti aPTT-a i ukupnog porasta aPTT-a nakon uzimanja dabigatrana ($r = 0,96$ za dozu od 150 mg i $r = 0,83$ za dozu od 110 mg).

Za procenu efekta dabigatrana aPTT je koristan test i može se koristiti kao skrining test kod bolesnika kod kojih je potrebno hitno odrediti efikasnost leka.

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Ključne reči: *dabigatran, aPTT, antikoagulans, krvarenje*