Effectiveness and Safety Assessment of Citrate Anticoagulation During Albumin Dialysis in Comparison to Other Methods of Anticoagulation

Agnieszka Dyla, Wojciech Mielnicki, Joanna Bartczak, Tomasz Zawada, and Piotr Garba

Anesthesiology 4th Military Clinical Hospital, Wroclaw, Poland

Abstract: Liver failure is a serious and often deadly disease often requiring MARS (Molecular Adsorbent Recirculating System) therapy. Choosing the safe and effective method of anticoagulation during artificial liver support systems seems to be very difficult and extremely important. The aim of this study was to assess effectiveness and safety of regional anticoagulation with citrate in liver failure patients during MARS. We used a single center observational study. We analyzed 158 MARS sessions performed in 65 patients: 105 (66.5%) sessions in 41 patients with heparin anticoagulation, 40 (25.3%) sessions in 19 patients with citrate, and 13 (8%) sessions in only five patients without anticoagulation, that were excluded from part of the analysis. To determine the effectiveness of regional anticoagulation with citrate, probability of filter survival and changes in laboratory parameters were analyzed according to the applied method of anticoagulation. The safety of citrate was determined by Ca/Ca$^{2+}$ ratio, acid-base balance, bleeding complications, and the need for blood product transfusions. The probability of filter survival in the citrate group was 94% and in the heparin group 82% ($P = 0.204$). There was no relationship between the method of anticoagulation and effectiveness of MARS therapy in lowering the levels of the analyzed parameters. Only one patient had a Ca/Ca$^{2+}$ ratio higher than the safety margin. There were no statistically significant changes in pH and lactate level irrespective of anticoagulation; bicarbonate dropped significantly only in the heparin group ($P = 0.03$). The frequency of bleeding complications and the need for transfusions did not differ significantly between groups. Regional anticoagulation with citrate can be an effective and safe method of anticoagulation during MARS therapy, but requires attentive monitoring and further studies in liver failure patients.

Key Words: Liver failure—Molecular adsorbent recirculating system therapy—Regional citrate anticoagulation—Ca/Ca$^{2+}$ ratio—Liver support system—Albumin dialysis.

Liver failure, irrespective of its etiology and cause, is a serious and frequently deadly disease. The role of a clinician focuses on prevention and treatment of liver failure symptoms and complications. Certain clinical situations justify using artificial liver support systems, which help to stabilize the patient’s condition until liver function improvement is achieved, or liver transplantation, when the liver is irreversibly damaged (1,2).

The most frequently used liver support system is Molecular Adsorbent Recirculating System (MARS) (3) which uses albumin to transport and remove hydrophobic substances. Albumin dialysis takes place on a special filter combined with a standard membrane used during renal replacement therapy (4,5). Smooth and long-lasting blood flow through the system requires effective suppression of clotting cascade and use of adequate anticoagulation during all extracorporeal toxin removing systems. Anticoagulants used in extracorporeal systems are: heparin, hirudin, or a new direct thrombin inhibitor like argatroban or bivalirudin, citrate, prostacycline, and coumarin derivatives (6–11).

The balance of clotting cascade is very fragile in liver failure. Even the smallest derangement caused by anticoagulant use can substantially increase the
risk of severe coagulopathy, bleeding, or thrombosis (2,12). Choosing the safe and effective method of anticoagulation during artificial liver support systems seems to be very difficult and extremely important (2).

The most frequent anticoagulation method used during albumin dialysis is continuous infusion of unfractionated heparin. Even if properly monitored, it can lead to serious coagulopathy or heparin-induced thrombocytopenia. The alternative for systemic drugs is regional anticoagulation with citrate (9,13). The principle of regional anticoagulation is based on clotting inactivation by binding ionized calcium. Also, this method of anticoagulation has its limitations. Sodium citrate, which is metabolized in liver to bicarbonate, can lead to metabolic alkalosis and hypernatremia. The biggest potential danger in liver failure patients is the accumulation of calcium citrate due to metabolism impairment. It can lead to metabolic acidosis, low ionized calcium, and eventually to serious bleeding (14,15).

Considering those limitations, using regional anticoagulation with citrate in liver failure patients is still controversial and needs further research. The aim of this study was to assess effectiveness and safety of regional anticoagulation with citrate in liver failure patients during albumin dialysis with MARS.

