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TECHNICAL NOTE

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Proof of fatal air embolism

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Abstract Venous air embolism is a rare cause of death. Entry of gas into the circulation is caused by trauma, mostly surgical or therapeutic, and sometimes resulting from criminal intervention. The detection of air embolisms requires special precautions during autopsy. An aspirometer has to be used for the detection, measurement and storage of gas originating from the heart ventricles. The aspirometer has to be filled completely with distilled water containing two drops of Tween 80 to reduce the surface tension of the water and to prevent adherence of small air bubbles to the wall of the aspirometer. Subsequently the gas has to be analysed by gas chromatography. When the results correspond with the main criteria defined by Pierucci and Gherson [2] the diagnosis "air embolism" is justified. The technique for the detection of air embolism is simple but requires a careful procedure which is described in detail.

Key words Venous air embolism · Diagnostic procedure · Storage of gas · Gas analysis · Criteria for air embolism

Introduction

In cases of air embolism the entry of air into the circulation occurs via an injured blood vessel, mostly a vein. The bubbles of air block the circulation by filling lung arteries and/or the right atrium and ventricle, leading to suffocation and/or to pump failure of the heart. The air usually remains in the right side of the heart, pulmonary trunk and arteries [1]. Only under special circumstances can air be found in the left ventricle (e.g. open foramen ovale, intrapulmonary shunts). Special procedures are necessary to diagnose an air embolism as the cause of death. One of the main problems is differentiation between embolised gas and gases of putrefaction [2–6].

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Detection of air embolism

External inspection

If the circumstances indicate a possible air embolism, Knight [7] recommends as the first step a pre-autopsy chest radiograph, which can reveal larger quantities of air in the heart and the blood vessels [8, 9]. This is also necessary in cases of barotrauma [7]. Circumstances indicating a possible air embolism include injuries to the great veins of the upper extremities and the neck, pneumothorax, basal skull fractures, sudden death during surgical or therapeutic intervention (infusion, transfusion [10], endoscopy [11, 12]), during birth [13], criminal abortion [14], injection marks of unclear origin, e.g. in drug abuse; also after resuscitation attempts, in cases with unclear previous history, and tissue emphysema or tissue swelling [7].

Detection, measurement and storage of gas from the heart ventricle

The technique of air detection in the heart ventricles was first described by Mercier in 1837 [15] and further developed by Richter [16]. Before opening the skull a pneumothorax test should be carried out [7]. Then the thoracic cavity has to be opened by a short cut of about 20 cm length, extending from the lower part of the sternum to the second rib without damaging the great veins. The sternum has to be severed transversely between the second and third ribs and removed (Fig. 1). The pericardium is opened for about 3–4 cm in a longitudinal direction, beginning at the frontal base, and filled by water before the right and left ventricles are separately punctured under the fluid surface.

For measurement and storage of the gas Dyrenfurth [17–19] constructed an aspirometer. This apparatus was subsequently modified by different authors [20, 21], and particularly by Schmidt [22], who developed an aspirom-

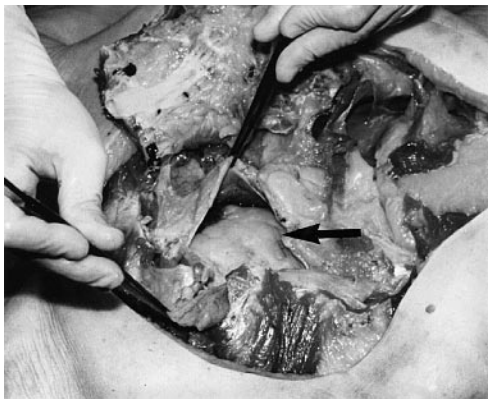

Fig. 1 The thoracic cavity is opened, the lower part of the sternum is removed and the pericardium is opened. The heart is swollen by gas in the ventricle (*arrow*)

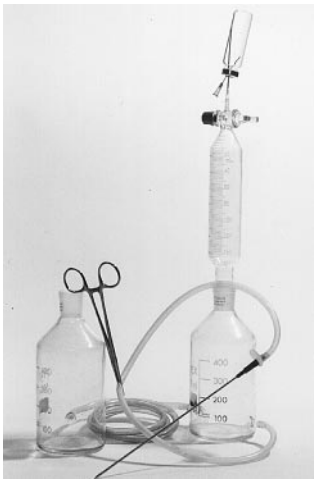

Fig. 2 Aspirometer consisting of two glass cylinders, one with a flask on the top for the measurement of gas amount, one tube connecting the cylinders and one tube with the needle to puncture the heart. On the top of the flask there is the head space tube

eter which is easy to handle and allows exact measurement of the gas amount in a scaled glass cylinder.

