G45 Distribution Pattern of Pulmonary Surfactant Protein A (SP-A) in Drowning and Opiate-Related Deaths

Carlo P. Campobasso, MD, PhD, Alessandro S. Dell'Erba, MD, PhD, Annalisa Addante, MD, PhD, Fiorenza Zotti, PhD, Sara Sblano, MD, and Massimo F. Colonna, MD, LLB, Section of Legal Medicine (Di.M.I.M.P.), University of Bari, Piazza Giulio Cesare, Policlinico di Bari, Bari, 70124, Italy*

After attending this presentation, attendees will understand the immunohistochemistry of pulmonary surfactant and the usefulness of SP-A staining as diagnostic marker of alveolar injury in drowning compared with agonal changes such as pulmonary edema in opiate-related deaths.

This presentation will impact the forensic community and/or humanity by demonstrating to the forensic pathology community a finding supporting the final diagnosis of drowning by routinely using SP-A staining.

Pulmonary surfactant covers the surface of the alveoli and prevents alveolar collapse by lowering surface tension. It is composed of phospholipids (90%) and proteins (10%). Four surfactant-associated proteins (SPs) have been identified: hydrophilic SP-A and -D, and hydrophobic SP-B and -C. SP-A is the most prevalent form, and is produced in the alveolar type II cells. Under normal conditions, SP-A is immunohistochemically detected in the alveolar type II cells, but also on the alveolar interior surface since a small quantity of SP-A is secreted into the alveoli. The immunohistochemical distribution pattern of SP-A in the intra-alveolar space has been previously reported as a useful tool to distinguish mechanical asphyxia from other hypoxic cases (Zhu *et al*., 2000). It can also be considered a valuable marker of the pulmonary dysfunction in drowning, showing partial differences in pulmonary pathophysiology depending on the immersion medium (Zhu *et al*., 2002). Many prominent, massive aggregates of granular SP-A staining observed in the intra-alveolar space have been considered the result of an enhanced secretion caused by strong forced breathing that often takes place in the mechanical asphyxia, or by over-excitement of the autonomic nervous system or, even more, by the Ca^{2+} ions in the edema fluid. The above-mentioned aggregated form may also indicate an early biochemical alteration of SP-A in asphyxial deaths.

To evaluate the role of plasma components exuded into the alveolar space and its relationship with the distribution pattern of SP-A, the authors have retrospectively investigated a total of 48 forensic autopsy cases. They have been divided into three main groups: 18 cases of drowning (12 in salt water and 6 cases in fresh water), 20 cases of opiate-related deaths showing gross pulmonary edema and, as a control group, 10 cases of rapid deaths by gunshot injuries to the head without pulmonary edema. The study was carried out on paraffin tissue blocks from which serial sections (4 ?mthick) were used for hematoxylin-eosin and immunostaining. For immunohistochemistry, anti-human SP-A mouse monoclonal antibody (Novo Castra Laboratories Ltd; U.K.) was used at 150-fold dilution, with a 1-hour incubation at room temperature, using the universal Avidin-Biotin Complex (ABC). The expression of the SP-A staining was scored semiquantitatively based on two staining patterns: membranous or linear staining on the interior surface of alveolar epithelia, and the interface of intra-alveolar effusion and granular staining showing many prominent massive aggregates of SP-A within the intra-alveolar space.

The results show that aggregated granular SP-A staining in the intraalveolar space was frequently observed in drowning victims. A high intensity of this pattern was frequently found in these victims, suggesting a molecular alteration caused by a direct effect of aspirated water and/or subsequent metabolic disturbance in the alveolar type II cells. Granular deposits of SP-A in the intra-alveolar space were never observed in the control group of non-asphyxial deaths (10 cases of fatal gunshot injuries). The group of gross pulmonary edema observed in narcotic deaths showed a prevalent distribution of membranous or linear pattern staining and only scattered SP-A aggregates in the intra-alveolar space. The granular SP-A staining detected in pulmonary edema is consistent with previous findings of SP-A positive staining in lungs with secondary damage such as acute respiratory distress syndrome (ARDS) or a bronchial lavage causing both a biochemical alteration of pulmonary surfactant. These results suggest some molecular alterations of SP-A due to abnormal surfactant metabolism caused by edema fluid. Based on the comparison of SP-A distribution pattern between drowning, agonal changes such as pulmonary edema, and non-asphyxial deaths, the expression of intra-alveolar SP-A aggregates can significantly support the final diagnosis of drowning.

Pulmonary Surfactant, Drowning, Opiate-Related Deaths

 ** Presenting Author*