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CASE REPORT

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Immunohistochemical identification of syncytiotrophoblastic cells and megakaryocytes in pulmonary vessels in a fatal case of amniotic fluid embolism

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Abstract The histological diagnosis of amniotic fluid embolism (AFE) is based on finding amniotic fluid components in the pulmonary microvasculature. In addition to the distinctive constituents of AFE, placental and decidual tissue fragments as well as isolated trophoblastic cells and megakaryocytes are potentially detectable within pulmonary vessels. The identification of single syncytiotrophoblastic cells (STC), and their differentiation from circulating megakaryocytes (MK) within the lumen of small and medium-sized pulmonary vessels is difficult by classical morphological methods. In a fatal case of AFE, we have successfully detected the simultaneous presence of STC and MK in the pulmonary microvasculature by means of a panel of specific monoclonal (CD61-GpIIIa, β-hCG) and polyclonal (FVIII-vW, hPL) antibodies. The immunohistochemical analysis for identification of STC and MK should provide more precise data on their incidence and distribution in physiological and pathological conditions as well providing new insights into their physiopathological implications and their correlation with AFE and other gynaecological complications.

Key words Amniotic fluid embolism · Syncytiotrophoblastic cells · Megakaryocytes · Immunohistochemistry

Introduction

Amniotic fluid embolism (AFE) is a rare but severe complication of pregnancy, characterized by the passage of amniotic fluid and its components into the maternal circulation through tears in the utero-placental sinusoids or endocervical veins, which are then trapped within the pulmonary microcirculation. Since the first report of AFE by Meyer in 1926, over 300 cases have been described in the literature [1], recently also in forensic journals [2–4].

The post-mortem diagnosis of AFE is based on the histological demonstration of the components of amniotic fluid i.e. squames from the stratum corneum, lanugo hairs, meconium, mucin, and sebum in pulmonary vessels. The majority of such components are easily recognizable by classical cytomorphological criteria and staining methods. Immunohistochemistry has even been recently performed in fatal cases of AFE to indicate some of its components (cytokeratin, mucin-type glycoprotein and fetal isoantigen) [2, 5–7]. In addition to such distinctive constituents of AFE, isolated trophoblastic cells (cytotrophoblastic and syncytiotrophoblastic cells) and megakaryocytes are potentially detectable in the pulmonary microcirculation [8–12], even though their identity is difficult to confirm in routine samples.

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Fig. 1 Squames from the stratum corneum and granular debris distending an alveolar capillary. Attwood's stain modified, × 230 ▶

Fig. 2 Mucoid material in an alveolar capillary. Attwood's stain modified, × 170

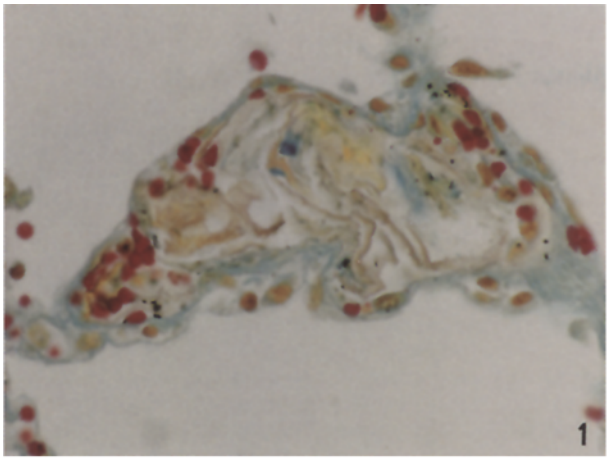
Figs. 3, 4 Squames from the stratum corneum positive to CK in alveolar capillaries. In Fig. 4 note a convoluted giant cell trapped in a capillary at the left margin (*arrow*). Paraffin, ABC method, × 95

Fig. 5 STC trapped in a pulmonary capillary with granular hPL-positive cytoplasm located at the lower pole of the cell. Paraffin, ABC method, × 610

Fig. 6 MK with a multilobed nucleus and diffuse FVIII-vW-positive cytoplasm in a pulmonary vessel. Note endothelial lining positive to FVIII-vW. Paraffin, ABC method, × 160

Fig. 7 MK with a pale nucleus and diffuse FVIII-vW-positive cytoplasm in a pulmonary vessel. Note myeloblastic and erythroblastic precursors in the vessels. Paraffin, ABC method, × 120