The aspirometer (Fig. 2) consists of two cylinders and two tubes. The first cylinder has a scale for the measurement of the gas volume and an outlet on the top where the gas can be filled into head space tubes for storage. The second one is necessary to decrease the pressure in the first cylinder during the sampling and to increase it for storage. One of the tubes connects the two cylinders and the other joins the puncture needle to the first cylinder (Fig. 2). Before use the aspirometer should be carefully cleaned and all remnants of fat have to be removed from the inner surface of the cylinders and tubes. The device has to be filled completely with distilled water, to which one to two drops (about 100 µl/l water) of Tween 80 (polyoxyethylenesorbitan monooleate; Merck, Frankfurt) should be added. This reduces the surface tension of the water and also the number of small air bubbles clinging to the glass surface. After filling the system even the smallest air bubbles have to be removed by careful mechanical agitation.

Keil et al. [4] found that the O_2 concentration in gas samples stored at room temperature was not influenced by the separating fluid (distilled water, saline) and recommended the use of distilled water.

For gas aspiration, the ventricle has to be punctured, using the needle, while still under the water surface. Decreased pressure in the first cylinder can be produced by reducing the height of the second one (20–25 cm; Fig. 3 A). The outlet has to be closed during this phase, and the clamp squeezing the tube between the first cylinder and the needle must be opened (Fig. 3 B, C). After the whole gas volume has been aspirated, the clamp is closed and the gas volume can be read on the scale. Subsequently, the gas can be transferred to head space tubes (20 ml volume) by opening the outlet at the top of this cylinder and then turning back the pressure in the first cylinder (Fig. 4 A–C). It is important that at least 3 ml of distilled water remain in the head space tubes and that the tubes containing the gas be stored upside down so that the water covers the stopper to avoid any contamination by atmospheric air. Furthermore, the gas volume should be divided and filled into two tubes if the total volume is greater than 20 ml.

The gas analysis should be carried out immediately or on the next day after storage of the samples in a refrigerator, although experiments have shown that the composition of the gases does not change significantly during storage over a period of 2 weeks at room temperature [4].

In some institutes a “good” 50-ml syringe fitted with a two- (or three-) way stopcock with one canula for aspiration and a second canula with tubing for the head space tube is used to aspirate gas from the heart ventricles. This equipment is totally unsuitable, for the following reasons:

1. It is not possible to exclude unnoticed contamination by atmospheric air due to a plunger which is not air-tight.

2. The pressure for gas aspiration is defined to be about 20–25 cm water using the aspirometer. The pressure for gas aspiration using a syringe alone is unknown. It is easily possible to produce a 10- to 20-fold higher pressure by the syringe than by the aspirometer, possibly leading to an opening of other bypasses (e.g. vessels injured by trauma, injection or due to autopsy; reduced tissue resistance, especially if the fluid surface over the tissue is very thin or if the autopsy technique used prior to the storage of gas is not absolutely correct), also leading to contamination by air.

Gas analysis

Gas analysis has to be carried out by gas chromatography (HP 5890 series II) using a thermoconducting detector (TCD). To separate oxygen (O_2), nitrogen (N_2), carbon dioxide (CO_2), methane (CH_4), hydrogen (H_2) and the carrier gas helium (He), two different columns are necessary:

The separation of O_2, N_2 and CH_4 can be performed by a Chrompack Plot fused silica column (25 m × 0.53 mm coating molsieve 5Å) under isotherm conditions at a temperature of 65 °C, an injector temperature of 150 °C and a detector temperature of 250 °C. The prepressure of the carrier gas is 40 kPa. Because CO_2 cannot pass through the column if a molecular sieve column has been used, the samples have to be separated for a second time using a

