Preporuke za laboratorijsku dijagnostiku DOAK lijekova: prijeanalitički, analitički i poslijeanalitički čimbenici

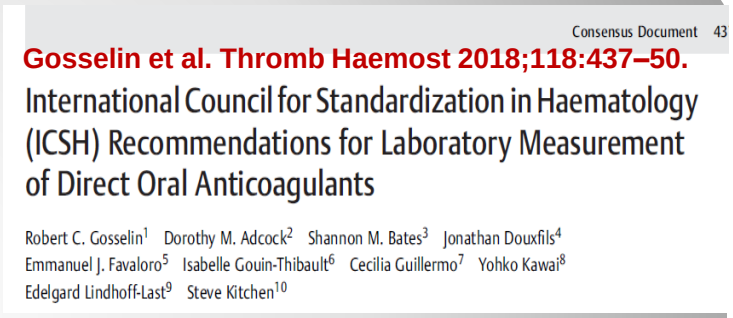
Sandra Margetić
Odjel za laboratorijsku hematologiju i koagulaciju
Klinički zavod za kemiju
KBC Sestre milosrdnice, Zagreb

4. listopada 2025.

Uvod

Preporuke za lab. dg. DOAK lijekova:

1. Međunarodni savjet za standardizaciju u hematologiji (*International Council for Standardization in Hematology, ICSH*)



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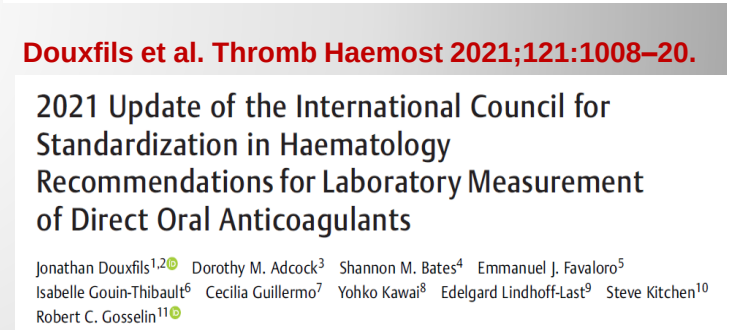
Gosselin et al. Thromb Haemost 2018;118:437–50.

International Council for Standardization in Haematology (ICSH) Recommendations for Laboratory Measurement of Direct Oral Anticoagulants

Robert C. Gosselin[1] Dorothy M. Adcock[2] Shannon M. Bates[3] Jonathan Douxfils[4] Emmanuel J. Favaloro[5] Isabelle Gouin-Thibault[6] Cecilia Guillermo[7] Yohko Kawai[8] Edelgard Lindhoff-Last[9] Steve Kitchen[10]



Douxfils et al. Thromb Haemost 2021;121:1008–20.

2021 Update of the International Council for Standardization in Haematology Recommendations for Laboratory Measurement of Direct Oral Anticoagulants

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2. Istraživački projekt Hrvatske zaklade za znanost

Novi oralni antikoagulansi: odnos između koncentracije i antikoagulantnog učinka lijeka LAB-NOAC; IP-206-06-8208

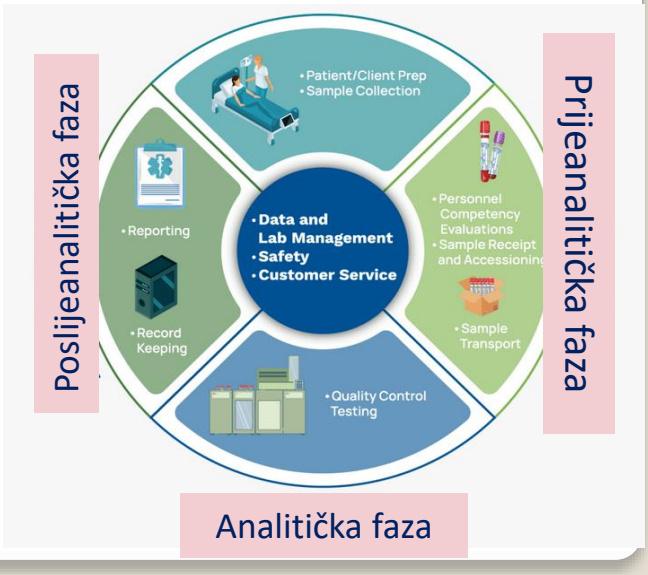
- **Trajanje projekta: 5 godina (2017. – 2022.)**
- **Nositelj projekta: Klinički zavod za kemiju KBC Sestre milosrdnice, Zagreb**
- **Klinika za kardiologiju KBCSM**
- **Klinika za neurologiju KBCSM**
- **Klinika za traumatologiju KBCSM**

Preporuke za laboratorijsku dijagnostiku DOAK lijekova

- **Prijeanalitička faza**
- postupak uzorkovanja
- analitički uzorak
- stabilnost uzorka
- pohrana uzorka…

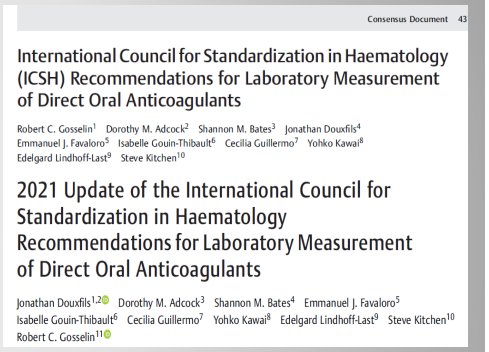
- **Analitička faza**
- probirne koagulacijske pretrage i DOAK lijekovi
- kvantitativne metode određivanja DOAK lijekova
- ostale metode (polukvantitativne, kvalitativne)
- kontrola kvalitete

- **Poslijeanalitička faza**
- izražavanje rezultata
- očekivane terapijske vrijednosti (lijek/doza/kl. indikacija)
- interpretacija rezultata



Preporuke za laboratorijsku dijagnostiku DOAK lijekova

1. Prijeanalitički čimbenici



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Uzorak

1. Metoda LC/MS-MS

- Analitički uzorak: serum ili plazma
- Očekivane terapijske vrijednosti u serumu i plazmi se razlikuju!
  - objavljene u ICSH preporukama – odnose se na uzorak plazme

2. Metode: koagulometrijske, kromogene na automatiziranim koagulometrima

- Uzorkovanje krvi: 3,2% natrijev-citrat
- Analitički uzorak: plazma (PPP)

Preporuke za laboratorijsku dijagnostiku DOAK lijekova

Stabilnost uzorka (plazme)

