Journal of Visualized Experiments

Technique of veno-venous extracorporeal membrane oxygenation in a mouse

--Manuscript Draft--

Article Type:	Methods Article - JoVE Produced Video
Manuscript Number:	JoVE58146R3
Full Title:	Technique of veno-venous extracorporeal membrane oxygenation in a mouse
Keywords:	Extracorporeal membrane oxygenation, extracorporal circulation, ECMO, mouse, organ damage, surgery
Corresponding Author:	Nodir Madrahimov Hannover Medical School Hannover, GERMANY
Corresponding Author's Institution:	Hannover Medical School
Corresponding Author E-Mail:	Madrahimov.Nodir@mh-hannover.de
Order of Authors:	Nodir Madrahimov
	Abdurasul Khalikov
	Erin C. Boyle
	Ruslan Natanov
	Ann-Kathrin Knoefel
	Thierry Siemeni
	Klaus Hoeffler
	Axel Haverich
	Ulrich Maus
	Christian Kuehn
Additional Information:	
Question	**Response**
Please indicate whether this article will be Standard Access or Open Access.	Standard Access (US$2,400)
Please indicate the **city, state/province, and country** where this article will be **filmed**. Please do not use abbreviations.	Hannover Medical School, Carl-Neubergstr. 1, 30625 Hannover, Germany

Medizinische Hochschule Hannover

Department of Cardiothoracic, Transplantation, and Vascular Surgery
Hannover Medical School
Carl-Neuberg-Straße 1
30625 Hannover
www.mh-hannover.de

Telephone: +49-511-532-2782
Fax: +49-511-532-5404
E-Mail: Madrahimov.Nodir@mh-hannover.de

July 4, 2018

Dear Editors,

Please find enclosed our revised manuscript, "**Technique of veno-venous extracorporeal membrane oxygenation in a mouse**" (JoVE58146R2_RE) by Madrahimov et al. originally submitted on March 15, 2018.

We have now addressed all comments of the editor and have provided responses. We hope it is now acceptable for publication in *JoVE*.

Thank you for considering our manuscript.

Sincerely,

Nodir Madrahimov

TITLE:

Veno-Venous Extracorporeal Membrane Oxygenation in a Mouse

AUTHORS & AFFILIATIONS:

Nodir Madrahimov[1], Abdurasul Khalikov[1], Erin C. Boyle[1], Ruslan Natanov[1], Ann-Kathrin Knöfel[1], Thierry Siemeni[1], Klaus Höffler[1], Axel Haverich[1], Ulrich Maus[2]*, Christian Kühn[1]*

[1]Department of Cardiothoracic, Transplantation, and Vascular Surgery, Hannover Medical School, Hannover, Germany

[2]Department of Pneumology, Hannover Medical School, Hannover, Germany

*These authors contributed equally

Corresponding Author:

Nodir Madrahimov (Madrahimov.Nodir@mh-hannover.de)

Email Addresses of Co-Authors:

Nodir Madrahimov (Madrahimov.Nodir@mh-hannover.de)
Abdurasul Khalikov (Khalikov.Abdurasul@mh-hannover.de)
Erin C. Boyle (Boyle.Colleen@mh-hannover.de)
Ruslan Natanov (Natanov.Ruslan@mh-hannover.de)
Ann-Kathrin Knöfel (Knoefel.Ann-kathrin@mh-hannover.de)
Thierry Siemeni (Siemeni.Thierry@mh-hannover.de)
Klaus Höffler (Hoeffler.Klaus@mh-hannover.de)
Axel Haverich (Haverich.Axel@mh-hannover.de)
Ulrich Maus (Maus.Ulrich@mh-hannover.de)
Christian Kühn (Kuehn.Christian@mh-hannover.de)

KEYWORDS:

Extracorporeal membrane oxygenation, extracorporal circulation, animal model, mouse, organ damage, surgery

SUMMARY:

Here we present a protocol describing the technique of veno-venous extracorporeal membrane oxygenation (ECMO) in a non-intubated, spontaneously breathing mouse. This murine model of ECMO can be effectively implemented in experimental studies of acute and end-stage lung diseases.

ABSTRACT:

The use of extracorporeal membrane oxygenation (ECMO) has increased substantially in recent years. ECMO has become a reliable and effective therapy for acute as well as end-stage lung diseases. With the increase in clinical demand and prolonged use of ECMO, procedural optimization and prevention of multi-organ damage are of critical importance. The aim of this protocol is to present a detailed technique of veno-venous ECMO in a non-intubated, spontaneously breathing mouse. This protocol demonstrates the technical design of the ECMO

and surgical steps. This murine ECMO model will facilitate the study of pathophysiology related to ECMO (*e.g.,* inflammation, bleeding and thromboembolic events). Due to the abundance of genetically modified mice, the molecular mechanisms involved in ECMO-related complications can also be dissected.

INTRODUCTION:

Extracorporeal membrane oxygenation (ECMO) is a temporary life support system that takes over functions of the lungs and heart to allow adequate gas exchange and perfusion. Hill et al[1] described the first use of ECMO in patients in 1972; however, it only became widely used after its successful application during the H1N1 influenza pandemic in 2009[2]. Today, ECMO is routinely used as a lifesaving procedure in end-stage heart and lung diseases[3]. Veno-venous ECMO is increasingly employed as an alternative to invasive mechanical ventilation in awake, non-intubated, spontaneously breathing patients with refractory respiratory failure[4].

