Published in final edited form as:

Neurotox Res. 2018 February ; 33(2): 300–308. doi:10.1007/s12640-017-9791-0.

Blood Glutamate Reducing Effect of Hemofiltration in Critically Ill Patients

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Abstract

Glutamate toxicity plays a well-established role in secondary brain damage following acute and chronic brain insults. Previous studies have demonstrated the efficacy of hemodialysis and peritoneal dialysis in reducing blood glutamate levels. However, these methods are not viable options for hemodynamically unstable patients. Given more favorable hemodynamics, longer treatment, and less needed anticoagulation, we investigated whether hemofiltration could be effective in lowering blood glutamate levels. Blood samples were taken from 10 critically ill patients immediately before initiation of hemofiltration and after 1, 2, 4, 6, and 12 h, for a total of 6 blood samples. Samples were sent for determination of glutamate, glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), hemoglobin, hematocrit, urea, creatinine, glucose, sodium, potassium, platelet, and white blood cell (WBC) levels. There was a statistically significant reduction in blood glutamate levels at all time points compared to baseline levels. There was no difference in levels of GOT or GPT. Hemofiltration can be a promising method of reducing blood glutamate levels, especially in critically ill patients where hemodialysis and peritoneal dialysis may be contraindicated.

Keywords

Hemofiltration; Glutamate; Brain injury; GOT; GPT

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Evgeni Brotfain and Ruslan Kutz equally contributed to this work.

Compliance with Ethical Standards: This experiment was conducted according to the recommendations set forth by the Helsinki Committee and was approved by the Ethics Committee at Soroka University Medical Center, Beer Sheva, Israel.

Conflict of Interest: The authors declare that they have no conflict of interest.

Introduction

It is well established that glutamate (L-glutamate), an excitatory amino acid, is neurotoxic at high concentrations (Zauner et al. 1996) and plays an important role in secondary brain damage following acute (Zauner et al. 1996; Castillo et al. 1996; Johnston et al. 2001; Zlotnik et al. 2007, 2008) and chronic (Ferrarese et al. 2001; Shaw et al. 1995; Spranger et al. 1996) brain insults. Consequently, tight regulation of brain glutamate concentrations is vital in preventing the neurodegenerative effects of excess brain glutamate. The exact mechanism of glutamate release following brain injury remains unclear, and the search for viable treatment options to reduce the secondary brain damage associated with excess brain glutamate levels continues to be of great interest.

There have been various attempts to safely reducing brain glutamate levels described in the literature. While *N*-methyl-D-aspartate (NMDA) receptor antagonists showed initial promise for neuroprotection in animal models (McCulloch 1992; Lee et al. 1999), studies with antagonists of astrocytic and glial glutamate receptors have failed to demonstrate clinical efficacy in human clinical studies (Ikonomidou and Turski 2002; Muir 2006).

Another potential therapeutic modality is to utilize known glutamate transporters in the brain capillaries that allow glutamate to be removed from the brain into the blood (O’Kane et al. 1999; Teichberg et al. 2009; Danbolt 2001; Berl et al. 1962). Blood glutamate scavengers, such as oxaloacetate, pyruvate, glutamate oxaloacetate transaminase (GOT), and glutamate pyruvate transaminase (GPT), have been shown to reduce blood glutamate concentrations, thereby increasing the driving force of the brain to blood glutamate efflux and subsequently reducing brain glutamate levels (Gottlieb et al. 2003). Previous studies have demonstrated that infusions of blood glutamate-lowering drugs in rats are associated with improved neurological outcomes after traumatic brain injury (TBI), ischemic stroke, and subarachnoid hemorrhage (SAH) irrespective of the mechanism by which this reduction was achieved (Zlotnik et al. 2007, 2008, 2009; Boyko et al. 2011, 2012a; Zlotnik et al. 2012).

