Results of the HepZero study comparing heparin-grafted membrane and standard care show that heparin-grafted dialyzer is safe and easy to use for heparin-free dialysis

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Heparin is used to prevent clotting during hemodialysis, but heparin-free hemodialysis is sometimes needed to decrease the risk of bleeding. The HepZero study is a randomized, multicenter international controlled open-label trial comparing no-heparin hemodialysis strategies designed to assess non-inferiority of a heparin grafted dialyzer (NCT01318486). A total of 251 maintenance hemodialysis patients at increased risk of hemorrhage were randomly allocated for up to three heparin-free hemodialysis sessions using a heparin-grafted dialyzer or the center standard-of-care consisting of regular saline flushes or pre-dilution. The first heparin-free hemodialysis session was considered successful when there was neither complete occlusion of air traps or dialyzer, nor additional saline flushes, changes of dialyzer or bloodlines, or premature termination. The current standard-of-care resulted in high failure rates (50%). The success rate in the heparin-grafted membrane arm was significantly higher than in the control group (68.5% versus 50.4%), which was consistent for both standard-of-care modalities. The absolute difference between the heparin-grafted membrane and the controls was 18.2%, with a lower bound of the 90% confidence interval equal to plus 7.9%. The hypothesis of the non-inferiority at the minus 15% level was accepted, although superiority at the plus 15% level was not reached. Thus, use of a heparin-grafted membrane is a safe, helpful, and easy-to-use method for heparin-free hemodialysis in patients at increased risk of hemorrhage.

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Hemodialysis treatment requires anticoagulation to prevent thrombosis of the dialyzer and the extracorporeal circuit (bloodlines or cassette system). Anticoagulation is usually achieved with unfractionated or low-molecular-weight heparin. In clinical practice, it is not unusual to have to perform hemodialysis sessions for patients with active bleeding or those at an increased bleeding risk conditions in which heparin anticoagulation is contraindicated.[1] These situations are generally of short duration (gastrointestinal hemorrhage, hemorrhagic stroke, surgery). According to team practices, various strategies have been implemented to prevent clotting of the extracorporeal circuit.

Regional anticoagulation (i.e., heparin administration into the arterial line and protamine into the venous line) or tight

heparinization (the use of minimal dose of heparin) is not confidently safe for patients with active bleeding or at those at a risk of bleeding.[2] No-heparin infusion using regular saline flushes is one of the methods of choice.[3,4] Alternatively, regional citrate anticoagulation (RCA) can be used. Both methods are currently recommended by the 2002 European Best Practice Guidelines for hemodialysis.[2] The use of RCA is limited by the need for additional pumps for citrate and calcium infusion, and the potential risk of electrolyte disorders (hypocalcemia, hypernatremia, and acid–base disorders).[5] Moreover, the level of evidence is low, as no randomized controlled study evaluating regular saline flushes has ever been reported.[6–13] No-heparin hemodialysis (NH-HD) treatment with predilution is another procedure used in some dialysis facilities.[14] However, fluid infusion is far from optimal because of the increased volume load that has to be removed during the current dialysis session, and an additional logistic burden for dialysis nurses owing to the need for closer one-to-one monitoring.[3]

Another alternative for NH-HD is to bind heparin on the blood side of the dialyzer membrane.[3,5] A new dialyzer (Evodial, Gambro-Hospal, Meyzieu, France) contains a heparin-grafted membrane (HGM) composed of a polyacrylonitrile sodium methallylsulfonate copolymer; the manufacturing process includes a surface treatment with high-molecular-weight polyethyleneimine before heparin grafting. *In vitro* and *in vivo* data requested for CE marking (conformity with European Medical Device Directive 93/42/EEC) have shown the stability of heparin grafting with absolutely no release (Supplementary Appendix online). In a study conducted in 45 regular dialysis patients, Kessler *et al.*[15] found that the systemic heparin dose could be reduced by 45 ± 13% without any coagulation issues. In addition, Sanchez-Canel *et al.*[16] have observed, in six patients treated with postdilution hemodiafiltration, no increase in complete clotting event frequency with HGM without systemic anticoagulation, compared with polysulfone membrane and standard anticoagulation with nadroparin.

The present international, multicenter, randomized, controlled, open-label trial (HepZero study) was designed to test the hypothesis that, in patients at risk of bleeding, NH-HD treatment with HGM can be performed easily (without saline flushes or blood predilution) and is at least not inferior, or even superior, to the standard of care NH-HD treatment.

RESULTS

Briefly, this clinical study was a prospective, multicenter, international, open-label, controlled, randomized, clinical study. Consecutive eligible patients were enrolled in the study and treated during a maximum of three consecutive NH-HD treatments. Two types of methods were evaluated in parallel: the study group comprised NH-HD treatment with HGM, and the control group comprised NH-HD treatment according to the standard of care defined by the usual procedure in place at each study site (i.e., saline flushes or predilution). The full study protocol was published recently,[17] the methods are detailed in the corresponding section, and extended methods are provided in a Supplementary Appendix online.

