



Multicentric case series of scuba diving fatalities: The role of intracardiac gaseous carbon dioxide in the forensic diagnosis

T. Keller^a, B. Desgraz^b, M. Lossois^c, E. Baccino^c, J.M. Casadesus^{d,e}, L. Tuchtan^f, M.D. Piercecchi^f, P. Klinguer^g, M. Zarattin^g, J.L. Gassend^{a,g}, V. Varlet^{a,*}

^a Swiss Human Institute of Forensic Taphonomy, University Centre of Legal Medicine Lausanne Geneva, Switzerland; Lausanne University Hospital and University of Lausanne, Lausanne, Switzerland

^b University Centre of Hyperbaric Medicine (CURMedHyp), Lausanne, Geneva, Switzerland

^c Legal Medicine Department, Lapeyronie Hospital, Montpellier, France

^d Institute of Legal Medicine and Forensic Sciences of Catalonia, (Division of Girona, Spain), Spain

^e Research Group on Clinical Anatomy, Embryology and Neuroscience (NEOMA), Department of Medical Sciences, University of Girona, Girona, Spain

^f Legal Medicine Department, Marseille La Timone University Hospital, Marseille, France

^g Forensic pathology unit, University Centre of Legal Medicine Lausanne Geneva, Switzerland; Lausanne University Hospital and University of Lausanne, Lausanne, Switzerland

ARTICLE INFO

Keywords:

Scuba diving fatality
Carbon dioxide
Intracardiac gas
Drowning
Gas embolism
Diving pathology

ABSTRACT

Scuba diving fatalities post-mortem diagnosis presents a higher level of forensic complexity because of their occurrence in a non-natural human life environment. Scuba divers are equipped with diving gas to breathe underwater. It is essential for them to be fully trained in order to be able to manage their dive safely despite the varying increase of ambient pressure and temperature decrease. Throughout the dive, the inhaled diving gas is dissolved in the diver's tissues during the descent and if the decompression steps are not respected during the ascent, the balance between the dissolved gas and the tissues (including blood) is disrupted, leading to a gaseous release in the organism. Depending on the magnitude of this gaseous release, free gas can occur in blood and tissue. Venous or arterial gas embolism can also occur as a consequence of decompression sickness or barotraumatism. It can also induce drowsiness that consequently leads to drowning. As a result, the occurrence of gas in dead scuba divers is very complex to interpret, as is the difficulty to distinguish it from resuscitation maneuver artifacts or body decomposition. Although the literature is scarce in this domain, significant work has been done to provide a precise intracardiac gas sampling method to enlighten the cause and circumstances of death during the dive. The aim of this study is to obtain higher statistical significance by collecting a number of cases to confirm the gas sampling protocol and analysis and gain more information about the cause of death and the events surrounding the fatality through the establishment of clear management guidelines.

1. Introduction

Scuba diving fatalities post-mortem (PM) diagnosis presents a higher level of forensic complexity as the fatality occurs underwater in a non-natural human life environment.

Drowning is the main cause of scuba diving death and occurs when water has entered the respiratory system [1–4]. The PM diagnosis is based on the finding of drowning signs such as foam cone allowing to differentiate an in-vivo drowning from a PM submersion [5]. The causes of drowning are very various including mainly loss of consciousness induced by gaseous releases, cardiorespiratory diseases, hypoxia,

hyperoxia, hypercapnia, nitrogen narcosis, CO poisoning, drug consumption or immersion pulmonary edema (IPE) [6,7].

Gaseous releases constitute another frequent cause of death among diving fatalities. When scuba diving, the diver is expected to control the speed of ascent and make decompression stops in order to avoid barotraumas and excessive desaturation of the diving gas initially dissolved in the tissues during the dive. If these safety rules are not respected, the dissolved gases are released which can cause a decompression sickness (DCS), whose magnitude can evolve to pulmonary barotrauma (PBT) which can also lead to a fatal arterial gas embolism (AGE).

DCS is generally rather uniformly distributed in the body represented

* Corresponding author.

E-mail address: vincent.varlet@chuv.ch (V. Varlet).

<https://doi.org/10.1016/j.forensiint.2023.111845>

Received 30 June 2023; Received in revised form 14 September 2023; Accepted 19 September 2023

Available online 22 September 2023

0379-0738/© 2023 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

by Henry's law, described as spreading to the muscle and joints first and then to the nervous system, audiovestibular and vascular and lead to a loss of consciousness and/or drowning [8–11]. Barotrauma is represented by Boyle's law as damage to tissues caused by an inability to maintain near equivalence between the pressure in body cavities and ambient pressure. It tends to occur during rapid ascent, when the gas expands, or if the diver does not exhale during ascent and affects mainly the inner ear, sinuses but can also cause Pbt. Pbt is a disruption of the airway or alveolar wall that allows air to enter the pulmonary vessels or interstitial tissue and spread to the pleura, mediastinum or subcutaneous tissues. If the barotrauma reaches the vascular system (i.e. pulmonary arteries) and allows air to escape into the pulmonary venous system, thereby accessing the left side of the heart, it is then requalified in arterial gas embolism (AGE) whose magnitude seems to be proportional to the maximum depth, the duration of the dive and the rapidity of the ascent. As result, the understanding of the fatal mechanism is mainly focused on the gases behaviour during the dive and their effects on the organs and tissues at different levels [12]. However, the body taphonomy can rapidly induce intracardiac gaseous artefacts.

Until recently, the presence of intra-arterial gas was systematically related to barotrauma but other causes such as decomposition, off-gassing and resuscitation maneuvers can induce gas release [13]. Indeed, resuscitation maneuvers can cause subcutaneous emphysema. The chest compressions and/or positive pressure ventilation accelerate the gas collection to the arterial system and can imitate barotrauma.

