Dialyzer Performance During Hemodialysis Without Systemic Anticoagulation Using a Heparin-Grafted Dialyzer Combined With a Citrate-Enriched Dialysate

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Dialyzer performance during hemodialysis without systemic anticoagulation using a heparin-grafted dialyzer combined with a citrate-enriched dialysate: results of the randomized crossover non-inferiority EVOCIT study

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Running headline: Hemodialysis without systemic anticoagulation

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ABSTRACT

Rationale and Objective

The EVOCIT study was designed to evaluate dialyzer performance during hemodialysis with and without systemic anticoagulation.

Study Design

Randomized, crossover, non-inferiority trial. Noninferiority was defined as a difference of ≤10% for the primary outcome.

Setting and Participants

Single hemodialysis center; 26 prevalent patients treated with 617 hemodialysis sessions.

Interventions

Hemodialysis using a heparin-grafted dialyzer combined with a 1.0 mmol/L citrate-enriched dialysate ("Evocit") without systemic anticoagulation was compared to hemodialysis performed with a heparin-grafted dialyzer with systemic heparin ("Evohep"). Patients were randomly allocated to a first period of 4 weeks and crossed over to the alternative strategy for a second period of 4 weeks.

Outcomes

The primary end point was the difference in Kt/V_{urea} between Evocit and Evohep. Secondary end points were urea reduction ratio (RR), middle molecule removal, treatment time, thrombin generation and reduction in dialyzer blood compartment volume.

Results

The estimated difference in Kt/V_{urea} between Evocit and Evohep was -0.03 (95%CI, -0.06 to -0.007) establishing non-inferiority with mean Kt/V_{urea} of 1.47 ± 0.05 (SE) for Evocit and 1.50 ± 0.05 for Evohep. Non-inferiority was also established for urea RR and beta-2 microglobulin RR. Premature discontinuation of dialysis was required for 4.2% of sessions

among 6 patients during Evocit and no sessions during Evohep. Effective treatment time was 236±5 minutes for Evocit and 238±1 minutes for Evohep. Thrombin generation was increased, and there was greater reduction in dialyzer blood compartment volume after treatments with Evocit compared with Evohep.

Limitations

The effects of avoiding systemic anticoagulation on clinical outcomes were not evaluated.

Conclusions

Evocit is non-inferior to Evohep with respect to solute clearance but results in a greater number of shortened treatments, more membrane clotting and greater thrombin generation.

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Trial Registration

NCT03887468

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Heparin-free hemodialysis

Anticoagulation

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PLAIN-LANGUAGE SUMMARY

The EVOCIT study is a clinical trial that compared dialysis adequacy and coagulation activation during hemodialysis with and without systemic anticoagulation. This randomized, crossover, non-inferiority study showed that the combined use of a heparin-grafted dialyzer with a citrate-enriched dialysate offers solute removal that is less than 10% inferior compared to hemodialysis using heparin for systemic anticoagulation of the patient. The avoidance of systemic anticoagulation resulted in shortened treatment times in 13/307 Evocit sessions, higher thrombin generation and more loss of blood compartment volume of the dialyzers. Future research is needed to explore clinical outcomes of heparin-free hemodialysis beyond blood purification results.

INTRODUCTION

Platelets, leucocytes and the coagulation cascade are activated during hemodialysis when blood comes into contact with the extracorporeal circuit[1-4]. The standard of care to prevent clotting of the extracorporeal circuit during hemodialysis is to use unfractionated heparin or low-molecular-weight-heparins, strategies which also result in systemic anticoagulation[5-7]. In patients with an increased bleeding risk, several methods of hemodialysis avoiding systemic anticoagulation have been described. Recommended methods are dilution techniques and regional citrate anticoagulation[6]. Both methods are labor-intensive and require close nursing supervision. The use of a heparin-grafted dialyzer has been proposed as a strategy to reduce systemic heparin requirements by approximately 50% without increase in the risk of macroscopic clotting of the extracorporeal circuit[8]. Studies indicate that, as a strategy to avoid systemic anticoagulation, the heparin-grafted dialyzer is inferior to regional citrate anticoagulation in terms of successful treatment termination[9] but has a lower risk of circuit clotting compared with dilution techniques[10]. The use of a dialysate enriched with a low dose citrate enables a 33% reduction of systemic heparin requirements while not affecting dialysis adequacy and without safety issues regarding calcium homeostasis[11]. The combined use of a heparin-grafted dialyzer with a citrate-enriched dialysate without systemic anticoagulation has been described as a feasible hemodialysis strategy in an ICU setting[12]. A prospective study showed that this heparin-free hemodialysis strategy is not inferior to regional citrate anticoagulation in terms of completion of 4 hours hemodialysis treatments in prevalent hemodialysis patients[13]. To our knowledge, there has not been a comparison of the performance of heparin-grafted dialyzers with and without systemic anticoagulation. The EVOCIT study was conducted to compare solute clearance and other outcomes with the use

