RESULTS

Table 2. Demographic and baseline data (intention-to-treat population)

	Control	Peritoneal dialysis	P value
Number of patients	5	5	
Median age [IQR]	80 [19]	70 [23]	n.s.
Sex: Male (%)	2 (40)	4 (80)	n.s.
Hypertension (%)	4 (80)	2 (40)	n.s.
Atrial fibrillation (%)	2 (40)	1 (20)	n.s.
Diabetes (%)	0	1 (40)	n.s.
Current smoker (%)	1 (20)	2 (40)	n.s.
Former smoker (%)	0	1 (20)	n.s.
Previous stroke >3 months	0	0	n.s.
Previous TIA	0	0	n.s.
Median baseline NIHSS score [IQR]	11 [7]	15 [10]	n.s.
Median baseline ASPECTS [IQR]	6 [4]	6 [3]	n.s.
Intravenous thrombolysis (%)	0	1 (20)	n.s.
Previous treatment:			
Aspirin (%)	0	1 (20)	n.s.
VKA (%)	2 (40)	0	n.s.
ACE inhibitors (%)	1 (20)	1 (20)	n.s.
ARB (%)	1 (20)	1 (20)	n.s.
Diuretics (%)	4 (80)	0	0.01
Calcium antagonists (%)	2 (40)	1 (20)	n.s.
Insulin (%)	1 (20)	0	n.s.
Statins (%)	4 (80)	2 (40)	n.s.

Data show number of patients with an event (percentage of events) and median of age, NIHSS and ASPECTS scores [IQR], in the intention-to-treat population. IQR, interquartile range. TIA, transient ischaemic attack; NIHSS, National Institutes of Health Stroke Scale. ASPECTS, Alberta Stroke Programme Early CT Score. VKA: Vitamin K oral antagonists. ACE, Angiotensin-converting enzyme. ARB, angiotensin II receptor blocker. n.s., not significant. Mann-Whitney test was used for continuous variables and X2 test was used for qualitative variables. The minimal level of significance accepted was $P<0.05$.

Baseline demographic characteristics of patients

Ten patients were recruited between June 2013 and December 2015 (5 patients for each group). Baseline characteristics are shown in table 2. All patients were included in the safety (intention-to-treat) analysis. There were no significant differences in baseline characteristics between the two groups, except for the percentage of patients taking diuretics ($P<0.05$), *which was considered by the researchers as not clinically relevant in this context.*

Feasibility of applying peritoneal dialysis to stroke patients in acute phase

In order to reduce Glu levels increased by cerebral ischaemia in stroke patients in acute phase, we designed a PD protocol consisting of 6 dialysis cycles with 2000 cm^3 exchanges during the first 24 hours and performing an exchange every 4 hours.

In one patient (treatment group, case 02), the procedure could not be performed due to inadequate catheter placement in the preperitoneum, which impeded the infusion of the dialysate. The catheter was removed without complications but a new catheter could not be placed due to poor patient tolerance. This case was included in the intention-to-treat population and was reported to the Spanish Medicines Agency as a non-medication-related serious adverse event (SAE).

Only one of the patients under dialysis completed all 6 exchanges according to the protocol.

The number of dialysis exchanges performed was 1-2 for the remaining patients. The PD cycle system does not allow the introduction of dialysis solution until the fluid stored in the peritoneum has been completely drained. This complete drainage was not easily achieved in stroke patients, in our conditions. Accordingly, the rest of the cycles programmed by the study protocol could not be performed.

Table 3. Serious adverse events in acute stroke patients.

Group	Case	SAE			
		Number	Type	Time after dialysis	Time after stroke onset
Control	01	2	Haemorrhagic transformation	-	3 days
			Death	-	12 days
Dialysis	02	1	Discontinuation of the intervention*	PD onset	16h
			Infarction recurrence	2 months	
	07	2	Ascites	PD onset	17h
			Death	3 months	

Data show number of events in the intention-to-treat population. *Case 02 was a randomised patient who did not undergo PD and was included in the intention-to-treat analysis. These serious adverse events (SAE) were reported to the Spanish Drug Agency as SAEs not related to medication.

Safety analysis of the use of peritoneal dialysis in stroke patients

Serious, non-treatment-related adverse events are listed in table 3.

One case of haemorrhagic transformation of the infarct was described in the control group, in the context of the natural evolution of cardioembolic stroke.

The procedure was interrupted in one patient in the PD group because the catheter was inadvertently placed outside the peritoneal cavity (see Treatment section). There were no complications arising from the procedure. This patient suffered a new cardioembolic cerebral infarction 2 months after stroke onset.