Lijek	Sobna temp. (h)	5°C	-20°C
Dabigatran	24 h**	24 h	min. 30 dana*
Rivaroksaban	8 h	48 h	min. 30 dana
Apiksaban	8 h	48 h	min. 30 dana
Edoksaban**	4 h	?***	min. 30 dana

***Dabigatran – stabilnost do 14 mjeseci**
****Dabigatran – ako se koristi modifikacija TV testa (dTV) – 4h na sobnoj temp.**
****Edoksaban - pohranom na sobnoj temp. 8 sati stabilnost se smanjuje (18% niže vr.) !**
*****Edoksaban – 14 dana za LC/MS-MS m. – nije poznato može li se stabilnost primijeniti za funkcionalne anti-Xa testove**

- McGrail R, Revsholm J, Nissen PH, Grove EL, Hvas AM. Stability of direct oral anticoagulants in whole blood and plasma from patients in steadystate treatment. ThrombRes 2016;148:107–110.
- Gous T, et al. Measurement of the direct oral anticoagulants apixaban, dabigatran, edoxaban, and rivaroxaban in human plasma using turbulent flow liquid chromatography with high-resolution mass spectrometry. Ther Drug Monit 2014;36(05):597–605.

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Preporuke za laboratorijsku dijagnostiku DOAK lijekova

Stabilnost uzorka (plazma)

prema proizvođaču…

Lijek	Sobna temp. (h)*	5°C**	-20°C*** (d)	-74°C*** (mj)
Dabigatran	48	/	30 d	6 mj
Rivaroksaban	4	/	30 d	6 mj
Apiksaban	4	/	30 d	6 mj
Edoksaban	4	/	30 d	6 mj

- * Plazma pohranjena na stanicama i plazma odvojena od stanica
- ** Nema dostupnih podataka
- *** Plazma odvojena od stanica

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Lijek	Sobna temp. (h)	5°C	-20°C
Dabigatran	24 h	24 h	min. 30 dana
Rivaroksaban	8 h	48 h	min. 30 dana
Apiksaban	8 h	48 h	min. 30 dana
Edoksaban	4 h	?	min. 30 dana

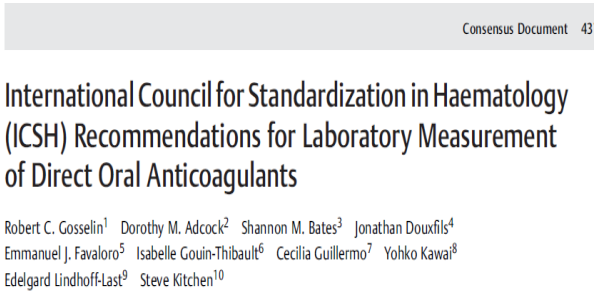
Preporuke za laboratorijsku dijagnostiku DOAK lijekova

Stabilnost uzorka (plazma)

Svi DOAK lijekovi:

- ✓ **ukoliko nije moguće odrediti koncentraciju unutar deklariranog prihvatljivog vremena preporuča se zamrzavanje plazme na ≤ -20°C do 30 dana**
- ✓ **dugotrajna pohrana uzorka plazme (> 30 dana) – preporuča se zamrzavanje na ≤ -60 °C**
- ✓ **ciklus zamrzavanja i odmrzavanja – ispitivanja višekratnih (3x) ciklusa zamrzavanja i odmrzavanja nisu pokazala razlike u stabilnosti uzorka plazme**
- ✓ **proturječni podaci za dabigatran i apiksaban – preporuka: jednokratno zamrzavanje i odmrzavanje**

- *McGrail R, Revsholm J, Nissen PH, Grove EL, Hvas AM. Stability of direct oral anticoagulants in whole blood and plasma from patients in steadystate treatment. ThrombRes 2016;148:107–110.*
- *Gous T, et al. Measurement of the direct oral anticoagulants apixaban, dabigatran, edoxaban, and rivaroxaban in human plasma using turbulent flow liquid chromatography with high-resolution mass spectrometry. Ther Drug Monit 2014;36(05):597–605.*



Preporuke za laboratorijsku dijagnostiku DOAK lijekova

1. Prijeanalitički čimbenici

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Vrijeme uzorkovanja za specifične pretrage hemostaze na čiji rezultat DOAK lijekovi imaju interferirajući učinak:

- ✓ **najmanje 3 dana nakon prestanka uzimanja DOAK lijeka kod bolesnika s urednom bubrežnom funkcijom (ili dulje u bolesnika s bubrežnom insuficijencijom)**

- ✓ **Alternativni postupak:**
  - **neutralizacija lijeka u plazmi primjenom medicinskog aktivnog ugljena (komercijalni pripravci, *in house* metoda) – omogućuje određivanje pretraga tijekom DOAK terapije**

Preporuke za laboratorijsku dijagnostiku DOAK lijekova

1. Prijeanalitički čimbenici

Vrijeme uzorkovanja za određivanje konc. DOAK-a: MK i/ili VK???

MK (min., ostatna konc.) = neposredno prije sljedeće doze

VK (vršna konc.) = 2 (1 - 3) sata nakon zadnje doze

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Nehitna stanja:

- > 80 god - MK
- bolesnici s bubrežnom insuficijencijom s/bez hemodijalize - MK
- sumnja na interakciju s drugim lijekovima – MK
- BMI > 40 kg/m2 – MK

Važno: minimalno 5 uzastopnih doza lijeka (ravnotežno stanje konc. lijeka u krvi)!

- prije planiranog (elektivnog) zahvata s visokim rizikom krvarenja – unutar nekoliko sati prije zahvata u svrhu isključivanja klinički značajne konc. (≥30 ng/mL) uz uvjet: zadnja doza prije najmanje 12 ili 24 sata (ovisno 1x ili 2x/dan)

IP-2016-06-8208 LAB-NOAC

Nehitna stanja:

- > 80 god – MK i VK
- bolesnici s bubrežnom insuficijencijom s/bez hemodijalize – MK i VK
- sumnja na interakciju s drugim lijekovima – MK i VK
- BMI > 40 kg/m2 – MK i VK

Minimalno 5 uzastopnih doza lijeka (ravnotežno stanje konc. lijeka u krvi)!

Preporuka: odrediti i MK i VK u svim nehitnim stanjima !!!

Primjer 1.

Margetić S, Ćelap I, Lovrenčić-Huzjan A, Bosnar Puretić M, Roje Bedeković M, Razum M, Mihić R, Šupraha-Goreta S. **Presentation of the patient with low peak dabigatran levels in plasma suggests the importance of quantitative measurement of DOAC drugs in clinical decision making and treatment.** Res Pract Thromb Haemost 2021;5(Suppl 2):12589:285.

Table 1. The results of laboratory testing in a patient treated with dabigatran followed by replacement therapy with rivaroxaban.