Study population

Patients were enrolled in the study between November 4th, 2011 and February 29th, 2013. The study flowchart is presented in Figure 1. A total of 265 patients were randomized, among whom 251 were considered in the intention-to-treat and 231 in the per-protocol analyses (Supplementary Figure S1 online). The baseline features of the intention-to-treat population are presented in Table 1 and the bleeding risk characteristics in Table 2. Enrolled patients mostly displayed a high (68.1%) or very high (11.6%) bleeding risk

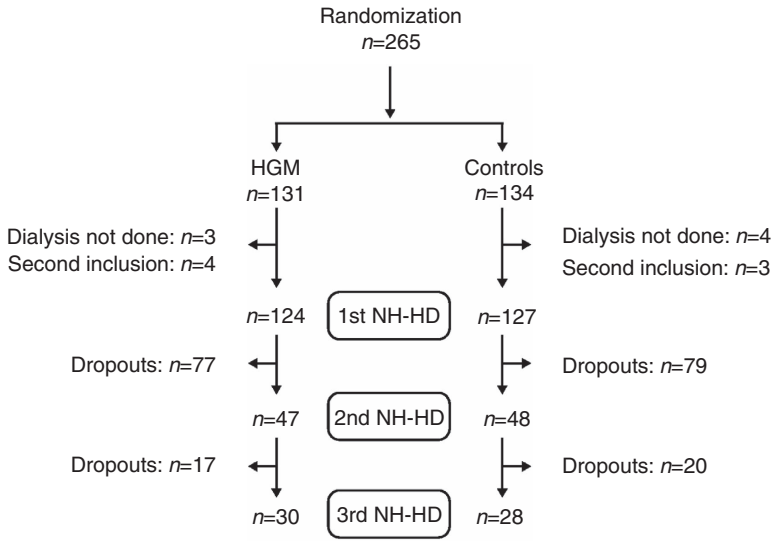

Figure 1 | Flowchart of the intention-to-treat population. Dropouts mean discontinuation after the first or second no-heparin hemodialysis (NH-HD) treatment. HGM, heparin-grafted membrane.

Table 1 | HepZero intention-to-treat population baseline features

Characteristic	Heparin-grafted membrane group N	Heparin-grafted membrane group Mean ± s.d. or *n* (%)	Control group N	Control group Mean ± s.d. or *n* (%)
Demography				
Age (years)	124	64 ± 16	127	62 ± 15
Male sex	124	78 (63)	127	70 (55)
Primary renal disease				
Glomerulonephritis	124	22 (18)	127	18 (14)
Pyelonephritis	124	5 (4)	127	4 (3)
Polycystic kidney disease	124	7 (6)	127	14 (11)
Diabetic nephropathy	124	26 (21)	127	33 (26)
Hypertension	124	27 (22)	127	22 (17)
Vascular nephropathy	124	13 (10)	127	15 (12)
Other renal disease	124	31 (25)	127	27 (21)
Unknown cause	124	17 (14)	127	21 (17)
Renal replacement history				
Hemodialysis duration (years)	123	3.1 ± 3.4	126	3.0 ± 4.1
Vascular access type	124		127	
Native arteriovenous fistula		76 (61)		71 (56)
Prosthetic graft		4 (3)		6 (5)
Central venous catheter		44 (35)		50 (39)
Main comorbidities				
Charlson index	81	5.7 ± 2.5	83	6.1 ± 2.4
Coronary heart disease	124	33 (27)	127	30 (24)
Peripheral arterial disease	124	24 (19)	127	29 (23)
Diabetes	124	44 (35)	127	51 (40)
Hyperlipidemia	124	44 (35)	127	53 (42)
Cancer/hematologic malignancy	124	25 (20)	127	41 (32)[†]
Concomitant medications				
Antiplatelet agents	124	55 (44)	127	56 (44)
Antacid preparations	124	90 (73)	127	89 (70)
Antidiabetics	124	35 (28)	127	37 (29)
Vitamins	124	71 (57)	127	65 (51)
Calcium supplements	124	60 (48)	127	71 (56)
Antianemic preparations	124	66 (53)	127	67 (53)
Diuretics	124	44 (35)	127	60 (47)
β-Blockers	124	49 (40)	127	57 (45)
Renin-angiotensin system antagonists	124	30 (24)	127	43 (34)
Blood laboratory values before the 1st no-heparin hemodialysis session				
Hemoglobin (g/dl)	120	10.2 ± 1.5	125	10.2 ± 1.4
Albumin (g/l)	110	34 ± 6	109	33 ± 6
Calcium (mmol/l)	119	2.12 ± 0.22	116	2.16 ± 0.23
Magnesium (mmol/l)	112	0.91 ± 0.26	107	0.85 ± 0.15*
Phosphate (mmol/l)	112	1.59 ± 0.59	112	1.61 ± 0.54
C-reactive protein (mg/l)	109	55.0 ± 70.1	110	54.9 ± 58.8
aPTT[a]	97	1.11 ± 0.22	95	1.14 ± 0.27
INR[a]	117	1.2 ± 0.6	113	1.1 ± 0.2
INR[a] > 1.5	117	6 (5%)	113	5 (4%)

*$P < 0.05$.
[a]aPTT, activated partial thromboplastine time; INR, international normalized ratio.