In clinical practice, however, glutamate scavengers have some crucial limitations. Studies with rat models of TBI, stroke, and SAH have all demonstrated that the therapeutic window of glutamate scavengers is very short, from 30 to 60 min, and ineffective after 120 min (Zlotnik et al. 2007, 2008; Boyko et al. 2011, 2012a, b). Glutamate scavengers also have a potential for systemic toxicity, with side effects and pharmacokinetic properties that may limit their use in clinical practice.

A potential therapeutic modality that has shown great promise in reducing blood, and thus brain glutamate concentrations, is by extracorporeal methods in which glutamate is filtered from the blood and definitively eliminated. Extracorporeal methods are well established and are widely used in filtering various substances from the blood. These modalities may reduce brain glutamate in a similar manner to that observed with the treatment of pharmacological blood glutamate scavengers with the advantage of avoiding the systemic effects of blood glutamate scavengers (Boyko et al. 2014). Each of these modalities have relative advantages and disadvantages that should be considered, which are summarized in Table 1.

Compared with healthy controls, patients with end-stage renal failure on hemodialysis (HD) have higher concentrations of blood glutamate (Rogachev et al. 2012). Prior studies have shown that during HD, especially in the first hour, there is a reduction in glutamate concentrations regardless of the size of the filter pores, blood flow rate, or gender. Similarly, peritoneal dialysis has resulted in a decrease of blood glutamate (Rogachev et al. 2013; Godino Mdel et al. 2013), with a corresponding increase of glutamate in the dialysis solution (Rogachev et al. 2013). In a rat model of stroke, the reduction of blood glutamate levels observed with peritoneal dialysis was associated with a decreased infarct area (Godino Mdel et al. 2013). This method is especially promising because its blood glutamate-reducing effects were long-lasting, compared with the transient effects observed after administration of blood glutamate scavengers.

Unfortunately, HD may be limited in the setting of acute brain injury. First, many of the patients admitted with acute brain injury suffer some degree of hemodynamic instability, typically due to hypovolemia or shock. Furthermore, anticoagulation is required for HD therapy to prevent clot formation in the set's tubing, which may be detrimental for patients suffering from multiple trauma or isolated head injury due to risk of hemorrhage. For these patients, continuous HF may offer a preferable therapeutic approach. In contrast to dialysis, which utilizes diffusion to transport solutes, hemofiltration (HF) uses principles of convection to create a positive hydrostatic pressure to drive solutes from the blood to the filtrate via a filter membrane (Friedrich et al. 2012). There is evidence that HF may be superior to HD in clearing medium-sized and larger molecules and may be more effective in clearing amino acids like glutamate in critically ill patients (Ronco et al. 2003). Considering that the diameter of pores in the membranes of filter used for HF is larger than those used for HD, we postulated that HF may be a surrogate for HD to decrease blood glutamate concentrations. HF results in a lower incidence of hemodynamic instability compared with HD and may be utilized with only minimal anticoagulation. Moreover, in contrast to HD, which typically lasts for only 4 h, HF may be utilized continuously for long periods of time (up to several days). HF may therefore provide a longer lasting glutamate-reducing effect, thereby promoting optimal neuroprotection. HF is widely used in critically ill patients for both renal and non-renal indications. It is possible to perform HF even in hemodynamically unstable patients. Early initiation of HF for the removal of excess glutamate from the plasma may be a useful adjuvant therapy for acute neurodegenerative conditions. To date, there are no published reports that examine the effects of HF on blood glutamate levels and neurological outcome after acute brain insults. The goal of the present study was to investigate whether HF may effectively reduce blood glutamate levels in critically ill patients and to investigate the pattern of blood glutamate reduction.

Materials and Methods

Population

This experiment was conducted according to the recommendations set forth by the Helsinki Committee and was approved by the Ethics Committee at Soroka University Medical Center, Beer Sheva, Israel. A total of 10 critically ill patients from the ages of 25 to 82 years with any health condition that required HF clinically decided by their physician were identified

and prospectively studied. All patients that started HF were candidates for participation in the study, regardless of their condition, initial pathology, or indications for starting HF. This study was an audit of current practice in our institution and did not require any specific interventions other than documentation and blood sampling for glutamate determination.

