Quantifying Residual Rivaroxaban Plasma Concentration after Antagonization with Andexanet Alfa: A Difficult Task in Routine Clinical Practice

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Abstract

We describe the case of a 38-year-old man with a history of chronic portal vein thrombosis who presented with abdominal pain after a transjugular intrahepatic portosystemic shunt procedure. Under anticoagulation therapy with rivaroxaban, he experienced active splenic bleeding, leading to hemodynamic instability. Emergency interventions, including andexanet alfa and nanoparticle administration, successfully stopped the bleeding. However, routine tests showed persistently high rivaroxaban levels despite reversal with andexanet alfa. This case report shows that next to standard anti-Xa activity assay, high-performance liquid chromatography is as well unreliable in this regard. In contrast, viscoelastic tests might better serve as indicators of the efficacy of the reversal. The availability of modified anti-Xa tests is urgently needed, to monitor the effects of andexanet alfa reversal.

Keywords
- rivaroxaban
- andexanet alfa
- DOAC reversal
- HPLC measurement

Introduction

Andexanet alfa is an inactive factor X molecule, acting as selective antidote to the Xa-inhibitory direct oral anticoagulants (DOACs) rivaroxaban and apixaban. Routine chromogenic anti-Xa activity assays are unsuitable for assessing its DOAC-antagonizing efficacy. Our case report shows that regarding this, high-performance liquid chromatography (HPLC) is equally uninformative, and does not correlate well with rotational thromboelastometry (ROTEM) in a setting of acute bleeding.

Case Presentation

A 38-year-old man was admitted to our emergency department at 8:00 PM with abdominal pain. Three weeks before, he had undergone a transjugular intrahepatic portosystemic shunt (TIPSS) procedure because of a chronic portal vein thrombosis. He had a history of thrombocytopenia, type 2 diabetes mellitus, and a duodenal ulcer. His medication upon admission included the following: rivaroxaban 20 mg (1–0–0), metformin/empagliflozin 1,000 mg/5 mg (1–0–1), glimepiride 2 mg (1–0–0), carvedilol 6.25 mg (1–0–0), and pantoprazole 40 mg (1–0–0). He reported the intake of his last rivaroxaban dose at around lunchtime of that day.

A computed tomographic scan was performed shortly after admission and revealed active diffuse bleeding originating from the spleen. The patient's hemoglobin level decreased from 133 to 85 g/L indicative of a significant blood loss. ►Table 1 summarizes the initial coagulation assessment. The patient became hemodynamically unstable, and required the administration of norepinephrine up to

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Table 1 Initial coagulation analyses

Parameter	Value	Normal range
Hemoglobin	85 g/L	134–170 g/L
Platelets	60 G/L	143–400 G/L
INR/Prothrombin time	1.8/18.8 s	<1.2/10–13 s
APTT	47 s	24–36 s
Thrombin time	14 s	<22 s
Factor XIII	42%	>60%
Anti-XA (rivaroxaban)	393.3 µg/L	N/A
ROTEM EXTEM	CT 149 s/MCF 50 mm	CT 38–79 s/MCF 50–72 mm
ROTEM INTEM	CT 275 s/MCF 46 mm	CT 100–240 s/MCF 50–72 mm
ROTEM FIBTEM	CT 163 s/MCF 7 mm	CT 38–62 s/MCF 9–25 mm

Abbreviations: APTT, activated partial thromboplastin time; CT, clotting time; INR, international normalized ratio; MCF, maximum clot firmness; ROTEM, rotational thromboelastometry.

10 µg/min. Hemostatic resuscitation was initiated, including the empiric administration of tranexamic acid 1 g, fibrinogen concentrate 2 g, and calcium gluconate 2.25 mmol. As soon as factor XIII activity was available, factor XIII concentrate of 1,250 IU was administered. In addition, andexanet alfa was given to reverse the residual rivaroxaban effect, according to guidelines on life-threatening bleeding in patients with a high residual DOAC plasma concentration.[1] Andexanet alfa was given as an initial intravenous (IV) bolus of 800 mg, and a subsequent IV infusion of 8 mg/min for 2 hours, equaling a total of 960 mg. ►**Table 2** summarizes the coagulation and transfusion management in the emergency room, and ►**Table 3** gives a before-and-after comparison.

