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Title: Diagnosis of arterial gas embolism in SCUBA diving: Modification suggestion of autopsy techniques and experience in eight cases.

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Abstract

Purpose: Suggest modifications of autopsy techniques in order to improve post-mortem diagnosis of arterial gas embolism (AGE) based on multidisciplinary investigation of SCUBA diving fatalities.
Methods: Five human adult specimens from the voluntary donation program of the Human Anatomy Laboratory, and eight judicial autopsied corpses of SCUBA divers from the Forensic Pathology Service were used. Before performing the autopsies, we accessed the diving plan and the divers' profiles for each case. The technical procedure included identification, isolation and manipulation of carotid, vertebral and thoracic arterial systems.
Results: Using the suggested technical procedure, those vascular structures allowing to optimally isolate the systemic arterial circulation were identified and ligated. In three of eight judicial cases, we had a strongly suggestive history of arterial gas embolism following pulmonary barotrauma (PBt/AGE). In these cases, the proposed technical modifications allowed us to clearly diagnose AGE in one of them. The autopsy of the rest of the cases showed other causes of death such as asphyxia by drowning and heart attack. In all cases we were able to reject decompression sickness, and in some of them we showed the presence of artefacts secondary to decomposition and resuscitation maneuvers.
Conclusions: These results allow us to suggest a specific autopsy technique divided into four steps, aimed at confirming or excluding some evidence of dysbaric disorders according to a re-enactment of the incident. We have demonstrated the presence of large volumes of intravascular air, which is typical of PBt/AGE.

Keywords: SCUBA-diving; autopsy; barotrauma, arterial gas embolism.

Word count of the manuscript: 5003 (5409 including Title page); Abstract word count: 241; Word count Title Page: 406

1. Introduction

Diving is one of the most popular underwater activities on the Spanish coast. Despite all precautions taken, death cases related to this activity are reported every year. Drowning is the main cause of death, but other reasons are observed, such as decompression illness, natural pathology, and trauma.

Decompression illness is caused by intravascular or extravascular bubbles that are formed as a result of reduction in environmental pressure. The term covers both arterial gas embolism (AGE) and decompression sickness (DCS) [1]. Arterial gas embolism (air embolism) following pulmonary barotrauma (PBt/AGE) has been described as the cause of death in SCUBA diving in 13-24% of cases [2], and it is well documented in diving medical texts [3-10].

When a SCUBA diver goes up to the surface too quickly, the gas retained in his/her lungs over-expands as the pressure decreases rapidly, thus causing a PBt. If lung damage due to overexpansion occurs, the incoming air may enter the pulmonary venous system and access the left side of the heart [11-12]. If air bubbles reach cerebral arterial circulation, they can interrupt encephalic blood flow with ischemia and brain anoxia and cause death [13, 14]. PBt/AGE is given as the pathological cause of death if the following four criteria are met [2]: history of rapid ascent followed by loss of consciousness; air in the left side of the heart and circle of Willis; low probability of post-mortem decompression artefact (PMDA) or decomposition; and mediastinal or subcutaneous emphysema limited to the perithoracic area and/or pneumothorax.

In order to establish a PBt/AGE post-mortem diagnosis, it is essential to know the dive profile and use specific autopsy techniques and/or image diagnoses [7,15-17]. The use of plain radiography and, more recently, post-mortem computerized tomography (PMCT) and magnetic resonance imaging (MRI) is recommended prior to diving autopsies [18,19-21]. In case these technologies are not available, some authors describe different specific autopsy techniques for the diagnosis of PBt/AGE [7,8,12,17,22-26]. These autopsy techniques are not always easy or reliable enough to prove the existence of intravascular air, justifying the need for modifications [16].

The aim of this work is to suggest modifications of autopsy techniques in order to exclude or confirm any evidence of dysbarism or barotrauma based on multidisciplinary investigation of fatal diving incidents with compressed breathing gas (SCUBA diving).

2. Material and Methods

The study has been developed in collaboration with the Institute of Legal Medicine and Forensic Sciences of Catalonia (IMLCFC), the Special Group for Underwater Activities (GEAS) of the Spanish Civil Guard, and the Department of Medical Sciences of the University of Girona. The study was authorized on

October 10, 2013 by the Teaching and Research Commission of IMLCFC (Registry number 0316S-81/13).

The study includes two groups of subjects:

Study subjects 1: cadavers from the human anatomy laboratory

Five human adult cadavers from the voluntary body donation program of the University of Girona, pursuant to legal procedures and ethical framework governing body donation programs in Spain. Specimens were preserved by intra-arterial injection of 10% formalin and/or frozen at -25 ºC. Skull and thoracic wall anatomical dissections were made in every specimen, except for one, where the skull had been previously studied by anatomical procedures.

Study subjects 2: cadavers from the forensic pathology service

SCUBA divers disease cases detected in Girona coast (North-East of Spain) from January 2014 to December 2016. A total of 8 autopsies were conducted at the Forensic Pathology Service. Before starting autopsies, a technical file for each case with preliminary data about the testing of the diving equipment, the diving plan, and the divers' profiles were provided. For this, the police diving team analyzed diving computer records, the accompanying divers' reports, and the application of emergency and evacuation plans. For the study of the equipment, the police laboratory was asked to run a toxicological analysis of the breathing gas content of the bottles.

Technical Procedures

The major purpose in all these cases is to show the existence of air in the left heart cavity and the circle of Willis; to do this, optimal identification, isolation and manipulation of the carotid, vertebral and thoracic arterial systems were performed, proceeding with the following parameters for their dissection:

First: Identification, isolation and ligature at the cranial base of internal carotid arteries, superior cerebellar arteries and basilar artery (Figs. 1a, b).

Second: Identification, isolation and ligature of both right and left internal thoracic arteries in the second intercostal space (Fig. 2).

Third: Pericardium opening, forming a cavity or sac; filling of pericardial cavity with water; and finally puncturing of left ventricle (Fig. 3).

