ORIGINAL ARTICLE

Hypofibrinogenemia Caused by Hemocoagulase Injection: A Retrospective Study on Clinical Laboratory Findings

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Objective Hemocoagulase injection based on the venom of *Agkistrodon halys Pallas* is widely used in the treatment of hemorrhagic disorders. This study aimed to characterize the clinical laboratory findings of hemocoagulase-induced hypofibrinogenemia as the associated adverse reaction of hemocoagulase injection.

Methods We retrospectively enrolled 27 in-patients who were treated with hemocoagulase injection for hemoptysis and developed hypofibrinogenemia during the period of January 1, 2015 to March 31, 2018. Clinical data were collected and investigated, including clinical manifestations, hemostatic and fibrinolytic parameters, dosage of hemocoagulase, the medication time, and the cryoprecipitate blood product infusion. Differences in fibrinogen, D-dimer, and fibrin/fibrinogen degradation products (FDP) before, during, and after the application of hemocoagulase injection were analyzed statistically.

Results Plasma fibrinogen level during medication of hemocoagulase injection decreased significantly compared to that before the treatment ($F=1.80$, $P<0.001$), with the average decrease of 2.28 g/L (0.63-3.9 g/L). After withdrawal, fibrinogen level increased significantly compared to that during the medication ($F=-1.20$, $P<0.001$), but was still lower than that before the medication ($F=0.59$, $P=0.03$). The D-dimer level and the FDP level after withdrawal decreased significantly compared to the levels during the medication ($F=0.83$, $P=0.002$; Wilcoxon-test, $Z=-4.54$, $P<0.001$). Spearman's correlation analyses did not find either fibrinogen change during-before the administration or FDP change after-during the administration was associated with the dosage of hemocoagulase ($r=-0.17$, $P=0.40$; $r=-0.28$, $P=0.15$; respectively) and the time of recovery from hypofibrinogenemia ($r=-0.45$, $P=0.05$; $r=0.13$, $P=0.61$; respectively).

Conclusion Monitoring both clotting and fibrinolysis parameters is essential in the management of hemoptysis patients treated with hemocoagulase injection. Clinicians should be aware of hypofibrinogenemia and consider discontinuation of the administration of hemocoagulase whenever necessary.

FIBRINOGEN, a soluble protein, is synthesized in hepatocytes and is the main substrate of thrombin.^[1,2] Serum fibrinogen level lower than 1.5 g/L suggest hypofibrinogenemia.^[3,4]

Congenital hypofibrinogenemia is a rare and hereditary hemorrhagic disorder that is induced by heterozygous mutations in fibrinogen-encoding gene,^[3] while acquired hypofibrinogenemia is most frequently caused by consumption of clotting factors, impaired hepatic function, or hemodilution.^[5]

Hemocoagulase injection is a mixture containing thrombin-like and thromboplastin-like enzymes which have been isolated, purified, and refined from the venom of *Agkistrodon halys Pallas*.^[6] In China, it has been widely applied in the treatment of bleeding disorders, *e.g.*, hemoptysis, hematochezia, as well as bleeding in trauma or surgery, especially in late-onset bleeding patients.^[7] The associated adverse reactions are increasingly encountered clinically, and cases with life-threatening coagulopathy and hypofibrinogenemia have been reported recently.^[6,7] However, there has been few report on the clinical laboratory characteristics of hypofibrinogenemia caused by hemocoagulase injection. To address this gap, we herein retrospectively studied on the patients with hemoptysis who developed hypofibrinogenemia in the treatment with hemocoagulase injection.

MATERIALS AND METHODS

Study patients and data collection

This study was approved by the Ethics Committee of the Shengjing Hospital China Medical University. Informed consents were waived. We retrospectively reviewed in-patients with symptom of hemoptysis who developed hypofibrinogenemia in the treatment with hemocoagulase injection during period of January 1, 2015 to March 31, 2018. Exclusion criteria were abnormal liver function, having received transfusion therapy, signs of thrombophilia, and congenital hypofibrinogenemia.

