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Original Research

Utility of Biomarkers for Postpartum Hemorrhage Transfusion Requirements Relating to Amniotic Fluid Embolism

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Abstract

Background: We aimed to evaluate the utility of C1 esterase inhibitor, squamous cell carcinoma antigen, and complements C3 and C4 in the prediction of fresh frozen plasma transfusion requirements in postpartum hemorrhage and characterize the involvement of amniotic fluid embolism in the pathophysiology of postpartum hemorrhage. **Methods**: In this single-centered prospective observational study, consecutive patients with postpartum hemorrhage were evaluated for C1 esterase inhibitor, squamous cell carcinoma antigen, C3 and C4. We analyzed the relationship between the fresh frozen plasma transfusion volume and the above-mentioned biomarkers. The interaction of low C1 esterase inhibitor and squamous cell carcinoma antigen with respect to the fresh frozen plasma transfusion volume was analyzed. **Results**: The analysis included 132 patients with postpartum hemorrhage. In univariate analysis, C1 esterase inhibitor (correlation coefficient: –0.368, $p < 0.001$), C3 (correlation coefficient: –0.376, $p < 0.001$) and C4 (correlation coefficient: –0.291, $p < 0.001$) were negatively correlated with fresh frozen plasma transfusion volume. In multivariate analysis, only C3 was an independent factor associated with fresh frozen plasma transfusion volume (regression coefficient: –0.173, $p = 0.022$). C3 had the highest area under the curve (0.843) and cut-off value (80 mg/dL) for the prediction of fresh frozen plasma transfusion of $\geq$15 units. The fresh frozen plasma transfusion volume was higher in patients with low C1 esterase inhibitor and high squamous cell carcinoma antigen in the interaction analysis. **Conclusions**: Measuring C1 esterase inhibitor, squamous cell carcinoma antigen, and complements C3 and C4 in postpartum hemorrhage may allow assessment of the extent of anaphylactoid reaction and the requirement for fresh frozen plasma transfusion.

Keywords: amniotic fluid; biomarkers; blood transfusion; embolism; postpartum hemorrhage

1. Introduction

Postpartum hemorrhage (PPH) is one of the most frequent, unpredictable, and life-threatening childbirth complications. One of the pathogeneses is uterine focused amniotic fluid embolism (AFE) [1,2].

Amniotic fluid embolism is a syndrome characterized by the sudden onset of hypoxia, hypotension, seizures, or disseminated intravascular coagulation (DIC) occurring during labor, delivery, or immediately postpartum, related to the inflow of amniotic components into the maternal circulation [3]. Amniotic fluid embolism is one of the most serious obstetric complications, accounting for 5–15% of all maternal deaths and having a mortality rate of 37–80%. Only 10–15% of AFE cases are of the cardiopulmonary collapse-type, causing severe maternal shock and loss of consciousness. On the other hand, when the inflow of amniotic fluid components is localized to the uterus, it causes uterine atony and rapidly progressing DIC, which is called uterine focused AFE. This accounts for approximately half of maternal deaths due to PPH [4].

The diagnosis of AFE is traditionally made by pathologists through the presence of fetal squamous cells in the maternal pulmonary circulation. However, the passage of amniotic fluid components into the maternal circulation is thought to occur regularly without harming either the mother or the fetus. Since amniotic fluid components can be detected in 21–100% of obstetric patients without AFE, the presence of amniotic fluid components in the maternal pulmonary vessels is not a reliable diagnostic criterion for AFE [3]. Similarly, the presence of amniotic fluid components in the uterine vasculature is unlikely to be a definitive indicator of uterine focused AFE [5].

Recently, various biomarkers have been reported as alternatives to traditional pathological diagnosis. C1 esterase inhibitor (C1INH) is a protein that inhibits the complement, coagulation, and kinin pathways, thereby inhibiting the progression of the anaphylactoid reaction, which is involved in the pathophysiology of AFE [6]. Squamous cell carcinoma (SCC) antigen is a substance that is more abundant in amniotic fluid than in maternal serum. Elevated SCC antigen in maternal serum has been reported to be an indicator of amniotic fluid inflow into the maternal circulation [7]. Furthermore, complement activation is thought to reflect the pathology of AFE. Both C3 and C4 are usually involved in the antibody-dependent classical complement pathway, which normally requires antigen-antibody complexes for activation. However, it has been shown that complement levels can also decrease as a result of antibody-independent

anaphylactoid reactions [8].

