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Amniotic Fluid Embolism Induces Uterine Anaphylaxis and Atony following Cervical Laceration

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Established Facts

- Amniotic fluid embolism (AFE) is a rare, high-risk obstetric complication primarily found in the lungs and potentially related to anaphylaxis.
- High serum levels of tryptase and complements have been thought to support anaphylaxis and complement activation as mechanisms underlying the pathogenesis of AFE.

Novel Insights

- Immunostaining for AFE markers, tryptase release and complement receptor demonstrated the uterus as the focus of anaphylaxis and complement activation resulting from AFE.
- The findings on anaphylaxis and complement activation appeared to be related to the findings on cervical laceration, uterine atony and lethal hemorrhage.

Key Words
Amniotic fluid embolism · Tryptase · Anaphylaxis · Mast cell · Uterine atony · Cervical laceration

Abstract
Amniotic fluid embolism (AFE) is a rare, high-risk obstetric complication primarily found in the lungs and potentially related to anaphylaxis. Tryptase release from the mast cell reflects anaphylaxis. Case report and findings: A female, aged over 40 years, presented with uterine atony and lethal hemorrhage after induced vaginal labor. Cervical laceration was accompanied by severe hemorrhage. Stromal edema and myometrial swelling were consistent with uterine atony. Alcian blue staining and zinc coproporphyrin immunostaining disclosed AFE, which was more prominent in the uterus than in the lungs. Tryptase immunostaining was diffuse and prominent around the activated mast cells (halos) in the uterus, including the cervix. Similar distribution of findings on the AFE markers, tryptase halos, complement receptor C5aR, and atony in the uterus suggested the causality of AFE to anaphylaxis, complement activation and atony. It is probable that disseminated intravascular coagulation (DIC), induced by AFE, uterine atony and cervical laceration, caused the lethal hemorrhage. It is likely that AFE, in association with cervical laceration, induces uterine anaphylaxis, complement activation, atony, DIC and lethal hemorrhage.

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Introduction

Amniotic fluid embolism (AFE) is a rare, high-risk obstetric complication that primarily occurs in the lungs and is potentially related to anaphylaxis [1, 2]. High serum levels of mast cell-derived tryptase and complements have supported the contribution of anaphylaxis and complement activation to the pathogenesis of AFE, though the results have been somewhat inconsistent [3]. Meanwhile, immunohistochemistry demonstrated mast cell degranulation with tryptase-positive materials outside the cells in AFE lungs [4]. However, there is no report on uterine anaphylaxis related to AFE.

Case Report

A woman, aged over 40 years, was admitted to a clinic after a normal 39-week pregnancy. She was healthy except for a successful liver transplantation. Labor began 3 h after administration of $PGF_{2\alpha}$, and following premature rupture of the membrane a healthy baby was born 3 h thereafter. The cervical laceration with 1,500 ml hemorrhage was sutured 30 min after delivery, but the woman soon became hypotensive, with hemorrhage amounting to 2,400 ml. The atonic uterus was compressed manually. Despite transfusion, and the administration of ergometrine and vasopressors, hemorrhage and hypotension worsened. Normal O_2 saturation and lack of respiratory distress at this time ruled out pulmonary embolism.

By 1.5 h after delivery, the woman received additional cervical suturing but became pulseless, with hemorrhage amounting to

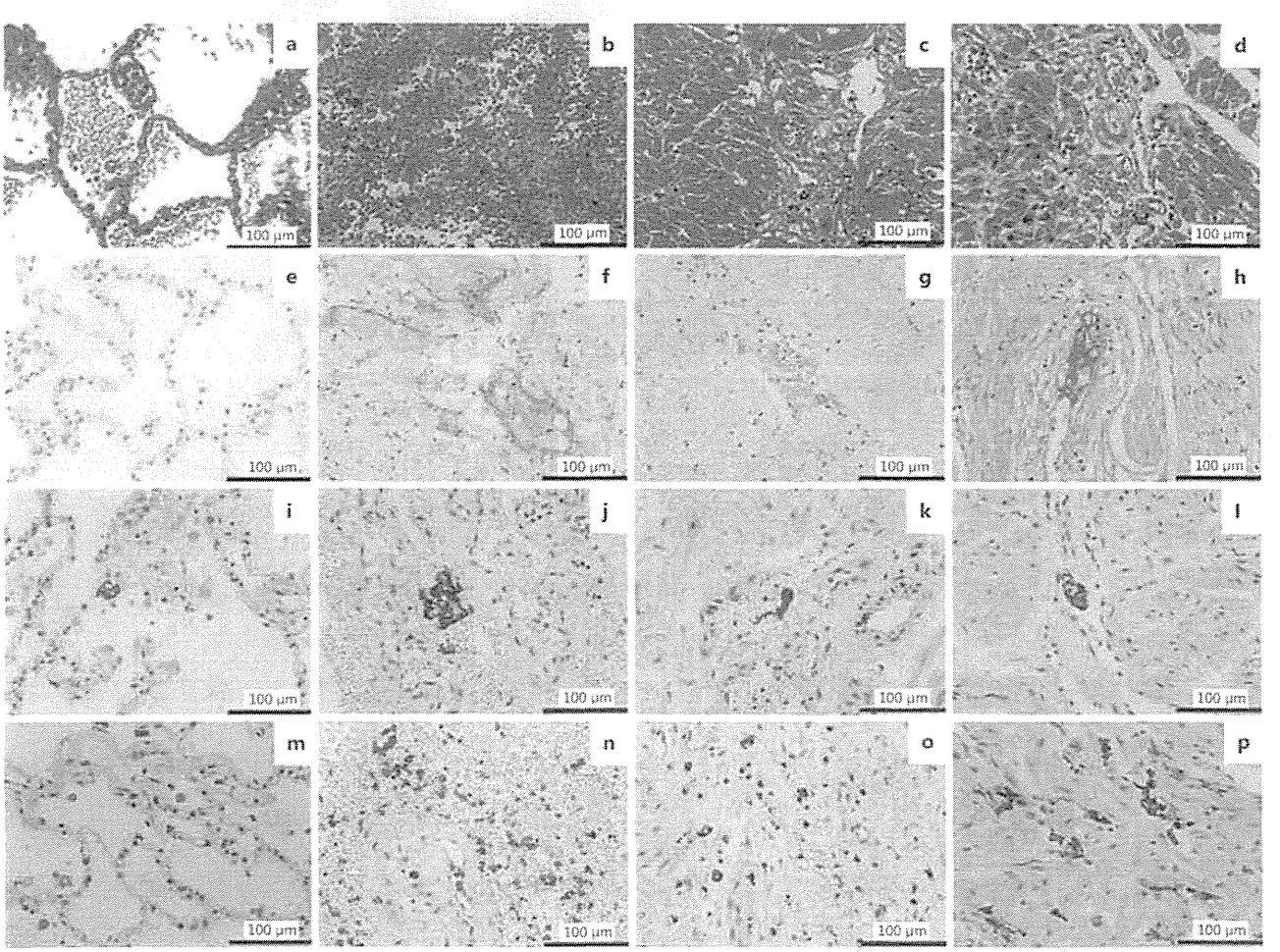

Fig. 1. Staining of lung and uterus with HE (**a–d**), Alcian blue (**e–h**), zinc coproporphyrin (**i–l**) and C5aR (**m–p**) in the lungs (**a**, **e**, **i**, **m**), cervix uteri (**b**, **f**, **j**, **n**), isthmus uteri (**c**, **g**, **k**, **o**) and corpus uteri (**d**, **h**, **l**, **p**). Scale bars indicate 100 µm.

3,065 ml. She collapsed 2 h after delivery with severe anemia (Hb 4.7 g/dl), was transferred to a university ER, and died 5 h after delivery with total hemorrhage of 5,746 ml.

Autopsy results revealed pulmonary edema and anemic organs. A dye infusion test in the inner iliac artery showed effusion from the cervical laceration and intrauterine cavity. Histological examination confirmed massive hemorrhage in the cervix (fig. 1). Immunostaining of zinc coproporphyrin derived from meconium [5] was detected in the uterine and pulmonary vessels (fig. 1). Additionally, AFE-derived mucin materials were detected only in the uterine vessels with Alcian blue staining [6].

C5aR immunolabeling was positive on the leukocytes in the pulmonary alveoli and uterine stroma (fig. 1). Tryptase is released from activated mast cells [3]. Immunostaining showed prominent tryptase halos around activated mast cells without significant regional differences between the cervix (fig. 2b), isthmus (fig. 2f) and body of the uterus (fig. 2j), but was much less prominent in the fundus (fig. 2k). The tryptase halos and reduced mast cell numbers compared with nonpregnant cases (fig. 2a, e) were related to stromal edema and myometrial swelling (fig. 1). Of the pregnant uteri harvested during hysterectomy from 2 other cases, 1 showed mild mast cell activation (fig. 2c, d), whereas the other lacked it (fig. 2g, h). Moreover, no difference was observed in the mast cell activation before (fig. 2c, g) and after (fig. 2d, h) amniotic membrane disruption. Tryptase-positive cells were also found in the pulmonary capillaries but were much fewer than in the uterus (fig. 2i).

