12. Cheung HY. *Strategies to Improve and Stabilize Extended Ex Vivo Lung Perfusion. Master of Health Science Thesis*. Toronto: University of Toronto; 2017.
13. Aboelnazar NS, Himmat S, Hatami S, White CW, Burhani MS, Dromparis P, et al. Negative pressure ventilation decreases inflammation and lung edema during normothermic ex-vivo lung perfusion. *J Heart Lung Transplant*. 2018;37:520-30.
14. Noda K, Tane S, Haam SJ, Hayanga AJ, D'Cunha J, Luketich JD, et al. Optimal ex vivo lung perfusion techniques with oxygenated perfusate. *J Heart Lung Transplant*. 2017;36:466-74.
15. Iskender I, Cosgun T, Arni S, Trinkwitz M, Fehlings S, Yamada Y, et al. Cytokine filtration modulates pulmonary metabolism and edema formation during ex vivo lung perfusion. *J Heart Lung Transplant*. 2018;37:283-91.
16. Ordies S, Frick AE, Claes S, Schols D, Verleden SE, Van Raemdonck DE, et al. Prone positioning during ex vivo lung perfusion influences regional edema accumulation. *J Surg Res*. 2019;239:300-8.
17. Sadaria MR, Smith PD, Fullerton DA, Justison GA, Lee JH, Puskas F, et al. Cytokine expression profile in human lungs undergoing normothermic ex-vivo lung perfusion. *Ann Thorac Surg*. 2011;92:478-84.
18. Kakishita T, Oto T, Hori S, Miyoshi K, Otani S, Yamamoto S, et al. Suppression of inflammatory cytokines during ex vivo lung perfusion with an adsorbent membrane. *Ann Thorac Surg*. 2010;89:1773-9.
19. Machuca TN, Cypel M, Yeung JC, Bonato R, Zamel R, Chen M, et al. Protein expression profiling predicts graft performance in clinical ex vivo lung perfusion. *Ann Surg*. 2015;261:591-7.
20. Poli EC, Rimmelé T, Schneider AG. Hemoadsorption with CytoSorb®. *Intensive Care Med*. 2019;45:236-9.
21. Cosgun T, Iskender I, Yamada Y, Arni S, Lipiski M, van Tilburg K, et al. Ex vivo administration of trimetazidine improves post-transplant lung function in pig model. *Eur J Cardiothorac Surg*. 2017;52:171-7.
22. Inci I, Ampollini L, Arni S, Jungraithmayr W, Inci D, Hillinger S, et al. Ex vivo reconditioning of marginal donor lungs injured by acid aspiration. *J Heart Lung Transplant*. 2008;27:1229-36.
23. Yamada Y, Iskender I, Arni S, Hillinger S, Cosgun T, Yu K, et al. Ex vivo treatment with inhaled N-acetylcysteine in porcine lung transplantation. *J Surg Res*. 2017;218:341-7.
24. Smith AJ, Clutton RE, Lilley E, Hansen KEA, Brattelid T. PREPARE: guidelines for planning animal research and testing. *Lab Anim*. 2018;52:135-41.
25. Yeung JC, Cypel M, Machuca TN, Koike T, Cook DJ, Bonato R, et al. Physiologic assessment of the ex vivo donor lung for transplantation. *J Heart Lung Transplant*. 2012;31:1120-6.
26. Slama A, Barta M, Schillab L, Mitterbauer A, Jaksch P, Hoetzenecker K, et al. Metabolic assessment of marginal donor lungs during ex-vivo perfusion (EVLP): new parameters for decision making. *J Heart Lung Transplant*. 2015;34(Suppl):S97.
27. de Perrot M, Liu M, Waddell TK, Keshavjee S. Ischemia–reperfusion–induced lung injury. *Am J Respir Crit Care Med*. 2003;167:490-511.
28. Suzuki K, Shimazaki M, Kutsuki H. Beta2-microglobulin-selective adsorbent column (Lixelle) for the treatment of dialysis-related amyloidosis. *Ther Apher Dial*. 2003;7:104-7.
29. Iskender I, Sakamoto J, Nakajima D, Lin H, Chen M, Kim H, et al. Human α1-antitrypsin improves early post-transplant lung function: pre-clinical studies in a pig lung transplant model. *J Heart Lung Transplant*. 2016;35:913-21.
30. Diamond JM, Arcasoy S, Kennedy CC, Eberlein M, Singer JP, Patterson GM, et al. Report of the International Society for Heart and Lung Transplantation working group on primary lung graft dysfunction, part II: epidemiology, risk factors, and outcomes-A 2016 consensus group statement of the International Society for Heart and Lung Transplantation. *J Heart Lung Transplant*. 2017;36:1104-13.
31. Gelman AE, Fisher AJ, Huang HJ, Baz MA, Shaver CM, Egan TM, et al. Report of the ISHLT working group on primary lung graft dysfunction Part III: mechanisms: A 2016 consensus group statement of the International Society for Heart and Lung Transplantation. *J Heart Lung Transplant*. 2017;36:1114-20.
32. Netea MG, van de Veerdonk FL, van der Meer JWM, Dinarello CA, Joosten LA. Inflammasome-independent regulation of IL-1-family cytokines. *Annu Rev Immunol*. 2015;33:49-77.
33. Turner MD, Nedjai B, Hurst T, Pennington DJ. Cytokines and chemokines: at the crossroads of cell signalling and inflammatory disease. *Biochim Biophys Acta*. 2014;1843:2563-82.