Diagnosis of hypofibrinogenemia was confirmed by hematologists based on the standard hemostasis assessment that fibrinogen level dropped below 1.5 g/L.^[3,4] We collected data of coagulation and fibrinolytic parameters before, during, and after the treatment with hemocoagulase. Hemocoagulase injection was intravenously administered at a dosage of 2 KU twice per day.^[6]

Coagulation studies

Coagulation assays were performed using an automatic coagulation analyzer (Instrumentation Laboratory, ACL-TOP 700, USA). The supplementary reagents used were as follows: fibrinogen (Clauss method, Instrumentation Laboratory, USA), D-dimer (DDU) (Latex enhanced immunoassay method, Instrumentation Laboratory, USA), and the fibrin/fibrinogen degradation products (FDP) (Latex enhanced immunoassay method, Instrumentation Laboratory, USA).

Statistical analysis

Quantitative variables of abnormal distribution were described as medians and interquartile range (IQR), qualitative variables were described as numbers and percentage. Friedman-test or Wilcoxon-test were performed to assess differences of coagulation and fibrinolytic parameters. Spearman's correlation analyses were applied to assess the relationship between the dosage of hemocoagulase and changes in fibrinogen and FDP, as well as the relationship between time of recovery from hypofibrinogenemia (without cryoprecipitate blood product infusion) and changes in fibrinogen and FDP. SPSS (version 22.0, IBM, USA) was applied to perform statistical analyses. $P < 0.05$ indicated statistical significance.

RESULTS

Patient characteristics

Totally there were 27 patients enrolled in this study. The condition of bleeding on admission improved with timely hemostatic therapy including hemostatic drugs and vascular embolism. After long-term use of hemocoagulase injection, 8 patients experienced bleeding again, which were converted by infusion of cryoprecipitate blood product. The gender, age, underlying disease, dosage of hemocoagulase, medication time, recovery time from hypofibrinogenemia, and percentage of patients who improved by cryoprecipitate blood product were summarized in **Table 1**.

Blood coagulation and fibrinolytic parameters changes

The coagulation and fibrinolytic parameters of 27 hemoptysis patients before, during, and after the application of hemocoagulase injection were shown in **Table 2**. During medication period, fibrinogen level decreased significantly compared with that before the medication (Friedman test, $F = 1.80$, $P < 0.001$). The average of

fibrinogen level changes in 27 patients was 2.28 g/L, ranging from 0.63 g/L to 3.9 g/L. After withdrawal of the hemocoagulase medication, fibrinogen level upgraded to normal level, with significant difference compared to that during the treatment (Friedman test, $F=-1.20$, $P<0.001$). Meanwhile, the fibrinogen level after withdrawal was also lower than that before the administration (Friedman test, $F=0.59$, $P=0.03$). There was no difference in D-dimer between the levels before administration and during administration (Friedman test, $F=-0.50$, $P=0.07$). The FDP levels decreased significantly after the withdrawal of hemocoagulase (Wilcoxon-test, $Z=-4.54$, $P<0.001$).

Correlation with recovery time and dosage of hypofibrinogenemia

The correlation analyses of hemocoagulase dosage and time of recovery from hypofibrinogenemia with changes of laboratory parameters were shown in **Table 3**. Spearman's correlation analysis found the dosage of hemocoagulase was independent of the changes in fibrinogen before and during the medication ($r=-0.17$, $P=0.40$) and changes in FDP during and after the medication ($r=-0.28$, $P=0.15$). In patients with no cryoprecipitate blood product infusion, the recovery time from hypofibrinogenemia was not associated with the change in fibrinogen during administration compared to that before ($r=-0.45$, $P=0.05$) and the change in

FDP levels after withdrawal compared to that during administration ($r=0.13$, $P=0.61$).

DISCUSSION

Fibrinogen plays a major role in hemostasis and is considered as the main substrate for thrombin; its

Table 1. Clinical characteristics of patients with hemoptysis on medication of hemocoagulase ($n=27$)

Clinical features	Results
Sex [n (%)]	
male	16 (59)
female	11 (41)
Age [years, median (min-max)]	64 (31-94)
Underlying disease [n (%)]	
bronchiectasis	20 (74)
lung cancer	3 (11)
pneumonia	4 (14.8)
Total dosage of hemocoagulase [KU, median(min-max)]	14 (12-28)
Medication time of hemocoagulase [days, median(min-max)]	3.5 (3-7)
Time to recover from hypofibrinogenemia [days, median(min-max)]	4 (2-7)
Cryoprecipitate blood product infusion [n (%)]	8 (29.7)

Table 2. Blood coagulation and fibrinolytic parameters of hemoptysis patients before, during and after administration of hemocoagulase ($n=27$)

