BRIEF REPORT

Assessing Coagulation by Rotational Thromboelastometry (ROTEM) in Rivaroxaban-Anticoagulated Blood Using Hemostatic Agents

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Keywords: Celox; factor Xa inhibitors; hemostatic techniques; Kaolin; thromboelastography

Abbreviations:
CFT: clot formation time
CT: clotting time
DOAC: direct oral anticoagulant
ED: emergency department
FXa: factor Xa
FXII: factor XII
MCF: maximum clot firmness
NATEM: Non-Activated Thromboelastometry
PCC: prothrombin complex concentrate
ROTEM: rotational thromboelastometry

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Abstract

Introduction: The use of direct oral anticoagulants (DOACs) such as rivaroxaban (Xarelto) is increasingly common. However, therapies for reversing anticoagulation in the event of hemorrhage are limited. This study investigates the ability of hemostatic agents to improve the coagulation of rivaroxaban-anticoagulated blood, as measured by rotational thromboelastometry (ROTEM).

Hypothesis/Problem: If a chitosan-based hemostatic agent (Celox), which works independently of the clotting cascade, is applied to rivaroxaban-anticoagulated blood, it should improve coagulation by decreasing clotting time (CT), decreasing clot formation time (CFT), and increasing maximum clot firmness (MCF). If a kaolin-based hemostatic agent (QuikClot Combat Gauze), which works primarily by augmenting the clotting cascade upstream of factor Xa (FXa), is applied to rivaroxaban-anticoagulated blood, it will not be effective at improving coagulation.

Methods: Patients (age >18 years; non-pregnant) on rivaroxaban, presenting to the emergency department (ED) at two large, university-based medical centers, were recruited. Subjects (n = 8) had blood drawn and analyzed using ROTEM with and without the presence of a kaolin-based and a chitosan-based hemostatic agent. The percentage of patients whose ROTEM parameters responded to the hemostatic agent and percent changes in coagulation parameters were calculated.

Results: Data points analyzed included: CT, CFT, and MCF. Of the samples treated with a kaolin-based hemostatic agent, seven (87.5%) showed reductions in CT, eight (100.0%) showed reductions in CFT, and six (75.0%) showed increases in MCF. The average percent change in CT, CFT, and MCF for all patients was 32.5% (Standard Deviation [SD]: 286; Range:-75.3 to 740.7%); -66.0% (SD:14.4; Range: -91.4 to -44.1%); and 4.70% (SD: 6.10; Range: -4.8 to 15.1%), respectively. The corresponding median percent changes were -68.1%, -64.0%, and 5.2%. Of samples treated with a chitosan-based agent, six (75.0%) showed reductions in CT, three (37.5%) showed reductions in CFT, and five (62.5%) showed increases in MCF. The average percent changes for CT, CFT, and MCF for all patients were 165.0% (SD: 629; Range:-96.9 to 1718.5%); 139.0% (SD: 174; Range: -83.3 to 348.0%); and -8.38% (SD: 32.7; Range:-88.7 to 10.4%), respectively. The corresponding median percent changes were -53.7%, 141.8%, and 3.0%.

Conclusions: Rotational thromboelastometry detects changes in coagulation parameters caused by hemostatics applied to rivaroxaban-anticoagulated blood. These changes trended in the direction towards improved coagulability, suggesting that kaolin-based and chitosan-based hemostatics may be effective at improving coagulation in these patients.

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Introduction

Direct oral anticoagulants (DOACs) are indicated for a number of medical conditions, including non-valvular atrial fibrillation and venous thromboembolism.[1] Their adoption into clinical practice has been rapid, accounting for 62% of new anticoagulant prescriptions for atrial fibrillation and 98% of anticoagulant-related drug costs between 2010 and 2013.[2]

