Ex vivo reversal of effects of rivaroxaban evaluated using thromboelastometry and thrombin generation assay

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Abstract

Background: In major bleeding events, the new direct oral anticoagulants pose a great challenge for physicians. The aim of the study was to test for ex vivo reversal of the direct oral anticoagulant rivaroxaban with various non-specific reversal agents: prothrombin complex concentrate (PCC), activated prothrombin complex concentrate (aPCC), recombinant activated factor VII (rFVIIa), and fibrinogen concentrate (FI).

Methods: Blood was obtained from healthy volunteers and from patients treated with rivaroxaban. Blood samples from healthy volunteers were spiked with rivaroxaban to test the correlation between rivaroxaban concentration and coagulation tests. Patient blood samples were spiked with various concentrations of the above-mentioned agents and analysed using thromboelastometry and thrombin generation. 

Results: When added in vitro, rivaroxaban was significantly (P<0.05) correlated with ROTEM® thromboelastometry EXTEM (extrinsic coagulation pathway) clotting time (CT), time to maximal velocity (MaxV−t), and with all measured thrombin generation parameters. In vivo, CT, MaxV−t, lag time, and peak thrombin generation (Cmax) were significantly correlated with rivaroxaban concentrations. Regarding reversal of rivaroxaban, all tested agents significantly (P<0.05) reduced EXTEM CT, but to different extents: rFVIIa by 68%, aPCC by 47%, PCC by 17%, and FI by 9%. Only rFVIIa reversed EXTEM CT to baseline values. Both PCC (+102%) and aPCC (+232%) altered overall thrombin generation (area under the curve) and increased Cmax (+461% for PCC, +87.5% for aPCC).

Conclusions: Thromboelastometry and thrombin generation assays do not favour the same reversal agents for rivaroxaban anticoagulation. Controlled clinical trials are urgently needed to establish doses and clinical efficacy of potential reversal agents. 

Clinical trial registration: EudracCT trial no. 213-00474-30.

Key words: blood, anticoagulants; complications, haemorrhage; thromboelastography

Rivaroxaban (Xarelto®; Bayer, Germany) is used as thrombosis prophylaxis and therapy instead of anticoagulants such as unfractionated heparin, low molecular weight heparin, or vitamin K antagonists, because it is considered to have a wider therapeutic range and a more predictable dose–response relationship.1–3 According to the manufacturer, rivaroxaban does not require routine drug monitoring.4–6 In the European Union, Canada, and the USA, rivaroxaban is approved for the prevention of stroke and systemic embolism in patients undergoing hip- or knee-replacement surgery.

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Editor’s key points

• There is limited information on the best treatment of bleeding in patients taking newer direct oral anticoagulants.
• The effects of various procoagulant factors on laboratory coagulation parameters were assessed on blood treated ex vivo with rivaroxaban or blood from rivaroxaban-treated patients.
• Reversal of rivaroxaban anticoagulant effects ex vivo was possible, but varied between reversal agents and the coagulation assay, indicating that clinical trials are needed to validate reversibility in vivo.

Methods

Ethics committee approval

This study was approved by the Human Subjects Review Board of the Medical University of Innsbruck, Austria (reference: UN4984_LEK) and by the national competent authority (Bundesamt für Sicherheit im Gesundheitswesen, BASG, Vienna, Austria; reference: LCM-717978) and registered with EudraCT (reference: 213-00474-30). Written informed consent was obtained from all study participants before study-related procedures were performed. The study was performed in compliance with the Declaration of Helsinki guidelines regarding ethical principles for medical research involving human subjects and followed Good Clinical Practice as defined by the International Conference on Harmonization (ICH-GCP).

Study population

Healthy volunteers

Blood samples were obtained from healthy volunteers aged 18–85 yr. Exclusion criteria were pregnancy, concomitant medication with influence on anticoagulant or platelet activity, or presence of an inherited or acquired bleeding disorder.