For uncontrolled PPH secondary to AFE, massive transfusion should be initiated before surgical intervention. The transfusion of sufficient fresh frozen plasma (FFP) is prioritized above the transfusion of red blood cells [9]. However, if the cause of hemorrhage is not addressed, empirical early transfusion of FFP for persistent PPH does not reduce maternal death, hysterectomy or arterial embolization compared with no or later FFP transfusion [10]. Massive transfusion is associated with adverse effects, such as infection, allergic reactions, posterior reversible encephalopathy syndrome, pulmonary injury, and thromboembolism [11]. Therefore, developing tools for estimating appropriate blood transfusion targets and doses is vital. Such tools may identify early cases of coagulopathy in persistent PPH.

Since the concept of an anaphylactoid reaction as the pathophysiology of AFE is based on retrospective pathological studies, prospective studies are needed to identify biomarkers that enable prompt diagnosis in clinical practice. This study aimed to assess the involvement of AFE in PPH using C1INH, SCC antigen, C3, and C4 and evaluate whether these markers can be used to predict FFP transfusion requirements.

2. Materials and Methods

2.1 Participants and Study Procedures

This study was a single-centered prospective observational study conducted at a perinatal center in a hospital providing tertiary emergency care. Consecutive patients with PPH who met the following conditions were recruited: (1) Cases within 24 hours from the onset of PPH (delivery) to blood sampling, (2) Patients transferred from another hospital due to PPH, or (3) Inpatients with a PPH volume of $\geq$1500 mL during vaginal delivery, or $\geq$2000 mL during cesarean delivery.

Blood sampling was performed immediately after arrival at the hospital in transferred patients, or when the volume of blood loss met the study criteria in the inpatients. C1INH, SCC antigen, C3 and C4, fibrinogen, fibrin degradation product, D-dimmer, antithrombin-III, albumin, hemoglobin, platelets and human chorionic gonadotropin were evaluated.

The causes of PPH were retrospectively classified into uterine inversion, birth canal laceration, placenta accreta and previa, and cases that did not clearly fit into the above conditions were classified as atonic hemorrhage.

2.2 Treatment Strategy for PPH

Among the transferred patients, treatment strategies differed depending on the presence of continuous hemorrhage and signs of shock on arrival. General care was performed concurrently with an ultrasonographic search for the source of PPH, and if possible, hemostasis was achieved through minimally invasive treatment, e.g., manual repositioning for uterine inversion, or manual removal of the placenta for mild placenta accreta. In other cases, transcatheter arterial embolization (TAE) was the first-line treatment, interventional radiology being available 24 hours a day at this facility. If the patient was not clinically shocked, initial contrast-enhanced computerized tomography was performed to locate bleeding points, and if extravasation was observed, TAE was performed. Computerized tomography was omitted in shocked patients.

Blood transfusions were started promptly based on the results of laboratory investigations performed on arrival, with the goal of exceeding 150 mg/dL for fibrinogen and 7.0 g/dL for hemoglobin (the volume of hemorrhage was not used to guide the transfusion volume). When vital signs did not stabilize and hemostasis was not obtained by the above treatment, laparotomy (including surgical compression sutures and hysterectomy) was performed.

Among inpatients with PPH, intrauterine balloon tamponade was the first-line treatment for hemostasis. Blood transfusions were initiated based on the same criteria as those used for transferred patients. In the case of cesarean delivery, surgical compression sutures were used when hemostasis was insufficient despite the administration of a uterotonic during surgery. When the hemorrhage persisted, TAE was performed, followed by hysterectomy if unsuccessful.

2.3 Statistical Analysis

The blood transfusion volume was calculated assuming that one unit of packed red blood cells is 140 mL and one unit of FFP is 120 mL. The Kolmogorov–Smirnov test was used to test the normality of the distribution.

The relationship between FFP transfusion volume and laboratory investigation results was evaluated by Pearson's correlation coefficient as a univariate analysis. Logistic regression analysis was used for multivariate analysis of factors affecting the FFP transfusion volume.

A receiver operating characteristic (ROC) curve was constructed to estimate the predictive value of C1INH, SCC antigen, C3 and C4 for FFP transfusions of $\geq$15 units. The area under the curve (AUC) was also calculated. The interaction analysis of low C1INH and SCC antigen with respect to the FFP transfusion volume was performed with reference to the cut-off value of C1INH.

Statistical analyses were performed using EZR [12], a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria). Statistical significance was set at $p < 0.05$.