Table 2 | HepZero intention-to-treat population bleeding risk characteristics

Characteristic	Heparin-grafted membrane group N	Heparin-grafted membrane group Mean ± s.d. or *n* (%)	Control group N	Control group Mean ± s.d. or *n* (%)
Gastrointestinal hemorrhage	124	14 (11)	127	15 (12)
Invasive procedure	124	18 (15)	127	24 (19)
Perioperative risk	124	73 (59)	127	67 (53)
Cerebral hemorrhage	124	2 (2)	127	2 (2)
Other bleeding risk	124	15 (12)	127	20 (16)
Bleeding risk (Lohr-Schwab definition)				
Low	121	6 (5)	126	8 (6)
Moderate		20 (17)		13 (10)
High		81 (67)		90 (71)
Very high		14 (12)		15 (12)

score. Antiplatelet agents included chiefly aspirin (91%) and clopidogrel (9%), without difference between groups. Two potentially relevant baseline characteristics were found to be significantly unbalanced between allocation groups: a previous history of cancer or hematologic malignancy, and blood magnesium level. Blood flow rate during the first NH-HD session was 318 ± 42 ml/min in the HGM group versus 320 ± 40 ml/min in the control group ($P = 0.56$). Patients in the control group were treated using dialyzers from several manufacturers, the surfaces of which ranged between 1.4 and 2.1 m^2, and membranes were polyaryether-sulfone/polyamide in 79 patients (62.2%: predilution 44 and saline flushes 35), polysulfone in 21 patients (16.5%: predilution 19 and saline flushes 2), polyethersulfone in 21 patients (16.5%: predilution 0 and saline flushes 21), polyarylethersulfone in 4 patients (3.1%: predilution 0 and saline flushes 4), and cellulose triacetate in 2 patients (1.6%: predilution 2 and saline flushes 0).

Primary outcome

Using an intention-to-treat analysis, patients treated with HGM displayed a significantly higher success rate than controls (HGM: 85/124: 68.5% [95% CI 59.5–75.7%] vs. controls: 64/127: 50.4% [95% CI 41.4–58.6%], $P = 0.005$), with a difference between HGM patients and controls of 18.2%, with a 7.9% lower bound of the 90% CI. Because that value was greater than the prespecified –15% noninferiority threshold, the noninferiority hypothesis was demonstrated at the 5% error level. Because that value was lower than the prespecified + 15% superiority threshold, the prespecified criteria for superiority were not reached (Figure 2). Of note, the per-protocol analysis confirmed the results observed in the intention-to-treat analysis (HGM success rates: 77/114: 67.5% [95% CI 58.0–75.0%] vs. controls: 59/117: 50.4% [95% CI 41.1–58.9%] with a difference of 17.1% and a lower bound of the 90% CI of 6.4%; $P = 0.012$). The events that led to the recording of a primary outcome are described in Table 3. Agreement of the clotting grades assessed by the two raters (see Methods) was found to be excellent, with 1351/1366 concordant scores (98.9%).

There was no significant interaction between the treatment effect and (1) the two potentially relevant baseline

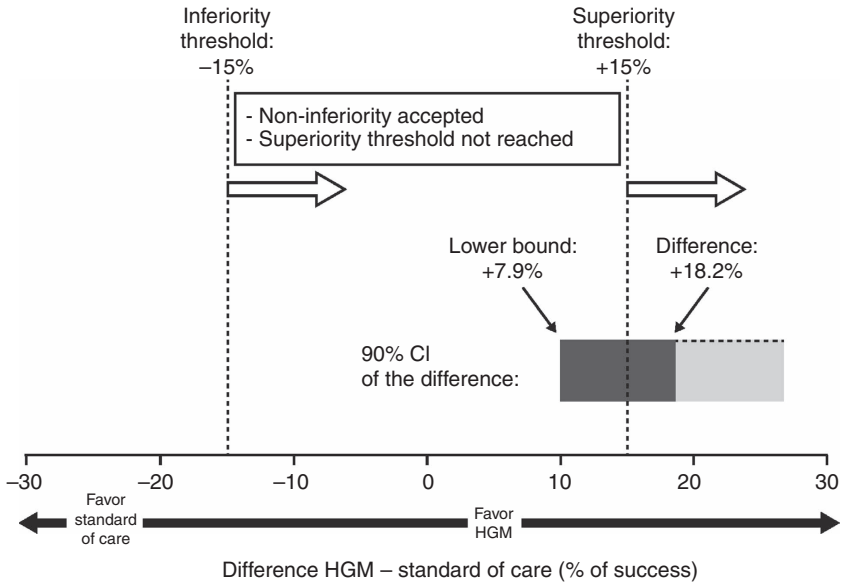

Figure 2 | Primary outcome (first no-heparin hemodialysis session). HGM, heparin-grafted membrane.