Abundant abdominal free ascites fluid was evident in another case of the PD group. Treatment did not continue after 2 PD cycles, but the blood samples for Glu analysis for those cycles were collected. Incidentally, peritoneal carcinomatosis was diagnosed later. In the 24 hours following ascites appearance, haemodynamic decompensation was evidenced requiring treatment with vasoactive amines. The patient was stabilized but presented a confirmed diagnosis: metastatic gastric and colonic cancer.

Mortality at 3 months was equal in both groups (20%). One control patient died during the study period. This death was secondary to a major stroke due to an extensive middle cerebral artery infarction, 90 days after the haemorrhagic

transformation event. One PD patient died as a consequence of cancer diagnosed, during the study but not related to the clinical trial treatment.

Non-serious adverse events observed at 3 months were aspiration pneumonia and respiratory infection in controls, and urinary tract infection and intracraneal hypertension in the context of the natural evolution of a large infarct in the PD group.

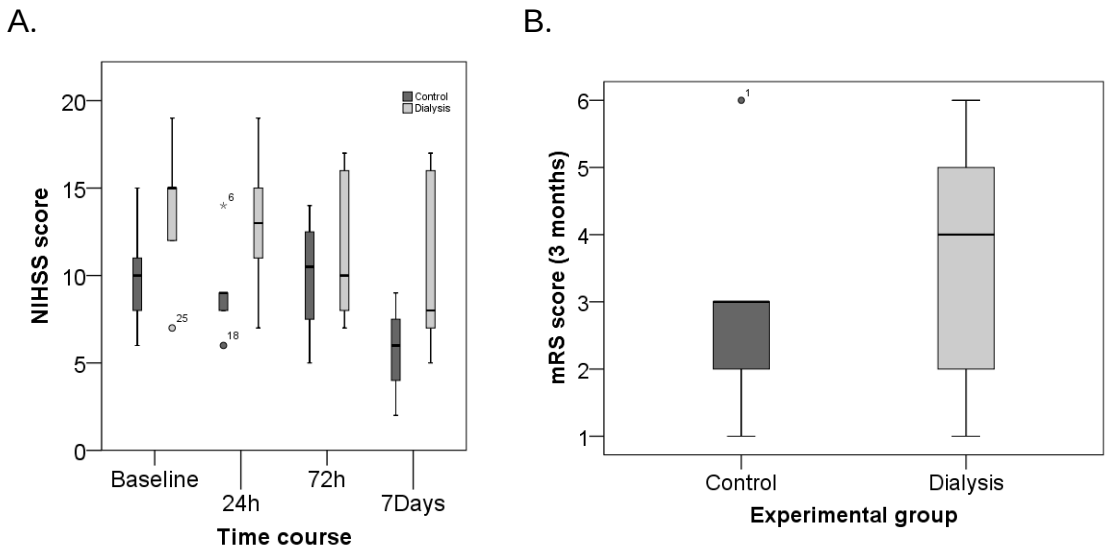

Fig 2. NIHSS and mRS scores data are showns as box plot. **A**. Time course of National Institutes of Health Stroke Scale (NIHSS) scores in control (dark grey boxes) and peritoneal dialysis (PD) groups. **B**. Modified Rankin scale (mRS) scores at 3 months in control and PD groups. Scores ranged from 0 to 6: *0, no symptoms; 1, no clinically significant disability; 2, slight disability (patients able to take care of themselves without assistance but unable to perform all previous activities); 3, moderate disability (patients require some help but are able to walk without assistance); 4, moderately severe disability (patients are unable to take care of bodily needs or walk without assistance); 5, severe disability (patients require constant nursing care and assistance); 6, death.*

Table 4. ASPECTS score and infarct volume at different time points.

	Time points	Group		P value
		Control	Dialysis	
ASPECT [IQR]	Baseline	6 [4]	6 [3]	>0.9
Infarct volumen (ml) [IQR]	24h	29.9 [54.5]	54 [69.6]	0.142
	72h	89.2 [38.4]	55.2 [106.8]	0.655

Data are shown as median and IQR. Infarct volume was missing for 1 control patient at 24h and 2 control patients at 72h. ASPECTS, Alberta Stroke Programme Early CT Score. IQR, interquartile range. Differences were analysed by Mann-Whitney test and P<0.05 was considered significant.

Neurological and functional outcomes in stroke patients after the use of peritoneal dialysis

To study the clinical benefit of peritoneal dialysis in stroke patients, we assessed ASPECT score, infarct volume, mortality and neurological and functional status of patients.

Table 4 shows ASPECT score and infarct volume. There were no significant differences in these parameters between control and treatment groups measured in basal conditions. Neither there were statistically significant differences in infarct volume between 24 and 72h within each group.

The median time from stroke onset to the first clinical evaluation (baseline) of patients before treatment was 15h (range 4-22h) for the control group and 13h (range 5-16h) for the PD group (P=0.295).

The median difference in the NIHSS score (Fig.2A) between both groups over time was 5 points at baseline (P=0.169), 3 at 24h (P=0.173), 0.5 at 72h (P=0.712) and 3.5 at 7days (P=0.064).

