

# Detection of fetal keratin with high molecular weight cytokeratin immunostaining in lung biopsy material from a patient with amniotic fluid embolism

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**Abstract.** Amniotic fluid embolism (AFE) is a rare and fatal disorder in which the diagnosis can be challenging for clinicians and pathologists. A healthy 36-year-old woman (gravida 4, para 2) was admitted for delivery in the 40th week of gestation. At the fifth minute following birth, during expulsion of the placenta, the patient suddenly collapsed with bradycardia, shallow respiration, and loss of consciousness. After evaluation, an emergent pulmonary embolectomy for acute thrombo-embolism was performed, however the patient could not be weaned from cardiopulmonary bypass, and died of severe right ventricular dysfunction following the operation. Microscopic examination of the biopsy material detected clearly visible fetal epithelial squames inside pulmonary vessels, both with routine hematoxylin-eosin (HE) staining and immunostaining for high molecular weight cytokeratin (HMW-CK). The diagnosis of amniotic fluid embolism (AFE) was made, which was confirmed as the cause of death. We show that HMW-CK staining can be a useful means of detecting amniotic fluid-derived fetal keratin within alveolar tissue. We suggest that this technique, used in addition to HE staining and in combination with sudden-onset clinical findings, may increase the accuracy of diagnosis in AFE.

Key words: Amniotic fluid embolism, cardiovascular collapse, cytokeratin, high molecular weight cytokeratin, HMW-CK

## 1. Introduction

Amniotic fluid embolism (AFE) is a rare and fatal disorder with an incidence of 1:13,000–1:50,000 and a perinatal mortality rate of 9–44% (1, 2). Although the term was first introduced by Meyer in 1926 (3), little is known about the pathogenesis and diagnosis of AFE. The diagnosis of AFE can be challenging for clinicians and pathologists. A likely diagnosis

can be made based on biopsy findings, often from autopsy material, in combination with clinical findings. Typical clinical features of AFE include sudden onset of cardiovascular collapse, respiratory symptoms, disseminated intravascular coagulation (DIC), and seizures in previously asymptomatic women. There is no specific therapy for AFE and supportive care is the mainstay of treatment. A reliable means of definitive diagnosis would have a significant effect on the management and outcome for these patients.

Due to its abrupt onset and catastrophic course, there is generally no time to take biopsy material from living tissues. Herein, we report a fatal case of amniotic fluid embolism in which fetal keratin was detected using both hematoxylin-eosin (HE) staining and high molecular weight cytokeratin

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(HMW-CK) immunostaining within the pulmonary vasculature of biopsied material.

## 2. Case report

A healthy 36-year-old woman (gravida 4, para 2) was admitted to our hospital for delivery in the 40th week of gestation. The patient's medical history was unremarkable, and the current pregnancy course was realized to be uneventful from her private physician. Physical examination findings, vital signs, and laboratory work-up were all normal on admission. Early labor was uneventful. One hour prior to delivery, clear amniotic fluid was seen after artificial rupture of the membranes. The patient delivered a live 2600 g baby normally without any complications. In the fifth minute following birth, during the expulsion of placenta, she suddenly collapsed with bradycardia, shallow respiration, and loss of consciousness. Immediate cardiopulmonary resuscitation (CPR) was initiated, endotracheal intubation was done, and atropine and adrenaline were administered as needed. No signs of hemorrhagic complications were noted. An emergent bedside echocardiogram revealed hyperechoic mobile intracardiac particles and a moderate right-sided dilatation of the heart (Figure 1). Since pulmonary thromboemboli could not be ruled out and the patient's condition deteriorated, we decided on an emergency operation for pulmonary thrombo-embolotomy, and the patient was transferred to the operating room while CPR continued.

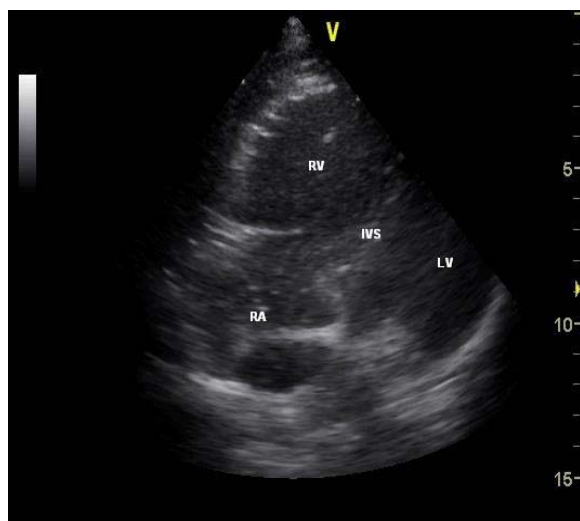


Fig.1. Bedside echocardiogram showing hyperechoic mobile intracardiac particles in the long-axis transthoracic position, which most likely correspond to fetal amniotic fluid elements. The right ventricle and the right atrium appear moderately enlarged, consistent with right ventricular pressure overload.

