Effect of activated charcoal in removing interference on several thrombophilia assays: resistance to activated protein C, activity of coagulation factor VIII and antithrombin activity

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INTRODUCTION

Treatment with direct oral anticoagulants (DOACs) has significant impact on results of many specialized coagulation tests, such as thrombophilia assays, due to possible false negative (FN) or false positive (FP) results.

AIM

To investigate the impact of dabigatran, rivaroxaban and apixaban on thrombophilia assays: resistance to activated protein C (APCR), coagulation factor VIII (FVIII) and antithrombin (AT) activities and to evaluate the efficiency of our own optimized method using activated charcoal (AC) in removing interference.

METHOD

DOACs concentrations and thrombophilia assays were firstly determined in native plasma samples. Secondly, 100 mg of AC was added to 500 µL plasma allowing DOACs adsorption for 10 minutes. Treated samples were centrifuged 20 minutes at 1800xg and all measurements were repeated in supernatant. All assays were performed using commercial methods on BCSXP analyzer (Siemens Healthineers, Germany): Innovance anti-FXa assay with drug specific calibrators for rivaroxaban and apixaban (HYPHEN BioMed, France); Innovance DTI assay for dabigatran; ProC Global with FV deficient plasma for APCR; one-stage coagulation assay for FVIII activity and Innovance Antithrombin for AT activity. Wilcoxon test was used to test differences between pairs of samples. The study was funded by the Croatian Science Foundation as part of the research project IP- 2016-06-8208.

RESULTS

All three DOACs have shown significant and different interfering effect on particular thrombophilia assays evaluated in this study, as shown in Table 1, indicating that most of these assays could not be performed in patients on DOACs, and suggesting the possible solution in removing interference by application of procedure with AC (Figure 1). In AC treated samples, concentrations of DOACs were below limit of detection.

Table 1. Results for thrombophilia assays i.e. resistance to activated protein C (APCR), coagulation factor VIII (FVIII) and antithrombin (AT) activities before and after addition of medical activated charcoal (AC) in patients treated with dabigatran, rivaroxaban and apixaban.

DOAC drug	DOAC conc. (ng/mL) **before addition of AC** Median (95%CI); IQR	DOAC conc. (ng/mL) **after addition of AC#**	APCR (normalized ratio)* Positive results < 0.86 **(N, ratio)**		AT (% activity) Median (95%CI); IQR		FVIII (% activity) Median (95%CI); IQR	
			Before AC	**After AC**	**Before AC**	**After AC**	**Before AC**	**After AC**
Dabigatran N = 24	**120 (86-186) 70-203**	**<2.8**	**23 (0.96)**	**0 (0)**	**NA****	**NA****	**106 (84-121) 76-129**	**161 (150-171) 149-174**
			P < 0.001		**NA****		**P < 0.001**	
Rivaroxaban N = 13	**168 (33-238) 39-237**	**< 5.9**	**11 (0.85)**	**2 (0.15)*****	**106 (101-123) 101-122**	**95 (85-98) 86-98**	**130 (115-150) 117-150**	**135 (121-152) 121-152**
			P < 0.001		**P=0.001**		**P = 0.787**	
Apixaban N = 28	**151 (127-182) 108-203**	**<4.1**	**11 (0.73)**	**0 (0)**	**116 (109-125) 107-129**	**94 (90-104) 89-106**	**202 (176-206) 167-206)**	**199 (168-206) 159-206**
			P < 0.001		**P < 0.001**		**P = 0.599**	

*APCR<0.86 represents cut-off value for positive normalized APCR ratio for method in use (Pro C Global, Siemens); 95%CI = 95% confidence interval; IQR = interquartile range; AC = activated charcoal; **NA= not aplicable since dabigatran does not interfere in AT determination based on FX principle; *** true positive result (positive before and after AC addition); #-limit of detection obained in our laboratory

Figure 1. Interpretation and recommendations based on the study results for determination of evaluated thrombophilia assays resistance to activated protein C (APCR), coagulation factor VIII (FVIII) and antithrombin (AT) activities in patients treated with DOACs.

DOAC drug	Resistance to activated protein C	FVIII activity	Antithrombin activity
Dabigatran	Dabigatran has strong interfering effect on APCR in terms of false positive result. Medical AC is an effective in vitro agent in removing interference on APCR testing in patients treated with dabigatran.	Dabigatran has interfering effect on FVIII in terms of false negative result (falsely lower FVIII activity)* Medical AC is an effective in vitro agent in removing interference on FVIII testing in patients treated with dabigatran.	Evaluation not performed since dabigatran does not interfere in AT determination based on FX principle. Testing for AT activity based on FXa principle can be performed in patients treated with dabigatran.
Rivaroxaban	Rivaroxaban has strong interfering effect on APCR in terms of false positive result. Medical AC is an effective in vitro agent in removing interference on APCR testing in patients treated with rivaroxaban.	Rivaroxaban has no impact on FVIII measurement. Testing for FVIII activity can be performed in patients treated with rivaroxaban.	Rivaroxaban has a slight interfering effect on AT testing in terms of false negative result (falsely higher AT activity) showing statistically significant difference, although without clinically significant difference. Medical AC is an effective in vitro agent in removing interference on AT testing in patients treated with rivaroxaban.
Apixaban	Apixaban could have an interfering effect on APCR testing in some patients in terms of false positive result. Medical AC is an effective in vitro agent in removing interference on APCR testing in patients treated with apixaban.	Apixaban has no impact on FVIII measurement. Testing for FVIII activity can be performed in patients treated with apixaban.	Apixaban has a slight interfering effect on AT testing in terms of false negative result (falsely higher AT activity) showing statistically significant difference, although without clinically significant difference. Medical AC is an effective in vitro agent in removing interference on AT testing in patients treated with apixaban.

***FVIII activity >150% represents an independent thrombophilia risk factor**

CONCLUSIONS

Activated charcoal has been found an effective *in vitro* agent to overcome effect of dabigatran, rivaroxaban and apixaban on particular thrombophilia assays, confirming its potential for using before testing in patients treated with DOACs.

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