Patients

Blood samples were obtained from patients aged 18–90 yr receiving rivaroxaban (Xarelto®; Bayer, Germany). Exclusion criteria were pregnancy, non-compliance in taking medication, concomitant medication with an influence on rivaroxaban activity, or presence of haemophilia or an acquired or hereditary coagulation disorder.

Collection and preparation of blood samples

Blood samples were obtained by peripheral venipuncture 3 h after the patient took rivaroxaban (15 or 20 mg) in order to obtain peak plasma concentrations. Blood coagulation assays and determination of blood coagulation factor concentrations were performed using vacutainer tubes containing 1.106 mol l\(^{-1}\) trisodium citrate solution (Sarstedt, Nümbrecht, Germany). Coagulation analyses in whole blood were performed within 4 h after blood sampling. Plasma for thrombin generation assays was immediately frozen at −80°C (or −20°C for a maximum of 7 days) and thawed immediately before being analysed.

Reagents

Rivaroxaban was purchased from Bayer Pharma AG (Wuppertal, Germany) and diluted with 100% dimethyl sulphoxide (Fluka, Neu-Ulm, Germany), before being added in various concentrations (0, 100, 200, 300, 400, 500, 600, and 700 ng ml\(^{-1}\) final concentrations) to blood samples from healthy volunteers. Adding rivaroxaban solutions to whole blood resulted in dimethyl sulphoxide concentrations <5% and did not influence assays. To exclude diluting effects, PBS (Dulbecco’s phosphate-buffered saline; Bio Whittaker®, Lonza, Belgium) was added to blood samples to produce equal volumes.

Reagents used for reversal of rivaroxaban effects were dissolved according to the instructions in product specification leaflets and added ex vivo to patient blood in concentrations corresponding to doses applied for clinical indications (lower doses) and maximal doses according to product specification leaflets (higher doses).

Spiking procedure

Blood samples from patients on rivaroxaban were spiked immediately after blood sampling to achieve the following final concentrations (assuming a body weight of 75 kg and blood volume of 5 litres): 0.3 or 1 U ml\(^{-1}\) PCC (corresponding to 1500 or 5000 U PCC); 1.5 or 2.25 U ml\(^{-1}\) aPCC (100 or 150 U kg\(^{-1}\) aPCC); 1.5 or 4.05 µg ml\(^{-1}\) rFVIIa (100 or 270 µg kg\(^{-1}\) rFVIIa); and 0.6 or 3 mg ml\(^{-1}\) Fl (40 or 200 mg kg\(^{-1}\) Fl).

Coagulation assays

Thromboelastometry

Within 4 h after blood collection, untreated baseline and spiked blood samples were analysed using thromboelastography (ROTEM®). ROTEM® parameters were determined using a ROTEM® gamma analyser (TEM Innovations GmbH, Munich, Germany). ROTEM® measurements were run at least until A30 values (clot firmness after 30 min) were reached, and all tests were performed according to manufacturers’ instructions using the specific reagents provided by the manufacturer for EXTEM (extrinsically activated assay with tissue factor), INTEM
Thrombin generation assay

Thrombin generation measurements were performed using the Innovance ETP assay (Siemens, Marburg, Germany) on an automated coagulation analyser (BCS XP; Siemens). Coagulation was activated by adding phospholipids, human recombinant tissue factor, and calcium ions to platelet-poor plasma. The generated thrombin cleaves a chromogenic substrate (H-b-Ala-Gly-Arg-pNA), and the turnover of the substrate is recorded over time. The final concentration of substrate was 733 nM l⁻¹ with CaCl₂ 19 mM l⁻¹. The original curve was corrected for estimated α-macroglobulin-bound thrombin activity. From this curve, the following parameters can be obtained: total amount of generated thrombin in the reaction from initiation until return to baseline, also known as ‘endogenous thrombin potential’ (ETP), indicated as ‘area under the curve’ (AUC in mE, as a measure of the total endogenous generated thrombin); peak thrombin generation (Cmax, in mE min⁻¹), which is the maximum of the first derivation of the ETP AUC; lag phase until initiation (tlag); and time to peak thrombin activity (tmax).