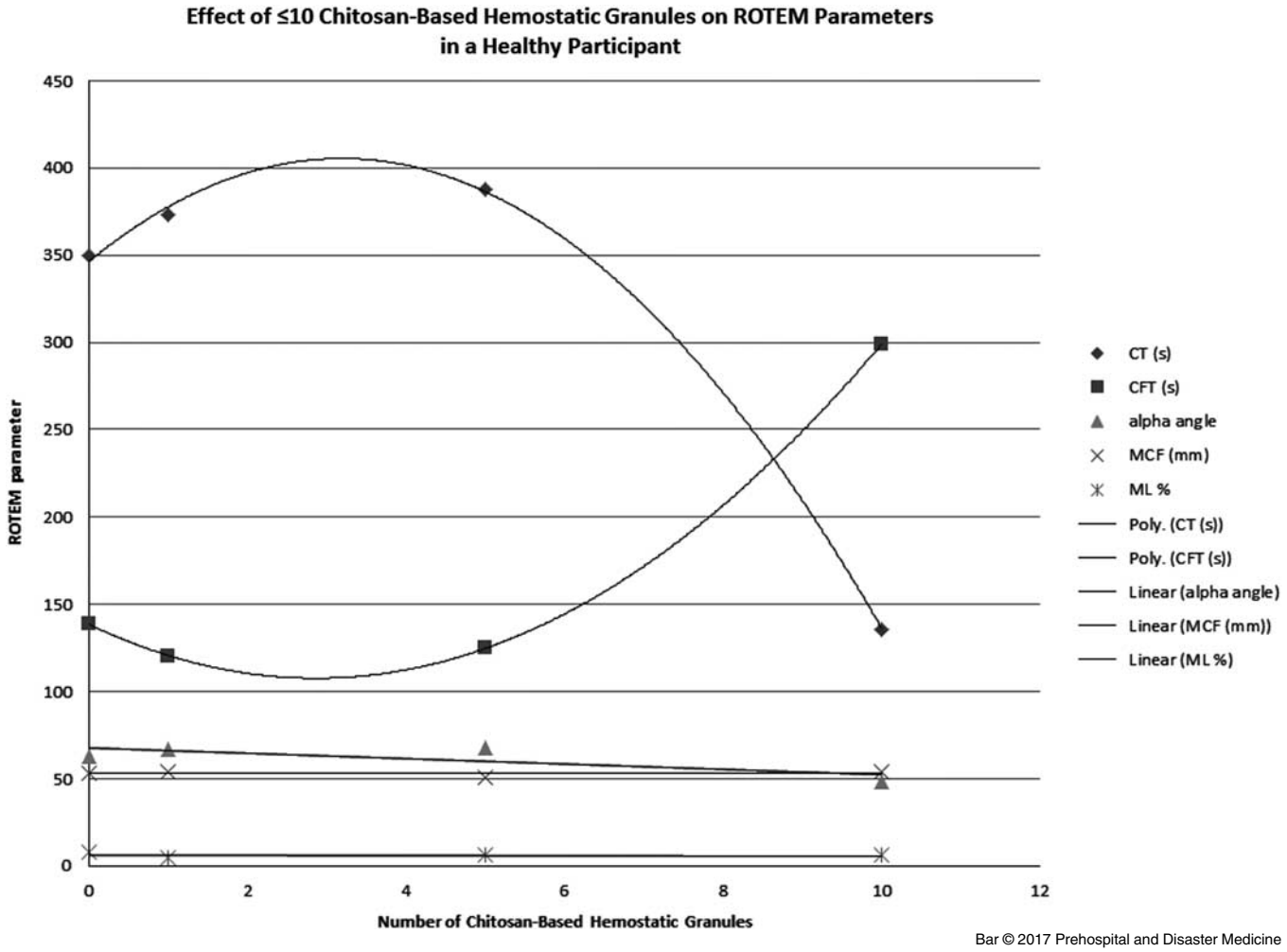

Figure 1. Data from Empiric Determination of Chitosan-Based Hemostatic "Dose" for Subsequent Experiments (≤10 granules).
Abbreviations: CFT, clot formation time; CT, clotting time; MCF, maximum clot firmness; ROTEM, rotational thromboelastometry.

These numbers are expected to grow as more studies analyze the safety of DOACs in other patient populations, including pregnant patients and patients with end-stage renal disease.[3,4]

While the risk of bleeding with DOACs may be lower compared to a vitamin K antagonist,[5] the use of any anticoagulant still poses an increased bleeding risk. A satisfactory solution to bleeding in patients taking DOACs has yet to be developed.[6] Therapies for reversing anticoagulation include drug removal through dialysis and administration of prothrombin complex concentrates (PCCs).[6] Dialysis may take too long to be effective in an emergency, and PCCs may lead to thrombotic complications in a vulnerable population.[7,8] In October of 2015, the Food and Drug Administration (FDA; Silver Spring, Maryland USA) approved idarucizumab, a specific antidote for the DOAC, dabigatran, a direct thrombin inhibitor. While idarucizumab appears effective, it is costly[9] and may not be practical when needed in the emergency department (ED) or prehospital setting. It also does not address the reversal of the DOACs that inhibit factor Xa (FXa).

This study explores the ability of kaolin-based and chitosan-based topical hemostatic agents to reverse the effects of a representative DOAC, rivaroxaban. Rivaroxaban binds to the active site of FXa and inhibits FXa's ability to convert prothrombin to thrombin, preventing amplification of the coagulation cascade.[10] Inhibiting FXa also prevents platelet activation.[10]

Topical hemostatic agents have been used by military and Emergency Medical Service providers for the control of external hemorrhage.[11] The agents are available at sporting goods stores, military surplus vendors, or online marketplaces without a prescription. They are applied as impregnated bandages or powders, and their onset of effect is extremely rapid.[11] The various hemostatic agents work by different mechanisms.

Chitosan-based products cause platelet activation and erythrocyte adhesion, due to their positively charged carbohydrate components binding negative charged moieties on erythrocytes.[12,13] This mechanism is independent of the clotting cascade and should be unhindered by FXa inhibitors. Thus, chitosan-based products should improve coagulation in blood anticoagulated with rivaroxaban.

Kaolin, the key component of other hemostatic products, works by two mechanisms. First, the inert minerals in the product act by rapidly dehydrating their substrate.[8] Kaolin also initiates the intrinsic coagulation pathway by activating factor XII (FXII).[14,15] Since FXII is upstream of the actions of the rivaroxaban, the authors predicted that this product would not be effective at promoting coagulation in blood anticoagulated with rivaroxaban.