In an interdisciplinary discussion in the emergency room, we aimed at determining an effective approach to stop the bleeding. Emergency angiography was then performed. Because of the previous TIPSS procedure, complete embolization of the splenic artery was not possible. Nanoparticles were therefore administered into a splenic artery branch, using a Chuang catheter via the femoral artery. This intervention was performed under mild sedation with remifentanil. Repetitive laboratory assessment was then conducted, to determine the patient's hemostatic status. A detailed overview is given in ►**Table 4**.

The described coagulation management, together with the angiographic delivery of nanoparticles, proved successful in stopping the bleeding and stabilizing the patient's condition. Concomitantly, the clotting time (CT) of the ROTEM EXTEM test normalized after the administration of andexanet alfa. However, a routine anti-Xa activity assay calibrated for rivaroxaban as well as the HPLC test still showed markedly elevated rivaroxaban levels, apparently displaying an unsatisfactory effect of andexanet alfa.

The patient was transferred to the ICU after the embolization with a norepinephrine infusion rate of 1 µg/min. There were no signs of recurrent bleeding, and no further hemostatic therapy was required. However, during the post-acute phase in the ICU (days 1–3), the patient required an additional three units of red blood cells, probably due to volume-induced hemodilution. The patient presented with a distended and painful abdomen while on prophylactic antibiotic therapy with piperacillin/tazobactam, and was febrile up to 39 °C. A follow-up computed tomographic scan revealed the presence of hemoperitoneum, with the TIPSS in the portal vein and the splenic vein remaining patent. Superinfection of the intra-abdominal hematoma was assumed. The patient underwent laparoscopic lavage that led to a significant improvement of clinical symptoms and inflammatory

Table 2 Coagulation and transfusion management in the emergency room

Timeline	Drug	Dose	Laboratory result (at admission)
8:50 pm	Tranexamic acid	1 g	(empiric)
9:00 pm	Calcium gluconate	2.25 mmol	Ca^{2+}, 1.01 mmol/L
9:10 pm	Fibrinogen	2 g	FIBTEM MCF, 7 mm
11:00 pm	Factor XIII	1,250 IU	Factor XIII activity 42%
11:00 pm	Andexanet alfa	800 mg + 960 mg (bolus followed by continuous infusion over 2 h)	Anti-Xa activity calibrated for rivaroxaban, 393.3 µg/L
11:00 pm	Red blood cell concentrate	1	Hemoglobin 63 g/L

Abbreviation: MCF, maximum clot firmness.

Table 3 Key laboratory results before and after hemostatic resuscitation in the emergency room

Laboratory value	Pre-correction	Post-correction
Calcium	1.01 mmol/L	1.26 mmol/L
Hemoglobin	63 g/L	76 g/L
ROTEM	EXTEM CT: 149 s EXTEM MCF: 50 mm FIBTEM MCF: 7 mm	EXTEM CT: 58 s EXTEM MCF: 49 mm FIBTEM MCF: 15 mm
Factor XIII	42%	74%

Abbreviations: CT, clotting time; MCF, maximum clot firmness; ROTEM, rotational thromboelastometry.

Table 4 Coagulation parameters to assess residual DOAC plasma concentration

Timeline	ROTEM (EXTEM CT)	Anti-Xa (RXA)	HPLC
8:20 pm (D1)		393.3 µg/L	
8:37 pm	149 s		
10:16 pm	116 s		
10:30 pm	Start andexanet alfa bolus followed by continuous infusion		
11:00 pm			688.0 µg/L[a]
11:53 pm	58 s[a]		
1:00 pm (D2)	End of andexanet alfa infusion		
1:30 am			711.4 µg/L[b]
5:30 am			417.4 µg/L[b]
12:15 pm		200.9 µg/L[b]	
1:30 pm			172 µg/L[b]
6:00 pm		132.5 µg/L[b]	
0:00 am (D3)		118.7 µg/L[b]	
01:30 am			118.1 µg/L[b]
5:00 am		91.6 µg/L[b]	
1:30 am			45.6 µg/L[b]
2:00 am		41.2 µg/L[b]	

Abbreviations: CT, clotting time; D1, day 1; D2, day 2; D3, day 3; HPLC, high-performance liquid chromatography; ROTEM, rotational thromboelastometry; RXA, calibrated for rivaroxaban.
[a]Results during andexanet alfa administration.
[b]Results after andexanet alfa administration.

markers. Following the cessation of the hemorrhage, prophylactic anticoagulation with heparin was commenced and before discharge transitioned to apixaban. In total, the patient was treated in the ICU for 3 days, in the intermediate care unit for 2 days, and in the ward for another 7 days. No thromboembolic event occurred during this time.