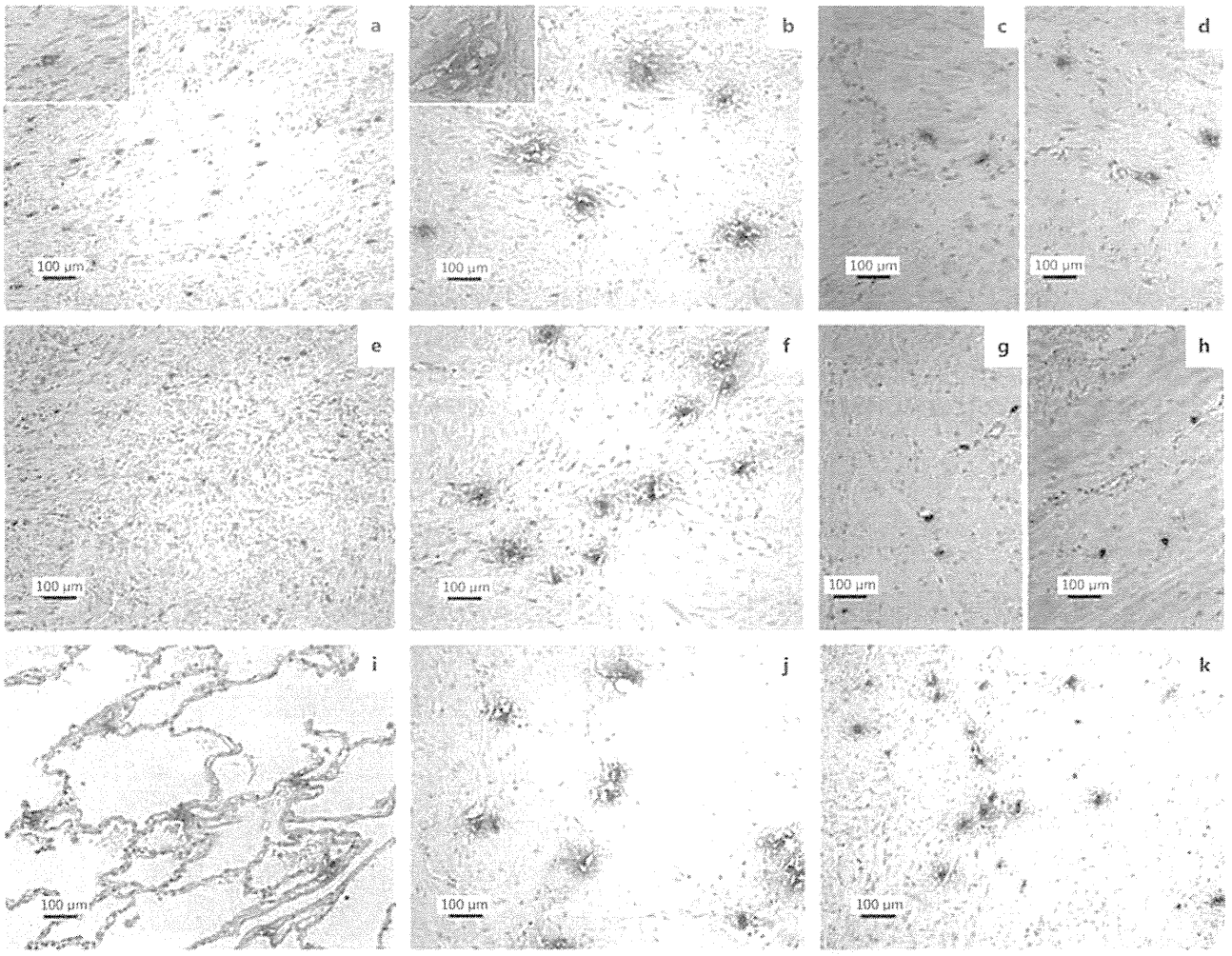

Fig. 2. Tryptase immunostaining of the uterus from nonpregnant cases (**a**, **e**), the present case (**b**, **f**, **j**, **k**) and hysterectomy cases before (**c**, **g**) and after (**d**, **h**) amniotic membrane disruption, and in the cervix (**b**), corpus (**a**, **c**–**h**) and lungs of the present case (**i**).

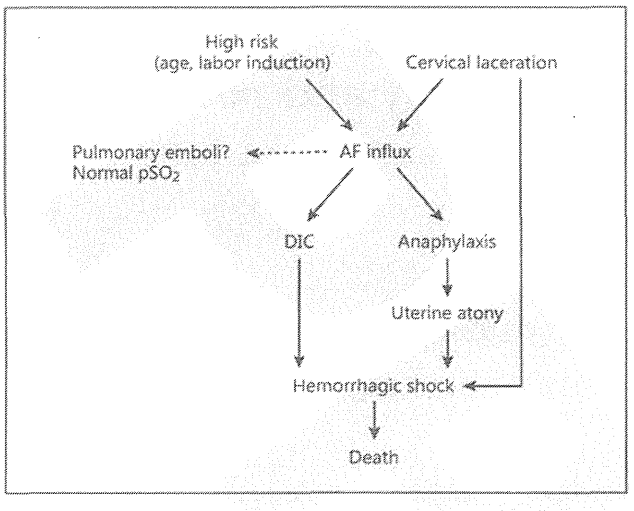

Fig. 3. Schema indicating the mechanism of pathogenesis in this case.

Discussion

Immunostaining of AFE markers and tryptase halos showed that the focus of the AFE and anaphylaxis was the uterus rather than the lungs, which is consistent with uterine atony with massive hemorrhage and absence of respiratory dysfunction. The distribution of tryptase halos in the uterus, including the cervix and sparing the fundus, supported that not only ablation of the placenta but also cervical laceration allowed entry of the amniotic fluid. Tryptase immunostaining was valuable in evaluating the focus of tissue anaphylaxis and the relation to pathological findings such as hemorrhage, myometrial swelling, stromal edema and leukocyte infiltration. In contrast, serum tryptase measurement has provided inconsistent results in AFE, and is unable to find the source (organ) of tryptase [3].

There has been only 1 case report on the causality between AFE and uterine atony [7]. We found a similar distribution of the AFE markers (Alcian blue and zinc coproporphyrin staining) to the stromal edema and myometrium swelling, which would reflect uterine atony. Enhanced C5aR immunostaining on inflammatory cells was found to be related to the edematous stroma and swollen myometrium, suggesting a relation of the complement activation and inflammation to the uterine atony. Consistent with the interpretation, in a porcine model of cardiac anaphylaxis, C5a induces contractile dysfunction, neutrophil aggregation, microvascular thrombi, and ischemia in the myocardium [8].

Figure 3 summarizes the interpretation on the relation between cervical laceration, AFE, anaphylaxis and uterine atony. From a medicolegal point of view, cervical laceration and AFE have been supposedly mutually exclusive and may lead to opposite legal responsibility of the gynecologist, but this case challenges this concept. Meanwhile, the AFE-derived tissue factor would have induced disseminated intravascular coagulation (DIC). Moreover, the massive hemorrhage due to cervical laceration, uterine atony and DIC, but not cardiopulmonary collapse, caused her death, which is consistent with the clinical course.

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Amniotic fluid embolism: Pathophysiology and new strategies for management

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Abstract

The registry program of amniotic fluid embolism (AFE) in Japan started in 2003. More than 400 hundred clinical diagnosed amniotic fluid embolism has been accumulated. Those data showed that there were two etiologies of AFE: the fetal materials create physical obstructions in the maternal microvessels in various organs, such as the lung; and (ii) the liquids cause an anaphylactoid reaction that leads to pulmonary vasospasm and activation of platelets, white blood cells and/or complements. The clinical findings showed that AFE was characterized mainly by cardiopulmonary collapse, the other involves the presence of disseminated intravascular coagulation (DIC) and atonic bleeding. Zinc coproporphyrin-1, sialyl Tn antigen (STN), complement C3, C4 and interleukin-8 have been used as serum markers of AFE. The levels of zinc coproporphyrin-1 and STN were increased in cardiopulmonary collapse type AFE, and a marked reduction of C3 and C4 was observed in DIC type AFE. At the primary medical institution, initial treatments for shock airway management, vascular management, fluid replacement, administration of anti-DIC therapy such as antithrombin, and administration of fresh frozen plasma should be provided. C1 esterase inhibitor activity in AFE cases was significantly lower than those of normal pregnant women. C1 esterase inhibitor may be a promising candidate of treatment of AFE.

Key words: amniotic fluid embolism, anaphylactoid reaction, atonic bleeding, C1 esterase inhibitor, disseminated intravascular coagulation, serum marker, rupture of the membranes.

Introduction

Amniotic fluid embolism (AFE) is one of the most serious complications of obstetrics, anesthetics and critical care. Despite earlier recognition and intensive critical care, the mortality of AFE remains high and has been estimated at between 5% and 15% of all maternal deaths.[1] Maternal mortality rates due to AFE have been estimated at between 37% and 80%.[2,3] Maternal death has been decreasing year by year in Japan, however, the incidence of maternal death due to AFE has remained unchanged. The maternal mortality rate due to AFE has increased to 24.3% in Japan.[4]

In August 2003, an AFE registry program was launched in Japan. Approximately 50 AFE cases are registered each year. The incidence of AFE seems to be five in every 100 000 deliveries, as approximately 1 million deliveries are reported each year. There is no marked difference in the incidence of AFE in Japan, Europe and the USA.

