HISTOMORPHOMETRIC STUDY FOR THE DIFFERENTIAL DIAGNOSIS BETWEEN PULMONARY BAROTRAUMA IN SCUBA DIVING AND PULMONARY TAPHONOMIC CHANGES

Final Degree Project

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Lastly, I would like to thank my family who has been my pilar of strength and has given me unconditional support always.
ABBRVIATIONS

AGE: Arterial gas embolism
BMI: Body mass index
CNS: Central nervous system
CO₂: Carbon dioxide
DI: Decompression illness
DCS: Decompression sickness
DM: Diabetes mellitus
H: Hydrogen
IMLCFC: Institut de Medicina Legal i Ciències Forenses de Catalunya
N₂: Nitrogen
msw: Meters of sea water
µm: Micrometres
µm²: Square micrometres
%: Percentage
PFA: Paraformaldehyde
PB: Phosphate buffer
PM: Post-mortem
PMDA: Post-mortem decompression artifact
PMI: Post-mortem interval
PBT: Pulmonary barotrauma
PBT/AGE: Arterial gas embolism following pulmonary barotrauma
SCUBA: Self-contained underwater breathing apparatus

STR: Servei Tècnic de Recerca de la Universitat de Girona
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ABSTRACT

**Background:** Diving is a popular recreational sport on the Spanish coast. Despite the safety measures, deaths related to this activity are frequently reported. PBt/AGE has been described as the cause of death in SCUBA divers in 13-24% of the cases. The main impediment in the diagnosis of PBt/AGE is the existence of entities that can mask it and divert the cause of death, especially putrefaction. Studies have delved into this field to reinforce macroscopic autopsy, imaging, and gas analysis techniques. Microscopic studies have fallen short in refining the diagnoses. Therefore, we propose the histomorphometric analysis of lung tissue to provide objective quantitative data and more reliable information for the diagnoses of PBt/AGE and its differentiation from putrefaction.

**Objectives:** To describe and quantify the histomorphological changes observed in lung tissue samples for the differential diagnosis between pulmonary barotrauma in deaths due to SCUBA diving and taphonomic lung damage secondary to putrefaction.

**Design:** Cross sectional and descriptive study to be performed in Girona from 2020-2021.

**Population:** The study population will be divided in three groups: 1) Divers deaths from PBt/AGE, 2) Non-diving-related deaths and in an emphysematous evolutionary period, and 3) Non-diving-related deaths with low probability of putrefaction. All samples will be obtained from the Forensic Pathology Service of the Legal Medicine and Forensic Sciences Institute of Catalonia.

**Methods:** Pulmonary histological preparations will be sampled and analysed. The histomorphometric analysis will be done through a FIJI ImageJ2 (National Institutes of Health) image analysis programme.

**Keywords:** SCUBA-diving · Pulmonary Barotrauma, Arterial Gas Embolism · Histomorphometry.
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1. INTRODUCTION

1.1 EPIDEMIOLOGY

Diving is one of the most practiced underwater activities, having recently become an important business in many countries all over the world. Approximately 7 million divers are active worldwide and half a million more are training every year (1). It is one of the most popular underwater activities on the Spanish coast. The Girona coast in the Northeast of Spain, located 44 miles north of Barcelona, has optimal conditions for recreational diving with more than 150,000 dives using compressed gas registered per year (2). The most common breathing methods used in diving activities are SCUBA (self-contained underwater breathing apparatus) diving, rebreather (recirculating underwater breathing apparatus) diving, and surface – supplied breathing gas. Although it is considered to be a safe activity, serious accidents and fatalities are reported with each passing year (3).

Drowning has been the main cause of the increased fatalities in this ambit. It is defined as the process of experiencing respiratory impairment from submersion or immersion in a liquid medium, usually water (4). Yet, there is an array of possibilities that can lead a submersion to a fatal situation, and to reach the core cause a detailed examination is needed (see Annex 1). Although drowning has been reportedly the main cause of death of these recreational sport members; there are also other reasons due to which fatalities are reported each year, as mentioned previously. Some of them are arterial gas embolism (AGE), decompression sickness (DCS), natural pathology and trauma (3).

Decompression illness (DI) is caused by intravascular or extravascular bubbles that are formed as a result of a reduction in environmental pressure. This term includes AGE and DCS (3). Since time there has been an ongoing confusion when it comes to the terminology and the characteristics of both, DCS and AGE. But there are significant differences regarding both concepts which are important to differentiate as we aim to focus on AGE. At the same time, in order not to be confused by the terminology, it is important to know that AGE is the consequence that derives from pulmonary barotrauma (Pbt).
Therefore, the differentiation must take place between AGE following pulmonary barotrauma (PBT/AGE) and DCS (see Table 1). A study that took place in 1990 differentiated both terms with the following differential diagnosis (5).

**Table 1.** Differential diagnosis between DCS and PBT/AGE. Adapted from (5).

<table>
<thead>
<tr>
<th></th>
<th>Decompression Sickness (DCS)</th>
<th>Pulmonary Barotrauma (PBT/AGE)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Causal factor</strong></td>
<td>Inert gas supersaturation</td>
<td>Lung overexpansion</td>
</tr>
<tr>
<td><strong>Location of physiological disorder</strong></td>
<td>Fat-rich tissues</td>
<td>Air cell</td>
</tr>
<tr>
<td><strong>Initial bubble constitution</strong></td>
<td>Nitrogen (N₂)</td>
<td>Air</td>
</tr>
<tr>
<td><strong>Situation of extravascular bubbles</strong></td>
<td>Infiltrators throughout the anatomy: muscle, fat, bone, etc.</td>
<td>Subcutaneous emphysema “in cape”</td>
</tr>
<tr>
<td><strong>Preferred path of bubbles</strong></td>
<td>Venous circulation</td>
<td>Supra-aortic arterial circulation</td>
</tr>
<tr>
<td><strong>Preferred destination of bubbles</strong></td>
<td>Lumbar spinal cord</td>
<td>Brain</td>
</tr>
<tr>
<td><strong>Fat embolism</strong></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td><strong>Technical requirements</strong></td>
<td>Long deep dive</td>
<td>2-3 m deep and having breathed once</td>
</tr>
<tr>
<td><strong>Free time interval</strong></td>
<td>0-24 hours</td>
<td>Immediate</td>
</tr>
<tr>
<td><strong>Skin lesion</strong></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td><strong>Pneumothorax or pneumomediastinum or pneumopericardium</strong></td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Subcutaneous emphysema</strong></td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Associated abdominal pathology</strong></td>
<td>Rare</td>
<td>Possible</td>
</tr>
<tr>
<td><strong>Predominant clinical picture</strong></td>
<td>Monoparesis or paraplegia</td>
<td>Hemiplegia or tetraplegia</td>
</tr>
<tr>
<td><strong>Severity of hemodynamic condition</strong></td>
<td>+</td>
<td>+++</td>
</tr>
</tbody>
</table>

* Both phenomena can be intermingled, although the most frequent is the dissociation mentioned in the table.