Study Protocol

The following data were collected from medical records of patients on HF: age, sex, background morbidity, duration of hospitalization in ICU, the reason for initiation of HF, length of HF session, the type of filter, type of line, type of dialyzer, and the use of heparin. All patients received conventional intensive care therapy in accordance with standard protocols, such as intubation, mechanical ventilation, and sedation according to clinical requirements. Additionally, GOT, GPT, hemoglobin, hematocrit, urea, creatinine, glucose, sodium, potassium, platelet, and white blood cell (WBC) levels were collected and hemodynamic and respiratory parameters of patients were recorded.

Blood samples for determining the glutamate levels were collected from an arterial line or central venous line. Baseline blood samples were obtained before initiation of HF. Additional samples were collected at 1, 2, 4, 6, and 12 h after the start of HF, for a total of six blood samples. Blood samples for evaluation of GOT, GPT, hemoglobin, hematocrit, urea, creatinine, glucose, sodium, potassium, platelet, and WBC levels were collected simultaneously with glutamate samples at baseline, 6, and 12 h after initiation of HF.

CRRT: HF

We used the continuous veno-venous HF as a method of continuous renal replacement therapy (CRRT) (Nikkiso Dialysis Machine, Tienen Belgium). Main indications for CRRT included acute or chronic renal failure in septic and trauma critically ill patients during the study period. The main parameters of HF were a predilution rate 2000–3000 mL/h and postdilution rate of 1000–2000 mL/h with blood flow rate around 140–150 mL/min. Heparin dilution was given when indicated at 1000 units/h. Two patients with signs of fluid overload secondary to acute renal failure were treated with fluid removal at 100 mL/h. For five patients, anticoagulation was not provided with the HF due to severe coagulopathy.

Blood Sample Analysis

Whole blood (200 μ L aliquot) was deproteinized by adding an equal volume of ice-cold 1 mol/L perchloric acid and then by centrifuging at $10,000\times g$ for 10 min at 4 °C. The pellet was discarded and supernatant collected, adjusted to pH 7.2 with 2 mol/L K_2CO_3 , and, if needed, stored at –80 °C for later analysis. Glutamate concentration was measured using the fluorometric method of Graham and Aprison (1966). A 20- μ L aliquot from the perchloric acid supernatant was added to 480 μ L of a 0.3-mol/L glycine, 0.25-mol/L hydrazine hydrate buffer adjusted to pH 8.6 with 1 mol/L H_2SO_4 , and containing 15 U of glutamate dehydrogenase in 0.2 mmol/L NAD. After incubation for 30 to 45 min at room temperature, the fluorescence was measured at 460 nm with excitation at 350 nm. A glutamate standard curve was established, with concentrations ranging from 0 to 6 μ mol/L. All determinations were done at least in duplicates.

Other Blood Measurement Levels

The levels of GOT and GPT were determined in the biochemical laboratory of Soroka Medical Center via a fluorescent method (Olympus AU2700 Chemistry-Immuno Analyzer, MN, USA) and were determined based on the conversion of glutamate into alanine and aspartate in the presence of GOT and GPT, respectively. Na, K, urea, creatinine, and glucose levels were determined in the biochemical laboratory of Soroka Medical Center using a fluorescent method (Olympus AU2700 Chemistry-Immuno Analyzer, MN, USA). Hb, Ht, WBC, and PLT levels were determined in the hematology laboratory of Soroka Medical Center using an automated hematology analyzer (Sysmex XE-2100 Automated Hematology Scan, IL, USA).

Statistical Analysis

Descriptive statistics were used to characterize the study population; continuous variables are presented as means with standard deviations or medians with first and third quartiles depending on the normality of the data. Categorical variables are presented as counts with relative frequencies. For repeated measurement in the different glutamate levels in the time points at 1, 2, 4, 6, and 12 h and baseline, we applied the Wilcoxon signed rank test. Two-tailed level alpha 0.05 was considered a threshold for statistical significance. Statistical analyses were performed using IBM SPSS Statistics 20 (IBM Corp, New York, USA).