Key Words: ex vivo lung perfusion, perfusate adsoprtion, cytosorb, lung transplantation, ischemia-reperfusion injury, inflammatory response

THOR

Discussion

Dr Siva Raja *(Cleveland, Ohio)*. I would like to start by commending the authors for this work but also for their prior work in this field. As EVLP is increasing, it has the potential to increase the availability of this precious resource, the organ. In this work you are describing the use of adsorbers in the EVLP circuit and showing that there is decreased inflammatory response that corresponds to an improved function. I have the following 3 questions.

As you mentioned, these are relatively nonspecific adsorption beads. How do you think this will affect future studies involving other molecules that are anti-inflammatory even though this takes up proinflammatory molecules? The reason I ask this is, one, in your own study you show that some markers such as IL-10 are actually anti-inflammatory, are also decreased, and your own prior work looking at trimetazidine, which is an anti-ischemic agent, which when used in the EVLP circuit decreases inflammation and produces the exact same result. How do you think that this technology of taking everything out will play in the role of adding things that could also decrease the effect?

Dr Ilker Iskender *(Zurich, Switzerland)*. I think this could be explained along with the side effects of medications. Using medications has advantages and disadvantages. How can we tackle the unwanted effects of perfusate-adsorption related to drug removal? I think one way could be administration of high-doses of medications or agents. EVLP is an isolated platform. Interventions will affect only the lungs, not all the body. One simple way is to tackle this side effect is to administration of an increased amount of therapeutics while using this adsorber in the setting of EVLP.

Dr Raja. There was an article by Kakishita and colleagues[18] in 2010 in the *Annals of Thoracic Surgery* that also used an adsorbent membrane in the EVLP circuit and showed that decreasing cytokines like IL-8 and tumor necrosis factor-alpha that were chemically detected to be decreased but had no physiologic effect.

So the beads that you were using, were they provided by the company or were they commercially available for other groups to test? I am trying to figure out, what's different

about your beads that the last adsorption membrane was not able to show a physiologic effect?

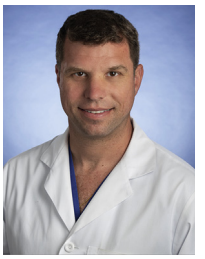
Dr Iskender. I don't know the mechanistic difference between the 2 membranes. It's difficult for me to answer this question from that point of view at the moment. But the experimental model they used was different than this study. The EVLP was initiated after a short warm-ischemic time, whereas we have used a clinically relevant, prolonged cold ischemic injury model in this study. Together with the difference in membrane technology, the use of different experimental models may yielded discordant results between the 2 studies.

This device is available for patient use in Europe with a CE mark. The company has provided the adsorber for research purposes in this study. I think the device is currently under investigation on other parts of the world.

Dr Raja. In this study, it's a very manicured setting where the harvesting of the organ is done in a pristine fashion; there is no trauma to the lungs and so on and so forth. My interpretation of the data is that by binding or removing these inflammatory mediators and decreasing the inflammatory response, you are able to preserve better graft function.

But life is not so pristine. The organs are usually placed on EVLP because there is something not quite right with the organ. So how do you think these data would pan out in a setting where already the inflammatory cascade has been started? How do you think this would work in terms of reversing some of the effects? It may work in not starting it, but what are your thoughts?

Dr Iskender. True, we have tested the device in a controlled setting for research purposes, which may not simulate the real-life conditions. Nevertheless, this broad-spectrum adsorbent may be advantageous, especially in the clinical setting where the donor lungs are also injured with microorganisms or aspiration, involving other endotoxins or some other harmful substances that could be further cleared with this adsorber during EVLP. This would be my speculation.



Dr Marcelo Cypel *(Toronto, Ontario, Canada)*. Very nice work. I have 2 questions. One is, in your EVLP data in the 1-hour time point you already saw a significant difference between the 2 groups. Do you think the effect already happened early on that showed that difference at a 1-hour baseline?

I think the more interesting finding of this study to me is the postreperfusion cytokines in the lung tissue, because that really shows you did something to EVLP that decreased reperfusion injury. But is that true or was it just a matter of accumulation of those cytokines during EVLP that were different in one group than the other and just persisted in the first few hours after reperfusion or was it truly reperfusion injury? I wonder if you did, for example, percutaneous coronary intervention to look at gene expression levels rather than just protein accumulation?

Dr Iskender. To answer your first question, we started to see a physiologic improvement from the first hours of EVLP, which is actually identical to what we saw in the first article. It appears that this strategy is affecting the EVLP physiology early with the initiation of EVLP.

Regarding the second question, we have only looked at the plasma levels of cytokines, not tissue levels. All of the studied analytes were comparable except for IL-1ra, which has been classified as an anti-inflammatory cytokine. It has also been secreted as an acute-phase protein from liver. We believe that elevated IL-1ra plasma levels are a surrogate marker of enhanced inflammation regulated by proinflammatory cytokines, which is in favor of the treatment effect.