Definition of AFE in Japan

Amniotic fluid embolism was defined based on the Japan consensus criteria for the diagnosis of AFE based on the US/UK criteria (Table 1). Because the above diagnosis of AFE depended on clinical manifestations, we say that meeting these criteria is clinically diagnosed AFE (clinical AFE). If fetal debris and amniotic fluid components were found in the maternal

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Table 1 Japanese criteria of amniotic fluid embolism (AFE)

(1) If symptoms appeared during pregnancy or within 12 h of delivery.
(2) If any intensive medical intervention was conducted to treat one or more of the following symptoms/diseases:
  A) Cardiac arrest
  B) Severe bleeding of unknown origin within 2 h of delivery (≥1500 mL)
  C) Disseminated intravascular coagulation
  D) Respiratory failure
(3) If the findings or symptoms obtained cannot be explained by other diseases.

A clinical diagnosis of AFE can be made if the pathological condition meets the above three criteria. Because these diagnostic criteria serve the purpose of making a clinical diagnosis and being able to promptly provide treatment, the pathological conditions that meet them may include those other than AFE.

pulmonary arteries in addition to clinical AFE, we say that it is pathologically diagnosed AFE (AFE). As for clinical AFE, consumptive coagulopathy/disseminated intravascular coagulation (DIC) due to evident etiologies such as abnormal placentation (e.g. placental abruption), trauma during labor and delivery, and severe pre-eclampsia/eclampsia should be excluded from the criteria.

Definition of AFE in other countries

There are the variations in definitions of AFE used between countries and between data sources. The definitions of AFE in three nations are listed as below.[5] Basically, many countries diagnose by clinical symptoms.

The Netherlands

1 Reported as maternal mortality or severe maternal morbidity with AFE as diagnosis or in differential diagnosis.
2 One or more of the following severe enough to require medical treatment: hypotension (and/or cardiac arrest); respiratory distress; DIC; and coma and/or seizures.
3 Absence of any other clear medical explanation for the clinical course.

UK

In the absence of any other clear cause, either:

1 Acute maternal collapse with one or more of the following features: acute fetal compromise; cardiac arrest; cardiac rhythm problems; coagulopathy; hypotension; maternal hemorrhage; premonitory symptoms (e.g. restlessness, numbness, agitation, tingling); seizure; or shortness of breath. (Excluding: women with maternal hemorrhage as the first presenting feature, in whom there was no evidence of early coagulopathy or cardiorespiratory compromise.)

Or:

2 Women in whom the diagnosis was made at postmortem examination with the finding of fetal squames or hair in the lungs.

Australia

1 If not fatal, the hospital record had to include a diagnosis of one or more of the following: cardiac arrest; hypotension syndrome; respiratory distress; coagulation defects; coma and/or seizure; and an absence of other medical conditions or potential explanations of the symptoms and signs.
2 Where death was the outcome, AFE had to be listed as the cause of death.

Etiology

The condition that facilitates the inflow of amniotic components into maternal blood can be regarded as a risk for development of AFE. Other risk factors include amniocentesis, artificial amniotic fluid injection, multiple pregnancies, laceration during delivery, uterine scarring, induction of delivery, cesarean section and placenta previa. According to a report published in the UK in 2010, critical risk factors associated with the development of AFE were induction of delivery (odds ratio = 3.86), multiple pregnancies (10.9) and cesarean section (8.84). According to the total data reported in Japan in 2010, AFE associated with induction of delivery or cesarean section accounted for slightly more than 60% of all cases.

According to our data, onset of AFE seems to require two necessary conditions: (i) an influx of amniotic fluid into maternal circulation; and (ii) pulmonary embolus or anaphylactoid symptoms against the inflow of amniotic fluid. AFE develops after a relatively large amount of amniotic fluid containing fetal materials (e.g. meconium, squamous cells, lanugo, vernix, mucin) and liquids (e.g. protease in the meconium,

tissue factors) flows into the maternal circulatory system.[2] Amniotic fluid flows into the maternal circulatory system as follows. The amniotic components leak out of the lacerated egg membrane, flow out of the egg membrane and enter the ruptured vessels that are exposed in the lacerated uterine muscles or intrauterine cavity. The amniotic components flow into the maternal blood in accordance with the following scenarios. The fetal materials create physical obstructions in the maternal microvessels in various organs, such as the lung, while the liquids cause an anaphylactoid reaction that leads to pulmonary vasospasm and activation of platelets, white blood cells and/or complements.[6–8] AFE formed by the fetal materials as a physical embolus in the maternal pulmonary artery is shown on the left side in Figure 1. AFE resulting from an anaphylactoid reaction is shown on the right side in Figure 1. Accumulation of many inflammatory cells around the Alcian blue-positive image that is presented on the right side in Figure 1 reflects an anaphylactoid reaction caused by the amniotic fluid. The cases of AFE associated with a physical embolus are relatively few, while those of pulmonary vasospasm due to an anaphylactoid reaction are more frequently reported.

Amniotic fluid embolism also develops as a result of the flow of amniotic fluid into the uterus. The local flow of amniotic fluid into uterine tissues may cause an anaphylactoid reaction in the uterus, resulting in DIC or atonic bleeding. The severe cases of DIC or atonic bleeding which are refractory to various treatments are considered as mild AFE. The contact of amniotic fluid with allergy-associated cells, including mast cells in the cervix, results in the production of large amounts of bradykinin and inflammatory cytokines such as interleukin (IL)-8. Consequently, the uterine muscles relax and become edematous. Within the vessel, an anaphylactoid reaction promotes excessive coagulation and a fibrinolytic state. DIC progresses in this manner. From a clinical viewpoint, atonic bleeding derived from AFE accompanies sometimes incoagulable vaginal bleeding.

Pathological Conditions

One type of AFE is characterized mainly by cardiopulmonary collapse, with dyspnea, chest pain and symptoms of shock; the other involves the presence of DIC and atonic bleeding.[3,9–11] Patients with AFE with cardiopulmonary collapse (cardiopulmonary collapse type AFE) may suddenly complain of chest distress, become restless, and also develop cyanosis, dyspnea, cough and convulsive seizures. In some cases, this type occasionally starts with fetal distress before these cardiopulmonary symptoms. These patients account for 10–15% of those with AFE in our data. This type of AFE is serious because it develops into a life-threatening condition within a short time of onset. The laboratory findings on this type of AFE typically include left ventricular insufficiency associated with increased pulmonary wedge pressure, while the left ventricular work index and systemic vascular resistance decrease. During this process, pulmonary edema accompanying coarse rales rapidly progresses in the lungs. Few characteristic findings are observed in the chest X rays taken immediately after onset. Generally, edematous infiltration gradually expands from the center uniformly on both sides.

The characteristic course of AFE that starts with atonic bleeding and DIC (DIC type AFE) is as follows: incoagulable vaginal bleeding after delivery, which progresses to atonic bleeding and then severe bleeding, and then to shock. This type occasionally starts with fetal distress of an unknown origin accompanying

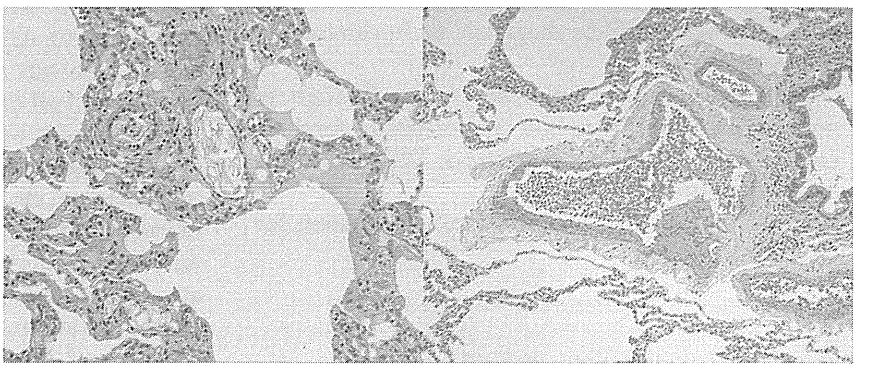

Figure 1 Left (hematoxylin–eosin), amniotic fluid embolism due to fetal debris. Right (Alcian blue staining), amniotic fluid embolism due to anaphylactoid reaction. Alcian blue-positive substance and many inflammatory cells are observed in pulmonary arteries (original magnifications: [left] × 200; [right] × 100).

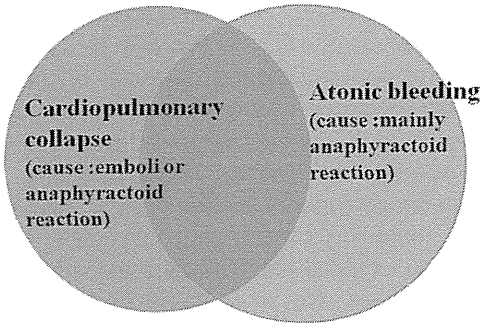

Figure 2 Pathophysiology of amniotic fluid embolism.

lower abdominal pain at the time of delivery. Following a detailed investigation of the autopsied cases, these patients were diagnosed with AFE, although generally they had been diagnosed with atonic bleeding/DIC of unknown origin in the ante mortem findings. AFE can be divided into the following two categories based on initial symptoms: (i) AFE that starts with cardiopulmonary collapse and characterized by pulmonary/respiratory symptoms; and (ii) AFE that starts with atonic bleeding/DIC. In the AFE registry program in Japan, one-third of the AFE cases reported are considered to be in the former category; the remaining two-thirds in the latter. The conceptual diagram of AFE is presented in Figure 2.