An emergent sternotomy was performed, and cardiopulmonary bypass (CPB) was initiated by internal cardiac massage. The pulmonary embolotomy procedure was performed on both pulmonary arteries through the main pulmonary artery under total CPB; however, no thromboembolic material was noted within the pulmonary bed, and both lungs were normal on gross inspection. Following the initiation of CPB, intense vaginal bleeding was observed and was mostly attributed to the high dose of heparin given for CPB although a subsequently developed intraoperative DIC was not ruled out. High dose heparin was given as an initial loading dose of  $350 \text{ U.kg}^{-1}$ , and additional doses to maintain the activated clotting time above 400 seconds during the extracorporeal circulation. At that time, despite administration of packed red blood cells, fresh frozen plasma, and fresh blood, the hemoglobin level was 3 g/dL with increasing vaginal blood loss. Patient's condition was deteriorated because of acute vaginal blood loss, and to save the patient's life, an emergent subtotal abdominal hysterectomy was performed, together with ligation of both hypogastric arteries while the patient was on extracorporeal circulation support.

Following several unsuccessful attempts to wean the patient from CPB, severe right ventricular dysfunction involving severe hypokinesis and acute dilatation of the right ventricle was observed intraoperatively despite high-dose inotropic support. Although CPB was prolonged to provide extracorporeal circulation support for several hours, cardiac arrest developed as a result of acute right ventricular dysfunction when CPB was terminated, and the patient was considered to die due to severe right ventricular dysfunction.

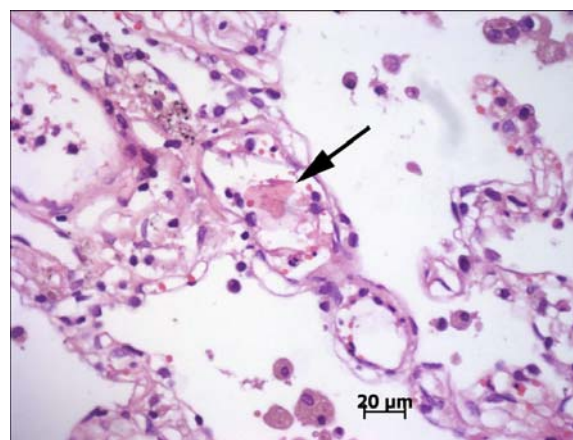


Fig. 2. Alveolar tissue section. A fetal squamous epithelial cell (arrow) derived from amniotic fluid is seen in the pulmonary vasculature (HE,  $\times 40$ ).

A lung biopsy was obtained before termination of surgery. Microscopic examination of the biopsy specimen detected clearly visible fetal epithelial squames inside pulmonary vessels, both with routine HE staining and with immunostaining for HMW-CK (Figure 2-3).

These results unequivocally demonstrate the presence of amniotic fluid-derived fetal keratin. Based on these data in combination with the clinical picture, a diagnosis of AFE was made, and the cause of death was determined to be AFE.

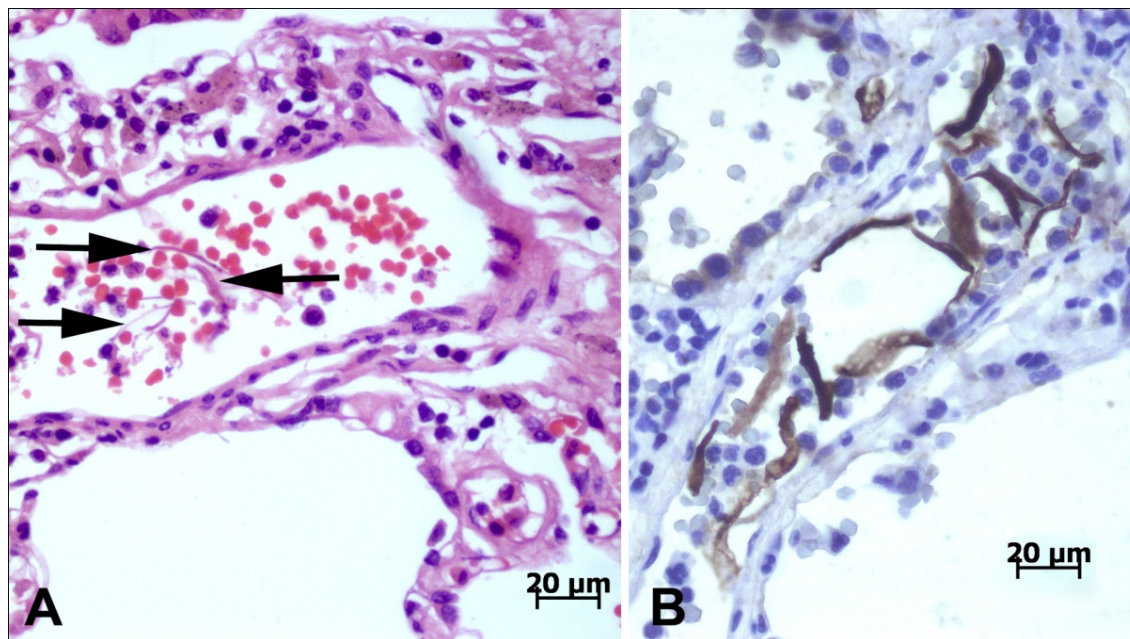


Fig. 3. Amniotic fluid embolism. (A) Arrows indicate fetal epithelial squames (HE,  $\times 40$ ). (B) Immunostaining for HMW-CK demonstrating intravascular fetal epithelial squames (black-brown areas).