Fig. 8 MK trapped in a pulmonary capillary with a small rim of FVIII-vW-positive cytoplasm surrounding the nucleus. Paraffin, ABC method, × 240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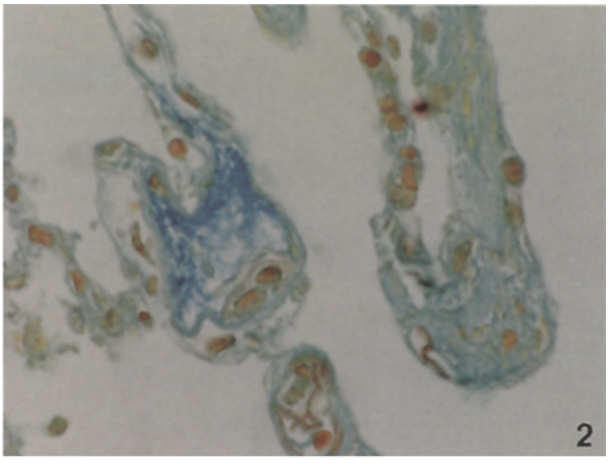
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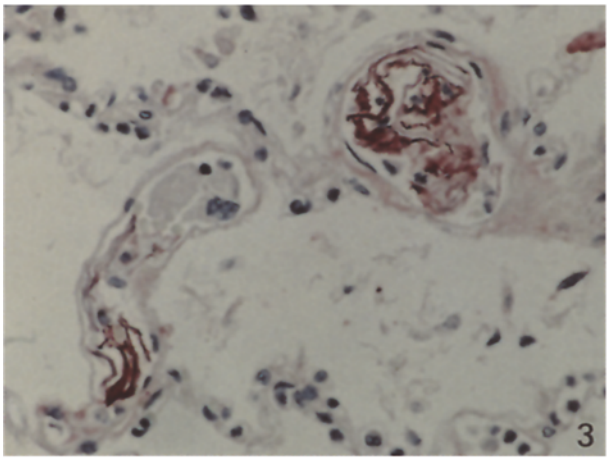
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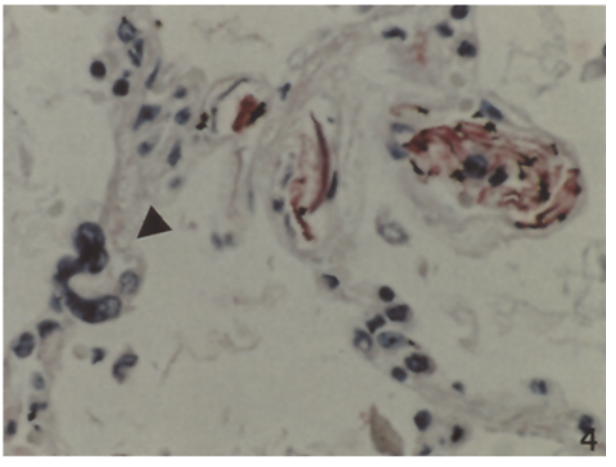
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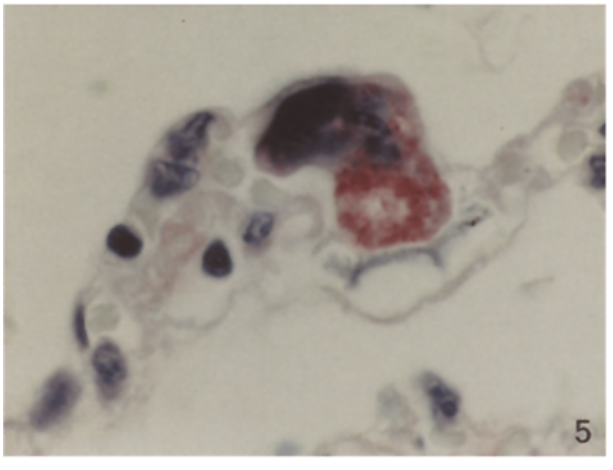