of 1) a heparin-grafted dialyzer with a citrate-enriched dialysate and no systemic anticoagulation, and 2) a heparin-grafted dialyzer with systemic anticoagulation.

METHODS

Study population and study design

Prevalent adult hemodialysis patients were recruited at the Universitair Ziekenhuis Brussels' hemodialysis facility for a randomized crossover non-inferiority trial between March 2018 and October 2019. Patients with known vascular access dysfunction, $spKt/V_{urea}$ <1.35 during the last three months prior to study entry, a contraindication for systemic anticoagulation, planned surgery or planned dialysis modality switch, chronic anticoagulant therapy, and those with a known hypercoagulable state were excluded from study participation. Use of an ACE-I was defined as an exclusion criterion because of historical reports of anaphylactoid reactions during hemodialysis using AN69 and AN69 ST dialyzers for patients receiving this medication class[14-19]. Patients were 1:1 randomized in permuted blocks of four to a first treatment period of 4 weeks receiving either the intervention or comparator treatment. After a 2-day long interdialytic interval, each patient crossed over to the alternative hemodialysis strategy for 4 weeks. Allocation sequence was generated using the Excel random number generator and prepared in the form of sealed envelopes by a person otherwise unrelated to the conduct of the study. There was no masking of study assignments. Each study period consisted of 4 weeks of thrice-weekly 4-hours hemodialysis treatments. Procedures during both study periods were performed using 1.65 m^2 heparin-grafted AN69 ST dialyzers (Evodial®, Baxter Belgium, Braine l'Alleud, Belgium) holding the same lot number; dialyzers were not reused. The dialyzer was combined with a 1.0 mmol/L citrate-enriched dialysate (Citrasate®, Fresenius Medical Care,

Bad Homburg, Germany) during intervention treatments ("Evocit") without adding any systemic anticoagulation during priming or hemodialysis treatment. In the comparator period, the same dialyzer was combined with a conventional bicarbonate-based dialysate ("Evohep"). During Evohep sessions, unfractionated heparin was administered as a push bolus of 20 IU/kg (dry weight) at session start and a maintenance dose of 15 IU/kg/hour through the prefilter port of the extracorporeal circuit. Heparin therapy was stopped 1 hour before session end in patients with an arteriovenous access and continued until the end of the dialysis session in patients with a tunneled catheter. Per protocol, two consecutive episodes of dialysis session length shortening due to thrombotic complications required a loading dose of unfractionated heparin (20 IU/kg) during the following Evocit hemodialysis sessions.

In both study periods, calcium and bicarbonate concentrations in the dialysate were fixed at 1.5 mmol/L and 32 mmol/L respectively. Sodium and potassium concentrations were set according to the most recent dialysis prescription with adjustments during the study as per routine clinical care. Each patient finished both study periods using the same vascular access. Single needle dialysis was not allowed. A vascular access dysfunction occurring during study participation lead to premature study end because access dysfunction would impair paired outcome comparisons after crossover. Blood and dialysate flow rates were fixed at 320 mL/min and 700 mL/min respectively. The same type of blood tubing was used during both study periods. All study sessions were executed using a Fresenius 5008 dialysis machine (Fresenius Medical Care, Bad Homburg, Germany) with a standardized priming procedure per center's standard operational protocol. Ultrafiltration was set according to the patient's dry weight and supported ultrafiltration rate.

The study was approved by the Universitair Ziekenhuis Brussels' independent Institutional Review Board (Medical Ethics Committee O.G.016) and performed in accordance with the ICH-

GCP guidelines. The trial adheres to applicable CONSORT guidelines and was registered to CT.gov (NCT03887468). Participants provided written informed consent before entering the study.