	Drug	Dabigatran*	Dabigatran*	Dabigatran*	Rivaroxaban**
Dabigatran (ng/mL)	Peak	35	16	19	/
	Trough	34	<5	14	/
Rivaroxaban (ng/mL)	Peak	/	/	/	215
	Trough	/	/	/	30
Fibrinogen (g/L)	Peak	2.8	/	/	[illegible]
	Trough	2.6	/	/	4.0
PT (% act.)	Peak	49	52	51	126
	Trough	49	49	51	131
INR	Peak	1.4	1.4	1.4	0.9
	Trough	1.4	1.4	1.4	0.9
aPTT (s)	Peak	45	38	33	26
	Trough	38	38	30	25
TT (s)	Peak	145	30	33	15
	Trough	108	30	28	14
D-dimer	Peak	/	0.35	0.36	/

ISTH VIRTUAL CONGRESS 2021 JULY 17–21 ISTH2021.ORG HOSTED FROM PHILADELPHIA SCIENCE CONQUERING DISEASE

Presentation of the patient with low peak dabigatran levels in plasma suggests the importance of quantitative measurement of DOAC drugs in clinical decision making and treatment

Margetić Sandra[1], Ćelap Ivana[1], Lovrenčić-Huzjan Arijana[2], Bosnar Puretić Marijana[2], Roje Bedeković Marina[2], Razum Marija[1], Mihić Roman[1], Šupraha Goreta Sandra[3]

[1]Department of Clinical Chemistry, Sestre Milosrdnice University Hospital Center, Zagreb, Croatia
[2]Department of Neurology, Sestre Milosrdnice University Hospital Center, Zagreb, Croatia
[3]Department of Biochemistry and Molecular Biology, Faculty of Pharmacy and Biochemistry, University of Zagreb, Croatia

HRZZ Croatian Science Foundation

INTRODUCTION In special clinical conditions quantitative measurement of direct oral anticoagulants (DOACs) in plasma, should be performed to help clinical decision making and treatment.

AIM To present a case report of a patient treated with dabigatran in whom low peak drug concentrations in plasma suggested inadequate anticoagulation.

METHOD

A 88 YEARS OLD MALE WAS HOSPITALIZED DUE TO RECURRENT SYMPTOMS OF CEREBRAL ISCHEMIA FOR LAST FEW DAYS AND BRAIN ISCHEMIA WAS CONFIRMED ON CT SCAN. BEFORE HOSPITALIZATION THE PATIENT WAS TAKING DABIGATRAN (110 MG TWICE DAILY) DUE TO PERSISTENT ATRIAL FIBRILLATION.

DABIGATRAN WAS MEASURED BY INNOVANCE DTI ASSAY AND RIVAROXABAN WAS MEASURED BY ANTI-FXA ASSAY ON BCSXP ANALYZER (SIEMENS HEALTHINEERS, GERMANY). THE STUDY WAS FUNDED BY THE CROATIAN SCIENCE FOUNDATION AS A PART OF THE RESEARCH PROJECT IP-2016-06-8208.

RESULTS

Peak (two hours after the last dose) concentrations of dabigatran measured at three consecutive days were 35, 16 and 19 ng/mL, whereas trough (before the next dose) concentrations were 34, <5 and 14 ng/mL, respectively. Low peak dabigatran concentrations obtained in three consecutive measurements suggested anticoagulation undertreatment that might result with embolic complications. This observation led to the decision to replace dabigatran therapy with rivaroxaban (1x20 mg/day). Measurement of rivaroxaban plasma levels after the second day of administration has shown both peak and trough rivaroxaban concentrations within expected therapeutic values i.e., 215 and 30 ng/mL, respectively. No new ischemic symptoms occurred and the patient was discharged home with rivaroxaban therapy. Results of laboratory testing are presented in Table 1.

Table 1. The results of laboratory testing in a patient treated with dabigatran followed by replacement therapy with rivaroxaban.

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PT (% act.)	Peak	49	52	51	126
	Trough	49	49	51	131
INR	Peak	1.4	1.4	1.4	0.9
	Trough	1.4	1.4	1.4	0.9
aPTT (s)	Peak	45	38	33	26
	Trough	38	38	30	25
TT (s)	Peak	145	30	33	15
	Trough	108	30	28	14
D-dimer (mg/L FEU)	Peak	/	0.35	0.36	/
	Trough	/	0.34	0.30	/
creatinine (μmol/L)		74	/	73	/
eGfR (CKD-EPI (ml/min/1,73m2)		78	/	79	/
hemoglobin (g/L)		133	/	150	/

PT – prothrombin time; INR – international normalized ratio; aPTT – activated partial thromboplastin time; TT – thrombin time; CKD-EPI – Chronic Kidney Disease - Epidemiology Collaboration, * measurements performed during the three consecutive days on dabigatran therapy; **measurements performed two days after switching dabigatran with rivaroxaban therapy

CONCLUSIONS

Persistently low peak dabigatran concentrations could contribute to inadequate anticoagulation and consequent embolic complications in our patient. This case report strongly suggests the importance of quantitative measurement of DOACs levels in plasma in selected clinical conditions confirming as an effective approach in clinical decision making and treatment.

REFERENCES

1. JIGNESH P. PATEL ET AL. PROGRESS IN THE MONITORING OF DIRECT ORAL ANTICOAGULANT THERAPY. B J HAEM 2019; 184, 912–24.
2. CONNORS JM. TESTING AND MONITORING DIRECT ORAL ANTICOAGULANTS. BLOOD 2018, 132:2009–15.

CONTACT INFORMATION

margeticsandra@gmail.com

Primjer 2.

Margetic S, Celap I , Razum M, Tomic F, Šupraha-Goreta S. **Presentation of Four Real-Life Patients with Persistently Low Both Peak and Trough Direct Oral Anticoagulant Levels in Plasma: Potential Effect of Drug-Drug Interactions**. Res Pract Thromb Haem 2023;7(Suppl 2):466.