Table 3 | Details of events leading to a primary outcome

Failure criteria	HGM group	Control group
Failure	39 (31.5%)	63 (49.6%)
Premature stop	2 (1.6%)	9 (7.1%)
Line change	—	4 (3.1%)
Line + dialyzer change	—	1 (0.8%)
Additional flush(es)	5 (4.0%)	1 (0.8%)
Flush(es) + premature stop	3 (2.4%)	2 (1.6%)
Flush(es) + line change	1 (0.8%)	—
Flush(es) + dialyzer + line change	—	1 (0.8%)
Occlusion (grade 4)	8 (6.5%)	6 (4.7%)
Occlusion + premature stop	13 (10.5%)	26 (20.5%)
Occlusion + flush(es)	—	1 (0.8%)
Occlusion + line change	1 (0.8%)	—
Occlusion + flush(es) + stop	4 (3.2%)	8 (6.3%)
Occlusion + flush(es) + line change	—	2 (1.6%)
Occlusion + flush + line change + stop	2 (1.6%)	1 (0.8%)
Occlusion + flush + dialyzer + line + stop	—	1 (0.8%)

Table 4 | Efficacy according to the usual practice of the center

Usual practice	Treatment	Success	Success rate (95% CI)	*P*-value
Predilution	Evodial	36/63	57.1 (44.1–67.9)	
	Controls	26/65	40.0 (28.3–51.4)	0.078
	Difference E-C[a]		17.1 (2.6–30.7)	
Saline flushes	Evodial	49/61	80.3 (67.8–87.7)	
	Controls	38/62	61.3 (48.0–71.7)	0.034
	Difference E-C[a]		19.0 (5.4–32.0)	
Interaction[b]			−1.9 (−24.9; +20.9)	0.64[c]

[a]Difference E-C: Evodial-controls. CI, confidence interval. The 95% CIs are 2-tailed for intragroup success rates (in agreement with *P*-value), 1-tailed for the intergroup difference (in agreement with the noninferiority/superiority analysis).
[b]Interaction: between usual practice and treatment, i.e., difference between differences Evodial-controls.
[c]*P*-value of the Breslow-Days test of homogeneity of odds ratios.

characteristics significantly unbalanced between allocation groups (data not shown); or (2) center results (meta-analysis of the individual results of the 10 centers: Cochran's test for heterogeneity: $P = 0.48$; absolute risk reduction: 20.1% [95% CI 9.2–31.0%]; $P = 0.0003$; number of patients needed to treat for benefit: 5 [95% CI 4–11 patients]); or (3) usual practice of the center (interaction $P = 0.64$, Table 4). Of note, it was observed in a *post-hoc* analysis that centers using saline flushes achieved significantly better global (i.e., in both study and control groups) success rates compared with predilution (saline infusion: 87/123 = 70.7% vs. predilution: 62/128 = 48.4%, $P = 0.0005$).

Secondary outcomes

The success rates did not significantly differ between HGM and controls during the second (36/47: 76.6% [95% CI 61.6–85.6%] vs. 33/48: 68.8% [95% CI 53.6–79.2%], respectively; $P = 0.53$) and third (26/30: 86.7% [95% CI 68.4–93.7%] vs. 21/28: 75% [95% CI 54.8–86.0%]; $P = 0.43$) sessions. Owing to high drop-out rates after the first session (Table 5), these results are based on a smaller number of dialysis sessions.

Among all the successful NH-HD session subgroups, the mean clotting grade was found to be numerically lower in the HGM group, but this difference was not statistically significant (HGM: $n = 85$, mean ± s.d. = 1.54 ± 0.49; controls: $n = 64$, mean ± s.d. = 1.67 ± 0.51; $P = 0.14$).

Within the whole population, the dialysis duration achieved was significantly longer in the HGM group (HGM: $n = 124$, mean ± s.d. = 3.65 ± 0.75 h; controls: $n = 127$, mean ± s.d. = 3.45 ± 0.86 h; $P = 0.018$).

No significant difference in the efficacy of NH-HD treatment between HGM and the controls was observed, as

Table 5 | Reasons for discontinuation after the first or second no-heparin hemodialysis treatment

	Heparin-grafted membrane group	Control group
Stop after first session	77	79
Adverse event	2	2
Clotting	6	14
Heparin/anticoagulant treatment	6	5
Patient discharged	14	19
Heparin-free dialysis no longer needed	46	37
Patient decision	3	2
Stop after second session	17	20
Heparin/anticoagulant treatment	0	1
Discharge	5	5
Heparin-free dialysis no longer needed	8	11
Patient decision	0	1
Organization reasons	4	2

assessed either by Kt/V (HGM: median [interquartile range] 1.15 [0.95–1.37] vs. controls: 1.19 [0.98–1.39]; $P = 0.47$) or by ultrafiltration achieved (HGM: 2.0 l [1.0–2.5] vs. controls: 1.8 l [1.1–2.9]; $P = 0.73$).

To assess the ease of use, the number of unscheduled saline flushes was compared and found not to be statistically different between the two groups (HGM: 0.3 ± 0.6 vs. controls: 0.3 ± 0.8 flushes per session; $P = 0.72$). Of note, the saline control subgroup received 6.4 ± 1.3 flushes among the 7 scheduled (1 flush every half-hour), with a mean volume of 137 ± 70 ml. The predilution control subgroup achieved a predilution rate of 1.57 ± 1.40 l/h for a total reinfusion volume of 5.5 ± 6.3 l, for the entire dialysis session.