Results

Demographic data and underlying diseases of the study population are presented in Table 2. There were 10 patients over the course of the study who underwent HF (4 women and 6 men, see Table 3). The average age was 61.8 ± 18.9 years. A Wilcoxon signed rank test showed that there was a statistically significant reduction in blood glutamate levels by 1 h ($P < 0.005$, median = 338 μM , $Z = -2.845$, $r = 0.61$, $n = 10$) after starting HF compared to baseline levels (median = 352 μM) and continued to decrease reaching significance at 4 h ($P < 0.05$, median = 296 μM , $Z = -2.1$, $r = 0.48$, $n = 8$) and 6 h ($P < 0.05$, median = 284 μM , $Z = -2.028$, $r = 0.478$, $n = 7$; Fig. 1). While there was a reduction in blood glutamate levels at 2 and 12 h after starting HF, values did not reach significance (median at 2 h was 352 μM , $Z = -1.682$, $P < .093$, $r = 0.367$, $n = 3$, and median at 12 h was 260 μM , $Z = -1.826$, $P < .068$, $r = 0.472$, $n = 4$).

There was no difference in levels of blood GOT or GPT before or throughout HF (Table 4). Levels of hemoglobin, hematocrit, urea, creatinine, glucose, sodium, potassium, platelets, or WBC count before and throughout HF are displayed in Table 5. Gender, indication for HF, or underlying disease did not influence glutamate clearance by HF. HF was discontinued in three patients prior to 12 h, as there was no longer any clinical indication to continue.

Discussion

The principal finding of this study was that HF effectively reduces blood glutamate levels. Measured concentrations of glutamate in the blood were consistently reduced during the 12 h following HF, decreasing by more than 20% of the baseline. This effect was seen as soon

as 1 h after initiation of HF. Gender, indication for HF, and underlying disease did not influence glutamate clearance by HF.

Utilizing extracorporeal methods of blood glutamate reduction to promote neuroprotection has potential clinical implications. Previous studies have shown the efficacy of HD and peritoneal dialysis in reducing blood glutamate concentrations, but the observed patterns were different (Rogachev et al. 2012, 2013; Godino Mdel et al. 2013). During HD, there was a prominent decrease in blood glutamate level during the first hour of HD. However, blood glutamate levels remained stable during the second and third hour and rose slightly during the fourth hour (Rogachev et al. 2012). Similarly, a reduction in blood glutamate was seen by 1 h after starting peritoneal dialysis, remaining stable for the following 3 h (Rogachev et al. 2013).

An osmotic rebound syndrome may explain the pattern of glutamate change by dialysis, in which removal of urea from the plasma by dialysis exceeds its removal from tissue. This produces an osmotic gradient between the cells and the plasma and subsequently leads to the development of an osmotic disequilibrium. Glutamate, an organic osmolyte, plays a significant role in osmotic adaptation (Soupart et al. 2002; McLaggan et al. 1994). Thus, it can be assumed that neurons secrete glutamate into the cerebrospinal fluid and subsequently into the plasma in response to the hypoosmotic stress during dialysis. The late elevation in glutamate levels seen during HD may be a defense mechanism to counteract this disequilibrium syndrome. The hypoosmotic stress phenomenon has been described during both HD and peritoneal dialysis (Rogachev et al. 2012, 2013).

The findings of this study suggest a different pattern of glutamate reduction by HF. Similar to HD and peritoneal dialysis, there was a significant reduction in blood glutamate after the first hour. However, our findings suggest that blood glutamate levels continue to decline as long as 12 h after starting HF, likely from the continuous nature of the procedure. This observation is significant in the clinical setting when choosing an optimal method for glutamate reduction, especially in the treatment of critically ill patients after brain insults.