		Patient 1	Patient 2	Patient 3	Patient 4
Age (years)		65	87	82	22
Sex		male	male	female	male
1.DOAC drug	Conc. (ng/mL) Peak Trough	rivaroxaban 29 / 50* 21 / 30*	dabigatran 45 / 49* 34 /35*	dabigatran 46 / 54* 4 / 38*	dabigatran 31 / 0* 26 / 0*
DOAC dose		1x20 mg	2x110 mg	2x110 mg	2x150 mg
Diagnoses		St. post CVI Atrial fibrillation Art.hypertension Dyslipidemia	St. post CVI Atrial fibrillation Art.hypertension Dyslipidemia Ca prostatae Mb. coronarius	St. post CVI Atrial fibrillation parox. Art.hypertension Osteoporosis Thrombophlebitis	Epilepsy Traumatic subarachnoid hemorrhage (CVI)
Concomitant drugs		Pantoprazole Ramipril Atorvastatine Bisoprolol	Metildigoxin Simvastatine Triapine	Perindopril Oxazepam	Carbamazepine Levetiracetam
Possible drug-drug interaction	P-gp inducer	Atorvastatine	Metildigoxin Simvastatine	-	Carbamazepine
Adverse event during therapy		Recurrent CVI	Recurrent CVI	Recurrent CVI	No adverse event
2. DOAC drug applied	Conc. (ng/mL) Peak Trough	dabigatran 2x150 mg 95 **/ 114*** 26** / 43***	rivaroxaban 1x20 mg 101** /129*** 28** / 24***	No drug replacement No data	rivaroxaban 1x20 mg 91**** 14****

*two independent measurements; **2-5 days after DOAC drug replacement; ***1-2 months after DOAC drug replacement; ****1 month after DOAC replacement
DOAC = direct oral anticoagulant; CVI = cerebrovascular insult; P-gp = P-glycoprotein

- **DOAK + P-gp induktor: (pacijenti 1, 2 .4.) – interakcija s DOAK lijekom objašnjava se trajno niskim MK i VK DOAK-a !!!**
- **Određivanje VK uz MK potvrđuje koncentracije značajno niže u odnosu na preporučene terapijske vrijednosti !!!**

	Dabigatran		
Indikacija	NVAF (10 – 90 percentil)	VTE medijan (IQR)	p
Doza			
MK, ng/mL	91 (40-215)	60 (39-95)	
VK, ng/mL	175 (74-383)	175 (117-275)	

Preporuke za laboratorijsku dijagnostiku DOAK lijekova

1. Prijeanalitički čimbenici

Vrijeme uzorkovanja za određivanje konc. DOAK-a

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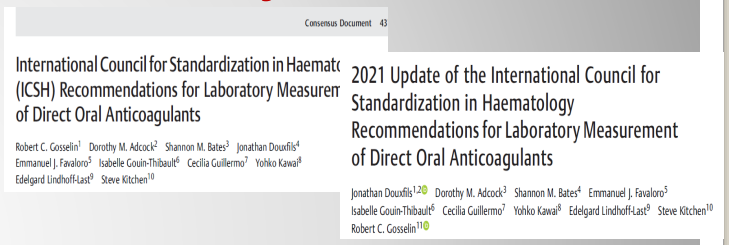
Hitna stanja:

1. Akutno krvarenje tijekom terapije
2. Tromboembolija tijekom terapije
3. Donošenje odluke o vremenu izvođenja hitnog zahvata – isključivanje klinički značajne konc. lijeka ($\geq$ 30 ng/mL) prije hitnog zahvata ili $\geq$ 50 ng/mL kod pacijenata s krvarenjem s/bez potrebe za hitnim zahvatom
4. Donošenje odluke o potrebi hitnog poništavanja učinka lijeka (antidot)
5. S povećanim rizikom krvarenja (bubrežna insuficijencija, bolest jetre, sumnja na predoziranje lijekom, odluka o trombolitičkoj terapiji)
6. Ostala hitna stanja prema procjeni kliničara za pojedinog bolesnika

- **Uzorkovanje tijekom hitne obrade pacijenta (neovisno o zadnjoj dozi)**

Preporuke za laboratorijsku dijagnostiku DOAK lijekova

2. Analitički čimbenici - metode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International Council for Standardization in Haemat
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Robert C. Gosselin[11]

PV, APTV

- rezultati unutar referentnog intervala ne isključuju terapijske konc. DOAK lijeka u cirkulaciji
- neosjetljive na terapijske koncentracije apiksabana (unutar RI i kod vršnih konc. lijeka)
- različit utjecaj ovisno o dozi, lijeku, sastavu reagensa, vremenu uzorkovanja u odnosu na zadnju dozu…

TV

- unutar referentnog intervala upućuje na odsutnost klinički značajne konc. (>30 ng/mL) dabigatrana
- produženo već kod minimalnih konc. dabigatrana/nemjerljiva vrijednost kod terapijskih koncentracija DOAK-a
- pretraga neosjetljiva na DOAK lijekove iz skupine inhibitora FXa

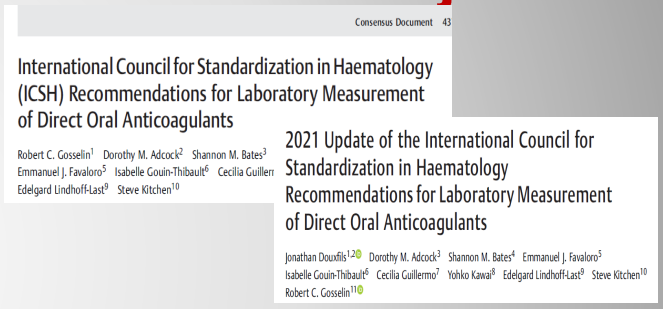
Probirne koagulacijske pretrage PV, APTV, TV ne smiju se koristiti za procjenu koncentracije i/ili antikoagulacijskog učinka DOAK lijekova !

Za određivanje koncentracije DOAK lijekova koristiti isključivo specifične kvantitativne metode!

Preporuke za laboratorijsku dijagnostiku DOAK lijekova

2. Analitički čimbenici

Kvantitativne metoda određivanja DOAK lijekova


Consensus Document 43

International Council for Standardization in Haematology (ICSH) Recommendations for Laboratory Measurement of Direct Oral Anticoagulants

Robert C. Gosselin[1] Dorothy M. Adcock[2] Shannon M. Bates[3] Emmanuel J. Favaloro[5] Isabelle Gouin-Thibault[6] Cecilia Guillen Edelgard Lindhoff-Last[9] Steve Kitchen[10]

2021 Update of the International Council for Standardization in Haematology Recommendations for Laboratory Measurement of Direct Oral Anticoagulants

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1. LC-MS/MS

(spektrometrija masa spregnuta s tekućinskom kromatografijom visoke djelotvornosti)

- metoda zlatnog standarda (granice detekcije i kvantifikacije 0,02 – 3 ng/mL, mjerni raspon 5 – 500 ng/mL
- neprikladna za svakodnevni rad (tehnički zahtjevna, skupa oprema, poznavanje analitičke metodologije, nedostupna u većini laboratorija…)

2. Kvantitativne metode na automatiziranim koagulometrima –

- koagulometrijske i kromogene

Direktni inhibitor trombina

dabigatran

- ✓ dTT - diluirano trombinsko vrijeme
- ✓ ECT - ekarinski koagulometrijski test
- ✓ ECA - ekarinska kromogena metoda
- ✓ anti - FIIa test

