DIAGNOSIS OF DROWNING, AN EVERLASTING CHALLENGE IN FORENSIC MEDICINE: REVIEW OF THE LITERATURE AND PROPOSAL OF A DIAGNOSTIC ALGORITHM

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ABSTRACT

Introduction*: The diagnosis of drowning is one of the major challenges in the field of forensic medicine and usually it is a "diagnosis of exclusion." Traditionally, the post-mortem diagnosis of drowning is based on the combination in one diagnosis both the alterations, caused by the type of asphyxia, and those due to the thanatological changes, caused by the duration of the corpse in water. Due to the development of technologies in forensic sciences this approach results actually obsolete. The purpose of this study was to design and propose an algorithm that diagnoses drowning.*

Materials and method*: A review of the papers published in forensic pathology literature was performed using multiple combinations of search terms were used to select scientific research about drowning on PubMed.*

Conclusion*: The methodology used up to now, which was based on external and internal findings, is not very effective, bringing together in one analysis the alterations in the body depending on the type of asphyxia and the thanatological expression of the permanence of the corpse in water. The technological evolution in the medicolegal field has, therefore, encouraged us to use more sophisticated investigations by assigning the diagnosis not only according to autopsy finding. The algorithm we propose has the purpose of guaranteeing a diagnosis based on uniform criteria that contribute to formulating a diagnosis of drowning that is reliable and coherent, while using the available survey tools.*

Keywords*: drowning, autopsy, forensic pathology, histology, diatom test, algorithm.*

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Introduction

The diagnosis of drowning is one of the major challenges in the field of forensic medicine[(1)]. In most instances, it is a "diagnosis of exclusion." To date, we have relied on the classic autopsy to diagnose drowning as well as histological, microbiological, and biochemical methods[(2-15)]. The traditional medicolegal treatises distinguished the external and the internal findings observable on the cadaver to arrive at diagnostic criteria in cases of corpse recovery from water. This approach, however, seems not particularly effective because it requires that we combine in one diagnosis both the alterations, caused by the type of asphyxia, and those due to the thanatological changes, caused by the duration of the corpse in water. This subdivision of issues is now obsolete due to the technological evolution in the medicolegal field. Thus, a methodological approach seems more effective, starting from the macroscopic findings observed at the autopsy followed by the laboratory results from the histological, microbiological, and biochemical investigations.

Materials and methods

Multiple combinations of search terms were used to select scientific research about drowning on PubMed (research source of the US National

Library of Medicine - National Institutes of Health, widely used worldwide for medical-scientific bibliographic research). The terms and combinations of terms used were as follows: drowning, drowning AND diagnosis, drowning AND autopsy, drowning AND histological examination, drowning AND biochemical alterations, drowning AND microbiological examination, diatoms, diatoms AND drowning diagnosis. We limit our search strategy to the journals of forensic pathology [International Journal of Legal Medicine, Medicine, science, and the law, Forensic science international, Journal of forensic and legal medicine, Legal medicine (Tokyo, Japan), Journal of forensic sciences, Australian Journal of Forensic Sciences, the American journal of forensic medicine and pathology]. No temporal limit was set. Among the publications identified (873), all the papers were written in English. The selected papers were analyzed on the basis of the abstract ensuring that they highlighted the methods used to diagnose drowning, such as autopsy, histological examination, microbiological examination, and biochemical examination.

Finally we selected 65 papers. During our analysis we requested other 12 papers selected between the references cited. Finally, after studying all the selected scientific literature, a drowning diagnostic algorithm was elaborated.