Primary end point

The primary end point was single pool Kt/V_{urea} evaluated during each midweek dialysis session using the Daugirdas formula = -ln([ur240/ur0]-0.008*[treatmenttime_minutes/60])+([4-3.5*(ur240/ur0)]*[UFvolume/weight240])[20].

Secondary end points

Secondary dialysis adequacy markers are online Kt of all sessions, weekly midweek urea reduction ratio (URR), and midweek β2microglobulin and myoglobin reduction ratios during first and fourth treatment weeks of every study period. Extracorporeal circuit clotting was assessed for all study sessions: the number of sessions with premature treatment end due to clotting was recorded as well as the total number of sessions complicated by inability to return the extracorporeal blood to the patient at the end of the dialysis session. Processed blood volume was measured during every dialysis session. Dialyzer membrane clotting was assessed by blood compartment volume measurement of the used dialyzer after every midweek dialysis session using a Renatron-II device® (Medivators Renal Systems, Cantel Medical Corporation, Little Falls, New Jersey, USA), initially developed to prepare used dialyzers for re-use. Dialyzer patency after treatment is quantified by filling and sweeping the blood compartment and by measuring the swept mass using a calibrated precision scale. The theoretical blood compartment volume of the Evodial 1.65 m^2 dialyzer is 100 mL according to the product leaflet. Thrombin generation was evaluated by thrombin-antitrombin-complex

(TAT) and prothrombin fragments 1+2 (PF1+2) measurements before, during and after the midweek dialysis session of the first and last treatment week of every study period.

Although citrate-enriched dialysate has proven to be safe in terms of calcium-chelating effects[11], safety outcomes included biologically confirmed and symptomatic hypocalcemia and intradialytic hypotension. The latter was defined by a drop in systolic blood pressure of 15 mmHg or more or a drop in diastolic blood pressure of 10 mmHg or more compared to the previous measurement, or a declining blood pressure associated with clinical symptoms of cerebral anoxia. Predialysis serum bicarbonate was monitored to evaluate effects of acetate-free citrate-enriched dialysate on acid-base status.

Biological analyses

Blood was sampled during midweek sessions before dialysis start and heparin administration and at session end. If applicable, additional blood sampling was performed 60 and 120 minutes after dialysis start. Predialysis blood samples were taken through the vascular access. Per- and postdialysis samples were taken through the prefilter port of the extracorporeal circuit between patient and heparin infusion site. At treatment end, the dialysis solution was placed in the bypass mode and blood flow rate was lowered to 100 mL/min during at least 2 minutes before sampling the blood. Chemistry and hematology measurements were performed per routine laboratory processes. To evaluate thrombin generation, blood was sampled into 3.2% citrate blood collection tubes, centrifuged at 1500g at room temperature for 10 minutes followed by storage of the plasma at -70°C. Commercial enzyme-linked immunosorbent assays were used for the measurement of TAT (Enzygnost TAT micro, Siemens Healthcare Diagnostics, Marburg, Germany) and PF1+2 (Enzygnost F 1+2 monoclonal, Siemens

Healthcare Diagnostics). All coagulation assays were performed in duplicate on stored plasma samples and according to manufacturer's instructions.

Statistical analyses

We designed a non-inferiority trial allowing a reduction of 10% in Kt/V_{urea} during Evocit compared to Evohep. Kt/V_{urea} results of our unit's hemodialysis cohort in 2017 (1.58±0.16) were used for sample size estimation. Sixteen patients provided more than 90% power (two-sided α=0.05) to reject a null hypothesis with a 10% non-inferiority margin (See Statistical Analysis Plan [Item S1]). The sample size was arbitrarily fixed to 25 patients to provide an adequate number of patients to investigate the secondary end points. Means were generated from repeated measures within patients using linear mixed models that incorporated within-participant variation, correlation over time, and period effects, allowing for unbalanced numbers of observations between groups. A 10% non-inferiority hypothesis was also tested for the differences between Evocit and Evohep in URR, online Kt, beta-2 microglobulin reduction ratio and myoglobin reduction ratio using linear mixed model analysis. Extracorporeal circuit coagulation, dialyzer blood compartment volume after use and thrombin generation were analyzed using descriptive statistics. The sessions with an additional anticoagulation dose were excluded from the intention-to-treat dataset for on-therapy analyses. The latter were only performed for outcomes assessed every hemodialysis session given the lack of difference between the two populations in midweek outcomes.

