Peer Review File

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Reviewer comments

The clinical relevance of inflammation induced by elective perioperative Extracorporeal Membrane Oxygenation usage as an integral part of modern lung transplantation remains elusive. In the manuscript "Transient perioperative inflammation following lung transplantation and major thoracic surgery with elective extracorporeal support: a prospective observational study", authors determined the perioperative cytokine response accompanying major thoracic surgery employing different extracorporeal devices comprising extracorporeal-membrane-oxygenation, cardiopulmonary bypass or no extracorporeal circulation in relation to inflammation, clinically tangible as increased Sequential Organ Failure Assessment score, called SOFA.

Couple questions are required to be answered before accepted.

Comment 1:

(1) In the introduction, please supplement the introduction of ECMO and SOFA.

Reply 1:

We thank Reviewer 1 for this comment. We did not explain ECMO and SOFA in detail.

Changes 1:

(Page 5, line 81-82).

ECMO is a technology capable of providing short- and long-term mechanical support to the heart, lungs or both (1).

(Page 5, line 94-97).

The SOFA score was designed to clinically measure organ dysfunction by assessing the respiratory, coagulation, hepatic, cardiovascular, renal and neurological function in patients admitted to the ICU (2).

Comment 2:

(2) Are there any complications after using CPB? Are there any causes leading to inflammatory response after using of CPB?

Reply 2:

Are there any complications after using CPB?

Reviewer 1 raised an important point. Cardiothoracic surgery on CPB triggers a systemic inflammatory response due to several reasons including surgical trauma, activation of blood components in the extracorporeal circuit, ischemia/reperfusion

injury, and endotoxin release (3, 4). This broad wave of systemic inflammatory activation has been linked to adverse clinical outcomes ranging from mild adverse effects (fever or diffuse tissue edema), to moderate adverse effects (pathological hemodynamic instability or coagulopathy), to severe complications (acute organ injury requiring mechanical support), and even mortality (5).

Are there any causes leading to inflammatory response after using of CPB?

We thank Reviewer 1 for this question. As we pointed out in our introduction, the exposure of a patient's blood to foreign surfaces of the CPB circuit is known to imbalance the inflammatory system via blood flow shear stress, expression of cytokines, activation of the complement system and dysfunctions of the coagulation system.

Changes 2:

(Page 5, line 100-104)

This broad wave of systemic inflammation has been linked to adverse clinical outcomes ranging from mild adverse effects such as fever or diffuse tissue edema, to moderate adverse effects comprising pathological hemodynamic instability or coagulopathy, to severe complications including acute organ injury requiring mechanical support, and even mortality (6, 7).

Comment 3:

(3) What is the meaning of “ECC” in the introduction, and “CTEPH” in the methods?

Reply 3:

We thank Reviewer 1 for this comment; we did not explain the abbreviation of ECC and CTEPH in the appropriate section.

Changes 3:

(Page 6, line 115)

Extracorporeal circulation (ECC)

(Page 7, line 133)

Chronic Thromboembolic Pulmonary Hypertension (CTEPH)

Comment 4:

(4) What are the inclusion and exclusion criteria for enrolled patients?

Reply 4:

We agree with Reviewer 1, we did not mention the inclusion and exclusion criteria. In our study we enrolled all consecutive patients undergoing LUTX, PEA and major lung resection in a period of 12 months, from May 2018 until April 2019. We excluded

pregnant women, patients younger than 18 years and patients who did not give written informed consent.

Changes 4:

(Page 7, line 131-137)

We included forty-two consecutive patients with end-stage pulmonary disease{COPD(n=15), CF(n=15), 123IPF(n=7), IPAH(n=5)} undergoing LUTX on ECMO; fifteen consecutive Chronic Thromboembolic Pulmonary Hypertension (CTEPH) patients undergoing PEA on CPB and 15 consecutive lung cancer patients undergoing major lung resections without ECC during a period of 12 months, from May 2018 until April 2019. We excluded pregnant women, patients who were younger than 18 years and patients who did not give written informed consent.