Functional outcomes (mRS) are shown in Fig. 2B. Functional independence (mRS=0-2) was observed in 40% of patients in both groups. No significant differences in mRS were observed at 3 months between patients in PD and control groups (median=4 [IQR=4] *vs* 3[3]; P=0.672).

According to the aetiology of the infarction, the proportion cardioembolic: atherothrombotic: cryptogenic infarcts was 4: 0: 1, in the control group and 2: 2: 1, in the PD group. No signs of haemorrhagic transformation were identified in the CT at 24-72 h (Fig. 3) except for the case 01 in the control group (Fig. 3a).

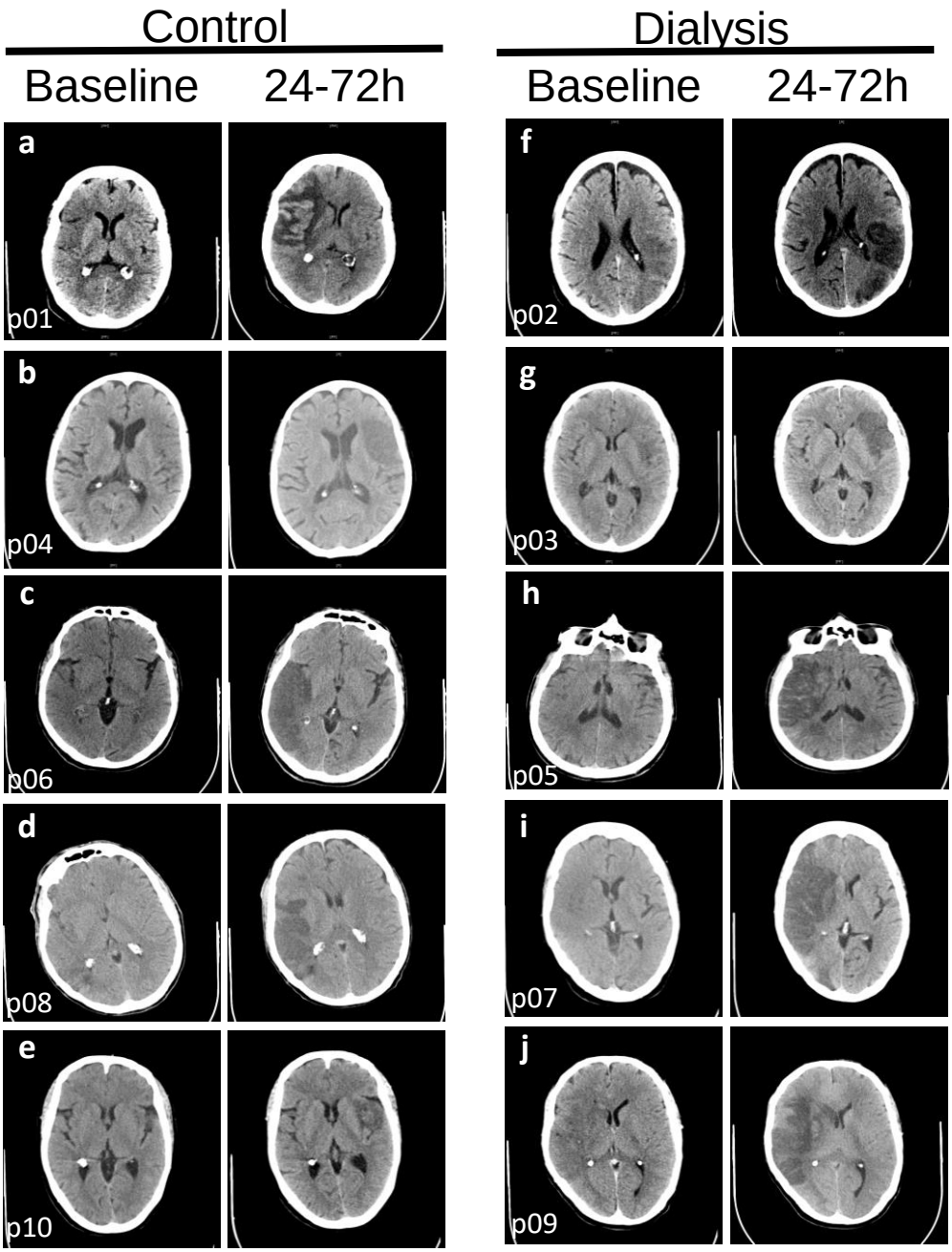

Fig 3. Brain CT of every patient after stroke onset. Axial slices show the area with higher damage in control (**a-e**) and PD groups (**f-j**) at baseline (prior to initiation of dialysis in the active group and after completion of randomization in the control group) and after 24-72h. CT at 24-72h shows: **a**) Hypodense area in the frontal region with inner hyperdense material in relation to infarction established in the territory of the right middle cerebral artery (MCA). **b**) Hypodense area that affects the left anterior insular region as well as the left frontal operculum, with grey-white blurring, in relation to infarction in evolution in the territory of the anterior division of the left MCA. **c**) Hyperacute infarction in the right temporal-occipital cortical territories, with defined hypodensity. **d**) Acute infarction affecting the right parietal cortex with progression to the anterior parietal cortex, insular and right external capsule. **e**) Hypodense cortico-subcortical lesion with loss of grey-white differentiation and discrete effacement of grooves in the frontal opercular region, insula, lenticular nucleus and external capsule of the left hemisphere, in relation to established ischaemic lesion of the ipsilateral MCA. **f**) Extensive hypodensity with loss of grey-white differentiation in the insula and left parieto-temporal region which produces mass effect with collapse of the adjacent subarachnoid space in relation to acute infarction in the left MCA territory.