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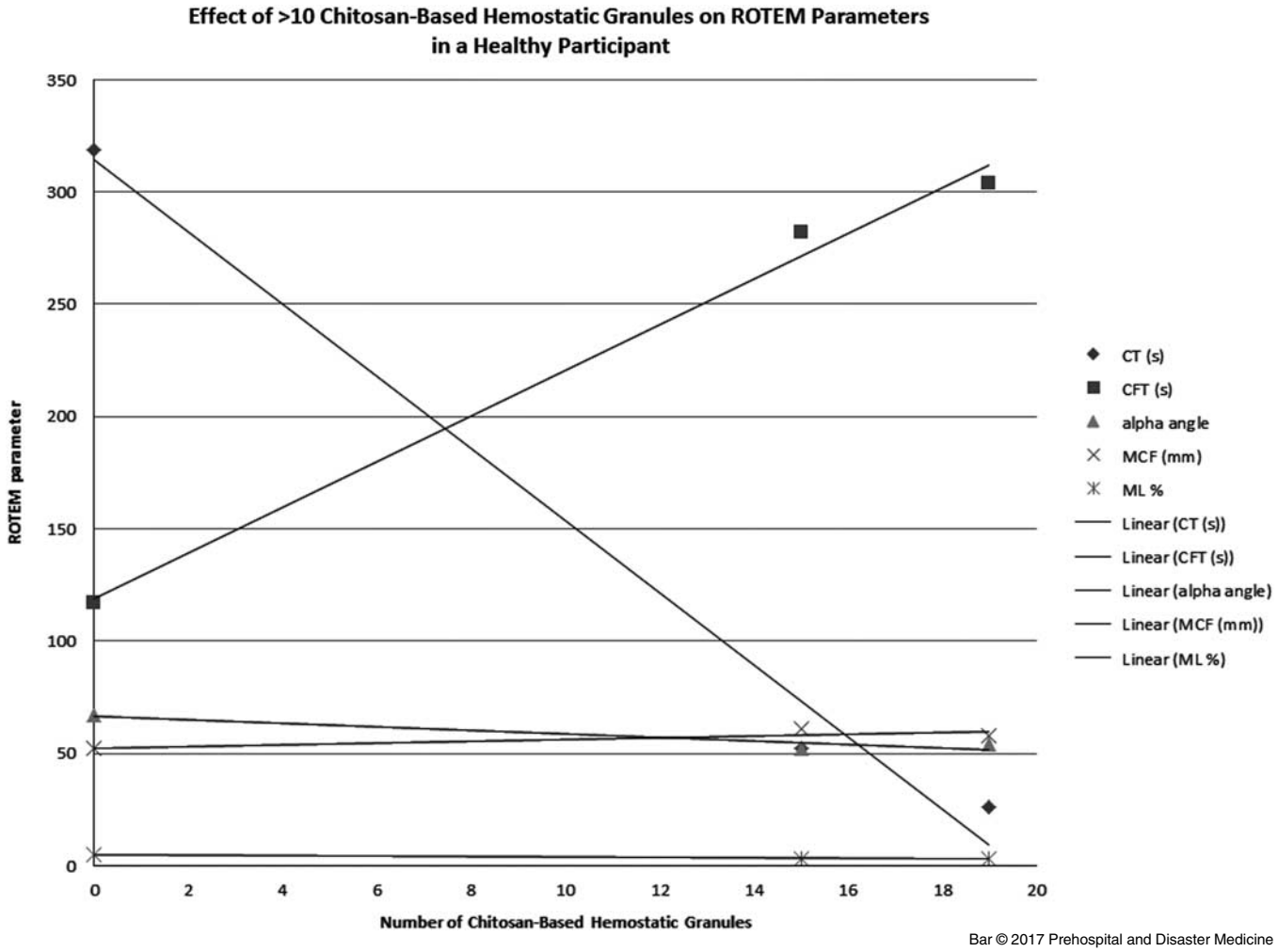

Figure 2. Data from Empiric Determination of Chitosan-Based Hemostatic "Dose" for Subsequent Experiments (>10 granules).
Abbreviations: CFT, clot formation time; CT, clotting time; MCF, maximum clot firmness; ROTEM, rotational thromboelastometry.

Methods

The authors conducted a prospective, experimental pilot study at two university-based EDs with a combined annual adult volume of over 270,000 patients. This study was approved by the Institutional Review Boards of both Columbia University Medical Center (New York, New York USA) and Weill Cornell Medical College (New York, New York USA). Patients who presented to the ED and met the following criteria were eligible for the study: (1) taking rivaroxaban for any indication; (2) age ≥18 years; (3) non-pregnant; and (4) not taking any other anticoagulant or anti-platelet medication other than aspirin. Eligible subjects were identified using the electronic medical record system or by direct patient interview with informed consent obtained by one of the authors. Participants completed a health history questionnaire which assessed their medications, medication compliance, past medical history, and reasons for the current ED visit. Participants then had one tube of citrated blood (2.7 mL) drawn for analysis.

Both the kaolin-based hemostatic agent, QuikClot Combat Gauze (Z-Medica, LLC; Wallingford, Connecticut USA), and the chitosan-based agent, Celox Hemostatic Granules (Medtrade Products Ltd; Crewe, United Kingdom), were stored in sealed plastic bags in a secure location after opening and handled only with forceps and nitrile gloves to avoid contamination.

Blood samples were analyzed by rotational thromboelastometry (ROTEM) using a ROTEM *delta* (TEM Innovations GmbH; Munich, Germany) analyzer within two hours of acquisition. For each sample, three tests were run simultaneously at 37°C using Non-Activated Thromboelastometry (NATEM), following the manufacturer's instructions, except where otherwise stated. The NATEM test was chosen for two reasons: first, there are no additives to the blood other than the reagent (0.2 mol/l $CaCl_2$ in HEPES buffer pH 7.4 and 0.1% sodium acide) used to re-calcify the citrated blood; and second, it is sensitive to any change in the coagulation system. Clotting time (CT), clot formation time (CFT), and maximum clot firmness (MCF) were analyzed. For each citrated tube, the first test used the participant's blood only. During the second test, 15 granules of chitosan-based hemostatic were transferred, using a folded slip of paper, to the participant's blood in the step immediately after adding the re-calcifying agent. Finally, a 0.5cm x 0.5 cm square of kaolin-impregnated gauze was added to the remaining citrated blood. Since the NATEM tests were always performed in the same order, the volume of remaining blood was constant at 2.02mL, which is equivalent to the volume of