Despite its widespread adoption, diverse complications have been reported for ECMO[5,6,7]. Complications that can be experienced by patients on ECMO include bleeding, thrombosis, sepsis, thrombocytopenia, device-related malfunctions, and air embolism. Moreover, a systemic inflammatory response syndrome (SIRS) resulting in multi-organ damage is well-described both clinically and in experimental studies[8,9]. Neurological complications such as brain infarction are also frequently reported in patients undergoing long-term ECMO therapy. To confuse matters, it is often difficult to distinguish whether complications are caused by ECMO itself or arise from the underlying disorders accompanying acute and end-stage diseases.

To specifically study the effects of ECMO on a healthy organism, a reliable experimental animal model must be established. There are very few reports on performance of ECMO on small animals and are all limited to rats. To date, no mouse model of ECMO has been described in the literature. Due to the availability of a large number of genetically modified mouse strains, establishment of a mouse ECMO model would allow further investigation of the molecular mechanisms involved in ECMO-related complications[10,11].

Based on our previously described murine model of cardiopulmonary bypass (CPB)[12], we have developed a stable method of veno-venous ECMO in non-intubated, spontaneously breathing mice. The ECMO circuit (**Figure 1**), containing outflow and inflow cannulas, a peristaltic pump, oxygenator, and air-trapping reservoir, is similar to our previously described model of murine CPB[12] with the exception of having a smaller priming volume (0.5 mL). This protocol demonstrates the detailed techniques, physiological monitoring, and blood gas analysis involved in a successful ECMO procedure.

PROTOCOL:

Experiments were performed on male C57BL/6 mice, aged 12 weeks. This study was conducted in compliance with guidelines of the German Animal Law under Protocol TSA 16/2250.

1. Materials Preparation

Note: All steps are performed under clean, non-sterile conditions.

1.1. Introduce 3 fenestrations into a 2-Fr polyurethane tube using a surgical blade under a microscope with 16X magnification.

Note: All fenestrations must be located in the distal third of the cannula to ensure optimal blood drainage.

1.2. Prepare the priming solution (**Materials Table**). Include 30 IU/mL heparin and 2.5% v/v of an 8.4% solution of $NaHCO_3$. Refrigerate this solution at 4 °C until it is ready to use. Prime the circuit with 500 uL of priming solution.

1.3. Place the outflow cannula into the priming solution and fill the ECMO machine by switching on the peristaltic pump. Continue to circulate the priming solution through the machine for the next 30 min at a flow rate of 1 mL/min.

1.4. Give 0.5 L/min of 100% oxygen to the oxygenator.

2. Anesthesia

2.1. Place the animal in an induction chamber filled with a 2.5% v/v isoflurane/oxygen mixture. Provide 0.5 L/min of 100% oxygen to the vaporizer. Before surgery, check that full anesthesia is achieved by testing pedal withdrawal and pain reflexes. Apply eye gel to prevent drying damage.

2.2. Use a warming pad to maintain the body temperature at 37 °C.

2.3. Perform inhalation mask anesthesia using an isoflurane vaporizer and inject 5 mg/kg carprofen subcutaneously.

2.4. Regularly observe spontaneous breathing and adjust the concentration of isoflurane so that it is between 1.3 and 2.5%.

3. Surgery

3.1. Expose the left jugular vein by using a lateral skin incision of 4 mm with the help of fine scissors on the left side of the neck. Together with sharp and blunt preparation using micro-forceps and cotton swabs, use bipolar coagulation of the small vessels.

3.2. Once the left jugular vein is exposed, ligate the distal part using an 8-0 silk suture with the help of micro-forceps.

3.3. Place a slip knot at the proximal end of the vein. Incise the anterior wall of the vein using micro-scissors.

3.4. To achieve full heparinization, inject 2.5 IU/g heparin into the jugular vein via a 26 G braunula.

3.5. Raise the head side of the animal pad by 30° to avoid excessive blood loss from the vein during insertion of the cannula.

3.6. Insert a 2-Fr polyurethane (PU) cannula into the proximal part of the jugular vein, rotating it slightly while pushing it to a depth of 4 cm; while doing so, the iliac bifurcation of inferior vena cava (IVC) will be reached.

3.7. Secure the cannula with 8-0 silk knots using microforceps.

3.8. Expose the right jugular vein using the steps described in 3.1, 3.2, and 3.3.

3.9. Cannulate the right jugular vein with a 1-Fr PU cannula and gently move it 5 mm towards the direction of right atrium.

3.10. Repeat step 3.7.

3.11. Catheterize the left femoral artery with another 1-Fr PU cannula and use it for invasive pressure monitoring as well as blood sampling for blood gas analysis (BGA).

3.12. Insert electrocardiogram (ECG) needles connected to a data acquisition device subcutaneously into both forelimbs and into the left thoracic wall.

3.13. Insert a rectal thermometer connected to a data acquisition device.

4. Veno-Venous Extracorporeal Membrane Oxygenation and Blood Gas Analysis

Note: For a schematic of the complete ECMO circuit, see **Figure 1**.

4.1. Initiate ECMO on the animal by turning on the pump with an initial flow rate of 0.1 mL/min. Adjust the flow rate of the pump within the next 2 min to 3-5 mL/min.

4.2. In case of air suction in the outflow cannula via the cannulation site, reduce the flow and add 0.1 mL of priming solution to the circuit via an air-trapping reservoir.

4.3. Under stable flow, continue to monitor in real-time mode all vital parameters via the data acquisition device.

4.4. Constantly observe backflow from the venous drainage and monitor the level of the blood in the air-trapper reservoir.

4.5. Collect any blood leaking from wounds into a 1 cc syringe with the tip of a 24 G branula and

return it to the ECMO circuit via the air-trapping reservoir.

