Ned Tijdschr Klin Chem Labgeneesk 2014; 39: 156-157

Impact of dabigatran on routine and specific coagulation assays in patients treated by dabigatran

E.F.A. GEMEN[1], M.A. KARIMAN[1], J.H.A.W. van DIJK[1], J.W.M. van ECK[2] and N.C.V. PÉQUÉRIAUX[1]

Dabigatran etexilate, as a new oral anti coagulant (NOAC), is a direct thrombin inhibitor, which is used in patients with non-valvular atrial fibrillation, to reduce the risk of stroke. With a half-life-time of 14-17 hours and a renal clearance of 80%, no need of follow up of dabigatran plasma concentration is necessary in normal situations. In case of bleeding, overdose and before (urgent) surgery, it is recommended to investigate plasma levels of dabigatran and activated partial thromboplastin time (APTT). For other purpose coagulation tests are required and the clinician and the laboratory specialist must be aware of the impact of the NOAC's on the coagulation assays to avoid misinterpreting test results.

The aim of our study was to investigate the effect of dabigatran on the thrombin enzyme or -substrate depended routine and specific coagulation assays in patients treated by dabigatran. Until now, most of the studies are in vitro studies with dabigatran spiked plasma.

Methods

Nineteen patients with non-valvular atrial fibrillation treated by dabigatran (> 5 years) were included. Before the initial prescription of dabigatran, patients with coagulation disorders (abnormal coagulation tests) were excluded. The daily oral dose of dabigatran was twice 110 mg or 150 mg. Blood samples were collected in the morning during a doctor's visit. After centrifugation, citrated platelet poor plasma was obtained and stored at -70 °C.The following coagulation assays with corresponding reagents were measured on a STA-R Evolution® coagulation analyser (Diagnostica Stago, France): International normalized ratio (INR)/STA Hepato Quick®, prothrombin time (PT)/STA Neoplastin Plus®, activated partial thromboplastin time (APTT)/ STA APTT Kaolin®, fibrinogen/STA fibrinogen®, antithrombin III (AT-III)/STA antithrombin III®, lupus anticoagulant (LAC)/STACLOT DRVV® screen and confirm and PTT-LA®, and the extrinsic and intrinsic pathways factors II-V-VII-X-VIII-IX-XI-XII/STA factors®. Also we measured activated protein C resistance (APCR)/Pefakit APC-R factor V Leiden® (Pentapharm, Basel, Swiss) and dabigatran concentration with Hemoclot Thrombin-Inhibitors® (Hyphen Biomed, Neuville-sur-Oise, France) on the STA-R Evolution®. Figures show the influence of dabigatran increasing level on the different routine and specific coagulation tests. Ethical approval was obtained for conducting the study at the Jeroen Bosch Hospital.

Laboratory of Clinical Chemistry and Haematology[1] and Cardiology department[2], Jeroen Bosch Hospital 's-Hertogenbosch, The Netherlands

Correspondence: N. Péquériaux, MD, Laboratory of Clinical Chemistry and Haematology, Jeroen Bosch Hospital, 's-Hertogenbosch, The Netherlands

E-mail: n.pequeriaux@jbz.nl

Results

The dabigatran plasma concentration in our population ranged from 16 to 639 ng/ml.

APTT rises from 31 sec to 80 sec (Reference value: 26-36 sec) as dabigatran concentration increases (Fig. 1A). PT is prolonged from 14 sec to 35 sec (Reference value: 12-15 sec) (Fig. 1A). Factors of the intrinsic pathway (VIII-IX-XI-XII) were strongly underestimated as dabigatran concentration increases (Fig. 1C). Extrinsic factors II-V-VII-X were less underestimated (Fig. 1D). LAC diagnostic tests were strongly prolonged (Fig. 1B). APC-R was extremely influenced and was not measurable in our patients (data not shown). All affected assays seemed to respond in a dose-dependent manner. INR, fibrinogen (Clauss method) and AT-III (based on inhibition of thrombin) were not affected in most patients (Fig. 1A).

In one patient with a dabigatran plasma concentration of 639 ng/ml, all assays except INR and fibrinogen, were strongly affected (not shown).

Discussion

This study is the first study that investigate the influence of dabigatran on routine and specific coagulation assays in patients treated by dabigatran. Until now most of the studies have used plasma samples spiked with dabigatran and have evaluate the effect on dabigatran mostly on routine coagulation assays.

The dabigatran plasma concentrations in our patients corresponds to the expected concentrations found in patients treated with dabigatran in several published studies where the mean peak plasma concentration in patients who received a 150 mg twice daily regimen was approximately 180 ng/ml and ranged from 80 to 200 ng/ml.

As expected the APTT increases with the increase of the dabigatran concentration. Almost all patients showed a prolonged APTT even at trough concentrations of dabigatran. Intrinsic factors based on an APTT measurement are also influenced. Even at low dabigatran concentration the intrinsic factor activities are clearly