PM off-gassing corresponds to the passive gas release in the body of a diver found dead at depth and brought to the surface. Pathologists are often mistaken by artifactual bubbles seen in an autopsy of a dead diver who never ascended from the bottom. The dive profile is of utmost importance to interpret the symptoms or absence of expected signs.

Finally, the gas in dead divers can be the consequence of body decomposition (microbial metabolism products). Nowadays, forensic imaging tools such as multi-detector computed tomography (MDCT) is reliably used to detect the presence of gas in cadavers. This method provides distribution patterns but cannot distinguish scuba diving gas from PM decomposition gas [14]. A radiological alteration index (RAI), illustrating the distribution of gas in the body, has been developed and found to be relevant to assist in PM diagnosis [11,15]. An RAI of 85–100 is usually found in Pbt/AGE. However, the radiologists can misinterpret the gaseous distribution and magnitude if they do not take into account the measurements of gaseous concentrations. Therefore, the forensic imaging expertise must be completed by a toxicological expertise demonstrating the absence of decomposition gas contribution to this calculation in order to fully attribute the lesions to Pbt/AGE [16,17].

These past 10 years, research has been made concerning the gas analysis in scuba diving fatalities. Porcine model was used to determine distribution and time of onset of PM gas collections [13]. Stranded marine mammals were analysed to describe gas composition analysis and scoring of the gas bubbles confirming the decompression hypothesis [18,19]. Systematic rabbits necropsies at different PM times allowed to identify how putrefaction gas can mask in vivo gas embolism [20]. RAI calculated from CT-scan [16] and precise gaseous CO₂ concentration measurement to confirm RAI [21] from which the ratio between carbon dioxide (CO₂) and nitrogen (N₂) has proven to orientate a diagnosis toward embolism or alteration [22]. Cadaveric alteration of human body producing hydrogen, hydrogen sulfide or methane were identified as possible result distortion of the CT-scan interpretation to locate vital embolism gases [20]. As result, past studies have clearly established that when drowning occurs at depths greater than 30–40 m sea water (msw), the off-gassing is related to intracardiac gaseous CO₂ composition proportional to the diving parameters and between 9 and 12 µmol/ml [11]. Compression/decompression studies have highlighted that the gas release spread rapidly to the venous system then to the arterial system after 3 h PM [23]. In case of AGE, the body is mainly found at surface with bloody signs of barotrauma and an intracardiac gaseous CO₂ composition proportional to the magnitude of the AGE between 10 and

16 µmol/ml [11].

Consequently, the intracardiac gaseous CO₂ composition seems to be a helpful forensic indicator to corroborate the witness of other divers, to strengthen radiological findings and complete the autopsy results. This information is crucial, as the recording of investigation data is segmented over time due to the sequential handling of the body by different specialists. Indeed, there are currently no standardized forensic protocols to manage a scuba diving fatality. However, the added value of the contribution of forensic underwater experts is well described [24]. It is therefore important to suggest a protocol that could easily be followed by any police/ hospital/ forensic department to obtain all the information needed and avoid any bias concerning artifacts or missing details for the diagnosis even if the center does not have access to the latest technologies.

The main aim of this multicentric work is to strengthen the statistical significance of the preliminary gaseous parameters hypothesized as to confirm the diving profile or the PM diagnosis. Additionally, this study aims to present intracardiac gaseous CO₂ concentrations collected in IPE fatal diving cases for the first time. The second goal is constituted by the development of a medico-legal check-list for all professionals surrounding a scuba diving accident. A controlled protocol gathering all information necessary for a correct diagnosis is mandatory to increase the understanding and preventing of scuba diving fatalities.

2. Material and methods

This study was carried out on the basis of a collaboration with three forensic centers located in nearby countries to obtain a more important and varied amount of scuba diving fatality cases. The cases included in this study concerned SCUBA dives (no rebreather) in the Mediterranean Sea under the jurisdiction of the following forensic medicine departments: Geneva / Lausanne hospitals (Switzerland, 2 cases), Marseille La Timone hospital (France, 1 case), Montpellier Lapeyronie hospital (France, 1 case) and Tarragona / Girona / Barcelona hospitals (Spain, 1 case).

2.1. Sampling

The intracardiac gas sampling was performed according to the methodology developed by the University Centre of Legal Medicine (CURML). The method consists of detecting the gas by multi-detector computed tomography (MDCT) and to collect intracardiac gas under laser CT scan guidance by the insertion of needle mounted on a three-way valve and a 20 ml Luer-Lock syringe [11,16]. However, as access to a CT-scanner is not guaranteed by all partners, a video tutorial has been developed to perform intracardiac gas sampling during autopsy (Fig. 1). The forensic doctors of each partner institute were thus able to be trained in advance to perform these samples. Sampling kits were also sent to ensure homogeneity of the equipment and to gather the same information from the different countries.

Intracardiac gas sampling is based on the classical underwater heart dissection [17]. After opening the rib cage and incising the pericardial sac, a volume of water is introduced into the pericardial sac, which leads to some floating of the heart if it contains gas. The gaseous sample is taken by a 20 ml Luer-Lock syringe equipped with a three-way valve and a standard needle inserted in the emergent part of the heart avoiding the transfer of any biological fluid that could induce contamination. The sample is then transferred to a 20 ml crimped vial previously filled with pure water. The samples are then stored upside down in a refrigerator and analyzed on site or sent by mail to CURML for analysis.

2.2. Analysis

The analysis of intracardiac gas samples is performed by micro gas chromatography coupled with a thermal conductivity detector (Micro



Fig. 1. Tutorial for intracardiac gas sampling (a. Opening of thoracic cage and chest plate release, b. Incision of pericardium and filling the pericardial sac with water, c. Superficial needle insertion in the upper heart cavity and gas sampling, d. Insertion of a needle through vial septum, e. Gas transfer from syringe to vial: insertion of sampling set-up through vial septum, opening of the three-way valve, pushing on the syringe plunger while water is evacuating by the other needle, f. Gas sample ready for analysis).