RESULTS

Patient flow and baseline demographics

Twenty-six patients underwent 307 Evocit and 310 Evohep sessions (Figure 1). Relevant demographic and medical data of the study population are depicted in Table 1.

Non-inferiority of Kt/V_{urea} during Evocit compared to Evohep

Overall, mean±SE Kt/V_{urea} was 1.47±0.05 during Evocit and 1.50±0.05 during Evohep according to linear mixed model analysis. The prespecified non-inferiority margin of 10% Kt/V_{urea} reduction was thus set at -0.15. The linear mixed model analysis resulted in an estimated difference in Kt/V_{urea} of -0.03 (95%CI -0.06 to -0.007) between Evocit and Evohep. Non-inferiority was established given the lower bound of the 95%CI >-0.15 (p<0.001) (Figure 2, Table 2).

Small and middle molecule clearances

Mean±SE URR was 72.1±1.0% during Evocit and 72.8±1.0% during Evohep with a difference of -0.7% (95%CI -1.3% to -0.1%). Online Kt was 47.1±0.6L and 48.0±0.5L for Evocit and Evohep respectively. Difference in online Kt between Evocit and Evohep was -0.9L (95%CI -1.6L to -0.2L). Beta-2 microglobulin reduction ratio (RR) was 37.4±1.3% for Evocit and 37.8±1.3% for Evohep with a difference of -0.4% (95% CI -3.2% to 2.3%). The non-inferiority using a 10% margin was thus established for URR, online Kt and beta-2 microglobulin RR (Table 2). Myoglobin RR was 31.2±1.6% and 33.8±1.6% for Evocit and Evohep respectively with a difference of -2.6 (95%CI -5.4% to 0.2%). The lower bound of the confidence interval being lower than the predefined margin of -3.4%, non-inferiority for Evocit compared to Evohep could not be established for myoglobin RR (Table 2).

Clotting complications and reduction in dialysis time

Activated partial thromboplastin times were not prolonged during Evocit sessions while clotting times increased during Evohep sessions (Supplementary Table). Out of 26 patients participating in the trial, 6 patients (3 men and 3 women; all AV-access) had clotting of the extracorporeal circuit requiring premature ending of 13 Evocit sessions (4.2%). None of the Evohep sessions were prematurely ended because of circuit clotting. Blood return from the extracorporeal circuit to the patient was impossible in only 3 Evocit sessions (0.98%). Overall, mean±SD treatment time was 236±5 minutes for Evocit sessions and 238±1 minutes for Evohep sessions (p=0.05). The median treatment time reduction in the 13 Evocit sessions complicated by clotting was 36 (IQR 20-46) minutes. Patients who experienced a clotting complication with premature session end (n=6) had a mean Evocit treatment time of 230±8 minutes whereas the patients that did not present clotting complications (n=20) received 238±1 minutes Evocit hemodialysis (p<0.001). Treatment times did not differ significantly between the on-therapy and the intention-to-treat analyses (232±24 versus 236±5 minutes; p=0.3). On-therapy analysis confirmed that treatment times between Evocit and Evohep did not differ (232±24 versus 238±1; p=0.2). Processed blood volume was 75.4±1.1L for Evocit sessions and 75.8±0.5L for Evohep sessions (p=0.06). Evocit processed blood volume did not differ between on-therapy and intention-to-treat analysis.

Postdialysis blood compartment volume of used dialyzers was 77±12mL for Evocit and 88±8mL for Evohep sessions. Linear mixed model analysis estimating the difference in blood compartment volume after Evocit and Evohep showed a significant procedure effect (p<0.001). Within each treatment arm, loss of blood compartment volume did not differ significantly between the four weekly measurements (Figure 3).

Thrombin generation

Predialysis TAT and PF1+2 did not differ between Evocit and Evohep sessions. Thrombin generation markers rose significantly during Evocit but not during Evohep, with the rise starting the first hour of Evocit dialysis. Postdialysis levels of thrombin generation markers were significantly higher after Evocit than after Evohep. Predialysis TAT and PF1+2 levels were not different between the fourth and the first Evocit treatment week. The difference in postdialysis TAT and PF1+2 levels between the fourth and the first Evocit treatment week was not significant after adjustment for multiple hypothesis testing (Table 3 and Figure 4).