PATIENTS AND METHODS

All MARS sessions performed in patients between 2007 (introduction of MARS in our department) and 2015 were analyzed. The following data were collected: demographics, diagnosis and etiology of liver disease, Model for End-Stage Liver Disease (MELD) (16) and Simplified Acute Physiology Score (SAPS) II (17) scores, and laboratory results. Blood tests were routinely taken just before each MARS session and within 1 h of albumin dialysis completion. Additional blood tests taken to evaluate safety of the treatment are described in detail later in the section. We thoroughly analyzed duration and complications during every MARS session.

Subjects

Liver failure was diagnosed using the following definitions:

1 Acute liver failure (ALF) was diagnosed in patients without previously known liver disease and with acute deterioration of liver function, shown as elevation of the international normalized ratio (INR) >1.5 and encephalopathy (18).

2 Acute-on-chronic liver failure (ACLF) was considered in patients with acute deterioration of previously existing chronic liver disease, usually related to a precipitating event and associated with increased mortality at 3 months due to multisystem organ failure (19).

3 Acute decompensation (AD) was defined by the acute development of one or more major complications of liver disease (i.e., ascites, encephalopathy, esophageal varices, hemorrhage, or infection) without organ dysfunction (20).

Eligibility for MARS treatment

Patients with ALF, ACLF, and AD were screened for eligibility for MARS therapy after ineffective symptomatic treatment of liver disease or triggering factor of liver decompensation. MARS was recommended provided that liver disease or triggering factor was treatable or if the patient was qualified for liver transplantation. Uncontrolled systemic infection was regarded as contraindication for MARS treatment.

Performance of MARS therapy

MARS therapy was performed using combined MARS and Prismaflex sets (Gambro AB, Lund, Sweden). During therapy two types of anticoagulation were used: systemic anticoagulation with heparin, and regional anticoagulation with citrate. In a few patients MARS therapy was conducted without anticoagulation. Flow rates during MARS treatment are shown in Table 1.

Dialysis was performed in continuous venovenous hemodiafiltration (CVVHDF).

PBP (Pre Blood Pump) was the amount of fluid infused before the filter. In the citrate group we used Prisrocitrato 18/0 (Na⁺ 140 mmol/L, Cl⁻ 86 mmol/L, citrate 18 mmol/L, Cl⁻ 109.5 mmol/L, Ca²⁺ 1.75 mmol/L, Mg²⁺ 0.5 mmol/L, HCO₃⁻ 32 mmol/L, LA 3 mmol/L, 287 mOsmol/L). CVVHDF dose, also known as effluent dose, was the total of PBP, replacement fluid, dialysate, and patient fluid removal flow rates, normalized to the patient’s body weight. It is expressed in mL/kg/h. Heparin anticoagulation was conducted with continuous infusion of unfractionated heparin. At the beginning of each MARS session a bolus of 5000 IU of heparin was given, followed by continuous
infusion to reach activated clotting time (ACT)/
activated partial thromboplastin time (APTT) level
of 2–2.5 times the normal range. Citrate anticoagu-
lation was used to achieve the theoretical concen-
tration of 3 mmol/L of trisodium citrate in blood.
To achieve this goal, we used blood flow to Prismo-
citrate 18/0 flow in 6:1 ratio. Ionized calcium levels
of inflow blood and outflow blood were not taken.
Citrate concentration of 3 mmol/L was considered
enough to protect the filter.

Effectiveness and safety assessment

Anticoagulation was considered effective when
lifespan of the circuit reached at least 10 h. Anoth-
er way of assessing effectiveness was analysis of
changes in laboratory parameters during therapy.
Safety of heparin anticoagulation was monitored
with ACT or APTT taken every 2–4 h during treat-
ment. This was our local policy based on our expe-
rience with continuous renal replacement therapy
(CRRT).

Safety of regional anticoagulation was monitored with:

- Calcium citrate accumulation using ratio Ca/
  Ca^{2+} taken every 4 h (ratio <2.5 was consid-
ered safe). Total calcium and ionized calcium
  concentrations were expressed in mmol/L.
- Acid-base balance analysis.
- Electrolyte derangements analysis.
- Clinical assessment based on looking for signs
  of bleeding or necessity to transfuse blood
  products.