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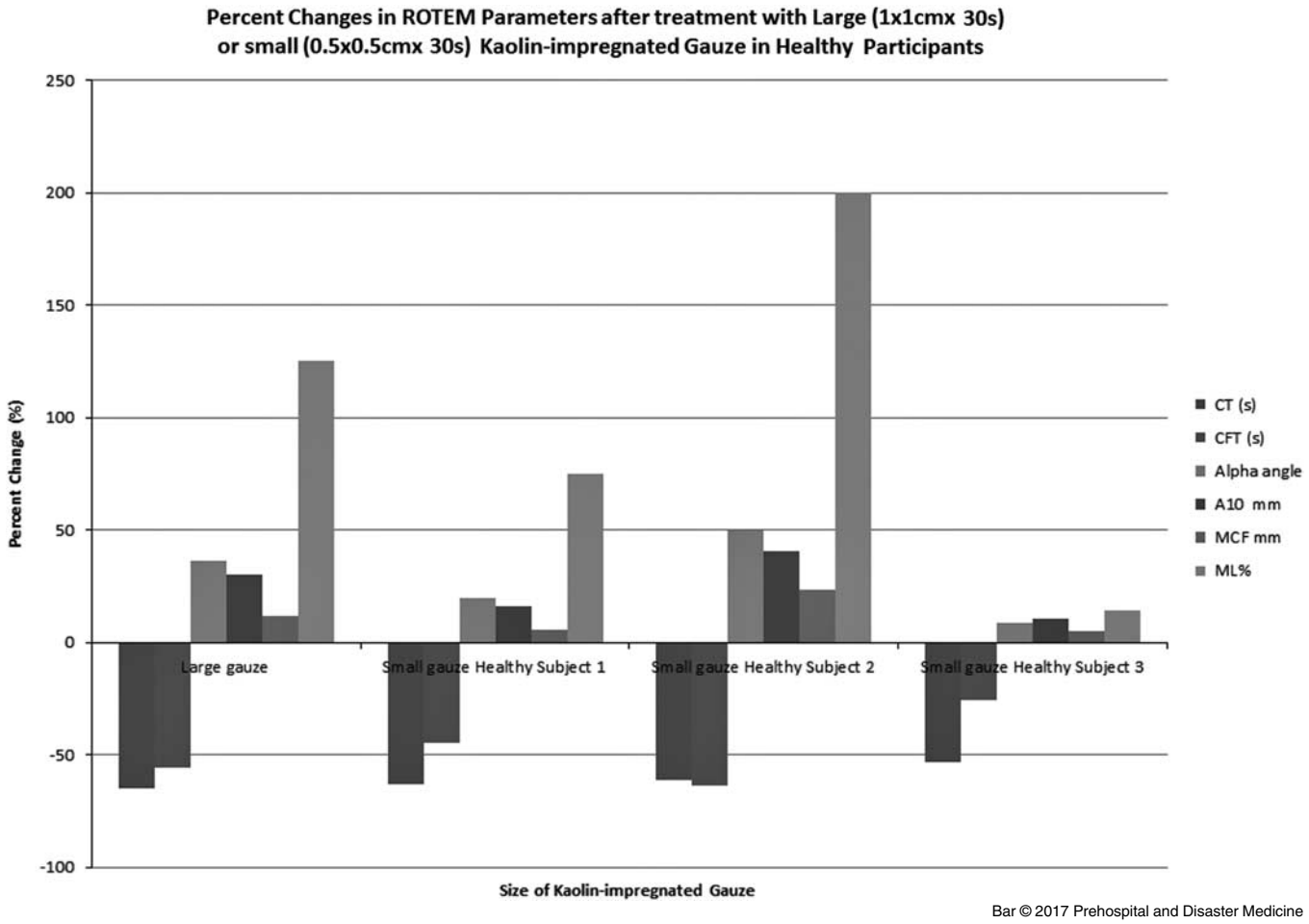

Figure 3. Data from Empiric Determination of Kaolin-Based Hemostatic "Dose" for Subsequent Experiments.
Abbreviations: CFT, clot formation time; CT, clotting time; MCF, maximum clot firmness; ROTEM, rotational thromboelastometry.

blood in the citrated tube (2.7mL), less the blood used in the two previous tests (340µL per test). The tube was inverted gently back and forth for 30 seconds to expose the blood to the gauze prior to being added to the analyzer. After the measurements were taken, the data were entered into a Microsoft Excel 2010 (Microsoft Corporation; Redmond, Washington USA) spreadsheet. The percentage of patients whose ROTEM parameters responded to the hemostatic agent and the average percent changes in ROTEM parameters were determined.

To the authors' knowledge, the coagulation effects of topical hemostatic agents have not been previously evaluated using ROTEM. To determine the amount of hemostatic agent needed for each ROTEM test, it was necessary to detect an effect on coagulation parameters while simultaneously preventing the blood from clotting too quickly for the machine to measure. For the chitosan-based hemostatic agent, the test dose of 15 granules was determined empirically. The number of granules was increased until the machine could not perform an analysis because the sample clotted too quickly (Figure 1 and Figure 2). The method for testing the kaolin-impregnated gauze was also devised empirically, using the smallest practical area to demonstrate an effect. Initially, a 1x1cm piece of kaolin-impregnated gauze was added to a tube of citrated blood and inverted gently for 30 seconds just prior to adding the exposed blood to ROTEM. The gauze itself is not added to the ROTEM, since doing so is not technically possible in the analyzer. After testing the 1x1cm gauze, a smaller piece of gauze (0.5x0.5cm) was tested, and it was noted that it could also demonstrate a similar effect on ROTEM parameters. This size of gauze was then tested with three different tubes of citrated blood from healthy participants, showing a consistent direction of effect (Figure 3).

Results

A summary of participant characteristics is given in Table 1. Figure 4 through Figure 6 illustrate the changes in coagulation parameters for individual patients. Notably, the clotting times for Participant 8 were outliers (>1.5 interquartile ranges above the 3rd quartile) for unknown reasons. For this reason, both the mean and median of the dataset are reported.