Analysis of AFE by the Japan Maternal Mortality Evaluation Committee

The causes of maternal deaths reported to the Japan Association of Obstetricians and Gynecologists have been analyzed by the Maternal Mortality Evaluation Committee (hereinafter referred to as the Evaluation Committee), organized by the members of Health and Labor Sciences Research. Maternal deaths have been registered, and almost all of them have been subjected to an analysis for cause of death since 2010. Of 75 maternal deaths analyzed from January 2010 to November 2012, 21 were caused by AFE. Of these cases, 10 involved cardiopulmonary collapse and 11 atonic bleeding/DIC. The clinical characteristics of each type were analyzed and the results are summarized below.

Clinical characteristics of cardiopulmonary collapse type AFE

The patients' mean age was 34.8 ± 4.1 years; four of them were primiparous. The initial symptoms were respiratory discomfort ($n = 4$), loss of consciousness ($n = 5$) and restlessness ($n = 1$). The time from symptom onset to cardiac arrest was extremely short (0–140 min; 37 min on average). Three patients experienced early rupture of the membranes, five induced delivery and three cesarean sections. Fetal distress of unknown origin was noticed before the appearance of cardiopulmonary collapse symptoms in four patients. Accordingly, AFE that involves cardiovascular collapse is a pathological condition that quickly becomes severe. As noted above, on average it rapidly progresses to cardiac arrest within 37 min. Because of the extremely short period from symptom onset to cardiac arrest, physicians find it difficult to provide life-saving medical treatment. Five patients experienced loss of consciousness as the initial symptom. Some patients who lost consciousness died because this symptom, which was taken for cerebral hemorrhage or eclampsia, was exacerbated during computed tomography scanning. Therefore, we should emphasize that the initial symptoms include loss of consciousness as well as respiratory discomfort and restlessness. Fetal dysfunction was noticed before the development of cardiopulmonary collapse in a relatively large number of cases. When we encounter a case of fetal dysfunction of unknown origin, we should consider AFE in the process of making a differential diagnosis list.

Clinical characteristics of DIC type AFE

In every patient, the initial symptom was incoagulable vaginal genital bleeding after placental delivery (or during cesarean section). Uterine atony developed nearly simultaneously. The time from symptom onset to cardiac arrest was 102 min, on average. The typical laboratory finding was rapid and marked decrease in fibrinogen levels (to <100 mg/dL within 2 h after initial symptom onset in all six patients in whom it was measured: 81, 47, <50, <30, <50 and <50 mg/dL, respectively). Five of the 11 patients did not undergo an examination of the coagulation system, including the measurement of fibrinogen. In a conference to investigate cause of death, delay in transfusion therapy (particularly when using fresh frozen plasma [FFP]) was pointed out in nine of 11 patients. Five patients suffered DIC/atonic bleeding accompanied by pulmonary edema.

Labor-inducing/promoting drugs were used in six of 11 patients and Caesarean section was performed in two of them.

In patients with AFE involving atonic bleeding/DIC, the mean time from initial symptom onset to cardiac

arrest was 107 min, slightly longer than that in the patients with AFE involving cardiopulmonary collapse. Premature ablation of a normally implanted placenta is said to progress to serious DIC within 5–6 h. Compared with this case, DIC associated with AFE progresses rather quickly. Unless any appropriate action is taken to control DIC within 2 h after incoagulable vaginal bleeding, a fatal outcome can be expected. Immediate control of DIC is indispensable in ensuring patients' survival. In the typical laboratory findings, fibrinogen levels decreased in the early stages. In all six patients whose fibrinogen levels were measured, the levels declined to less than 100 mg/dL within 2 h of bleeding. If incoagulable bleeding is noticed after delivery, blood cell count and measurement of fibrinogen should be performed as the first step of treatment. In nine of 11 patients, delayed transfusion therapy (particularly using FFP) was pointed out. Early transfusion with higher doses of FFP is indispensable for controlling DIC associated with AFE. To be able to administer FFP without delay, we should measure fibrinogen levels and make a diagnosis of DIC as soon as possible.

Autopsy Findings on Patients with AFE

Of 21 patients with AFE, five with AFE involving cardiopulmonary collapse (cardiopulmonary collapse type AFE) and four with DIC were subjected to autopsies. The pathological findings were evaluated and compared. The findings from the lungs and uteruses of patients with AFE involving cardiopulmonary collapse are listed in Table 2. In all of the patients with AFE of this type, amniotic and fetal materials were detected in the pulmonary vessels. The conventional or typical pathological image of AFE was found in these patients.

The clinical and pathological findings of the lungs and uteruses obtained from patients with the type of AFE with DIC (DIC type AFE) preceding are listed in Table 3. Amniotic components/fetal materials were detected in the pulmonary vessels in all of the patients with AFE involving cardiopulmonary collapse. The conventional or typical pathological image of AFE was observed in these. No remarkable findings in the uterus were obtained from four of the five patients with AFE involving cardiopulmonary collapse. Uterine atony and inflammatory cell infiltration were clearly recognized in the one remaining patient. In cardiopulmonary collapse AFE, the amniotic components/fetal materials probably formed emboli primarily in the pulmonary artery, which resulted in shock or loss of consciousness. A relatively large volume of amniotic fluid is likely to flow into the maternal circulatory system with this type of AFE.

Uterine atony (a large, edematous uterus) was macroscopically observed and amniotic components microscopically observed in the uterine vessels in all of the patients with DIC type AFE. Uterine atony reflects a marked edematous uterus. Histologically, in some cases, edema existed as if it divided the uterine smooth muscle cells. Amniotic components were detected in the lungs in only one patient with DIC type AFE; however, pulmonary edema was detected in 50% of these patients. Compared to the presence of amniotic components, pulmonary edema seemed to be a more suitable pulmonary finding that characterizes DIC type AFE. In a conference to investigate cause of death, a

Table 2 Autopsy findings from patients with cardiopulmonary collapse type amniotic fluid embolism

	Lungs	Uterus	Time from symptom onset to cardiac arrest
Case A	Many amniotic materials were detected in the branches of the pulmonary artery and capillaries throughout the lungs.	No remarkable findings.	76 min
Case B	Dilation of pulmonary capillaries, congestion and fetus-derived cells were recognized.	No remarkable findings.	35 min
Case C	Fetus-derived keratin was observed in the pulmonary microvessels.	No remarkable findings.	13 min
Case D	Alcian blue-positive parenchymas were detected in the pulmonary artery and microvessels.	No remarkable findings.	3 min
Case E	Alcian blue-positive, cytokeratin-positive and sialyl Tn antigen-positive images were observed in the pulmonary vessels. Marked inflammatory cell infiltration was recognized.	Uterine atony and inflammatory cell infiltration was definitely observed.	60 min

Table 3 Autopsy findings of DIC type amniotic fluid embolism (AFE) patients

Clinical symptoms	Lungs	Uterus	Time from symptom onset to cardiac arrest
Case A Initial event of disseminated intravascular coagulation (DIC); no respiratory symptoms other than coughing until death.	Pulmonary edema	Uterine atony, many Alcian blue-positive images in the uterine veins	Approximately 60 min
Case B Initial event of DIC; no respiratory symptoms until death.	No pulmonary finding	Uterine atony, many Alcian blue-positive images in the uterine veins	Approximately 60 min
Case C Initial event of DIC; subsequent events (chest pain, respiratory failure).	Keratin-positive images in the pulmonary vessels	Uterine atony, keratin-positive images in the uterine veins	115 min
Case D DIC during cesarean section due to placenta previa.	Pulmonary edema, no amniotic materials in the lungs.	Uterine atony, Alcian blue-positive and keratin-positive cells in the vessels from the cervix to the isthmus of the uterus, small round cell infiltration under the vascular endothelium	63 min

pathologist described a pulmonary finding for a case of DIC type AFE as 'pulmonary edema that looked like a damp rag wrung out'. The findings that characterize DIC type AFE are edematous lesions mainly in the uterus and lungs. The amniotic fluid flows into the maternal circulatory system and comes in contact with certain organs, including the uterus and lungs. Edematous changes are more frequently observed in these organs. Even in patients with DIC type AFE for whom autopsies were not performed, atonic bleeding was observed nearly consistently. The characteristic of DIC type AFE is edematous changes mainly in the uterus or lungs resulting from the inflow of amniotic fluid. The possibility exists that the mechanism of the sudden occurrence of edematous changes may be closely related to the etiology of DIC type AFE.

