

Supplementary information for:

Optimized detection of lung IL-6 via enzymatic liquefaction of low respiratory tract samples: application for managing ventilated patients

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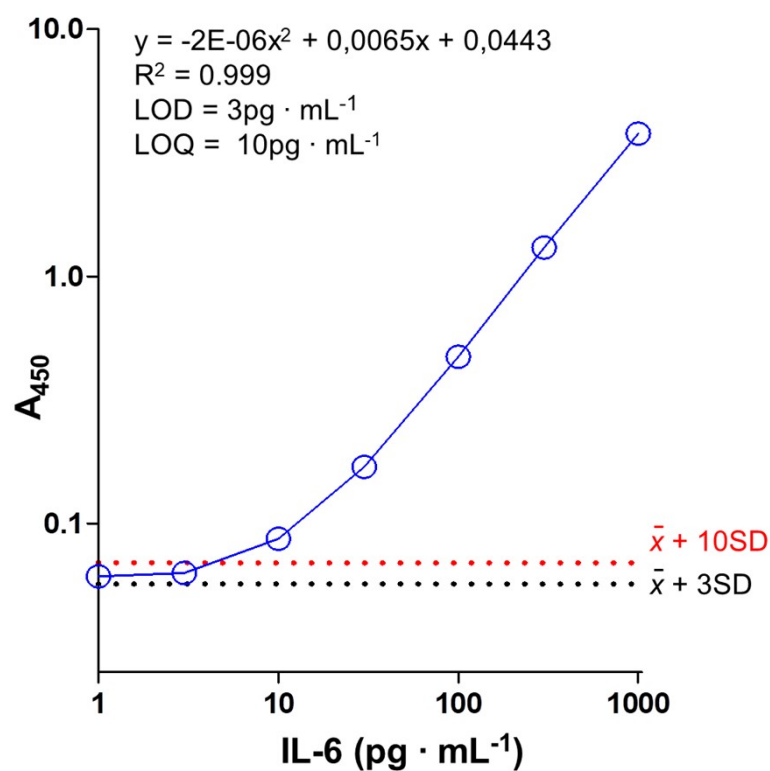


Figure S1. Calibration curve of IL-6 yielded by sandwich ELISA protocol. Absorbance at 450 nm (logarithmic Y axis) as a function of increasing amounts of IL-6 (logarithmic X axis). Circles represent the mean (n=3), dotted lines show the signal above 3 (black) or 10 (red) times the standard deviation of the blank. LOD, limit of detection; LOQ, limit of quantification.