Continuous HF is a highly effective system for renal replacement therapy in critically ill patients. Traditionally, HF has been used in patients with acute renal failure associated with several conditions, including heart failure, volume overload, chronic liver failure, and brain swelling (Patel et al. 2010). Non-renal indications include systemic inflammatory response (SIRS), hyperkalemia, sepsis and septic shock, multi-organ failure, and adult respiratory distress syndrome (Patel et al. 2010). Middle and larger molecular weight substances are more efficiently removed using HF compared to dialysis. During HF, hydrostatic pressure causes the filtration of plasma across a semi-permeable membrane. Solutes cross the membrane along with the plasma resulting in the convective transport of solutes in the same direction as water.

On the surface, HF has many similarities to HD. In both techniques, access to the circulation is required and blood passes through an extracorporeal circuit that includes either a dialyzer or a hemofilter. However, the mechanisms by which the composition of the blood is modified differs markedly (Forni and Hilton 1997; Barton and Hilton 1993). In the simplest

form of HF, blood under pressure passes down one side of a highly permeable membrane allowing both water and substances up to a molecular weight of about 20,000 to pass across the membrane by convective flow, as in glomerular filtration. The control of biochemical characteristics is constant, allowing the patient to avoid unwanted alterations of extracellular-fluid volume, even if large quantities of fluid are administered (for nutritional or other purposes). The use of HF has been shown to be tolerated in patients with hemodynamic instability, with a substantial reduction in mortality (Forni and Hilton 1997; Barton and Hilton 1993).

Levels of plasma GOT and GPT did not changed significantly throughout the experiment. In 3 of 10 patients, the levels of GOT and GPT were elevated at baseline due to their critical illness and severe comorbidities but remained constant throughout the experiment. These enzymes were of particular interest given their blood glutamate scavenging effects. In the presence of their cosubstrates oxaloacetate and pyruvate, GOT and GPT convert glutamate to 2-ketoglutarate and its metabolites. Peripheral injections of GOT and GPT in naïve rats have been shown to result in a reduction of blood glutamate levels (Zlotnik et al. 2007; Boyko et al. 2012a, b). Levels of hemoglobin, hematocrit, urea, sodium, platelets, and WBC count also remained constant. This important finding further emphasizes the advantage HF for blood glutamate reduction in patients with brain injury, especially in patients with no other primary indication for HF.

We observed no gender differences in blood glutamate level. Previous clinical studies have shown that in healthy volunteers, blood glutamate levels are inversely correlated to estrogen and progesterone levels (Zlotnik et al. 2011a). It was shown that during the menstrual cycle in women, the increase in estrogen and progesterone levels was accompanied by a corresponding decrease in blood glutamate level (Zlotnik et al. 2011a). Similarly, other studies have demonstrated that men have a significantly higher blood glutamate level than women (Zlotnik et al. 2011b), which may explain why women generally tend to have a better prognosis compared with men after traumatic brain injury and stroke (Green and Simpkins 2000). In this study, however, women were postmenopausal which may account for the lack of gender differences.

It should be noted that we observed elevations in GOT and GPT levels in three patients; in two of these patients, the baseline enzyme levels were elevated prior to initiation of HF and were subsequently moderately decreased. In one patient, however, the baseline GOT and GPT values were normal prior to HF initiation but increased significantly afterwards. While this rise in GOT and GPT might be due to the HF, it is difficult to draw this conclusion on the basis of a single observation. To the authors' knowledge, there is no evidence in the literature that HF itself may result in hepatic failure or elevation in blood liver enzymes. More likely, the patient's comorbidities (abdominal aorta aneurism, complicated by compartment syndrome and rhabdomyolysis) contributed to the elevations in GOT and GPT observed here.