Treating Clinical AFE with no Evidence of Amniotic Components in the Lungs

Deceased patients can be definitively diagnosed with AFE if the presence of amniotic components/fetal materials in the lung tissue is confirmed during autopsy. Alcian blue staining and the measurement of zinc coproporphyrin-1 (Zn-CP1) levels are useful in detecting acidic mucin in amniotic fluid.[12] Moreover, fetal skin-derived keratin staining and staining of TKH-2, glycoprotein derived from mucin contained in the meconium/amniotic fluid (sialyl-Tn [STN] staining) are also useful.[13] Villus cell-derived cells are occasionally detected in maternal lungs during a normal pregnancy. Amniotic components, however, are rarely detected or stained in maternal lungs during the normal course of pregnancy. Therefore, Alcian blue-positive or Zn-CP1-positive result in the lungs is an important finding suggestive of AFE.[14]

As mentioned before, neither amniotic components nor fetal materials were detected in the lungs of some patients with DIC type AFE, among clinical cases of AFE. In the UK, detection of amniotic components/fetal materials in the pulmonary vessels is required for making a pathological diagnosis of AFE. According to UK diagnostic criteria, a pathological condition that meets the criteria for clinical AFE but has no evidence of amniotic components in the lungs is not regarded as AFE. A pathological condition with no evidence of amniotic components/fetal materials in the lungs (no amniotic components/fetal materials detected in the excised section) but meets the criteria for clinical AFE (uterine atony and amniotic components in the uterine

vessels) should be differentiated from conventional types of AFE. We propose that this form of AFE be called amniotic fluid embolism of uterine type (uterine type AFE) (Fig. 3). Onset of uterine type AFE resembles uterine atony, however, uterine type of AFE rapidly advances DIC and shock, and its histological findings mentioned above are different from those of conventional uterine atony. A patient may undergo a total hysterectomy because of clinical AFE. We propose this case should be called uterine type AFE if the above uterine findings are obtained. Based on the results of an investigation by the Evaluation Committee, many cases of uterine type AFE could be clinically judged to be DIC type AFE. If a patient undergoes a hysterectomy, a diagnosis of uterine AFE can be made according to the pathological findings of the uterus and clinical evidence.

Disseminated intravascular coagulation type AFE is characterized by uterine atony that can be macroscopically observed in the pathological examination, with a large, edematous uterus. Histologically, Alcian blue-positive amniotic components were observed in the uterine vessels in all of the patients with DIC type AFE. Amniotic components were detected in the pulmonary vessels in one of four patients. Pulmonary edema was recognized in two of four patients.

Serum Markers

Serological methods are available for making an auxiliary diagnosis of AFE. In Japan, we use Zn-CP1 and STN to detect the substances specific to amniotic fluid in maternal blood.[12,15] Currently, Legrand *et al.* recommend other various useful amniotic markers, such as insulin-like growth factor binding protein 1.[16]

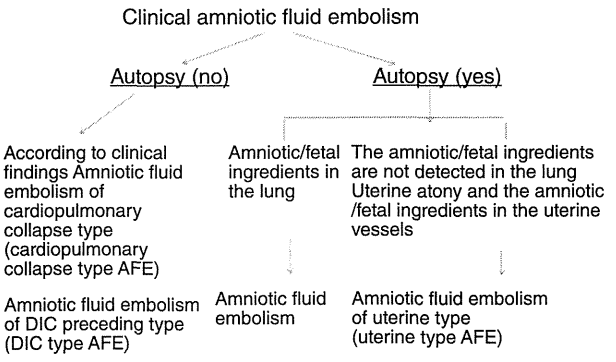

Figure 3 Classification of amniotic fluid embolism (AFE) in Japan. AFE, disseminated intravascular coagulation.

We simultaneously measure serum levels of complement C3 (C3) and complement C4 (C4) to examine whether an anaphylatoxin-like reaction is involved, and IL-8 to check for increased cytokine (Table 4). Exposure of the blood sample to light results in degeneration of Zn-CP1. Therefore, serum should be obtained from the blood sample collected and the serum sample should be covered with aluminum foil for light shielding. Although these marker levels frequently increase in cardiopulmonary collapse type AFE, these are not sensitive markers for DIC type AFE. On the other hand, C3 and C4 levels generally become extremely low regardless of AFE type (Fig. 4). Our data suggest that the excessive activation of complement system may be crucially involved in pathophysiology of AFE. The measurement of C3 and C4 is important in assessment of severity of AFE. Benson *et al.* reported that serum levels of C3 and C4 complement had a sensitivity between 88% and 100%, and a specificity of 100% for the diagnosis of AFE.[17] We propose that if AFE is suspicious from clinical symptoms and amniotic markers, a marked decrease of C3 and C4 would strongly confirm AFE. Benson described that the presence or the absence of fetal material in the maternal circulation of living women cannot either confirm or refute the diagnosis of AFE and that it is unclear why there should be a difference in the sensitivity and specificity of intravascular fetal material between the living and the dead. He commented that previous serum markers do not seem entirely satisfactory on

Table 4 Serum markers for an auxiliary diagnosis of amniotic fluid embolism

(1) Zinc-coproporphyrin-1 (Zn-CP1). Normal value, <1.6 pmol/mL.
High-performance liquid chromatography is used to measure Zn-CP1 contained in the meconium. This substance emits fluorescence at wavelengths of 580 nm and 630 nm with excitation light having a wavelength of 405 nm.
(2) Sialyl-Tn. Normal value: <46 IU/mL.
This sugar chain of the mucin-type glycoprotein recognizes mucin in the meconium.
(3) Complement C3 and C4. Normal values: 80–140 and 11–34 mg/dL, respectively.
These enzymes that complement antigen–antibody reaction are activated by inflammation or allergy.
(4) Interleukin-8. Normal value: <20 pg/mL.
This inflammatory cytokine increases in the event of disseminated intravascular coagulation, systemic inflammatory response syndrome or acute respiratory distress syndrome.

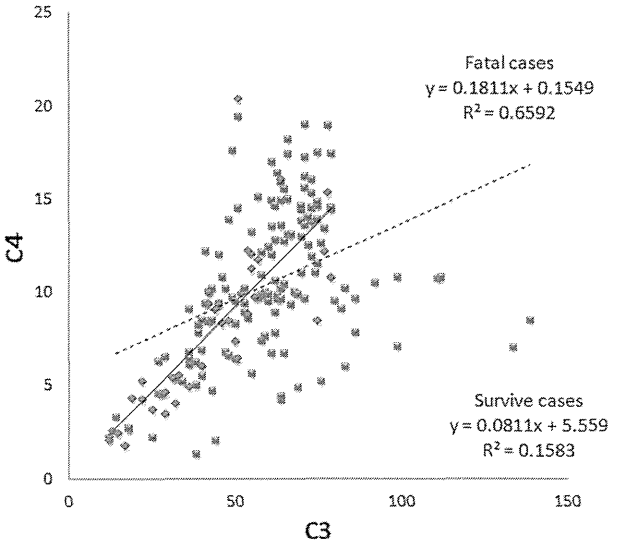

Figure 4 Levels of C3 and C4 in clinical amniotic fluid embolism. ■, survived; ◆, died.

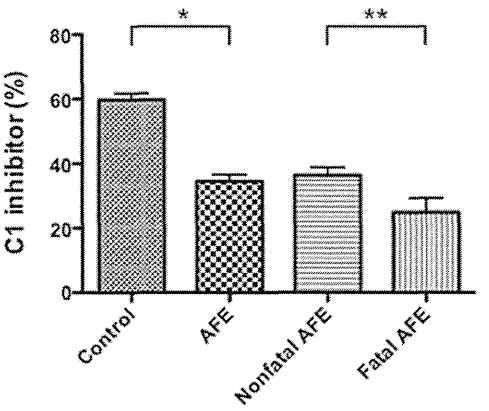

Figure 5 C1 inhibitor level and amniotic fluid embolism (AFE). Non-fatal AFE, surviving AFE patients; fatal AFE, deaths due to AFE. $^{**}P < .001$, $^{*}P < 0.05$.

further examination because the amount of fetal material in fatal AFE cases was not necessarily massive and indeed present only in microscopic amounts.[18]

New Marker: C1 Esterase Inhibitor

C1 esterase inhibitor (C1INH), which is mainly synthesized in hepatocytes and endothelial cells and belongs to the serpin family, is a major inhibitor not only of C1 esterase, but also of FXIIa and kallikrein.[19–21] Its deficiency is know to be a specific cause of hereditary angioedema (HAE).[22] Because C1INH is capable of not only inhibiting the complement system, but also modulating the coagulo-fibrinolytic and kallikrein-kinin systems, we hypothesized that C1INH was key in the pathophysiology of AFE.[23] We discovered that C1 esterase inhibitor (C1 inhibitor) decreased in patients with AFE.[24] We found that many deceased patients had C1 inhibitor levels far below 25% (Fig. 5). C1 inhibitor inhibits the complement system and has a direct effect on the kinin and fibrinolytic systems. We found that a decrease in C1 inhibitor resulted in the development of various pathological conditions associated with AFE, including uterine atony (uterine edema), DIC and an anaphylactoid reaction. Although early FFP transfusion therapy has been known to be effective for treating AFE, C1 inhibitor contained in FFP seems to contribute to improvement of the pathological condition. Because a C1 inhibitor (Berinert P, CSL Behring, Marburg, Germany) is included as a drug for hereditary angioedema on the National Health Insurance (NHI) list, its application for AFE can be expected.