Safety

Thirty-two patients experienced one to three adverse events within each treatment group during the study (Supplementary Table S1 online). No obvious imbalance, especially regarding hypotensive episodes, was observed. No adverse event was related to the investigational device.

DISCUSSION

To the best of our knowledge, the HepZero study is the first ever international, multicenter, randomized, controlled study comparing two methods of NH-HD, including the gold standard, in two parallel groups, in an open-label design. Its findings show, first, a 20% higher success rate in the HGM arm compared with the control group, with a very small number ($n = 5$) of patients needed to treat to avoid a primary outcome. Second, the use of HGM is firmly determined to be noninferior but not superior to the standard of care, as the prespecified 15% superiority margin was not reached. Third, the current standard of care has a high 50% failure rate. Finally, *post-hoc* analysis showed that saline flushes achieved higher success rates than predilution method.

This result was achieved without loss of dialysis efficacy: there was no significant difference of Kt/V between HGM and standard of care sessions. As the duration of HGM sessions was significantly 12 min longer than standard of care sessions, equivalence of Kt/V likely results from an increase in convective transfers to eliminate the fluids injected as bolus or predilution in standard of care sessions.

Hemodialysis for patients with contraindications to heparin anticoagulation is challenging. Regional anticoagulation or tight heparinization lowers the risk of bleeding compared with the standard method, but there is still a notable risk of bleeding (5–50%).[5] Current guidelines state that 'In patients with increased bleeding risks, strategies that can induce systemic anticoagulation should be avoided. Treatment strategies that avoid this include: no use of anticoagulants with regular flushing or RCA.'[2] To date, the use of RCA is restricted to specialized units, because its application is cumbersome,[18] as it requires additional pumps that are not provided by standard dialysis machines, as well as the need for careful monitoring of plasma electrolytes. These issues not only increase the complexity of the dialysis procedure but also the likelihood of complications, the most concerning being severe symptomatic hypocalcemia and related life-threatening arrhythmias.[19] Therefore, fluid infusion (flushes or predilution) NH-HD is currently the gold standard of care in patients with high bleeding risk.[3,4] However, this technique does not provide reliable prevention of severe clotting, which occurs in 15–35% of sessions[20,21] and, in addition to blood loss, it may lead to inadequate dialysis because of frequent and/or premature termination of the session.

There is little evidence to guide the best practice of fluid infusion NH-HD. Several protocols are used in practice, including saline flushes (delivered at different intervals and different volumes), which require close monitoring by dialysis staff and increase the burden on nurses, or online predilution with either saline or dialysate at different volume rates. In appreciation of this heterogeneity, and with objectives to compare the study method with the standard of care at its best and to increase the external validity of study results, we took the decision to allow both types of NH-HD fluid infusion according to routine practice at each site. This was accompanied by loose guidelines relative to volume and rate of fluid infusion to avoid outlying practices among controls. In this HepZero trial, our *post-hoc* comparisons of both control techniques showed significantly lower success rates of the predilution method.

There are few reported results of saline infusion assessed in randomized controlled trials. Recently, a prospective, randomized crossover study examined ten stable patients during intermittent hemodialysis with (1) regular saline flushes of extracorporeal circuit, (2) RCA, and (3) online heparin-primed membrane. All ten procedures with RCA were successfully completed after 4 h, whereas six of ten procedures with saline flushes and five of ten procedures with heparin-primed membrane were terminated prematurely because of clotting ($P < 0.05$).[22] In the HepZero trial, none of the study centers was using RCA as a routine method for anticoagulation-free hemodialysis, but these favorable results

of RCA should lead to a further head-to-head comparison of RCA with hemodialysis using the HGM.

In contrast, in a recent single-center Australian study, 50 NH-HD treatments were randomized into two treatment arms, namely, continuous saline infusion (where normal saline was infused into the dialysis extracorporeal circuit at a rate of 200 ml/h throughout the duration of dialysis) and intermittent saline flushing (where 100 ml saline was infused via the arterial line every 30 min while occluding the blood inlet line). Patients treated with continuous saline infusion were less likely to have clotted dialysis extracorporeal circuits (odds ratio 3.4, 95% CI 1.04–11.2; $P = 0.04$).[14] Whether the seven-times-higher continuous infusion rate (1.57 ± 1.40 l/h) and 37% higher mean saline flush volume (137 ± 70 ml) used in the HepZero trial compared with Zimbudzi's trial may have contributed to such discrepancies is speculative. Intermittent occlusion of the blood inlet line in the saline flushes group could have increased the risk of clotting. The unexpected, but *post-hoc*, finding of differential failure rate between saline flushes and predilution infusion in the present HepZero study warrants further research.

