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ScienceDirect
Forensic Science International 163 (2006) 1–9
Forensic Science International
www.elsevier.com/locate/forsciint

Drowning: Still a difficult autopsy diagnosis

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Received 18 October 2004; accepted 20 October 2004
Available online 27 December 2005

Presented at the 8th Cross Channel Conference (Bruges, April 20th–24th, 2004).

Abstract

Investigation of bodies recovered out of water comprises an important proportion of the medico-legal requests. However, the key question whether the victim died due to ''true'' drowning can frequently not easily be solved. In addition, the diagnosis of hydrocution is even more difficult. In this manuscript, a review of reported diagnostic methods is discussed in order to provide guidelines, which can be used in current forensic practice. In particular, the (dis)advantages of various biological and thanatochemical methods, described in literature during the last 20 years, will be confronted with the classical techniques such as the detection of diatoms and algae. Indeed, the diatom test is still considered as the ''golden standard''.

In conclusion, the ideal diagnostic test as definite proof for drowning still needs to be established. At present, the combination of the autopsy findings and the diatom test is a good compromise in arriving at a conclusion. Additional biochemical and technical methods could be useful. Unfortunately, the cost–benefit analysis in current practice could be hard to defend. However, the importance of this subject asks for further scientific approaches and research.

Keywords: Drowning; Diagnosis; Review; Diagnostic methods; Diagnostic markers

1. Introduction

Several definitions of drowning are available and in fact all of them carry some wit. One of the most classical definitions is provided by Roll: ''death by drowning is the result of a hampering of the respiration by obstruction of mouth and nose by a fluid medium (usually water).'' [1]. In fact there are a lot of definitions and all of them carry some truth. About true drowning there are no substantial controversies: a common pathway is the inhalation of water which can pass the alveolo-capillary membrane and reach the circulation. Classically, a distinction between fresh and saltwater is made, although this is essentially based on animal experiments. However, huge controversies exist about immersion death other than true drowning, such as dry lung drowning, immersion death, hydrocution and ''Badetod''. Furthermore, the pathophysiological phenomena are hard to prove. Indeed, reflex cardiac arrest by vasovagal stimulation and laryngospasm cannot be demonstrated during autopsy, but favourising factors such as an ethanol intoxication and cold water should be taken into account. By definition, immersion death other than true drowning can never be substantiated by autopsy techniques. Those deaths can in fact be natural deaths. Modell et al. stated that ''to ascribe drowning as a cause of death to a body found in water without some evidence of the effect of having aspirated water is risky'' and concluded that ''in this situation, it may be more accurate to list a differential diagnosis rather

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doi:10.1016/j.forsciint.2004.10.027

than a specific cause of death'' [2]. Police information such as eye-witnessing can, as an example, be much more reliable.

Drowning has extensively been discussed in medico-legal literature and historical reviews can be found by Reh [3], Thomas et al. [4], Timperman [5], Keil [6], Saukko and Knight [7], Ludes and Fornes [8], Brinkmann [9] and Giertsen [10].

The aim of this manuscript is to provide a critical overview of reported diagnostic methods of ''true drowning'' which can be used as a guideline in daily medico-legal practice. Following a theoretical consideration of the general principles of drowning, relevant literature data of the last 20 years was reviewed. First, in brief, the classical diagnostic autopsy findings (such as Paltauf sign, emphysema aquosum, pleural effusion) and more recent microscopical techniques are considered.

Thereafter, various biological and thanato-chemical methods described in literature and their (dis)advantages are discussed.

At first, the detection of foreign plant elements such as diatoms, algae, chlorophyl is assessed and the value of diatom test as the ''golden standard'' is evaluated. Moreover, thanato-chemical markers such as strontium, magnesium, chloride, haemoglobin, pulmonary surfactant proteins A and D as well as other various investigations will be envisaged.

2. General principles

Fig. 1 shows the general principles in the assessment of true drowning.