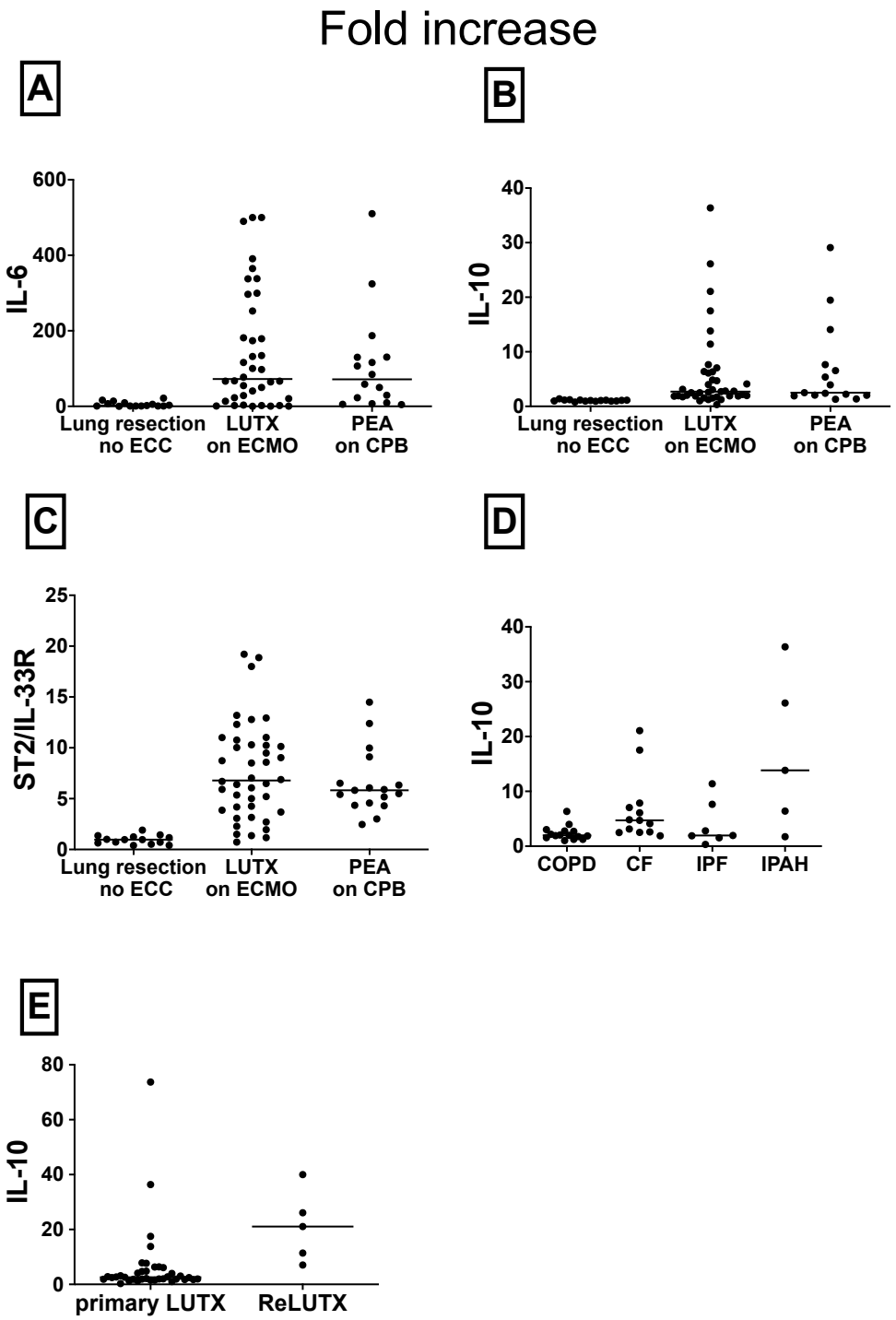
Comment 5:

(5) In the figure 1, the histogram should be changed to scatter plots.

Reply 5:

Reviewer 1 made a valid point. Showing figure 1 as a scatter plot provides more information on individual values.

Changes 5: We changed figure 1 to a scatter plot as shown below:



Comment 6:

(6) The ELISA method should be described detailed in the methods.

Reply 6:

We are thankful to Reviewer 1 for this comment. We did not include the ELISA technique in detail in the methods section.