Study limitations

The first limitation lies in an apparently small number of patients and dialysis sessions analyzed. Precalculation according to previously reported data on heparin-free dialysis showed that a sample size of 126 patients per arm (252 for the entire sample) will provide the trial with 80% power to first conclude to noninferiority and then to superiority, with a one-tailed 5% error rate.[17] Indeed, the power of the study to demonstrate superiority with the observed $+7.9\%$ difference between success rates in the control and HGM groups was about 35%.

Second, for obvious logistic and technical reasons, the study was conducted as an open-label study, as the primary end point could not be assessed blindly. Indeed, neither the predilution process nor the saline flushes could have been masked, and the dialyzers could be easily differentiated by the nursing staff (different housings, different membrane colors, and transparency). Moreover, as they had to be carefully examined during the dialysis treatment, they could not be completely covered by a label aimed at preventing any differentiation between the two groups. To minimize potential bias due to such an open-label design, it was decided to have the primary end point of the study evaluated using a standardized visual semiquantitative clotting scale, as used in several researches,[23–27] and independently rated by two observers possibly assisted by a third authorized person in case of disagreement. Remarkably, the concordance between the two raters was almost perfect, which does not preclude a certain degree of subjectivity in the assessment of primary outcome—as the decisions to perform additional saline flushes and to terminate dialysis based on level 2 or 3 clotting was often made by the dialysis nurses.

Third, the current standard of care has a high 50% failure rate. This figure was higher than that previously reported,[20,21] but it is highly consistent with the failure rate of 48% observed in a more recent trial using intermittent saline flushing.[14] Moreover, a complete occlusion was only one of the criteria for failure, and it accounted for 35% of failures in the Control group.

Finally, a *post-hoc* analysis disclosed that the global rate of success (i.e., in both study and control groups) was higher in centers using saline flushes as compared with centers using predilution. However, the inclusion of centers using predilution for routine NH-HD sessions did not result in a disadvantage for the control group, as the difference of success rates between HGM and control groups was comparable regardless of the method used in the center: 17 vs. 19% in saline flushes versus predilution users, respectively.

In conclusion, this first large, international open-label, randomized, controlled trial evaluating the NH-HD treatment options of HGM and standard of care for patients with end-stage renal disease showed a statistically significant noninferiority of HGM over the controls (primary outcome). Moreover, the success rate in the HGM group was 20% higher, with a very small number ($n = 5$) of patients needed to treat to avoid one failure. Remarkably, the failure rate of current standard of care practices was high (50%), emphasizing the need for technical improvements.

We therefore suggest that HGM is a safe, helpful, and easy-to-use method for NH-HD in end-stage renal disease patient at a high bleeding risk.

MATERIALS AND METHODS

The full study protocol was published recently,[17] and extended methods are provided in a Supplementary Appendix online. Briefly, this clinical study was a prospective, multicenter (ten centers), international (seven countries), open-label, controlled, randomized, clinical study. Consecutive patients were screened by the investigators, and when eligible they were enrolled in the study and treated during a maximum of three consecutive NH-HD treatments, without any switch allowed between arms.

Two types of methods were evaluated in parallel:

- Study group: NH-HD treatment with HGM (Evodial 1.6);
- Control group: NH-HD treatment according to standard of care.

As there is no unique standard NH-HD procedure, the control group was defined by the usual procedure in place at each study site (i.e., saline flushes or predilution), with guidelines aimed at standardizing practices within the control group. It could be either saline flushes during dialysis treatment (100–300 ml per flush every 30 min, as stated in the European Guidelines)[2] or predilution (online or bags, with a predilution rate between 1 and 2 l/h). To allow the comparison, a dialyzer with roughly the same surface area as the HGM dialyzer ($1.65\,m^2$) was used in the control group. Otherwise (i.e., except for dialyzer and anticoagulation), dialysis sessions were conducted in the usual manner for each patient, particularly in terms of vascular access or duration.

Primary objective

The primary objective was to determine the effectiveness of the HGM dialyzer, compared with the standard of care in terms of successful treatments during the first NH-HD session. If the

noninferiority of HGM was demonstrated, we planned to test the superiority of HGM over the standard of care.

Secondary objectives

The secondary objectives were as follows:

- To compare the success rate during the second and the third consecutive NH-HD treatment with HGM versus the standard of care;
- To compare clotting grades in air traps every hour during all treatments with HGM versus standard of care;
- To assess the efficacy of NH-HD treatment with HGM versus standard of care;
- To assess the ease of use of NH-HD treatment with HGM;
- To follow up the safety of NH-HD treatment with HGM versus standard of care.

Study population

Main inclusion criteria were as follows:

- Patients with end-stage renal disease treated with maintenance hemodialysis for at least three months
- and requiring NH-HD treatments; the reasons for NH-HD prescription were recorded and the level of hemorrhagic risk was classified according to Lohr and Schwab.[28]

Main exclusion criteria were as follows:

- Patients in intensive care unit settings;
- Patients with acute kidney injury;
- Known heparin contraindication (heparin-induced thrombocytopenia type II);
- Transfusion of blood and other labile blood products required during hemodialysis treatment;
- Patients receiving oral anticoagulants or more than one antiplatelet agent, or unfractionated or low-molecular-weight heparin to prevent deep-vein thrombosis.