3. Results

3.1 Patient Characteristics

During the study period, 132 patients (61 transferred patients and 71 inpatients) met the recruitment criteria.

Table 1 shows the characteristics, causes of postpartum hemorrhage, and hemostatic procedures for the in-

cluded cases. Thirty-two percent of the participants were included after cesarean delivery. The proportion of causes was 5% for uterine inversion, 15% for birth canal laceration, 14% for placenta accreta, 20% for placenta previa and 46% for those that did not clearly fall into these conditions (classified as atonic hemorrhage).

Table 1. Characteristics, causes of postpartum hemorrhage, and hemostatic procedures of the participants.

	n = 132
Characteristics	
Maternal age (years)	33 ± 5
Gestational age	38w5d ± 24d
Birth weight (g)	3066 ± 448
Male	58 (44%)
Cesarean section	42 (32%)
Causes of postpartum hemorrhage	
Uterine inversion	6 (5%)
Birth canal laceration	20 (15%)
Placenta accreta	19 (14%)
Placental previa	26 (20%)
Atonic hemorrhage	61 (46%)
Hemostatic procedures	
Intrauterine balloon tamponade	21 (16%)
Transcatheter arterial embolization	26 (20%)
Hysterectomy	6 (5%)

Mean ± Standard deviation/number of cases (%).

Intrauterine balloon tamponade was performed as a hemostatic procedure in 16%, transcatheter arterial embolization in 20% and total hysterectomy in 5%.

3.2 Laboratory Investigation Results and Treatments

Table 2 shows the blood loss, FFP transfusion volume and blood investigation results for each cause of postpartum hemorrhage.

The mean blood loss before blood sampling was highest for placenta previa (2550 ± 1294 mL), and lowest for birth canal laceration (1349 ± 609 mL).

FFP transfusion volume before blood sampling was highest in placenta previa (180 ± 589 mL), as transfusions were often initiated intraoperatively. All cases of uterine inversion were transport cases, and no FFP transfusions were performed before blood sampling due to the short time between onset and transport. FFP transfusions of >15 units were most often required for uterine inversion (33% of cases) and atonic hemorrhage (13% of cases).

Blood investigation results showed that the mean values of C1 inhibitor, C3 and C4 were lowest in uterine inversion (40.8 ± 8.8%, 59 ± 10 mg/dL, and 14 ± 5 mg/dL, respectively) and SCC antigen was lowest in placenta previa (2.5 ± 2.0 ng/mL).

3.3 Predicting FFP Volume

Table 3 shows the results of univariate and multivariate analyses of laboratory investigation results related to FFP transfusion volume.

In univariate analysis, C1INH (correlation coefficient (CC): –0.368, 95% confidence interval (CI) lower: –0.514, upper: –219, $p < 0.001$), C3 (CC: –0.376, 95% CI lower: –0.514, upper: –0.219, $p < 0.001$), C4 (CC: –0.291, 95% CI lower: –0.44, upper: –0.127, $p < 0.001$), fibrinogen (CC: –0.462, 95% CI lower: –0.587, upper: –0.317, $p < 0.001$), and hemoglobin (CC: –0.254, 95% CI lower: –0.407, upper: –0.085, $p = 0.003$) were negatively correlated with FFP transfusion volume. Squamous cell carcinoma antigen was not significantly correlated with FFP transfusion volume. In multivariate analysis, only C3 was independently extracted as an independent factor associated with FFP transfusion volume (regression coefficient: –0.173, 95% CI lower: –0.322, upper: –0.025, $p = 0.022$).

Fig. 1A,B show the ROC curves of C1INH, SCC antigen, C3 and C4 for the prediction of FFP transfusion of 15 units or more. The AUC of C1INH was 0.819 (95% CI lower: 0.724, upper: 0.914), and the cut-off value, sensitivity, and specificity were 56%, 53.8% and 100%, respectively. The AUC of SCC antigen was 0.598 (95% CI lower: 0.407, upper: 0.789), and the cut-off value, sensitivity, and specificity were 4.4 ng/mL, 69.7% and 61.5%, respectively. C3 had the highest AUC of 0.843 (95% CI lower: 0.769, upper: 0.917), and the cut-off value, sensitivity, and specificity were 80 mg/dL, 65.5% and 100%, respectively. The AUC of C4 was 0.76 (95% CI lower: 0.65, upper: 0.87), and the cut-off value, sensitivity, and specificity were 15 mg/dl, 58.8% and 84.6%, respectively. The FFP transfusion volume was higher in patients with low C1INH and high SCC antigen in the interaction analysis (Table 4) when a C1INH of 55 or less was set as low, referencing the cut-off value.