GC 490, Agilent Technologies) or following a similar previously published methodology for case 1 [11]. For helium analysis, hydrogen was used as carrier gas. The equipment used is a portable model that can be deployed directly in the partner institutes and perform the analysis very quickly after collection. The device was set up with a dual channel for calibration and analysis of hydrogen (H_2), oxygen (O_2) and nitrogen (N_2) with a column MS 5 A PLOT 10 m and methane (CH_4), carbon dioxide (CO_2) and hydrogen sulfide (H_2S) with a PPQ 10 m. This system allows the acquisition of all the gases in a single run after a manual injection. The injector was fitted with a LuerLock adapter and a filter Genie type 170. The injector was set at 70 °C and flushed by an amount of 1–2 ml of gaseous sample to guarantee the filling of the injection loop commuted to the microGC columns for an injection time of 40 ms. The carrier gas was helium at a pressure of 200 kPa and the total runtime was 170 s. The oven conditions were isothermic at 60 °C. Finally, data was retreated with OpenLab EZChrom.

The deployment of the technique on site was notably carried out with one of our Mediterranean partners to validate the emergency analysis approach if such circumstances required it. However, this degree of urgency is rarely achieved and samples would likely have to be forwarded to the laboratories with a delay, so other cases were sent to us by standard mail.

Check-list

A form was sent to all the partners in order to collect a maximum of standardized information about each anonymized case. This form is presented in Table 1. The diving profile (when available) and the main elements of the context of the dive (sex/age of victim, diving gas, resuscitation protocol, water temperature, PM delay, storage temperature, autopsy conclusions) were asked in order to interpret the intracardiac gaseous CO_2 concentration. As resuscitation procedures and decomposition can also introduce some distortions in the diagnosis of intracardiac gas occurrence and composition, it is also very important to collect this essential information.

3. Results and discussion

The two first cases (Case 1 and Case 2) concern lake dives and

Table 1
Scuba diving fatality checklist.

Age	Sex	Diving experience
		- level:
		- n° dives:
Medical history		
Diving plan and context		
- Recreational dive, other:		
- Dive entry (offshore, boat):		
- Fresh water dive / marine dive:		
Accompanying divers reports		
- Onsite symptoms: loss of consciousness, foam cone, etc.:		
- Resuscitation protocol: YES/NO		
- Description:		
- Equipment (SCUBA, rebreather, etc.):		
Divers profile		
- Duration of dive:		
- Maximum depth:		
- Decompression stop:		
- Kinetic graphic/description		
Police diving team analysis of computer records		
- Water T°:		
- Atmospheric T°:		
- Corpse discovery location and depth:		
- Discovery of the body (dd/mm/yy and hh/mm):		
Breathing gas content of bottles with toxicological records		
- Diving gas:		
Diving equipment testing		
Medicolegal center		
- Arrival to medicolegal institute (dd/mm/yy and hh/mm):		
- Autopsy / Examination (dd/mm/yy and hh/mm):		
- Storage T° of cadaver:		
- Preliminary findings (foam cone, blood, etc):		
Miscellaneous / toxicological analysis		
- Xenobiotic:		
- Gas composition:		

Case information contact (doctor, officer.):

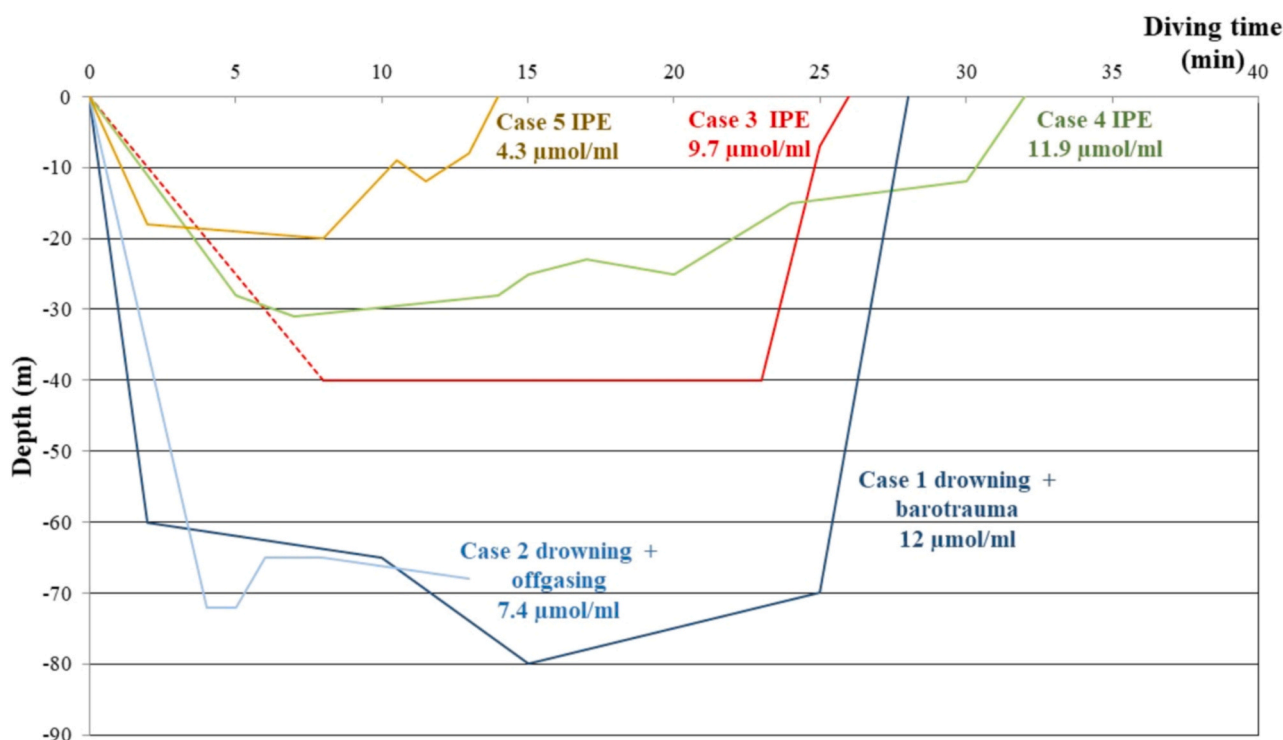


Fig. 2. Diving profiles of the cases.

showed similarities in the circumstances of death. Both described deep dives with loss of consciousness at depth leading to fatal drowning (Fig. 2) of experienced divers.