Fourth: Section of internal carotid, superior cerebellar and basilar arteries proximally to the ligature. Brain removal and placement in a container filled with water, showing the basal face of the encephalon (Fig. 4); section of the internal carotid artery ligation.

3. Results

Human Anatomy Laboratory

Dissection of the cranial base

The cerebral part of both internal carotid arteries extends from the origin of the ophthalmic artery to its terminal division, into the anterior and middle cerebral arteries. Superior cerebellar arteries originate a few millimeters proximal to the terminal end of the basilar artery and go around the mesencephalon and up to the upper surface of the cerebellum. The basilar artery lies in a longitudinal groove on the anterior aspect of the pons, close to the base of the skull. It runs from the junction of the right and left vertebral arteries and divides into the posterior cerebral arteries. In all specimens, both the identification and isolation of the internal carotid arteries, the superior cerebellar arteries and the basilar artery were optimal.

Thoracic dissection

The internal thoracic artery arises from the first part of the subclavian artery and vein. It passes downward, forward and medially, behind the sternocleidomastoid, the clavicle, and the subclavian and internal jugular veins. It goes down through the thorax behind the upper six costal cartilages and the internal intercostal muscles, just laterally of the sternum. It is accompanied by two venae and ends at the sixth intercostal space. In all specimens, the internal thoracic artery and veins have been identified and isolated in the second intercostal space lateral to the sternum. The topographic disposition of the internal thoracic artery and vein showed some variability, since in one of the specimens the right internal thoracic vein was in a retrosternal position.

Forensic Pathology Service

Case 1

Police technical report: 63-year-old female, with no significant medical history, SCUBA diving in open waters. Recreational diving; group dive. According to witnesses' statements, she indicated something was wrong with signs approximately at 12 m depth. The dive computer profile showed that at 5 m depth she started a quick ascent to the surface. Later on, she lost consciousness, the body was immediately rescued and cardiopulmonary resuscitation (CPR) maneuvers were performed. The victim was taken to a medical center and admitted in intensive care unit. A computerized tomography (CT) scan showed bilateral pulmonary condensation and massive pneumoperitoneum. She died 48 hours after admission to the hospital.

Autopsy: Non-specific autopsy techniques were used. The heart weighed 408 g and there was no left ventricular hypertrophy. The lungs were heavy, weighing 855 g and 689 g, and markedly edematous. Histological examination revealed a non-specific alveolar edema. Toxicology results were negative for alcohol and drugs. Carboxyhemoglobin level was 1%. Cause of death was confirmed as near-drowning.

Case 2

Police technical report: 57-year-old male, with unknown medical history, SCUBA diving in open waters. Recreational diving; group dive. The dive computer profile indicated a maximum depth of 20 m. At 12 m depth, he started a rapid ascent to the surface at a higher speed than indicated. Once aboard, he vomited foam and quickly lost consciousness. CPR maneuvers were unsuccessfully performed and the patient died on the site of the accident.

Autopsy: Internal examination revealed subcutaneous emphysema of the upper chest and left pneumothorax, and mass air bubbles in the left side of the heart and coronary arteries (Fig. 5). The heart weighed 472 g and there was no left ventricular hypertrophy. The lungs weighed 580 g and 185 g. Examination of the brain revealed air bubbles in the circle of Willis. We observed atherosclerosis of the abdominal aorta, hepatomegaly and splenomegaly. The histological examination revealed alveolar edema, interstitial pulmonary emphysema and moderate-to-severe coronary atherosclerosis of the right coronary artery. Toxicology results were positive for alcohol (0.10 g/L) and tricyclic antidepressants. Carboxyhemoglobin level was negative. Cause of death was given as pulmonary barotrauma/cerebral arterial gas (air) embolism due to diving.

Case 3

Police technical report: 62-year-old male, with unknown medical history, SCUBA diving inside a cave. Recreational diving; group dive. Once the immersion was over, the group reported the disappearance of two divers to the emergency services. One of the divers' bodies was found inside a cave after a 20-hour search.

Autopsy: Internal examination revealed absence of air in left heart cavities and in the vessels of the circle of Willis. The heart weighed 408 g and there was no left ventricular hypertrophy. The lungs were heavy, weighing 855 g and 689 g, and markedly edematous. There was transudate fluid in pleural cavity bilateral (>400 ml), cardiomegaly, generalized visceral congestion, cerebral edema and atherosclerosis of the abdominal aorta. The histological examination revealed a marked alveolar edema, moderate atherosclerosis of the descending anterior and right coronary arteries and moderate hepatic steatosis.

Toxicology results were positive for alcohol (0.31 g/L) and negative for all drugs tested. Carboxyhemoglobin level was negative. Cause of death was asphyxia by drowning.

Case 4

Police technical report: 72-year-old male, with unknown medical history, SCUBA diving in open waters. Recreational diving; pair diving. The body was found by the rescue team at the bottom of the sea a week after the diver's disappearance.

Autopsy: Internal examination revealed putrefaction in emphysematous period; subcutaneous emphysema and putrefaction gases in heart cavities; absence of air in the vessels of the circle of Willis. The heart weighed 590 g and there was left ventricular hypertrophy. Coronary arteries showed moderate atherosclerosis. The lungs were heavy, weighing 818 g and 720 g, and exuded hemorrhagic fluid. Both hepatomegaly and hepatic steatosis were detected. Histology showed focal scar in the rear wall of the left ventricle, but there were no changes in other locations. Toxicology results were negative for alcohol and all drugs tested. Carboxyhemoglobin level was 1%. Cause of death was asphyxia by drowning / cardiac event.

Case 5

Police technical report: 24-year-old female, with unknown medical history. SCUBA diving in open waters. Recreational diving; pair diving. The dive computer profile showed a maximum depth of 16 m. At 5 m depth, the ascent was interrupted and she fell to the bottom of the sea. Immediate rescue of the corpse was performed, but CPR maneuvers were unsuccessful.

