

Supplemental digital content 2:

Myeloperoxidase activity measurement.

Myeloperoxidase assay:

After broncoalveolar lavage, the lungs were removed en bloc for determination of myeloperoxidase activity. After excision, lungs were washed with sterile saline, separately weighed and stored at -80°C until myeloperoxidase dosage. The lungs were homogenized in 0.5% hexadecyltrimethylammonium bromide in 50 mM phosphate buffer (pH 6.0; 1 ml hexadecyltrimethylammonium bromide/100mg tissue). The homogenate was sonicated twice, frozen, and then centrifuged at 12,000g for 15 min at 4°C, and myeloperoxidase activity was assayed according to the method of Goldblum et al.¹ modified as follows: 50 µl homogenate was added with 1,405 µl phosphate buffer, 50 mM; 30 µl hydrogen peroxide, 0.03%; and 15 µl O-dianisidine dihydrochloride, 16.7 mg/ml (Sigma-Aldrich, Milan, Italy). Absorbance at 460 nm was read every 10 s for 2 min. Data were expressed as difference of optical density in the first minute per lung.

Results:

We analyzed 8 lung (right and left side) for HCl-SB (mice instilled with HCl in right bronchus and left spontaneous breathing for 7 hours), 7 for HCl-VILI₁₅ group (mice instilled in the right bronchus with HCl and subjected to mechanical ventilation with V_T of 15 ml/kg for 7 hours), 5 for HCl-VILI₂₅ (mice instilled in the right bronchus with HCl and subjected to mechanical ventilation with V_T of 15 ml/kg for 7 hours) and VILI₂₅ (mice only ventilated with V_T of 25 ml/kg for 7 hours). Since the lungs analyzed underwent to broncoalveolar lavage before excision, the measured myeloperoxidase is an index of neutrophilic infiltration within the interstitial spaces and it does not include alveolar infiltration. Myeloperoxidase levels resulted elevated in all study group both in the right and in the left lung, in particular in HCl-VILI₁₅ right lungs (being significantly higher as

compared to right lung of HCl-VIL125) (see Fig 1). This result is very different from what we have shown about polymorphonuclear cells in bronchoalveolar lavage which were consistently increased in right and left lung of HCl-VIL125 group. We could hypothesize that there was a difference in leukocyte migration between groups but further specific studies are necessary for clarifying this discrepancy.

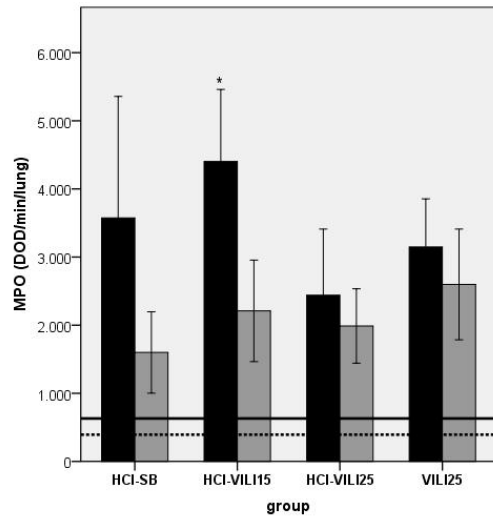


Fig 1: Myeloperoxidase (MPO) activity express as delta of Optical Density within the first minute for right or left lung (mean±SD). Baseline values: horizontal continuous line represents right lung of 3 healthy mice, dotted line represents left lung. * $p < 0.05$ vs right lung of HCl-VIL125

References:

1. Goldblum SE, Wu KM, Jay M: Lung myeloperoxidase as a measure of pulmonary leukostasis in rabbit. *J Appl Physiol* 1985; 59:1978–85