

## **Research Protocol**

**TITLE:** the Change of R-spondin Proteins Plasma Concentration Level caused by Mechanical Ventilation and its Effect on Mechanical Ventilation Induced Lung Injury

**PROTOCOL ID:** XH-17-015

**NCT number:** NCT03315702

**SPONSOR:** Xinhua Hospital, Shanghai Jiao Tong University School of Medicine

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## **Materials and methods**

### **1. Patients recruitment**

Research protocol was approved by Xinhua Hospital Ethics Committee Affiliated to Shanghai Jiao Tong University School of Medicine. With written informed consents given by patients undergoing elective surgery, individual information and blood samples were collected.

Inclusion Criteria is shown as followed: patients undergo elective surgery with mechanical ventilation lasting for > 4 hours; classified as physical status I to III according to the American Society of Anesthesiologists Physical Status Classification System; Written informed consent is approved.

Participating subjects with any one of criteria shown as followed were excluded: chronic lung disease; recent lung infection; recent anaesthetics or mechanical ventilation treatment; hemodilution with massive fluid supply during surgery; children; women during pregnancy or lactation; being involved in other clinical subjects.

### **2. Mechanical ventilation**

Mechanical ventilation protocol: tidal volume 6-8 ml/kg, positive end-expiratory pressure 5 cmH<sub>2</sub>O, oxygen concentration 40%; respiratory rate 10-15/min, inspiratory/expiratory ratio 1:1.5.

### **3. Blood samples collection and detection**

With written informed consents given by patients undergoing elective surgery, collect 2ml venous blood samples each time before endotracheal intubation and third hour after, thus mechanical ventilation for one patient lasts for four hours. Store samples in EDTA plus blood collection tubes. All samples were stored in 4 °C refrigerator and centrifuged in 1 hours after being collected. Plasmas were separated from venous blood samples via centrifugation at 4 °C 1000rpm for 10 min, and stored in -80 °C before being analyzed with Elisa.

### **4. Statistical analysis**

Analyze R-spondin proteins plasma concentration levels of two samples from the same patient collected relatively before endotracheal intubation and fourth hour after as paired-samples. Quantitative data were expressed as mean and 95 % confidence interval (CI) for mean. Differences between paired-samples are assessed by paired *t*-test, performed using the SPSS13.0 package.  $P < 0.05$  is considered as statistically significant criteria.