Direktni inhibitori FXa

rivaroksaban, apiksaban, edoksaban

- ✓ **Anti – Xa test**

uz kalibraciju specifičnim lijekom (R, A, E)

Preporuke za laboratorijsku dijagnostiku DOAK lijekova

2. Analitički čimbenici

Kvantitativne metoda određivanja DOAK lijekova


Consensus Document 43

International Council for Standardization in Haematology (ICSH) Recommendations for Laboratory Measurement of Direct Oral Anticoagulants

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Direktni inhibitor trombina
dabigatran

- ✓ dTV - diluirano trombinsko vrijeme
- ✓ ECT - ekarinski koagulometrijski test
- ✓ ECA - ekarinska kromogena metoda
- ✓ anti - FIIa test (kromogena)

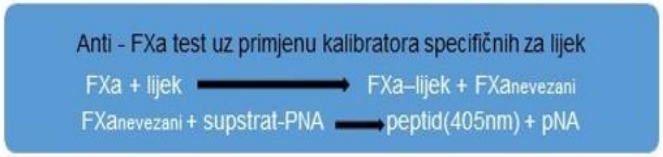
sve navedene metode su preporučene kao prikladne za određivanje koncentracije DTI

- ograničenja: koagulometrijske – interferencije (ECT – FII, fib), dTV – mjerni raspon….
- Metode izbora (preporučene): kromogene

Direktni inhibitori FXa
rivaroksaban, apiksaban, edoksaban

- ✓ **Anti – Xa test**

uz kalibraciju specifičnim lijekom (R, A, E)



metoda je preporučena za određivanje koncentracije inh. FXa

Preporuke za laboratorijsku dijagnostiku DOAK lijekova

Analitički čimbenici

Kvantitativne metoda određivanja DOAK lijekova

Direktni inhibitor trombina
dabigatran

- ✓ dTV - diluirano trombinsko vrijeme
- ✓ ECT - ekarinski koagulometrijski test
- ✓ ECA - ekarinska kromogena metoda
- ✓ anti - FIIa test (kromogena)

Direktni inhibitori FXa
rivaroksaban, apiksaban, edoksaban

- ✓ Anti – Xa test

uz kalibraciju specifičnim lijekom koji se određuje (R, A, E)

- Linearnost: 20 – 500 ng/mL – omogućuju određivanje MK i VK
- Svi automatizirani koagulometri
- Dostupnost: 24/7
- TAT: 60 min. (30 min.) – sva hitna stanja

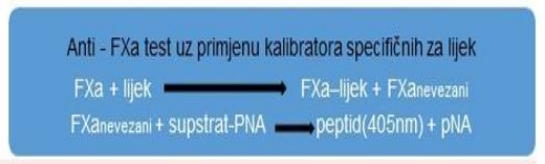
Preporuke za laboratorijsku dijagnostiku DOAK lijekova

2. Analitički čimbenici

Kvantitativne metode određivanja DOAK lijekova - ograničenja

Direktni inhibitori FXa
rivaroksaban, apiksaban, edoksaban

✓ **Anti – Xa test**
uz kalibraciju specifičnim lijekom (R, A, E)



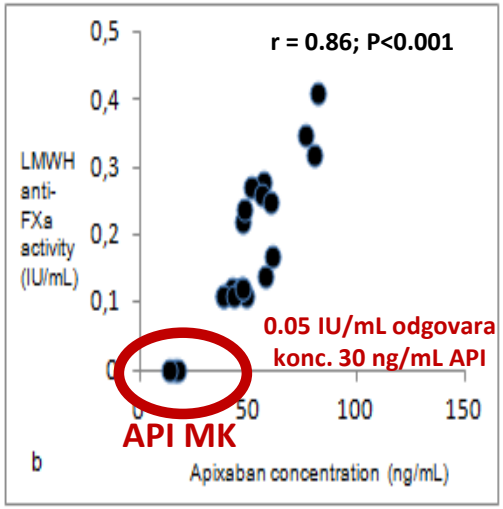
1. **Primjena anti-Xa metode kalibrirane za niskomolekularni heparin (LMWH) nije prikladna za procjenu antikoag. učinka/konc. DOAK lijekova iz skupine inh. FXa**

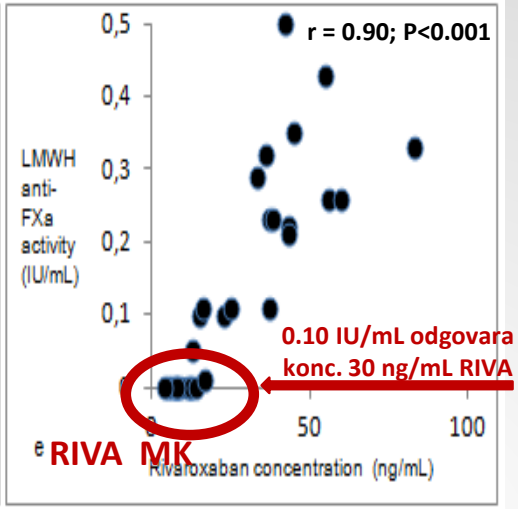
Ćelap I, Margetić S, Mihić R, Obuljen J, Linarić I, Leniček Krleža J. Is Low Molecular Weight Heparin-Calibrated Chromogenic Anti-Xa Assay Suitable for Assessing Anticoagulant Effect of Apixaban in Adolescents? Res Pract Thromb Haemost 2020;4 (Suppl 1):1185.

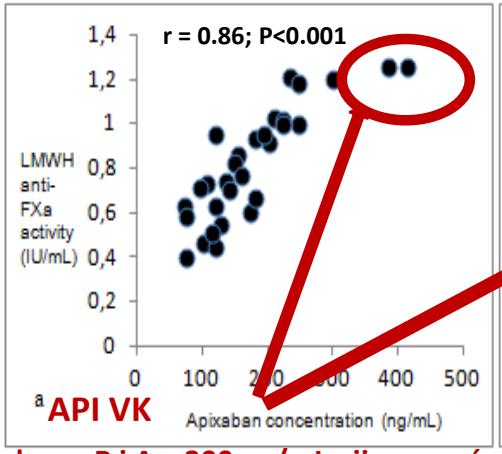
Margetić S, Ćelap I, Delić Brkljačić D, Pavlović N, Šupraha Goreta S, Kobasić I, Lovrenčić-Huzjan A, Bašić Kes V. Chromogenic anti-FXa assay calibrated with low molecular weight heparin in patients treated with rivaroxaban and apixaban: possibilities and limitations. Biochem Med 2020;30(1):010702.