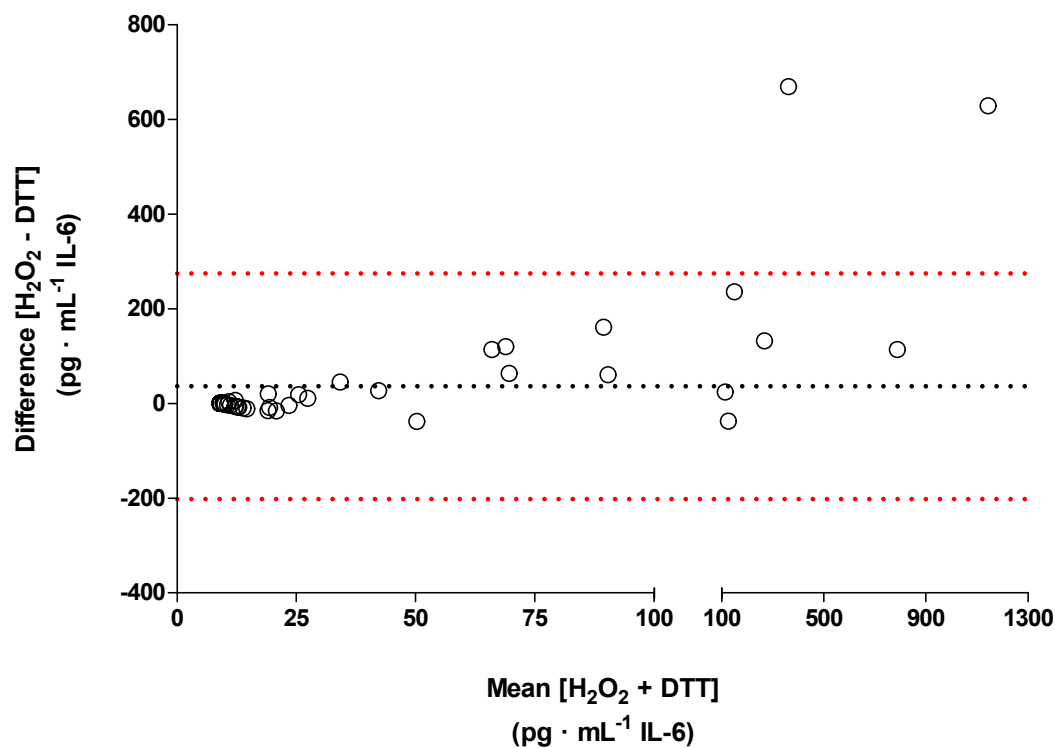


Figure S2. Bland-Altman accordance analysis of lung IL-6 levels yielded by DTT and enzymatic liquefaction methods. Difference (Y-axis) *versus* average (X-axis) of lung IL-6 levels detected after liquefaction of respiratory samples by the reducing (DTT) or enzymatic (H₂O₂) method. Dotted lines represent the bias (black) and the 95% limits of agreement (red).

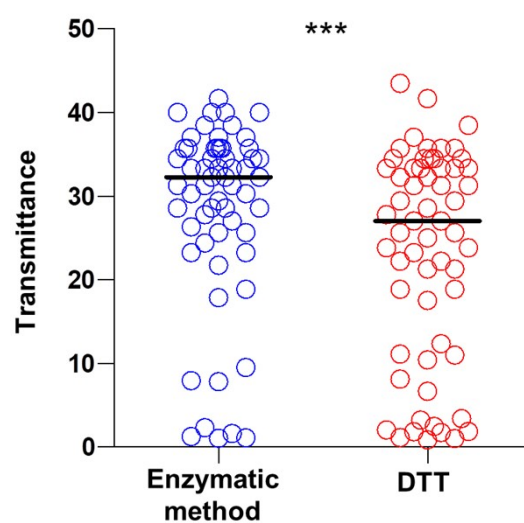


Figure S3. Liquefaction of respiratory samples after reducing and enzymatic procedures. Turbidimetry (transmittance) of low respiratory tract samples after enzymatic (blue) or reducing (red) treatments. Horizontal bars represent the median. *** p -value <0.0001 was obtained with a Wilcoxon paired test.

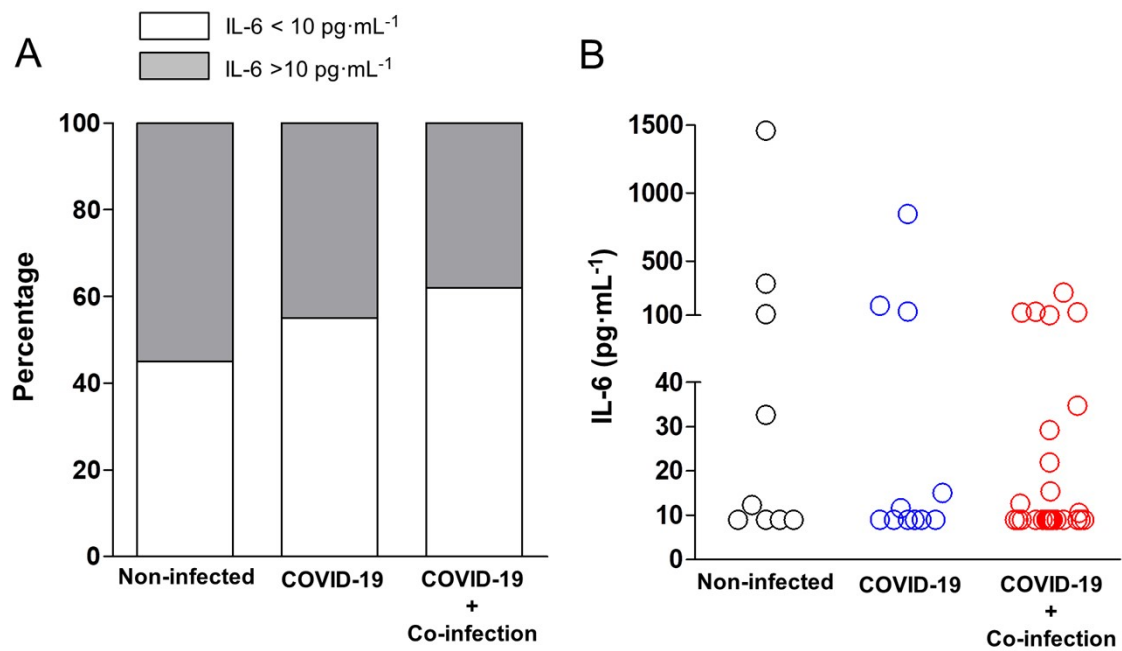


Figure S4. IL-6 detection on low respiratory tract samples from mechanically ventilated patients depending on infection status. (A) Negative (white) and positive (grey) rates (%) for IL-6 test in low respiratory tract samples from patients without respiratory infection (n=9) and COVID-19 patients with (n=26) or without (n=11) bacterial co-infection (Fisher exact test, $p>0.05$). (B) Similar IL-6 levels in low respiratory tract samples with a positive IL-6 test (>10 pg·mL⁻¹) between patients without infection (black), COVID-19 patients (blue) and COVID-19 patients with a bacterial co-infection (red) (Kruskall Wallis test, $p>0.05$). Data refer to IL-6 detected on low respiratory tract samples after enzymatic liquefaction.

