INTERNATIONAL JOURNAL OF
MEDICAL BIOCHEMISTRY

DOI: 10.14744/ijmb.2019.33043
Int J Med Biochem 2019;2(2):35-40

Research Article

New generation oral anticoagulants may cause unreliable results in routine coagulation testing

Asuman Gedikbasi, Yuksel Gulen Cicek, Sebnem Tekin Neijmann, Soner Erdin, Nilgun Isiksacan, Zeynep Levent Cirakli, Alev Kural

Health Science University Bakirkoy Doctor Sadi Konuk Training and Research Hospital, Biochemistry Laboratory, Istanbul, Turkey

Abstract

Objectives: The aim of this study was to raise awareness among laboratory staff and physicians that prothrombin time-international normalized ratio (PT-INR) tests are not reliable in patients receiving direct oral anticoagulant treatment and, in particular, dabigatran.

Methods: PT-INR tests were performed on 151 plasma specimens using 3 coagulation analyzers with different methodologies: (i) the Thrombolyzer XRM (Behnk Elektronik GmbH & Co. KG, Norderstedt, Germany), which uses the optomechanical method; (ii) the Diagon CoagXL (Diagon Ltd., Budapest, Hungary), which uses the optical method; and (iii) the reference method for the study, the Stago STA Compact (Diagnostica Stago SAS., Asnières sur Seine Cedex, France), which uses the mechanical method. All of the results were analyzed using the intraclass correlation coefficient (ICC), Passing-Bablok regression analysis, and Bland-Altman analysis to examine the consistency of measurement techniques. Unacceptable results were observed for accuracy and precision when the analysis included 23 patients who were using the new generation oral anticoagulant dabigatran.

Results: The Stago STA Compact-Thrombolyzer PT-INR ICC was higher than that of Stago STA Compact-Diagon CoagXL, with a narrow 95% confidence interval. Passing-Bablok regression analysis of all of the results revealed a significant deviation from linearity ($p<0.01$), but there was no significant deviation from linearity when the results of the patients using dabigatran were excluded. Once the patients using dabigatran were excluded, the results of the comparison studies reached more acceptable limits.

Conclusion: It was determined that the use of a new generation oral anticoagulant (dabigatran) was a source of pre-analytical error. It will be of great benefit for clinicians to communicate with laboratory specialists in the follow-up of patients who are other than traditional anticoagulation cases.

Keywords: Coagulation, dabigatran, direct oral anticoagulants

Direct oral anticoagulants (DOACs) are widely used in routine clinical practice for the treatment of various thromboembolic diseases, including the prevention and management of venous thromboembolism and secondary prevention of stroke and systemic embolism in patients with non-valvular atrial fibrillation. Unlike vitamin K antagonists, DOACs have a rapid onset and provide more predictable pharmacokinetics and pharmacodynamics without requiring routine coagulation monitoring of constant dose regimens [1]. Treatment with a DOAC has been reported to significantly reduce the risk of major bleeding. In addition, intracranial hemorrhage, fatal bleeding, and clinically relevant major hemorrhage have occurred significantly less in DOAC users [2]. Dabigatran is a new generation oral anticoagulant drug that is excreted through the kidneys. Prothrombin time (PT) and activated partial thromboplastin time (aPTT) have been recommended

Address for correspondence: Yuksel Gulen Cicek, MD. Health Science University Bakirkoy Doctor Sadi Konuk Training and Research Hospital, Biochemistry Laboratory, Istanbul, Turkey
Phone: +90 555 346 87 00 **E-mail:** yukselgulencicek@gmail.com **ORCID:** 0000-0002-0328-0234

Submitted Date: April 02, 2019 **Accepted Date:** May 11, 2019 **Available Online Date:** June 13, 2019

to measure the effects of these drugs in the early stages [3]. At the time these tests were recommended for follow-up, there was not enough experience to know about possible sources of interference. Liquid chromatography (LC)-mass spectrometry (MS) is widely applied to measure the quantity of drugs. This technique has better selectivity than activity-based coagulation analysis [4, 5].