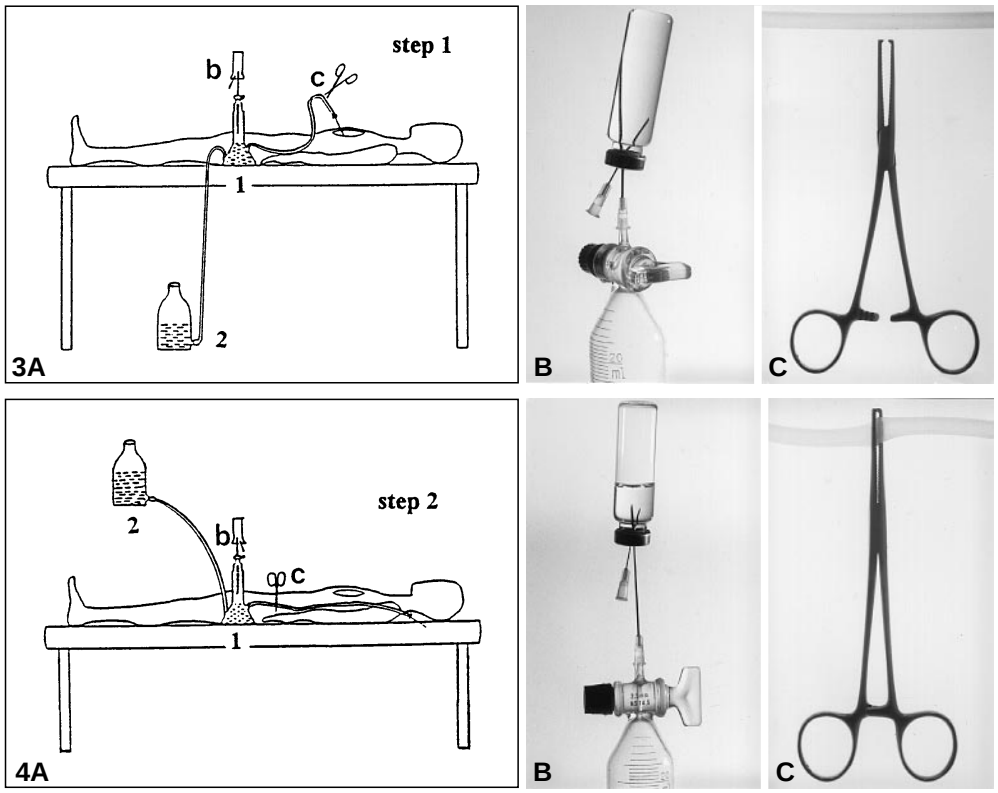

Fig. 3 Scheme showing the technique of gas sampling in two steps. **A** The gas from the heart is sucked in the aspirometer by reducing pressure in the cylinder 1 by lowering the height of cylinder 2 (20–25 cm). **B** The outlet is closed, the head space tube is placed on the top of cylinder 1 and a second needle, necessary in step 2, is placed in the head space tube. The system including the head space tube is completely filled with distilled water. **C** The clamp must be opened after the needle is placed into the ventricle under the water surface

Fig. 4 **A** The clamp has to be closed when the whole volume of gas was aspirated and before storage in the head space tube (**C**). The aspirated gas is forced into the head space tube on top of cylinder 1 by increasing pressure by lifting cylinder 2. **B** The outlet is open and the water in the head space tube is partially displaced by gas. Important: the tip of the second needle must remain under the water surface after completing the gas intake

Carboxen 1006 Plot fused silica capillary column 30 m × 0.53 mm (Supelco). This procedure does not allow the separation of O_2 and N_2 but does allow the separation of these two gases from CH_4 and CO_2 (kiln temperature: 35 °C, 2 min; –24 °C, 1 min; 150 °C, 2 min; injector temperature 230 °C, detector temperature 230 °C, He prepressure 25 kPa).

Because H_2 does not give a peak using both columns, the amount of H_2 in the gas mixture can be calculated to be the difference between the sum of all the other components up to 100%.

Alternatively, the real amount of H_2 can be determined directly using N_2 as carrier gas in the first column. Because H_2 gives a negative peak in the TCD its polarity has to be changed before measurement.

Gas analysis showed that the composition of embolised air differs from that of atmospheric air [2, 6, 26]. The main criteria for the diagnosis of air embolism are an amount of CO_2 in the embolised gas less than 15 vol% and an amount of N_2 higher than 70 vol%, leading to a ratio of < 0.1 [2] or < 0.2 [6]. The amount of O_2 can vary, but amounts between 8 and 15 vol% are typical [6]. The detection of CH_4 and H_2 excludes the diagnosis of a pure air embolism. If small amounts of CH_4 and H_2 are determined and the other parameters show concentrations typical for air embolism, a mixture of embolised gas and gases of putrefaction is possible.

The amount of gas in the heart has no significance for the diagnosis [1, 4, 6].

Morphological signs of air embolism

Walcher [23] particularly emphasised the diagnostic significance of the frothy consistence of the blood in the ventricles. He explained this as the result of an intravital mixture of embolised air and blood.

Histological changes “typical” for air embolism such as separation of leukocytes and thrombocytes, small hollow spaces in blood clots surrounded by leukocytes and thrombocyte aggregates, and “air bubbles” in lung capillaries have been described in the literature [24, 25], but their evidential value is controversial [1]. Ritz-Timme et al. [28] produced leukocyte and platelet accumulations experimentally and interpreted this phenomenon as result of a vital flotation process.

Conclusions

It is strongly recommended to practise the application of the equipment in artificially produced cases. For a valid diagnosis of fatal air embolism the entry site of about 70–130 ml [27] air into the circulation must be found and embolised gas in the right ventricle has to be detected. Furthermore, the diagnosis requires a gas analysis giving a typical gas composition. The sampling method is easy but requires the use of an aspirometer and some training of the pathologist, who has to avoid any contamination of the embolised gas by atmospheric air. The aspirated gas should be filled in two head space tubes for storage. The gas analysis also needs some training and should be car-

ried out immediately after storage by GC using a TCD. The analysis should be established as a routine method in the laboratory. If the results of gas analysis seem to indicate contamination by atmospheric air, gas in the second tube can be analysed as a control.

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