Discussion

Anticoagulation with DOAC is now routinely administered for the prevention and treatment of venous thromboembolism as well as for stroke prevention in nonvalvular atrial fibrillation.[2] Consequently, the rate of major bleeding episodes in patients taking DOAC is increasing, reaching 3 to 5% patient-years.[3–7] Thus, more patients require urgent DOAC reversal. An elevated perioperative bleeding risk was shown for a residual rivaroxaban plasma concentration above 100 µg/L in patients undergoing surgery with an expected high blood loss.[8] Two antidotes are recommended for reversal of DOAC during major bleeding episodes, andexanet alfa, and four-factor prothrombin complex concentrate.[1,9]

Standard chromogenic anti-Xa assays are unsuitable for determining the residual anti-Xa activity after administration of andexanet alfa, to assess the efficacy of the reversal.[10] This is explained by a higher sample dilution (1:44) when using routine anti-Xa assays (e.g., the STA-Liquid assay). This causes dissociation of the andexanet–DOAC complex and an implausibly high residual anti-Xa activity, suggestive of an unsatisfactory andexanet effect even when the in vivo concentration of free DOAC is low.[10] Modified anti-Xa assays are more accurate.[10] The overestimation of the residual DOAC plasma concentration is reduced and accuracy is improved when using an assay setup with a lower sample dilution.[10] However, these modified anti-Xa

assays are not yet established in routine laboratories or commercially available.

In the case presented here, we observed contrasting results of HPLC as well as anti-Xa assay versus ROTEM. Although a positive correlation was demonstrated between plasma concentrations of rivaroxaban measured by HPLC and CT ROTEM,[11] we observed a negative correlation between these parameters in our case. The viscoelastic test revealed a marked improvement of coagulation (EXTEM CT decreased from 149 to 58 seconds, see ►**Table 4**), which correlates with the improvement of the patient's clinical condition. The administration of fibrinogen could also contribute to this marked improvement. After the administration of 2 g fibrinogen, we performed another ROTEM showing an initial decrease of the CT EXTEM from 149 to 116 seconds (see ►**Table 4**). After the second ROTEM, however, only andexanet was additionally administered, leading to the normalization of the CT EXTEM (from 116 to 58 seconds, see ►**Table 4**). In contrast, the HPLC showed a different picture with an increase of rivaroxaban plasma levels despite its reversal with andexanet alfa and a reduced CT in ROTEM. This indicates that HPLC is not suitable for monitoring the effect of andexanet either, as it results in underestimation of its efficacy, at least when standard high sample dilutions are applied. So far, this is the first report showing that HPLC plasma concentration determination of rivaroxaban also shows falsely elevated levels resulting in significant underestimation of andexanet alfa reversal activity.

The difference in rivaroxaban plasma concentration between the first anti-Xa measurement and the HPLC measurement was conspicuous. We examined this in detail because we suspected that andexanet and rivaroxaban might interfere in the HPLC measurement. However, andexanet and rivaroxaban have significantly different molecular sizes: rivaroxaban is much smaller, with ~435 Da, compared with andexanet, with ~40,000 Da. Therefore, it is unlikely that andexanet was falsely measured as a rivaroxaban molecule. A detailed analysis of the raw data from the HPLC measurement was conducted and it showed ion suppression in the measurement signals of the two samples taken during the andexanet infusion. The andexanet carrier solution may have caused this disturbance. The excipients of andexanet that accompany the infusion include tromethamine hydrochloride, L-arginine hydrochloride, sucrose, mannitol, and polysorbate. However, we cannot definitively determine which of these components is responsible for the above discrepancy of results. Additional studies are required to thoroughly investigate the interference of the carrier solution with the HPLC signal.

In this regard, viscoelastic tests might be better suited. Assessing the effect of DOAC reversal should primarily rely on clinical judgment, using clinical indicators of hemostasis or of an unsatisfactory reversal effect, and identifying adverse events such as thromboembolism.

Conclusion

Determination of the residual rivaroxaban plasma concentration after reversal with andexanet alfa remains a challenge. Our case report shows that next to standard anti-Xa activity assay, HPLC is as well unreliable in this regard. In contrast, viscoelastic tests might better serve as indicators of the efficacy of the reversal. The availability of modified anti-Xa tests is urgently needed, to monitor the effects of andexanet alfa reversal.

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Conflicts of Interest

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