In clinical laboratories, when one method is replaced with another, or evaluation of a new or alternative method is needed and there is the possibility of compliance problems between 2 devices, the differences should be measured and assessed. Generally, to analyze the agreement between 2 quantitative measurements, the Bland-Altman (B&A) plot is used, and to solve data distribution problems and to detect a fixed or proportional difference between 2 methods, Passing-Bablok regression analysis is recommended [6]. In our laboratory, during the transition period to replace the coagulation analyzer in use, incompatible results were encountered in comparison and reproducibility studies. Consultations with the requesting clinicians revealed that the use of a new generation oral anticoagulant, dabigatran, was a source of preanalytical error. Thus, the aim of this study is to raise awareness among laboratory staff and physicians that PT-international normalized ratio (PT-INR) tests are not reliable in patients taking DOACs, and in particular, dabigatran therapy.

Materials and Methods

In a method comparison and reproducibility study conducted at the Bakirkoy Doctor Sadi Konuk Training and Research Hospital biochemistry laboratory between May 15, 2017 and May 23, 2017, PT-INR tests were performed for 151 plasma specimens. The specimens were collected in Vacuette 3.2% sodium citrate tubes (Greiner Bio-One, Kremsmünster, Austria). Non-hemolyzed samples taken in sufficient quantity from individuals without a bleeding diathesis, those who had a routine laboratory examination for a surgical procedure, and follow-up patients using anticoagulants were included in the study. This research was approved by the Bakirkoy Dr Sadi Konuk Training and Research Hospital Clinical Research Ethics Committee on December 17, 2018 (no: 2018-447).

Analysis was performed using 3 coagulation analyzers with different methodologies: (i) the Thrombolyzer XRM (Behnk Elektronik GmbH & Co. KG, Norderstedt, Germany), which uses the optomechanical method; (ii) the newly installed analyzer, the Diagon CoagXL (Diagon Ltd., Budapest, Hungary), which uses the optical method; and (iii) as a reference method, the Stago STA Compact (Diagnostica Stago SAS., Asnières sur Seine Cedex, France), which uses the mechanical method. The sampling conditions were standardized based on the Clinical and Laboratory Standards Institute H21-A5 publication "Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays, 5th Edition." All of the results were examined using the intraclass correlation coefficient (ICC), Passing-Bablok regression analysis, and B&A analysis methods to assess the consistency of the measurement techniques. Values that were unacceptably high for precision were observed in some of the plasma samples with a high INR value (>3). As a result of consultations with the clinics requesting the test, it was determined that 23 patients were using the new generation oral anticoagulant dabigatran. Other patients included in the study were not using a DOAC.

In this study, statistical analysis was performed using NCSS 2007 statistical software (NCSS, LLC, Kaysville, UT, USA). ICC was used to determine the consistency of Stago STA Compact, Diagon CoagXL and Thrombolyzer XRM measurements, and descriptive statistics (mean, SD) were also calculated. Linearity regression graphs and analysis were performed using Passing-Bablok regression, which is one of the Type II regression techniques. The Mann-Whitney U test was used to compare the differences in measurement. The results were evaluated as significant at $p<0.05$. Statistical analyses were performed using both the original results and after the exclusion of the 23 inconsistent results.

Results

The initial PT-INR measurements of the 2 comparison methods examined were determined to be greater than 0.700, which is the reliability acceptance limit for the Stago STA Compact, the reference method for this study. The Stago STA Compact-

Table 1. Intraclass correlation coefficient (ICC) analysis of Thrombolyzer XRM, Diagon CoagXL CoagXL, and Stago STA Compact hematology analyzers

	ICC-1 n=151	95% CI	ICC-2 n=128	95% CI
Stago STA Compact-Diagon CoagXL INR	0.875	(0.800-0.923)	0.980	(0.963-0.989)
Stago STA Compact-Thrombolyzer XRM INR	0.942	(0.899-0.966)	0.991	(0.981-0.996)
Stago STA Compact-Diagon CoagXL PT	0.873	(0.811-0.922)	0.975	(0.955-0.986)
Stago STA Compact-Thrombolyzer XRM PT	0.932	(0.883-0.961)	0.996	(0.993-0.998)

