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Abstract Title:	**Injury Severity Validation With Bronchoalveolar Lavage Cell Analysis: Expression Of HMGB1 And TLR 4 After Smoke Inhalation Injury And Burns Treated With Extra Corporeal Life Support**
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Objective:	1) Describe the utility of immunocytochemistry to identify damage associated molecular pattern changes after smoke inhalation injury and burns.
Abstract:	**Introduction:** Acute Respiratory Distress Syndrome (ARDS) is the most severe form of acute lung injury (ALI), and is characterized by diffuse pulmonary inflammation, hypoxemia, bilateral radiographic pulmonary infiltrates and increased pulmonary vascular permeability. The primary clinical diagnostic tool for ARDS is the PaO2/FiO2 ratio (PFR); however, further diagnostic tools are necessary to improve understanding of injury severity and patient response to therapy. The high mobility group box 1 protein (HMGB1) is a damage associated molecular pattern (DAMP) released from damaged cells that promotes expression of pro-inflammatory cytokines and toll like receptor tor 4 (TLR4), which contribute to inflammation and disease progression in ARDS and are associated with poor outcomes. Bronchoalveolar lavage (BAL) is widely accepted as a key diagnostic procedure in lung disease. In this study, we quantified HMGB1 and TLR4 positive cells from BAL fluid using a quick immunocytochemistry (ICC) method in a porcine model of smoke inhalation and burn induced lung injury. We then applied this measure to assess disease progression over 72 hours in animals treated by extracorporeal life support (ECLS) devices. We hypothesized that HMGB1 and TLR 4 expression in BAL cells would be increased after injury. **Methods:** Female Yorkshire pigs (weight, 50.83±1.27 kg) (n=10) were anesthetized and received arterial and venous catheters and tracheostomy. Following smoke inhalation injury (SII), animals received a full-thickness flame burn covering 40% total body surface. After injury subjects were treated with ECLS (Cardiohelp console with ECLS SET 2.8, MAQUET, Getinge, Germany) using an extracorporeal CO2 removal (ECCO2R) device. BAL fluid was collected at baseline (BL), post-injury

	(PI), 24 hours (24H), 48 hours (48H) and 72 hours (72H) post-injury. Filtered BAL cell count of the supernatant was adjusted to 1x106 cells/mL with phosphate buffered saline. BAL white blood cell count and differential were determined by ADVIA 2120 hematology system (Simens Healthineers, Erlangen, Germany). Slides were cytocentrifuged and incubated with anti HMGB1 and TLR4 (Abcam; Cambridge, MA) followed by incubation with Alex fluor dyes (Thermofisher, MA, USA) for visualization. Images were taking by Olympus BX43 microscope (Olympus, Japan) and quantified using ImageJ software (NIH, MD, USA). **Results:** HMGB1 positive cells were significantly higher at PI and 24H; TLR4 positive cells were significantly higher at 24H and 48H following injury when compared to BL ($p<0.05$). HMGB1 and TLR4 were shown to correlate with each other ($r2=0.5552$, $p<0.0001$). **Conclusions:** Successful detection of changes in HMGB1 and TLR4 expression in BAL cells by the ICC method is an important finding, as this a relatively quick technique (approx. 1 hour) that could be used to provide rapid clinical feedback to guide treatment. Additional studies are required to further understand the role of HMGB1 and TLR4 as injury severity and disease progression markers in lung injury. Further studies are needed to confirm the correlation between elevated BAL HMGB1, TLR4 and clinical indicators of ARDS such as PFR. *The first two authors contributed equally.