2.1. Water enters the blood circulation by diffusion and osmosis (haemodilution)

This has been proven by animal experiments at the end of the 19th century by, e.g., Brouardel and Vibert [11]. Several chemical and physical methods have been presented in order to point out this haemodilution. An overview of the literature focussing this item can be found [12]. However, these methods can only be applied when autolysis and putrefaction have not taken place. As the autopsy is usually only performed 24 h post mortem and referring to the fact that bodies retrieved out of the water usual show an earlier putrefaction, these dilution experiments are hardly performed in current forensic practice. In addition, cardiopulmonary reanimation interferes with haemodilution tests [13]. Some researchers consider these techniques as obsolete [14]. Only the more recent haemodilution tests will be mentioned.

2.2. Suspended particles in the drowning water enter into the blood circulation

This implies that during the drowning process, in addition to diffusion and osmosis, small lesions in the alveolar membranes take place and, as a result, particles present in the drowning water can enter the blood circulation. Corin and Stockis demonstrated that crystalline elements suspended in drowning water can be found in the left heart [15–18]. The methods of Corin and Stockis passed into disuse and a few decades later, the diatom investigation in blood and organs was introduced. The uptake of corpuscular substances suspended in drowning water was more recently demonstrated by means of latex and gold tracers in a rat animal model [19].

The diatom technique was auspicious and induced flourishing scientific research and debate (e.g. [20]). Soon after the establishment of the diatom method, unfavourable criticism raised and, at present, advocates and opponents are not reconciled. Herewith, the risk of contamination matters a lot [21–23]. In addition, due to the pollution of the surface waters, diatoms could vanish in the future [24]. Indeed, an obvious impoverishment of the diatom species was observed and, e.g., in Belgian mere, only one species survives, namely *Eunotia exigua* [25]. An alternative but never established method is the prospecting of ciliated protozoa in blood in case of freshwater drowning [26].

2.3. Chemical substances, which are dissolved in the drowning water and which are found in very low concentrations in the human body (trace elements), can enter the blood circulation

Theoretically, the perfect method should correspond to following premises. First, the marker should have the possibility to pass the alveolo-capillary membrane to get into the circulation. Second, the substance should be present in large amounts in the drowning water and in very small amounts in the blood of healthy persons. In addition, there should be no entering into the circulation by other ways such as via the gastro-intestinal tract or due to post-mortem diffusion.

The merit of this theoretical approach can be assigned to Icard [27]. Experiments in animals proved the benefit of this method [28]. However, reported medico-legal cases to prove the value in current practice are scarce.

2.4. Corpuscular or cellular elements present in the lung alveoli can be encountered in the blood circulation following drowning

The examination of these elements was reported a few decades ago [29–31]. However, the published case series are small and are not suitable for reliable statistical analysis. In addition, these methods are not applicable to all drowning cases, e.g., smoker cells can only be determined in persons having an obvious smoking behaviour. Furthermore, the study of asbestos bodies and surfactant factor could be taken into account.

To investigate how the above-mentioned general principles are applied in research, a literature review was performed in order to provide an update of the last 20 years. This search is far from complete as a lot of articles are