decreased. PT is also affected at therapeutic concentrations. For PT assays the dilution of the sample is important, effect on the PT will decrease with increasing sample dilution. Our assay is based on a final sample dilution of 1:3. The INR is not influenced since the final sample dilution in the assay is 1:40. PT based factor activity assays are less influenced but at dabigatran over-dosing concentration, factors are markedly reduced. Fibrinogen shows normal results in our Clauss method based assay. In the reagent a high amount of human thrombin (> 70 NIH units/ml) is used. Underestimation of fibrinogen is described with reagent with a lower concentration of thrombin (< 50 NIH units/ml). Unlike overestimated results previously reported with AT-III assay based on thrombin inhibition, AT-III results seems not affected at therapeutic concentrations. On the other hand, one patient with over-dosing (dabigatran plasma concentration of 639 ng/ml) shows overestimation of AT-III (170 %.). As expected with a thrombin inhibitor, APC resistance measurements based on prothrombinase activation are grossly affected. The effect on LAC tests has still not be completely investigated but it can be expected that thrombin inhibitors influenced LAC measurements. In our patients clotting times for dilute russel viper venom time and APTT based test are dose dependently prolonged. Our findings confirm the observations made with dabigatran spiked samples that dabigatran shows clinically significant interference in various thrombin enzyme or substrate dependent coagulation assays.
Falsely overestimation or underestimation of a specific test could lead to mistaken diagnosis. Laboratory specialist and clinician must be aware of these factitious test results to avoid inappropriate result interpretation, misdiagnosis and patient mismanagement.

Conclusion

Our results are in accordance with previous results reported in studies with dabigatran spiked plasma. We showed the effect of dabigatran on routine and specific coagulation assays. Most of the assays are influenced, sometimes strongly, which makes the daily interpretation of routine and specific coagulation results a challenge. Each laboratory will have to investigate the influence of dabigatran on the locally used coagulation assays and will have to develop a strategy about the request and the interpretation of coagulation assays in patients treated by dabigatran.

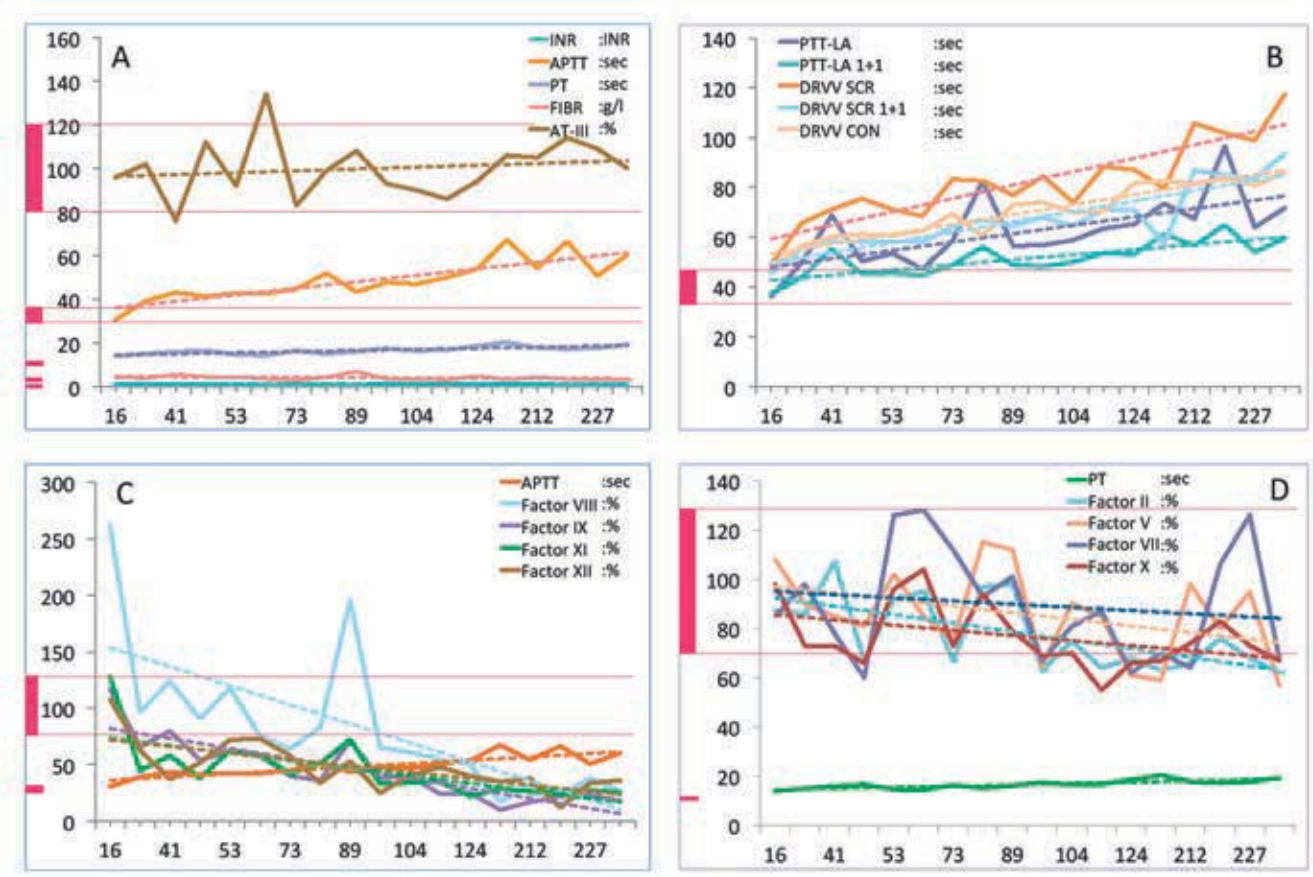

Figure 1. Effect of Dabigatran on A: Routine screening tests, B: LAC diagnostic tests, C: Intrinsic factors VIII, IX, XI, XII and D: Extrinsic factors II, V, VII, X. X-axis: dabigatran plasma concentration in ng/ml, Y-axis: results of the different coagulation assays with their own reference values.