

### H35 The Immunohistochemical Analysis in the Diagnosis of Freshwater Versus Saltwater Drowning: A Case Report and a Review of the Literature

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**Learning Overview:** After attending this presentation, attendees will be able to describe which investigations are most useful in cases of death due to drowning.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the importance of immunohistochemical analysis and the need for the research of a pathognomonic parameter for the differential diagnosis between freshwater and saltwater drowning.

Drowning is one of the most frequent causes of death. According to recent epidemiologic research, the incidence is about 400,000 deaths per year in the world, of which 30,000 occur in Europe.<sup>1</sup> In most cases, these are accidental deaths or suicides.<sup>2</sup> Drowning is a form of asphyxia. The liquid filling the airways replaces air contents resulting in pulmonary edema and anoxic-ischemic encephalopathy. The pathophysiology of drowning depends on the characteristics of the inhaled liquid. If it is hypertonic (i.e., saltwater), the consequences will be hemoconcentration with hypovolemia, sodium, calcium, and magnesium increase, impaired cardiac function (without ventricular fibrillation), and massive pulmonary edema. Death occurs in 8–9 minutes. If the liquid is hypotonic (i.e., freshwater), it will inactivate the surfactant and is rapidly absorbed into the bloodstream, inducing hypervolemia, dilution anemia, and hemolysis with the release of intracellular potassium and lethal ventricular fibrillation. Death occurs in 3–5 minutes. Foaming at the mouth, cyanosis, bright red color hypostasis, and goosebumps are some indicative external signs. Histologic examination plays an essential role. It can establish the presence of irregular areas of dilatation and rupture of alveolar trabeculae, hemorrhage, and edema. The purpose of this work is to compare forensic investigation results in freshwater versus saltwater drowning.

Two forensic cases are presented. The first case is that of a man who fell from a boat into the Ionian Sea. The second case is that of a man who fell into the water of a lake. Autopsy, cartometric testing, and histopathologic and toxicologic examination were performed in both cases. In both cases, external examination showed signs indicative of drowning. In the first case, the lungs were emphysematous and crackling with evidence of petechiae and pulmonary edema at autopsy. Cartometric testing showed greater hemodilution of blood within the right ventricle as compared to the left. In the second case, the lungs appeared congested and edematous. Cartometric testing showed greater hemodilution of the blood within the left ventricle as compared to the right. The renal and cerebral immunohistochemical analyses showed a different expression of aquaporins in the two presented cases. The results obtained from these investigations were compared with those found in a non-systematic review of the literature conducted via the PubMed NCBI search engine.

The diagnosis of drowning remains a challenge for the forensic pathologist. There is no single pathognomonic autopsy finding, especially with regard to distinguishing between freshwater and saltwater drowning. As presented in these cases, the cartometric test and, above all, the immunohistochemical analysis are fundamental in the forensic investigation. The cartometric test entails dropping blood taken from the ventricles onto a sheet of bibula paper and subsequently observing the size and color tone of the marks. In cases of hemodilution, the blood from the left ventricle will leave a larger and lighter red-colored mark than the right (e.g., as in the second presented case). The opposite occurs in cases of drowning in saltwater (e.g., as in the first presented case). Immunohistochemical investigations already play an important role in distinguishing between drowning and other types of asphyxia.<sup>3,4</sup> In differentiating between freshwater and saltwater drowning, it is important to evaluate the expression of aquaporins, a protein-channel family responsible for transmembrane passage of liquids. Their expression at tissue level varies according to changes in plasma osmolarity. In particular, in cases of freshwater drowning, aquaporin-2—which is normally found on the renal collecting ducts—is hypo-expressed, while the aquaporin-4—normally found in astrocytes—is hyper-expressed.<sup>5,6</sup> The opposite pattern is seen in cases of saltwater drowning. The two cases presented here confirm these expression patterns. Literature highlights how the study of aquaporin-5—normally expressed in type I pneumocytes—is more useful to distinguish between drowning in fresh water and postmortem immersion.<sup>7</sup> At present, the investigations described herein are the most reliable and accurate means of distinguishing fresh versus saltwater drowning, as there is not yet a single pathognomonic histopathologic marker.

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#### Forensic Sciences, Drowning, Immunohistochemical Analysis