4.6. For BGA, use a blood sampling cartridge to collect approximately 75 µL of arterial blood at the following time points and from the following locations:

4.6.1. 10 min after the initiation of ECMO, collect blood from the IVC before the oxygenator, directly after oxygenator (control), and from the femoral artery.

4.6.2. 30 min after the initiation of ECMO, collect blood from the femoral artery.

4.7. Give an extra 0.1 mL of priming solution to compensate for intravasal liquid loss every 45 min via the air-trapper or femoral artery catheter or by sucking the air bubbles through the blood draining cannula.

4.8. For BGA, use a blood sampling cartridge to collect approximately 75 µL of arterial blood:

4.8.1. 1 h after the initiation of ECMO from the femoral artery.

4.8.2. 2 h after the initiation of ECMO from the oxygenator, IVC, and the femoral artery.

4.9. After 2 h, reduce the flow rate on the pump gradually (over the course of 5 min), thereby stopping ECMO.

4.9. Continue to record vital parameters for another 10 min.

4.10. Finish the experiment by exsanguinating the animal and harvesting the blood and organs.

REPRESENTATIVE RESULTS:

This protocol describes the method of veno-venous ECMO in a mouse. This model is reliable and reproducible, and compared to our previously described model of CPB with respiratory and circulatory arrest[12,13], it is less technically demanding to establish.

ECMO flow in the venous system was maintained between 1.5 and 5 mL/min. The mean arterial pressure was kept between 70 and 85 mmHg by adding extra priming solution into the ECMO circuit. Usually, the adding of 0.1 mL of priming solution to the circuit during ECMO allows substitution of blood volume. All volume substituting or buffering solutions were given via the femoral artery or air-trapping reservoir.

Physiological parameters were recorded every 10 min and data from a representative ECMO experiment are presented in **Figure 2**. BGA data from a successful ECMO are shown in **Table 1**.

Hematological parameters showed relevant hemodilution during ECMO; however, no blood transfusion was necessary to compensate for moderate anemia (**Table 1**). Oxygenation parameters from BGA demonstrated proper performance of the oxygenator at an oxygen/air

mixture at FiO_2 1.0 (**Table 1**).

Metabolic changes during ECMO showed respiratory alkalosis at the start and moderate acidosis at the end of the experiment (**Table 1**). No extra buffering of the blood was performed.

FIGURE AND TABLE LEGENDS:

Figure 1: ECMO layout in a mouse. Blood is drained from the inferior vena cava (IVC) via the left jugular vein and oxygenated blood is pumped into the superior vena cava (SVC) via the right jugular vein.

Figure 2: Physiological parameters measured during 2 h of ECMO. A = heart rate, B = mean arterial pressure (VS = volume substitution), and C = rectal temperature.

Table 1: BGA results over the course of the experiment. O = oxygenator, FA = femoral artery, and IVC = inferior vena cava.

DISCUSSION:

Previously, we described a successful model of CPB in a mouse[12,13]. To implement such a model for acute or end-stage lung disorders we developed an easy-to-use veno-venous ECMO circuit for mice. Different to the CPB model, veno-venous ECMO does not require complicated surgical procedures such as sternotomy and clamping of the aorta, thus reducing the risk of wound bleeding in a fully heparinized animal. To avoid embolization of the oxygenator with blood clots, 2.5 IU of heparin/kg is given to each animal. This dose was based on previous measurements of the activated clotting time (ACT) that showed full anticoagulation of the blood (ACT > 800 sec). Due to the absence of heparin coating in the micro-oxygenator, our anticoagulation protocol was kept similar to our CPB procedure.

In comparison to the CPB circuit, we could reduce the overall priming volume to 0.5 mL by reducing the volume of the air-trapper and micro-oxygenator. Moreover, a slower flow was necessary to keep adequate oxygenation of the animal. Intravasal loss of the blood volume resulted in a gradual drop of mean arterial pressure. Adding an extra 0.1 mL of priming volume to the animal led to an increase in blood pressure over 20 mmHg, but a small linear reduction in arterial pressure over the next 30 min was always present. Volume substitution was called for if air was sucked through the drainage cannula or there was a drop in blood pressure below 75 mmHg.

The most difficult challenge in the surgical procedure for mouse ECMO model is the placement of the cannula via the left jugular vein into the IVC. To establish this method, different types of cannulas were tested, and a laparotomy was performed in mouse cadavers to perfect the positioning of the cannula tip into the IVC just before the iliac bifurcation. Sometimes, in bigger animals, placement of the cannula can lead to dislocation of the cannula into the right kidney vein. Nevertheless, the whole blood from all segments of the IVC could be well-drained due to side fenestrations of the cannula.

In preliminary trials, we performed cannulation via the femoral vein. Unfortunately, only a 1-Fr cannula can be placed into the femoral vein, which results in inadequate blood flow (≤ 1 mL/min). 1-Fr catheters pushed into the IVC all displayed insufficient backflow. To achieve substantial backflow, both femoral veins would need to be cannulated; therefore, we abandoned this procedure and achieved adequate draining via a 2-Fr cannula placed in the IVC via the jugular vein. Blood loss during placement of the cannula into jugular vein is very typical. Therefore, before placement, the head end of the animal pad is raised 30-40°, so backflow from the vein is significantly reduced.

A gradual reduction in hemoglobin and hematocrit is explained by hemolysis and repetitive blood samplings taken to demonstrate the performance of the device. For survival experiments, to avoid blood transfusions, blood sampling should be extremely limited or even avoided. Moreover, at the end of the experiment, the blood from the ECMO circuit should be returned into the animal. However, survivability of the model has to be studied in a separate project using a less invasive protocol.