4. Discussion

Interaction analysis suggested that the FFP transfusion volume was higher in patients with low C1INH and high SCC antigen. The utility of C1INH and C3 was shown in the prediction of PPH cases requiring FFP transfusions of 15 units or more.

In this study, postpartum hemorrhage was classified into five pathological conditions. However, actual postpartum hemorrhage is believed to be a complex combination of various pathological conditions. In particular, the cases classified as atonic hemorrhage in this study may have a variety of causes and degrees, ranging from mild atony due to uterine muscle fatigue to severe atony due to uterine focused amniotic fluid embolization.

AFE is difficult to predict or prevent due to its rare prevalence and broad spectrum of clinical symptoms. In addition, definitive and objective diagnostic criteria are lacking. However, early diagnosis is very important for im-

Table 2. Blood loss, FFP transfusion volume, and laboratory investigation results for each case of postpartum hemorrhage.

	Uterine inversion	Birth canal laceration	Placenta accreta	Placental previa	Atonic hemorrhage
n	6	20	19	26	61
Blood loss					
Before blood sampling (mL)	1422 ± 766	1349 ± 609	1784 ± 1052	2550 ± 1294	1846 ± 916
Total (mL)	2625 ± 619	2043 ± 911	2505 ± 1313	2540 ± 1265	2214 ± 1752
Fresh frozen plasma transfusion					
Before blood sampling (mL)	0	54 ± 167	25 ± 110	180 ± 589	155 ± 682
Total (mL)	880 ± 953	294 ± 735	240 ± 560	457 ± 624	716 ± 1861
>15 units (%)	2 (33%)	1 (5%)	1 (5%)	1 (4%)	8 (13%)
Laboratory investigation results					
C1 inhibitor (%)	40.8 ± 8.8	60.6 ± 18.3	52.1 ± 15.9	59.7 ± 10.2	55.9 ± 17.5
C3 (mg/dL)	59 ± 10	83 ± 24	85.7 ± 25.7	90 ± 18	87 ± 32
C4 (mg/dL)	14 ± 5	17 ± 5	18.2 ± 8.4	18 ± 7	18 ± 10
SCC antigen (ng/mL)	3.6 ± 2.5	4.1 ± 2.7	4.2 ± 3.1	2.5 ± 2.0	4.7 ± 3.6

Mean ± Standard deviation/number (%).

FFP, fresh frozen plasma; SCC, squamous cell carcinoma.

Table 3. Univariate and multivariate analysis of blood test findings related to FFP transfusion volume.

Blood test findings	Univariate analysis				Multivariate analysis			
	CC	95% CI lower	95% CI upper	*p*	RC	95% CI lower	95% CI upper	*p*
C1 inhibitor (%)	–0.368	–0.507	–0.21	<0.001	–0.049	–0.208	0.108	0.533
C3 (mg/dL)	–0.376	–0.514	–0.219	<0.001	–0.173	–0.322	–0.025	0.022
C4 (mg/dL)	–0.291	–0.44	–0.127	<0.001	0.037	–0.287	0.363	0.817
SCC (ng/mL)	0.107	–0.065	0.273	0.223				
Fibrinogen (mg/dL)	–0.462	–0.587	–0.317	<0.001	–0.005	–0.03	0.018	0.633
FDP (μg/mL)	0.709	0.612	0.785	<0.001	0.002	–0.041	0.493	0.936
D-dimer (μg/mL)	0.708	0.611	0.784	<0.001	0.225	–0.041	0.493	0.096
Anti-thrombin III (%)	–0.318	–0.463	–0.155	<0.001	0.182	–0.016	0.381	0.072
Albumin (g/dL)	–0.326	–0.484	–0.146	<0.001	2.198	–3.359	7.756	0.434
Hemoglobin (g/dL)	–0.254	–0.407	–0.085	0.003	–0.219	–1.345	0.906	0.699
Platelet ($10^3/\mu$L)	–0.378	–0.516	–0.221	<0.001	–0.012	–0.044	0.019	0.429

FDP, fibrin degradation product; SCC, squamous cell carcinoma; FFP, fresh frozen plasma; CC, correlation coefficient; RC, regression coefficient; CI, confidence interval.