Results

Autopsy

A finding traditionally described in corpses found in water is froth around the mouth and nostrils, consisting of a mixture of pulmonary fluid and the ventilated mucus that occurs at the level of the bronchi and trachea. It surfaces at the respiratory orifices after the body has been removed from the water, the result of a gaseous putrefactive phenomenon that gives the diaphragm a push upward. If the froth is not apparent, it is sometimes sufficient to exert light pressure on the chest to expel it. This finding, for the purpose of diagnosis, is an external sign indicative of drowning because it is an expression of respiratory activity. The presence of froth around the mouth and nostrils alone, however, is not enough to formulate a diagnosis of death by drowning because it may also be observed following other asphyxial deaths. An association of froth, pulmonary emphysema, and overlapping of the polar margins has been identified by some as a useful diagnostic indication of drowning[16]. Among other autopsy findings, pulmonary alterations, dilution of the blood, the presence of pleural effusion, and the weight of the lungs are particularly significant, whereas the amounts of fluid present in the lungs, stomach, and bowel are of little importance. The first noticeable and immediately identifiable macroscopic finding is "acute aqueous pulmonary emphysema." Upon opening the chest, one may observe that the front margins of the lungs cover the cardiac wall and sometimes overlap one another. The lungs, because of their considerable increase in air content, are of increased volume, adherent to the chest wall, of pale pink or gray-whitish color, and of cottony, inelastic consistency. A characteristic crackling may be appreciated, and the surfaces of the section may appear dry ("dry-lung drowning") or edematous with the presence of a mucous-like fluid mixed with froth. In the case of pulmonary edema, the "drowning liquid" may give rise to hyperhidrosis. When examining the lungs, it is also possible to detect subpleural petechiae, or "Paltauff spots," which are found on the anterior surfaces and, more frequently, on interlobar surfaces. Blood dilution, if present, is due to passage of the drowning liquid in vessels at the pulmonary level. Thus, blood in the left ventricle, arriving from the lungs, is more diluted than that in the right ventricle. This phenomenon is easily demonstrated at autopsy using the "cartometric test." The test consists in dropping drops of blood, obtained separately from the right and left ventricles, on sheets of blotting paper and observing the size of the relative halos. In the case of left ventricular blood dilution, the halo of the left ventricle should be paler red and have a relatively larger diameter than that of blood from the right ventricle. The dilution of the blood and its altered salt concentration, due to the drowning liquid, also involves modification of the cryoscopic delta (lowering of the freezing temperature) and electrical conductivity. The freezing point of the blood is lowered if the salt content is high, thereby increasing electrical conductivity.

Other macroscopic internal findings that can be investigated for diagnostic purposes are pleural effusion and increased lung weight. Pleural effusion has diagnostic value when it presents with the characteristics of a serum-hematic transudate. This phenomenon is characterized by passage of the drowning liquid from the lungs in the pleural cavities and for the filtration of the plasma from the walls of small-caliber vessels[17] consequent to degradation of phospholipids in pulmonary surfactant. Passage of fluid into the pleural cavities, which begins during drowning, is accentuated by a prolonged postmortem period. The amount of pleural effusion is closely related to the degree of pulmonary hyperhidrosis and depends on the length of time the body has been in the drowning liquid[18]. The correlation between pleural effusion and lung weight has not yet proved to be of significance diagnostically in drowning cases. Some authors, however, have proposed that a lung/pleural fluid weight and spleen weight ratio of 14.1 (Drowning Index) could be a standard diagnostic value[19,20]. Others suggested that the relation between lung weight and body weight (L/B ratio), identifying 19.5 g/kg as the cutoff value, could be a useful tool for diagnosing death by drowning with some accuracy[21].

Histological examination

Histological examination, supplementing the macroscopic data, can be helpful in arriving at a drowning diagnosis. The histological examination reveals dilation of the alveoli associated with thinning of the alveolar walls and rupture of the interalveolar septa. In addition, the elastic fibers are fragmented, the pneumocytes are swollen and broken, and siderocytes may be found in the interaleveolar septa. Hemorrhage may also be observed in peribronchial sites. Histological examination performed on lung samples from drowning victims have shown decreased alveolar macrophages and a low interstitial tissue ratio[22]. Immunohistochemical tests have indicated an increase in myelomonocytes at the level of the alveolar capillaries.

Experimental drowning models have revealed cytological and histological alterations due to osmotic phenomena[23]. The most significant histological alterations discovered in conjunction with drowning and other asphyxial deaths are aqueous emphysema and alveolar hemorrhage[24]. Delmonte and Capelozzi[25] specified the histological aspects of the lung in cases of drowning, underlining the recurrence of intra-alveolar edema and dilatation of the alveolar spaces with compression of the septal capillaries. Other authors have noted the importance of histological examination of the temporal bone - seeking hemorrhage in the inner ear and mastoid cells - for differentiating between drowning and other types of asphyxia[26, 27]. In such cases, tympanomastoid hemorrhage, in the absence of other explicable causes, is a factor strongly indicative of death by drowning[28-30]. The analysis of aquaporins (also called "water channels")[31], which are integral membrane proteins that facilitate the flow of water molecules inside or outside cells, has provided useful indications for differentiating between saltwater and freshwater drowning. A significantly high immunohistochemical positivity for aquaporin 4 in astrocytes[32] and aquaporin 2 in kidney cells[33] is an indication of freshwater, rather than saltwater, drowning.