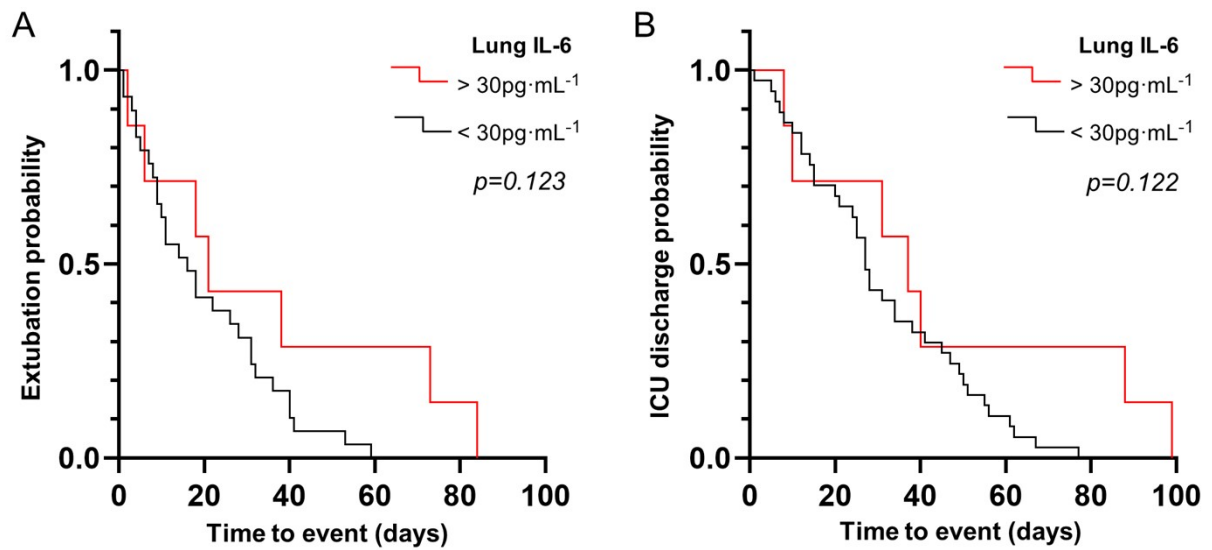


Figure S5. Impact of DTT on lung IL-6 assessment as predictive marker in mechanically ventilated patients. Cutoff IL-6 value yielded by AUC-ROC method was used to classify patients in a survival analysis to evaluate the expected time of (C) mechanical ventilation needs and (D) ICU admission. Data refer to IL-6 detected on low respiratory tract samples after liquefaction by reducing DTT.