Statistical analysis

Analysis of differences in the level of quantitative
parameters, depending on the type of anticoagu-
lation, was performed by means of Kruskal–Wallis
test, and in the case of qualitative parameters, by
means of Fisher’s exact test. In the event of signifi-
cant results, post hoc analysis was performed by
means of multiple repetitions method with Holm’s
adjustment. Multifactorial analysis of the impact of
anticoagulation type and MARS on a number of lab-
oratory parameters was carried out by two-way
analysis of variance (ANOVA) test. Statistical analy-
sis was performed using R statistical package.

RESULTS

Between 2007 and 2015, there were 65 patients
qualified for MARS therapy included in the analysis.
Thirteen (20%) patients had ALF, 34 (52.3%) had
acute-on-chronic liver failure (ACLF), and 18
(23.1%) had AD of liver function (AD). Etiological
factors included: active or chronic viral infection—10
(15.4%) patients, autoimmune liver and biliary dis-
bane—13 (20%) patients, toxic liver impairment due
to alcohol, xenobiotics and medications, other rare
diseases or unknown factor—12 (18.5%) patients.

In the analyzed group, 158 MARS sessions were
performed: 105 (66.5%) sessions in 41 patients were
performed using anticoagulation with heparin, 13
(8%) sessions in 5 patients without anticoagulation
and 40 (25.3%) sessions in 19 patients using regional
anticoagulation with citrate. Patients qualified for
MARS treatment without anticoagulation presented
signs of coagulopathy, often with active bleeding,
and circulatory failure. Simplified acute physiology
score II (SAPS II) was significantly higher in this
group. Otherwise, all the groups did not differ as far
as important demographic and clinical characteristics
are concerned, as is shown in Table 2.

Effectiveness

To determine the effectiveness of regional anti-
coagulation with citrate, probability of filter survi-
val was analyzed according to the applied method of
anticoagulation. One hundred fifty-eight MARS
sessions were performed lasting 1.3–20.5 h (median
10.2 h). MARS therapy was planned for 10–12 h. In
the filter survival analysis, filter clotting and ongo-
ing clotting, leading to premature therapy disap-
pearance with blood returned to the patient, were
considered adverse events. Loss of observation
was determined as planned therapy completion,
prefixed completion due to patient’s death during
treatment, or system breakdown.
Probability of filter survival >12 h in the group without anticoagulation reached 81%, but because of a small number of patients and not random selection of patients, MARS sessions without anticoagulation were excluded from further analysis.

Probability of filter survival >12h in the heparin group reached 82% and in the citrate group 94%. The difference did not reach statistical significance ($P = 0.204$). It might be caused by the limited number of observations. On the other hand, 6% filter loss is a satisfactory result and allows to consider regional anticoagulation with citrate as effective (Fig. 1).

Another way of assessing effectiveness of different methods of anticoagulation was analysis of changes in laboratory parameters. They were analyzed according to their change and its significance during therapy in heparin and citrate groups.

Both heparin and citrate groups had statistically significant drop in bilirubin, gamma-glutamyl transpeptidase (GGTP), and creatinine levels during treatment. There were no changes in alanine aminotransferase and aspartate aminotransferase levels. To analyze the relationship between changes in the laboratory parameters and anticoagulation, two-way ANOVA test was performed for parameters with significant change during treatment. There was no relationship between the method of anticoagulation and effectiveness of MARS therapy in lowering the levels of the analyzed parameters (Fig. 2A–C).

There was a statistically significant drop in albumin level during treatment only in the heparin group ($P = 0.01$), and a statistically significant drop in urea level only in the citrate group ($P = 0.04$). Two-way ANOVA test did not show a statistically significant relationship between these observations and method of anticoagulation. It might be related to a small number of observations and needs further research.

Safety

Citrate accumulation (Ca/Ca$^{2+}$)

Safety of citrate anticoagulation in liver failure patients was monitored using total calcium to
ionized calcium ratio (Ca/Ca\(^{2+}\)). A ratio <2.5 was considered safe. Due to the differences in therapy duration, only the highest ratio during treatment was taken into account. In the analyzed 40 MARS sessions, the ratio was 1.41–2.63 (median 2.13). Only one patient had a ratio (2.63) higher than the safety margin. It led to reduction of citrate flow and bore no clinical consequences of citrate accumulation or hypocalcemia.

Considering citrate metabolism, the hypothesis that patients with higher level of lactate are at higher risk of citrate accumulation was analyzed. Assessment of this potential correlation could answer the question of whether higher level of lactate predicts worse liver function. Thereby, is there a group of patients with higher risk of citrate accumulation? In the analyzed group, there was no correlation between lactate level before treatment and highest Ca/Ca\(^{2+}\) ratio during treatment (Fig. 3).