In addition, the chronological assessment of C1INH activity levels in two AFE patients indicated that their basal C1INH activity levels before onsets were also lower than those of healthy pregnant controls during labor. Thus, we proposed that low C1INH activity levels before onset of AFE could be a predictive factor, as well as low levels at onset and the persisting low levels of C1INH activity could be prognostic factors of AFE.

Treatment

At the primary medical institution, initial treatments for shock (airway management, vascular management, fluid replacement, administration of an antishock agent) and DIC (administration of antithrombin, administration of FFP if possible) should be provided. The patient should then be transferred to an advanced medical institution. At the secondary medical institution, the patient should be treated in the intensive care unit as soon as possible. The treatment protocol should be in accordance with guidelines for managing critical obstetric bleeding. In the case of hypotension, epinephrine, dopamine hydrochloride or dobutamine hydrochloride (1–5 μg/kg per min) should be administrated to maintain blood pressure and urine volume. High-dose adrenocorticosteroid hormone (500–1500 mg in the form of hydrocortisone) is occasionally effective.

Antithrombin (3000 units) should be administrated as soon as possible because severe DIC frequently develops in the early stages. FFP (≥10–15 units) should be administrated. FFP is preferable to red blood cell products. Red blood cell products should be administrated while monitoring bleeding volume. The target ratio between FFP and red cell concentrate should be adjusted to a level exceeding 1.5. In instances of DIC associated with AFE or the premature ablation of a normally implanted placenta, coagulation factor replacement therapy is first priority because the activation of coagulation factors enables the production of intravascular thrombi.

Recently, we reported that C1INH activity levels in AFE cases were significantly lower than those of normal pregnant women. Furthermore, when we compared fatal cases to non-fatal cases of AFE, the C1INH activity of fatal cases was significantly lower than that of non-fatal cases.[24] Many published works have reported that the rapid administration of FFP or cryoprecipitates was sufficient to extricate the patient from a critical situation. According to our study, the meaning of administration of FFP does not only supply coagulating factors but also C1INH. One hundred units of C1INH are contained in FFP derived from 200 mL blood. Clinically, the use of 500–1500 units of human plasma-derived C1INH concentrates can revert HAE in C1INH-deficient patients. Because AFE patients certainly have significant lower levels of C1INH activity, similar to a C1INH deficiency, the clinical application of human plasma-derived C1INH concentrates may become one of the promising candidates for the treatment of AFE.

Platelet transfusion should be considered while monitoring DIC. If the platelet count exceeds 20 000/μL, platelet transfusion in not needed immediately. If the above treatments are ineffective for improving DIC, the use of recombinant factor VII, which is not included on the NHI list but whose efficacy has been demonstrated in Japan and overseas, can be considered. Conventionally, heparin has been recommended for treating patients suspected of having AFE with DIC. Generally, DIC associated with AFE rapidly progresses and causes severe bleeding, and thus the use of heparin is not recommended.

Actions to be Taken in the Event of a Fatal Outcome

An autopsy should be performed on all deceased patients. Their families may decline to accept this proposal; however, the importance of the investigation into the cause of death should be explained and sufficient efforts should be made to receive their consent. Notification of maternal death is submitted to the Japan Association of Obstetricians and Gynecologists and Prefectural Associations of Obstetricians and Gynecologists. An additional form with the specific details of the case is submitted to the Japan Association of Obstetricians and Gynecologists. The treating physician reports the case to the head of the medical institution and takes the appropriate actions in accordance with the institution's investigation protocol.

Prevention

Risk factors found in several studies to be significantly associated with an increased risk of AFE included maternal age of 35 years or older, cesarean delivery, forceps/vacuum delivery, placenta previa, abruption placenta, eclampsia and fetal distress.[5] It is assumed that the rupture of the membranes followed by the inflow of amniotic fluid into maternal circulation occurs easily in cases of cesarean delivery, forceps/vacuum delivery, placenta previa, abruption placenta and eclampsia because these conditions are ascribed to injury of the birth canal or injury of trophoblasts. The amniotic membrane and chorion membrane form an important barrier that prevents amniotic fluid from flowing into the maternal circulatory system. The egg membrane, especially the amniotic membrane, restricts contact of the amniotic fluid with maternal allergy-associated cells. In the event of rupture of the egg membrane, an anaphylactoid reaction is likely to occur in the mother's body, and routine delivery management should be employed. Unless the membrane is ruptured, the maternal allergy-associated cells, including mast cells, eosinophils and basophils, are not exposed to a large amount of amniotic fluid. If the membrane is ruptured and amniotic fluid flows into the vagina, the possibility of contact with maternal allergy-inducing cells decreases because, as with the skin, the vagina is lined by thick, stratified, squamous epithelium and rarely comes into contact with maternal immune cells. If the membrane is ruptured, and amniotic fluid comes into contact with the cervical tissues or uterine muscles, either major or minor allergic-like reactions are induced in some parts of the uterus. In the event of rupture of the membrane, the contact between the amniotic fluid and the mother's body should be prevented as much as possible. Timely rupture of the membrane, which is defined as 'complete dilation of

the uterine cervix followed by rupture of the membrane', is regarded as normal. This conventional process ensures safe delivery management. Artificial rupture of the membrane at the higher station of the presenting part of the fetus or rupture of the membrane without effacement of the cervix enables contact with the endocervical columnar epithelium or stroma (in the presence of laceration) and increases the possibility of allergic reaction. Therefore, obstetricians should recognize the increased risk of inflow of amniotic fluid into maternal blood in the event of an injury, vacuum delivery and forceps delivery. The pathological conditions that increase the risk for development of AFE include pregnancy accompanying allergy-related condition, pregnancy-induced hypertension syndrome, low-lying placenta and placenta previa. Treating obstetricians should carefully follow-up with pregnant women with these risks and direct special attention to the progress in the event of rupture of the membrane.

Important Considerations for Preventing AFE

Based on those risk factors of AFE we manage the patients during labor to prevent AFE as below:

1 Measuring the volume of bleeding is not reliable. Always consider the possibilities of internal bleeding and blood leakage toward the back, which makes it difficult to measure volume accurately. Measuring the volume of bleeding during delivery also is occasionally inaccurate.
2 Direct special attention to the pulse and shock index. Carefully consider pulse rate. Make it a rule to calculate the shock index as a matter of routine practice. A timely diagnosis of shock cannot be made if attention is directed only to blood pressure.
3 Restlessness, respiratory discomfort, severe lower abdominal pain and fetal dysfunction of unknown origin appear before AFE manifests.
4 Check for atonic bleeding and incoagulable vaginal bleeding of unknown origin. In the obstetric field, patients suffer severe bleeding characteristic of DIC after uterine atony or incoagulable vaginal bleeding resulting from consumption of coagulation factors.
5 Pay particular attention to a rupture of the membrane that can result in AFE. Carefully observe the mother and her baby for some time after the rupture of the membrane.
6 Do not rupture the membrane using a non-physiological or artificial technique. As mentioned before, contact of the amniotic fluid with the lumen of the cervix entails the risk for AFE. Therefore, rupture of the membrane at stations higher than station 1 and artificial rupture in a state of insufficient dilation of the uterine cervix (<5 cm) should be avoided.
7 In the event of early rupture of the membrane, induced delivery should be carefully monitored. Because amniotic fluid easily flows into maternal blood, this type of delivery should be managed as a high-risk delivery.

In conclusion, there are two etiologies of AFE: (i) the fetal materials create physical obstructions in the maternal microvessels such as lung; and (ii) an anaphylactoid reaction that leads to pulmonary vasospasm and activation of platelets, white blood cells and/or complements. The clinical findings showed two types of AFE: (i) cardiopulmonary collapse type AFE; and (ii) DIC type AFE. Zinc coproporphyrin-1, STN, C3 and C4 have been used as serum markers in Japan. In addition to them, C1INH could be a sensitive marker to reflect the pathophysiology of AFE. C1INH may be a new treatment for AFE. Regarding prevention of AFE, obstetricians must take care of the appropriate time of the rupture of membranes and reduce lacerations of the birth canal.

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Disclosure

The authors declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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C1 Esterase Inhibitor Activity in Amniotic Fluid Embolism*

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Objectives: Amniotic fluid embolism exhibits activation of the complement system and the kallikrein-kinin and coagulofibrinolytic systems. C1 esterase inhibitor is a major inhibitor of C1 esterase and can inhibit plasma kallikrein and also factors XIIa and XIa. Its activity has been shown to be significantly lower in pregnancy and labor than in the nonpregnant state. The purpose of this study was to determine C1 esterase inhibitor activity levels in amniotic fluid embolism.

Design: Retrospective study.

Setting: A single university-based center.

Patients: One hundred six cases with amniotic fluid embolism in a total of 194 singleton pregnant women between January 2010 and December 2011.

Interventions: None.