Patients were considered enrolled in the study when the informed consent had been signed.

Study end points

Primary end points. To evaluate the rate of successful treatments, clotting in the air traps was scored using a visual semiquantitative scale:[23–27]

- Grade 1: No detectable clotting;
- Grade 2: Minimal clot formation (presence of fibrinous ring);
- Grade 3: Clot formation (up to 5 cm) but dialysis still possible;
- Grade 4: Complete occlusion of air traps or dialyzer rendering dialysis impossible.

Clotting scoring was performed hourly by two independent observers. Depending on the organization at each study site, the evaluation was performed by two nurses (the nurse in charge of the patient and a second nurse not in charge of the patient) or by the nurse in charge of the patient and a co-investigator. In case of discordance between the two observers or in the eventuality of premature session termination (grade 4), the final adjudication was made by a third authorized and trained person (the principal investigator or registered co-investigators). Dialysis nurses and investigators were specifically trained and certified with regard to grading.

The first NH-HD treatment was considered successful when there was:

- No complete occlusion of air traps or dialyzer, rendering dialysis impossible (grade 4 of the scale);
- No additional saline flushes to prevent clotting;
- No change of dialyzer or bloodlines because of clotting;
- No premature termination (early rinse-back) because of clotting.

Secondary end points

- Hourly follow-up of the clotting during the first NH-HD treatment and evaluation of success rate, and of the clotting during the second and the third consecutive NH-HD treatments. Treatments were assessed using the same criteria and assessment methods as for the first heparin-free dialysis treatment.
- Hemodialysis session efficacy assessment: the ultrafiltration achieved and urea Kt/V (computed from urea changes as K/tV index $= \ln [C0 - C1]$) were documented;
- Ease of use: frequency and volume of saline flushes;
- Safety: adverse events/serious adverse events were recorded during the following two days after the last study dialysis session.

Ethical issues

Inclusion in the study was initiated in 2011 after approval of the appropriate ethics committees and competent authorities if applicable. The study protocol was recorded before any enrollment at ClinicalTrials.gov NCT01318486.

Statistics

All analyses were performed using the SAS 9.2 software (SAS Institute, Cary, NC, USA). The significance level was set to $P < 0.05$, one-tailed for the primary objective and two-tailed for others.

The primary objective was to assess the noninferiority and, if accepted, the superiority of HGM over the standard of care (saline flushes or online predilution) on success rate using a two-step procedure. The noninferiority and the superiority margins were set to the same value of 15%. In the hypothesis of a 65% success rate in the control group,[20,21] a sample size of 126 patients per arm gave the trial 80% power to conclude with one-tailed 5% error rate, first, to noninferiority and, second, to superiority. The 90% CI was constructed using the Wilson score method.[29]

DISCLOSURE

Maurice Laville, Marc Dorval, Joan Fort Ros, and Patrick Rossignol received honoraria for working as members of the study Steering Committee, and were reimbursed for travel and housing expenses related to meetings of the Steering Committee and Investigators. Marc Dorval: within the last 3 years, consultancies: Gambro; honorarium: Gambro, Servier, Merck, Takeda; research grants: Gambro, Baxter, Amgen. Laurent Juillard: consultant for Hospal, Hemotech, Roche, Amgen. Alicja Dębska-Ślizień received honorarium from Gambro for lecture—HD in high bleeding risk patients. Michael Schulz as member of the initial Advisory Board member meeting received an honorarium from Gambro, and he received travel and hotel expenses for attending two study group meetings from the study sponsor. He was not paid to write this article. Frédérique Moureau and Nathalie Loughraieb are employed by Gambro.

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SUPPLEMENTARY MATERIAL

Figure S1. Flowchart of the per-protocol population (HGM: heparin-grafted membrane; NH-HD: no-heparin hemodialysis).

Table S1. Membranes and surfaces of the dialyzers used in the control group.

Table S2. Total number of adverse events during the study.

Supplementary material is linked to the online version of the paper at http://www.nature.com/ki