Changes 6:

(Page 7, line 141-152)

Sandwich- ELISA technique

IL-6, IL-10, ST2/IL-33R, TNF-α and Transforming Growth Factor (TGF)-β serum concentrations were measured by commercially available enzyme- linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, Minnesota, United 130States) according to the manufacturers` instructions. 96-well microplates were incubated with capture antibodies (Mouse anti-human IL-6, IL-10, ST2, TNF-α and TGF-β) overnight at room temperature. Blocking was done with assay buffer. After incubation with serum samples and washing, HRP-conjugated detection antibodies were added (Biotinylated goat anti-human IL-6, IL-10, ST2- TNF-α and TGF-β). A colour reaction was obtained with peroxidase reagent tetramethylbenzidine (TMB) (Sigma-Aldrich Corp., St. Louis, MO, USA) and the optical density (OD) was read at 450 nm using an absorbance microplate reader for ELISA, the Infinite F50 (Tecan, Männedorf, Switzerland).

Comment 7:

(7) Why to focus on IL-6 and IL-10 cytokines? Which extracorporeal device is the best for patients with lung transplantation?

Reply 7:

Why to focus on IL-6 and IL-10 cytokines?

We thank Reviewer 1 for this important question. It is well accepted that cardiothoracic surgery on CPB triggers a pro-inflammatory response followed by a second phase of immune suppression (8). We therefore hypothesized that LUTX on ECMO induces similar to cardiothoracic surgery on CPB a pro-and anti-inflammatory immune reaction. We therefore measured pro-inflammatory cytokines such as IL-6 and TNF-α and anti-inflammatory cytokines comprising IL-10 and ST2/IL-33R.

Which extracorporeal device is the best for patients with lung transplantation?

With this question Reviewer 1 highlights an important topic: During the reperfusion phase, a newly implanted graft is very vulnerable. Gradually opening the clamp on the pulmonary artery over a time period of 5 to 10 minutes is nowadays a standard procedure in lung transplantation. However, controlled reperfusion over a longer period of time can only be achieved by using CPB or ECMO support. Nevertheless, the potential beneficial effect of CPB is hindered by an increased intraoperative blood turnover and an augmented risk for postoperative bleeding. Therefore, ECMO is the best extracorporeal device to provide controlled reperfusion without increasing those risks (9-11).

Changes 7:

(Page 20, line 491-497)

The institutional lung transplantation experience clearly showed better outcomes with intraoperative ECMO support, since controlled reperfusion of a newly implanted pulmonary graft over a time period of 5 to 10 minutes saves the vulnerable organ. However, controlled reperfusion can only be achieved by using CPB or ECMO support. While, the potential beneficial effect of CPB during LUTX is hindered by an augmented intraoperative blood turnover and an increased risk of postoperative bleeding, ECMO support seems the best option to provide controlled reperfusion without increasing those risks (9-11).

Comment 8:

(8) What are your suggestions for inflammation following lung transplantation? Please supplement in the discussion.

Reply 8:

We assume that similar to the CPB conduit the exposure of a patient's blood to foreign surfaces of the ECMO circuit is known to imbalance the inflammatory system via blood flow shear stress, expression of cytokines, activation of the complement system and dysfunctions of the coagulation system.

In our study we pointed out that in patients undergoing elective thoracic surgery on ECC have an immediate rise and concomitant fall of inflammation as observed in serum cytokine release and SOFA criteria.

Changes 8:

(Page 16, line 286-395)

We highlight what we mentioned already in the discussion:

In this study we revealed evidence for enhanced Th1 as well as Th2 responses at end of surgery in patients undergoing elective LUTX on ECMO and PEA on CPB, which we did not observe in patients undergoing major pulmonary resections without ECC. The following observations point to an on/off phenomenon concerning SOFA and cytokine expression following major thoracic surgery on ECC support(CPB and ECMO): We did not observe perioperative differences in the quantitative and qualitative cytokine response or SOFA between PEA on CPB and LUTX on ECMO(stressing that no *t*-statistic was employed because of the inherent differences between patient groups and their respective surgery); Concerning the use of ECC the

reasons for this on/off phenomenon may lie purely in the contact of blood components with tubing of ECC circuits.