ICC-1: The results of all of the study patients were included; ICC-2: The results when 23 patients receiving dabigatran were excluded; ICC: Intraclass correlation coefficient; INR: international normalized ratio; PT: prothrombin time; Thrombolyzer XRM (Behnk Elektronik GmbH & Co. KG, Norderstedt, Germany), Diagon CoagXL (Diagon Ltd., Budapest, Hungary), Stago STA Compact (Diagnostica Stago SAS., Asnières sur Seine Cedex, France).

Thrombolyzer PT-INR ICC was higher than that seen for Stago STA Compact-Diagon CoagXL, with a narrow 95% confidence interval (CI) (Table 1). The Stago STA Compact-Diagon CoagXL INR B&A results yielded a mean of -0.27 and a 95% CI of 0.95 to -1.48.The Stago STA Compact-Thrombolyzer XRM INR B&A mean was 0.21 with a narrower 95% CI of 1.16 to -0.73. The

Table 2. Comparison of 2 analyzers with reference method

Bland-Altman Test	Mean-1 n=151	95% CI	Mean-2 n=128	95% CI
Stago STA Compact-Diagon CoagXL INR	-1.3	2.4/-5.1	-0.27	0.95/-1.48
Stago STA Compact-Thrombolyzer XRM INR	0.60	2.7/-1.5	0.21	1.16/-0.73
Stago STA Compact-Diagon CoagXL PT	-14.6	18.2/-47.5	-5.5	4.6/-15.7
Stago STA Compact-Thrombolyzer XRM PT	3.2	22.1/-15.7	-0.5	3.7/-4.7

Mean-1: The results of all of the study patients were included; Mean-2: The results when 23 patients receiving dabigatran were excluded; INR: International normalized ratio; PT: Prothrombin time; Thrombolyzer XRM (Behnk Elektronik GmbH & Co. KG, Norderstedt, Germany), Diagon CoagXL (Diagon Ltd., Budapest, Hungary), Stago STA Compact (Diagnostica Stago SAS., Asnières sur Seine Cedex, France).