Measurements and Main Results: One hundred six cases of amniotic fluid embolism had applied to the Japan amniotic fluid embolism registration center in Hamamatsu University School of Medicine between January 2010 and December 2011. In amniotic fluid embolism cases, 85 cases were nonfatal and 21 cases were fatal. Eighty-eight women who delivered without amniotic fluid embolism were regarded as a control. C1 esterase inhibitor activity levels were significantly lower in amniotic fluid embolism patients (30.0% ± 1.8%) than in control women (62.0% ± 2.0%) ($p < 0.0001$). C1 esterase inhibitor activity levels in fatal amniotic fluid embolism cases (22.5% ± 3.4%) were significantly lower than those in nonfatal amniotic fluid embolism cases (32.0% ± 2.1%) ($p < 0.05$).

Conclusions: These results demonstrated that low C1 esterase inhibitor activity levels were closely associated with the pathogenesis of amniotic fluid embolism suggesting that C1 esterase inhibitor activity levels have potential as a prognosis factor of amniotic fluid embolism. (*Crit Care Med* 2014; 42:1392–1396)

Key Words: amniotic fluid embolism; C1 esterase inhibitor; disseminated intravascular coagulopathy; kallikrein; postpartum hemorrhage; serpin

*See also p. 1548.

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Amniotic fluid embolism (AFE) is one of the most serious complications of obstetrics, anesthetics, and critical care. Despite earlier recognition and intensive critical care, the mortality of AFE remains high and has been estimated at between 5% and 15% of all maternal deaths (1). Maternal mortality rates due to AFE have been estimated at between 37% and 80% (2, 3). Maternal death has been decreasing year by year in Japan; however, the prevalence of maternal death due to AFE has remained unchanged. The maternal mortality rate due to AFE has increased to 24.3% in Japan (4).

AFE is recognized as a kind of syndrome characterized by the abrupt onset of hypoxia, hypotension, and disseminated intravascular coagulopathy (DIC) (5). Benson et al (6) reported that maternal complement levels were significantly decreased in AFE. These findings suggested a disorder in the coagulofibrinolytic system as well as the complement system that may play important roles in the pathogenesis of AFE.

We developed the Japan AFE registration system in 2003 and collected clinical data, maternal serum, and uterine tissue from nearly all cases of fatal AFE in Japan (4, 7). Under the system, maternal serum has been applied to determine mainly the levels of specific amniotic fluid complements such as Sialyl Tn and zinc coproporphyrin 1 (8, 9). These clinical and histopathological observations demonstrated that AFE was frequently associated with uterine atony due to angioedema (N. Tamura and N. Kanayama, unpublished data, 2013).

C1 esterase inhibitor (C1INH), belonging to the serpin group/family, is a major inhibitor not only of C1 esterase but also of kallikrein and factors XIIa and XIa (10–12). Its

deficiency has been known to be a direct cause of hereditary angioedema (HAE) as well as acquired angioedema (13). Since C1INH has the potential to regulate the coagulofibrinolytic system, complement system, and kallikrein-kinin system, we have become greatly interested in C1INH activity levels in AFE in Japan.

MATERIALS AND METHODS

Definition of AFE

AFE was defined based on the Japan consensus criteria for the diagnosis of AFE based on the United States/United Kingdom criteria as shown in **Figure 1** (7, 14). A pathological diagnosis was determined when fetal debris was found in the maternal pulmonary arteries. The diagnosis of nonfatal AFE depended on clinical manifestations and was done when factors B1–B3 were all present, but more than one of the signs and symptoms listed in B1 needed to be present. Consumptive coagulopathy/DIC due to evident etiologies such as abnormal placentation (placental abruption, etc.), trauma during labor and delivery, and severe preeclampsia/eclampsia should be excluded from the criteria.

Patients

The Japan AFE registration system was started in 2003 (7). This system has included the procedure of consent to apply and analyze their clinical data and blood samples. Consent was obtained from patients or patient's family, when physicians regarded their patients with significant symptoms as AFE based on the diagnostic criteria of AFE. Clinical data and serum from nearly all cases of AFE have accumulated in Hamamatsu University School of Medicine. Subjects of the present study were extracted from entry cases in the Japan AFE registration center in Hamamatsu University School of Medicine, Shizuoka, between January 2010 and December 2011. Women with multiple pregnancies, preeclampsia, thrombophilia, preterm labor, uterine disorder such as uterine myoma, and a history of systemic disease were excluded from this study. Cesarean section was carried out due to obstetrical indications, such as breech presentation, history of cesarean section, arrest of labor, and nonreassuring fetal status. Women, who delivered at Hamamatsu University Hospital between April 2011 and September 2011, without AFE and any medical intervention other than general birth and surgical assistances were analyzed as the control subjects. One hundred six cases of AFE and 88 cases of control were defined (**Table 1**). Among the AFE cases, 85 cases survived and 21 cases died due to AFE.

The Japan consensus criteria for the diagnosis of AFE

A. Pathological confirmation: A diagnosis is made on the basis of clinical presentation after excluding differential diagnosis and at autopsy in the event of death of the parturient. The diagnosis is confirmed by histochemical studies.

B. Clinical manifestation: The patients has the hallmark clinical manifestations of AFE following 1, 2, and 3:

1. Signs and symptoms: Cardiac arrest/ Respiratory arrest/ Consumptive coagulopathy
2. Onset of all of the signs and symptoms during pregnancy, labor, or cesarean section or within 12 hours of delivery
3. Absence of other illness that could explain the signs and symptoms described above

Figure 1. The Japan consensus criteria for the diagnosis of amniotic fluid embolism (AFE). A pathological diagnosis was determined when fetal debris was found in the maternal pulmonary arteries. The diagnosis of nonfatal AFE depended on clinical manifestations and was done when factors B1–B3 were all present, but more than one of the signs and symptoms listed in B1 needed to be present.

Blood Collection and Measurement of C1INH Activity

Blood samples from registered AFE patients were collected at the Japan AFE registration center in Hamamatsu, and serum and plasma samples were then kept at –30°C until use. Time points of blood samples obtained were at onset of and before interventions against AFE. Control blood samples were obtained at the completion of labor. The determination of C1INH activity was performed using the Berichrom C1 inhibitor kit (Siemens Healthcare Diagnostics, Deerfield, IL) according to the manufacturer's instructions. The intra-assay coefficients of variation (CV) ranged between 1.8% and 7.9% and the interassay CV were between 3.2% and 6.6%. We analyzed all the samples at the same time under a blind fashion. In the present study, we demonstrated the measurement of C1INH activity in serum. Furthermore, C1INH activity was measurable in serum as well as plasma; there were no significant differences ($p < 0.0001$, $R^2 = 0.9881$) in the activity level between serum and plasma under the Berichrom C1 inhibitor kit (data not shown).

Approval

Written informed consent was obtained after full explanation of the study. The study was carried out under the approval of the Ethics Committee of Hamamatsu University School of Medicine (Number 24–130 and 25–107), which conforms to the provisions of the Declaration of Helsinki (as revised in Tokyo 2004).

Data Analysis

Values of C1INH activity (%) were presented as the median ± SE. Significant differences were assessed with the Mann-Whitney *U* test. A *p* value of less than 0.05 was considered significant.

RESULTS

As shown in **Figure 2**, C1INH activity levels in the controls and AFE cases were 62.0% ± 2.0% and 30.0% ± 1.8%, respectively. C1INH activity levels in the AFE cases were significantly lower than those in the controls ($p < 0.0001$). C1INH activity levels in fatal and nonfatal AFE cases were 22.5% ± 3.4% and 32.0% ± 2.1%, respectively. A significant difference was observed between the two groups ($p = 0.0121$).

Changes in C1INH activity levels in one survivor case and one case that died due to AFE are shown in **Figure 3**. Both cases were defined as AFE by the Japan consensus criteria for the diagnosis of AFE shown in Figure 1. C1INH activity levels were potentially very low before the onset of AFE. C1INH activity in the survivor case was at its lowest level at the onset 3 hours after the selective cesarean section due to history of cesarean section when AFE was defined due to the development of DIC. Immediate replacement therapy with fresh frozen plasma successfully increased the activity of C1INH. In the case that died,

TABLE 1. Clinical Characteristics of Patients in This Study

Patient Characteristics	Control	Total AFE	Nonfatal AFE	Fatal AFE
No. of subjects	88	106	85	21
Age (yr)	31.0±4.8	33.8±5.8	33.3±5.4	35.6±3.8
Gravida[a]	1.27±1.02	1.64±1.77	1.74±1.82	1.23±1.47
Parity[a]	0.72±0.63	0.83±1.06	0.89±1.12	0.57±0.72
Nulliparous (%)	28 (31.8)	52 (49.0)	41 (48.3)	11 (52.4)
Multiparous (%)	60 (68.2)	54 (51.0)	44 (51.7)	10 (47.6)
Gestational period (d)	273±12	268±19	267±20	270±17
Delivery methods (%)				
Vaginal delivery	60 (68.2)	52 (49.0)	44 (51.7)	10 (47.6)
Cesarean section	28 (31.8)	54 (51.0)	41 (48.3)	11 (52.4)
Blood loss at delivery (mL)				
Vaginal delivery	395±170	4,864±3,039	5,038±3,111	4,097±2,569
Cesarean section	840±279	4,270±2,988	4,314±2,657	4,107±3,961

AFE = amniotic fluid embolism.
[a]Woman without previous history of pregnancy and delivery was determined as gravida 0 and parity 0, respectively.