In case 1, the body was found at the surface of a lake where resuscitation maneuvers were undertaken without success by the fisherman who found him. The tandem diver testifies to confusion of the victim as the visibility was very poor due to water turbidity. Finally, the tandem diver has described the last vision of his colleague, apparently unconscious, regulator out of mouth, as the body seemed to slowly rise to the surface due to the dilatation of the life jacket. The PM CT and autopsy were carried out at our institute with a time to autopsy less than 24 h.

The death was attributed to deep water drowning followed by immediate off-gassing while the victim was probably already dead. The intracardiac gaseous composition was 12 µmol/ml CO₂, 26 µmol/ml N₂, < 2 µmol/ml He and < 1 µmol/ml O₂ without decomposition gas (Table 2). The presence of He in intracardiac gas is a clear evidence that Trimix has been used. Indeed, based on previous works, intracardiac gaseous CO₂ concentration is found independent of the nature of the embolism gas but linked to desaturation kinetics and volumes of gas embolism [11].

Taking into consideration the diving profile, Case 1 constitutes a deep dive of 25 min followed by a rapid ascent. The diving computer

Table 2

Diving information, autopsy findings and intracardiac gaseous CO₂ concentrations of the compiled 5 fatal cases.

Case	Sex and age	Dive and diving gas	Autopsy findings	Intracardiac CO ₂ concentration (µmol/ml)	Intracardiac CO ₂ concentration in drowning / Off-gasing cases (µmol/ml)	Intracardiac CO ₂ concentration in barotrauma / AGE cases (µmol/ml)
1	Male, 38 yo	Freshwater dive and air, trimix (He/N ₂ /O ₂) and nitrox as diving gas	tracheal foam cone and pulmonary edema	12	9 (20 min, 30 mfw) to 12 (40 min, 95 mfw)	10 (32 min, 45 mfw) to 16 (28 min, 80 mfw)
2	Male, 33 yo	Freshwater dive and air as diving gas	foam cone, Paltauf's spots and hydroaeric emphysema, and non-specific acute signs, including cerebral edema.	7.4		
3	Female, 48 yo	Sea water dive and air as diving gas	resuscitation maneuvers and an asphyxia syndrome with polyvisceral congestion, major pulmonary edema, cyanosis of the integuments and a dilatation of the cardiac chambers, possibly related to the lethal mechanism	9.7		
4	Male, 63 yo	Sea water dive and air as diving gas	minimum amount of air in the left heart cavities (pericardial water test), but absence of air in the vessels of the circle of Willis polygon (cerebral water test) and an absence of pneumothorax (thoracic water test). Absence of crepitus indicative of subcutaneous emphysema and no foam cone	11.2		
5	Female, 57 yo	Sea water dive and air as diving gas	Absence of barotrauma or drowning signs	4.3		

shows a descent to 60 m fresh water (mfw) in 3 min, 80 mfw after 25 min and followed with a rapid ascent to surface in 3 min. A high concentration of intracardiac gaseous CO₂ is then expected. Bodies of dead scuba divers found at the surface usually show clear signs of barotrauma depending to the dive duration, the depth reached and the speed of ascent. Based on the diving profile and intracardiac gases composition, an intracardiac gaseous CO₂ concentration higher than 14 µmol/ml was expected. As the intracardiac gaseous CO₂ concentration was 12 µmol/ml, this hypothesis appears at first sight not confirmed. In the other hand, drownings followed by later off-gassing display intracardiac gaseous CO₂ concentration between 9 and 12 µmol/ml, for dives respectively of 20 min at 30 mfw and 40 min at 95 mfw [11]. Even if the intracardiac gaseous CO₂ concentration is 12 µmol/ml, the diving profile does not match with a dive of 40 min at 95 msw. As result, the death should have been caused by a combination of drowning and barotrauma.

Therefore, when the information from autopsy, witness audition and intracardiac gaseous composition are combined, it becomes easier to interpret the fatality. The death could be attributed to a deep drowning simultaneously followed by PM off-gassing because the body is brought up to the surface due to the dive jacket dilatation. The consecutive off-gassing is strengthened by the witness of the tandem diver. Therefore, we cannot precisely specify if the drowning occurred at 80 mfw or during the rapid ascent of the body, however the drowning almost certainly occurred at the beginning of the ascent because the water in airways has counterbalanced the magnitude of an expected barotrauma from 80 mfw after 25 min of total dive. The confirmatory importance of intracardiac gaseous CO₂ is fully demonstrated in this case: Trimix use, witness of the tandem diver, autopsy findings are all supported by the intracardiac gaseous CO₂ concentration.

In case 2, the body was found at 68 mfw depth of a lake. The tandem diver reported that they confirmed by sign that everything was under control once arrived at 72 mfw. Immediately after, the victim became confused, and pulled the regulator from the mouth of the tandem diver in a probable act of panic. The tandem diver then used the rescue regulator and they started to ascend but the divers got lost during the ascent. The tandem diver continued his ascent to the surface and called for help. The search in the evening was unsuccessful and the body of the victim was found the next morning at 68 mfw and brought up by the police. The PM CT and autopsy were carried out at the institute with a time to autopsy less than 24 h. The death was attributed to a deep drowning followed by secondary off-gassing with the ascent of the body the following day. The intracardiac gaseous composition was 7.4 µmol/ml CO₂, 34.2 µmol/ml N₂, traces of O₂ without putrefaction gas.