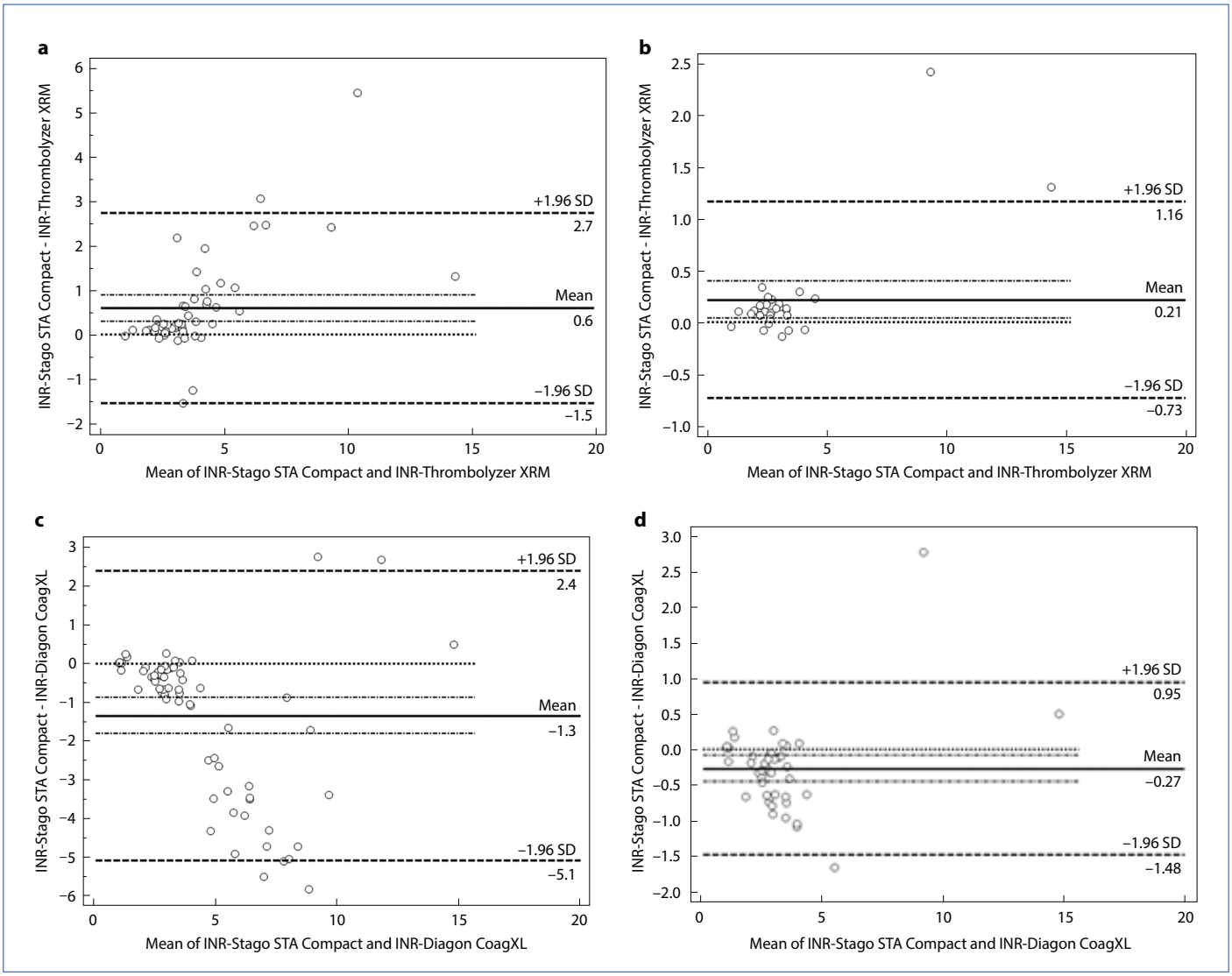

Figure 1. Bland-Altman plots for INR comparison. Plot of differeces Stago STA Compact-Thrombolyzer XRM (a, b) and Stago STA Compact-Diagon CoagXL (c,d) vs. the mean of the two measurements. Graphs a and c show the results of the whole group, while graphs b and d demonstrate the analysis when the 23 debigatran patients were excluded. INR: international normalized ratio. Thrombolyzer XRM (Behnk Elektronik GmbH & Co. KG, Norderstedt, Germany), Diagon CoagXL (Diagon Ltd., Budapest, Hungary), Stago STA Compact (Diagnostica Stago SAS., Asnières sur Seine Cedex, France).

Stago STA Compact -Diagon CoagXL PT B&A had a mean of -5.5 and a 95% CI of 4.6 to -15.7, and the Stago STA Compact-Thrombolyzer XRM PT B&A mean was -0.5 and the 95% CI was a narrower 3.7 to -4.7 (Table 2, Fig. 1). The Passing-Bablok regression analysis of all of the results revealed a significant deviation from linearity ($p<0.01$), but there was no significant deviation from linearity when the results of the patients using dabigatran were excluded (Fig. 2). Once the patients using dabigatran were excluded, the results reached more acceptable limits.

Discussion

The aim of this study was to perform method comparison research to evaluate the validity of a new analyzer in our laboratory against a reference technique during the exchange of the previous analyzer we use in routine coagulation analyses. In some patients, the PT-INR results in the comparison between devices were incompatible. We contacted the requesting clinics and found that 23 of the patients used dabigatran. These patients had high INR values and were followed up in the emergency medicine and gastroenterology clinic. We excluded these patients from the study and re-analyzed the results. The subsequent results were in a more acceptable range. Our goal was to raise awareness in both laboratories and clinics. Routine coagulation screening assays, including PT and activated partial thromboplastin time (aPTT), are widely performed on a routine and emergent basis in most clinical laboratories. These tests do not reliably reflect the effect of the DOAC anticoagulant effect. The sensitivity of PT and aPTT varies considerably with the reagents used and the DOAC being measured [7].