g) Hypodensity affecting the left insular cortex with progression to the left frontal operculum. **h**) Hyperacute infarction in right base ganglia and in right frontoparietal cortical rotations, establishing at 24-72h a definite hypodensity without progression of ischaemia but with the presence of small haemorrhagic bleedings in the affected cortex. **i**) Extensive area of hypodensity compatible with ischaemic infarction in evolution in the territory of the right MCA that affects the grey and white fronto-parieto-temporal substance, insula and ganglia of the base, partially respecting the caudate nucleus. **j**) Infarction established in most of the surface and deep territories of the right MCA.

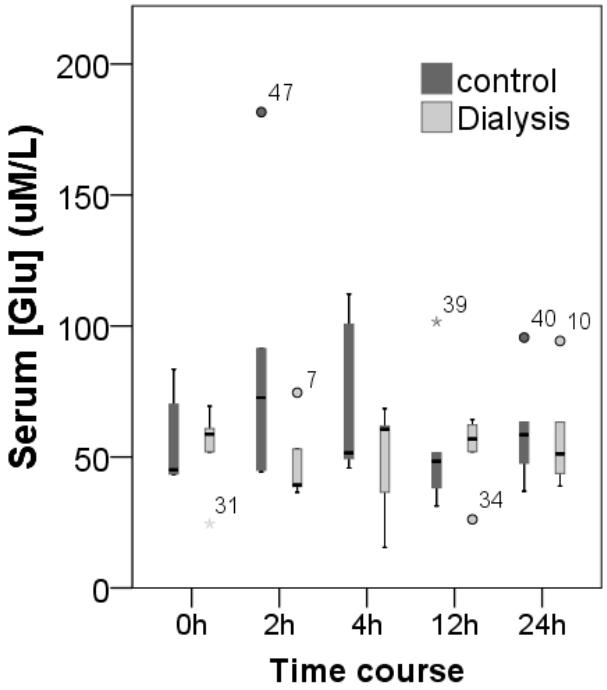

Figure 4. Time course of serum Glu concentration in control and PD groups. Data represent mean ± SE (N=5).

Serum and dialysate glutamate levels after peritoneal dialysis in stroke patients

To study the potential efficacy of peritoneal dialysis in reducing blood Glu levels we measured Glu concentration in serum and dialysate fluid after PD in patients with acute phase stroke.

The median time from stroke onset to the first blood sampling to measure Glu levels (T_Glu_{0h}) was 18h (range 15-22.5h) for the control group and 13.5 (range 8-17.5h) for the PD group (P=0.059).

In the intention-to-treat analysis (N=5 per group), no significant differences in serum Glu levels were found at any time studied. Although median serum Glu concentration at 2h in controls was reduced by 46% in PD group (72.650 [91.8] vs 39.320 [26.2]) this difference did not reach statistical significance (P>0.05; Fig. 4A).

However, excluding case 02 –in which the PD procedure could not be performed– from statistical analysis, levels of Glu were 72.650(91.8) in control group and 39.030(12.5) in treatment group with P=0.05.

Glu was detected in the PD liquid after a dwell time of 4h in the all cases in which PD treatment was performed (N=4 patients; *PD treatment was not performed in case 02*). On the other hand, excluding the patient with ascites where Glu concentrations were 46.2uM in the drained fluid after the first PD cycle (4h), Glu concentration ranged from 1.4-11.3uM in all other patients (N=3) at that time. Moreover, in the cases 03 and 09, in which 2 and 6 PD cycles were carried out, 11-fold and 22-fold increases of Glu concentrations were observed, respectively, after 24h.

AST, ALT, Urea, Creatinine, Glucose, Alkaline phosphatase

There were no statistically significant differences in median values of AST, ALT, Urea, Creatinine, Glucose and Alkaline phosphatase over time throughout the study ($P>0.05$).