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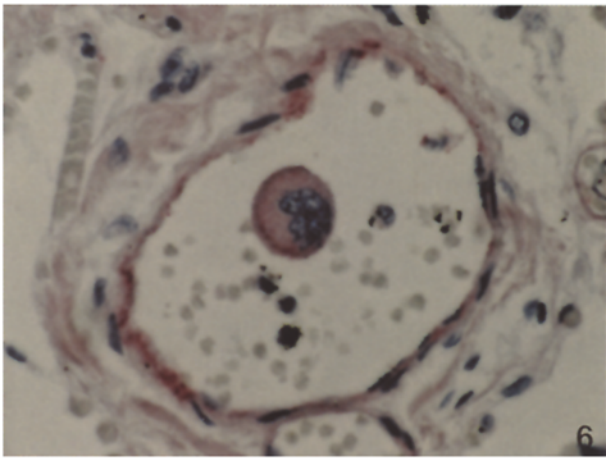
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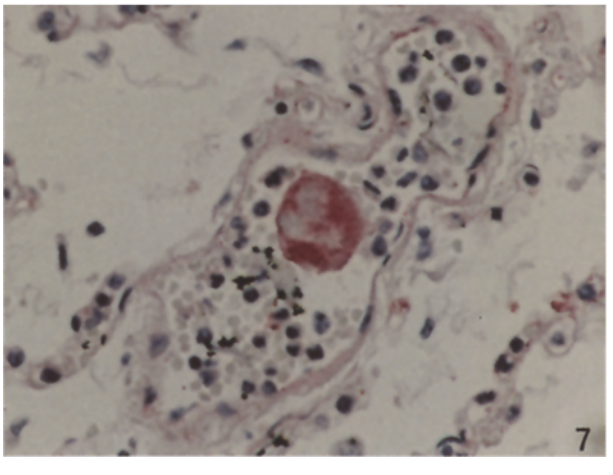
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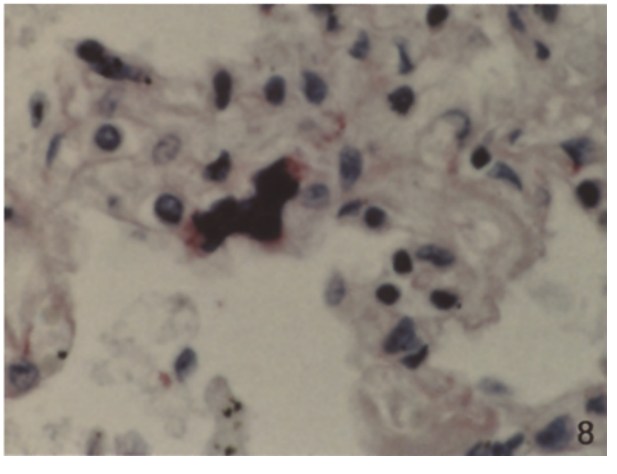
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We describe a fatal case of AFE in which identification of and differentiation between STC and MK in small and medium-sized pulmonary vessels were achieved by means of a specific panel of antibodies (Abs) and the avidin-biotin complex (ABC) method.