Of the samples treated with kaolin-impregnated gauze, seven (87.5%) showed reductions in CT, eight (100.0%) showed reductions in CFT, and six (75.0%) showed increases in MCF. Additionally, the average percent change in CT, CFT, and MCF for all patients was 32.5% (Standard Deviation [SD]: 286; Range: -75.3 to 740.7%); -66.0% (SD:14.4; Range: -91.4 to -44.1%); and 4.70% (SD: 6.10; Range: -4.8 to15.1%), respectively. The corresponding median percent changes were -68.1%, -64.0%, and 5.2%, respectively.

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Patient Number	Age	Gender	Xarelto Dose	Other Medications	Pertinent Medical History	Reason for ED Visit
1	58	Female	20mg daily	Tofacitinib, methotrexate, prednisone, simvastatin, rabeprazole, metformin, famciclovir, calcium, multivitamin	RA, herpes zoster, high cholesterol, hx of clots	Left-sided facial droop
2	41	Female	15mg BID	Fentanyl, ondansetron, metoclopramide, gabapentin	Spinal cord injury, hx of clots	N/V/D Hx of C. diff
3	58	Female	20mg daily	Levothyroxine, digoxin	A. fib, hypothyroidism	Dizziness, stomach pain
4	51	Female	Once daily (pt. did not know exact dosage)	Diltiazem, dofetilide	A. fib	A. fib
5	72	Male	20mg daily	Metoprolol, lubisartan, fish oil, vitamin D, chondroitin, wheat grass	A-fib, hypertension	Dizziness and numbness
6	73	Male	25mg daily	Dexamethasone, metformin, lenalidomide	Multiple myeloma, colon cancer, blood clots	Referred for low blood pressure
7	88	Male	10mg daily	Allopurinol, furosemide, olmesartan	Spinal stenosis, arthritis, gout, edema, A. fib	Pneumonia
8	90	Female	15mg daily	Diltiazem, sitagliptin, furosemide, omeprazole, rosuvastatin, montelukast, budesonide/ formoterol	Non-valvular A-fib, Diabetes mellitus, asthma, COPD, history of right leg arterial thrombus	Pleuritic Chest pain

Table 1. Patient Characteristics
Abbreviation: ED, emergency department.

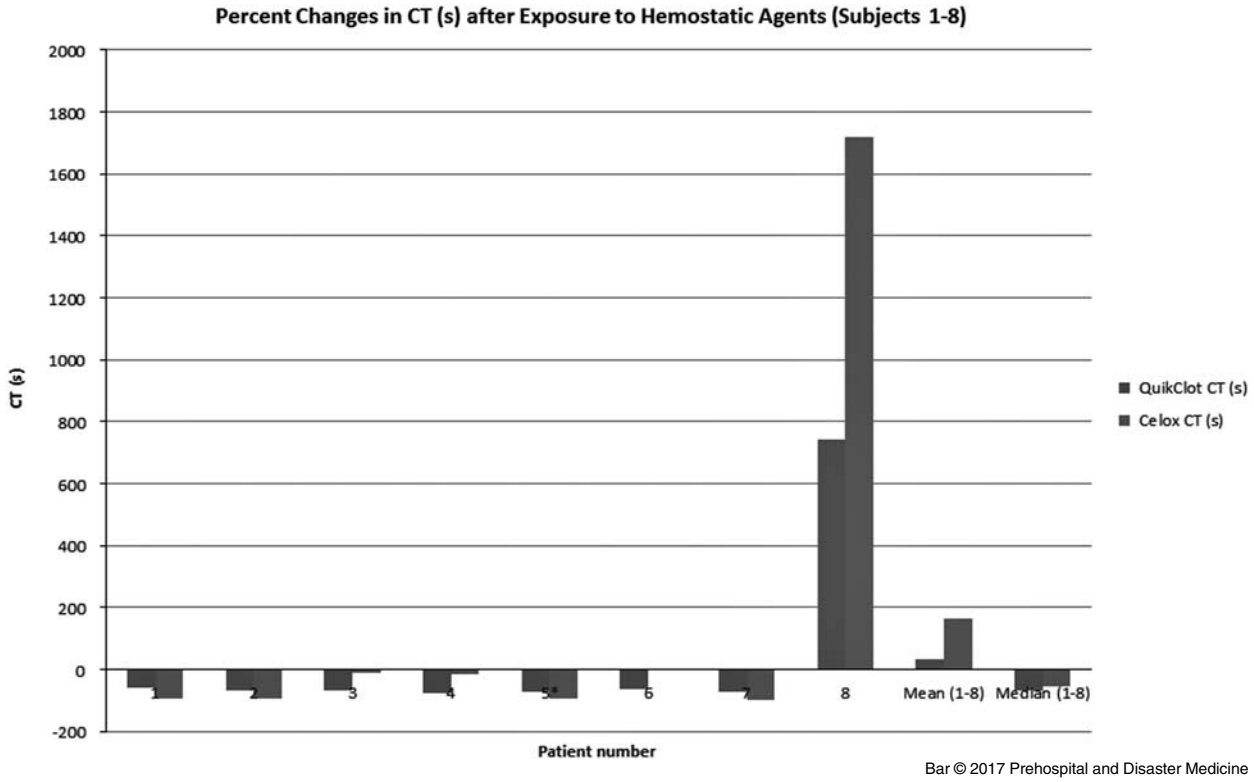

Figure 4. Percent Changes in Clotting Time (seconds) after Exposure to Hemostatic Agents.
Note: During the test of the chitosan-based hemostatic agent, the ROTEM recorded error code 6033, which means that the measurement was influenced by drying of the sample (likely due to chitosan-based hemostatic test effect). During the kaolin-impregnated gauze test, the ROTEM recorded error code 4033, which is a motion timeout error. This usually occurs when the firmness cannot be determined due to mechanical failure.
Abbreviations: CT, clotting time; ROTEM, rotational thromboelastometry.