Laboratory characteristics	Before administration	During administration	After discontinuation	F^*	P^*	$F^†$	$P^†$	$F/Z^‡$	$P^‡$
Fibrinogen (g/L)	3.0(2.3, 3.7)	0.6(0.5, 0.9)	2.0(1.7, 2.6)	1.80	<0.001	0.59	<0.05	-1.20	<0.001
D-dimer (μ g/L)	161(105, 333)	262(129, 473)	135(117, 245)	-0.5	>0.05	0.33	>0.05	0.83	<0.01
FDP (mg/L)	n/a	22.9(19.7, 29.1)	4.4(3.4, 6.1)	n/a	n/a	n/a	n/a	-4.54 [#]	<0.001 [#]

* compare between values during administration and before administration; [†] compare between values after discontinuation and before administration; [‡] compare between values after discontinuation and during administration; [#] by Wilcoxon-test. FDP, fibrin/fibrinogen degradation products.

Table 3. Correlation analysis of blood laboratory parameters changes with dosage of hemocoagulase and recovery time from hypofibrinogenemia ($n=27$)

Variables	Dosage of hemocoagulase		Time to recover from hypofibrinogenemia [‡]	
	r	P	r	P
Δ Fibrinogen* (g/L)	-0.17	0.40	-0.45	0.05
Δ FDP [†] (mg/L)	-0.28	0.15	0.13	0.61

* value change before and during administration; [†] value change during administration and after discontinuation; [‡] refers to 19 patients who did not receive cryoprecipitate blood product.

half-life is 3-4 days.^[5,8] In primary hemostasis, platelets are activated by cross-linked fibrinogen. Thrombin is generated when the coagulation pathway is triggered during secondary hemostasis, and it cleaves soluble fibrinogen to form fibrin, which are then polymerized to form a solid meshwork conferring stability and elasticity to blood clots.^[7,8] Fibrinogen, with molecule weight of 340 kDa, occurs in a 45-nm hexameric arrangement of peptide chains (A α , B β , γ) connected by disulfide bonds. It is composed of two outer D domains and a central E domain, and is encoded by chromosome 4.^[5] Concentration of plasma fibrinogen normally ranges from 2-4 g/L,^[9-11] whereas hypofibrinogenemia is identified when plasma fibrinogen drops below 1.5 g/L.^[3,4] In our study, acquired hypofibrinogenemia was identified due to the administration of hemocoagulase injection, as in all enrolled patients, fibrinogen levels before hemocoagulase treatment were greater than 1.5 g/L.

Acting on bleeding sites without affecting intravascular coagulation status, hemocoagulase injection has a good targeting capability and does not trigger thrombosis.^[6] In China, it is widely used in the treatment of bleeding due to its high safety. Despite its beneficial effects on hemostasis, the associated adverse events deserve attentions. Zhou^[6] reported seven cases of hypofibrinogenemia caused by hemocoagulase after excision of colon polyps, whereas no hypofibrinogenemia occurred in patients who did not receive intravenous hemocoagulase administration. Wang *et al.*^[11] reported three hypofibrinogenemia cases caused by long-term use of hemocoagulase. Qi *et al.*^[7] proposed that hemocoagulase could not control, but worsen gastrointestinal bleeding. In our study, plasma fibrinogen levels decreased after the administration of hemocoagulase injection, and the levels returned to normal after withdrawal. In contrast, other drugs, such as antibiotic and expectorant, had rarely been reported to cause hypofibrinogenemia as an adverse reaction. This result suggested that the decrease in fibrinogen was an adverse reaction associated with hemocoagulase injection.

The mechanism through which hemocoagulase injection provokes hypofibrinogenemia is ambiguous. According to literature, hemocoagulase degrades the Arg16-Gly17 scissile bonds in amino acid chain of fibrinogen, and releases fibrinopeptide A or both fibrinopeptide A and B, which then form unstable fibrin monomer I. At the site of vascular injury, the unstable

soluble fibrin monomer I can be assembled into fibrin I polymer, which promotes and accelerates platelet aggregation and thrombus formation, and finally results in hemostasis. Nevertheless, hemocoagulase exerts its hemostatic effect by hydrolyzing A chain of fibrinogen; therefore, it continuously consumes fibrinogen, leading to reduce of its plasma concentrations.^[11-14] Moreover, the unstable fibrin monomer I in blood can be quickly broken down. Therefore, the risk of thrombosis is low under treatment with intravenous administration of hemocoagulase because there is a further depletion of fibrinogen.^[6] According to the instructions of hemocoagulase injection, probability of reducing fibrinogen level at dosage of 50-100 KU is low.^[6] In our study, fibrinogen levels dramatically decreased to the level of severe hypofibrinogenemia when patients received hemocoagulase therapy at dosage exceeding 12 KU, or when medication time exceeded 3 days.