To evaluate the acid-base balance and frequency of hypernatremia during MARS therapy with regional anticoagulation, acid-base balance parameters were analyzed: pH, lactate, bicarbonate (HCO\(_3^-\)), and Na\(^+\) in both groups of patients.

![Image](image_url)
There was no statistically significant difference in sodium level, irrespective of the method of coagulation. There was also no correlation between changes in sodium concentration in plasma and method of anticoagulation in two-way ANOVA test ($P = 0.41$).

There were no statistically significant changes in pH and lactate level in the course of treatment. Bicarbonate level dropped significantly only in the heparin group, such correlation was not observed in the citrate group. Two-way ANOVA test showed a trend towards larger decline in bicarbonate level during treatment with heparin ($P = 0.06$). Lack of statistical significance seems to be influenced by the small number of patients. Acid-base balance is also very stable during therapy (Fig. 2D).

The last analyzed aspect of citrate anticoagulation safety was assessment of bleeding complications and need for blood product transfusion. A few statistical tests were performed.

Incidents of bleeding complications in both groups were compared. In the heparin group, nine (8.6%) such incidences occurred. There were three variceal bleedings, two airway bleedings, and four bleedings from central and dialysis catheters. The risk of catheter bleeding was not only hemoglobin loss but also formation of hematoma and neck edema that led to potential airway obstruction. In the citrate group, no incidence of bleeding was observed. Although statistical analysis showed only a trend in correlation ($P = 0.063$), the observation seems to be promising.

Analyzing frequency of blood product transfusions in both groups, there was no statistically significant difference between the groups. In the heparin group, blood products were transfused in 21 (20%) patients, in the citrate group in 4 (10%) patients. The difference is not statistically significant ($P = 0.22$).

Need for transfusions could also be related to losses on filter. To assess that, hemoglobin, platelet, and fibrinogen changes during MARS therapy were analyzed.

While fibrinogen level did not decline in any of the groups after treatment, platelet ($P = 0.003$) and hemoglobin ($P = 0.002$) levels significantly declined only in the heparin group. In patients with citrate anticoagulation, such significant changes were not observed. Two-way ANOVA test did not show a statistically significant correlation between decline in platelet count, hemoglobin level, and method of anticoagulation. It could be related to the small number of patients. Statistically significant loss of blood components during MARS treatment in the heparin group could be related to subclinical clotting in the extracorporeal circuit (Fig. 4).

**DISCUSSION**

Liver failure patients often develop coagulation cascade impairment. Reduced synthesis of pro- and anticoagulation factors, reduced clearance of active clotting factors, impaired platelets, hyperfibrinolysis, and increased intravascular anticoagulation form the pathophysiological mechanism of this disorder (21,22). In this condition, every method of anticoagulation could pose a risk of bleeding complications (23).

Heparin inducts systemic anticoagulation and intensifies liver failure coagulopathy (2). In patients with cirrhosis, with low serum concentration of AT III, heparin often leads to simultaneous filter clotting and bleeding (24). These problems pose a challenge in the context of looking for other solutions and performing MARS therapy without heparin.

A few retrospective studies describe such protocols. Faybic et al. write about using prostaglandin 2 during 61 MARS sessions in 33 patients, but 17 patients also received unfractionated heparin (25). Some of the studies describe MARS sessions...
without anticoagulation (26–30), but all of them mention clotting (0–25%) or bleeding (1–18%) complications during treatment.

Regional anticoagulation with citrate has become an alternative to systemic methods. Randomized, controlled studies unequivocally proved that citrate prolongs filter lifespan and reduces risk of bleeding and thrombotic complications during CRRT (31–34). Despite that, clinicians avoid using citrate in liver failure patients for fear of toxicity.

Literature review reveals the prospective study of Faybik et al. (23) where 20 patients in ALF with high MELD score underwent MARS therapy with citrate anticoagulation during 77 sessions. Another prospective, randomized study with citrate during MARS was performed by Meijers et al. (2). Unfortunately, it was used only in 10 patients, in whom in total 27 MARS sessions were performed.

Our analysis, comprising the review of different anticoagulation protocols, is based on a relatively large group of 65 patients and 158 MARS sessions. Citrate anticoagulation was used in 40 MARS sessions in 19 patients. After excluding a small group of patients without anticoagulation, we compared regional anticoagulation with citrate to standard protocol with unfractionated heparin. We focused on safety and effectiveness of citrate anticoagulation.