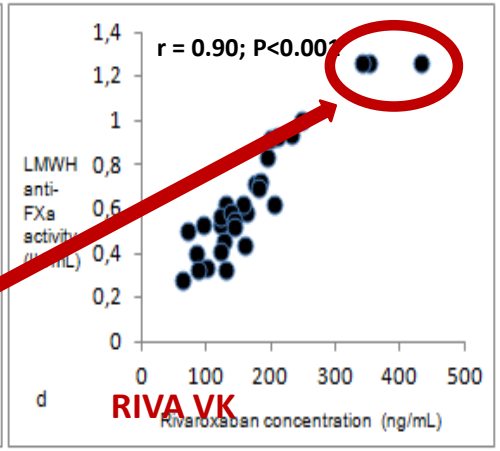
IP-2016-06-8208 LAB-NOAC

HRZZ Croatian Science Foundation









konc. R i A > 300 ng/mL nije moguće kvantitativno odrediti primjenom metode uz kalibraciju LMWH-om

- Mjerni raspon anti FXa uz LMWH kalibraciju (0.05 – 1.26 IU/mL) odgovara konc. R i A u rasponu od oko 30 - 300 ng/mL
- Izražavanje rezultata IU/mL
- Anti-FXa test uz kalibraciju LMWH-om – isključiva potencijalna primjena je u hitnim kliničkim stanjima za isključivanje klinički značajne konc. inh. FXa u cirkulaciji (≥30 ng/mL) ukoliko nije dostupna metoda uz kalibraciju odgovarajućim lijekom!!!
- Anti-FXa test uz kalibraciju LMWH-om nije prikladna metoda za procjenu koncentracije i/ili antikoagulacijskog učinka DOAK lijekova

IP-2016-06-8208 LAB-NOAC

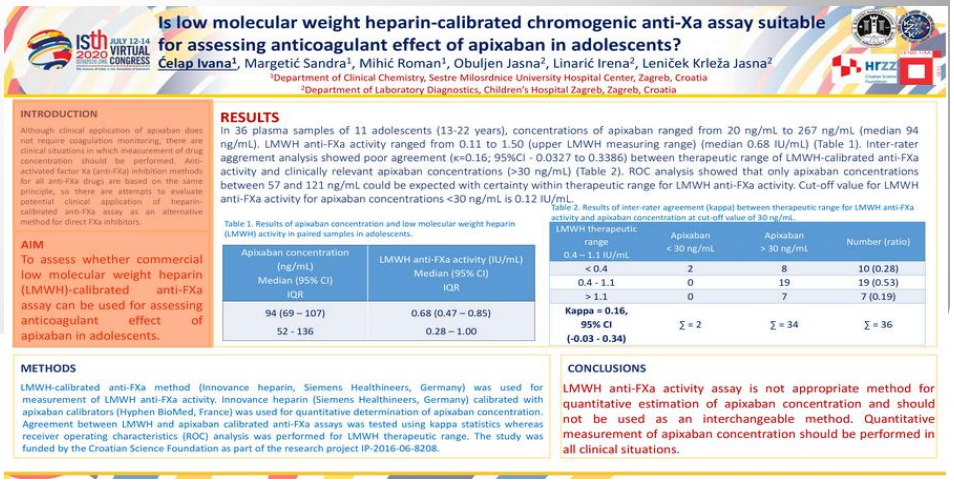
Margetić S, Ćelap I, Delić Brkljačić D, Pavlović N, Šupraha Goreta S, Kobasić I, Lovrenčić-Huzjan A, Bašić Kes V. Chromogenic anti-FXa assay calibrated with low molecular weight heparin in patients treated with rivaroxaban and apixaban: possibilities and limitations. Biochem Med 2020;30(1):010702.

Preporuke za laboratorijsku dijagnostiku DOAK lijekova

2. Analitički čimbenici

Primjena anti-Xa metode kalibrirane za niskomolekularni heparin (LMWH) nije prikladna za određivanje konc. i/ili procjenu antikoagulacijskog učinka DOAK lijekova iz skupine inh. FXa

Ćelap I, Margetić S, Mihić R, Obuljen J, Linarić I, Leniček Krleža J. **Is Low Molecular Weight Heparin-Calibrated Chromogenic Anti-Xa Assay Suitable for Assessing Anticoagulant Effect of Apixaban in Adolescents?** Res Pract Thromb Haemost 2020;4 (Suppl 1):1185.



Is low molecular weight heparin-calibrated chromogenic anti-Xa assay suitable for assessing anticoagulant effect of apixaban in adolescents?

Ćelap Ivana[1], Margetić Sandra[1], Mihić Roman[1], Obuljen Jasna[2], Linarić Irena[2], Leniček Krleža Jasna[2]

[1]Department of Clinical Chemistry, Sestre Milosrdnice University Hospital Center, Zagreb, Croatia
[2]Department of Laboratory Diagnostics, Children's Hospital Zagreb, Zagreb, Croatia

INTRODUCTION

Although clinical application of apixaban does not require coagulation monitoring, there are clinical situations in which measurement of drug concentration should be performed. Anti-activated factor Xa (anti-FXa) inhibition methods for all anti-FXa drugs are based on the same principle, so there are attempts to evaluate potential clinical application of heparin-calibrated anti-FXa assay as an alternative method for direct FXa inhibitors.

AIM

To assess whether commercial low molecular weight heparin (LMWH)-calibrated anti-FXa assay can be used for assessing anticoagulant effect of apixaban in adolescents.

RESULTS

In 36 plasma samples of 11 adolescents (13-22 years), concentrations of apixaban ranged from 20 ng/mL to 267 ng/mL (median 94 ng/mL). LMWH anti-FXa activity ranged from 0.11 to 1.50 (upper LMWH measuring range) (median 0.68 IU/mL) (Table 1). Inter-rater aggrement analysis showed poor agreement (κ=0.16; 95%CI - 0.0327 to 0.3386) between therapeutic range of LMWH-calibrated anti-FXa activity and clinically relevant apixaban concentrations (>30 ng/mL) (Table 2). ROC analysis showed that only apixaban concentrations between 57 and 121 ng/mL could be expected with certainty within therapeutic range for LMWH anti-FXa activity. Cut-off value for LMWH anti-FXa activity for apixaban concentrations <30 ng/mL is 0.12 IU/mL.

Table 1. Results of apixaban concentration and low molecular weight heparin (LMWH) activity in paired samples in adolescents.

Apixaban concentration (ng/mL) Median (95% CI) IQR	LMWH anti-FXa activity (IU/mL) Median (95% CI) IQR
94 (69 – 107)	0.68 (0.47 – 0.85)
52 - 136	0.28 – 1.00

Table 2. Results of inter-rater agreement (kappa) between therapeutic range for LMWH anti-FXa activity and apixaban concentration at cut-off value of 30 ng/mL.