Autopsy: Internal examination revealed lack of air in left heart cavities and in the vessels of the circle of Willis. The heart weighed 258 g and there was no left ventricular hypertrophy. The lungs were heavy, weighing 848 g and 600 g, with presence of tracheo-bronchial foam and generalized visceral congestion. Histology revealed a marked alveolar edema. Toxicology results were negative for alcohol and positive for benzodiazepines (0.11 mg/L), atropine and tetrahydrocannabinol carboxylic acid (THC-COOH). Carboxyhemoglobin level was 6.5%. Cause of death was asphyxia by drowning.

Case 6

Police technical report: 65-year-old male, with unknown medical history. SCUBA diving in open waters. Recreational diving; group dive. The dive computer profile showed a maximum depth of 10 m. At 9 m depth, he started a rapid ascent to the surface. According to witnesses' statements, the diver went out of the water and lost consciousness on the beach. CPR maneuvers were unsuccessful.

Autopsy: Internal examination revealed lack of air in left heart cavities and in the vessels of the circle of Willis polygon. The heart weighed 702 g with left ventricular hypertrophy. The lungs were heavy, weighing 910 g and 840 g. Atherosclerosis of the abdominal aorta. Histology revealed an alveolar edema, severe atherosclerosis of the anterior descending coronary artery, and showed a focal scar in the rear wall of the left ventricle, suggesting changes of acute ischemia in the interventricular wall. Toxicology results were positive for cocaine metabolites. Carboxyhemoglobin level was negative. Cause of death was lung acute edema and acute myocardial infarct.

Case 7

Police technical report: 31-year-old female, with unknown medical history. SCUBA diving in open waters. Recreational diving; group dive. The dive computer profile showed a maximum depth of 14 m. At 10 m depth, the ascent was interrupted and she fell down to the bottom of the sea. She was immediately rescued and successfully administered CPR maneuvers, transferring her to a medical center where she was admitted in intensive care unit. A CT scan showed bilateral pulmonary condensation. She died four days after admission to the hospital.

Autopsy: Judicial authorization for organs and tissue donation (heart and liver). Non-specific autopsy techniques were used. Macroscopic findings: massive bronco aspiration. The lungs weighed 380 g and 314 g. Toxicology results were negative for alcohol and all drugs tested. Carboxyhemoglobin level was negative. Cause of death was near-drowning.

Case 8

Police technical report: 52-year-old female, with unknown medical history. SCUBA diving in open waters. Recreational diving; group dive. According to witnesses' statements she gave indications of indisposition at 8 m depth. The dive computer profile showed that at 8 m depth, she started a controlled ascent to the surface. Then she lost consciousness, being immediately rescued and given advanced CPR maneuvers which were unsuccessful.

Autopsy: Internal examination revealed subcutaneous emphysema and small pneumothorax, as well as lack of air in left heart cavities and in the vessels of the circle of Willis, with pulmonary edema. Toxicology results were negative for alcohol and all drugs tested. Carboxyhemoglobin level was negative. Cause of death was asphyxia by drowning.

Investigators did not detect mechanical failures or signs of manipulation of the SCUBA diving equipment in any of these cases. In all cases, toxicological analysis of the breathing gas content of the bottles was negative. A brief summary of main findings of all cases is shown on Table 1.

4. Discussion

Autopsies of SCUBA diving-related deaths are a big challenge for any forensic pathologist [18]. It is advisable to have some knowledge about diving physiopathology and experience in the practice of special autopsy techniques [15-17]. Collaboration with field specialists in underwater activities is mandatory as well. Unfortunately, autopsy is partially or totally out of the investigative dive team's hands [17].

All autopsies described in this paper were performed by forensic pathologists with experience in diving fatalities, in the presence of the dive police. First, the existence of pneumothorax and/or mediastinal or subcutaneous emphysema limited to the perithoracic area was proven. Subcutaneous emphysema occurs due to the breaking of the alveolus or an injury with disruption of the bronchial mucosal surface, which makes the air stream go to the connective tissue [12]. In cases 2, 4 and 8, the examination revealed palpable crepitus of the perithoracic area, which is an indicative sign of subcutaneous emphysema. Chests were examined for pneumothorax after the initial T incision. The intercostal space was punctured under a pool of water held in the lateral skin flap [12,24,25]. In cases 2 and 8, this maneuver, that has been named *thoracic water test*, was successful, with the presence of bubbling.

The next step was useful to prove the presence of air in the left side of the heart and in the circle of Willis. Under-pressure air located on the left side of the heart is quickly distributed along the main arterial branches (carotid and vertebral), reaching the cerebral arterial system through the polygon of Willis. If the chest is opened before the head, some authorities [2, 23] support the tying of the carotid arteries at the base of the neck, but without tying the vertebral arteries. To prevent artificial air from entering the intracranial vessels, the skull should be opened before the thoracic and abdominal cavities [7, 8, 12]. Once the skull is opened, vessels must be tied up at the base of the brain [7,8,12,22,25]. Different special autopsy techniques are described to detect air in cerebral arteries; these techniques consist of isolating and tying the middle cerebral, basilar and vertebral arteries before removing the brain [12,25]. Based on these results, we choose to tie the internal carotid arteries, superior cerebellar arteries and basilar artery at the base of the brain. This allows greater and faster accessibility to vascular structures and the extraction of the brain, leaving the cerebellum in situ, reducing the possibility of artificial entrance of air in the cerebral vessels of the circle of Willis. Once cerebral vessels were isolated, the skull autopsy was interrupted without extracting the brain to perform the chest autopsy.

To test for the presence of air in the heart, internal thoracic arteries are clamped to prevent artificial air from entering the intrathoracic vessels [22]. Although variability in its anatomic disposition has been described [27], we isolated this artery in all cases by making an incision next to sternum at a bilateral intercostal second space level. Without disarticulating the clavicle, in order to avoid damage of the thorax big entry vessels, we carefully sectioned the manubrium of the sternum and the ribs. In cases 2 and 8, collapsed lungs were observed to a lesser extent, indicative of pneumothorax. Then we filled the pericardial sac with water to make an incision on the left side of the heart [7,8,12]. In cases 2 and 4, this *pericardial water test* was positive, observing bubbling inside the pericardial sac, which indicates the presence of air in the left ventricle of the heart. Before the pericardial water test, some protocols suggest to capture and analyze the air in the pleural and cardiac cavities [23,25]. This step, while desirable, is not necessarily as important as to prove there is air within the heart itself, since air will nearly always be analyzed as nitrogen with varying amounts of oxygen [17].