C1INH activity was also low before the manifestation of AFE symptoms. In this case, amniotic fluid, fetal substance, and gram-positive coccus were observed and autopsy diagnosis was AFE and bacteremia.

DISCUSSION

AFE is an unpredictable and serious disorder of pregnancy characterized by hypotension, hypoxia, and coagulopathy (5). In most pregnant women, the entry of small amounts of amniotic fluid into the maternal circulation may be innocuous; however, such exposure is associated with a fatal outcome in other women. Anaphylactic reactions have been suggested as a concept of AFE to explain such an individual difference in the response to amniotic fluid (2, 15). Benson (6, 16) reported that serum tryptase and urinary histamine increased and complement levels decreased in AFE patients, suggesting that contact and maternal immune activation played important roles in the pathophysiology of AFE.

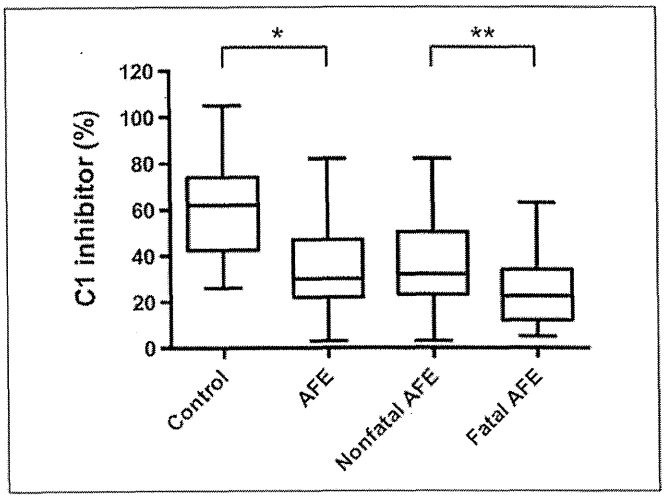

Figure 2. C1 esterase inhibitor (C1INH) activity levels in control, amniotic fluid embolism (AFE), nonfatal AFE, and fatal AFE cases. Columns indicate the medians and whiskers represent the minimum and maximum values. Significant differences were $^{*}p < 0.0001$ and $^{**}P = 0.0121$, respectively.

Clinically, DIC-type postpartum hemorrhage accompanying uterine atony is one of the recognized symptoms of AFE (4, 17). To explain this, coagulation factor XII (FXII) may be responsible for the pathological condition as it is activated by contact with various artificial or biological negatively charged surfaces, resulting not only in blood coagulation but also in the activation of the complement system and kallikrein-kinin system to produce bradykinin (18). We demonstrated that FXII inactivated plasminogen activator inhibitor 1 and enhanced fibrinolysis (19). Interestingly, bradykinin has strong vasodilation effects, a hypotensive effect, and causes an increase in vascular permeability resulting in a hypotonic uterus (20, 21). These findings suggest that FXII activation by contact triggers the subsequent catastrophic chain of AFE. We are continuing to investigate the possible role of FXII in AFE.

C1INH, which is mainly synthesized in hepatocytes and endothelial cells and belongs to serpin family, is a major inhibitor of not only C1 esterase but also FXIIa and kallikrein (11, 12). Its deficiency is known to be a specific cause of HAE (13). Since C1INH is capable of not only inhibiting the complement system but also modulating the coagulofibrinolytic and kallikrein-kinin systems (22, 23), we hypothesized that C1INH was key in the pathophysiology of AFE.

Halbmayer et al (24) reported that basal C1INH activity levels decreased markedly with pregnancy up to labor. Although the mechanism remains unclear, estradiol (E2) was shown to suppress the potential activity of C1INH (25, 26).

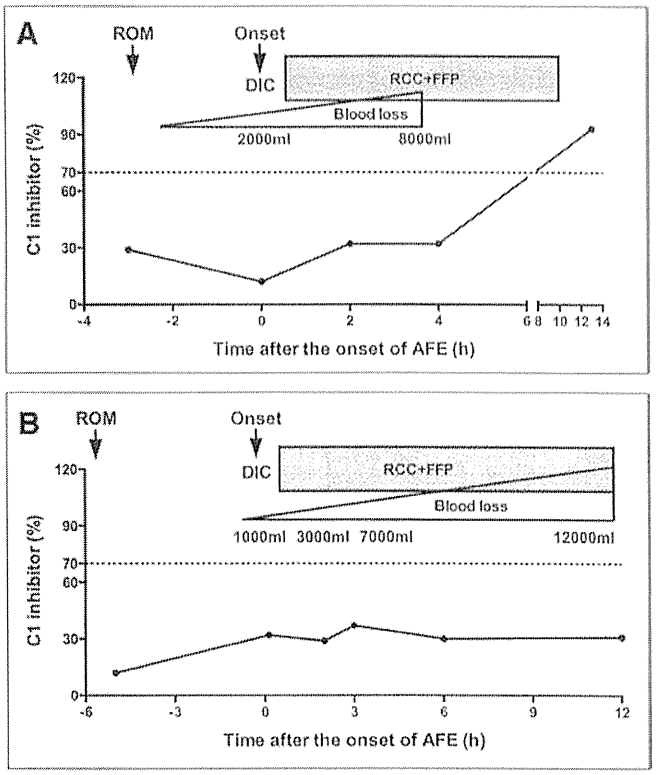

Figure 3. Chronological changes in C1 esterase inhibitor (C1INH) activity levels in amniotic fluid embolism (AFE) cases. A survivor of AFE (**A**) and a case that died due to the coexistence of AFE with bacteremia (**B**). Potential C1INH activity levels were low before the onset of AFE. In case A, the sudden onset of AFE presented disseminated intravascular coagulopathy (DIC) 3 hr after rupture of membrane (ROM) under a selective cesarean section. When AFE was recognized as abnormal coagulopathy, blood replacement therapy was immediately administrated resulting in an increase in C1INH activity levels. In case B, despite intensive care including adequate blood transfusions, C1INH activity levels did not recover. A *dotted line* indicates 70% of C1INH activity. Normal C1INH activity is more than 70%. RCC = red cell concentrates, FFP = fresh frozen plasma.

The increase in E2 levels during pregnancy may be associated with the decrease in C1INH activity levels in pregnant women. They also observed that C1INH activity levels were significantly lower in preeclampsia patients than in normal pregnant women (24). Increases in C3a and C5a have been reported not only in AFE patients but also in patients with preeclampsia and eclampsia (27), which suggests that the consumption of C1INH is due to activation of the complement system. This may explain the high risk of AFE associated with preeclampsia and eclampsia as risk factors of AFE (28, 29).

The present study demonstrated that C1INH activity levels in AFE cases were significantly lower than those of controls. Furthermore, when we compared fatal cases to nonfatal cases using Pearson chi-square test for C1INH activity less than 25% as a cutoff value almost comparable to "attack of angioedema," there was a significant difference with p equal to 0.026 (degree of freedom 1 and chi-square value 4.956). In addition, the chronological assessment of C1INH activity levels in two AFE patients indicated that their basal C1INH activity levels before delivery and onsets of AFE were also lower at 29% and 12% than that of healthy pregnant controls at 74.3% ± 15.5% during the third trimester (24). These results suggest that low C1INH activity levels before onset of AFE could be a predictive factor as well as low levels at onset and the persisting low levels of C1INH activity could be a prognostic factor of AFE.

It has been reported that the levels of C1INH may be increased during infection as an acute phase protein, then cleaved and inactivated by neutrophil elastase and bacterial proteases under developing inflammatory conditions due to bacteremia and sepsis resulting in a functional C1INH deficiency (30–32). As demonstrated in the fatal case in **Figure 3*B***, we should note here that not only C1INH consumption under AFE condition but also C1INH inactivation under inflammatory conditions due to bacteremia may be involved in the persistent low levels of C1INH activity (32).

As a treatment for DIC with AFE, the rapid administration of FFP or cryoprecipitate was sufficient to extricate the patient from a critical situation. FFP contains several essential proteins such as antithrombin III and fibrinogen. One hundred units of C1INH are contained in FFP derived from 200 mL blood. Our chronological assessment of C1INH activities in the AFE patient shown in **Figure 3*A*** demonstrated that a suited blood transfusion including FFP was able to improve C1INH activity. Clinically, the use of 500–1,500 U of human plasma–derived C1INH concentrates can revert HAE in C1INH-deficient patients (33–35). Since AFE patients certainly have significant lower levels of C1INH activity, similar to a C1INH deficiency, the clinical application of human plasma–derived C1INH concentrates may become one of the promising candidates for the treatment of AFE.

In summary, we reported here that C1INH activity levels were significantly lower in AFE cases, particularly in fatal cases. These results indicate that C1INH activity levels reflect the severity of AFE and can be a prognostic factor of AFE. We speculate that the clinical application of C1INH concentrates will be effective for the treatment of AFE. Although the chronological measurement of C1INH activity was small, our results suggest that pregnant women with potentially low C1INH activity levels may be at a high risk of the onset of AFE. Further clinical studies are required to elucidate the etiological role of C1INH in AFE and determine whether C1INH activity may be a predictive factor of AFE.

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