Taking into consideration the diving profile, Case 2 constitutes a short deep dive of 9 min followed by a PM off-gassing the following day. The diving computer shows a descent to 72 mfw in 5 min, then a stabilization around 65 mfw after 9 min. The diving computer did not record any depth change after those 9 min of dive. Bodies of dead scuba divers found drowned in deep water usually show clear signs of drowning (foam cone, etc.) and the concentration of intracardiac gaseous CO₂ is proportional to the dive duration and the depth reached. As previously reported, drownings followed by later off-gassing display intracardiac gaseous CO₂ concentration between 9 and 12 µmol/ml, for dives respectively of 20 min at 30 mfw and 40 min at 95 mfw [11]. As a result, the intracardiac gaseous CO₂ concentration of 7.4 µmol/ml conforms totally with the expected results (short deep dive) and the autopsy findings.

Thus, these two cases strengthen the existing intracardiac gaseous CO₂ indicators. Combining the autopsy findings with the data from the police investigation (auditions, diving profile, etc.), it becomes possible to deduce the magnitude of the intracardiac gaseous CO₂ concentration. Conversely, with the intracardiac gaseous composition and the autopsy findings, it becomes possible to deduce the diving profile and circumstances of death. However, the hypotheses become impossible as soon as decomposition gases appear in the intracardiac gaseous composition

because a part of CO₂ could be generated by the microbial activity.

The last three cases (Cases 3–5) concern sea dives and showed similarities in the circumstances of death. They all described medium-depth dives with drowsiness during the dive and loss of consciousness at the surface (Table 2). Considering these circumstances, drowning and barotrauma can be discarded as potential causes of death. In fact, all these three cases display the same mechanisms but only the last case (Case 5) was diagnosed formally with IPE as cause of death. Natural disease was hypothesized as cause of death but taking into account the diving profiles and background history of the victims, the cause of death for the cases 3 and 4 can be more likely identified as IPE (Fig. 2).

In case 3, the body of an experienced 48yo female, treated for hypertension, is recovered showing clear signs of discomfort at the surface followed by loss of consciousness, during a group dive in the Mediterranean sea (sea temperature 24 °C, external temperature 25 °C).

The diving computer shows a descent to 40 msw in 7 min while the victim indicated signs of breathlessness but tried to continue the dive. The diving teacher indicated a stabilization for 20 min and a strong stream at 40 msw. Finally, the victim became confused, showed signs of faintness and the ascent was undertaken. The victim was still confused at 7 msw but managed to perform the decompression stop. Once at the surface, the victim became unresponsive. Resuscitation maneuvers were performed on the boat by first aiders, then the emergency rescuers during 35–45 min without success and the diver was pronounced deceased during her transport to the hospital. The time to autopsy was about 48 h. A heart attack at depth after a dive seems to be at the origin of the death. The intracardiac gaseous composition was 9.7 µmol/ml CO₂, 28.8 µmol/ml N₂, traces of O₂ without putrefaction gases for a dive of 26 min with a maximal depth of 40 msw.

In case 4, the body of an experienced 63yo male is brought up to the surface during the last decompression stop at 8 msw, during a pair dive in Mediterranean sea (sea temperature 23 °C, external temperature 24 °C) with an experienced instructor. The diving computer shows a descent to 30 msw in 7 min, then a stabilization between 25 and 30 msw after 20 min. Then, the diving profile shows an ascent to 15 msw and a stabilization between 10 and 15 m during 10 min. Finally, by the end of the 8 msw decompression stop, the instructor described seeing the victim in an upright position, attached to the anchor line, suddenly starting to shake and rapidly becoming unresponsive with a loss of consciousness (eyes closed). The regulator was still in the mouth. The victim was brought up to the surface where immediate resuscitation maneuvers were performed on the boat by first aiders. Advanced resuscitation maneuvers during 30 min on the transfer boat to the port were unsuccessful. Taking into consideration the diving profile, the police was able to reject decompression sickness, i.e., the victim did not take long or go deep enough to develop this dysbaric disorder. Investigators did not detect mechanical failures or signs of manipulation of the scuba diving equipment and the analysis of the diving equipment was totally in order. The time to autopsy was less than 24 h. The minimum amount of air in the left heart cavities is found compatible with a minor pulmonary barotrauma during the ascent controlled in perimortal period (rescue) in the last 8 m but could be also compatible with the resuscitation maneuvers. Toxicology results were negative for alcohol and all drugs tested. The carboxyhemoglobin level was zero. The preliminary cause of death was hypothesized as a natural death (pending histopathological results). In this regard, although arrhythmias are often a differential diagnosis in non-monitored sudden deaths, a sudden death of non-cardiological origin compatible with immersion pulmonary edema was hypothesized. In case 4, the intracardiac gaseous composition was 11.2 µmol/ml CO₂, 31.1 µmol/ml N₂ and 2 µmol/ml of O₂, without trace of decomposition gases for a dive of about 32 min with a maximal depth of 30 msw.

In case 5, the body of a 57yo female is recovered showing clear signs of discomfort at the surface and then losing consciousness, during a dive in Mediterranean sea (sea temperature 23 °C, external temperature 26 °C). The diving computer shows a descent to 18 msw in 2 min

followed by a stabilization between 16 and 20 msw for 6 min while the victim began to express signs of breathlessness. As discomfort symptoms were increasing, an ascent to 10 msw was performed after 12 min of total dive. The victim reached the surface after 14 min where she became confused and underwent loss of consciousness. Resuscitation maneuvers were carried out by first aiders without success and the diver was pronounced deceased during her transport to the hospital. The time to autopsy was about 4 days. The intracardiac gaseous composition was 4.3 $\mu\text{mol/ml}$ CO_2 , 31.1 $\mu\text{mol/ml}$ N_2 and 6.2 $\mu\text{mol/ml}$ of O_2 , without trace of decomposition gases despite a 4 days storage in cold chamber, for a dive of 14 min with a maximal depth of 20 msw.

