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A promising microbiological test for the diagnosis of drowning

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ABSTRACT

A number of biological and chemical tests have been developed over the years to determine whether a person was drowned. This study focuses on the potential of a microbiological test for detecting common bacterial markers of water faecal pollution such as faecal coliforms (FC) and faecal streptococci (FS) as possible indicators of drowning. A promising previous study was carried out on central and peripheral blood samples of 42 drowned victims (20 cases in saltwater and 22 cases in freshwater) and 30 not-drowned bodies. To improve the accuracy of our previous results and also in order to investigate a possible cause of a false positive due to pulmonary passive diffusion and subsequently endogenous or exogenous bacterial invasion of the blood in the post-mortem interval (PMI), the FC and FS test was applied to bodies submerged in water but died from causes other than drowning. In the present study, blood samples collected from the left ventricle (LV), right ventricle (RV), femoral artery (FA) and, femoral vein (FV) of 10 drowned victims (5 cases in freshwater and 5 cases in seawater) and 3 not-drowned individuals with bodies submerged in water for a while after death have been analysed. Preliminary results are in agreement with other reports dealing with diatoms and marine bacteria that suggest to exclude the hypothesis of a passive penetration of sufficient quantities of drowning medium into circulation after death or during the agonal period. Based on our results there is also no evidence of a relevant dissemination of endogenous micro-flora from the gastrointestinal tract affecting the FS and FC test. There are still several other factors that could influence the applicability of post-mortem FS and FC cultures for the diagnosis of drowning and they need further investigations. The present article provides only a glimpse of the potential of the FS and FC test as bacteriological method for the diagnosis of drowning.

1. Introduction

From the forensic pathology point of view, drowning is still a difficult autopsy diagnosis and it is usually a diagnosis of exclusion [1]. At autopsy, there are no pathognomonic findings to indicate drowning as cause of death [2,3]. Macroscopic pathological signs suggestive of drowning include a plume of froth at the mouth and nostrils, frothy fluid in the airways, elevated lung weights along with overlapping of the anterior lung margins, pulmonary overinflation, pulmonary emphysema and oedema aquosum with oozing froth and/or large amounts of liquid from the cut sections of the lungs, pleural effusion and subpleural petechial haemorrhages [4]. Microscopic signs of drowning include hydropic swelling of the epithelial cells of the terminal airspaces with a wash-out effect of intra-alveolar macrophages, dilatation and rupture of alveoli with secondary compression of septal capillaries, thinning of the interalveolar walls and empty pulmonary capillaries [5,6]. All these external and internal signs are non-specific, and in combination are merely suggestive of a violent asphyxial process caused by drowning [1,3,4].

Many of the proposed biological and thanato-chemical markers of drowning [7] are not yet widely accepted [1]. Most of them are based on the physical and biochemical modifications that occur in the arterial blood compared to the venous blood due to the marked haemodilution caused by freshwater drowning (inhalation of large volumes of water passing through the alveolar–capillary interface and entering the circulation), or, to the hemoconcentration with electrolyte shifts in saltwater drowning. Actually the so many different chemical analyses of the plasma tested still do not provide any reliable evidence of drowning. Cardiac haemodilution methods are also strongly limited due to the lack of specificity and sensitivity caused by putrefaction [8].

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The first ion used to assay haemodilution was chloride (Cl) as described by Gettler since 1921 [9]. The Cl concentration of post-mortem blood is relatively stable and based on the Gettler chloride test if the Cl level is less on the right side of the heart than on the left side (a difference of 25 mg/100 ml being significant), the decedent can be assumed to have drowned in saltwater, otherwise in freshwater medium. A relevant increase of serum Cl along with magnesium (Mg) and calcium (Ca) in saltwater drowning has been recently confirmed by Zhu et al. [10,11]. Unfortunately, the post-mortem concentration of all plasma ions changes also deeply with post-mortem interval (PMI) [12,13].

Several more elements in the blood have been investigated for differentiation between freshwater and saltwater drowning. For example, iron (Fe) blood levels seem to be a good biochemical marker in freshwater drowning with a short PMI [14]. The mean difference of Fe concentration measured in the right and left ventricle of the heart ($RV_{Fe} - LV_{Fe}$) has been found significantly higher in drowning victims (mean value of 67.7 μmol/l) compared with control cases (1.36 μmol/l). Unfortunately, for bodies in advance stage of decay the Fe test can be unreliable or prevented by not enough blood in the heart [14].

Similarly, strontium (Sr) determination by spetrophotometry atomic absorption is very useful in cases of recent seawater drowning [15,16]. Because Sr is a common component of marine salts, drowning in seawater can be inferred when the differences between Sr concentrations in the left and right ventricle ($LV_{Sr} - RV_{Sr}$) are >75 μgSr/l while a value of <20 μgSr/l is considered to be indicative for atypical drowning [17]. The analysis of Sr absorbed into the bloodstream can be useful also for establishing the length of the agonal period in drowning victims [18] and it seems to be unaffected by pulmonary post-mortem diffusion or by gender and age of the victim [19]. However, the use of Sr as marker for drowning is complicated by the fact that the concentration of Sr in seawater varies according location and salinity, and because of a relatively high Sr level in mineral water drinkers or regular seafood users [1,20,21].