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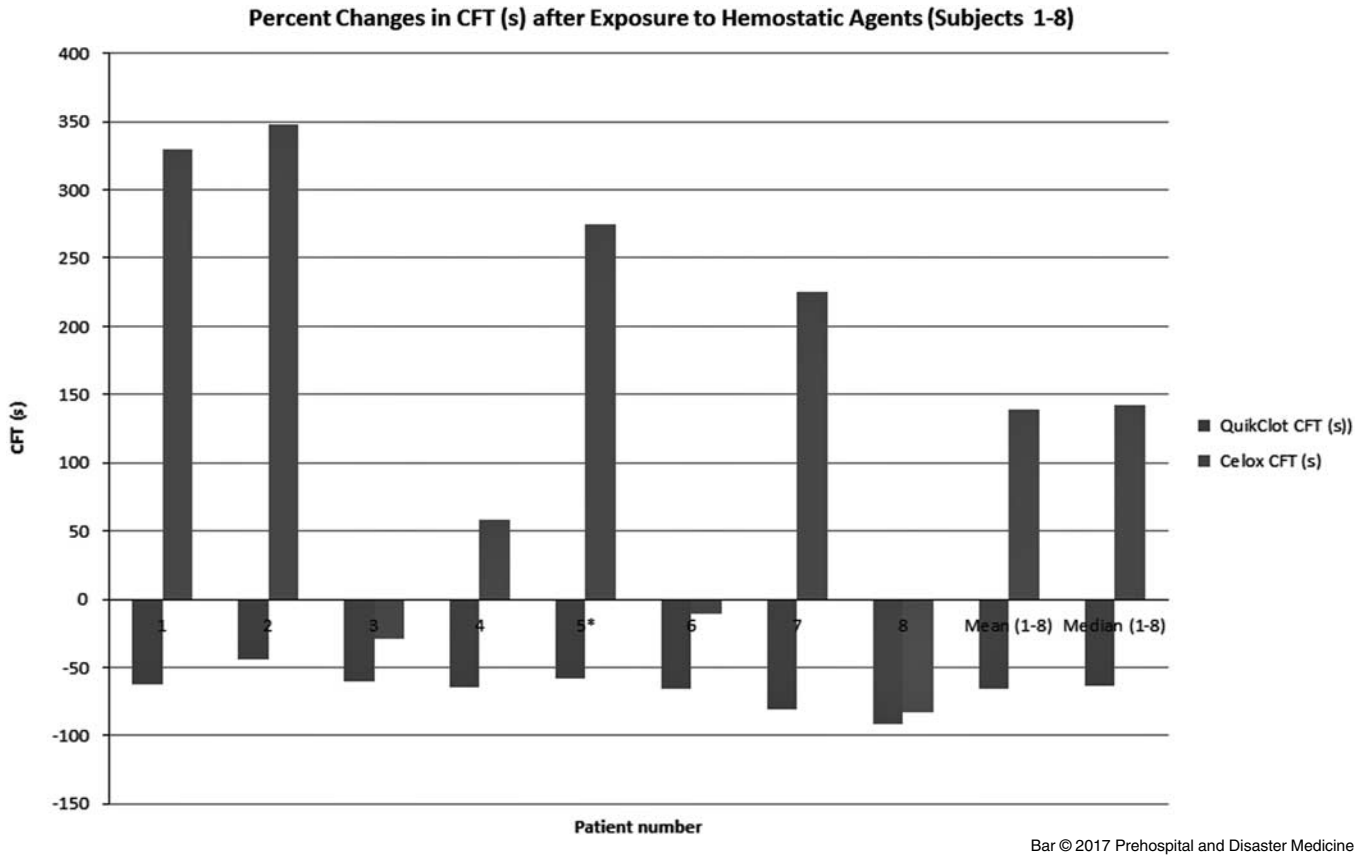

Figure 5. Percent Changes in Clot Formation Time (seconds) after Exposure to Hemostatic Agents.
Note: During the test of the chitosan-based hemostatic agent, the ROTEM recorded error code 6033, which means that the measurement was influenced by drying of the sample (likely due to chitosan-based hemostatic test effect). During the kaolin-impregnated gauze test, the ROTEM recorded error code 4033, which is a motion timeout error. This usually occurs when the firmness cannot be determined due to mechanical failure.
Abbreviations: CFT, clot formation time; ROTEM, rotational thromboelastometry.

Of the samples treated with chitosan-based hemostatic, six (75.0%) showed reductions in CT, three (37.5%) showed reductions in CFT, and five (62.5%) showed increases in MCF. The average percent changes for CT, CFT, and MCF for all patients were 165.0% (SD: 629; Range: -96.9 to 1718.5%); 139.0% (SD: 174; Range: -83.3 to 348.0%); and -8.38% (SD: 32.7; Range: -88.7 to 10.4%), respectively. The corresponding median percent changes were -53.7%, 141.8%, and 3.0%, respectively.

Discussion

The overall results of this study were encouraging. Because kaolin activates the clotting cascade prior to the activation of FXa,[14,15] the authors hypothesized that there would be no changes in CT, CFT, or MCF in blood treated with kaolin-based hemostatic. Therefore, seeing the majority of samples treated with kaolin-based hemostatic responding in a direction towards increased coagulability was unexpected. This may be the result of an incomplete blockade of FXa by rivaroxaban, or the kaolin may be working by an entirely different mechanism.