In fact, these three last cases conform with the diagnosis of IPE. IPE is described when absence of watery content in the stomach and conducting airways, and liquid filled lungs without hyperextension. Symptoms such as acute onset of coughing and/or progressing shortness of breath have been identified. IPE can lead to secondary drowning and has been proven to be more likely for hypertensive individuals around or older than 50 yo or young healthy individuals performing cold-water dives, women being more prone than men to the occurrence of this diving pathology [25–29]. For our cases, the ages of the victims were: 48 yo (Case 3: female, hypertension), 63 yo (Case 4: male), and 57 yo (Case 5: female). The diagnosis has to be considered in case of rapid respiratory deterioration preventing continuous breathing through an appropriate air source and no obvious signs of aspiration [7]. Intense physical effort causes an increase in the transpulmonary gradient by an increase in cardiac output and a heterogenous redistribution of pulmonary perfusion (favored in the declining areas). The symptomatology shows an apparition of discomfort to faintness at depth and worsens during the ascent. Moreover, the hydrostatic pressure gradient between the regulator and the pneumatic center (i.e. the point of confluence of the forces exerted by the respiratory system) is negative during ascent. This results in an increase in inspiratory effort and a decrease in alveolar pressure. The most common signs and symptoms are dyspnea, cough, and the presence of frothy sputum or even hemoptysis. Chest tightness without real pain and/or a feeling of imminent death is sometimes described. The severity of the symptoms can range from a cough without consequences to severe respiratory failure with a fatal outcome. In all our cases, dyspnea (tachypnea, hyperpnea, etc.) and respiratory rales have been reported. Ages near or above 50 yo and sometimes previous hypertensive pathologies have been also present. Finally, all the symptoms have been reported during the ascent when the hydrostatic pressure gradient requires more respiratory efforts.

Concerning the CO_2 concentration, the dive duration seems to be the most important factor to consider. Indeed, the intracardiac gaseous CO_2 concentration of Case 4 (11.2 $\mu\text{mol/ml}$) is higher than in Case 3 (9.7 $\mu\text{mol/ml}$) but the dive was slightly longer (32 min versus 26 min) whereas the dive in Case 3 was slightly deeper (40 msw) than Case 4 (30 msw). However, the important thing to consider is the magnitude of the intracardiac gaseous CO_2 concentration (from 10 $\mu\text{mol/ml}$ and higher for medium dive, i.e. 30 min at 30–40 msw) rather than the precise concentration. By way of comparison, the Case 5 is a short (14 min) and shallow dive (15–20 msw). The dive duration and the maximal depth were two times lower than Case 4 (11.2 $\mu\text{mol/ml}$). Consequently, an intracardiac gaseous CO_2 concentration about half the one obtained in Case 4 was expected. The final intracardiac gaseous CO_2 concentration of 4.3 $\mu\text{mol/ml}$ was then not surprising. A linear relationship seems to take shape but should be confirmed by additional cases.

Finally, another parameter could also explain these small variations in absence of decomposition artifacts: the resuscitation maneuvers. Indeed, cardiorespiratory procedures operated during twenty minutes or more could have caused the diving gas release from the tissues of the victims. However, the influence does not seem to be very significant because the results were close to the expected ones.

Concerning the check-list provided to the partners, they were all filled partially. It is very difficult to get complete information as the forensic works implies a team work of several expert groups whose

works are separated in space and time. The check-list should help the different experts to mutually learn about the case by the transmission of a form summarizing all the information needed for the case interpretation. The on-site parameters are crucial to be reported for the pathological, radiological and toxicological interpretations of the results, which can be different and induce the circumstances and cause of death to be falsely diagnosed. Similarly, the chain of custody concerning the body logistics (storage time, temperature, etc.) as well as the medical history of the victims constitute essential parts of the check-list as they can contain information that can influence the final diagnosis. A failure in storage or an important delay between body recovery and autopsy could promote internal body decomposition. The intracardiac gas sampling and analysis can become useless, or be used to make aware the radiological team that the intracardiac gases visible at imaging examination can come from decomposition and therefore cannot be attributed to the dive fatality only.

4. Limitations

The first limitations concerning the validation of intracardiac gas sampling and analysis as reliable dive indicators and predictors would be the number of cases. SARS-CoV-2 appeared as a contributory factor that has shortened the recreational diving access to the population. However, even if the multicentric work compiled only 5 cases during the period 2020 – 2022, all the results conform with the previous published indicators. This low number of fatalities can also be explained by a better and safer practice of diving by divers more and more trained.

Another limitation of this work is to be able to collect all the information needed. Since there is no international protocol for the moment, it is sometimes complicated to get exhaustive information from the forensic stakeholders including the police, rescuers, etc.

Other biases that are not always identified such as climate, decomposition and accidents that happen at the same time such as drowning and barotrauma for instance constitute additional limitations. The influence of these factors should be taken into account as they can impact the intracardiac gaseous composition.