Microbiological examination

An extremely important finding for the diagnosis of drowning is plankton in the lungs and internal organs derived from the drowning liquid. Particularly significant is the finding of phytoplankton with a siliceous shell, such as diatoms[34-39], which are easily recognized by their morphological aspects and for being resistant to usual laboratory chemicals. Among the many proposed methods for studying diatoms, a particularly effective one is based on enzymatic digestion mediated by proteinase K, as proposed by Ludes et al[40]. Its efficacy has been confirmed by others[41,42] and therefore improved. Takeichi and Kitamura[42], for example, introduced formalin fixation of the tissues obtained for examination to reduce the risk of contamination by pathogenic microorganisms during enzymatic digestion, and the incubation of samples with hydrogen peroxide for 6 h to improve the informational content of the diatom images. The finding of plankton in the peripheral areas of the pulmonary parenchyma and in other viscera (e.g., liver, kidneys) strongly suggests a drowning diagnosis. The ability of diatoms to cross the alveolus-capillary barrier, thereby entering the bloodstream, has been demonstrated[43].

The presence of plankton shows that when drowning liquid floods the lungs, the cardiocirculatory activity was effective, allowing transport of this material, through the blood circulation, into the viscera. The finding of plankton only in the lungs has little diagnostic value because water - and thus plankton - can passively enter the airways during submergence of the corpse. Hence, it must be emphasized that for a definitive diagnosis of death by drowning, the quantity of plankton found in the organs and viscera is relevant[44, 45] as a minimal amount may be present in all individuals, especially in those who live or work in dusty environments. Zhao et al. introduced a method for detecting diatoms. Called microwave digestion–vacuum filtration–automated scanning electron microscopy (MD-VF-ASEM), it allows us to find large quantities of diatoms that have reached the lungs during the forced inhalation that occurs during drowning[46]. A quantitative comparison analysis of diatoms in lung tissues (L) and the drowning liquid (D) as an indicator of drowning showed that the L/D ratio was higher in drowned bodies than in those immersed in water after death[47]. In the context of microbiological studies, research on bacteria must be included. These investigations make it possible to detect aquatic microflora in blood, which after passing through the alveolus–capillary membrane enters the circulatory system. For this purpose, studies based on bacteriological tests for research on Pseudomonas putida and Pseudomonas fluorescens[48] have been proposed, supplementing the analysis of the plankton composition[49, 50] in the diagnosis of drowning. Kakizaki et al.[51] established that the finding of blue and/or bioluminescent colonies in blood samples from the corpses, immersed in and then grown on TH agar with a 4% solution of NaCl, may be helpful for diagnosing drowning in seawater. The authors based their conclusions on the fact that 16S rRNA analysis detected as dominant colonies in TH agar containing 4% NaCl species of marine bacteria such as Photobacterium, Vibrio, Shewanella, and Psychrobacter.

This finding was confirmed in a subsequent study, which also showed how the proliferative capacity of bacterial species in human blood varies as a function of water salinity[52]. Technological development and the introduction of new molecular biology methods allowed us to identify and analyze other components of plankton[53]. Particularly, the polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE) has been used to evaluate the 16S rDNA of picoplankton and to identify the place of drowning[54, 55]. PCR is also associated with capillary electrophoresis to amplify the gyrB and 16S rRNA genes of Heromonas hydrophila in lung, liver, and kidney samples. Simultaneous detection of the gyrB and 16S rRNA genes has been suggested as a useful criterion for diagnosing drowning in freshwater[56]. The study of bacterial colonies has been useful for diagnostic purposes when associated with the study of diatoms[57]. The finding of bacterioplankton in the blood of drowned subjects appears to reflect the type of inhaled water. It is particularly important because the blood is not easily contaminated by postmortem bacteria even in decomposed bodies[58] The presence of the aquatic microbiota in organs dependent on the systemic circulation (e.g., kidneys, liver), and an increased concentration of surfactant protein A in the lungs have been identified as diagnostic markers of drowning[59].

Other studies have focused on the presence of fecal bacteria, coliforms, and streptococcal bacteria in the blood taken from the right and left ventricles and from the femoral artery and vein. Fecal bacteria are always present (in different amounts depending on the location of the sample) in subjects who drowned compared with those who died from other causes, although there is variability between those who drowned in freshwater versus those who drowned in seawater[60,61]. Aoyagi et al.[62] believed that bacteria are more reliable markers than plankton for diagnosing death by drowning. They therefore proposed a new diagnostic method based on the use of PCR to identify the DNA of Aeromonas sobria, one of the most common aquatic bacteria. Their method allowed us to observe DNA fragments of this bacterium in 27 of 32 subjects who drowned in freshwater. PCR is considered highly sensitive and specific for detecting bacterial DNA and therefore useful in cases of death by drowning in water that does not contain plankton[63]. Thus, the presence of phytoplankton DNA detected with PCR contributes autopsy data to the diagnosis of drowning even when diatoms are not found in organs supplied by the systemic circulation[64].