Coagulation assays and rivaroxaban concentration

The PT and aPTT were determined on an automated coagulation analyser (aPTT, pathromtin® SL; and PT, thromborel®; S BCSxp; Siemens) for patients receiving rivaroxaban. Rivaroxaban concentrations were also measured on the BCSxp, using a chromogenic assay calibrated for rivaroxaban (BIOPHEN® DiXa-1; CoaChrom Diagnostics, Neuville-sur-Oise, France).

Statistical methods

The Wilcoxon signed-rank test was used to evaluate differences between baseline untreated blood samples and spiked samples from the same patient. Statistical analyses were performed using STATISTICA 10 software (StatSoft Europe GmbH, Hamburg, Germany). Pearson’s correlation was used to evaluate correlations between rivaroxaban concentration and blood coagulation assays and coagulation factor activities. A value of P ≤ 0.05 was considered statistically significant.

Results

Healthy volunteers

General

Thirteen healthy volunteers (six female, seven male) aged 22–56 (mean 36) yr were enrolled.

Influence of rivaroxaban on coagulation measurement

Thromboelastometry.

Addition of rivaroxaban to blood samples from healthy volunteers prolonged EXTEM CT (clotting time) significantly in a dose-dependent manner (100–700 ng ml⁻¹ rivaroxaban, r = 0.76, P < 0.05) from a mean (sd) of 68 (6) s at baseline to up to 425 (146) s at the highest dose of rivaroxaban tested (700 ng ml⁻¹; Fig. 1A).

The EXTEM MaxV−t (time to maximal velocity) was also significantly prolonged in a dose-dependent manner (r = 0.43) from a mean (sd) of 197 (91) s at baseline to 416 (233) s at 700 ng ml⁻¹ rivaroxaban (Fig. 1B).

Thrombin generation.

The tlag was prolonged by rivaroxaban (r = 0.88) from 31 (6) s at baseline to 96 (17) s at a rivaroxaban concentration of 700 ng ml⁻¹ (Fig. 1C). The tmax was prolonged (r = 0.82) from 56 (7) to 233 (98) s (Fig. 1D). The AUC decreased in a dose-dependent manner with increasing rivaroxaban concentration (r = −0.46) from 329 (43) to 250 (36) mE (Fig. 1E). The Cmax was also reduced in a dose-dependent manner (r = −0.81) from 121 (22) to 48 (6) mE min⁻¹ (Fig. 1F).

Patients

Twenty patients (eight female, 12 male) receiving rivaroxaban and aged 21–90 (mean 68) yr were enrolled. For subject characteristics refer to Supplementary data SI.

Rivaroxaban concentrations in plasma compared with coagulation parameters

Subjects treated with rivaroxaban had a mean (sd) rivaroxaban concentration in blood plasma of 225 (100) ng ml⁻¹. Rivaroxaban concentrations correlated best with a decrease in Quick value prothrombin time (PT), and significantly correlated with an increase in aPTT (Table 1).

ROTEM analysis.

There was a significant, rivaroxaban-dependent prolongation and a clinically relevant increase in EXTEM CT and FIBTEM CT compared with normal values in healthy subjects. The MaxV−t was prolonged in a dose-dependent manner in both EXTEM and FIBTEM. The INTEM CT was also prolonged in a dose-dependent manner by rivaroxaban and showed a clinically relevant increase compared with normal values.

Thrombin generation.

Rivaroxaban produced a clinically relevant, significant decrease in Cmax compared with normal values (111–156 mE min⁻¹). There was also a clinically relevant prolongation of tlag compared with normal values (19.6–25.6 s). The AUC (P = 0.26) and tmax (P = 0.77) were not significantly correlated with rivaroxaban concentration, whereas AUC was still in normal range and tmax was prolonged (compare also Table 4).

Significant correlations observed between coagulation tests are shown in Table 2.

Reversal of rivaroxaban-induced changes ex vivo

Thromboelastometry.