Case report

A healthy 42-year-old woman, gravida 11, para 5, at 36 weeks (+ 6 days) of pregnancy was urgently admitted at 7.30 p.m. to hospital with sudden epistaxis, gingival and vaginal haemorrhage; a clinical diagnosis of partial detachment of the placenta and suspected disseminated intravascular coagulation (DIC) had been made. At 9.00 p.m. it was decided to proceed with a caesarean delivery with the patient under general anaesthesia. Some minutes after the incision a baby girl was delivered. During the caesarean section severe untreatable bleeding from the uterus led to the decision to perform a hysterectomy and a left ovariectomy. The estimated blood loss during surgery was about 1500 ml, and blood replacement was performed. At 11.00 p.m. the patient was transferred to the intensive care unit of a regional hospital, where she arrived in a deeply comatose state; about 500 ml of blood was collected at that time from the surgical drainage. At 11.40 p.m. blood values were: platelets 129 000/mm^3, Hb 5.0 g/100 ml, RBC 1 610 000 mm^3, WBC 21500/mm^3 and Ht 17%; 300 ml of whole blood and 2 U of plasma were infused. After progressive worsening of the clinical situation and haematological parameters, death occurred at 1.20 a.m. The autopsy was performed 32 h after death.

Autopsy findings

At external examination there was cannulation of the left subclavian area, needle therapeutic injection punctures in the antecubital fossa and in the dorsal regions of the wrists, and a surgically sutured wound in the pubic region from the caesarean section; no other signs of treatment or injury were detected. At internal examination the brain (1550 g) was oedematous, the lungs (right 830 g, left 760 g) were grey-yellowish, moderately firm, with red-brownish areas at the cut surface, especially in subpleural areas. Hypophysis, thyroid, myocardium, liver, pancreas and kidneys were markedly pale. Haemorrhagic infiltrates were observed in the mediastinal adipose tissue, in the pericardium, in perivesical and perirenal adipose tissue, in the parietal peritoneum, and in abdominal and pelvic muscles. Examination of the surgical area revealed the recent hysterectomy and left ovariectomy but did not disclose any signs of complications.

Histological samples were taken from brain, lungs, myocardium, liver, pancreas, adrenals, spleen, kidneys, skin and muscles and formalin-fixed and paraffin-embedded. Haematoxylin-eosin, Masson's trichrome, PAS and PTAH staining was performed on all samples and also a modified Attwood's stain [13] on the lung samples. Immunohistochemical staining with commercial monoclonal Abs anti-cytokeratin (CK; wide molecular weight range: 40–68 kDa; Biomeda, USA), anti-subunit β of human chorionic gonadotropin (β-hCG; Biogenex, USA), anti-glycoprotein IIIa molecule (CD61-GpIIIa, clone Y2/51; Dako, Denmark) and with polyclonal Abs anti-factor VIII-von Willebrand (FVIII-vW; Biomeda, USA) and human placental lactogen (hPL; Biomeda, USA) were performed on multiple semiserial 5 μm sections from formalin-fixed paraffin-embedded samples of the lungs. Monoclonal Abs anti-CK, anti-β-hCG and polyclonal Abs anti-FVIII-vW and hPL were commercially prediluted, whereas monoclonal Ab anti-CD61-GpIIIa were used at a dilution of 1:100. The avidin-biotin complex (ABC) alkaline phosphatase-based analysis was performed using the commercial reagents included in the UltraProbe Kit (Biomeda, USA) to identify the different antigens. Digestion with trypsin (0.05% at 37°C for 5 min) preceded the incubation with the primary Ab for 20 min. Biotinylated secondary antibody was then added, and the mixture was incubated at room temperature for 15 min. Finally the avidin-phosphatase complex was applied at room temperature for 15 min. The sections were rinsed several times with a buffer solution (pH 7.5) between incubation periods. Positive reactions were then visualized by a working chromogen included in the UltraProbe Kit (buffer solution, fast red and naphthol phosphate), and the sections were counterstained with haematoxylin. Positive controls were performed for all Abs (CK: skin; factor VIII-vW and CD61-GpIIIa: bone marrow; hPL and β-hCG: placenta), and immunoreactivity in pulmonary endothelium was considered an internal positive control for factor VIII-vW and CD61-GpIIIa.

The autopsy and microscopical findings confirmed the diagnosis of AFE suspected by the clinicians. Histological analysis with conventional staining methods revealed diffuse congestion of septa and the extensive presence of squames from the stratum corneum, mucin and meconium in small and medium-sized pulmonary vessels (Figs. 1, 2). Numerous isolated giant cells were seen in pulmonary vessels, sometimes moulded to the configuration of the alveolar capillaries and with a thin rim of cytoplasm ("seminaked" or "naked" nuclei: 30–35% of the giant cells). Squames from the stratum corneum were demonstrated by immunohistochemistry with a monoclonal Ab anti-CK having a wide molecular weight range of 40–68 kDa (Figs. 3, 4).

In immunohistochemical sections, a few STC in small vessels, strongly positive to hPL and slightly to β-hCG, were clearly detectable (Fig. 5). MK (85/cm^2) and large cytoplasmic fragments without a nucleus trapped in small vessels and capillaries, strongly positive for factor VIII-vW and slightly positive for CD61-GpIIIa were also identified (Figs. 6 and 7). Small portions of cytoplasm positively reacting for factor VIII were found surrounding apparent "naked nuclei" of MK trapped in capillaries in about 80% of the cases (Fig. 8). Two large but isolated aggregates of decidual cells, hPL and β-hCG negative, were also detected in pulmonary vessels. Other relevant changes were marked pericellular and perivascular oedema in the brain, mild centrolobular necrosis in the liver and abundant proteinaceous material in some tubuli recta in the kidney.