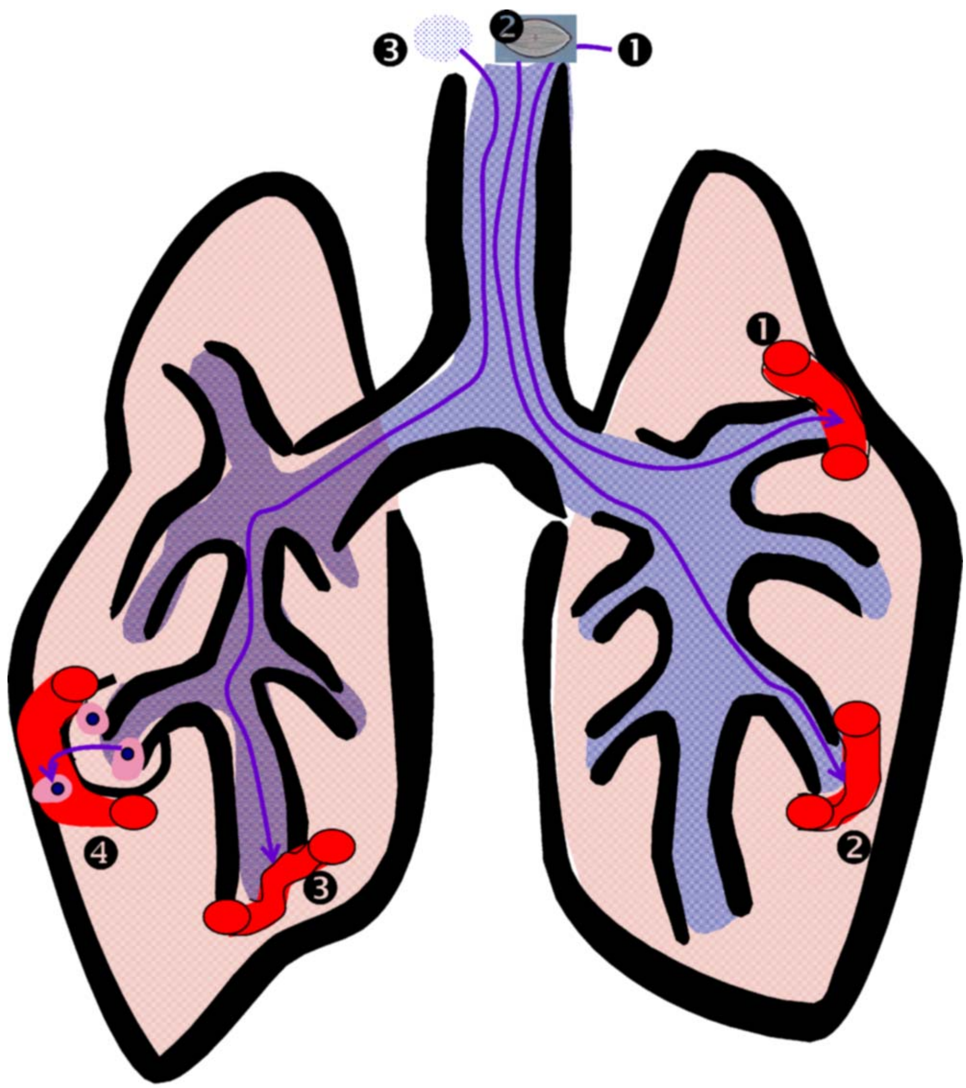

Fig. 1. ❶: Haemodilution; ❷: particles in drowning water (e.g., diatoms); ❸: soluted constituents in drowning water; ❹: particles or cells in alveoli.

written in less accessible languages such as Chinese, Japanese or Russian.

First, the classical macroscopical and microscopical characteristics will be presented. Thereafter, referring to the above-mentioned general principles, an overview of the biological and thanato-chemical tests and various tools which cannot be classified in this scheme, will be provided.

3. Autopsy data

3.1. Macroscopical autopsy findings

An interesting review of possible macroscopical findings in a large series of drowning cases is provided by Lunetta et al. [32].

At *external examination*, the plume of froth at the mouth and nostrils can be considered as a valuable indication. The pitfall is that this finding is non-specific, quite transient and can only be found in fresh drowned bodies. Moreover, all other signs are immersion signs and not pathognomonical for drowning.

At *internal investigation*, following thoracic findings can provide useful information: lung emphysema and oedema aquosum [33], indentations of ribs in lungs, Paltauf's spots, froth in the trachea, elevated lung weights and pleural effusions. Hadley and Fowler found that elevated lung and kidney weights are the result of both asphyxiation and the aspiration of water, whereas increased spleen and liver weights in drowning victims were only due to the asphyxiation effect [34]. However, a small, anaemic spleen was hypothesized to be a post-mortem phenomenon [34]. A significantly higher lung–heart weight ratio was found in fresh-/saltwater drowning victims compared with other asphyxiation fatalities [35]. Morild demonstrated that there was a link between the post-mortem interval and the presence of increased pleural fluid [36], which was also demonstrated by other authors [37,38]. In addition, a significant difference was found between salt- and freshwater drowning cases: due to the hyperosmolar properties leading to plasma leaking into alveolar spaces, more pleural fluid is produced in the saltwater drowning cases [36]. However, Yorulmaz et al. were not able to prove a significant difference between the amount of pleural fluid in freshwater and saltwater drowning cases [37].

Inspection of the stomach can reveal Wydler's sign (swallowing of water) or even the Mallory–Weiss' syndrome (oesophageal mucosal tears) [6].