All tested agents significantly (P < 0.05) reduced prolonged EXTEM CT. Of the agents tested, rFVIIa 1.5 µg ml⁻¹ reduced CT most, by 68%. Of all agents, rFVIIa was the only one to reach normal CT values. Activated prothrombin complex (1.5 U ml⁻¹) reduced EXTEM CT by 47%. Fibrinogen concentrate reversed CT prolongation by 9% (0.6 mg ml⁻¹ FII; compare also Fig. 2 and Table 3). The INTEM CT was also prolonged, and the highest dose of rFVIIa (4.05 µg ml⁻¹) was able to reduce INTEM CT significantly by 21% from baseline down to the normal range. Fibrinogen concentrate (P = 0.78 for 0.6 mg ml⁻¹; P = 0.07 for 3 mg ml⁻¹) and aPC (P = 0.06 for 0.3 U ml⁻¹; P = 0.49 for 1 U ml⁻¹) did not significantly influence INTEM CT, but FCC significantly prolonged CT by 7% compared with baseline at the lower dose (0.3 U ml⁻¹) and by 19% at the higher dose (1 U ml⁻¹ FCC).

For FIBTEM CT, please refer to Supplementary data SII.

Thrombin generation

Ex vivo administration of aPCC 2.25 U ml⁻¹ significantly (P < 0.05) increased the AUC by 232% from baseline. Adding aPCC 1.5 U ml⁻¹ increased it by 154% (P = 0.028). Also, FCC significantly increased AUC from baseline by 107%. Moreover, aPCC (2.25 U ml⁻¹) and
Fig 1 Correlation between various coagulation parameters and rivaroxaban plasma concentrations. Whole blood from healthy volunteers (n=13) was spiked with various doses of rivaroxaban (100–700 ng ml$^{-1}$). Significantly (P<0.05 for all) correlated parameters were as follows: (A) thromboelastometry EXTEM clotting time (CT, linear approximation for CT=47.7+51.1x); (B) thromboelastometry EXTEM time to maximal velocity ($\text{Max} V$–t, linear approximation for $\text{Max} V$–t=196.6+30.5x); (C) thrombin generation lag time ($t_{\text{lag}}$, linear approximation for $t_{\text{lag}}=24.3+9.5x$); (D) thrombin generation time to peak ($t_{\text{max}}$, linear approximation for $t_{\text{max}}=25.7+27.2x$); (E) thrombin generation area under the curve (AUC, linear approximation for AUC=34.5–8.5x); and (F) thrombin generation maximum of thrombin potential [$C_{\text{max}}$, logarithmic approximation for $C_{\text{max}}=114.8–78.2\times\log_{10}(x)$]. X refers to the respective value on the x-axis. The strength of correlation was interpreted by evaluating the correlation coefficient, as follows: $r \geq 0.9$ to $-0.9$=very strong correlation; $r \geq 0.7$ to $-0.7$=strong correlation; $r \geq 0.5$ to $-0.5$=moderate correlation; and $r \geq 0.3$ to $-0.3$=weak correlation.

Means I Cl 0.95
PCC (1 U ml\(^{-1}\)) increased \(C_{\text{max}}\) from baseline by 88 and 461%, respectively (Table 4). Fibrinogen concentrate (3 mg ml\(^{-1}\)) prolonged both \(t_{\text{lag}}\) (by 27%) and \(t_{\text{max}}\) (by 26%) and significantly reduced \(C_{\text{max}}\) (by 9%). At the lower concentration (0.6 mg ml\(^{-1}\)), FI significantly prolonged \(t_{\text{lag}}\) by 15%. Prothrombin complex concentrate (1 U ml\(^{-1}\)) also significantly prolonged \(t_{\text{lag}}\) by 33%. Activated prothrombin complex concentrate at 2.25 and 1.5 U ml\(^{-1}\) prolonged \(t_{\text{max}}\) from baseline by 96 and by 80%, respectively.