Safety outcomes

No adverse events related to hypocalcemia occurred throughout the study. Predialysis serum bicarbonate in the fourth treatment week was 21±3mmol/L for Evocit sessions and 22±2mmol/L for Evohep sessions (p=0.2). Episodes of hypotension defined by a drop in systolic blood pressure greater than 15 mmHg or a drop in diastolic blood pressure greater than 10 mmHg occurred in 71% of Evocit sessions and in 67% of Evohep session (p=0.3). None of the hypotensive episodes were complicated with symptoms of cerebral anoxia.

DISCUSSION

Twenty-six patients underwent 307 Evocit and 310 Evohep sessions. Kt/V_{urea} was reduced by 0.03 to 1.47 during Evocit as compared to 1.5 during hemodialysis with systemic anticoagulation. Although this reduction was larger than expected by chance, it appears of limited clinical relevance and remained well above the protocol-defined 10% non-inferiority threshold. European and US guidelines recommend monitoring dialysis dose using small

solute clearance[21,22] since urea clearance is a sensitive marker for changes in solute exchange across the dialyzer[23]. Because we were interested in Kt/V_{urea} as an indicator of dialysis clearance rather than as an indicator of optimal dialysis[24], the primary study end point was defined by the difference in Kt/V_{urea} between treatment periods rather than by the absolute Kt/V_{urea} level. Kt/V_{urea} and URR were measured during the midweek hemodialysis sessions. Online Kt recorded every dialysis session confirmed non-inferiority of Evocit compared to Evohep. Our study was not powered to investigate middle molecules removal. The Evocit and Evohep procedures differed little in the reduction ratios of beta-2 microglobulin and myoglobin: the 95% confidence intervals of the difference of the middle molecules reduction ratios between Evocit and Evohep included positive values and might therefore be the result of random variation. However, the study could not establish non-inferiority for the myoglobin reduction ratio.

Although the absence of systemic anticoagulation during Evocit had only limited impact in terms of small and middle molecules clearances, the strategy resulted in detectable activation of coagulation. Premature treatment end because of clotting only occurred during Evocit with circuit clotting rates and treatment times in line with previously published results of the prospective CITED trial[13]. Taking into account the prematurely ended study sessions because of clotting, the difference in treatment time between Evocit and Evohep was neither clinically nor statistically significant. Only 13 Evocit sessions (4.2%) in 6 patients (23%) failed to reach the prescribed session length of 240 minutes because of clotting with a median treatment time reduction of 36 minutes for the shortened sessions. The 20 patients who did not present clotting complications during Evocit received the same treatment duration during both treatment periods. The low number of patients and sessions presenting clotting complications did not provide sufficient statistical power to identify predictive factors for thrombotic

complications during Evocit dialysis. The identification of such predictive characteristics would require large scale and long-term clinical research. Our study suggests that measuring dialyzer blood compartment volume and thrombin generation are both reproducible methods to identify activation of coagulation in the absence of clinical signs of clotting. Dialyzers' blood compartment volume was lower after Evocit compared to Evohep however above 80% of the original volume in the majority of Evocit sessions. Dialyzer blood compartment volume after use of 80% or more of the original volume was reported to be associated with a dialyzer clearance within 10% of its original value[25]. The limited reduction in dialyzer blood compartment volume after treatment with Evocit probably explains the limited impact on solute clearance results. Late-onset thrombin generation has been previously shown during hemodialysis despite effective anticoagulation avoiding macroscopic clotting of the extracorporeal circuit[2,4]. In the current study, thrombin-antitrombin-complex (TAT) rises late during Evohep in contrasts with an earlier and significantly higher TAT increase during Evocit. The results of our study indicate that Evocit is an efficient dialysis regimen for patients at increased risk of bleeding, and provide a rationale to extend the indications given the potential benefits of avoiding systemic anticoagulation. However, before widespread implementation of this approach, larger and longer term trials have to investigate whether repeated low-level activation of the coagulation system does not result in adverse clinical outcomes. Although an earlier retrospective study showed an 85% success rate of Evocit in 309 sessions in 94 ICU-admitted patients dialyzed with a temporary vascular access in 90% of cases[12], our study results cannot conclude on the performance of Evocit in patients at increased risk of clotting. Nevertheless, a key contribution of the present study is the investigation of coagulation activation beyond clinically apparent clotting complications. Measurements of these parameters might help to select patient populations that need additional anticoagulation.