The use of DOACs has increased significantly as a result of the effects demonstrated in many thromboembolic disorders, such as recurrent venous thromboembolism and prevention of

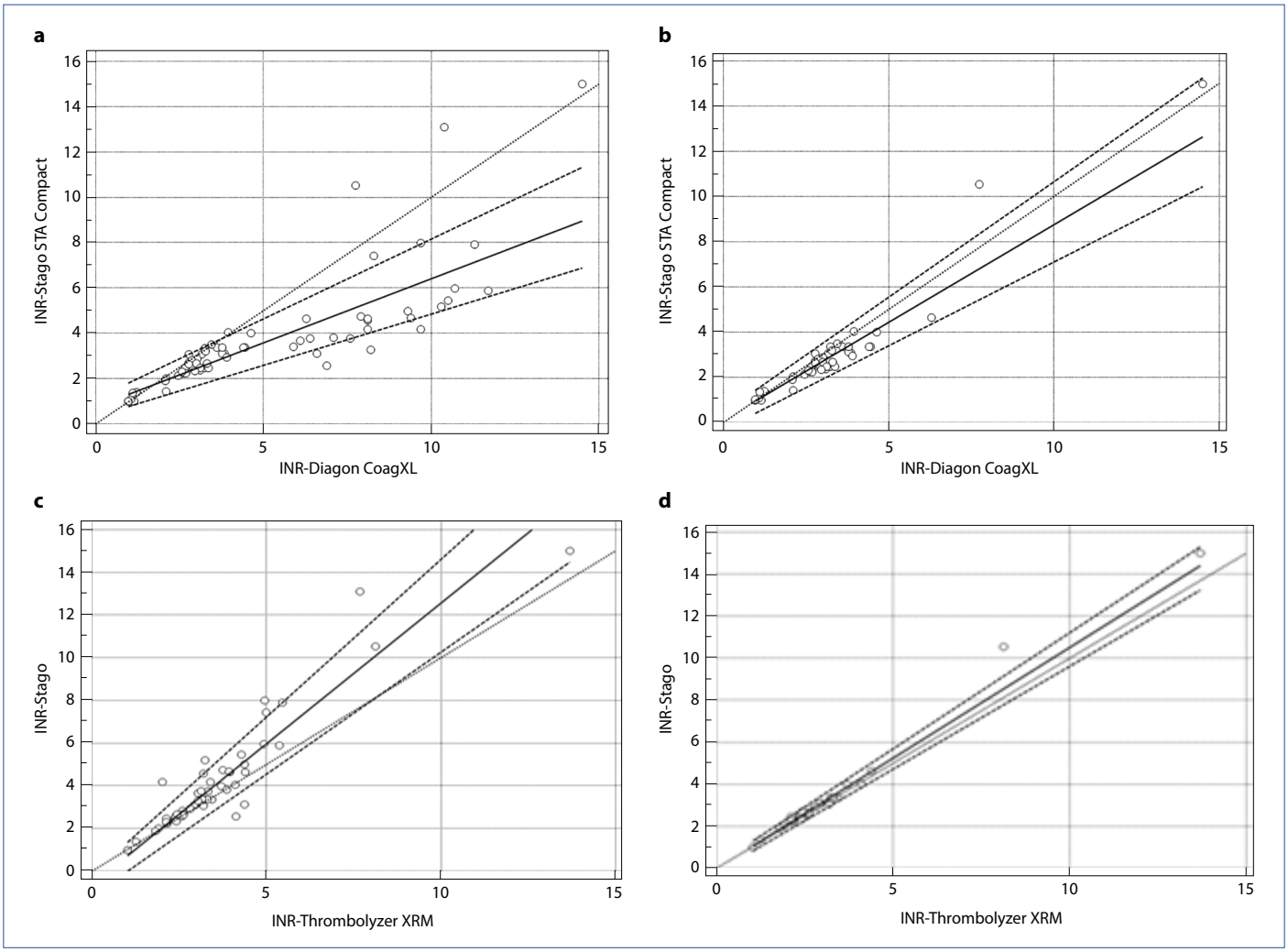

Figure 2. The regression line for INR obtained in the comparison of the Thrombolyzer XRM and Diagon CoagXL hematology analyzers with the Stago STA Compact. The regression equation is expressed as: y=a (95% CI)+b (95% CI)x(Passing-Bablok regression). Graphs a and c show the results of the entire group, while the b and d graphs illustrate the analysis once the 23 patients receiving dabigatran were excluded. INR: international normalized ratio. Thrombolyzer XRM (Behnk Elektronik GmbH & Co. KG, Norderstedt, Germany), Diagon CoagXL (Diagon Ltd., Budapest, Hungary), Stago STA Compact (Diagnostica Stago SAS., Asnières sur Seine Cedex, France).

stroke in patients with nonvalvular atrial fibrillation. These drugs are used in 1 or 2 doses per day with a fixed dose regimen. The dose to be used is determined by indications, age and/or creatinine clearance, body weight, and other drug use conditions [8]. DOACs exhibit more predictable pharmacokinetic and pharmacodynamic profiles than vitamin K antagonists (VKAs), and consequently, routine coagulation monitoring is not required [9]. The growth in the use of these drugs increases the need to know the effects on routine coagulation tests.

According to a systematic review performed by Bethany et al. [10], dabigatran can cause normal or prolonged values in routine coagulation tests like PT and aPTT. PT is used in the follow-up of VKAs. Dabigatran prolongs PT but is not associated with concentration; normal PT values do not exclude clinically sufficient dabigatran levels. aPTT is used in the follow-up of unfractionated heparin. Dabigatran can lead to excessive aPTT prolongation at low concentrations. It is aPTT can be useful to evaluate relative concentration in emergency conditions; however, it will not reflect supratherapeutic levels [11].