**1.2 PULMONARY BAROTRAUMA**

Recently, researchers are gaining interest in PBT/AGE for it has been described as the cause of death in SCUBA diving in 13-24% cases, being documented in renowned medical texts of diving pathology (3).

Barotrauma is the general name for an injury caused by pressure change (6). It is defined as a physical damage to body tissues caused by a difference in pressure between a gas space inside, or in contact with the body, and the surrounding fluid (7).
PBt occurs in the ascent phase of a dive using compressed air or other gas mixtures. Under increased ambient pressure, the air inhaled from the bottles decreases in volume in the lungs. Per Boyle’s Law, this volume is in inverse proportion to the ambient pressure. So, as ambient pressure decreases during ascent, the volume of air in the lungs increases accordingly (6,8). For a few moments, the thoracic cavity becomes a pressure container, which maintains the intrathoracic pressure higher than the ambient one (5). If this increased volume of air is not exhaled, over-distension of the lung is the result (6,8,9) and the trapped air then seeks an exit through different routes, entering canals and virtual spaces (5).

Interestingly, PBt is not necessarily confined to deep diving, and in fact, the risk of PBt is highest in shallow water, because the ambient pressure doubles between 0 and 10 meters of seawater (msw), and the relative volume change is greatest at that depth range (9). Therefore, depth cannot be a definitory aspect when it comes to the autopsy phase. So, when a SCUBA diver ascends to the surface too quickly, the gas retained in their lungs over-expands as the pressure decreases rapidly, causing PBt (3).

PBt not only occurs during a rapid ascent or a compromised situation where air is not able to be vented but, studies have shown that the development of PBt is attributable to four situations in total (10):

1. Involuntary laryngospasm on ascent caused by the water entering the airway, loss of consciousness, panic, etc.
2. Deliberate or accidental stoppage of exhalation while ascending, even for a short period (Valsalva manoeuvre, cough).
3. A sudden increase in the volume of the breathing mixture supplied by the diving apparatus.
4. An exceedingly rapid ascent, regardless of the reason.

Hence, divers encounter pressure-related lung injuries which are caused by the expansion of gas trapped in the lung during ascent with subsequent overexpansion and rupture of the alveolar air sacs (11). As mentioned, this can occur during ascent if divers are holding their breath or if a blockage in any part of their airway impedes exhalation. Buoyancy problems, panic, and out-of-air situations often predispose to a rapid ascent and a subsequent injury.
Underwater distractions such as photography, work, reduced visibility, new gear, etc., can also be contributing factors (6).

In all cases, the first step is rupture of the alveolus with a collection of air in the lung tissues, a condition known as interstitial emphysema (9,11). Interstitial emphysema causes no symptoms unless further distribution of the air occurs. Gas may find its way into the chest cavity or arterial circulation. If the latter situation takes place, potentially fatal AGE may occur. Pneumothorax arises when gas accumulates between the lung and chest wall and if this accumulation continues without any escape, then tension pneumothorax may ensue. These conditions are depicted in Figure 1 (11).

![Diagram of Pulmonary Barotrauma](image)

**Figure 1.** Clinical consequences of PBt. Adapted from (11).

So, as shown in Figure 1, overinflation of the lungs can result in air escaping from the lungs into surrounding tissues and can cause pneumomediastinum (air in the mediastinum), pneumopericardium (air in the sac surrounding the heart), subcutaneous emphysema (air trapped under the skin), pneumothorax (air between the lung and the chest wall) or AGE (6,8).
When alveoli rupture, the escaping gas can either enter the pulmonary venous system and access the left side of the heart (3) or escape into the lung interstitium. The former process will cause venous stasis, obstruct vessels, and cause ischaemic and endothelial injury of the tissues. Animal experiments suggest that gas bubbles not only may be formed in perivascular tissues and are admitted into vessels through endothelial gaps, but intravascular bubbles can also be formed as a result of PBt when expanding gas stretches and ruptures alveolar capillaries and enters the arterial circulation, commonly referred to as AGE (9).

Rarely, the volumes of gas are so great that the left ventricle becomes air-locked and the diver will die instantly (6). More commonly, smaller and variable amounts of gas are dragged into the arterial circulation (6,9). The bubbles tend to distribute with the flow; thus, those organs receiving a significant proportion of the cardiac output, particularly the brain, are likely to suffer the greatest exposure to bubbles. There is also some evidence that the distribution of bubbles in large blood vessels, particularly larger bubbles, can also be influenced by buoyancy. Therefore, in an upright diver (e.g. during ascent, when PBt is most likely to occur), larger bubbles tend to track around the roof of the aortic arch and are more likely to enter the vessels supplying the upper body and brain (6). Consequently, if air bubbles reach the cerebral arterial circulation, they can interrupt encephalic blood flow with resulting ischemia, brain anoxia, and death (3).

On the other hand, an escape into the interstitium allows gas to track along the outside of the pulmonary airways and blood vessels toward the hilum where the pleura is discontinuous. Its subsequent escape into the mediastinum produces mediastinal emphysema. From there, gas can track upward along the trachea to stay at the base of the neck, giving rise to subcutaneous emphysema. Finally, if there is a rupture of alveoli adjacent to the visceral pleura, then gas may enter the pleural cavity and produce a pneumothorax (6,9).

As mentioned previously, subcutaneous emphysema occurs due to the breaking of the alveolus or an injury with disruption of the bronchial mucosal surface, which makes the airstream go to the connective tissue. However, 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 manoeuvres (3). To sum up, Figure 2 depicts all the above-mentioned phenomena which can occur after a PBT.

![Figure 2: Physiopathology of clinical outcomes of PBT](image)

**Figure 2.** Physiopathology of the clinical outcomes of PBT. Adapted from (12)

### 1.3 ARTERIAL GAS EMBOLISM

The most feared and probably the most common event mentioned beforehand is the AGE. This occurs when air escapes from the alveoli enter into the pulmonary vasculature and the arterial system, where it blocks circulation (6). It is an obstruction of blood flow caused by gas emboli entering the arterial circulation. Once it enters the circulation, it will be dispersed to all organs of the body (11).

The passage of these bubbles through the circulation is interrupted by the systemic capillary beds. Bubble behaviour and effects at this point are largely influenced by their size. Bubbles that are large enough that their leading end occupies several branching arterioles
may stick and obstruct the flow. Smaller bubbles can redistribute through the microcirculation and thus cause minimal obstruction. Even larger bubbles redistribute in this way as the gas inside them is absorbed and they shrink (6).