In order to prove the presence of air in the circle of Willis, we sectioned the internal carotid arteries, superior cerebellar arteries and basilar artery proximally to the ligature. Once the brain was extracted, only in case 2 this *cerebral water test* was positive, observing some bubbling at arterial level, which indicates the presence of air in the circle of Willis. Autopsies were completed with macroscopic, histopathological, toxicological and biological studies based on specific recommendations for deaths in water given by the authors of this paper [28].

Unfortunately, the presence of intravascular air is very common in diving autopsies and it is not specific to PBt/AGE [2,16]; it can also be due to explosive decompression, PMDA, decomposition and/or resuscitation. Autopsy results should be complemented with pathological findings and a re-enactment of the incident with detailed and accurate information of the diving plan, the diver's profile, and equipment used [15, 16].

In all cases investigated, we were able to reject DCS and hypothesize a low probability of PMDA, that is to say, divers did not take long or go deep enough to develop this dysbaric disorder. Cases 1, 2 and 6 had a strongly suggestive history of PBt/AGE based on the technical report, because a history of rapid ascent followed by loss of consciousness was recreated. Furthermore, assuming that loss of consciousness occurred immediately after an immersion may be indicative of air embolism, case 8 might also be considered for this diagnosis. However, only in case 2 we have proved the macroscopic existence of large volumes of intravascular air, which is typical of AGE [2,16]. Case 1 clinically and pathologically died after drowning, but she also had intravascular air in secondary pneumoperitoneum formed when ascending quickly to the surface. Breath-holding during ascent from a dive is known to cause pulmonary barotrauma and subsequent pneumoperitoneum [29-31]. There can be pathologically no doubt that Case 6 died of acute myocardial infarct, with rapid ascent to the surface, but macroscopic absence of intravascular air. Case 8 pathologically died after drowning, with controlled ascent to the surface; however, subcutaneous emphysema and a small pneumothorax were demonstrated. We believe that secondary-to-CPR maneuvers due to resuscitation efforts rarely simulate the larger volumes usually seen with PBt [2, 16]. Well-controlled animal experiments suggest that in fatal SCUBA diving accidents, subcutaneous emphysema should not be mistaken as diagnostic criteria of barotrauma, because it can be caused by resuscitation maneuvers [21]. Case 4 died after drowning, albeit a cardiac event could not be ruled out; the intravascular gas described corresponds to an advanced decomposed body in emphysematous period, without being possible to assure its intravital origin, characteristic of gas embolism. Some authorities differentiate between *in vivo* gas embolism and putrefaction using gas composition analysis [32-35]. Cases 3 and 5 were undoubtedly due to drowning, whereas Case 7 was deemed to be near-drowning after a survival period. Case 3 was found in a cave and Cases 5 and 7 at the bottom of the sea. However, intravascular air was not observed in their autopsies because there was no possibility of rapid ascent and the bodies, once found, were brought to the surface in a controlled ascent by the rescue diver.

In conclusion, we present a representative study of SCUBA-diving fatalities, establishing a close correlation between police technical reports and pathological findings based on the suggested modifications of autopsy techniques and re-enactment of the incidents. However, the use of post-mortem image techniques (PMCT, MRI) and other technologies should not be left aside when applicable and available.

Conflict of interest

None

Funding

None declared

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the Institutional Research Committee and with the 1964 Helsinki Declaration and its later amendments, or comparable ethical standards.

Acknowledgements

We gratefully acknowledge the contribution of our colleagues and technical staff of the Institute of Legal Medicine and Forensic Sciences (A. Hospital, MD; FJ. San Miguel, MD; JM. Martínez-Calcerrada, and L. Cifuentes) for their help during autopsy conduct and the Anatomy Laboratory of Faculty of Medicine (J. San, MD, PhD and A. Puig, MD) for their help in the anatomic dissections. Gerard Carot-Sans, PhD, edited the final version of the manuscript.

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Figure captions.

Fig. 1. Image showing the circle of Willis' exposition during the autopsy. This maneuver allows to identify and ligate the internal carotid arteries, the superior cerebellar arteries and the basilar artery in the cranial base. **a.** Identification of arterial vessels. **b.** Ligature of vessels. 1. Internal carotid arteries; 2. Right ophthalmic artery; 3. Right middle cerebral artery; 4. Right anterior cerebral artery; 5. Basilar artery; 6. Superior cerebellar arteries; 7. Posterior cerebellar arteries; 8. Optic nerve; **infundibulum*.

Fig. 2. Image corresponding to the isolation and ligature of both right and left internal thoracic arteries in the second intercostal space. 1. Internal thoracic arteries; 2. Internal thoracic veins; 3. Second rib; 4. Third rib; 5. Parietal pleura; *sternum.

Fig. 3. Example of pericardium opening, forming a cavity or sac in order to fill it with water and posterior puncturing of the left ventricle (pericardial water test). 1. Pericardial sac; 2. Pericardial cavity; 3. Epicardium.

Fig. 4. Image showing the isolation of the circle of Willis. In the image, brain has been removed and placed into the water; the previously ligated vessels of the cranial base can be observed. 1. Basilar artery; 2. Superior cerebellar arteries; 3. Internal carotid arteries; 4. Posterior communicating arteries.

Fig. 5. Image showing an example of positive pericardial water test corresponding to case 2. 1. Pericardial sac; 2. Pericardial cavity; arrows: bubbling inside the pericardial sac after puncturing left ventricle.