### 3. Discussion

In this case presentation, we reported a case of fatal AFE in which supportive and surgical treatments were performed. Surgery was carried out due to possible pulmonary thrombo-embolism and addition of subtotal hysterectomy was done because of acute heavy vaginal bleeding.

The pathophysiology of AFE seems to be multifactorial but is poorly understood. The entrance of amniotic fluid into the systemic maternal circulation, triggering clinical symptoms of the disease, is accepted as the principal mechanism of the AFE syndrome. Since there is no routine diagnostic tool to detect AFE in living patients, a definitive diagnosis of AFE is difficult. The probable diagnosis of AFE is generally made by identifying characteristic signs and symptoms clinically as well as limited numbers of laboratory tests may support the probable diagnosis. Therefore, to further support a diagnosis of AFE, histological examination of various tissues are sometimes performed to test for amniotic fluid contents. Lung tissue (obtained from a biopsy or autopsy) is the most extensively

studied material in suspected AFE cases; in the present case, a lung biopsy sample was taken to demonstrate evidence of AFE, the diagnosis of which was supported clinically.

Amniotic fluid contains squamous epithelial cells from fetal skin or amnion, mucin secreted by the fetal intestine, fat derived from vernix caseosa, and lanugo hair. Demonstration of the presence of this fetal debris within the pulmonary vasculature is strong evidence for the diagnosis of AFE. The most common components of amniotic fluid found in pulmonary vasculature are squamous epithelial cells (4). These squamous cells in amniotic fluid can be identified by HE staining or with immunostaining for HMW-CK, which does not stain pulmonary capillary endothelium. Although routine HE staining can usually detect amniotic fluid elements, the technique is not highly sensitive or specific, and AFE can be easily missed. For more reliable diagnosis, various immunohistochemical markers are used to stain for fetal components. These include cytokeratin AE1/AE3 to detect fetal squamous cells, Alcian blue or mucicarmine staining to detect mucin, oil red O staining to

detect lipid droplets, and polarized light, which readily facilitates detection of lanugo hair.

The HMW-CK monoclonal antibody is typically used for the qualitative identification of human cytokeratin intermediate filament proteins by immunohistochemical staining. The method stains all layers of squamous epithelium in skin as well as some normal and tumor-derived tissues. We employed the technique to stain squamous cells from fetal skin in the maternal pulmonary vasculature.

Besides biopsied material, a cytological preparation of blood drawn from the lumen of a pulmonary artery catheter in the wedge position (i.e., pulmonary microvascular cytology) can also be used to precisely define the cellular elements of pulmonary microvasculature. This technique readily reveals fetal squames in an amniotic fluid embolism, providing significant diagnostic information by a relatively noninvasive route (5).

The histologic identification of squamous epithelial cells in the pulmonary circulation is sometimes performed in suitable patients and this technique *per se* does not confirm the definite diagnosis of AFE as well. Since, several studies have demonstrated that squamous cells, trophoblasts, and other debris of fetal origin may commonly be found in the central circulation of women with conditions other than amniotic fluid embolism or even normal pregnant women (6). This finding is thought to be neither sensitive nor specific, and may only support the clinical diagnosis of AFE.

Although DIC is a common finding in AFE, with an incidence of approximately 50%, we initially did not observe any hemorrhagic complications in the present case. However, acute vaginal blood loss during the operation made us consider a possible DIC also. For the treatment, fresh frozen plasma and fresh blood as well as packed red blood cells were administered as appropriate. The etiology of DIC in AFE is thought to be multifactorial, and whether the cause of bleeding is a consumption coagulopathy or massive fibrinolysis remains debated.

Transthoracic echocardiography in our patient showed hyperechoic mobile intracardiac particles. We believe these particles represent fetal amniotic fluid elements since we could not find any thrombi in the main pulmonary arteries on the surgery. Other investigators have also reported intracardiac mobile masses in AFE and

identified the particles as thrombi (7). More studies are needed to reveal the nature of these intracardiac particles in AFE.

Successful treatment of postpartum shock with CPB and pulmonary thrombo-embolotomy in AFE has been reported previously (8). In our case, we could not rule out thromboemboli initially and decided on an emergency procedure due to the patient's deteriorating condition. Although CPB and pulmonary embolotomy procedures were performed, the patient died of severe right ventricular dysfunction.

In conclusion, the definitive diagnosis of AFE is challenging for clinicians and pathologists. Fetal elements in AFE can be difficult to see on routine HE-stained sections. To increase the accuracy of diagnosis in AFE, HMW-CK immunostaining to detect amniotic fluid-derived fetal keratin within alveolar tissue may be diagnostically useful in addition to HE staining and in combination with sudden-onset clinical findings. Further studies are needed to demonstrate the diagnostic value of HMW-CK stain in other organs and tissues.

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