5. Conclusion

A direct link between the diving profile and the intracardiac gaseous CO_2 concentration is confirmed. This additional data can be used to enrich the forensic results of the autopsy and to support the findings of the police investigation. A testimony or a diagnosis of the cause of death can be supported by the intracardiac gaseous CO_2 concentration. In the case of deep drowning and PM off-gassing, there is a direct proportionality between dive duration, depth reached and intracardiac gaseous CO_2 concentration. A short, shallow dive will lead to less desaturation than a longer, deeper dive. Similarly, for an equivalent dive profile (duration and depth), a drowning followed by PM off-gassing will always lead to a lower magnitude of desaturation of diving gases compared to a rapid ascent promoting significant barotrauma. Intracardiac gaseous CO_2 analysis must be considered as mandatory for the forensic expertise. Judicial and forensic implications include the confirmation of witnesses auditions and diving data useful for the police investigation, the confirmation of radiological and medical findings deriving from CT-scans and autopsy. Finally, the results of this study also shed light on another cause of death in a diving context. In the case of IPE, a proportionality is also clearly identified with the presence of a higher intracardiac gaseous CO_2 concentration the longer and deeper the dive. In the context of forensic investigation of death in the context of diving, it is imperative to provide all possible information, hence the development of a checklist to be able to take into account all influencing parameters in the interpretation of the results. Indeed, the possible resuscitations, the duration of care and storage, the environmental conditions of the dive, etc. are parameters that can influence the forensic findings and the analysis results. It is very important to disseminate this

type of checklist so that forensic stakeholders become more autonomous in the management of their scuba diving fatalities. This study has also permitted to demonstrate the importance of multidisciplinary collaboration in the management of these cases and in particular to correctly preserve the means of proof (dive computer, bottles and other equipment, water sampling at the site, etc.) and to carry out the samples and analyses with the diligence that it requires. Other studies are obviously awaited to increase the statistical relevance of these indicators but the cases reported in this study and in those reported so far clearly identify the sampling and analysis of intracardiac gases as a clear progress in the management and forensic diagnosis of these accidents.

Compliance with ethical standards

none.

Ethical approval / informed consent

The presentation of the cases has been authorized by the prosecutions and there is no need of ethical agreement for the presentation of small number of cases coming from routine activity.

Funding

This study was partially supported by a SPARK grant from the Swiss National Science Foundation CRSK-3_190564 / 1 dedicated for the DIVE Project “Diagnosis and Interpretation of Vascular Embolism gas composition in Scuba Diving (2020–2022)”.

CRediT authorship contribution statement

Tanya Keller: Investigation; Methodology; Visualization; Project administration; Film recording; Writing – original draft preparation; Writing – review & editing. **Benoît Desgraz:** Investigation; Supervision; review & editing. **Maisy Lossois:** Investigation; Methodology; Data collection; Review & editing. **Eric Baccino:** Investigation; Methodology; Review & editing. **Josep Maria Casadesus:** Investigation; Methodology; Data collection; Review & editing. **Lucile Tuchtan:** Investigation; Methodology; Data collection; Review & editing. **Marie-Dominique Piercecchi:** Investigation; Methodology; Review & editing. **Paul Klingguer:** Investigation; Methodology; Data collection; Review & editing. **Margaux Zarattin:** Investigation; Methodology; Film recording; Data collection; Review & editing. **Jean-Loup Gassend:** Investigation; Methodology; Data collection; Review & editing. **Vincent Varlet:** Conceptualization; Investigation; Funding acquisition; Methodology; Project administration; Data acquisition; Supervision; Writing – original draft preparation; Writing – Review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