Blood flow during our ECMO runs was between 3 and 5 mL/min. Normal mouse cardiac output is reported to be between 6 and 9 mL/min; therefore, on average, we were able to achieve an ECMO flow of 54% of the mouse's cardiac output. Usually, veno-venous ECMO requires lower blood flow compared to veno-arterial ECMO, as overperfusion of the right atrium can lead to right ventricular overload and consequently, heart failure. Clinically, to achieve adequate oxygenation, a veno-venous ECMO flow of 50-75% of cardiac output is enough for sufficient oxygenation in ventilated or spontaneously breathing patients. Unnecessarily increasing the ECMO flow may lead to more damage caused by SIRS and hemolysis and useless recirculation of the major part of the venous blood between the IVC and SVC. Moreover, we observed that by increasing of the flow in the veno-venous ECMO, excessive negative pressure leads to air suction at the site of cannulation. Our animals received 100% oxygen under isoflurane anesthesia, and with the help of veno-venous ECMO, were hyper-oxygenated. In our model we have tried to reproduce conditions of "awake ECMO"[4] having less damage to lungs.

The molecular mechanisms involved in ECMO-related complications can now be investigated due to the plethora of genetically modified mouse strains available. There are also more than eighty strains of mice with lung disorders that may simulate ECMO in the context of these underlying diseases. Therefore, we believe that our veno-venous ECMO mouse model may be implemented in multiple synergistic projects.

ACKNOWLEDGMENTS:
This project was supported by KFO 311 Grant from Deutsche Forschungsgemeinschaft.

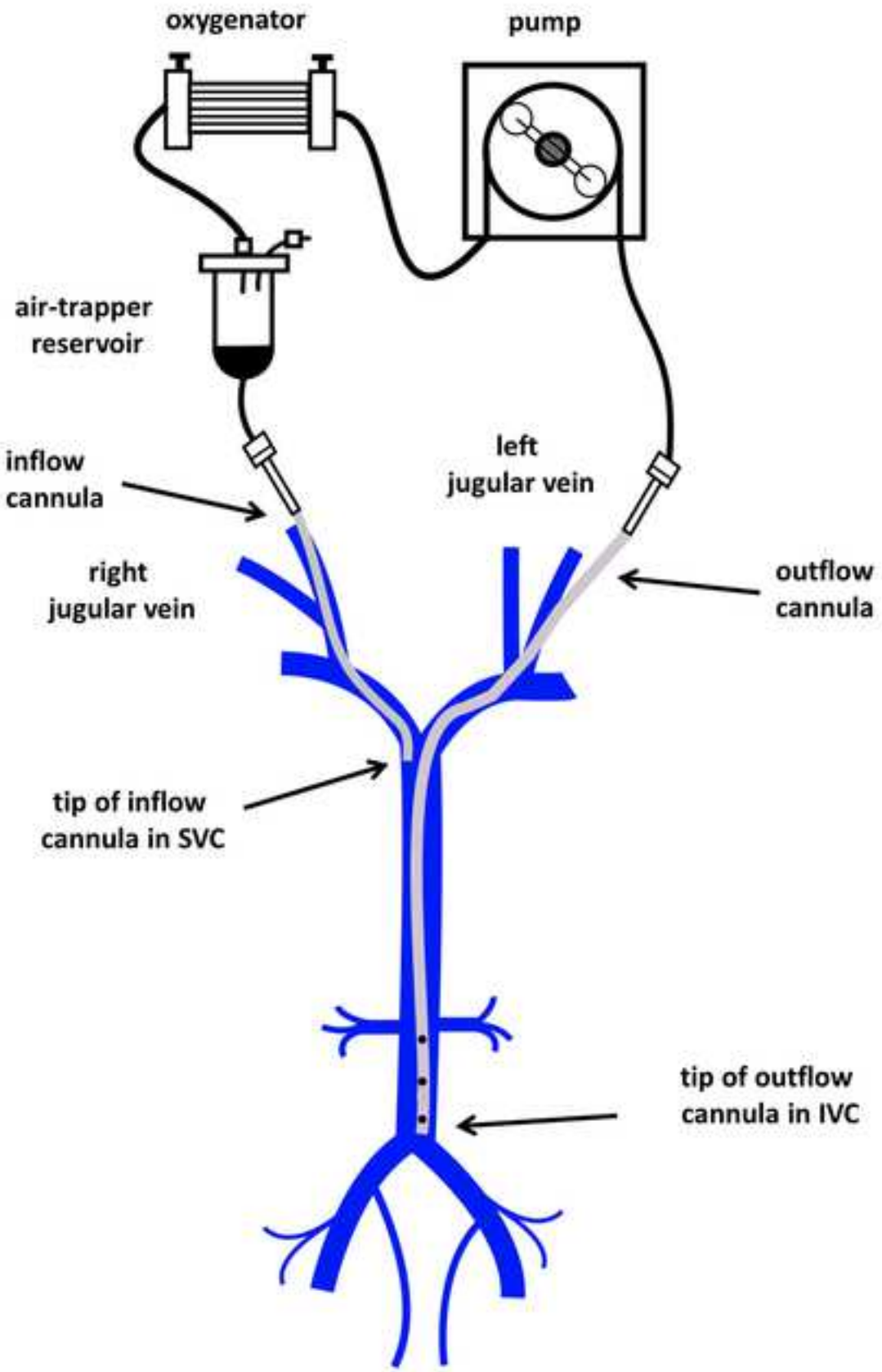
DISCLOSURES:
The authors have nothing to disclose.