The organs that are especially susceptible to AGE and that are responsible for the life-threatening symptoms are the central nervous system (CNS) and the heart (6,9,11). The resulting cerebral dysfunction manifests most commonly as sudden-onset unconsciousness and/or multifocal stroke-like events (6). In all cases of AGE, associated pneumothorax is possible and should not be overlooked. Exhaustion of air supply and the need for an emergency ascent is the most common cause of AGE (11).

1.4 DIAGNOSTIC CRITERIA OF PBT/AGE

It is demonstrated that PBT/AGE is given as the pathological cause of death if the following four criteria are met (3):

- 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 artifact (PMDA) or decomposition
- Mediastinal or subcutaneous emphysema limited to the perithoracic area and/or pneumothorax

Therefore, to establish a PBT/AGE post-mortem (PM) diagnosis, it is essential to know the descents dive profile and use specific autopsy techniques and/or image diagnoses (3). And, to establish that, expert personnel in underwater activities is required (9) (see Annex 2).

To begin with, the autopsy should be directed to confirm or exclude other aspects not related to PBT such as drowning, poisoning by gases and, natural or traumatic pathology (13). To follow, PM radiology of the thorax and abdomen is recommended as it allows us to show the existence of large volumes of intravascular gas (14) and clinical features that result from the expansion and leakage of gas (9).
External examination puts into evidence diverse alterations found on the corpse of a diver. Starting with the pink coloration of the skin, which is explained due to the effect of hypothermia (9,15). Other signs like, reddish linear marks, crepitus on palpation of the skin, eardrum examination and the variable distribution of the cadaverous lividities can be of guidance. Typical findings of drowning such as the presence of foam fungus, anserine complexion, skin maceration, etc., should be discarded (9,16).

The internal examination begins with an autopsy of the skull. The existence of air bubbles in the cerebral veins must be considered an artifact as it is impossible to remove the calvarium without aspirating some air into the vessels (9). To detect AGE, isolation of the circulation of the cerebral arteries (internal carotid arteries, basilar artery) is necessary. To investigate the presence of air in these arteries, we extract the brain and immerse it in water, observing the exit of bubbles when removing the forceps placed on the cerebral arteries. The existence of pneumothorax can be demonstrated by imaging or chest autopsy (3). We do the latter by conducting a thoracic fluid test. A sac is formed on the thorax and is filled with water, then a puncture is made at an intercostal space and if there is a pneumothorax, a fine bubbling will be observed. Also, the lungs will be collapsed and reduced in volume. A similar procedure can be performed with the pericardium (pericardial hydric test) (3,16,17). As most deaths related to diving are caused by drowning, we will proceed with the autopsy routine typical of submersion cases (9).

Complementary examinations are helpful. Histological and toxicological studies will be carried out to better understand the root cause. Also, Lawrence proposed a chemical analysis of the intrapericardial gas instead of the pericardial hydric test (14). Lastly, to confirm or exclude drowning as the cause of death, microscopic and biochemical tests are ought to be performed (13). Samples of lung fluid and other tissues should be taken into consideration for further demonstration of the possible existence of diatoms and other contaminants. And, if necessary, take samples from the immersion medium where the corpse was found (16).
The main interest of PM examination in diving accidents is to study gas collections, especially intravascular. However, many other phenomenon such as decomposition (putrefaction), resuscitation manoeuvres, DCS, trauma and PM tissue off-gassing can also cause PM release of gas which can hamper an accurate diagnosis of barotrauma (18,19). Apart from imaging, different techniques have been used to be able to reach a correct diagnosis but they also fall short when the results are overlaid with other phenomenon or they are time dependent (putrefaction). When studying the presence of intravascular air, unfortunately, it is very common in diving autopsies and it is not specific to PBt/AGE; it can also be due to decomposition (putrefaction), explosive decompression, PMDA and/or resuscitation. Studies have stated that when intravascular gas corresponds to an advanced decomposed body in the emphysematous period, it is not possible to assure its intravit origin which is characteristic of gas embolism (3). Researchers have also delved into gas composition analyses which are used to differentiate between gas embolism and putrefaction gases. But, these analyses are effective before a threshold PM time, making it critical to perform autopsies promptly (3,20). So, although newer techniques may provide alternative ways of viewing bodies, organs, and tissues, the artifacts induced by putrefaction still require interpretation (17).

It is important to comment on a field of knowledge which goes hand in hand with the forensic studies and should be considered when analysing the state of a cadaver, that is, taphonomy. Taphonomy is defined as the study of the environmental conditions affecting the preservation of animal or plant remains. Forensic taphonomy refers to the use of taphonomic models and analysis in forensic contexts to estimate the time since death, reconstruct the circumstances before and after deposition and discriminate the products of human origin from those created by biological, physical, chemical, and geological ways (21).

Decomposition refers to the variety of processes of degradation that commence as soon as an organism has died (17). During the process of the forensic study there is a key aspect which can intervene and distort the results, i.e., putrefaction, which forms one of the stages of decomposition.
Putrefaction is defined as a continual process based on tissue breakdown of the corpse by microorganisms such as bacteria, fungi, and protozoa, from the intestine and the environment, where a complete disintegration is going to take place following autolysis and this results in the production of gases, liquids, and simple molecules (22).

Putrefaction evolves in four phases (23,24):

1. **Colorative or chromatic period**: It begins with the first objective symptom of putrefaction, the green spot, initially located in the right iliac fossa where the concentration of microorganisms is higher. This period begins 24 hours after death, lasts several days and to it the phenomena of the second phase are added gradually.

2. **Emphysematous or gas development period**: It is characterised by the development of a large amount of gas that will cause the corpse to bulge. The gaseous infiltration invades all the subcutaneous cellular tissue. This period lasts for several days, sometimes up to a couple of weeks.

3. **Colliquative phase**: The epidermis detaches from the dermis. The gases go out through the natural orifices and the body starts losing the puffy appearance. This phase lasts for several months, generally 8 to 10.

4. **Skeletal reduction**: All the soft parts of the corpse will gradually disappear throughout from 2 to 5 years, remaining only the skeletal parts of the body.

According to studies, putrefaction is evident after about 24 hours if the body is not refrigerated, although the onset varies from 3 to 72 h, depending on the environmental conditions and the gas volumes being detected (22). Concerning the putrefaction gas, studies (18) have shown that this gas is not present on the arterial topography, at least for the first 24 h but it provokes intravenous and portal gas collections starting around 6 h after death. Hence, to affirm a death by barotrauma followed by a gas embolism, PM scanner should be conducted very early. And that is one of the limitations when relying on imaging. Also, subcutaneous emphysema should not be mistaken as diagnostic criteria of barotrauma because it can also be caused by resuscitation manoeuvres.
Bernaldo de Quirós et al., took out a study on rabbits so as to determine the relation between the appearance of gases, its composition, and its PMI. It is a useful study for researchers to have an idea of the importance of this field and the necessity of further studying. The study revealed that the first bubbles in putrefaction appeared in 6 hours but were minimal, the maximum and significant amount appeared 67 hours PM. Whereas in AGE, gas was detected immediately after death and with time it redistributed all around the body. The absence of gas bubbles in rabbits necropsied immediately or within a few PM hours is the most important result obtained from the putrefaction model in the study. This indicates that a certain level of putrefaction must be reached to produce enough gas to favour the gas separating from fluids, or at least to be large enough to be macroscopically identified.