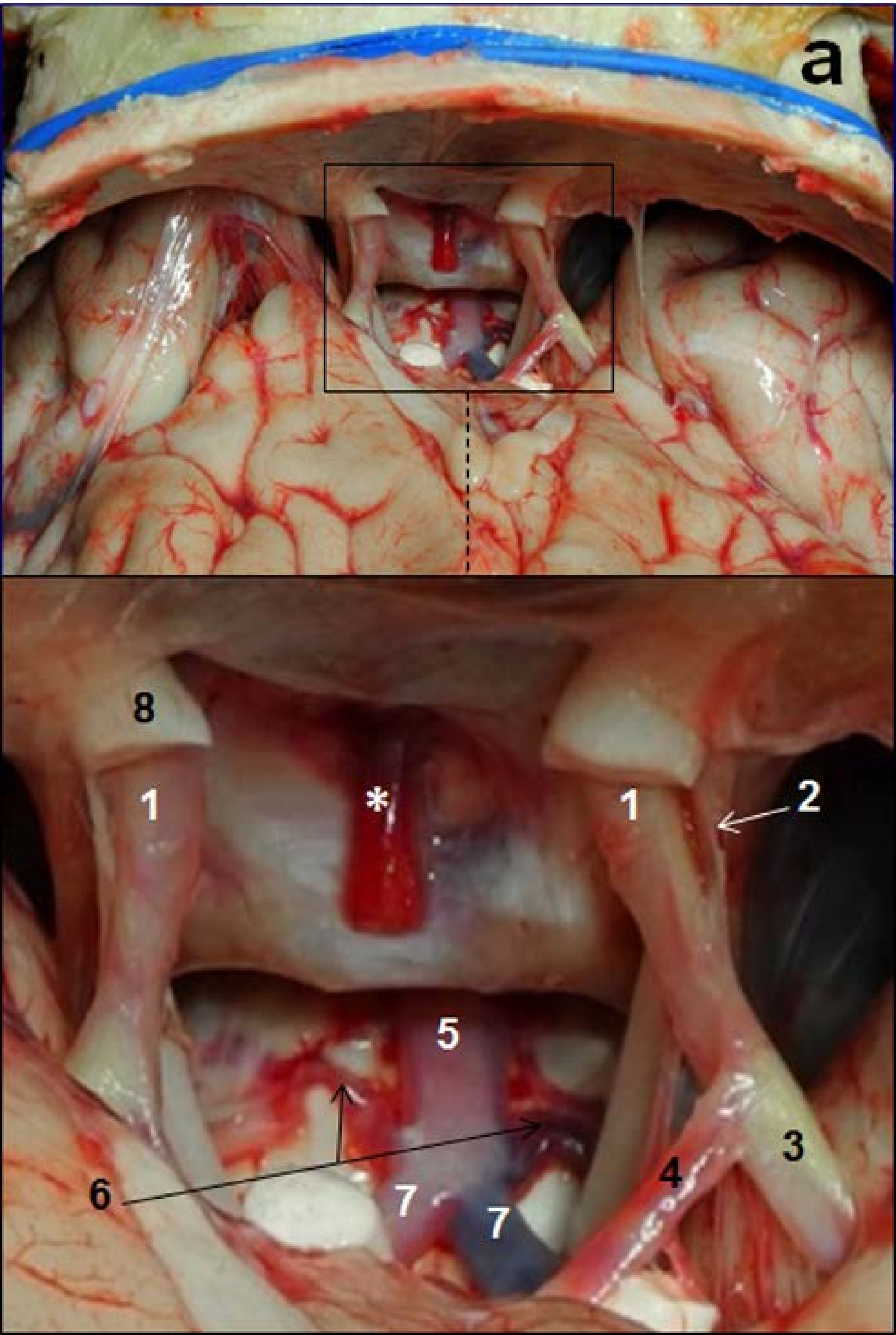
Table caption.

Table 1. Diver profile and pathological findings following autopsy examination.

Key points

1. When investigating diving fatalities, a detailed diving profile must be considered before the autopsy.
2. A systematic autopsy technique minimizes artifacts during diagnosis.
3. Whenever possible, the diagnosis should be supported by post-mortem imaging techniques and other technology
4. The re-enactment of the incident is advisable and requires a multidisciplinary team.