LMWH therapeutic range 0.4 – 1.1 IU/mL	Apixaban < 30 ng/mL	Apixaban > 30 ng/mL	Number (ratio)
< 0.4	2	8	10 (0.28)
0.4 - 1.1	0	19	19 (0.53)
> 1.1	0	7	7 (0.19)
Kappa = 0.16, 95% CI (-0.03 - 0.34)	∑ = 2	∑ = 34	∑ = 36

METHODS

LMWH-calibrated anti-FXa method (Innovance heparin, Siemens Healthineers, Germany) was used for measurement of LMWH anti-FXa activity. Innovance heparin (Siemens Healthineers, Germany) calibrated with apixaban calibrators (Hyphen BioMed, France) was used for quantitative determination of apixaban concentration. Agreement between LMWH and apixaban calibrated anti-FXa assays was tested using kappa statistics whereas receiver operating characteristics (ROC) analysis was performed for LMWH therapeutic range. The study was funded by the Croatian Science Foundation as part of the research project IP-2016-06-8208.

CONCLUSIONS

LMWH anti-FXa activity assay is not appropriate method for quantitative estimation of apixaban concentration and should not be used as an interchangeable method. Quantitative measurement of apixaban concentration should be performed in all clinical situations.

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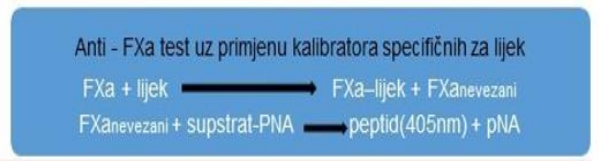
Preporuke za laboratorijsku dijagnostiku DOAK lijekova

2. Analitički čimbenici

Kvantitativne metoda određivanja DOAK lijekova - ograničenja

Direktni inhibitori FXa
rivaroksaban, apiksaban, edoksaban

✓ **Anti – Xa test**
uz kalibraciju specifičnim lijekom (R, A, E)



2. Zamjena terapije DOAK-a niskomol. heparinom u terapijskoj dozi (preoperativno):

- **lažno veće konc. DOAK. lijeka = interferencija heparina zbog istog načela metode!!!**
- **klinički značajno kod određivanja ostatne konc. DOAK-a preoperativno!!!**

Preporuka:

- ✓ **odrediti konc. DOAK-a neposredno prije slijedeće doze LMWH**
- ✓ **ako je ostatna konc. DOAK-a > 30 ng/mL – interpretativni komentar (upozorenje o nepouzdanom rezultatu konc. DOAK lijeka zbog interferencije LMWH)**

Preporuke za laboratorijsku dijagnostiku DOAK lijekova

Analitički čimbenici

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POCT metode za DOAK lijekove

- kvalitativne ili polukvantitativne
- nisu prikladne za procjenu koncentracije i/ili antikoagulacijskog učinka DOAK lijekova
- primjena: u hitnim stanjima – isključivanje kl. značajne konc. DOAK-a u slučaju nedostupnosti kvantitativnih metoda

1. DOAK Dipstick (*Doasense GmbH, Heidelberg Njemačka)*

- kvalitativna, određivanje prisutnosti/odsutnosti DOAK-a u urinu
- isključivanje klinički značajne konc. DOAK-a u krvi (≥ 30 ng/mL)
- za sada jedina preporučena POCT metoda u hitnim stanjima
- 2025. god. IVD certifikat

DOAC Dipstick Testing Can Reliably Exclude the Presence of Clinically Relevant DOAC Concentrations in Circulation

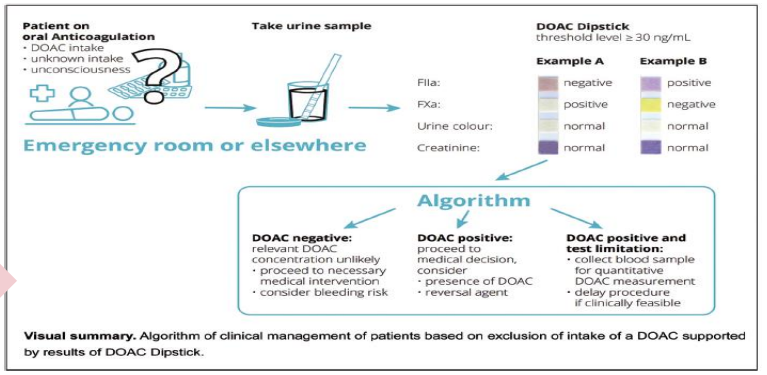
Sandra Margetić[1] Ivana Ćelap[1] Arijana Lovrenčić Huzjan[2] Marijana Bosnar Puretić[2] Sandra Šupraha Goreta[3] Anesa Čajević Glojnarić[1] Diana Delić Brkljačić[4] Pavao Mioč[4] Iob Harenberg[5,6] Svetlana Hetiens[7] Christel Weiss[7]

Thromb Haemost 2022;122:1542–1548.

Algorithm for Rapid Exclusion of Clinically Relevant Plasma Levels of Direct Oral Anticoagulants in Patients Using the DOAC Dipstick: An Expert Consensus Paper

Job Harenberg[1,2] Robert C. Gosselin[3] Adam Cuker[4] Cecilia Becattini[5] Ingrid Pabinger[6] Sven Poli[7,8] Jeffrey Weitz[9,10] Walter Ageno[11] Rupert Bauersachs[12] Ivana Celap[13,14] Philip Choi[15,16] James Douketis[10] Jonathan Douxfils[17,18] Ismail Elalamy[19,20] Anna Falanga[21,22] Jawed Fareed[23] Emmanuel J. Favaloro[24,25] Grigorios Gerotziafas[26,27] Harald Herkner[28] Svetlana Hetjens[29] Lars Heubner[30] Robert Klamroth[31] Florian Langer[32] Gregory Y. H. Lip[33,34] Brian Mac Grory[35] Sandra Margetić[12,36] Anne Merrelaar[28] Marika Pikta[37,38] ameis[28] Daniel Strbian[42] eanine Walenga[46] Christel Weiss[29]

Thromb Haemost 2024;124:770–777.