The interpretation of haemorrhages in the neck tissues can be controversial. In the absence of trauma in that region, these bleedings are most probably due to hypostasis or to an extension [39] or the Prinsloo and Gordon artefact (post-mortem haemorrhage on the posterior surface of the oesophagus) [7]. In a minority of the cases, these haemorrhages can be explained by violent neck movements during the drowning process [39]. This was supported by Püschel et al. who attributed the intramuscular haemorrhages in the neck of drowning victims to agonal convulsions, hypercontraction and overexertion of the skeletal muscles of the neck, anterior/posterior trunk and the upper extremities [40]. Moreover, they notified that these haemorrhages can be regarded as an additional tool in the diagnosis of drowning [40]. In cases of doubt, histological examination can be more informative [41]. However, Keil et al. reviewed 2060 autopsies and concluded that haemorrhages in the posterior cricoarytenoid muscles are not indicative for the cause or manner of death [42].

Several years ago, drowning fluid was described during examination of the sphenoidal sinus (Svechnikov's sign) [6]. Bohnert et al. studied the liquid amounts in the sphenoid sinuses: the average volume of the aspirate was smaller in the control group than in the drowning victims [43]. Hotmar found that both the investigation of liquid in sphenoid and maxillary sinuses can provide auxiliary information in the diagnosis of drowning [44].

In fact, all these macroscopical signs are non-specific but external and internal froth combined with acute emphysema in a victim retrieved from water can be considered as mere suggestive of drowning.

3.2. Microscopical autopsy findings (lungs)

Classical histological examination (hematoxylin eosin staining) shows obvious intra-alveolar oedema and dilatation of the alveolar spaces with secondary compression of the septal capillaries [3]. Delmonte and Capelozzi could differentiate between other modes of asphyxiation [45]. However, putrefaction obnubilates all fine histological structures.

A wash-out effect of intra-alveolar macrophages in case of drowning has been proven by Betz et al. [46]. In addition, an increase of macrophage subtypes (myelomonocyte subtypes) in the alveolar-intracapillary compartment by means of an immunohistochemical method was demonstrated [47]. However, the interpretation of these tests is quite difficult.

Several years ago, a scanning electron microscope study pointed out to distinguish chronic emphysema from the acute emphysema of drowning [48]. However, when both emphysema aquosum and chronic emphysema coexist, the diagnosis becomes difficult to establish [48]. Here as well, post-mortem alterations will hamper a correct interpretation.

4. Biological and thanato-chemical tests (see Fig. 1)

4.1. Haemodilution tests

Haemodilution tests are valuable only for fresh bodies recovered from water, namely within the first 24 h after death. Dilution of ureum or total proteins can sometimes be demonstrated [49,50]. Haemodilution methods are mostly abandoned due to lack of specificity and sensitivity caused by post-mortem biochemical autolytic and putrefactive deluge [13]. Furthermore, the interpretation can be influenced by cardiac resuscitation, which lowers the values [13]. However, the comparative determination of iron in both cardiac cavities is proposed as evidence of haemodilution [51]. Further research has to be done.

4.2. Micro-organisms

Various micro-organisms diluted in the drowning water can be studied, e.g., diatoms and algae.

4.2.1. Diatoms

Diatom investigation in the diagnosis of drowning engendered a lot of criticism and controversial. First, diatoms can be absent when macroscopical diagnosis of drowning is obvious such as when the victim died following a very short agony or when monthly variations due to climatic influences are interfering. This explains the lack of sensitivity as diatoms are demonstrated in only one-third of freshwater drownings [52–54]. Even in the open sea, diatoms can be expected not to function as a reliable marker [55]. Furthermore, diatoms can be present when people died due to other non-drowning causes of death. Diatom examination in tissues is a very tough and constrained analysis which asks for cooperation with an algae specialist [56,57]. However, the threat of contamination can be regarded as the mean reason for criticizing the diatom analysis method [58].

The standard principles include qualitative and quantitative diatom analysis in organs associated with diatom analysis of the drowning water. In addition, dangerous and destructive methods should be avoided [53–61].

Some technical improvements have been proposed, such as presented by Funayama et al. [62], Kobayashi et al. [63], Matsumoto and Fukui [64] and Sidari et al. [65]. In fact, these methods could counteract the destructive properties of the classical digestion methods by strong acids as, e.g., seawater diatoms are relatively fragile [66]. On the other hand, Soluene-350 is also destructive for seawater diatoms [65]. The techniques should thus be adapted to the search for fresh- or seawater diatoms.