Further research should assess the benefits and risks of using or avoiding systemic anticoagulation during hemodialysis taking into account patient characteristics such as vascular access, inflammation, clotting risk and bleeding risk.

Reducing heparin administration during hemodialysis[26-28] and reducing acetate-concentration in the dialysate[29,30], both incorporated in the Evocit strategy, have been associated with decreased oxidative stress. Future larger scale long-term trials of heparin-free dialysis should assess whether heparin-free hemodialysis strategies impact clinical outcomes such as residual kidney function preservation, volume status, nutritional status and patient-reported outcome measures. The current study supports the ongoing quest for heparin-free hemodialysis[31] by comparing solute clearance and coagulation activation during a hemodialysis strategy without systemic anticoagulation to the standard of care, i.e. hemodialysis using systemic anticoagulation. The lack of high-quality evidence regarding current anticoagulation practices during hemodialysis[32] requires validation of anticoagulant strategies by adequately designed trials, including cost-effectiveness analyses.

Our study is limited by its single-center design, chosen because of the small sample size and the availability of only one Renatron-II device to measure blood compartment volume of used dialyzers, and by restricting the population to patients with a stable vascular access. The study results should inform the design of multicenter trials with longer follow-up that evaluate clinical end points among a less-selected patient population. Although adequately powered for the primary end point, statistical power was limited for the evaluation of secondary outcomes. The high-flux heparin-grafted AN69 ST dialyzer is not routinely used for chronic hemodialysis. Its use in both study periods enabled to assess the effects of avoiding unfractionated heparin and citrate-enriched dialysate use on clearance ratios. Different dialyzer characteristics in the intervention and control period would have hampered the

evaluation of the primary efficacy end point. The combined use of a heparin-grafted dialyzer and full dose systemic administration of unfractionated heparin provided a control treatment with very efficient prevention of thrombosis.

In conclusion, avoidance of systemic anticoagulation by using a heparin-grafted dialyzer combined with a citrate-enriched dialysate offers Kt/V_{urea}, URR, online Kt and beta-2 microglobulin reduction ratios that are not inferior to hemodialysis using systemic anticoagulation. The avoidance of systemic anticoagulation is nevertheless associated with a modestly increased number of shortened dialysis treatments without a clinically significant impact on overall treatment time, with higher thrombin generation, and with higher losses in blood compartment volume of the dialyzer after use. Long-term benefits of heparin-free hemodialysis have to be evaluated in prospective trials with clinical end points.

Table 1: Baseline characteristics of the study population (n=26)

Age (years)	65.3±14.2
Sex (M/F (%male))	17/9 (65)
Nephropathy (N,(%))	
Diabetes nephropathy	8 (30.8)
Nephrangiosclerosis	7 (26.9)
Interstitial nephritis	2 (7.7)
Glomerulonephritis	1 (3.8)
Polycystic kidney disease	1 (3.8)
Other	7 (26.9)
Diabetes mellitus (N,(%))	15 (58)
KRT vintage (months)	66 (39-166)
Access type (N,(%))*	
Arteriovenous fistula	17 (65.4)
Arteriovenous graft	4 (15.4)
Tunneled central venous catheter	5 (19.2)
Antiplatelet use (N,(%))	
Overall	22 (84.6)
Aspirin	20 (76.9)
Aspirin + clopidogrel	1 (3.8)
Aspirin + dipyridamole	1 (3.8)
Dry weight (kg)	77.5±18.2

Relevant demographic, medical and drug therapy data at study start.
Data are given as mean±SD, median (IQR) or proportions (%) as appropriate.
M= male; F= female; KRT= kidney replacement therapy
*All tunneled central venous catheters were located in the right jugular vein

Table 2: Differences in small and middle molecule clearances between Evocit and Evohep sessions according to linear mixed model analysis

