Title of the study:

Impact of renal replacement therapy modality on coagulation and platelet function in critically ill patients: Intermittent hemodialysis vs. continuous renal replacement therapy

The National Clinical Trial (NCT) number: NCT04512131

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Study Protocol wih Statistical Analysis Plan (SAP)

Study Protocol

Study design and patients

This was a single-center prospective observational study. The institutional review board at Seoul National University Hospital approved the protocol (H-2007-102-1141). Patients or their surrogates provided written informed consent before enrollment.

Adult patients admitted to a surgical or medical intensive care unit (ICU) and were underwent a session of iHD or at least 48 hours of CRRT or both modalities between August 2020 and January 2023 were included. Patients were excluded if they fulfilled one of the following criteria: duration of CRRT was less than 48 hours, received platelet concentrates, fresh frozen plasma, or cryoprecipitate, discontinuation or initiation of the antiplatelet agents or anticoagulants during the study period, technical errors in laboratory tests.

Renal replacement therapy

All patients underwent either iHD or CRRT or both, according to the clinical situation. The detailed setting of RRT including blood flow rate, dialysis flow rate, ultrafiltration rate, duration, and use of anticoagulants was determined by nephrologists and intensivists. Patients were divided into the iHD or CRRT group according to the applied RRT modality. If both methods were applied to a patient, data from both modalities were included in the analysis. Each patient was exposed to a single episode of iHD or at least 48 hours of continuous venovenous hemodiafiltration or both. In the iHD group, hemodialysis was applied for 2 to 4 hours depending on the clinical condition of patients. Since maintenance iHD is usually performed 3 times a week, 48 hours of CRRT was deemed comparable to a single session of iHD.

Laboratory investigations

In the iHD group, blood samples were obtained immediately before and after the hemodialysis session. In the CRRT group, blood samples were taken within 24 hours of initiation of CRRT and the second samples were taken 48 hours after collection of the first samples.

Rotational thromboelastometry (ROTEM, ROTEM® delta, Pentapharm GmbH, Munich, Germany) was performed to evaluate the impact of RRT on the coagulation system. The Extrinsically Activated ROTEM (EXTEM) assay, which focuses on the extrinsic coagulation pathway and the Fibrin-based EXTEM (FIBTEM) assay, which highlights the fibrinogen function, were performed simultaneously. The clotting time (CT), clot formation time (CFT), α angle, maximum clot firmness (MCF), and maximum lysis measured parameters were recorded for both assays.

The impact on platelet function was assessed by electrical impedance aggregometry

with Multiplate® analyzer (Roche, Rotkreuz, Switzerland). To test different pathways of platelet aggregation, aggregation was stimulated 1) by arachidonic acid, the substrate of cyclooxygenase, which subsequently forms the potent platelet activator thromboxane A2 (ASPItest), 2) via adenosine-diphosphate (ADP) receptors by ADP (ADPtest). The area under the curve (AUC) values which is considered the best parameter to reflect overall platelet aggregation of both tests were recorded.

Platelet count and conventional coagulation tests including prothrombin time (PT), activated partial thromboplastin time (aPTT) and fibrinogen assay were performed before and after dialysis. The concentration of D-dimer was measured, which reflects fibrinolysis-specific degradation. The activity levels of protein C and protein S were also measured at two time points. The levels of these vitamin K-dependent anticoagulants were compared between pre- and post-dialysis to evaluate the regulation of clot formation.

Study outcomes

The primary study outcome was changes in CT of EXTEM after dialysis. Secondary outcomes included changes in other EXTEM and FIBTEM parameters, AUC of platelet aggregometry, platelet count, PT, aPTT, fibrinogen concentration, D-dimer concentration, the activity level of protein C and protein S, bleeding events, and transfusion requirements after dialysis. Laboratory test results were compared before and after the application of each dialysis modality. To identify differences in changes according to dialysis modality, a comparison of each study outcome was made between the iHD group and the CRRT group. Bleeding events and transfusion requirements within 24 hours after dialysis in the iHD group or within 24 hours after the second blood sample collection in the CRRT group were also compared between the two groups. The severity of bleeding events were graded

by the modified World Health Organization bleeding scale.

SAP

Based on a previous study regarding the reproducibility of ROTEM results, the mean (± standard deviation) CT of EXTEM was set as 55 ± 6.9 seconds before dialysis. An absolute difference of 5 seconds in CT after dialysis was deemed significant. With a two-sided type-I error of 0.05 and a power of 80%, the required sample size was calculated to be 60 patients. Considering a loss to follow-up of 40%, the sample size was increased to 50 patients in each group, resulting in a total sample size of 100 patients.

Normally distributed continuous data were described as means and standard deviation and non-normal distributed data were described as a median and interquartile range [25th–75th interquartile range]. The normality of continuous data was assessed through the Shapiro-Wilk test. Comparisons between groups were performed by using the Student t-test or the Mann-Whitney U test for the continuous variables normally or non-normally distributed, respectively. Categorical variables were reported as numbers and percentages. Categorical data were compared with a chi-square test. A *P* value less than 0.05 was considered statistically significant and statistical analysis was performed using IBM SPSS 27.0 (IBM Corp, Armonk, NY, USA).