In the putrefaction model, gas scores were very low during the first PM hours. Moderate scores were reached after 27h PM. As expected, gas composition analyses performed on the same animals showed statistical differences in the composition of the gas up to 27h PM. After this time putrefaction gases would mask the original gas composition of the emboli. Putrefaction gases were characterized by the presence of hydrogen (H), it was found in most of the samples taken after 27h PM, but it was absent in fresher samples. Therefore, both the gas score method and gas analysis showed similar results and they indicate the importance of performing autopsies in a timely manner in cases where air embolism or diving accidents are suspected.

As shown in Figure 3, it depicts the total gas score of each group in relation to the PM time at which each group was examined. In the air embolism model, gas score increased during the first PM hours, suggesting that the dissolved gas carrying capacity of the blood might be higher during circulation than in a static mode. Thus, some of the gas would come out from solution once the blood circulation has stopped (PM off-gassing). An alternative explanation would be that microscopic bubbles might coalesce to form larger macroscopic gas bubbles (20).
Therefore, timing is also relevant. Extra-alveolar gas from gas embolism derived from PBt is almost immediate and increases with time. DCS usually takes some minutes or up to an hour to develop, but then also increases over the next hours. PMDA is slower, usually developing over the first few hours, except in extreme exposures. Putrefaction is detectable by its odour and usually takes over 24 h to become clinically evident (19).

As mentioned, studies also suggest that gas analyses have been shown to be a valid technique to differentiate between putrefaction gases and gas embolism. Bernaldo de Quirós et al., demonstrated that infused air embolism and compression/decompression models had a similar gas composition prior to 27 h PM, being typically composed of around 70–80 % of nitrogen (N₂) and 20–30 % of carbon dioxide (CO₂). And, gas composition of samples from more decomposed animals and from the putrefaction model presented H, which was therefore considered as a putrefaction marker (22).
So, this study (22) which aimed to compare and analyse gas produced in AGE and putrefaction, concluded that differentiation of in vivo gas embolism from putrefaction gases was possible until 27 hours PM where H was absent. Previous studies and this study also agreed in the appearance of H after 42 hours PM in the case of an AGE, when a mix with putrefaction gases was produced. After 67 hours PM abundant subcapsular emphysema was also observed. Thus, the presence of putrefaction gases does not rule out the possibility of an in vivo gas embolism. Simply, it cannot be confirmed or denied through gas analysis.

These model studies can have altered results due to multiple factors so, it is important to address the existence of contributing factors that can alter the evolution of putrefaction and therefore, the result. Firstly, referring to the individual influences, the body mass index (BMI) has a great impact on the decomposition process, where corpses with higher BMI experience a rapid decomposition in comparison to those with normal or low BMI. As obesity speeds up decomposition it slows down body cooling rates. The temperature gradient, which drives cooling, varies with the mass of the body and the surface area as well as with the conducting properties of the tissues. The amount of subcutaneous and abdominal fat will affect the insulating properties and hence the temperature gradient, but there is no way of assessing or correcting accurately for obesity (25). Obesity may also be associated with diabetes mellitus (DM) and sepsis, both of which encourage microorganism growth within tissues. Therefore, accelerated decomposition at autopsy may occasionally provide a useful clue for the presence of an underlying condition such DM (17). Other complications of medical conditions other than DM such as cardiomyopathies, hypertension, congenital heart diseases, asthma and chronic obstructive lung diseases, and epilepsy have all been potentially associated with SCUBA diving fatalities and should be discarded during the autopsy (15). Additionally, the presence of pre-existing emphysema can be a sign of a smoker and it can alter the examination.

In terms of age, it is shown that putrefaction is faster in children and slower in the elderly, with adults evolving in an intermediate way (24). The post-mortem interval (PMI) plays an important role. As mentioned, when gas embolism is suspected as a cause of death, it is important to determine the origin and cause of gas embolism. However, a study that analysed marine mammals concluded that the main problem in these cases is to differentiate between
gas embolism and gases produced PM due to putrefaction since the time of death is frequently unknown (20). Therefore, determining the time of death cannot be done with any precision, and it is even difficult if we are dealing with a submerged corpse. PMI of in land corpses can be identified easily as entomological examination of insects and the temperature of maggot masses may provide some guide to this (17).

As stated, in the evolution of cadaveric putrefaction, it is well known that it is influenced by the temperature of the environment in which the corpse is submerged. It can be said that as long as the corpse remains submerged, the putrefaction seems to follow a slower rate than if it is found in the open air (12). Because divers who die underwater are exposed to environmental cooling influences, therefore the process is usually delayed in this group (19). Once the corpse has been recovered from the water, putrefaction accelerates considerably (26). Though the fact that a dead body becomes progressively colder after death, a uniform, homogeneous laboratory ‘body’ will cool according to Newton’s Law of Cooling, which states that the rate of cooling is proportional to the difference in temperature between the body surface and its surroundings (25).

As stated, temperature is the major determinant of the rate of putrefaction. Also, one study compared the length of time in the water and the time of year it occurred to see if there are significant changes in the decomposition process. The results confirm the impact of the time spent in the water on some of the morphological changes analysed (27).

On a positive note, internally, decomposition proceeds more slowly than at the surface. It is often quite surprising how valuable an autopsy on a putrefying corpse can be, as the internal organs may be in far better condition than the exterior would suggest (25).

All in all, PM time is unknown on many occasions, and putrefaction processes are dependent on the weather, especially temperature and humidity (22,25).
2. JUSTIFICATION

Autopsies of SCUBA diving-related deaths are a big challenge. It is advisable for people undertaking such autopsies to have knowledge about diving physiopathology and experience in special autopsy techniques (3). The main challenge lies in the fact that the presence of intravascular air is very common in diving autopsies and is not specific to PBT/AGE. It can also be due to decomposition (putrefaction), decompression sickness, PMDA and/or resuscitation (3). Also, decomposition carries many challenges for forensics because findings that may have been discriminant for the diagnosis of cause of death may disappear with decomposition (28).