For the samples treated with the chitosan-based hemostatic agent, the authors hypothesized that the CT and CFT would decrease and that the MCF would increase. This proved to be true in the majority of cases for the CT, but not for the CFT and MCF, with the CFT actually increasing. It is suspected that the CFT increased because the parameter is proportional to the rate of fibrin polymerization. As a clot forms in the analyzer, forming strands of fibrin resist the motion of the pin, which the machine detects to generate a measurement. Chitosan-based hemostatics do not act by polymerizing fibrin, but instead by aggregating erythrocytes to form a gel-like clot. This type of clot may be providing less resistance to pin motion, leading to higher CFT readings. This explanation is consistent with the observed results for the samples treated with kaolin-impregnated gauze, which showed reductions in CFT. Since kaolin-based hemostatic agent works by activating the clotting cascade and therefore increasing fibrin polymerization, the clots it generates cause increased motion restriction on the pin, leading to the observed results. The results for MCF in samples treated with chitosan-based hemostatic agent were variable. Since the MCF is dependent on the amount of clottable substrate, the results may be explained by the fact that each granule of chitosan-based hemostatic is not exactly the same size and shape. Therefore, each 15 granules tested may have had a different surface area and total volume upon which to build clot.

Based on this study, it is difficult to make head-to-head comparisons between the hemostatic agents. However, in general, the magnitude of the changes in CT and CFT were much higher with

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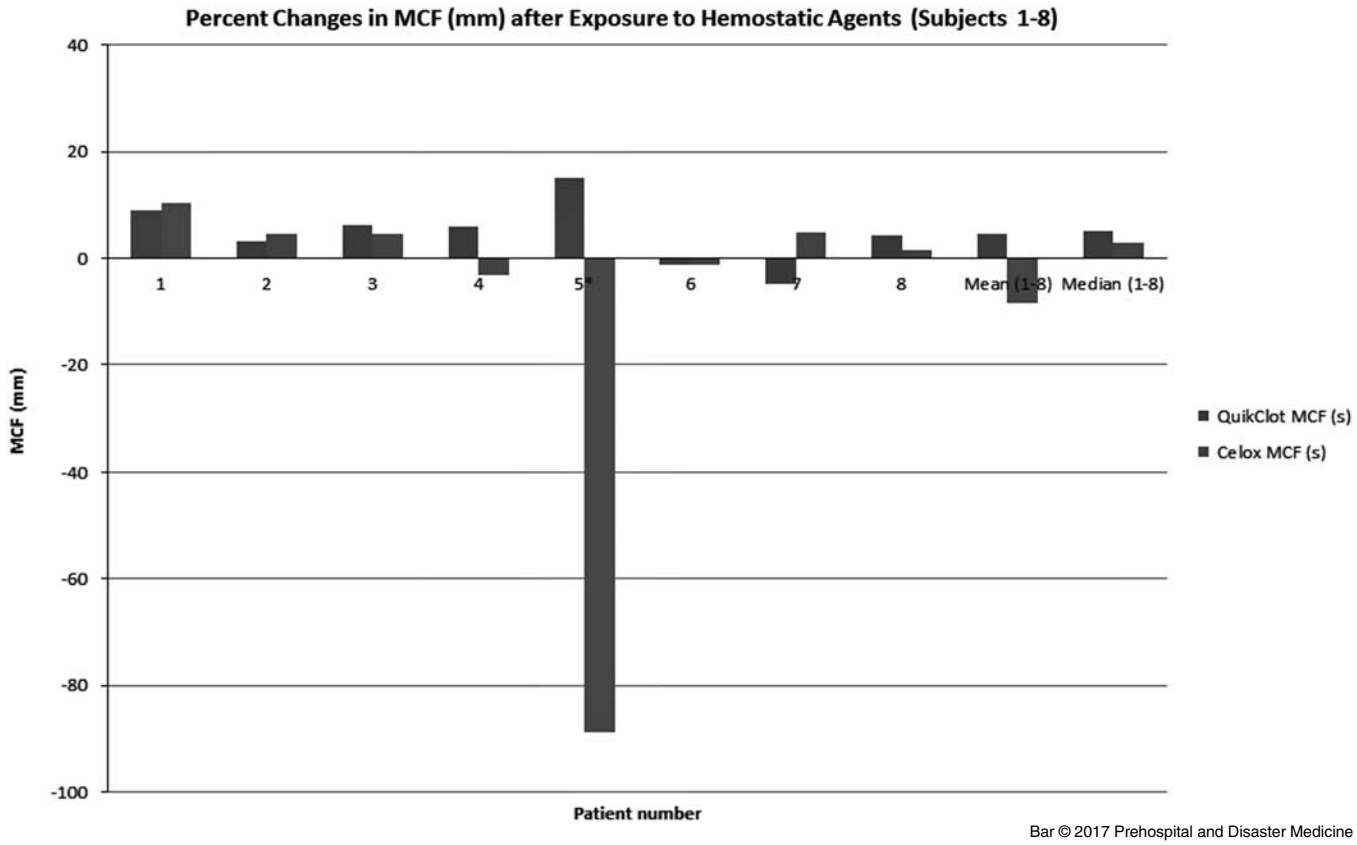

Figure 6. Percent Changes in Max Clot Firmness (millimeters) after Exposure to Hemostatic Agent.
Note: During the test of the chitosan-based hemostatic agent, the ROTEM recorded error code 6033, which means that the measurement was influenced by drying of the sample (likely due to chitosan-based hemostatic test effect). During the kaolin-impregnated gauze test, the ROTEM recorded error code 4033, which is a motion timeout error. This usually occurs when the firmness cannot be determined due to mechanical failure.
Abbreviations: MCF, maximum clot firmness; ROTEM, rotational thromboelastometry.

chitosan-based hemostatic agent compared to the kaolin-impregnated gauze, but the variability of response was also higher. This may be explained by the fact that such a small number of chitosan-based hemostatic granules were used in these tests. Since each granule is not the same size and shape, there is room for considerable variability. However, in the setting of an actual wound to which grams of granules are introduced, it is possible that the differences between granules would average out and a more consistent effect would be observed. For this reason, future studies using *in vivo* models of bleeding would help facilitate direct comparison and also help determine if changes in ROTEM parameters correspond to a clinically significant effect at a real wound site.