Discussion

The physiopathological migration of trophoblastic cells to the lungs during pregnancy and peripartum [8, 9] as well as the pulmonary "trapping" of circulating MK [10–12] can lead to difficulties in their identification and reciprocal differentiation, because of morphological similarities. The identification and differentiation of a single STC from a MK is particularly difficult, if not impossible, as both are giant and multinucleate/multilobate cells, especially in small pulmonary vessels where they lose their normal configuration and only small portions of cytoplasm are detectable. Because STC, as terminally differentiated cells, produce different steroid and polypeptide hormones (e.g. α-hCG, β-hCG, hPL) and during their maturation, MK line cells synthesize different cytoplasmic proteins (e.g. FVIII-vW) and membrane glycoproteins (e.g. glycoproteic Ia-IIa-IIb/IIIa complex) the utilization of a panel of monoclonal and polyclonal Abs specific for STC and MK appears to be a simple but a specific method for solving the problem of their identification and differentiation.

The utilization of anti-hPL and anti-β-hCG Abs made it possible to identify and differentiate isolated STC precisely (Fig. 5). STC showed a stronger positivity to hPL than to β-hCG in all the sections examined, which was

consistent with the sequence of hormonal synthesis during pregnancy, reflecting the trophoblastic maturation sequence with a higher production of hPL by STC during the last trimester of pregnancy [14]. The utilization of anti-factor VIII-vW and anti-CD61-GpIIIa Abs made it possible to count MK and large cytoplasmic fragments of MK without a nucleus ("enucleate" MK) accurately and to differentiate MK with small portions of cytoplasm from truly "naked" nuclei of MK (Fig. 8).

As far as we know no earlier reports on AFE have described the simultaneous presence of STC and MK in pulmonary vessels. Few authors report the post-mortem finding of isolated trophoblastic cells in cases of AFE, and in general no attention at all has been paid to MK. Only Janssen, in his textbook on "*Forensic Histopathology*" [15], mentions the possible difficulties in differentiating intravascular decidual and chorionic giant cells from circulating MK. Reasons for the lack of simultaneous descriptions of MK and STC could be related to the real absence and/or extremely low number of these cells (as in the case of AFE described by Hernandez and Bajanowski [2], where an Ab anti-hPL was without any positive results), but also to the difficulties of identifying the real nature of such giant cells with conventional staining methods.

It is well established that STC cells are physiologically present in the maternal circulation during pregnancy [8, 9]. MK can also be found during pregnancy in the pulmonary microcirculation, being physiologically released from bone marrow into the venous system and subsequently trapped in pulmonary vessels [10–12]. Under physiological conditions, the number of pulmonary MK is generally lower than 5–10/cm^2 of lung tissue. In a wide number of reactive or pathologic conditions (shock, haemorrhage, DIC, infections, liver insufficiency, malignancy, thromboembolism and after surgery) the bone-marrow release and pulmonary "trapping" of MK can increase markedly [10–12, 16]. The two major trigger mechanisms for increased release of MK are increased platelet consumption and severe bleeding, both frequently associated with AFE and other gynaecological complications. The increased platelet consumption is engendered by intravascular coagulation (isolated microthrombosis, local intravascular coagulation, or disseminated intravascular coagulation) that is an intermediary mechanism of disease closely related to several pathological conditions, e.g. shock, infection [11]. In cases of severe bleeding the stimulation of different bone-marrow precursors can lead to the release into the circulation of erythroblastic and myeloblastic cells, as was also observed in our case (Fig. 7).

Accurate quantitative studies on MK and STC are of great value, primarily in differentiating the borders between their numerical values in physiological, reactive and pathologic conditions. In addition, the value of studies of STC stems from their potential role in stimulation (or suppression) of maternal immunity against fetal histocompatibility antigens [17] and in induction of DIC in gynaecological complications: the potent thromboplastin-like effects of STC having been demonstrated [1]. The reported association of STC with toxaemia of pregnancy [18] should also be investigated. On the other hand, pulmonary MK could play an important role in extramedullary platelet production [19, 20]. Accurate quantitative studies on pulmonary MK should also furnish a basis to better assess the limits and the potential of this finding as one marker or criterion, among others, for the postmortem diagnosis of related pathologic conditions (e.g. shock), when clinical history and circumstances of death are unclear.

Because STC are physiologically present during pregnancy, and pulmonary trapping of MK can increase in different conditions associated with pregnancy (DIC, shock, haemorrhage), an immunohistochemical panel of specific Abs for MK and STC is recommended even for use retrospectively, not only in cases of AFE but in every maternal death occurring during pregnancy and peripartally.

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