	EVOCIT	EVOHEP	Non-inferiority threshold	Δ (95% CI)
Kt/V_{urea}	1.47±0.05	1.5±0.05	-0.15	-0.03 (-0.06 to -0.007)
Urea RR (%)	72.1±1.0	72.8±1.0	-7.3	-0.7 (-1.3 to -0.1)
Online Kt (L)	47.1±0.6	48.0±0.5	-4.8	-0.9 (-1.6 to -0.2)
Beta-2 microglobulin RR (%)	37.4±1.3	37.8±1.3	-3.8	-0.4 (-3.2 to 2.3)
Myoglobin RR (%)	31.2±1.6	33.8±1.6	-3.4	-2.6 (-5.4 to 0.2)

Kt/V_{urea}, urea reduction ratio (RR), online Kt, beta-2-microglobulin RR and myoglobin RR are expressed as mean estimates ± SE according to linear mixed model analysis for both Evocit and Evohep. The non-inferiority threshold was predefined as 10% reduction during Evocit compared to Evohep (-0.1*Evohep result). The Δ (95% CI) describes the estimate of the difference between Evocit and Evohep according to linear mixed model. Non-inferiority is established whenever the non-inferiority threshold is below the lower bound of the 95% confidence interval of the delta Δ and refuted whenever the lower bound of the 95% confidence interval of the delta Δ is below the non-inferiority threshold.
Kt/V_{urea} and urea RR were assessed every midweek dialysis session, online Kt was assessed every hemodialysis session and beta-2-microglobulin RR and myoglobin RR were assessed on the first and fourth midweek dialysis session of every study period.

Table 3: TAT and PF1+2 evolution over sessions and over study period

	Week 1	Week 4	*p*-value[2]	Week 1	Week 4	*p*-value[2]
	TAT before dialysis session start (mcg/L)			**TAT at dialysis session end (mcg/L)**		
EVOCIT	5.4 (3.7-10.6)	4.3 (3.7-6.3)	0.06	35.4 (26.4-47.6)	31.3 (24.2-42.5)	0.2
EVOHEP	4.5 (3.8-7)	5 (4.1-13.1)	0.2	6.9 (4.5-20.3)	12.2 (8.7-20.2)	0.9
p-value[1]	0.4	0.1		<0.001	<0.001	
	PF1+2 before dialysis session start (pmol/L)			**PF1+2 at dialysis session end (pmol/L)**		
EVOCIT	454 (402-674)	458 (340-611)	0.7	1155 (1024-1491)	983 (878-1247)	0.03
EVOHEP	428 (329-575)	442 (306-575)	0.7	451 (373-545)	450 (358-692)	0.9
p-value[1]	0.2	0.1		<0.001	<0.001	

TAT and PF1+2 values were not normally distributed and are presented as median (IQR).
TAT= thrombin-antithrombin complex; PF1+2= prothrombin fragments 1+2.
TAT and PF1+2 were assessed on the first and fourth midweek dialysis session of every study period.
[1] Hypothesis testing of differences between Evocit and Evohep both at start and end by Wilcoxon signed-rank test.
[2] Hypothesis testing of the null hypothesis of no difference in TAT and PF1+2 values between week 1 and week 4 by Wilcoxon signed-rank test.

Figure 1: Patient flow during the study

Figure 2: Non-inferiority plot of the difference between Evocit and Evohep

Figure 3: Blood compartment volume of the dialyzer after midweek dialysis sessions

Figure 4: Evolution of TAT and PF1+2 levels during midweek dialysis sessions

Legends to Figures

Legend to Figure 1:
Patient flow during the study. 30 patients were 1:1 randomized to start 4 weeks of thrice-weekly Evocit (heparin-grafted dialyzer, citrate-enriched dialysate, no unfractionated heparin) or 4 weeks of thrice-weekly Evohep (heparin-grafted dialyzer, no citrate-containing bicarbonate-based dialysate, unfractionated heparin).
[§] 4 patients dropped out of the study prior to crossover and could therefore not be included in the paired analysis comparing the two treatment periods. 26 patients crossed over after 4 weeks.
[£] 2 patients prematurely ended the second study period of 4 weeks of thrice-weekly hemodialysis. All dialysis sessions performed according to the allocated treatment protocol were included in the analysis.
[#] The 18 study sessions performed with an additional anticoagulation dose were excluded from the intention-to-treat (ITT) dataset for the on-therapy (OT) analysis. During the second Evocit period, the two patients had a heparin loading dose administered during 10 and 1 hemodialysis sessions respectively.