### Discussion

Rivaroxaban-spiked whole blood led to significant dose-dependent anticoagulant effects on various coagulation assay parameters. The AUC and EXTEM MaxV–t showed moderate correlation. Blood obtained from patients undergoing rivaroxaban treatment showed a strong correlation between rivaroxaban concentrations and Quick (PT) and moderate correlations with other coagulation assay parameters. All tested reversal agents significantly reduced EXTEM CT, but only aPCC and PCC were able to improve ETP parameters.

The results of the first part of the study are consistent with those of previous studies.\(^{20-22}\) Concerning in vivo analysis of rivaroxaban effects, our results are partly consistent with those of previous investigations.\(^{23-25}\) Oswald and colleagues\(^{26}\) also found a significant increase in ROTEM EXTEM CT and INTEM CT, as did another study conducted in 11 healthy male volunteers with significant prolongation of EXTEM and INTEM CT, whereas EXTEM was more strongly correlated with rivaroxaban concentrations than INTEM CTs.\(^{27}\) Rathbun and colleagues\(^{28}\) also observed only \(C_{\text{max}}\) and \(t_{\text{lag}}\) to be correlated with rivaroxaban, and Oswald and colleagues\(^{29}\) also found AUC to be unchanged. In contrast, several other studies revealed impairment of AUC and \(t_{\text{max}}\).\(^{21,24,29,30}\)

## Table 1

Correlation between rivaroxaban concentrations and blood coagulation parameters. Values are the mean (sd) of all coagulation tests and correlation with rivaroxaban concentrations at baseline. The Pearson correlation was used to detect correlations between various blood coagulation tests and rivaroxaban concentrations at baseline. Tests were sorted according to the strength of correlation. aPTT, activated partial thromboplastin time; \(C_{\text{max}}\), thrombin generation, peak thrombin generation; CT, clotting time; EXTEM, ROTEM, extrinsic coagulation pathway; FIBTEM, ROTEM, fibrinogen-dependent coagulation, thromboelastometry; INR, international normalized ratio; INTEM, ROTEM, intrinsic clotting time; MaxV–t, ROTEM, time from reaction start until the maximum of the first derivate of the curve is reached; PT, prothrombin time; Quick, Quick value; \(t_{\text{lag}}\), thrombin generation, lag time until initiation. Correlation (r) with rivaroxaban concentration in patient blood. The strength of correlation was interpreted by evaluating the correlation coefficient: \(r 0.9-1.0\) (very strong correlation); \(r 0.7-0.89\) (moderate correlation); \(r 0.5-0.69\) (weak correlation).

| Test                  | Units       | Mean (sd) | \(r\)   | Reference range |
|-----------------------|-------------|-----------|---------|-----------------|
| Quick (PT)            | %           | 63 (12)   | -0.78  | 70–130          |
| INR (PT)              | –           | 1.34 (0.15)| 0.75   | 0.8–1.2         |
| CT (FIBTEM)           | s           | 192 (83)  | 0.69   | 42–78           |
| \(C_{\text{max}}\)    | mE min\(^{-1}\)| 88 (21) | -0.66  | 111–156         |
| CT (EXTEM)            | s           | 215 (89)  | 0.63   | 42–78           |
| MaxV–t (FIBTEM)       | s           | 221 (88)  | 0.62   | –               |
| aPTT                  | s           | 43 (12)   | 0.57   | 26–37           |
| MaxV–t (EXTEM)        | s           | 260 (101)| 0.56   | –               |
| CT (INTEM)            | s           | 245 (31)  | 0.54   | 134–218         |
| \(t_{\text{lag}}\)   | s           | 55 (16)   | 0.52   | 19.6–25.6       |

## Table 2

Correlation between various blood coagulation assays. The Pearson correlation was used to detect the correlation between various blood coagulation tests. n.c., no significant correlation found; TG, thrombin generation; see Table 1 for definitions of other abbreviations. Only significant correlations (\(P<0.05\) for \(r\)) are shown.