Although gas analyses have shown to be a valid technique to differentiate putrefaction gases and gas embolism (22), there is little information regarding the histomorphological field. Therefore, the latter can be a useful tool to determine the morphological changes which can go by unseen and can be helpful during the diagnosis. There are cases where the conventional histopathological analysis takes place, but we consider that it does not allow an adequate evaluation of the dimension of air spaces. Hence, we propose the morphometric analysis to provide objective and more trustworthy data for the diagnosis. This technique has been employed in different studies, but it is yet to be utilized in deaths in context of PBT/AGE.

All this leads us to consider that differentiation between gas embolism and putrefaction gases might be PMI and decomposition status dependent. Therefore, this project might help us in those situations where time is not necessarily on our side. Plus, there is scarce literature when it comes to morphometric comparison between PBT and putrefaction and more research is necessary indeed. This project has the main objective of trying to find significant histomorphometric differences between PBT and putrefaction as both situations can be a source of gas production and can difficult the correct diagnosis. Maybe, this study will make way for a different yet effective approach for differentiating PBT from putrefaction specially when PMI is significantly high. The forensic diagnoses improvements will not only be essential for scuba diving fatality management but also very important to better understand the physiological mechanisms implied in fatal gas embolism in general.
3. BIBLIOGRAPHY


4. HYPOTHESIS

A pulmonary barotrauma in cases of deaths by SCUBA diving conditions histomorphological changes in the pulmonary tissue that must be distinguished from taphonomic changes secondary to putrefaction.

5. OBJECTIVES

With the aim of confirming the hypothesis, the following goal has been proposed:

1. Describe the histomorphological changes observed in lung tissue samples from forensic autopsies of PBt/AGE in context of SCUBA-diving as compared to the changes in taphonomic lung damage secondary to putrefaction.

2. Quantify the histomorphological changes and identify statistical differences in the histomorphological analysis between pulmonary barotrauma in cases of deaths due to SCUBA diving and taphonomic lung damage secondary to putrefaction.
6. METHODOLOGY

6.1 STUDY DESIGN

The design of the study is cross sectional and descriptive.

6.2 STUDY POPULATION

The target population of the study are people who died during SCUBA diving with a PM diagnosis of PBt/AGE and non-diving related deaths in an emphysematous evolutionary period.

6.3 STUDY SAMPLE

The study sample is based on deaths from diving diagnosed of PBt/AGE, non-diving-related deaths in a state of putrefaction and deaths not related to diving with low probability of pulmonary decomposition. All samples will be obtained from autopsies performed in the Forensic Pathology Service of the Legal Medicine and Forensic Sciences Institute of Catalonia.

The study will have 3 groups:

- Group 1: Eight divers who have been diagnosed with an arterial gas embolism after a pulmonary barotrauma as a cause of death according to specific protocols and recommendations for this type of death.
- Group 2: Eight cases of deaths not related to diving and in an emphysematous evolutionary period will be selected as a positive control group.
- Group 3: the negative control group will include eight cases of non-diving-related deaths, in which a low probability of pulmonary decomposition was expected (PM less than 30 hours).
### 6.4 INCLUSION AND EXCLUSION CRITERIA

<table>
<thead>
<tr>
<th>GROUP</th>
<th>INCLUSION CRITERIA</th>
<th>EXCLUSION CRITERIA</th>
</tr>
</thead>
</table>
| 1     | Deaths diagnosed of Pbt/AGE in a context of SCUBA diving by a macroscopic autopsy | - Age over 65 years  
- Use of high-pressure oxygen therapy in the context of advanced cardiopulmonary resuscitation (CPR) manoeuvres |
| 2     | Deaths not related to diving and in an emphysematous evolutionary period | - Admission to an intensive care unit of a hospital |
| 3     | Non-diving-related deaths with low probability of pulmonary decomposition (PMI < 30 hours). | - The existence of chronic pulmonary emphysema in accordance with the review of the clinical history and the results of routine histological studies |

We consider the macroscopic diagnose of **Pbt/AGE** if the following four criteria are accomplished:

- History of rapid ascent followed by a loss of consciousness.
- Air in the left side of the heart and circle of Willis.
- Low probability of PMDA or decomposition.
- Mediastinal or subcutaneous emphysema limited to the perithoracic area and/or pneumothorax.

We consider the macroscopic and complementary diagnose of **putrefaction** if the following criteria are accomplished:

- Macroscopical description of the emphysematous evolutionary period of putrefaction which is characterised by the development of a large amount of gas that will cause the corpse to bulge. The gaseous infiltration invades all the subcutaneous cellular tissue. This period lasts for several days, sometimes up to a couple of weeks.
6.5 SAMPLING

SAMPLE SIZE

The size of the sampling has been calculated with the *GRANMO Sample size and Power calculator* for proportions’, means’, and medians’ comparison.

Assuming an alpha risk of 0.05 and a statistical power of 0.8 in a bilateral contrast, 8 subjects are needed in the group of deaths by Pbt/AGE, 8 subjects are needed in the group of non-diving-related deaths found in an emphysematous evolutionary period and 8 subjects are needed in the group of non-diving-related deaths with low probability of pulmonary decomposition (PMI < 30 hours).

Due to previous studies and forensic expertise, from all the above-mentioned dependent variables, we have chosen the total area occupied by airspace as the most important to calculate the sample size. The number of subjects mentioned are necessary to recognize as statistically significant a difference greater than or equal to 150,000 µm². The common standard deviation is assumed to be 102,849. As expected, a drop-out rate of 0% is anticipated.

SAMPLE COLLECTION

In all the autopsies, two randomly selected lung tissue samples of the upper pulmonary lobes will be taken, in non-hypostatic central and peripheral areas, and 10% will be fixed in formalin. We section the samples with scissors and select them with a measurement of 2x2.

For group 1, it has consisted in collecting lung tissue samples of all autopsied corpses diagnosed of Pbt/AGE recorded in the Mediterranean Sea (coast of Girona, northeast of Spain) between January 2009 and August 2020.

The PM macroscopic diagnosis of this group has been done in a previous study of our investigation group, to make sure it accomplishes the previous inclusion criteria.
For group 2 and 3, the samples will be collected from January 2021 onwards. We do not have availability of these samples used in a previous investigation therefore, the sample collection process commenced after the acceptance of the *Comité de Ética de Investigación Clínica del Hospital Universitario de Bellvitge*.

### 6.6 VARIABLES

**INDEPENDENT VARIABLE**

- Presence or absence of pulmonary barotrauma: A dichotomous categorical qualitative variable. It will be expressed as presence or absence.

**DEPENDENT VARIABLES**

For each field we will calculate the following:

- Airspace count: A continuous quantitative variable. It will be expressed in unities.
- Total area occupied by airspace: A continuous quantitative variable. It will be expressed in square micrometres ($\mu$m$^2$).
- Percentage of the field occupied by the airspaces compared to the rest of the parenchyma: A continuous quantitative variable. It will be expressed as a percentage (%).
- Maximum diameter of each air collection: A continuous quantitative variable. It will be expressed in micrometres ($\mu$m).
- Minimum diameter of each air collection: A continuous quantitative variable. It will be expressed in micrometres ($\mu$m).