Limitations

This study has several other limitations, notably the small sample size. The authors were also unable to enroll enough case-matched controls not taking rivaroxaban in the study. Performing the same test on blood samples from these participants would allow comparisons between the percent changes in these participants and the ones taking rivaroxaban, and if the hemostatic agents are either fully or partially overcoming the anticoagulation. It would also allow better control of other participant characteristics that might be influencing the action of kaolin-based hemostatic or chitosan-based hemostatic. Lastly, this study relied on self-report for assessing compliance with medications, so it is possible that some of the patients were not taking rivaroxaban as directed or were taking other undisclosed medications.

To help address these limitations, there are several next steps: (1) increase the study sample size; (2) recruit case-match controls to account for additional patient characteristics and alternate medication interactions; and (3) expand recruitment to other DOACs.

Despite the aforementioned limitations, this study adds two contributions to the literature. First, it demonstrates that ROTEM analysis can be used to detect changes caused by topical hemostatic agents. Second, it suggests that such hemostatic agents may be beneficial as an adjunct to other methods of hemorrhage control in patients taking rivaroxaban. This is likely to be valuable to prehospital providers and providers working in backcountry settings who need a field-expedient solution that requires minimal medical training to apply. It would also be particularly valuable to physicians who need local anticoagulation reversal at a wound site, without the systemic reversal of anticoagulation that an antidote would provide. Future studies may help determine which specific hemostatic agent is ideal in these circumstances.

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Conclusion

Rotational thromboelastometry can detect changes in coagulation parameters caused by topical hemostatic agents when these agents are applied to rivaroxaban-anticoagulated blood. These changes trended in the direction towards increased coagulability, suggesting that kaolin and chitosan-based hemostatic agents may be effective at improving coagulation in these patients.

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Author Contributions

Jonathan Bar: literature search, study design, data collection, data analysis, data interpretation, writing, and critical revision. Alexa David: data collection and critical revision. Tarek Khader: literature search, data collection, writing, and critical revision. Mary Mulcare: data interpretation, data analysis, and critical revision. Christopher Tedeschi: study design, data interpretation, data analysis, and critical revision.

References

1. Gómez-Outes A, Suárez-Gea ML, Lecumberri R, Terleira-Fernández AI, Vargas-Castrillón E. Direct acting oral anticoagulants: pharmacology, indications, management, and future perspectives. *Eur J Haematol*. 2015;95:389-404.
2. Desai NR, Krumme AA, Schneeweiss S, et al. Patterns of initiation of oral anti-coagulants in patients with atrial fibrillation quality and cost implications. *Am J Med*. 2014;127(11):1075.e1-1082.e1.
3. Young SK, Al-Mondhiry HA, Vaida SJ, Ambrose A, Botti JJ. Successful use of argatroban during the third trimester of pregnancy: case report and review of the literature. *Pharmacotherapy*. 2008;28(12):1531-1536.
4. Hart RG, Pearce LA, Aguilar MI. Meta-analysis: antithrombotic therapy to prevent stroke in patients who have nonvalvular atrial fibrillation. *Ann Intern Med*. 2007;146:857-867.
5. Kakkos SK, Kirkilesis GI, Tsolakis IA. Editor's Choice – efficacy and safety of the new oral anticoagulants dabigatran, rivaroxaban, apixaban, and edoxaban in the treatment and secondary prevention of venous thromboembolism: a systematic review and meta-analysis of phase III trials. *Eur J Vasc Endovasc Surg*. 2014;48: 565-575.
6. Sartori MT, Prandoni P. How to effectively manage the event of bleeding complications when using anticoagulants. *Expert Review of Hematology*. 2015: 37-50.
7. Sorensen B, Spahn DR, Innerhofer P, Spannagl M, Rossaint R. Clinical review: prothrombin complex concentrates–evaluation of safety and thrombogenicity. *Crit Care*. 2011;15:201.
8. Makris M, Van Veen JJ, Tait CR, Mumford AD, Laffan M. Guideline on the management of bleeding in patients on anti- thrombotic agents. *Br J Haematol*. 2013;160:35-46.
9. Yogaratnam D, Ditch K, Medeiros K, Doyno C, Fong JJ. Idarucizumab for reversal of dabigatran. *Ann Pharmacother*. 2016;pii: 1060028016659504.
10. Kubitza D, Haas S. Novel factor Xa inhibitors for prevention and treatment of thromboembolic diseases. *Expert Opin Investig Drugs*. 2006;15:843-855.
11. Gordy SD, Rhee P, Schreiber MA. Military applications of novel hemostatic devices. *Expert Rev Med Devices*. 2011;8(1):41-47.
12. Cox ED, Schreiber MA, McManus J, Wade CE, Holcomb JB. New hemostatic agents in the combat setting. *Transfusion*. 2009;49(Suppl):248S-255S.
13. Granville-Chapman J, Jacobs N, Midwinter M. Prehospital hemostatic dressings: a systematic review. *Injury*. 2011;42(5):447-459.
14. Rhee P, Brown C, Martin M, et al. QuikClot use in trauma for hemorrhage control: case series of 103 documented uses. *J Trauma*. 2008;64:1093-1099.
15. Walsh PN. The effects of Collagen and Kaolin on the intrinsic coagulant activity of platelets. Evidence for an alternative pathway in intrinsic coagulation not requiring factor XII. *Br J Haematol*. 1972;22:393-405.