Despite the promising results of the present study, there were several limitations. The authors were restricted by clinical indications to start and discontinue HF, resulting in a small sample size. The authors believe that the lack of significance at 2 or 12 h were likely

due to the limitation of a small number of HF patients at those time points ($n = 3$ and $n = 4$, respectively). Regardless, there remained a clear reduction of blood glutamate levels following HF in this study. This meaningful finding provides a basis for future experiments in the setting of acute brain injuries. Previous studies in humans demonstrated a strong relationship between elevated brain glutamate levels and worsened neurological outcomes after head trauma and stroke (Zauner et al. 1996; Castillo et al. 1996, 1997; Campos et al. 2011a; Aliprandi et al. 2005), and thus mechanisms of brain glutamate reduction in these conditions are of great clinical importance. Moreover, while it would have been interesting to compare the effectiveness of HF with HD in reducing blood glutamate concentrations, the risk of hemodynamic stability commonly observed with HD would have raised ethical concerns for conducting such a study in critically ill patients. Lastly, we did not perform HF in brain-injured patients, but rather studied patients where HF was initiated for a variety of indications. It would therefore be difficult to surmise from this study whether there is any correlation between HF and improved outcomes in these 10 patients with a wide spectrum of underlying pathology. While the effectiveness of HF in critically ill patients has been extensively studied in renal failure, sepsis, ARDS, and other conditions, determining whether HF improves outcomes after an acute brain insult should be determined by future investigations.

Utilizing extracorporeal methods of blood glutamate reduction to promote neuroprotection has significant advantages compared to pharmacological methods of blood glutamate scavenging. While various pharmacological treatment modalities based on the conversion of glutamate into 2-ketoglutarate have been shown to be effective in settings of rat models of TBI, stroke, and SAH (Zlotnik et al. 2007, 2008; Boyko et al. 2011, 2012a; Campos et al. 2011b), this treatment may be associated with toxic effects (Nagy et al. 2010). Furthermore, the glutamate-reducing effect may be reversible, because 2-ketoglutarate may again be converted to glutamate. In contrast, extracorporeal methods of glutamate reduction may definitively remove excess glutamate from the brain, while avoiding the possible toxic effects that may accompany the administration of blood glutamate scavengers.

The timing of initiation of therapy is important to consider. An elevation in brain glutamate concentrations in the extracellular fluid occurs within a few minutes after an acute brain insult (stroke, TBI, SAH) and reaches very high levels that often exceeds > 100 times the normal concentrations. However, there are significant differences in the patterns observed in humans and animals. For example, in rats, the elevation often does not last for the more than 2 h, whereas in humans, the elevation typically lasts 8 to 12 h or longer. In our previous studies (Zlotnik et al. 2007), we showed that earlier treatment with pharmacological blood glutamate scavengers, which reduced brain and blood glutamate concentrations, resulted in improved neurological outcomes. Specifically, the treatment was effective when initiated within the first 60 min after brain injury and was ineffective when initiated 120 min after injury. In humans, there are no scientific data that guide when the appropriate timing of such interventions is. Based on our understanding of patterns in how brain and blood glutamate change over time, we suspect that the therapeutic window would be longer in humans than in animals. However, we would argue that the treatment's effectiveness is likely optimal when initiated as early as possible (such as with thrombolytic therapy after stroke). While the effective timing of therapy should be determined by future investigations, we measured

the glutamate-reducing effects of HF within a 12-h interval based on our understanding of how glutamate in the brain changes over time after an acute insult. It should be pointed out, though, that the optimal treatment by HF may be longer or shorter and should be determined by future studies.

In conclusion, we demonstrated that HF may be a promising method of reducing blood glutamate levels, especially in critically ill patients where hemodialysis and peritoneal dialysis may be contraindicated. This may serve as a neuroprotective tool in patients suffering from acute and chronic brain insults. The findings of this study will hopefully pave the way for future clinical trials.

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Fig. 1. Blood glutamate concentrations. There was a statistically significant reduction in blood glutamate levels by 1 h after starting HF compared to baseline levels. Values continued to decline, reaching significance at 4 and 6 h after HF initiation

Table 1

Advantages and disadvantages of modalities

Method of blood glutamate reduction	Length of the glutamate-reducing effect (based on the current literature)	Necessity for anticoagulation	Preparation	Potential side effects
HD	At least 3 h	Necessary	Requires a dialysis line and associated equipment	Hypovolemia, hemodynamic instability
PD	At least 4 h	Unnecessary	Requires the insertion of a peritoneal cannula	Peritonitis
HF	At least 12 h	May be reduced or withdrawn when necessary	Requires a dialysis line and associated equipment	No major side effects
Pharmacological	At least 3 h (depends on the timing of infusion)	Unnecessary	Unnecessary	Allergic reactions, toxicity (no data available)