Case	Rapid ascent	Controlled ascent	Subcutaneous emphysema	Pneumothorax	Air in the left side heart	Air in the circle of Willis	Cause of death
1	✓						Drowning
2	✓		✓	✓	✓	✓	PBt/AGE
3							Drowning
4			✓		✓		Drowning
5		✓					Drowning
6	✓						Heart attack
7		✓					Drowning
8		✓	✓	✓			Drowning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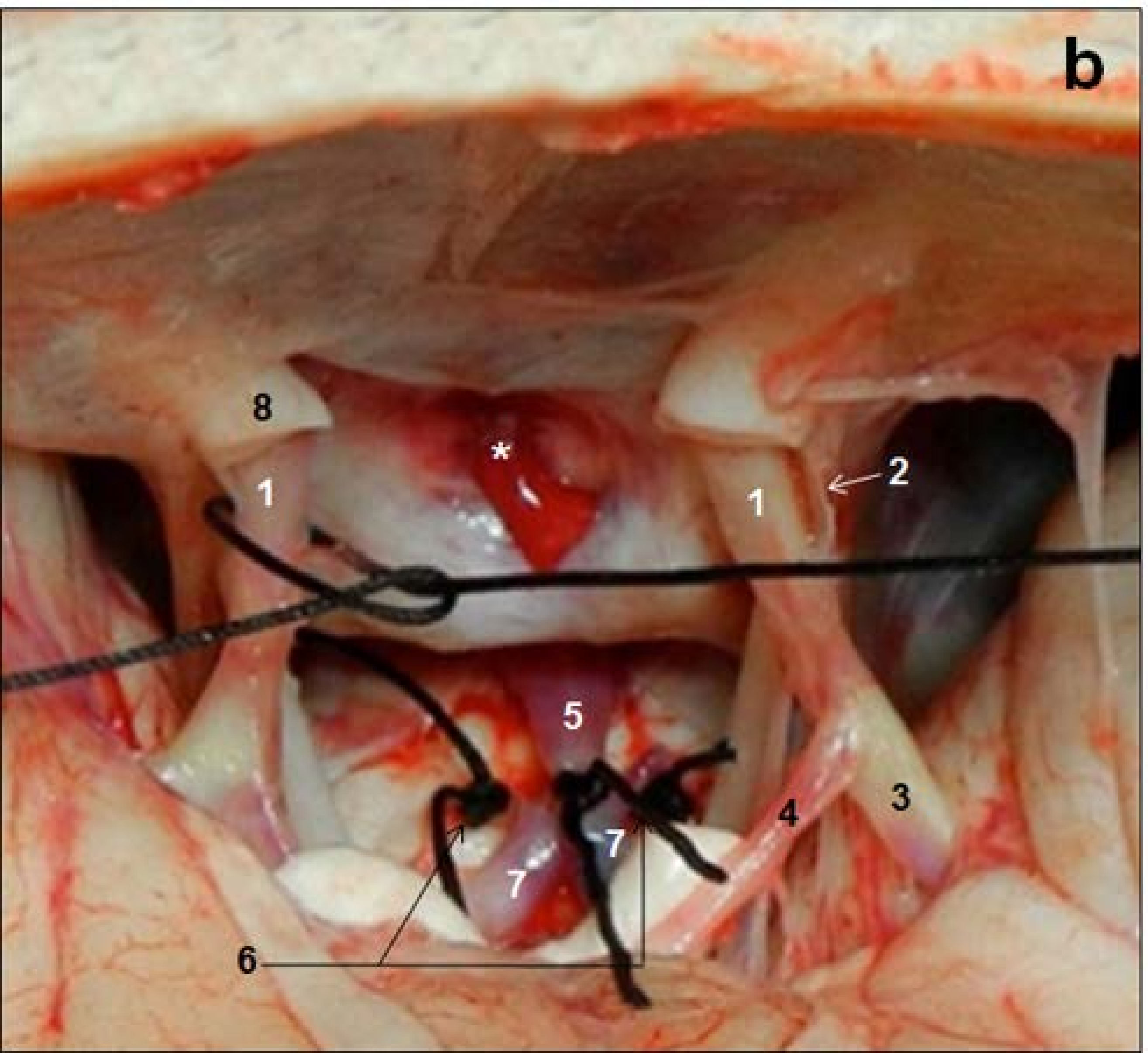
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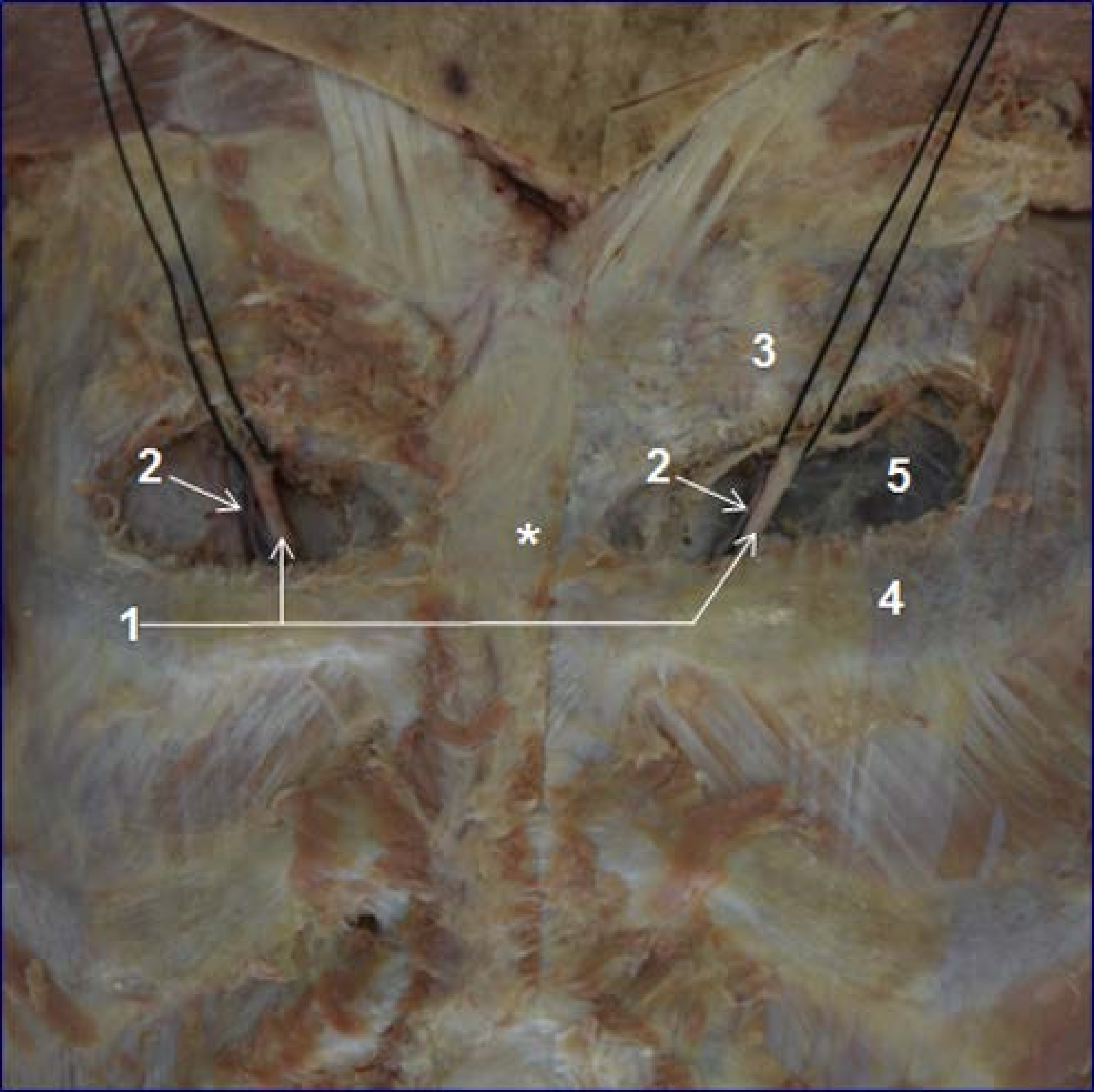