In recent studies, it was determined that DOACs are more effective and safer than warfarin in terms of bleeding risk. Because the pharmacokinetics of patients with a normal glomerular filtration rate (GFR) are predictable, no laboratory examination is required to control the effective dose. This increases the quality of life of patients. However, considering the age of the target population, although the GFR may initially be normal, it may be abruptly reduced by acute dehydration or the use of nonsteroidal drugs or angiotensin-converting enzyme inhibitor use. A reduction in renal clearance may lead to a toxic level of the drug [12, 13]. Gastrointestinal bleeding occurred in 14 of our patients who were using dabigatran, which may have reached a toxic dose, depending on the GFR reduction and the effect of the drug. The other patients were taking a normal dose and were admitted to the emergency department for other reasons.

The recommended screening assays for the anti-FIIa agent dabigatran are activated aPTT and/or thrombin time (TT), and the quantitative assays (using a dabigatran standard) are a diluted TT/direct thrombin inhibitor assay (e.g., Hemoclot thrombin inhibitor; Hyphen Biomed, Neuville-sur-Oise, France) or an ecarin-based assay, such as the ecarin clotting time [14]. The aPTT test has been reported to be more responsive to dabigatran levels in the literature. It is noteworthy that when using the PT-INR in this study, there were unacceptable results in terms of compatibility and reproducibility between devices in PT testing.

Conclusion

In some clinical situations, such as severe bleeding to critical organs (e.g., intracerebral hemorrhage), probable overdose intake, or emergency surgery, it is important for clinicians to assess the anticoagulant status of a patient under dabigatran therapy before deciding on future management strategies. Clinicians should communicate with laboratory specialists or provide information about the type of anticoagulant agent used.

Conflict of interest: There is no conflict of interest.

Ethics Committee Approval: This research was approved by the Bakirkoy Dr Sadi Konuk Training and Research Hospital Clinical Research Ethics Committee on December 17, 2018 (no: 2018-447).

Financial Disclosure: The authors declared that this study has received no financial support.

Peer-review: Externally peer-reviewed.