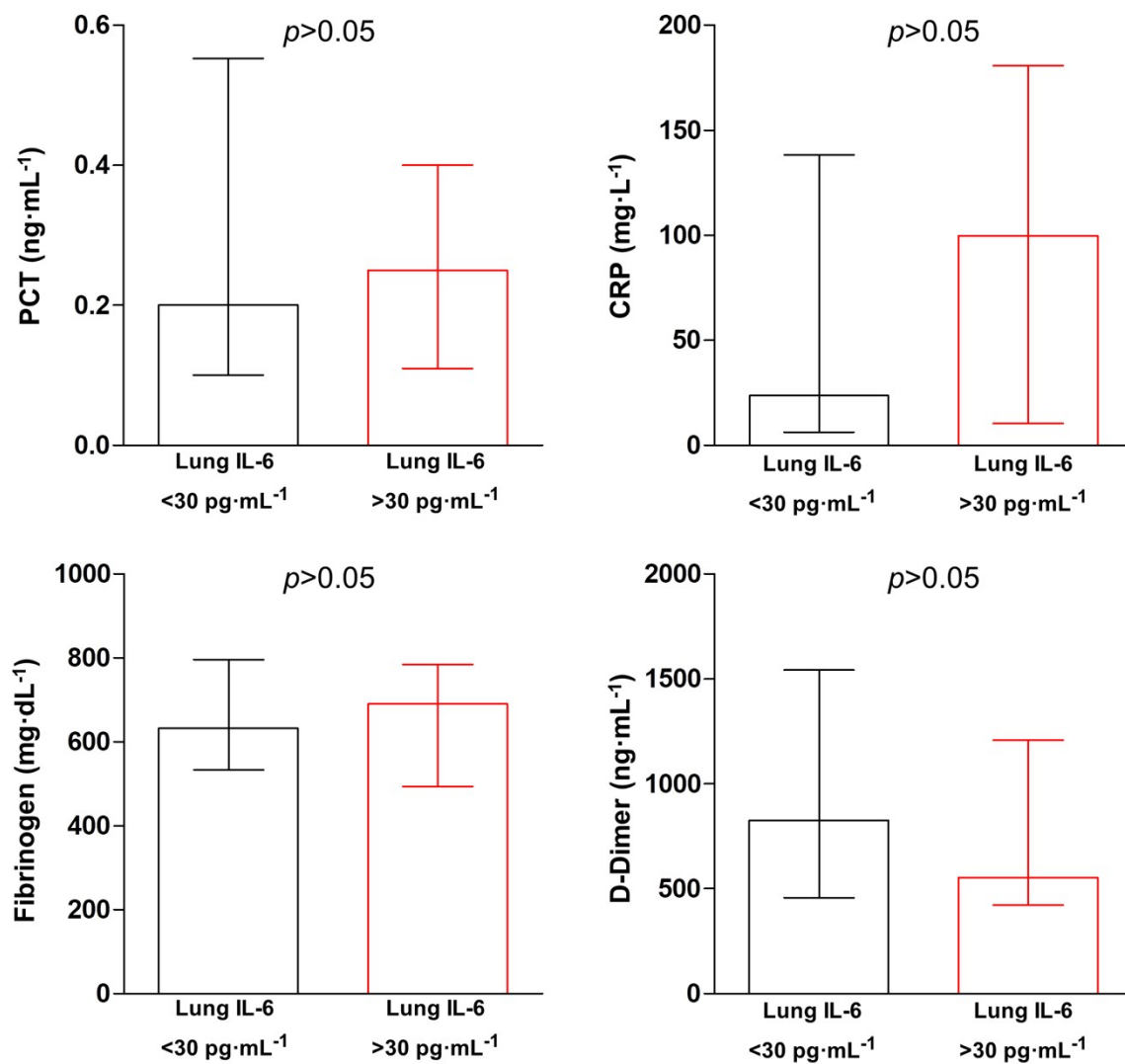


Figure S6. Association between lung IL-6 and systemic inflammatory biomarkers. Similar serum levels of procalcitonin (PCT), C-reactive protein (CRP), fibrinogen and D-Dimer between mechanically ventilated patients with lung IL-6 levels below (black) and above (red) the cutoff value proposed by the AUC-ROC analysis in Figure 4 of the main text (Mann-Whitney test, $p > 0.05$). Data are expressed as medians and interquartile range (25th and 75th percentiles).

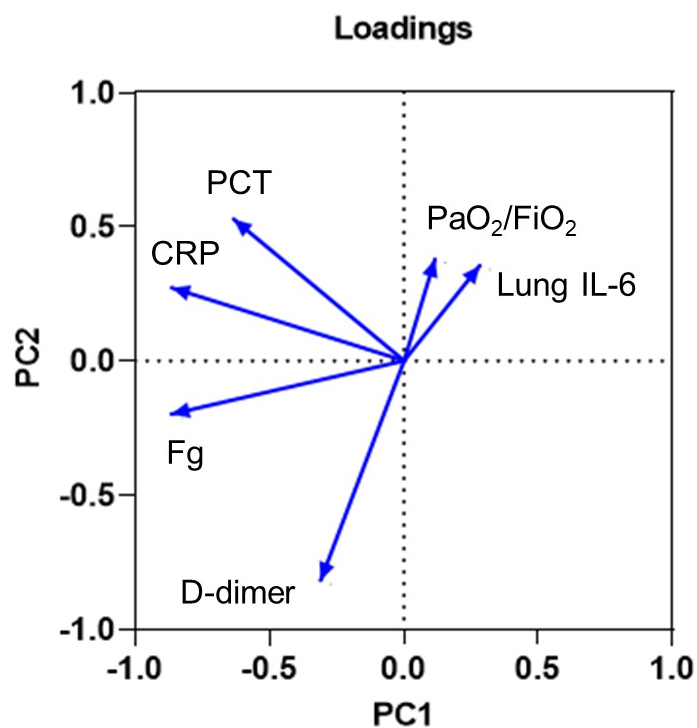


Figure S7. Principal component analysis by using lung IL-6 measures after DTT treatment. Relationships between local variables (lung IL-6 levels and PaO₂/FiO₂ values) and systemic variables [(D-dimer, fibrinogen (Fg), C-reactive protein (CRP) and procalcitonin (PCT)]. Data refer to IL-6 detection on low respiratory tract samples after liquefaction with DTT.