REFERENCES:

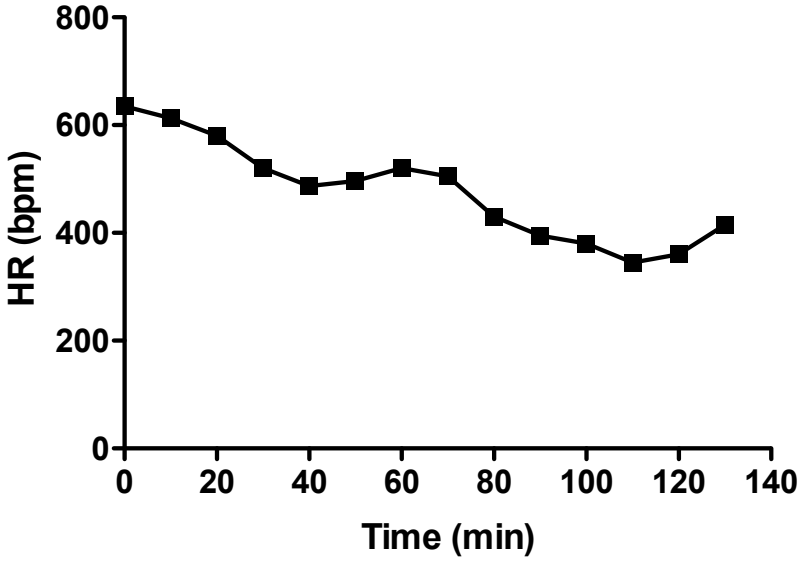
1. Hill, J. D., *et al.* Prolonged Extracorporeal Oxygenation for Acute Post-Traumatic Respiratory Failure (Shock-Lung Syndrome). *New England Journal of Medicine.* **286** (12), 629–634 (1972).
2. Noah, M. A., *et al.* Referral to an Extracorporeal Membrane Oxygenation Center and Mortality Among Patients With Severe 2009 Influenza A(H1N1). *Journal of the American Medical Association*. **306** (15), 1659 (2011).
3. Maslach-Hubbard, A., Bratton, S. L. Extracorporeal membrane oxygenation for pediatric respiratory failure: History, development and current status. *World Journal of Critical. Care Medicine.* **2** (4), 29–39 (2013).
4. Langer, T., *et al.* "Awake" extracorporeal membrane oxygenation (ECMO): pathophysiology, technical considerations, and clinical pioneering. *Critical Care*. **20** (1), 150 (2016).
5. Esper, S. A. Extracorporeal Membrane Oxygenation. *Advances in Anesthesia.* **35** (1), 119–143 (2017).
6. Millar, J. E., Fanning, J. P., McDonald, C. I., McAuley, D. F., Fraser, J. F. The inflammatory response to extracorporeal membrane oxygenation (ECMO): a review of the pathophysiology. *Critical Care.* **20** (1), 387 (2016).
7. Lubnow, M., *et al.* Technical complications during veno-venous extracorporeal membrane oxygenation and their relevance predicting a system-exchange--retrospective analysis of 265 cases. *Public Library of Science One.* **9** (12), e112316 (2014).
8. Passmore, M. R., *et al.* Inflammation and lung injury in an ovine model of extracorporeal membrane oxygenation support. *American Journal of Physiology - Lung Cellular and Molecular Physiology.* **311** (6), L1202-L1212 (2016).
9. Vaquer, S., de Haro, C., Peruga, P., Oliva, J. C., Artigas, A. Systematic review and meta-analysis of complications and mortality of veno-venous extracorporeal membrane oxygenation for refractory acute respiratory distress syndrome. *Annals of Intensive Care.* **7** (1), 51 (2017).
10. Houser, S. R., *et al.* Animal Models of Heart Failure A Scientific Statement From the American Heart Association. *Circulation Research.* **111** (1), 131–150 (2012).
11. Russell, J. C., Proctor, S. D. Small animal models of cardiovascular disease: tools for the study of the roles of metabolic syndrome, dyslipidemia, and atherosclerosis. *Cardiovascular Pathology.* **15** (6), 318–330 (2006).
12. Madrahimov, N., *et al.* Novel mouse model of cardiopulmonary bypass. *European Journal of Cardio-thoracic Surgery.* **53** (1), 186-193 (2017).
13. Madrahimov, N., *et al.* Cardiopulmonary Bypass in a Mouse Model: A Novel Approach *J. Journal of Visualized Experiments.* **127**, (2017).

Figure 1

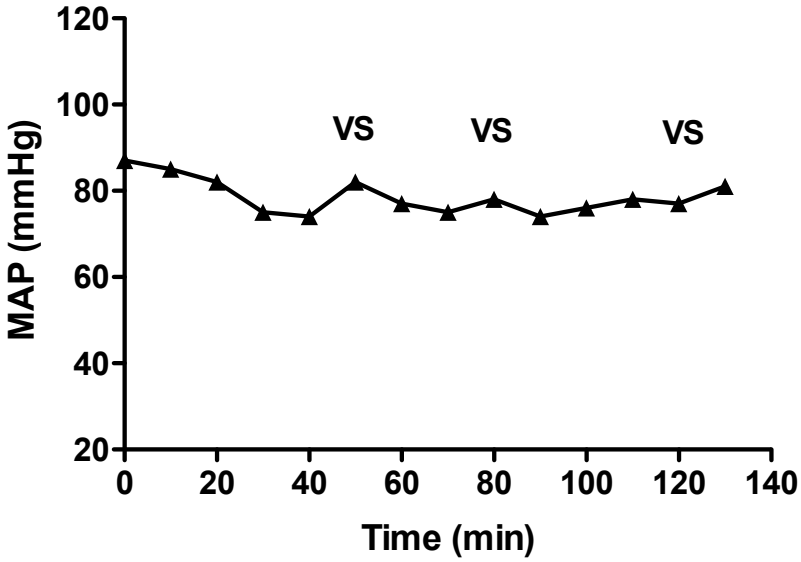
Click here to download Figure Figure 1.jpg



A.