In this article, plasma fibrinogen levels after discontinuation of hemocoagulase therapy was lower than that before the administration. The reasons may lie in that: firstly, fibrinogen as a validation index was prone to upward fluctuate in patients in acute stage of disease; secondly, with the control of bleeding symptoms, the underlying diseases gradually improved and fibrinogen levels gradually returned to normal after discontinuation of hemocoagulase injection. D-dimer levels did not change during administration of hemocoagulase, whereas FDP levels returned to normal after the withdrawal, indicating that fibrinolysis mainly occurs through the disassembly of fibrinogen instead of the polymer of fibrin. Correspondingly, plasma FDP levels rapidly raised, while d-dimer, as a specific decomposition product of cross-linked fibrin, did not change significantly. This phenomenon suggests that monitoring hemostatic and fibrinolytic parameters while applying hemocoagulase therapy for bleeding patients is necessary to prevent from the potential hypofibrinogenemia. The decrease of plasma D-dimer after discontinuation is considered to be related to the improvement of patient's underlying disease and hemoptysis symptom. In this study the median of D-dimer before and during administration was different but not statistically significant, which might due to the small sample size and sample selection bias.

According to the pharmacokinetics proposed in the instructions, hemocoagulase injection is to be completely cleared out after 3-4 days.^[7] However, before elimination of hemocoagulase, hypofibrinogenemia

could be potentially dangerous. In late-onset bleeding patients, it is more prone to bleed again because of decreased plasma fibrinogen. Therefore, the most effective treatment in these cases is fibrinogen supplements. Plasma fibrinogen below 1 g/L was advocated as the threshold to perform general transfusion to treat hypofibrinogenemia in bleeding patients.^[15] However, many experts prefer a higher threshold (e.g., 1.5 g/L), for supplementary therapy.^[5] The clinicians in our hospital considered cryoprecipitate blood product transfusion depending on signs of bleeding.^[16] In 27 patients with hypofibrinogenemia, 8 patients experienced recurrent bleeding because of hypofibrinogenemia, and recovered after transfusion of cryoprecipitate blood product; the other patients with no signs of re-bleeding recovered in 2–7 days without cryoprecipitate blood product transfusion.

In this study, it seems that the dosage of hemocoagulase was independent to the change of plasma fibrinogen and FDP. Meanwhile, in patients of hypofibrinogenemia with no transfusion of cryoprecipitate blood product, no correlation was found between recovery time and changes of plasma fibrinogen and FDP. Different volume of blood losses among patients could affect consumption of fibrinogen, which might mask the variation of plasma fibrinogen induced by hemocoagulase; moreover, the relatively small number of patients with hypofibrinogenemia in this study limited the sampling representativeness. Additionally, in this retrospective study, most clinicians focused on correcting coagulation situation at the sudden onset of hemoptysis, and immediately applied hemocoagulase injection without examining fibrinolysis index, so we lacked FDP data before administration of hemocoagulase and could not find the change in fibrinolysis status after the administration of hemocoagulase. Future studies with larger sample and better design are needed.

In summary, this study suggests that it is important to monitor both clotting and fibrinolysis parameters (fibrinogen, D-dimer, and FDP) when hemocoagulase injection is used in the treatment of hemoptysis patients. Clinicians should be alert to the potential hypofibrinogenemia and consider discontinuation of hemocoagulase if fibrinogen level drops below 1.5 g/L, FDP level elevates, hemocoagulase dosage exceeds 12 KU, or medication lasts over 3 days, even though there is no change in D-dimer. In case of recurrent bleeding caused by hypofibrinogenemia, infusion of cryoprecipitate blood product is suggested; in other

cases of hypofibrinogenemia, fibrinogen can return to normal level after discontinuation of hemocoagulase injection.

Conflict of interests

None.

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