Table 4. Interaction analysis of C1INH and SCC antigen on FFP transfusion volume.

	Regression coefficient	95% CI lower	95% CI upper	*p*
Low C1INH	–1.621	–7.671	4.43	0.597
Low C1INH & SCC	2.21	0.955	3.464	<0.001
SCC	–0.156	–0.854	0.543	0.66

CI, confidence interval; C1INH, C1 esterase inhibitor; FFP, fresh frozen plasma; SCC, Squamous cell carcinoma.

proving prognosis, and the pursuit of biomarkers that can be clinically applied is key.

Pregnant women with low C1INH may be at a high risk of AFE-associated PPH, but C1INH has previously been reported to be uncorrelated with blood loss at delivery [13]. This suggests that the pathophysiological mechanism may involve low C1INH as an indicator of predisposition to AFE, with amniotic fluid exposure acting as a trigger for onset. Interaction analysis shows that the FFP transfusion requirement is increased in patients with low C1INH and high SCC antigen. In these cases, it is speculated that the cause of PPH includes uterine focused AFE as a pathogenetic factor.

Squamous cell carcinoma antigen has been shown to be a potential indicator of amniotic fluid inflow into the maternal circulation. However, in most pregnant women, a small inflow of amniotic fluid to the maternal circulation may be harmless [6], suggesting that the presence of amniotic fluid components in the maternal uterine vasculature is not an indicator of AFE, but a common finding in the postpartum myometrium [5]. Anaphylactoid reactions have been proposed as the pathophysiology of AFE to explain this individual difference in response to amniotic fluid, and the interaction analysis in this study supports this hypothe-

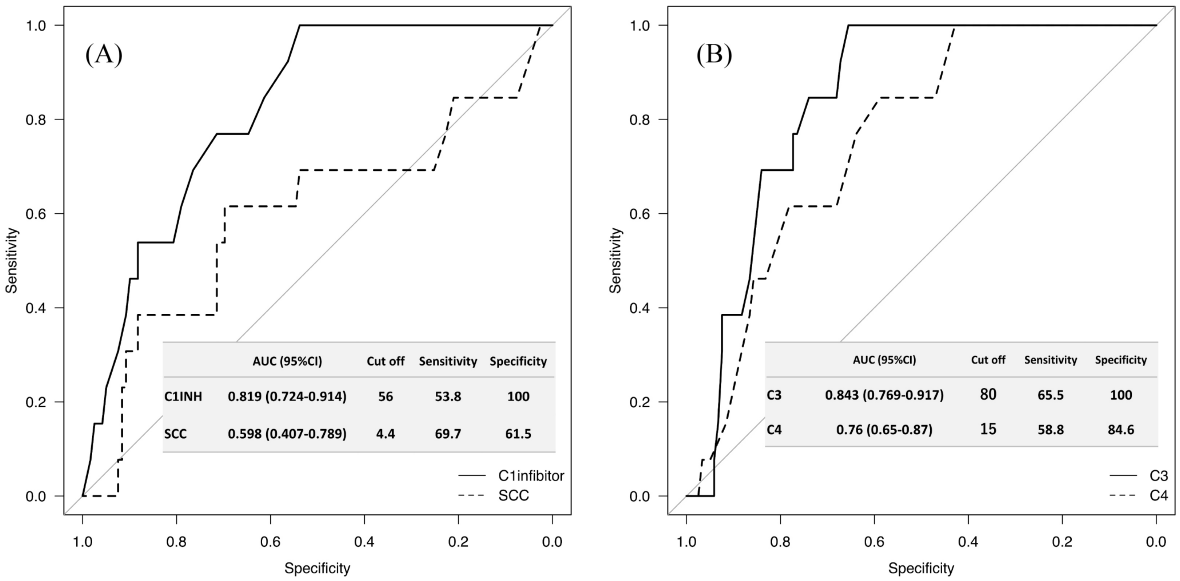

Fig. 1. Receiver operating characteristic curves of C1 esterase inhibitor, squamous cell carcinoma antigen, and complements for prediction of fresh frozen plasma transfusion of ≥15 units. (A) C1 esterase inhibitor and squamous cell carcinoma antigen. (B) Complement C3 and C4. SCC, squamous cell carcinoma; C1INH, C1 esterase inhibitor.

sis, as the FFP transfusion requirement was higher in those with low C1INH and high SCC antigen.