B.



C.

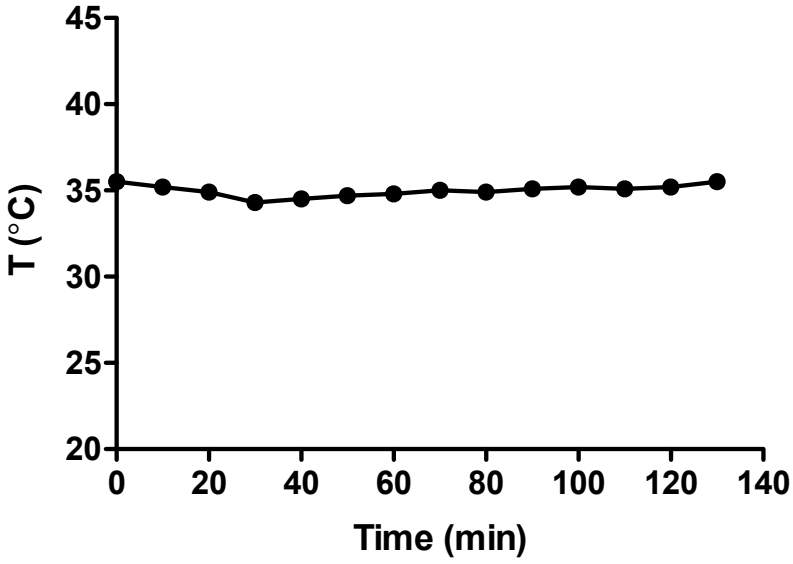


Table 1

Click here to download Table Table BGA.xlsx

	10 min			30 min	1 h	
Parameters	O	FA	IVC	FA	FA	O
pH	7.67	7.51	7.31	7.57	7.5	7.6
pCO2 (mmHg)	24.5	24	52	26	25	22
pO2 (mmHg)	707	656	135	643	621	638
HCO_3 (mmol/L)	28.3	25.3	26	24	23	27
sO2 (%)	100	100	99	100	100	100
HCT (%)	24	23	23	20	18	17
Hb (g/dl)	8.8	8.6	8.5	8	7.8	7.6
Lac (mmol/L)	1.9	1.7	1.8	2.1	2.4	3.2

2 h	
FA	IVC
7.57	7.34
26	51.1
573	101
23	25
100	98
17	16
7.2	7
3.1	3.3

Materials Table

Click here to download Table of Materials Madrahimov Materials.xlsx

Name of Reagent/ Equipment	Company	Catalog Number
Sterofundin	B.Braun Petzold GmbH	PZN:8609189
Tetraspan 6% Solution	B. Braun Melsungen AG	PZN: 05565416
Heparin Natrium 25.000	Ratiopharm GmbH	PZN: 3029843
NaHCO3 8,4% Solution	B. Braun Melsungen AG	PZN: 1579775
Carprofen	Zoetis Inc., USA	PZN:00289615
1 Fr PU Catheter	Instechlabs INC., USA	C10PU-MCA1301
2 Fr PU Catheter	Instechlabs INC., USA	C20PU-MJV1302
8-0 Silk suture braided	Ashaway Line & Twine Co., USA	75290
Isoflurane	Piramal Critical Care GmbH	PZN:9714675
Spring Scissors - 6mm Blades	Fine Science Tools GmbH	15020-15
Spring Scissors - 2mm Blades	Fine Science Tools GmbH	15000-03
Halsted-Mosquito Hemostat	Fine Science Tools GmbH	13009-12
Dumont #55 Forceps	Fine Science Tools GmbH	11295-51
Castroviejo Micro Needle Holder - 9cm	Fine Science Tools GmbH	12060-02
Micro Serrefines	Fine Science Tools GmbH	18555-01
Bulldog Serrefine	Fine Science Tools GmbH	18050-28
Isoflurane Vaporizer Drager 19.1	Drägerwerk AG & Co. KGaA	
Multichannel Data Aquisition Device with ISOHEART Software	Hugo Sachs Elektronik GmbH, Germany	
i-STAT portable device	Abbott Laboratories, Lake Bluff, Illinois, USA	
i-STAT CG4+ and CG8+ cartridges	Abbott Laboratories, Lake Bluff, Illinois, USA	
C57Bl/6 mice, male, 30 g, 14 weeks old	Charles River Laboratories	

Comments/Description
in 1:1 with Tetraspan
in 1:1 with Sterofundin
2,5 IU per ml of priming
3% in priming solution
5mg/kg/BW
carotide artery
jugular vein
ligature
narcosis
instruments
instruments
instruments
instruments
instruments
instruments
instruments
anesthesia 1,3 -2,5%
invasive pressure, ECG, t °C
blood gas analysis
blood gas analysis
housed 1 week before

jove JOURNAL OF VISUALIZED EXPERIMENTS
1 Alewife Center #200
Cambridge, MA 02140
tel. 617.945.9051
www.jove.com

ARTICLE AND VIDEO LICENSE AGREEMENT

Title of Article: TECHNIQUE OF VENO-VENOUS EXTRACORPOREAL MEMBRANE OXYGENATION W A MODIFIED

Author(s): N. MADRAHIMOV, A. KHALIKOV, E. BOYLE, R. NAZAROV, A.K. KNOEFEL, T. SIEMENI, K. HOEFFLER, A. HAUERICH, U. MAUS, CH. KURTH

Item 1 (check one box): The Author elects to have the Materials be made available (as described at http://www.jove.com/author) via: [X] Standard Access [ ] Open Access

Item 2 (check one box):

[X] The Author is NOT a United States government employee.