Table 2

Demographic and clinical information

Patient number	Age (years)	Gender	Weight (kg)	Diagnosis	Indication for HF
1	82	Male	86	Intraabdominal aortic aneurysm Infrarenal EVAR-aortic stent LT leg thrombectomy	Acute renal failure due to rhabdomyolysis Lt leg compartment syndrome
2	62	Male	95	Acute pancreatitis Retroperitoneal abscess Subphrenic abscess Severe sepsis Septic shock	Acute renal failure
3	67	Male	80	Severe intracerebral hemorrhage ARDS/pneumonia	Fluid overload Acute hypernatremia Acute renal failure
4	70	Male	90	Intraabdominal sepsis Duodenal perforation Low GI bleeding Sepsis	Acute renal failure
5	70	Female		Severe sepsis Septic shock	Acute renal failure
6	36	Male	80	Falling Multiple trauma	Acute renal failure
7	55	Female		Bilateral pneumonia ARDS	Acute renal failure
8	65	Female		LT U lobectomy Bilateral massive PE	Acute renal failure
9	86	Female	82	Acute cholangitis Sepsis	Acute or chronic renal failure
10	25	Male	75	Multiple trauma Gun shout wound of abdomen	Acute renal failure

Table 3

Values of HF parameters

Patient number	Heparin	Removal	Postdilution volume (mL)	Predilution volume (mL)
1	1000 units/h	0	1000	2000
2	1000 units/h	0	1000	2000
3	No	0	2000	3000
4	No	100 mL/h	1000	2000
5	No	100 mL/h	1000	2000
6	1000 units/h	0	1000	2000
7	1000 units/h	0	1000	2000
8	No	0	1000	2000
9	1000 units/h	0	1000	2000
10	No	0	1000	2000

Table 4

Absolute values of GOT and GPT in plasma at baseline and during HF

Patient number	GOT (IU/L)			GPT (IU/L)		
	T = 0	T = 6 h	T = 12 h	T = 0	T = 6 h	T = 12 h
1	16	597	5067	8	306	2676
2	23	44	48	11	24	23
3	10	9	18	17	21	43
4	81	38	36	97	61	61
5	34	37	68	44	45	53
6	94	87	88	126	115	105
7	34	39	50	23	56	50
8	1323	1120	1233	1941	1854	1700
9	109	112	156	63	65	69
10	2400	1898	1298	4800	4000	3245

Table 5

Blood levels of hemoglobin, hematocrit, urea, creatinine, glucose, sodium, potassium, platelet, and WBC count before and after HF

Parameters	<i>T</i> = 0	<i>T</i> = 6 h	<i>T</i> = 12 h
Hemoglobin (g/dL)	9.43 ± 1.34	9.1 ± 1.22	8.73 ± 0.76
Hematocrit (%)	29 ± 3.92	28.5 ± 3.87	30.4 ± 3.44
Urea (mmol/L)	153.3 ± 65.13	133.5 ± 55.38	177.8 ± 48.9
Creatinine (mg/dL)	3.25 ± 1.65	2.9 ± 1.3	2.59 ± 1.25
Glucose (mg/dL)	161.6 ± 50.33	147.7 ± 36.63	139.3 ± 46.68
Sodium (mEq/L)	139.5 ± 5.25	139.1 ± 4.3	139.5 ± 4.4
Potassium (mEq/L)	4.8 ± 0.5	4.3 ± 0.48	4.3 ± 0.49
Platelet (× 10 ³ /μL)	196.1 ± 54.28	195.1 ± 35.77	210.6 ± 59.42
WBC count (cells per microliter)	19,200 ± 10,932	15,100 ± 7203	13,950 ± 6785

The data is presented as average ± SD