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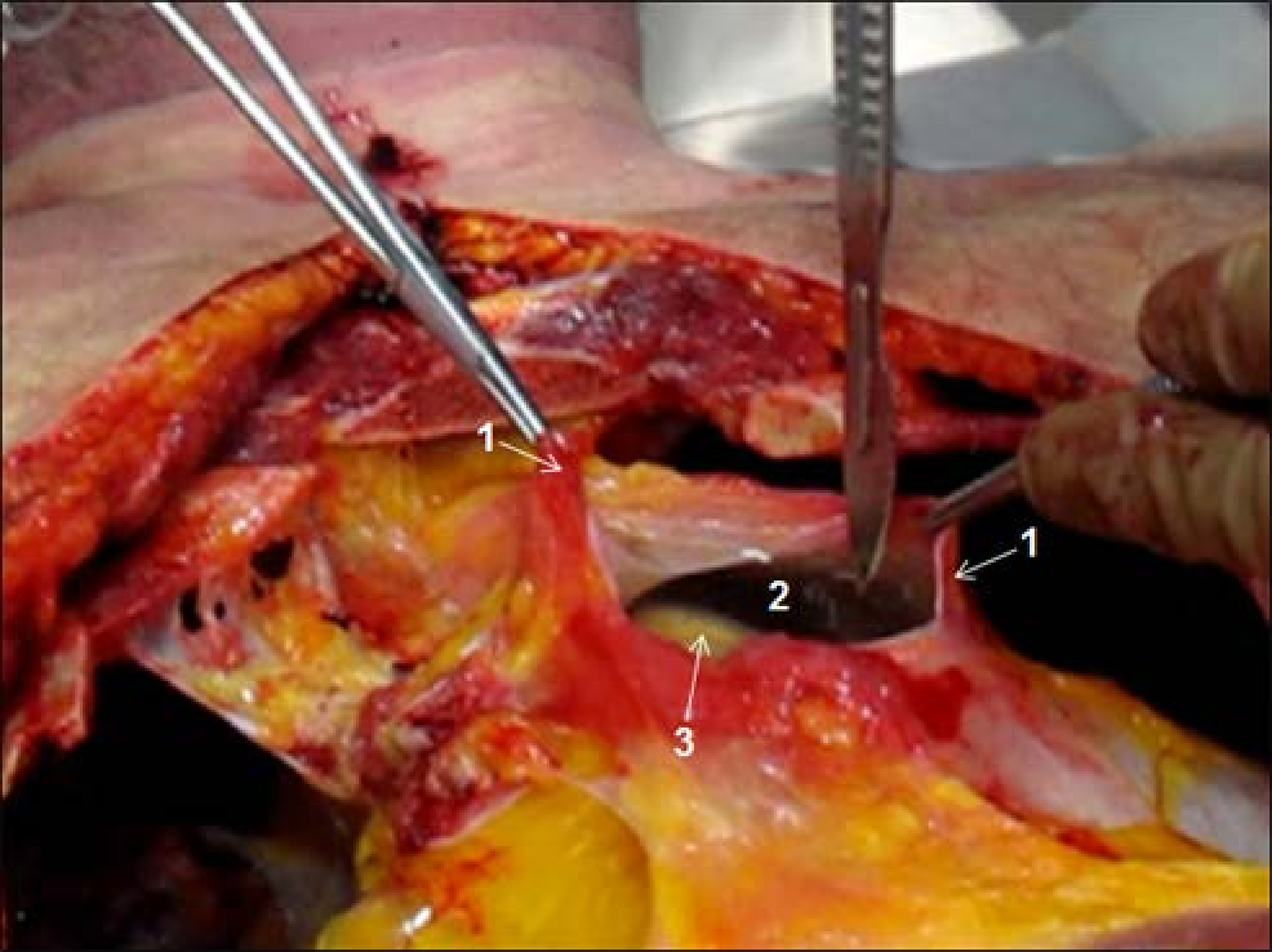
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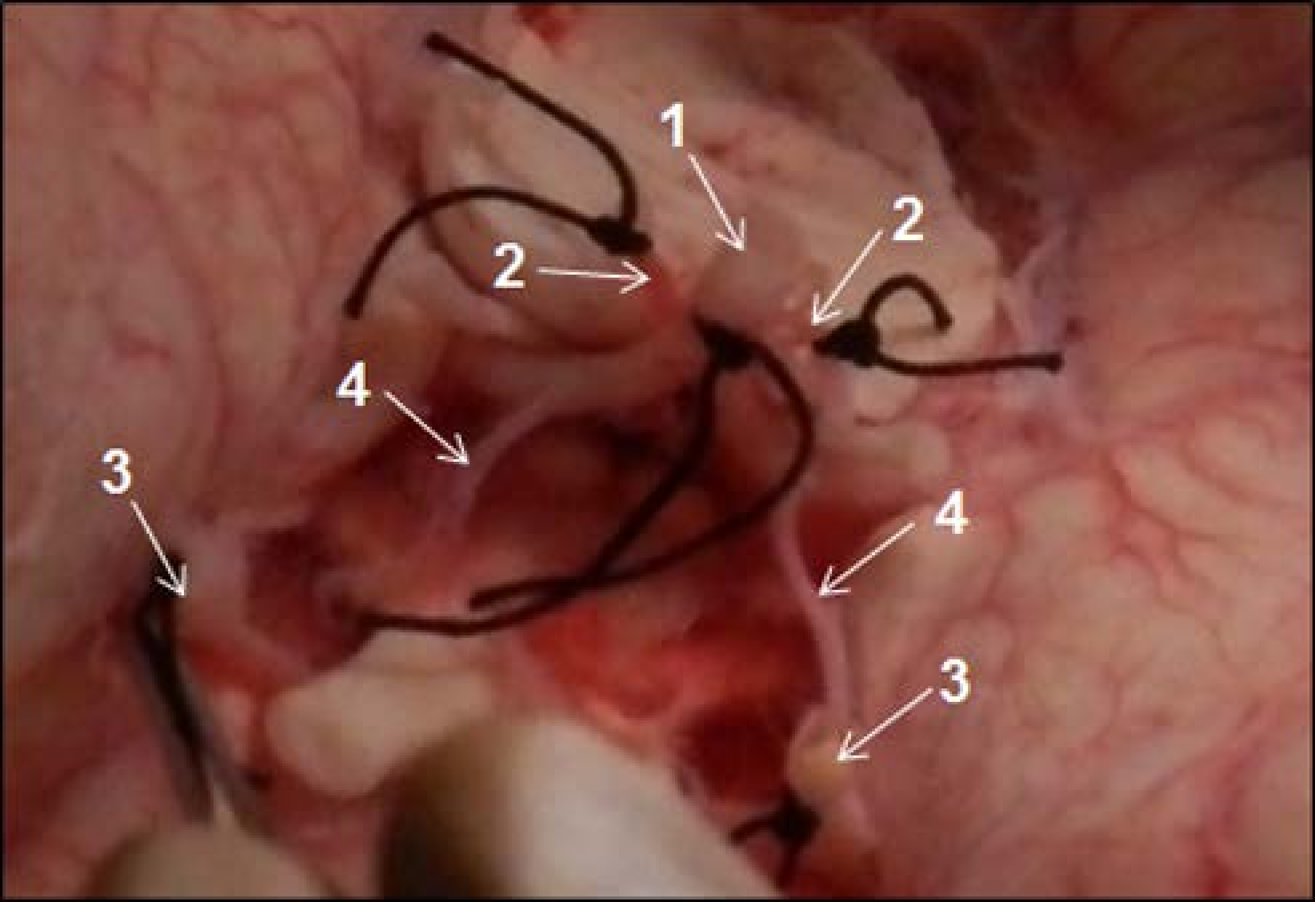