[ ] The Author is a United States government employee and the Materials were prepared in the course of his or her duties as a United States government employee.

[ ] The Author is a United States government employee but the Materials were NOT prepared in the course of his or her duties as a United States government employee.

ARTICLE AND VIDEO LICENSE AGREEMENT

1. Defined Terms. As used in this Article and Video License Agreement, the following terms shall have the following meanings: "**Agreement**" means this Article and Video License Agreement; "**Article**" means the article specified on the last page of this Agreement, including any associated materials such as texts, figures, tables, artwork, abstracts, or summaries contained therein; "**Author**" means the author who is a signatory to this Agreement; "**Collective Work**" means a work, such as a periodical issue, anthology or encyclopedia, in which the Materials in their entirety in unmodified form, along with a number of other contributions, constituting separate and independent works in themselves, are assembled into a collective whole; "**CRC License**" means the Creative Commons Attribution-Non Commercial-No Derivs 3.0 Unported Agreement, the terms and conditions of which can be found at: http://creativecommons.org/licenses/by-nc-nd/3.0/legalcode; "**Derivative Work**" means a work based upon the Materials or upon the Materials and other pre-existing works, such as a translation, musical arrangement, dramatization, fictionalization, motion picture version, sound recording, art reproduction, abridgment, condensation, or any other form in which the Materials may be recast, transformed, or adapted; "**Institution**" means the institution, listed on the last page of this Agreement, by which the Author was employed at the time of the creation of the Materials; "**JoVE**" means MyJove Corporation, a Massachusetts corporation and the publisher of *The Journal of Visualized Experiments*; "**Materials**" means the Article and / or the Video; "**Parties**" means the Author and JoVE; "**Video**" means any video(s) made by the Author, alone or in conjunction with any other parties, or by JoVE or its affiliates or agents, individually or in collaboration with the Author or any other parties, incorporating all or any portion of the Article, and in which the Author may or may not appear.

2. Background. The Author, who is the author of the Article, in order to ensure the dissemination and protection of the Article, desires to have the JoVE publish the Article and create and transmit videos based on the Article. In furtherance of such goals, the Parties desire to memorialize in this Agreement the respective rights of each Party in and to the Article and the Video.

3. Grant of Rights in Article. In consideration of JoVE agreeing to publish the Article, the Author hereby grants to JoVE, subject to **Sections 4** and **7** below, the exclusive, royalty-free, perpetual (for the full term of copyright in the Article, including any extensions thereto) license (a) to publish, reproduce, distribute, display and store the Article in all forms, formats and media whether now known or hereafter developed (including without limitation in print, digital and electronic form) throughout the world, (b) to translate the Article into other languages, create adaptations, summaries or extracts of the Article or other Derivative Works (including, without limitation, the Video) or Collective Works based on all or any portion of the Article and exercise all of the rights set forth in (a) above in such translations, adaptations, summaries, extracts, Derivative Works or Collective Works and (c) to license others to do any or all of the above. The foregoing rights may be exercised in all media and formats, whether now known or hereafter devised, and include the right to make such modifications as are technically necessary to exercise the rights in other media and formats. If the "Open Access" box has been checked in **Item 1** above, JoVE and the Author hereby grant to the public all such rights in the Article as provided in, but subject to all limitations and requirements set forth in, the CRC License.

jove JOURNAL OF VISUALIZED EXPERIMENTS
1 Alewife Center #200
Cambridge, MA 02140
tel. 617.945.9051
www.jove.com

ARTICLE AND VIDEO LICENSE AGREEMENT

4. Retention of Rights in Article. Notwithstanding the exclusive license granted to JoVE in **Section 3** above, the Author shall, with respect to the Article, retain the non-exclusive right to use all or part of the Article for the non-commercial purpose of giving lectures, presentations or teaching classes, and to post a copy of the Article on the Institution's website or the Author's personal website, in each case provided that a link to the Article on the JoVE website is provided and notice of JoVE's copyright in the Article is included. All non-copyright intellectual property rights in and to the Article, such as patent rights, shall remain with the Author.

5. Grant of Rights in Video – Standard Access. This **Section 5** applies if the "Standard Access" box has been checked in **Item 1** above or if no box has been checked in **Item 1** above. In consideration of JoVE agreeing to produce, display or otherwise assist with the Video, the Author hereby acknowledges and agrees that, Subject to **Section 7** below, JoVE is and shall be the sole and exclusive owner of all rights of any nature, including, without limitation, all copyrights, in and to the Video. To the extent that, by law, the Author is deemed, now or at any time in the future, to have any rights of any nature in or to the Video, the Author hereby disclaims all such rights and transfers all such rights to JoVE.

6. Grant of Rights in Video – Open Access. This **Section 6** applies only if the "Open Access" box has been checked in **Item 1** above. In consideration of JoVE agreeing to produce, display or otherwise assist with the Video, the Author hereby grants to JoVE, subject to **Section 7** below, the exclusive, royalty-free, perpetual (for the full term of copyright in the Article, including any extensions thereto) license (a) to publish, reproduce, distribute, display and store the Video in all forms, formats and media whether now known or hereafter developed (including without limitation in print, digital and electronic form) throughout the world, (b) to translate the Video into other languages, create adaptations, summaries or extracts of the Video or other Derivative Works or Collective Works based on all or any portion of the Video and exercise all of the rights set forth in (a) above in such translations, adaptations, summaries, extracts, Derivative Works or Collective Works and (c) to license others to do any or all of the above. The foregoing rights may be exercised in all media and formats, whether now known or hereafter devised, and include the right to make such modifications as are technically necessary to exercise the rights in other media and formats. For any Video to which this Section 6 is applicable, JoVE and the Author hereby grant to the public all such rights in the Video as provided in, but subject to all limitations and requirements set forth in, the CRC License.