| Assay                  | Quick (PT) | CT (FIBTEM) | \(C_{\text{max}}\) (TG) | CT (EXTEM) | MaxV–t (FIBTEM) | aPTT | MaxV–t (EXTEM) | CT (INTEM) |
|------------------------|------------|-------------|-------------------------|------------|----------------|------|---------------|------------|
| Quick (PT)             | –          | –           | –                       | –          | –              | –    | –             | –          |
| CT (FIBTEM)            | -0.781     | –           | –                       | –          | –              | –    | –             | –          |
| \(C_{\text{max}}\) (TG)| 0.696      | -0.682      | –                       | –          | –              | –    | –             | –          |
| CT (EXTEM)             | -0.747     | 0.978       | -0.596                  | –          | –              | –    | –             | –          |
| MaxV–t (FIBTEM)        | -0.795     | 0.972       | -0.647                  | 0.973      | –              | –    | –             | –          |
| aPTT                   | -0.768     | 0.637       | n.c.                    | 0.616      | 0.609          | –    | –             | –          |
| MaxV–t (EXTEM)         | -0.667     | 0.942       | -0.579                  | 0.972      | 0.944          | 0.514| –             | –          |
| CT (INTEM)             | -0.740     | 0.786       | n.c.                    | 0.804      | 0.759          | 0.657| 0.811         | –          |
| \(t_{\text{lag}}\) (TG)| -0.610     | 0.738       | -0.587                  | 0.741      | 0.684          | n.c. | 0.696         | 0.778      |
significantly increased by PCC. Fibrinogen concentrate seemed to have an impairing effect on thrombin generation in our study, but it was able to correct ETP in a rabbit model of rivaroxaban overdose. When comparing the first and the second part of this study, there is correlation with regard to ROTEM parameters but not thrombin generation parameters. This discrepancy could be caused by the high concentration range of rivaroxaban used in the first part of the study and the much narrower concentration range used in vivo. Moreover, platelet-poor plasma was used for thrombin generation assays, and patients concomitantly took other medication, which probably influenced the results.

The influence of rivaroxaban on PT, aPTT, and thromboelastometry assays differs widely depending on the reagents used and must be evaluated for each particular reagent. Consequently, normalizing effects of rivaroxaban on the various assays can also be expected to differ from reagent to reagent.

As expected, $t_{lag}$ was strongly correlated with ROTEM CT, which is in line with previous investigations; a study in 100 patients with thrombophilia or haemophilia revealed that both methods were comparable. After ex vivo addition of potential reversal agents, this correlation was not evident in the present study, which might be attributable to different methods and reagents or to the combination of in vivo rivaroxaban treatment and ex vivo administration of haemostatic interventions. Consequently, the efficacy of reversal of rivaroxaban seems to be highly dependent on the methods and parameters used and on whether experiments are conducted in vivo or not.

We showed that EXTEM and FIBTEM CT were comparable to plasma PT, whereas INTEM CT was comparable to plasma aPTT. Only rFVIIa was able to reverse INTEM CT to baseline values, which might be triggered by the ‘Josso loop’, in which FVIIa, Fibrinogen concentrate seemed to have an impairing effect on thrombin generation in our study, but it was able to correct ETP in a rabbit model of rivaroxaban overdose. PCC.

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Table 4 Thrombin generation assay. Blood samples were spiked with various non-specific reversal agents, as follows: fibrinogen concentrate (FI; FGTW), prothrombin complex concentrate (PCC; Beriplex®), activated prothrombin complex concentrate (aPCC; FEIBA), and recombinant activated factor VII (rFVIIa; NovoSeven®) and analysed with thrombin generation. AUC, area under the curve; C_{max}, peak thrombin generation; t_{lag}, lag time until initiation; t_{max}, time to peak thrombin activity. All values are means (sd). *P<0.05 compared with baseline values (Wilcoxon signed-rank test).