Biochemical analysis

Among the numerous proposed biochemical techniques, the amount of strontium in blood obtained from the right and left ventricles must be mentioned. It is particularly important because a difference in the concentration of this element in the two ventricles has been found with typical and atypical drowning, making it possible to establish cutoff values. If the difference between strontium concentrations in the right and left ventricles is >75 μg/L, it is likely that the drowning was "typical." If the concentration is < 20 μg/L, it can be viewed as "atypical"[65]. The differences in strontium concentration, in the blood of the right and left ventricles, have been suggested to be related to the phases that characterize the period of agony that precedes death from drowning in seawater[66]. Strontium concentration differences were also found in the blood of those drowned in freshwater and seawater[67, 68] and of those drowned in cold and hot water[69], so it could be used for differential diagnostic purposes. The study of strontium for diagnostic purposes was also performed on the teeth. In particular, the laser-induced breakdown spectroscopy technique allows us to perform both qualitative and quantitative analyses, by studying the optical spectrum emitted by the plasma generated by the interaction between a high-power laser radiation and a solid, gaseous, or liquid sample. A higher concentration of strontium was observed in the dentin of those drowned in seawater[70].

Other biochemical markers used for the diagnosis of drowning are calcium and magnesium. Higher levels of these two elements were observed in the blood present in the heart and peripheral blood of subjects drowned in seawater than in those drowned in freshwater[71]. Furthermore, a high Mg/Ca ratio was found in pericardial fluid in cases of drowning in seawater[72]. Deliligka et al.[73] observed low levels of troponin I in the pericardial fluid of those drowned in seawater and increased levels of calcium and magnesium in the pericardial fluid of those drowned in seawater and who had histological evidence of acute myocardial infarction. These results indicate that postmortem determination of troponin I levels in pericardial fluid is useful for diagnosing prior ischemic myocardial damage as a factor contributing to death by drowning and, in association with magnesium and calcium, for differentiating death from drowning versus death in water due to a myocardial infarction.

A significant difference in sodium and chlorine concentrations in the pleural fluid was observed between freshwater and seawater drownings[74]. Also, analysis of the summation of the sodium, potassium, and chlorine concentrations in the pleural fluid was useful for diagnosing death by drowning. Particularly, one study that examined variations in the summation of individual concentrations in pleural fluid established that, when the sum was <195.9 mEq/L it strongly suggested drowning in freshwater, and when it was >282.7 mEq/L it suggested drowning in freshwater. This method proved to be reliable for corpses found in water after 2 days or those with a pleural effusion amounting to >100 mL. In cases of drowning in the bathtub, the summation of the electrolyte concentrations exceeded 198.8 ± 40.0 mEq/L. This method has been suggested as useful when combined with anatomical an pathological data[75]. High concentrations of sodium, calcium, magnesium, and chlorine were detected in blood in the left cavities of the heart and in the pericardial fluid of those who drowned in saltwater, whereas sodium and chlorine levels measured in the blood of the left cavities of the heart were lower in those drowned in freshwater[76]. Another useful diagnostic indicator for differentiating between drowning in saltwater and freshwater was the different concentrations of sodium, chlorine, magnesium, and total protein in the liquid present in the sphenoid sinus[77]. Also, the presence of chlorine and bromine in the liquid contained in the sphenoid sinus has been indicated as a useful criterion for diagnosing drowning in saltwater[78].

Finally, combined sodium and chlorine concentrations of ≥259 mmol/L in vitreous has been identified as a drowning index when found in corpses submerged in saltwater for less than 1 h.[79] Other biochemical findings that have been proposed for diagnosing drowning concern the use of atrial natriuretic peptide[80], ureic nitrogen[81], myoglobin[82], iron[83], and bromine[84]. Particularly, an incresed blood concentration of atrial natriuretic peptide and a urinary concentration of myoglobin >100 ng/mL were observed in drowning cases, with peak levels of carboxyhemoglobin <60%.

In addition, lower urea nitrogen levels were found in the left cavities of the heart and pericardium than in the right cavities of the heart and peripheral blood. Studies on pulmonary surfactant showed an increase of phosphatidylcholine and phosphatidylinositol in drowning victims[85, 86]. Also, the blood concentration of protein D of the surfactant was increased for drownings in seawater and freshwater, with slight differences between the two groups, most likely depending on the survival time and the different duration of the agonic period[87]. An increase in the ratio of surfactant A1 and A2 proteins was observed in asphyxial deaths and drowning compared to control groups[88]. High levels of intra-alveolar aggregates of surfactant protein A were more frequently detected after drowning in freshwater than in saltwater[89, 90]. There were also increased concentrations of proteins A and D in the cardiac blood[91, 92].