- [1] J.M. Casadesus, F. Aguirre, A. Carrera, P. Boadas-Vaello, M.T. Serrando, F. Reina, Diving-related fatalities: multidisciplinary, experience-based investigation, *Forensic Sci. Med. Pathol.* 15 (2019) 224–232, <https://doi.org/10.1007/s12024-019-00109-2>.
- [2] C. Edmonds, J. Caruso, Recent modifications to the investigation of diving related deaths, *Forensic Sci. Med. Pathol.* 10 (2013) 83–90, <https://doi.org/10.1007/s12024-013-9491-x>.
- [3] P.J. Denoble, J.L. Caruso, G.L. Dear, C.F. Peiper, R.D. Vann, Common causes of open-circuit recreational diving fatalities, *Undersea Hyperb. Med.* 35 (2008) 393–406.
- [4] C. Lawrence, C. Cooke, Autopsy and the investigation of scuba diving fatalities, *Diving Hyperb. Med.* 36 (2006) 2–8.
- [5] J.M. Casadesus, F. Aguirre, A. Carrera, P. Boadas-Vaello, M.T. Serrando, F. Reina, Diagnosis of arterial gas embolism in SCUBA diving: modification suggestion of autopsy techniques and experience in eight cases, *Forensic Sci. Med. Pathol.* 14 (2018) 18–25, <https://doi.org/10.1007/s12024-018-9951-4>.
- [6] P.T. Wilmshurst, A. Crowther, M. Nuri, et al., Cold-induced pulmonary oedema in scuba divers and swimmers and subsequent development of hypertension, *Lancet* 8629 (1989) 62–65, [https://doi.org/10.1016/s0140-6736\(89\)91426-8](https://doi.org/10.1016/s0140-6736(89)91426-8).
- [7] J. Vinkel, P. Bak, P.J. Thiis Knudsen, O. Hyldegaard, Forensic case reports presenting immersion pulmonary edemas as a differential diagnosis in fatal diving accidents, *J. Forensic Sci.* 63 (2018) 299–304, <https://doi.org/10.1111/1556-4029.13526>.
- [8] E.W. Russi, Diving and the risk of barotrauma, *Thorax* 53 (1998) S20–S24, <https://doi.org/10.1136/thx.53.2008.s20>.
- [9] P. Siermuntowski, W. Kozłowski, A. Pedrycz, K. Krefft, D. Kaczerska, Experimental modeling of pulmonary barotrauma, *Undersea Hyperb. Med.* 42 (2015) 143–149.
- [10] R.D. Vann, F.K. Butler, S.J. Mitchell, R.E. Moon, Decompression illness, *Lancet* 377 (2011) 153–164, [https://doi.org/10.1016/S0140-6736\(10\)61085-9](https://doi.org/10.1016/S0140-6736(10)61085-9).
- [11] V. Varlet, A. Dominguez, M. Augsburg, M. Lossois, C. Egger, et al., Understanding scuba diving fatalities: carbon dioxide concentrations in intra-cardiac gas, *Diving Hyperb. Med.* 47 (2017) 75–81, <https://doi.org/10.28920/dhm47.2.75-81>.
- [12] J.M. Casadesus, J. Nieto-Moragas, M.T. Serrando, P. Boadas-Vaello, A. Carrera, F. Aguirre, R.S. Tubbs, F. Reina, Pulmonary barotrauma in SCUBA diving-related fatalities: a histological and histomorphometric analysis (Online ahead of print), *Forensic Sci. Med. Pathol.* (2023), <https://doi.org/10.1007/s12024-022-00567-1>.
- [13] P.E. Laurent, M. Coulangue, C. Bartoli, A. Boussuges, J.C. Rostain, M. Luciano, et al., Appearance of gas collections after scuba diving death: a computed tomography study in a porcine model, *Int. J. Leg. Med.* 127 (2013) 177–184, <https://doi.org/10.1007/s00414-011-0662-6>.
- [14] C. Egger, P. Bize, P. Vaucher, P. Mosimann, B. Schneider, A. Dominguez, et al., Distribution of artifactual gas on post-mortem multidetector computed tomography (MDCT), *Int. J. Leg. Med.* 126 (2012) 3–12, <https://doi.org/10.1007/s00414-010-0542-5>.
- [15] C. Egger, P. Vaucher, F. Doenz, C. Palmiere, P. Mangin, S. Grabherr, Development and validation of a post-mortem radiological alteration index: the RA-Index, *Int. J. Leg. Med.* 126 (2012) 559–566, <https://doi.org/10.1007/s00414-012-0686-6>.
- [16] V. Varlet, F. Smith, N. Giuliani, C. Egger, A. Rinaldi, A. Dominguez, et al., When gas analysis assists with postmortem imaging to diagnose causes of death, *Forensic Sci. Int.* 251 (2015) 1–10, <https://doi.org/10.1016/j.forsciint.2015.03.010>.
- [17] L. Comment, V. Varlet, K. Ducrot, S. Grabherr, A fatal case of oxygen embolism in hospital, *Forensic Sci. Res.* 2 (2017) 100–106, <https://doi.org/10.1080/20961790.2017.1329695>.
- [18] Y. Bernaldo de Quiros, O. Gonzalez-Diaz, M. Arbelo, E. Sierra, S. Sacchini, A. Fernandez, Decompression vs. decompression: distribution, amount, and gas composition of bubbles in stranded mammals, *Front. Physiol.* 3 (2012) 177, <https://doi.org/10.3389/fphys.2012.00177>.
- [19] Y. Bernaldo de Quiros, J.S. Seewald, S.P. Sylva, B. Greer, M. Niemeyer, A. L. Bogomolni, et al., Compositional discrimination of decompression and decompression gas bubbles in bycaught seals and dolphins, *PLOS ONE* 8 (2013), e83994, <https://doi.org/10.1371/journal.pone.0083994>.
- [20] Y. Bernaldo de Quiros, O. Gonzalez-Diaz, A. Mollerlokken, A.O. Brubakk, A. Hjelde, P. Saavedra, et al., Differentiation at autopsy between in vivo gas embolism and alteration using gas composition analysis, *Int. J. Leg. Med.* 127 (2013) 437–445, <https://doi.org/10.1007/s00414-012-0783-6>.
- [21] V. Varlet, F. Smith, S. De Froidmont, A. Dominguez, A. Rinaldi, et al., Innovative method for carbon dioxide determination in human postmortem cardiac gas samples using headspace-gas chromatography-mass spectrometry and stable labeled isotope as internal standard, *Anal. Chem. Acta* 784 (2013) 42–46, <https://doi.org/10.1016/j.aca.2013.04.046>.
- [22] A.D. Levy, H.T. Harcke, C.T. Mallak, Post-mortem imaging, MDCT features of post-mortem change and decomposition, *Am. J. Forensic Med. Pathol.* 31 (2010) 12–17, <https://doi.org/10.1097/PAF.0b013e3181c65e1a>.
- [23] P.E. Laurent, M. Coulangue, J. Mancini, C. Bartoli, J. Desfeux, P. Perich, et al., CT appearance of gas collections can predict the cause of death in scuba diving accidents, *J. Forensic Radiol. Imaging* 1 (2013) 84, <https://doi.org/10.1016/j.jofri.2013.03.015>.
- [24] I. Tarozzi, L. Franceschetti, G. Simonini, S. Raddi, D. Machado, V. Bugelli, Black box of diving accidents: contribution of forensic underwater experts to three fatal cases, *Forensic Sci. Int.* 346 (2023), 111642, <https://doi.org/10.1016/j.forsciint.2023.111642>.
- [25] B. Desgraz, C. Sartori, M. Saubade, F. Héritier, V. Gabus, Oedème pulmonaire d'immersion, *Rev. Med. Suisse* 13 (2017) 1324–1328 (In French).
- [26] M. Kumar, P.D. Thompson, A literature review of immersion pulmonary edema, *Phys. Sports Med.* 47 (2019) 148–151, <https://doi.org/10.1080/00913847.2018.1546104>.
- [27] E. Gempp, S. Demaistre, P. Louge, Hypertension is predictive of recurrent immersion pulmonary edema in scuba divers, *Int. J. Cardiol.* 172 (2014) 528–529, <https://doi.org/10.1016/j.ijcard.2014.01.021>.
- [28] N. Türkmen, O. Akan, S. Cetin, B. Eren, M.S. Güırses, U.N. Güındogmus, Scuba diver deaths due to air embolism: two case reports, *Soud. Lek.* 58 (2013) 26–28.
- [29] F. Evain, P. Louge, R. Pignel, T. Fracasso, F. Rouyer, Fatal diving: could it be an immersion pulmonary edema? Case report, *Int. J. Leg. Med.* 136 (2022) 713–717, <https://doi.org/10.1007/s00414-022-02809-x>.