The parameters studied in each image will be calculated automatically and exported to an MS Excel table.
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<thead>
<tr>
<th>VARIABLE</th>
<th>TYPE</th>
<th>TECHNIQUE</th>
<th>EXPRESSION</th>
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</thead>
<tbody>
<tr>
<td>Presence or Absence of PBt</td>
<td>Dichotomous categorical</td>
<td>Macroscopic autopsy</td>
<td>Presence or Absence of</td>
</tr>
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<td>qualitative variable</td>
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<td>PBt</td>
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<td></td>
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<td>Health</td>
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<tr>
<td>Total area occupied by airspace</td>
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<td>ImageJ National Institutes of</td>
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<td>Health</td>
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<tr>
<td>Percentage of the field occupied by the</td>
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<tr>
<td>Maxim diameter of each air collection</td>
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<tr>
<td>Minimum diameter of each air collection</td>
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<td></td>
<td>variable</td>
<td>Health</td>
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</tbody>
</table>

**COVARIABLES**

- **Sex:** Male or Female → A dichotomous categorical qualitative variable
- **Age:** The information will be obtained from the medical history of the corpse or through relatives. Years. → A continuous quantitative variable.
- **Body mass index:** The corpse is measured from head to toe and is weighed on the same table where it will be studied. Kg/m² → A continuous quantitative variable.
- **PMI:** It is considered from the time of death, which appears in the autopsy report, until the beginning of the autopsy. Hours → A continuous quantitative variable.
- **Smoking:** The information will be obtained from the medical history of the corpse or through relatives. Yes or No. → A dichotomous categorical qualitative variable.
- **Pre-existing health condition**: DM, cardiomyopathies, hypertension, congenital heart diseases, asthma and chronic obstructive lung diseases, and epilepsy. The information will be obtained from the medical history of the corpse or through relatives. Descriptive. → A non-dichotomous categorical qualitative variable.

- **Temperature of the water**: The information will be gathered from the database. °C. → A continuous quantitative variable.

### 6.7 DATA COLLECTION

#### HISTOLOGICAL SAMPLE

The technical processing used to make the histological preparations consist in:

1. **Fixing the samples of the different pulmonary lobes in a formalin solution**

   These tissues were perfused with a solution of 4% paraformaldehyde (PFA) in phosphate buffer (PB) 0.1 M pH 7.4. It is important to adjust the final pH of the solution to ensure good tissue preservation.

2. **Dehydration by alcohol**

   All tissues have been postfixed for a period of 10 hours. Before beginning to process them, they are washed at least for 12 hours in 70% ethyl alcohol.

   Dehydration is started by staining the samples with a 0.5% eosin solution dissolved in 96% ethyl alcohol for 30 minutes to facilitate the subsequent location of the sample in the paraffin blocks. This dehydration is carried out with a battery of alcohol of ascending gradient and xylol:
   - 2x1 hour alcohol 96%
   - 3x1 hour alcohol 100%
   - 3x1 hour xylol

   After that, they are placed in a paraffin bath at 62º for a period between 4 and 6 hours.
3. **Inclusion in paraffin**

After the dehydration, preparations are placed in a paraffin bath at 62º for a period of time between 4 and 6 hours. Samples are placed on blocks following a transversal orientation, the sections are 12 µm thick. Serial sections are placed on a slide that has a thin layer of Mayer’s albumin and with distilled water. Then, they stretch with distilled water on a plate at 40-45 ºC.

4. **Staining of the histological sections with hematoxylin-eosin**

We have done the staining following the next steps:

1. **De-parafination:** Xylol incubating 10 minutes (1 change).
   Xylol incubating 5 minutes (1 change).

2. **Hydration:** Ethanol 100 º, dipping it 5 minutes (1 change).
   Ethanol 96 º, dipping it 3 minutes (1 change).
   Ethanol 70 º, dipping it 3 minutes (1 change).
   Rinse with water, minimum 3 minutes
   (Before starting with hematoxylin it should be filtered).

3. **Hematoxilin:** Stain for 10 minutes.

4. **Differentiation:** Rinse water until it is slightly transparent.

5. **Virage:** With acidic alcohol (70º) and a hydrochloric acid between 3 and 4 dips.

6. **Partial dehydration:** With ethanol (80º), 3 minutes.

7. **Eosin floxin:** Stain for 5 minutes.

8. **Dehydration and differentiation:** Ethanol (80º) 3 minutes.
   Ethanol (96º) 3 minutes.
   Ethanol (100º) 5 minutes, 2 changes.

9. **Clarification:** Xylol, 2 changes (5-10 minutes each one).

   Finally, assembly with Xylol.
IMAGES ANALYSES TECHNIQUES

For each of the histological sections six microscopic fields will be randomly selected (magnification x10) obtaining a total of 288 fields. Each field will be photographed with a Point Gray Flea3 CMOS USB3.0 digital camera coupled to a Leica DMRXA optical microscope. The digital photographs obtained will be analysed using the FIJI ImageJ2 (National Institutes of Health) image analysis programme. This open access programme uses a recordable macro language and an extensible plug-in architecture.

This program can easily and accurately detect airspace in each field using the specific J Macro image. We have defined airspace as a collection of air with different shapes, located in an intra-alveolar or extra-alveolar space and with a minimum area of 100 µm². All airspaces present in each sample will be identified, including incomplete ones at the edges of the field. On the contrary, the presence of oedema, blood vessels or intra-alveolar cells will be automatically ruled out and will not be identified. In cases where an error occurs in the segmentation of airspace, these will be manually selected to exclude artifacts.

The following parameters will be measured for each field:

- Airspace count
- Total area occupied by airspace
- Percentage of the field occupied by the airspaces compared to the rest of the parenchyma
- Maxim diameter and minimum diameter of each air collection

Finally, these parameters will be calculated and exported automatically to an MS Excel table.
7. STATISTICAL ANALYSIS

Statistical analysis of the obtained data will be performed using Statistical Package for Social sciences (SPSS) and Microsoft Excel Windows to manage computed data.

p value of < 0.05 will be considered statistically significant.

7.1 DESCRIPTIVE ANALYSES

The qualitative variables (sex, smoking, pre-existing health condition) will be described using proportions and their confidence interval. Due to a small sample size and the consequent impossibility of assuming a normal distribution, we will summarize the continuous quantitative variables using medians (interquartile interval IQR): total area occupied by airspace, percentage of field occupied by the airspaces, maximum and minimum diameter of each air collection.

We will stratify it basing on the presence or absence of pulmonary barotrauma: Pulmonary barotrauma or putrefaction.