Preporuke za laboratorijsku dijagnostiku DOAK lijekova

Analitički čimbenici

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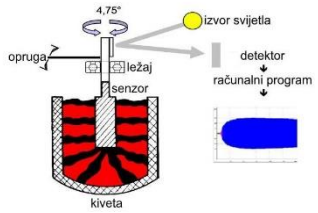
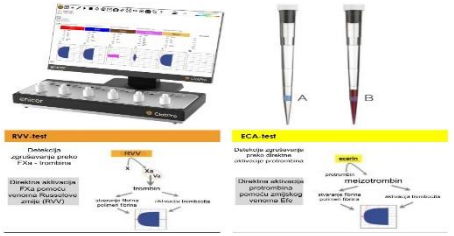
- nisu prikladne za procjenu koncentracije i/ili antikoagulacijskog učinka DOAK lijekova
- primjena: u hitnim stanjima – isključivanje kl. značajne konc. DOAK-a u slučaju nedostupnosti spec. kvantitativnih metoda

2. TEG, TEM, TGA... (procjena globalne koagulabilnosti i fibrinolitičke aktivnosti)



ROTEM



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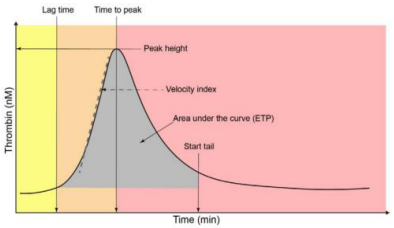

TGA

- nedovoljno osjetljive i specifične, nestandardizirane za DOAK lijekove
- za sada se primjena ovih metoda ne preporuča u hitnim stanjima za DOAK-e

Preporuke za laboratorijsku dijagnostiku DOAK lijekova

Poslijeanalitički čimbenici

- mjerna jedinica: ng/mL
- terapijski intervali nisu definirani, ali objavljene očekivane vršne i minimalne koncentracije ovisno o dozi i kliničkoj indikaciji treba izvještavati uz rezultat mjerenja
- rezultat interpretirati u skladu s kl. anamnezom bolesnika, dozom lijeka i vremenom uzorkovanja krvi u odnosu na zadnju dozu lijeka
- vrijeme izdavanja: za sva hitna stanja TAT = 30 min.

International Council for Standardization in Haematology (ICSH) Recommendations for Laboratory Measurement of Direct Oral Anticoagulants

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	Dabigatran		Rivaroxaban		Apixaban		Edoxaban	
Indication	Stroke prevention in NVAF	Treatment PE/VTE	Stroke prevention in NVAF	Treatment PE/VTE	Stroke prevention in NVAF	Treatment PE/VTE	Stroke prevention in NVAF	Treatment PE/VTE
Dose	150 mg bid	150 mg bid	20 mg qd	20 mg qd	5 mg bid	5 mg bid	60 mg qd	60 mg qd
Peak concentration, ng/mL	175[a] (117–275)	175[a] (117–275)	249[b] (184–343)	270[b] (189–419)	171[c] (91–321)	132[c] (59–302)	170[d] (125–245)	234[e] (149–317)
Trough concentration, ng/mL	91[a] (61–143)	60[a] (39–95)	44[b] (12–137)	26[b] (6–87)	103[c] (41–230)	63[c] (22–177)	36[e] (19–62)	19[e] (10–39)

Abbreviations: bid, twice daily; IQR, interquartile range; NVAF, non-valvular atrial fibrillation; PE, pulmonary embolism; qd, once daily; VTE, venous thromboembolism.
Notes: Other approved indications for DOACs include secondary prevention of PE/VTE, and post hip and knee replacement, which may have alternative dosing strategies. Additionally, changes in doses may occur after initiation phase of DOAC treatment. Consultation of regional DOAC labeling information is required before interpreting or using these peak and trough DOAC concentration data.
[a]Mean (25th–75th percentile).
[b]Mean (5th–95th percentile).
[c]Median (5th–95th percentile).
[d]Median (IQR).
[e]Median (IQR).

Gosselin et al. Thromb Haemost 2018;118:437–50.

2021 Update of the International Council for Standardization in Haematology Recommendations for Laboratory Measurement of Direct Oral Anticoagulants

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	Dabigatran		Rivaroksaban				Apiksaban					Edoksaban			
Indikacija	NVAF x̄ (10 – 90 percentil)	VTE medijan (IQR)	NVAF x̄ (5 – 95 percentil)	VTE x̄ (10 – 90 percentil)		ACS x̄ (10 –90 percentil)	NVAF medijan (5 – 95 percentil)		VTE medijan (5 – 95 percentil)			NVAF medijan (IQR)		VTE medijan (IQR)	
Doza, mg	2x150	2x150	1x20	1x20	1x10	1x2,5	2x5	2x2,5	2x10	2x5	2x2,5	1x60	1x30	1x60	1x30
MK, ng/mL	91 (40-215)	60 (39-95)	44 (12-137)	32 (6-239)	14 (4-51)	9 (4-18)	103 (41-230)	79 (34-162)	120 (41-335)	63 (22-177)	32 (11-90)	36 (19-62)	27 (15-45)	19 (10-39)	16 (8-32)
VK, ng/mL	175 (74-383)	175 (117-275)	249 (184-343)	215 (22-535)	101 (7-273)	47 (13-123)	171 (91-321)	123 (69-221)	251 (111-572)	132 (59-302)	67 (30-153)	170 (125-245)	85 (55-120)	234 (149-317)	164 (99-225)

MK=minimalna konc. lijeka, VK=vršna konc. lijeka, NVAF= ne-valvularna fibrilacija atrija, ACS = akutni koronarni sindrom; x̄ = srednja vrijednost; IQR – interkvartilni raspon

Douxfils et al. Thromb Haemost 2021;121:1008–20.

DABIGATRAN ng/mL (n = 37)	Medijan (95%CI)	IQR	Očekivane terapijske vrijednosti
Vršna koncentracija	165 (125 – 204)	102 - 249	175 (117 – 275)
Minimalna koncentracija	97 (61 – 120)	52 - 157	91 (61 – 143)
RIVAROXBAN ng/mL (n= 28)	**Medijan (95%CI)**	**IQR**	**Očekivane terapijske vrijednosti**
Vršna koncentracija	229 (168 -280)	124 - 296	249 (184 - 343)
Minimalna koncentracija	36 (23 – 93)	15 - 109	44 (12 – 137)
APIXABAN ng/mL (n = 36)	**Medijan (95%CI)**	**IQR**	**Očekivane terapijske vrijednosti**
Vršna koncentracija	180 (160 – 206)	142 - 224	171 (91 - 321)
Minimalna koncentracija	89 (67 – 125)	56 - 135	103 (41 – 230)

Usporedba vršnih i minimalnih koncentracija dabigatrana, rivaroksabana i apiksabana s očekivanim terapijskim vrijednostima kod pacijenata s NVAF

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Margetić S, Ćelap I, Brčić M, Mihić R. Comparison of peak and trough concentrations of dabigatran, rivaroxaban, and apixaban with the published expected values in patients with non valvular atrial fibrillation. Res Pract Thromb Haemost 2019;3(Suppl1):3-4.