Effectiveness of trisodium citrate, as anticoagulant during MARS therapy, was rated as high in the so far published studies. Faybik et al. (23) describe only two (2%) cases where it was necessary to finish MARS therapy prematurely: one due to clotting and the other one due to bleeding complication. In patients studied by investigators from Belgium, all MARS sessions with citrate finished as planned, and that was statistically significant in comparison to MARS without anticoagulation. The same group created a visual assessment of filter clotting, especially useful in subclinical situations, which showed statistically significant advantage of citrate over lack of anticoagulation (2). In our group of patients analyzed here, the probability of planned termination of MARS session with citrate was 96%, so loss of filters was only 4%.

Meijers et al. (2) underline the influence of filter loss in a group without anticoagulation on effectiveness of the whole treatment. MARS with citrate caused better clearance of some laboratory parameters in comparison to prematurely finished sessions without anticoagulation. When sessions terminated as planned, there was no difference in clearance between groups.

We compared differences in clearance of substances in citrate and heparin groups, not discriminating sessions that terminated prematurely or finished as planned. For parameters showing statistically significant change during therapy (bilirubin, GGTP, creatinine), we could not find association with the method of anticoagulation.

We found a significant drop in albumin level in the heparin group which is an interesting finding, but not easily explained. Heparin might induce structural modifications and oxidative cleavage of human serum albumin as was described in in vitro studies (35). Unfortunately, we cannot prove whether this modified albumin is adsorbed on the filter or dialyzed. Maybe there is a totally different explanation for this finding.

Concerns that follow clinicians who want to use citrate anticoagulation in liver failure patients are based not on lack of effectiveness, but on potential side effects.

A part of citrate compounds get to the patient’s systemic circulation and must be metabolized. Physiologically citrate is quickly metabolized to bicarbonate, the process takes place mainly in liver and to a lesser extent in skeletal muscles and other tissues (36). In liver failure patients there is potential risk of citrate accumulation and its toxicity (14,37,38). Circulating citrate binds ionized calcium leading eventually to symptomatic ionized hypocalcemia, despite the total hypercalcemia detected in blood tests. Due to less effective metabolism to bicarbonate, metabolic acidosis develops. Accumulation of free citrate ions may further worsen metabolic acidosis and lead to increased anion gap (38,39). Impaired citrate metabolism was described in patients with ALF (14,37) and during liver transplantation (40,41). Reduced citrate clearance concerned patients with liver cirrhosis (37).

A good marker of citrate accumulation is total calcium to ionized calcium ratio (Ca/Ca2+) (14,42,43) usually accompanied by increased need for calcium supplementation. Protocols describing the use of citrate anticoagulation in MARS relied on this ratio as a marker of safety (2,23,39). Even if the ratio was increased, symptoms of hypocalcemia, metabolic acidosis, or other side effects were not observed. Our observations confirm earlier studies.

After literature review and with our own experience, we gathered enough arguments to recognize regional anticoagulation with citrate as a safe alternative to other protocols during MARS treatment. Meijers et al. (2), who compared citrate anticoagulation to treatment without anticoagulation, underlined significant reduction in platelet count in the latter group. It is believed that hypercoagulation that accompanies liver failure complicates things.
even more. Further reduction in platelet count and coagulopathy due to filter clotting can increase the risk of bleeding complications in patients without anticoagulation (23).

We decided to compare loss of blood components during MARS treatment in the citrate and heparin groups. There was a statistically significant reduction in hemoglobin level and platelet count only in the heparin group. Faybik et al. underline that regional anticoagulation can decrease utilization of platelets. Regional anticoagulation impedes degradation of polynucleated cells and platelets and diminishes oxidative stress in comparison to the frequently used heparin (44).

CONCLUSIONS
Regional anticoagulation with citrate can be an effective and safe method of anticoagulation during Molecular Adsorbent Recirculating System therapy, but requires attentive monitoring in liver failure patients. With this method, there is low risk of filter loss, probably lower than in any other available alternative, but this observation requires more sessions and further studies. Effectiveness of regional anticoagulation is expressed as good clearance of substances removed during MARS treatment. Accumulation of citrate in liver failure patients happens rarely, it is simple to monitor, and usually bears no clinical consequences. Lesser blood component loss during MARS therapy with citrate anticoagulation is a big advantage, especially in patients with very high risk of bleeding.

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