Authorship contributions: Concept – A.G., S.T.N.; Design – A.G., Y.G.C., S.T.N.; Supervision – A.G., A.K.; Funding – S.E., N.I., Z.L.C., A.K.; Materials – A.G., S.T.N., Y.G.C.; Data collection &/or processing – A.G., Y.G.C., S.T.N.; Analysis and/or interpretation – A.G., Y.G.C., S.T.N.; Literature search – S.E., N.I., Z.L.C., A.K.; Writing – A.G., Y.G.C.; Critical review – A.G., Y.G.C.

References

1. Burnett AE, Mahan CE, Vazquez SR, Oertel LB, Garcia DA, Ansell J. Guidance for the practical management of the direct oral anticoagulants (DOACs) in VTE treatment. J Thromb Thrombolysis 2016;41:206–32. [CrossRef]
2. van Es N, Coppens M, Schulman S, Middeldorp S, Büller HR. Direct oral anticoagulants compared with vitamin K antagonists for acute venous thromboembolism: evidence from phase 3 trials. Blood 2014;124:1968–75. [CrossRef]
3. Favaloro EJ, Pasalic L, Curnow J, Lippi G. Laboratory Monitoring or Measurement of Direct Oral Anticoagulants (DOACs): Advantages, Limitations and Future Challenges. Curr Drug Metab 2017;18:598–608. [CrossRef]
4. Schmitz EM, Boonen K, van den Heuvel DJ, van Dongen JL, Schellings MW, Emmen JM, et al. Determination of dabigatran, rivaroxaban and apixaban by ultra-performance liquid chromatography - tandem mass spectrometry (UPLC-MS/MS) and coagulation assays for therapy monitoring of novel direct oral anticoagulants. J Thromb Haemost 2014;12:1636–46. [CrossRef]
5. Dale BJ, Chan NC, Eikelboom JW. Laboratory measurement of the direct oral anticoagulants. Br J Haematol 2016;172:315–36. [CrossRef]
6. Giavarina D. Understanding Bland Altman analysis. Biochem Med (Zagreb) 2015;25:141–51. [CrossRef]
7. Adcock DM, Gosselin R. Direct Oral Anticoagulants (DOACs) in the Laboratory: 2015 Review. Thromb Res 2015;136:7–12.
8. Douxfils J, Ageno W, Samama CM, Lessire S, Ten Cate H, Verhamme P, et al. Laboratory testing in patients treated with direct oral anticoagulants: a practical guide for clinicians. J Thromb Haemost 2018;16:209–19. [CrossRef]
9. Eikelboom JW, Quinlan DJ, Hirsh J, Connolly SJ, Weitz JI. Laboratory Monitoring of Non-Vitamin K Antagonist Oral Anticoagulant Use in Patients With Atrial Fibrillation: A Review. JAMA Cardiol 2017;2:566–74. [CrossRef]

10. Samuelson BT, Cuker A, Siegal DM, Crowther M, Garcia DA. Laboratory Assessment of the Anticoagulant Activity of Direct Oral Anticoagulants: A Systematic Review. Chest 2017;151:127–38. [CrossRef]
11. Mijovski MB. Advances in monitoring anticoagulant therapy. Advances in Clinical Chemistry 2019;90:1–17. [CrossRef]
12. Steiner T, Böhm M, Dichgans M, Diener HC, Ell C, Endres M, et al. Recommendations for the emergency management of complications associated with the new direct oral anticoagulants (DOACs), apixaban, dabigatran and rivaroxaban. Clin Res Cardiol 2013;102:399–412. [CrossRef]
13. Singh T, Maw TT, Henry BL, Pastor-Soler NM, Unruh ML, Hallows KR, et al. Extracorporeal therapy for dabigatran removal in the treatment of acute bleeding: a single center experience. Clin J Am Soc Nephrol 2013;8:1533–9. [CrossRef]
14. Lippi G, Favaloro EJ. Recent guidelines and recommendations for laboratory assessment of the direct oral anticoagulants (DOACs): is there consensus? Clin Chem Lab Med 2015;53:185–97. [CrossRef]