Conclusion and Diagnostic algorithm

The diagnosis of drowning, based on our examination of the literature and directly observed during our professional experience, presents many conundrums-so much so that most of the time we speak of the drowning diagnosis as a “diagnosis of exclusion.” In our attempt to guarantee a uniform assessment, these diagnostic uncertainties have pushed us to develop an algorithm that can contribute to formulating a reliable, coherent diagnosis of drowning using the investigative tools available. It derives from an in-depth study of the literature and our personal, collective experience as forensic pathologists.

The diagnostic algorithm (Figure 1) is based fundamentally on two levels:

• the necroscopic examination, which includes the external inspection of the cadaver and the autopsy, and the histological examination,

• laboratory tests.

As for the external inspection, the element of greatest significance is the froth around the mouth and nostrils. Of course, this finding alone does not allow a diagnosis of drowning, but it must be evaluated in relation to the data that emerge from the autopsy/histological examination and could assume definitive diagnostic value.

The literature and our professional experience tell us that the presence of pulmonary emphysema and hyperhidrosis are important for the diagnosis of drowning, especially if associated with Paltauff spots, dilution of the blood taken from the left ventricle, and serum-hematic pleural effusion. These three findings alone or even in association have no diagnostic value because they are too nonspecific. Among them, the most significant diagnostic finding was hemodilution, which assumes diagnostic value when associated with the presence of pulmonary emphysema and/or hyperhidrosis. After autopsy completion, the laboratory tests are performed. They are useful for confirming the diagnosis made based on autopsy findings or, in the absence of the latter, to diagnose drowning. Among the laboratory tests, the search of diatoms in the organs that depend directly on the systemic circulation (e.g., liver, kidneys), bacteriological tests, and biochemical analyses play a prominent role. On the basis of these findings, drowning can be diagnosed if high concentrations of diatoms are present in the organs of the systemic circulation (only small concentrations may be found in the general population) and/or if bacteria are found in the blood or viscera. Alterations in electrolyte concentrations (e.g., strontium, bromine, iron) do not have diagnostic value alone, as electrolyte changes may be present in the general population due to coexisting diseases. Such alterations must be evaluated relative to the results of all the other investigations, perhaps strengthening the diagnostic assessment. Finally, if the macroscopic and laboratory criteria considered in the algorithm are not present, the diagnosis of death by drowning must be excluded and further investigations carried out to establish the cause of death.

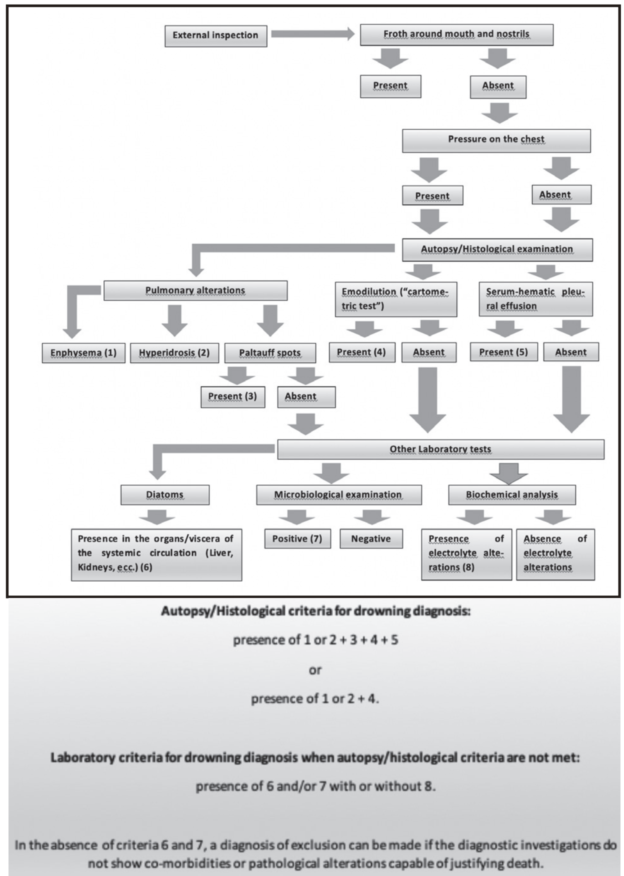

Figure 1. Diagnostic algorithm for drowning.

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