New perspectives are provided by the polymerase chain reaction (PCR) method for identifying diatoms by means of primers for chlorophyll-related genes, e.g., of *Euglena gracilis* and *Skeletonema costatum* [67]. Indeed, even in "diatom negative cases" with the use of the classical procedures, these tests could be positive. However, the enhanced sensitivity could be hampered by a higher risk for contamination.

4.2.2. Algae (Chlorophyceae)

The search for algae can be informative in diatom poor drowning waters but soft destruction methods such as using solubilizer Soluene-350 have to be employed [68]. Chlorophyll can also be determined in lungs by means of a spectrofluorometric method, but this is still in an experimental phase [69]. Phytoplankton gene detection (by means of a PCR method) can also be helpful [67,70,71]. However, the lung samples of non-drowned rabbits were also positive due to post-mortem plankton penetration into the respiratory system [71]. Kane et al. were able to demonstrate picoplankton in formalin-fixed lung tissue in drowning cases [70].

4.3. Chemical constituents

Various soluted constituents have been studied in an attempt to find a suitable marker for drowning, such as strontium, fluorine and other salts.

4.3.1. Strontium (Sr)

Several years ago, quantification of strontium in blood or serum has been investigated [28,72]. Azparren et al. noted that only in 32% of the bodies found in freshwater, the drowning diagnosis could be established using the strontium marker [73]. A difference of blood strontium concentrations between left and right ventricle can be helpful in the drowning diagnosis [74]. "Typical" drowning in seawater can be assumed when the strontium level between the right and the left side of the heart differ >75 μg Sr/l; a value of <20 μg Sr/l is considered to be indicative for "atypical" drowning [75]. In addition, the difference between Sr-levels in right and left ventricle could be indicative for the length of agony in drowning [76].

Following pitfalls should be taken into account. Some water types have a relatively low strontium concentration, e.g., rainwater, tap water [77]. On the other hand, strontium can be elevated in mineral water drinkers or regular seafood users [77]. Moreover, strontium can, especially in seawater, invade the organs and blood by, e.g., pulmonary post-mortem diffusion [77]. For comparison, Powitz also suggested that post-mortem uptake of bromine can occur in immersed dead bodies [78]. However, Azparren et al. could not demonstrate a substantial post-mortem strontium diffusion when the post-mortem interval was less than 5 days [76].

Strontium determinations can be particularly useful in seawater or brackish water drowning (fresh cadavers) [79,80]. In freshwater drowning, there is a unmistakable overlapping zone with non-drowned individuals [72]. However, Fornes et al. could not establish an overlap between freshwater drownings and non-drowned subjects [33]. Therefore, strontium determination in the drowning water is essential for comparison.

Fornes et al. used an interesting approach, namely the combination of computer-assisted histomorphometry of the lungs with blood Sr measurement [33].

4.3.2. Various markers

The quantitation of serum fluorine can be valuable in the diagnosis of drowning but is restricted to those areas in which fluorine is substantially present: this was shown in a rabbit animal model [81].

Zhu et al. studied left and right cardiac blood samples for several serum markers such as sodium (Na), chloride (Cl), magnesium (Mg) and calcium (Ca) [50]. In saltwater drowning victims, an obvious increase of serum Cl, Mg and Ca levels of the left heart blood is characteristic [50].

4.4. Normal or abnormal constituents of the lung alveoli which can be found in the circulation

4.4.1. Raucherzellen

Several years ago, Reiter demonstrated the presence of smoker cells in the left cardiac blood in drowning victims: these macrophages were washed from the pulmonary alveoli into the cardiac blood [31]. He suggests that this test could also be useful to diagnose the vitality of drowning and bath tube death cases in which there are hardly plankton and electrolytes [31]. However, few recent articles were published considering this item. Theoretically, other intra-alveolar constituents such as asbestos bodies could be retrieved in the blood circulation following drowning.