7.2 INFERENCE ANALYSES

We will contrast the difference of proportions and medians between pulmonary barotrauma or putrefaction according to the other variables. Considering that the presence or absence of PBt is a dichotomous categorical qualitative outcome, the Chi Square will be performed to determine the differences between groups to evaluate qualitative variables. Additionally, the Analysis of variance (ANOVA) test will be applied in case of the continuous qualitative variables.
7.3 MULTIVARIATE ANALYSES

A multivariate analysis will be performed adjusting the covariables to avoid confusion caused by the effect of these variables on the evolution of PBT or the emphysematous period during putrefaction. In this study an ANOVA test and post-hoc analysis will be performed to evaluate the association between the studied group and the total area occupied by airspace and other dependent variables, adjusted to each covariable. Therefore, they will be used to compare the means between the different groups.

7.4 TEST’S VALIDITY EVALUATION

The analysis of the Receiver Operating Characteristic (ROC) curve will be performed in order to establish a cut-off value to evaluate the sensitivity and specificity of the different parameters analysed.
8. ETHICAL CONSIDERATIONS

This study does not use invasive technical procedure on a corpse since we will work only with processed histopathological samples. During the research we will be using processed histological samples of corpses where a judicial autopsy ordered by an investigating judge has been performed. In the framework of this project, no clinical or identifying data of the subjects are used. The autopsies are exempt from an informed consent by the relatives or the source subject. It has been presented and approved by the Comisión de Docencia e Investigación del IMLCFC.

The IMLCFC Forensic Pathology Service does not have direct access to relatives of the study subjects, therefore, the request for consent for the use of the samples is not possible. In any case, it would be a disproportionate effort as established in article 58 of Law 14/2007 on Biomedical research. Hence, we presented the project to the Comité de Ética de Investigación Clínica del Hospital de Bellvitge and it has been shouldered by them therefore, we will be able to work without the authorisation of the subjects’ relatives (see Annex 3).

In any case, all data will be subject to the Organic Law of Data Protection 3/2018 as well as the Regulation (EU) 2016/679 General of Data Protection, that will also apply to the works and scientific publications that are derived from it.
9. STUDY LIMITATIONS

One of the limitations of this project is the scarce sample size with which this research is ought to take place. This is due to the minimal cases diagnosed of PBt/AGE therefore we can only include those with a definitive diagnosis. If the analysis gives statistically significant results a further investigation involving other Legal Institutes can take place and therefore, achieve bigger samples and more extrapolable results.

Additionally, environmental and personal conditions must be considered as a risk factor when analysing the different histomorphometric images as they are expected to vary with each case. Therefore, every situation must be individualised, and all the factors have to be taken into account when analysing the histomorphometry. Especially when determining the emphysematous evolutionary period in putrefaction, the PMI can be disturbed from its usual pattern due to factors such as the temperature of the water. We may find two corpse in the same PMI interval but in different phases of putrefaction. Therefore, this variability may difficult or slow down the study process.
10. CHRONOGRAM

The study began in **August 2020** when we started writing a memoir of what we wanted to do to present it to the *Comité de Ética del Hospital Universitario de Bellvitge* (Phase 0). Planification had been adapted to the approval of this Committee that arrived on the 14th of December 2020.

**January and February 2021** (Phase 1):
Observation, assessment, and selection of histopathological samples according to the type of death determined.
This work will be carried out in the Histopathology Section of the Forensic Pathology Service of the IMLCFC.
**Participating research members:** Dr. Josep Maria Casadesús, Dr. Joan Ignasi Galtes Vicente, and Mrs. Reena Samtani Bhagia.

**March, April, and May 2021** (Phase 2):
Digitization of the images by means of photography with high-resolution microscopic camera and morphometric analysis of the parameters previously referenced in each of the samples by means of image analysis software.
This task will be carried out in the morphology laboratory of the Faculty of Medicine of the University of Girona.
**Participating research members:** Dr. Josep Maria Casadesús, Dra. Maite Serrando Querol, Dra. Anna Carrera Burgaya, Dr. Francisco Reina de la Torre, and Mrs. Reena Samtani Bhagia.

**June 2021** (Phase 3):
Statistical analysis and multidisciplinary evaluation of the results.
This task will be developed in the Centre of Forensic Pathology of Girona of IMLCFC and in the Faculty of Medicine of the University of Girona.
**Participating research members:** Dr. Joan Ignasi Galtes Vicente, Dr. Josep Maria Casadesús, Dr. Ferrando Aguirre Lirón, Dra. Maite Serrando Querol, Dra. Anna Carrera Burgaya, Dr. Pere Boadas-Vaello, Dr. Francisco Reina de la Torre, and Mrs. Reena Samtani Bhagia.
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<td>Procedures for the ethical committee</td>
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<tr>
<td>1</td>
<td>Sample collection</td>
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<tr>
<td>2</td>
<td>Digitalization of images</td>
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<td>Morphometric analysis</td>
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<td>Statistical analysis</td>
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<table>
<thead>
<tr>
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## 11. BUDGET

### STAFF EXPENSES

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<tr>
<th>Description</th>
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<tbody>
<tr>
<td>Pathology staff</td>
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<tr>
<td>Qualifies statistician (14h x 35€/h)</td>
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Subtotal: 1.050 €

### HISTOLOGICAL MATERIAL (for all samples)

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<td>Formaldehyde solution 4L</td>
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<td>Alcohols for dehydration</td>
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<td>Paraffin for inclusion</td>
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<tr>
<td>Hematoxilin and eosin solution</td>
<td>198 €</td>
</tr>
</tbody>
</table>

(from Sigma-Aldrich)

Subtotal: 375,10 €

### IMAGING ANALYSIS

<table>
<thead>
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</thead>
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<tr>
<td>ImageJ license (free programme)</td>
<td>0 €</td>
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<tr>
<td>Digitalization - Servei Tècnic de Recerca de la UdG (STR)</td>
<td>120,82 €</td>
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<td>(14h distributed in 4 days x 8,63€/h)</td>
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<tr>
<td>Digital Technician (2h x 28,87€/h)</td>
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Subtotal: 178,56 €

### PUBLICATION AND DISSEMINATION

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<td>- Virtual congress</td>
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Subtotal: 450 €

### TOTAL COST

1.493,66 €
The team consists of the following members:

- Dr. Josep Maria Casaadesús Valbí and Dr. Joan Ignasi Galtes Vicente: Forensic doctors from the Institute of Forensic Medicine and Forensic Sciences of Catalonia (IM-LCFC). Experience in the field of forensic pathology and histopathology.