REFERENCES

1. Shen JI, Mittani AA, Chang TI *et al.* Use and safety of heparin-free maintenance hemodialysis in the USA. *Nephrol Dial Transplant* 2013; **28**: 1589–1602.
2. European Best Practice Guidelines Expert Group on Hemodialysis, European Renal Section. Section V. Chronic intermittent haemodialysis and prevention of clotting in the extracorporal system. *Nephrol Dial Transplant* 2002; **17**(suppl 7): 63–71.
3. Mujais SK, Chimeh H. Heparin free hemodialysis using heparin coated hemophan. *ASAIO J* 1996; **42**: M538–M541.
4. McGill RL, Blas A, Bialkin S *et al.* Clinical consequences of heparin-free hemodialysis. *Hemodial Int* 2005; **9**: 393–398.
5. Kim YG. Anticoagulation during haemodialysis in patients at high-risk of bleeding. *Nephrology (Carlton)* 2003; **8**(suppl): S23–S27.
6. Casati S, Graziani G, Ponticelli C. Hemodialysis without anticoagulants in patients with high bleeding risk. *Int J Artif Organs* 1982; **5**: 233–236.
7. Hathiwala S. Dialysis without anticoagulation. *Int J Artif Organs* 1983; **6**: 64–66.
8. Casati S, Moia M, Graziani G *et al.* Hemodialysis without anticoagulants: efficiency and hemostatic aspects. *Clin Nephrol* 1984; **21**: 102–105.
9. Agresti J, Conroy JD, Olshan A *et al.* Heparin-free hemodialysis with Cuprophan hollow fiber dialyzers by a frequent saline flush, high blood flow technique. *Trans Am Soc Artif Intern Organs* 1985; **31**: 590–594.
10. Sanders PW, Taylor H, Curtis JJ. Hemodialysis without anticoagulation. *Am J Kidney Dis* 1985; **5**: 32–35.
11. Caruana RJ, Raja RM, Bush JV *et al.* Heparin free dialysis: comparative data and results in high risk patients. *Kidney Int* 1987; **31**: 1351–1355.
12. Preuschof L, Keller F, Seemann J *et al.* Heparin-free hemodialysis with prophylactic change of dialyser and blood lines. *Int J Artif Organs* 1988; **11**: 255–258.
13. Keller F, Seemann J, Preuschof L *et al.* Risk factors of system clotting in heparin-free haemodialysis. *Nephrol Dial Transplant* 1990; **5**: 802–807.
14. Zimbudzi E. Intermittent saline flushes or continuous saline infusion: what works better when heparin-free dialysis is recommended? *Int J Nephrol Renovasc Dis* 2013; **6**: 65–69.
15. Kessler M, Gangemi C, Gutierrez Martone A *et al.* Heparin-grafted dialysis membrane allows minimal systemic anticoagulation in regular hemodialysis patients: a prospective proof-of-concept study. *Hemodial Int* 2013; **17**: 282–293.
16. Sanchez-Canel JJ, Pons Prades R, Salvetti ML *et al.* Evaluation of coagulation and anti-Xa factor when using a heparin-coated AN69ST dialyser. *Nefrologia* 2012; **32**: 605–612.
17. Rossignol P, Dorval M, Fay R *et al.* Rationale and design of the HepZero study: a prospective, multicenter, international, open, randomized, controlled clinical study with parallel groups comparing heparin-free dialysis with heparin-coated dialysis membrane (Evodial) versus standard care: study protocol for a randomized controlled trial. *Trials* 2013; **14**: 163.
18. Wright S, Steinwandel U, Ferrari P. Citrate anticoagulation using ACD solution A during long-term haemodialysis. *Nephrology (Carlton)* 2011; **16**: 396–402.
19. Kozik-Jaromin J, Nier V, Heemann U *et al.* Citrate pharmacokinetics and calcium levels during high-flux dialysis with regional citrate anticoagulation. *Nephrol Dial Transplant* 2009; **24**: 2244–2251.
20. Bertrand D, Hanoy M, Le Roy F *et al.* Comparaison de deux modalités de dialyse sans héparine à la technique de référence des rinçages. Abstract presented at the SFD annual congress. *Nephrol Ther* 2010; **14**: 281–299.
21. Guéry B, Servais A, Harrami E *et al.* Hémodialyse sans héparinisation systémique: comparaison de trois protocoles. Abstract presented at the SFD annual congress. *Nephrol Ther* 2010; **14**: 281–299.
22. Richtrova P, Rulcova K, Mares J *et al.* Evaluation of three different methods to prevent dialyzer clotting without causing systemic anticoagulation effect. *Artif Organs* 2011; **35**: 83–88.
23. Evenepoel P, Degagere T, Verhamme P *et al.* Heparin-coated polyacrylonitrile membrane versus regional citrate anticoagulation: a prospective randomized study of 2 anticoagulation strategies in patients at risk of bleeding. *Am J Kidney Dis* 2007; **49**: 642–649.
24. Kodras K, Benesch T, Neumann I *et al.* Comparison of two dialysers (AN69ST vs. FX100) for heparin-free dialysis in patients with oral anticoagulation. *Blood Purif* 2008; **26**: 226–230.
25. Ziai F, Benesch T, Kodras K *et al.* The effect of oral anticoagulation on clotting during hemodialysis. *Kidney Int* 2005; **68**: 862–866.
26. Yixiong Z, Jianping N, Yanchao L *et al.* Low dose of argatroban saline flushes anticoagulation in hemodialysis patients with high risk of bleeding. *Clin Appl Thromb Hemost* 2010; **16**: 440–445.
27. Cheng YL, Yu AW, Tsang KY *et al.* Anticoagulation during haemodialysis using a citrate-enriched dialysate: a feasibility study. *Nephrol Dial Transplant* 2011; **26**: 641–646.
28. Lohr JW, Schwab SJ. Minimizing hemorrhagic complications in dialysis patients. *J Am Soc Nephrol* 1991; **2**: 961–965.
29. Newcombe RG. Estimating the difference between differences: measurement of additive scale interaction for proportions. *Stat Med* 2001; **20**: 2885–2893.