The diatom test has been recently considered as the "golden standard" [1] even if it is still controversial and rather labour-intensive. Diatoms have been found in the tissues of people who died of causes other than drowning due to their widespread distribution throughout the environment, in soil, water supplies, food and in the air [4,22], and may be absent in case of drowning [3]. However, the presence of any aquatic micro-organism derived from the drowning medium in the tissues of a drowning victim (such as liver, kidney and bone marrow) is sufficient to prove the hematogenous dissemination of particles from the lung requiring a beating heart during the violent asphyxial process, especially if the same diatoms are observed in the drowning medium [23,24]. In this matter the value of water samples analysis needs to be strongly emphasized [25,26]. Qualitative and quantitative distribution of diatoms in the body is mainly dependent by the diatom density (number, species and dimensions of such organisms) in the drowning medium and the filtration with respect to size that occurs as the diatoms pass from the lungs into the blood [27]. The capacity of diatoms to penetrate the alveolar–capillary barrier along with the drowning medium has been experimentally demonstrated by electron and fluorescence microscopy [28,29], and should occur following the inhalation of large volumes of water.

Micro-organisms smaller than diatoms can easily enter the bloodstream along with the drowning medium. A microbiological test has been recently proposed [30] to investigate the effectiveness of marine bacteria as a new marker of drowning in seawater. In our previous research study [31] we have illustrated a similar microbiological test based on the analysis of faecal coliforms (FC) and faecal streptococci (FS) bacteria, common indicators of water faecal pollution. These aquatic micro-organisms are ubiquitous in the Adriatic sea [32] but also present in contaminated freshwater. They can be easily isolated from the intestines and/or feces but they are physiologically absent in the human blood. Post-mortem blood cultures taken from the left ventricle (LV) and right ventricle (RV) showed the presence of FC and FS (visualized as blue and red colonies respectively) among all the seawater victims analysed (20 bodies totally), the presence of FS among all the freshwater victims analysed (22 bodies total), while the presence of FC was demonstrated only in the 90.91% of the freshwater cases (20 bodies out of 22). FC and FS colonies were never observed in the control group of 30 individuals who died from a cause other than drowning, both in LV and RV blood cultures as well as in femoral artery (FA) and femoral venous (FV) blood cultures [31].

It seemed possible, however, that a corpse placed in water after death would yield a false positive FC and FS test for drowning because of pulmonary passive diffusion and subsequent bacterial invasion. Therefore, in the current study we examined bodies submerged in water following death from causes other than drowning. The results of the microbiological tests on submerged bodies were compared with data obtained from an additional group of drowning victims.

2. Materials and methods

2.1. Reagents, supplies and instruments

Culture media and saline solutions were provided by Biolife (Milan, Italy). Filtered membranes were obtained from Millipore (Moisheim, France). Filtration systems were provided by Sartorius (Goettingen, Germany). Sterile syringes and polypropylenes tubes were obtained from MPIM (Pescara, Italy). AAS instruments were PerkinElmer AAnalyst 600 and AAnalyst 100.

2.2. Drowned victims

The microbiological test was applied among a group of 10 drowned victims including 5 cases in freshwater and 5 cases in saltwater. Sex, age, drowning medium, toxicological data and immersion time interval (corresponding to the PMI) are reported in Table 1. The cause of death was determined as drowning by autopsy findings (see Section 1), circumstantial data, and toxicological screen and chemical tests. For all the drowning victims the following criteria were observed:

(a) Police reports, official statements, circumstantial elements suggestive for drowning,
(b) autoptic findings mainly based on the aspect of lungs (pulmonary overinfaltion, emphysema aquosum, pulmonary edema, frothy fluid in the airways, subpleural petechial haemorrhages, dilatation and rupture of alveoli, thinning of the interalveolar walls with empty septal capillaries, hydropic swelling of the epithelial cells) along with the absence of external injuries and/or other fatal organ failure;
(c) positive results of chemical tests suggestive of a marked haemodilution or hemoconcentration caused by drowning. In particular, the Fe test as marker for freshwater drowning and the Sr test for seawater drowning victims were performed;

Table 1
Data related to the drowned victims.

Case #	Sex	Age	Drowning medium	Toxicological results	Immersion time
1	M	42	Freshwater	Ethyl alcohol 2.52 g/l	05 d
2	M	60	Freshwater	Negative	04 d
3	M	47	Freshwater	Ethyl alcohol 1.65 g/l	12 h
4	F	72	freshwater	Venlafaxine 0.64 μg/ml	36 h
5	F	30	Freshwater	Negative	–
6	M	77	Saltwater	Negative	40 d
7	M	35	Saltwater	Ethyl alcohol 2.55 g/l	06 d
8	M	42	Saltwater	Flurazepam 0.18 μg/ml Ethyl alcohol 2.55 g/l	20 h
9	M	19	Saltwater	Negative	30 min
10	M	20	Saltwater	Negative	20 min