- Dr. Francisco Reina I de la Torre, Dra. Anna Carrera Burgaya, Dra. Maite Serrando Querol, Dr. Pere Boadas-Vello i Dr. Josep Maria Casadesús Valbí: Researchers from the University of Girona’s clinical anatomy, embryology and neuroscience (NEOMA) research group and professors from the University of Girona’s Department of Medical Sciences. Experience in the field of anatomy, morphology, digital histomorphometry, human physiology and in the investigation of deaths in diving.

- Dr. Ferrando Aguirre Liron: Member of the Group of Specialists in Underwater Activities (GEAS) of the Civil Guard. Experience in technopolitical investigation of diving accidents.

- Reena Samtani Bhagia: Medical student at the University of Girona. She is developing her final degree project on the investigation of deaths in diving, in the NEOMA research group. She is under the supervision of Dr. Josep Maria Casadesús Valbí and Rafael Ramos, the methodological tutor.

This team is exceedingly experienced in this topic. Their participation in previous studies in the investigation of deaths during the practice of diving has materialised in a total of:

- One national book chapter
- Three publications in national journals of the speciality
- Three publications in international and national journals
- And two presentations (oral communications and posters) in specialized and international conferences.
Annex 1: Diagnostic criteria of submersion. Adapted from (4)

MACROSCOPIC DIAGNOSTIC CRITERIA
(Characteristic but not pathognomonic)

EXTERNAL EXAMINATION

- Foam in the mouth and nostrils
- Cooling, pale skin and pink lividity
- Cutis anserina. Shrinkage of the penis, scrotum, and mammary papilla
- Maceration and skin whitening hands and feet
- Saponification (when remains > 2 months in water)

INTERNAL EXAMINATION

- Respiratory system
  - Foam in airway
  - Lung volume increased
  - Increased weight of the lungs (1400 – 2500g)
  - Subpleural Paltauff bleeding
  - Transudate liquid in pleural cavity (average volume 432 mL)

- Digestive system
  - Presence of water in stomach or duodenum (volume greater than 500 mL)
  - Tears in the mucosa of the cardia

- Other
  - Haemorrhages in anterior neck and chest muscle groups
  - Fluidity in the blood (mainly left cardiac cavities)

Correlation with the circumstances of the death

Traumatic pathology ruled out as cause of death

MACROSCOPIC Diagnosis of SUBMERSION
MACROSCOPIC findings characteristic of submersion

- Not evident, doubtful and/or non-specific
- No correlation with the circumstances of death

CONFIRM or EXCLUDE

- Death due to submersion
- Non-traumatic predisposing factors

Taking samples (Order JUS/1291/2010 of 13 May)

- Essential: Lung (5 lobes and hila) and whole heart.
  In addition, and depending on the autopsy findings: Brain, liver, spleen, kidney, pancreas, possible wounds, etc.
  Fixed with formol

- Peripheral blood (two 5 mL tubes, each with preservatives, anticoagulants, full and with no air pocket) and/or viscera (liver, kidney, and lung: 50 g of each, refrigerated and without fixative liquid)

- Left and right ventricular cardiac cavity blood (2 tubes with EDTA).
  Distal portions of the lung lobes (100-200 g), liver and spleen (100 g each), sternum or femur and CNS (dura mater, full cerebellum, choroid plexus) separated, refrigerated and without fixative liquid

- Histopathological studies: Study of lung submersion and sudden death

- Toxicological studies: Determination of drugs of abuse, ethanol, medicine and/or other (CO, etc.)

- Biological studies: Determination of strontium and/or study of diatoms

Institut de Medicina legal de Catalunya Laboratory Service
Instituto Nacional de Toxicología y Ciencias Forenses
Annex 2: Diagnostic criteria of PBt. Adapted from (13)

RADIOLOGICAL EXAMINATION
(Thorax and abdomen)

EXTERNAL EXAMINATION

Skin colour and appearance
Rule out subcutaneous crepitus
Integrity of the eardrum
Typical findings of drowning
Associated traumatic injuries

INTERNAL EXAMINATION

Cranial autopsy: Air in cerebral arterial vessels
Thoracic autopsy: Air in the left chamber of the heart
Abdominal autopsy: Air in the peritoneum and abdominal vessels

COMPLEMENTARY EXAMINATION

Histological studies
Toxicological studies
Biological studies: Diatom and hydremias studies and biochemical studies (determination of biventricular strontium)

Lung (5 lobes and hila), brain, heart, liver, spleen, pancreas, adrenal gland and kidney
Peripheral venous blood, bile, vitreous humor, and urine: Determination of ethanol, drugs of abuse and carboxyhemoglobin
INFORME DEL COMITÉ DE ÉTICA DE LA INVESTIGACIÓN
SOBRE PROYECTOS DE INVESTIGACIÓN BIOMÉDICA

El Dr. Enric Sospeda Martínez, responsable de la secretaría técnica del Comité de Ética de la Investigación del Hospital Universitario de Bellvitge,

CERTIFICA

Que el Comité de Ética de la Investigación, en su reunión de fecha 19/11/2020 (Acta 20/20), tras examinar toda la documentación presentada sobre el proyecto de investigación con nuestra Ref. PR418/20, titulado:

“ESTUDI HISTOMORFOMÈTRIC PEL DIAGNÒSTIC DIFERENCIAL ENTRE
EL BAROTRAUMATISME PULMONAR EN EL BUSSEIG AMB ESCAFANDRE AUTÒNOM I ELS
CANVIS TAFONÔMICS PULMONARS”

Presentado por el Dr. Josep Maria Casadesús Vaibl, del Servicio de Patología Forense (Centro de Patología Forense de Girona) y el Dr. Joan Ignasi Galtés Vicente, del Servicio de Patología Forense (Centro de Patología Forense del Institut de Medicina Legal i Ciències Forenses de Catalunya (IMLCFC), como promotores e investigadores principales, ha acordado emitir INFORME FAVORABLE al mencionado proyecto.

Que la composición actual del Comité de Ética de la Investigación es la siguiente:

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<tr>
<th>Puesto</th>
<th>Nombre</th>
<th>Cualificación</th>
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<td>Dr. Josep Manel Llop Talaveron</td>
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<td>Sra. Sonia López Ortega</td>
<td>Graduado Social - Atención a la Ciudadanía</td>
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<td>Dr. Sergio Marchón Ramos</td>
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<td>Dr. Joan Josep Quezall Jiménez</td>
<td>Jurista</td>
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Generalitat de Catalunya
Que este Comité cumple la legislación española vigente para este tipo de proyectos, así como las normas ICH y las Normas de Buena Práctica Clínica.

Que en dicha reunión del Comité de Ética de la Investigación se cumplió el quórum preceptivo legalmente.

Lo que firmo en L'Hospitalet de Llobregat, a 14 de diciembre de 2020.

SOSPEDRA MARTINEZ
ENRIQUE - 36986426
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Fdo. Dr. Enric Sospedra Martínez
Responsable de la secretaría técnica del CEIm