Complement increases with weeks of gestation during pregnancy, while not changing significantly during delivery, and decreases to within normal limits after delivery [14]. This study included patients whose levels of C3 or C4 were clearly lower than the normal range, which may reflect the presence of an anaphylactoid reaction in the pathophysiology of PPH. Furthermore, C3 is the most useful biomarker in predicting the FFP transfusion requirements, consistent with the fact that anaphylactoid reactions can lead to rapid progression of DIC.

Transfusions with an FFP: red blood cells ratio of 1 or higher are recommended for AFE with coagulopathy [15]. In addition, the administration of C1INH concentrate in cases of suspected uterine focused AFE may promote rapid uterine recovery and reduce transfusion requirements [16]. It has been reported that 8 units of FFP contain 1000 units of C1INH [6], and early FFP transfusion for uterine focused AFE is expected to not only replenish coagulation factors but also improve uterine atony by replenishing C1INH.

Postpartum hemorrhage involving an anaphylactoid reaction as the pathogenesis of AFE may require massive FFP transfusion. We believe that the discovery of biomarkers for anaphylactoid reactions would be very useful in developing a treatment strategy. C3 is expected to be a clinically useful biomarker, as the results can be easily and rapidly obtained in general hospitals. With a high predictive accuracy for FFP transfusion requirements, C1INH may also be useful, but it is difficult to obtain the results quickly in general hospitals. Squamous cell carcinoma antigen in isolation shows poor predictive value. In summary, the addition of C3 assessment to the management of PPH may provide useful information for the planning of treatment strategies through the estimation of FFP transfusion requirements, and the elucidation of the pathogenesis of AFE.

This study included three patients who underwent total hysterectomy for severe PPH and whose uterine pathology led to the diagnosis of AFE. In the first case, the patient was brought to the hospital with a decreased level of consciousness and circulatory failure within one hour after vaginal delivery. Despite prior TAE with permanent embolic material, total hysterectomy was required due to marked coagulopathy. The coagulopathy was treated with a massive blood transfusion and the patient survived, but the aftereffects of hypoxic encephalopathy remained. In the second case, the patient was transferred due to increased hemorrhage and rapid deterioration of level of consciousness after vacuum-assisted delivery. Massive blood transfusion, TAE and total hysterectomy were performed to save her life, but she developed Sheehan's syndrome after delivery. The third patient was a primiparous woman who developed hemorrhage and progressive loss of consciousness after an induced vaginal delivery for pre-eclampsia. A total hysterectomy was promptly performed, but the coagulopathy did not improve after the surgery and the patient was transferred to our hospital, where TAE and laparotomy were performed. In all cases, the pathology of the excised uterus led to the diagnosis of AFE. Fibrinogen and C1INH were both below the limit of quantification in the first and second cases. The third patient had a C1INH of 50% and a fibrinogen of 150 mg/dL despite the fact that 40 units of FFP was transfused before transfer to our hospital. The SSC antigen levels were

10.9, 9.1 and 7.2 ng/mL, respectively.

This study included patients with PPH of various causes, including cases requiring massive FFP transfusion due to coagulopathy caused by factors other than anaphylactoid reactions. In all deliveries, pregnant women are exposed to amniotic fluid and there is a possibility of developing AFE, the extent of which may be predicted by multiple factors such as amniotic fluid inflow and C1INH levels. Postpartum hemorrhage is not necessarily caused by a single factor, but by a combination of factors in varying degrees. We propose that measuring C1INH, SCC antigen, and complements in patients with PPH may allow us to assess the proportion of anaphylactoid reactions as the pathogenesis of PPH and the requirement for FFP transfusion. Further research is needed to determine the utility of biomarkers in determining treatment strategies for PPH.

5. Conclusions

Measuring C1INH, SCC antigen and complements C3 and C4 in postpartum hemorrhage may allow assessment of the requirement for fresh frozen plasma transfusion. These results may explain the pathophysiology of the discrepancy between blood loss and transfusion requirements in PPH.

Availability of Data and Materials

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

RS designed the research study and analyzed the data. RS, TS and KM performed the operation and collected the data. KM supervised the study. All the authors approved the final version of the manuscript.

Ethics Approval and Consent to Participate

Written informed consent was obtained from all study participants. The study was conducted in accordance with the Declaration of Helsinki, and the protocol for this study was approved by the ethics committee of Gifu University Hospital, Gifu, Japan (approval number: 2019-007).

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Conflict of Interest

The authors declare no conflict of interest.

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