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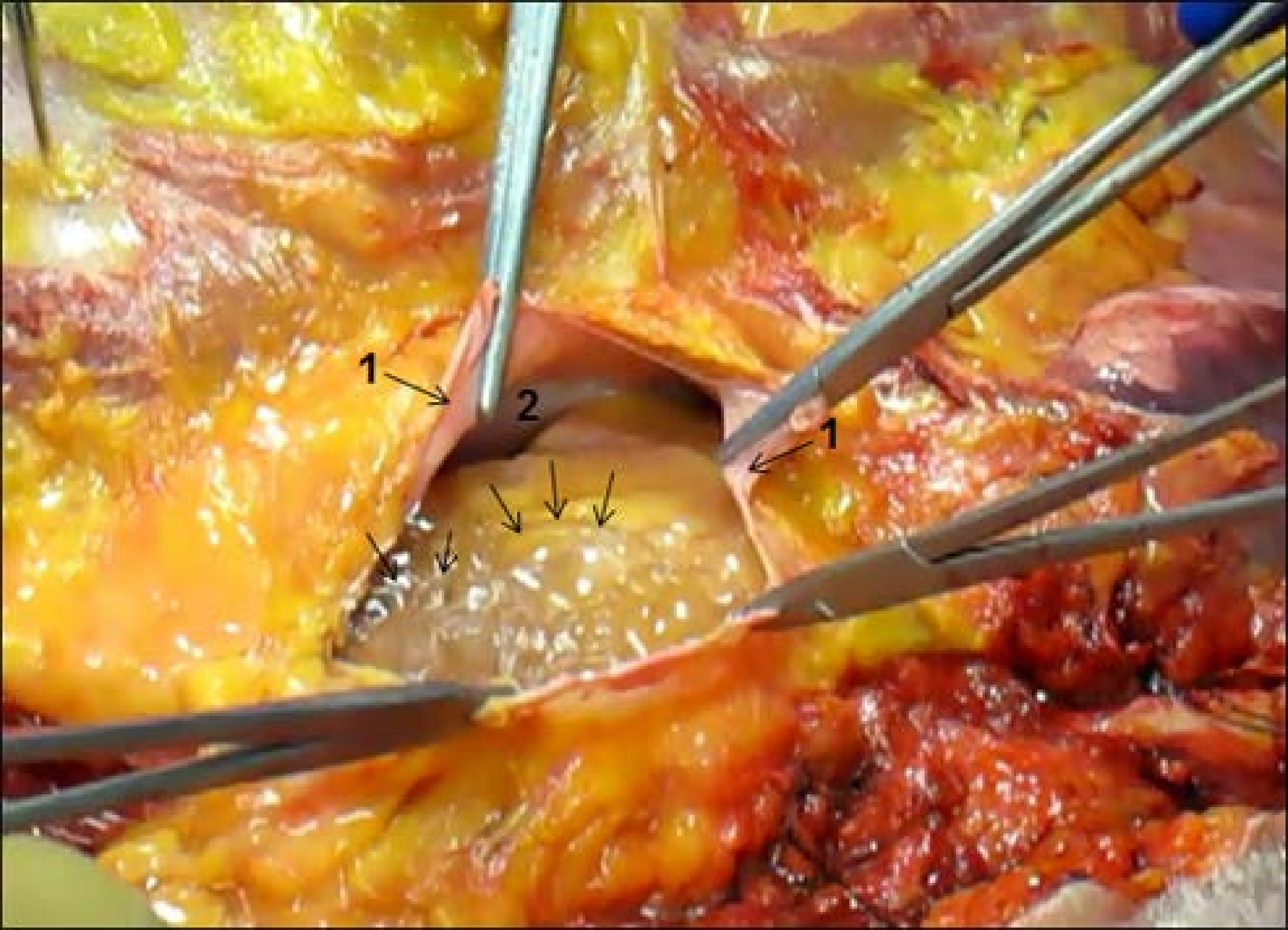
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