| Treatment | t_{lag} (n=13) | t_{max} (n=13) | AUC (n=13) | C_{max} (n=13) |
|-----------|---------------|---------------|------------|----------------|
| Baseline  | 19.6–25.6 s   | 50.8–72.0 s   | 312–441 mE | 111–156 mE min^{-1} |
| +Fl 3 mg ml^{-1} | 55 (16) | 114 (37) | 332 (20) | 88 (21) |
| +Fl 0.6 mg ml^{-1} | 70 (14)* | 144 (40)* | 325 (111) | 80 (22)* |
| +PCC 1 U ml^{-1} | 63 (16)* | 131 (29) | 339 (90) | 83 (15) |
| +PCC 0.3 U ml^{-1} | 73 (32)* | 180 (106) | 686 (297)* | 494 (1011)* |
| +aPCC 2.25 U ml^{-1} | 71 (15) | 175 (46) | 475 (204)* | 88 (29) |
| +aPCC 1.5 U ml^{-1} | 77 (10) | 223 (27)* | 1102 (434)* | 165 (60)* |
| +rFVIIa 4.05 µg ml^{-1} | 70 (8) | 205 (27)* | 842 (434)* | 136 (60) |
| +rFVIIa 1.5 µg ml^{-1} | 63 (12) | 139 (52) | 392 (206) | 96 (27) |

originally part of the extrinsic coagulation pathway, can activate factor IX or the intrinsic coagulation pathway. Prolongation of INTEM CT by PCC can be explained by the composition of PCC; PCC also contains anticoagulants, such as protein C, protein S, heparin, and antithrombin. Nilsson and colleagues found a significant increase in INTEM CT by recombinant human activated protein C. The heparin effect of PCC has been reported previously.

Prolongation of the thrombin generation assay parameters t_{lag} and t_{max} by addition of potential reversal agents can be explained by the test and reagents used, because other studies showed a reduction in t_{lag} after rFVIIa and aPCC for rivaroxaban. Moreover, the prothrombin content of PCC and aPCC is a major determinant of their potential to generate excessive thrombin, which clearly influences the test outcome of thrombin generation assays. This also depends on the type of assay and type of chromogenic substrate or tissue factor content used, as demonstrated in other studies.

All potential reversal agents in this study were added ex vivo, and dose-dependent reversal effects of potential reversal agents cannot be detected in vitro, as already reported by Perzborn and colleagues. This constitutes a major limitation of the present study, because the discrepancy between the in vitro and in vivo measurements and the ex vivo addition of potential reversal agents emphasizes the difficulty in demonstrating in vivo effects ex vivo. It also highlights the need for in vivo investigations and controlled clinical trials to evaluate the real potential of non-specific reversal agents.

The potential risk for thromboembolic events has to be evaluated before using any potential reversal agent to reverse rivaroxaban-induced bleeding. No controlled clinical studies in humans using reversal agents in bleeding situations are available. This illustrates a difficulty in evaluating potential reversal agents for the management of life-threatening bleeding in rivaroxaban-anticoagulated patients.

Our data indicate that great care must be taken when analysing data gained in vitro and translating such data to in vivo situations. It is not clear which reversal agent is best for the treatment of bleeding patients treated with rivaroxaban, and thrombin generation assay and ROTEM do not favour the same agents. Coagulation assays cannot predict the bleeding tendency, and animal models might not be representative for human models. Therefore, controlled clinical trials are needed, even if specific antidotes are in development, because not all hospitals will be able to store these antidotes, and there is still little knowledge about the real efficacy of alternative reversal agents.

Authors’ contributions
Study concept and design: W. Streif, D.F., M.B., B.S.
Application to ethics committee and authorities: B.S., D.F., M.B.
Patient recruitment: W. Sturm
Data acquisition: B.S., P.W., W. Streif, W. Sturm
Laboratory experiments: B.S., F.W., M.B.
Data analysis and interpretation: B.S., W. Sturm, D.F.
Writing of the first draft of the manuscript: B.S.
Responsible for data integrity and accuracy of data analysis: B.S.
Critical revision of the manuscript for important intellectual content: P.W., W. Streif, W. Sturm, D.F., M.B.

Supplementary material
Supplementary material is available at British Journal of Anaesthesia online.

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