Legend to Figure 2:
The red line indicates the non-inferiority margin of 10% of Evohep Kt/V_{urea} (-0.15). The point estimate and whiskers indicate the difference in Kt/V_{urea} (Evocit-Evohep) with 95% confidence interval (95%CI) according to linear mixed model analysis.
Non-inferiority is established because the lower bound of the 95%CI is superior to the non-inferiority margin.

Legend to Figure 3:
This graph shows the blood compartment volume (expressed in milliliters) measured after every midweek dialysis session for the overall cohort according to treatment period and treatment week, irrespective of treatment order.
For Evocit sessions, data were available for 25, 26, 23 and 25 dialyzers for week 1, 2, 3 and 4 respectively. For Evohep sessions, data were available for 25, 26, 25 and 25 dialyzers for week 1, 2, 3 and 4 respectively.
The *p*-value results from linear mixed model analysis estimating the difference between Evocit and Evohep.

Legend to Figure 4:
This graph shows the thrombin generation markers measured during the first and fourth week's midweek hemodialysis session according to treatment period and treatment week, irrespective of treatment order. Levels of thrombin-antithrombin complex (TAT) and prothrombin fragments 1+2 (PF1+2) were assessed before dialysis start (t0), 60 (t60) and 120 (t120) minutes after dialysis start and at session end (t240).
For Evocit sessions, TAT data were available for 22,19, 22 and 25 sessions in week 1 and for 23,22, 23 and 22 sessions in week 4 for t0, t60, t120 and t240 respectively.
For Evohep sessions, TAT data were available for 25,24, 23 and 26 sessions in week 1 and for 24,22, 23 and 24 sessions in week 4 for t0, t60, t120 and t240 respectively.

For Evocit sessions, PF1+2 data were available for 25,19, 21 and 25 sessions in week 1 and for 24,21, 23 and 22 sessions in week 4 for t0, t60, t120 and t240 respectively.
For Evohep sessions, PF1+2 data were available for 24,24, 23 and 26 sessions in week 1 and for 25,23, 23 and 25 sessions in week 4 for t0, t60, t120 and t240 respectively.
* The *p*-values result from Wilcoxon signed-rank test between t0 and t240 for each condition.

Supplementary material

- Supplementary item S1: Statistical analysis plan – Sample size estimation
- Supplementary Table S2

Supplementary Table S2: Systemic anticoagulant effect during Evocit and Evohep

aPTT (s)	**EVOCIT**	**EVOHEP**	*p*-value
before HD start	30±3	31±4	0.4
60 minutes after HD start	27±4	57±17	<0.001
120 minutes after HD start	24±13	53±18	<0.001
240 minutes after HD start	27±3	41±15	<0.001

aPTT= activated partial thromboplastin time (seconds); HD= hemodialysis.
Activated partial thromboplastin times were assessed during the first and fourth midweek dialysis session of every study period. For each individual patient, mean aPTT was calculated for the Evocit and Evohep period. The *p*-value results from hypothesis testing of the difference in sample means between Evocit and Evohep using a paired t-test.

ARTICLE INFORMATION

Author's Contributions

Designed study: KF, WC, KMW; recruited patients: KF, DDC, AT, M-LC; analyzed and interpreted data: KF, WC, KMW; carried out the hemodialysis study treatments: KF, DDC, AT, M-LC; carried out the biological analyses: CO, KJ. Each author contributed important intellectual content during manuscript drafting or revision and agrees to be personally accountable for the individual's own contributions and to ensure that questions pertaining to the accuracy or integrity of any portion of the work, even one in which the author was not directly involved, are appropriately investigated and resolved, including with documentation in the literature if appropriate.

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Prior presentation

Our manuscript is an original paper approved by all authors.

The results have been presented at the ERA-EDTA 2020 virtual congress, the ASN Kidney Week Reimagined 2020, the virtual annual BVN-SBN congress 2020 (Sep 29, 2020), the 100% Digital SFNDT 2020 congress (Oct 7, 2020).

Data Sharing

The authors will share the study protocol with the AJKD Editorial Board.

Deidentified participant data will be provided by the principal investigator on email request following publication of the current manuscript and according to a methodologically sound proposal for repeat analyses or for answering additional research questions.

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