4.4.2. Pulmonary surfactant factors

The value of lung surfactant factors was first studied by Lorente et al. in rabbits [82]. They demonstrated that the pulmonary surfactant, phosphatidyl choline, phosphatidyl ethanolamine and phosphatidyl glycerol may be useful markers in the drowning diagnosis, namely to distinguish true drowning and post-mortem immersion on the one hand, and to differentiate fresh- and saltwater drowning cases on the other [82]. In addition, in rats, an acceptable post-mortem stability of the lung surfactant phospholipids was shown in the first 24 h after death, followed by a progressive decrease from 48 h on until 96 h post mortem [83]. Kamada et al. [84] used a sandwich enzyme immunoassay to determine blood pulmonary surfactant protein D (SP-D) in drowning victims. Blood SP-D levels both from saltwater and freshwater drowning cases were increased, and the mean concentration was highest in the saltwater fatalities [84]. However, blood SP-D concentrations are also increased in other asphyxia fatalities, but to a lesser degree [84]. Pulmonary surfactant-associated protein A (SP-A) can be elevated in blood and tissue specimens, not only in drowning victims, but also in case of, e.g., acute respiratory distress syndrome [85]. Therefore, SP-A can be useful as marker of asphyxiation, respiratory distress and alveolar injury [85]. Zhu et al. found that a significant elevated serum SP-A concentration can be found in freshwater drowning victims, but there was a considerable overlapping with saltwater drowning and acute myocardial infarction [50]. In conclusion, both SP-D and SP-A are not fully specific for drowning and are in fact good markers of alveolar injury.

5. Varia

Herewith we present some research data which cannot be classified in the above-mentioned categories of Fig. 1.

Atrial Natriuretic Peptide (ANP) was first studied in rabbits by Lorente et al. who found an increase in blood particularly in freshwater drownings [86]. No further publications were seen with human material. It should be noted that post-mortem stability is at present not demonstrated in human, but only in rats where the ANP was stable in the first 8 h after death [87]. Furthermore, physiological release of ANP in life head-out immersion in water was demonstrated [88]. In addition, a pathological ANP increase in heart disease and in all circumstances of hypervolaemia such as in pregnancy excludes ANP as a specific marker for drowning.

Ubiquitine in substantia nigra neurons was studied by Quan et al. [89]. However, there is only a single report and there are no confirmatory data. Furthermore, ubiquitine (heat shock protein) increase was also seen in other forms of violent asphyxia [89].

6. Discussion and conclusion

To demonstrate drowning unambiguously as the cause of death, remains a difficult issue in current forensic practice. Following the establishment of the fact that the person was alive before entering the water, the key question whether the victim died due to drowning has to be solved. Moreover, the diagnosis of hydrocution is even more difficult if not impossible. In the autopsy diagnosis of drowning we will thus only consider ‘‘true’’ drowning. When we cannot confirm the inhalation of water and its components, Modell et al. suggest to list a differential diagnosis rather than to conclude to a specific cause of death [2]. Moreover we will not consider the manner of death which is mainly the affair of the police investigators. Indeed, forensic pathologists need to be aware that often the manner of death remains undetermined [90].

The macroscopical and microscopical signs in the fresh drowned victim are non-specific and moreover putrefaction will vanish these autopsy findings quite rapidly. Putrefaction must indeed induce further laboratory investigations to substantiate drowning. After a review of these tests, we must conclude that biological and thanato-chemical markers of drowning are not yet accepted with a scientifically reasonable certitude. However, at present, the diatom analysis remains the golden standard, but is rather labour-intensive. Strontium analysis could be an auxiliary tool, especially in recent seawater drowning victims. The combination of all autopsy findings and the additional drowning tests in non-putrefied water bodies can provide useful indications for the tentative diagnosis of true drowning. In the majority of the cases, autolytic and putrefactive changes would preclude a scientifically sounded diagnosis of drowning. More and more, we are convinced that the diagnosis of true drowning is not exclusively the duty of the forensic pathologist. In fact, the law enforcement authorities, have to be involved in final statement of the cause of death by drowning [8,10,91]. Only in this philosophy, we could reduce judicial errors. Death by drowning remains one of the most difficult diagnosis in forensic medicine.

Acknowledgments

The authors gratefully thank Mrs. Thérèse De Vuyst and Mrs. Isabelle Cornelis for their assistance in preparing the manuscript.

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