7. Government Employees. If the Author is a United States government employee and the Article was prepared in the course of his or her duties as a United States government employee, as indicated in **Item 2** above, and any of the licenses or grants granted by the Author hereunder exceed the scope of the 17 U.S.C. 403, then the rights granted hereunder shall be limited to the maximum rights permitted under such statute. In such case, all provisions contained herein that are not in conflict with such statute shall remain in full force and effect, and all provisions contained herein that do so conflict shall be deemed to be amended so as to provide to JoVE the maximum rights permissible within such statute.

8. Likeness, Privacy, Personality. The Author hereby grants JoVE the right to use the Author's name, voice, likeness, picture, photograph, image, biography and performance in any way, commercial or otherwise, in connection with the Materials and the sale, promotion and distribution thereof. The Author hereby waives any and all rights he or she may have, relating to his or her appearance in the Video or otherwise relating to the Materials, under all applicable privacy, likeness, personality or similar laws.

9. Author Warranties. The Author represents and warrants that the Article is original, that it has not been published, that the copyright interest is owned by the Author (or, if more than one author is listed at the beginning of this Agreement, by such authors collectively) and has not been assigned, licensed, or otherwise transferred to any other party. The Author represents and warrants that the author(s) listed at the top of this Agreement are the only authors of the Materials. If more than one author is listed at the top of this Agreement and if any such author has not entered into a separate Article and Video License Agreement with JoVE relating to the Materials, the Author represents and warrants that the Author has been authorized by each of the other such authors to execute this Agreement on his or her behalf and to bind him or her with respect to the terms of this Agreement as if each of them had been a party hereto as an Author. The Author warrants that the use, reproduction, distribution, public or private performance or display, and/or modification of all or any portion of the Materials does not and will not violate, infringe and/or misappropriate the patent, trademark, intellectual property or other rights of any third party. The Author represents and warrants that it has and will continue to comply with all government, institutional and other regulations, including, without limitation all institutional, laboratory, hospital, ethical, human and animal treatment, privacy, and all other rules, regulations, laws, procedures or guidelines, applicable to the Materials, and that all research involving human and animal subjects has been approved by the Author's relevant institutional review board.

10. JoVE Discretion. If the Author requests the assistance of JoVE in producing the Video in the Author's facility, the Author shall ensure that the presence of JoVE employees, agents or independent contractors is in accordance with the relevant regulations of the Author's institution. If more than one author is listed at the beginning of this Agreement, JoVE may, in its sole discretion, elect not take any action with respect to the Article until such time as it has received complete, executed Article and Video License Agreements from each such author. JoVE reserves the right, in its absolute and sole discretion and without giving any reason therefore, to accept or decline any work submitted to JoVE. JoVE and its employees, agents and independent contractors shall have

jove JOURNAL OF VISUALIZED EXPERIMENTS
1 Alewife Center #200
Cambridge, MA 02140
tel. 617.945.9051
www.jove.com

ARTICLE AND VIDEO LICENSE AGREEMENT

full, unfettered access to the facilities of the Author or of the Author's institution as necessary to make the Video, whether actually published or not. JoVE has sole discretion as to the method of making and publishing the Materials, including, without limitation, to all decisions regarding editing, lighting, filming, timing of publication, if any, length, quality, content and the like.

11. Indemnification. The Author agrees to indemnify JoVE and/or its successors and assigns from and against any and all claims, costs, and expenses, including attorney's fees, arising out of any breach of any warranty or other representations contained herein. The Author further agrees to indemnify and hold harmless JoVE from and against any and all claims, costs, and expenses, including attorney's fees, resulting from the breach by the Author of any representation or warranty contained herein or from allegations or instances of violation of intellectual property rights, damage to the Author's or the Author's institution's facilities, fraud, libel, defamation, research, equipment, experiments, property damage, personal injury, violations of institutional, laboratory, hospital, ethical, human and animal treatment, privacy or other rules, regulations, laws, procedures or guidelines, liabilities and other losses or damages related in any way to the submission of work to JoVE, making of videos by JoVE, or publication in *JoVE* or elsewhere by JoVE. The Author shall be responsible for, and shall hold JoVE harmless from, damages caused by lack of sterilization, lack of cleanliness or by contamination due to the making of a video by JoVE its employees, agents or independent contractors. All sterilization, cleanliness or decontamination procedures shall be solely the responsibility of the Author and shall be undertaken at the Author's expense. All indemnifications provided herein shall include JoVE's attorney's fees and costs related to said losses or damages. Such indemnification and holding harmless shall include such losses or damages incurred by, or in connection with, acts or omissions of JoVE, its employees, agents or independent contractors.

12. Fees. To cover the cost incurred for publication, JoVE must receive payment before production and publication the Materials. Payment is due in 21 days of invoice. Should the Materials not be published due to an editorial or production decision, these funds will be returned to the Author. Withdrawal by the Author of any submitted Materials after final peer review approval will result in a US$1,200 fee to cover pre-production expenses incurred by JoVE. If payment is not received by the completion of filming, production and publication of the Materials will be suspended until payment is received.

13. Transfer, Governing Law. This Agreement may be assigned by JoVE and shall inure to the benefits of any of JoVE's successors and assignees. This Agreement shall be governed and construed by the internal laws of the Commonwealth of Massachusetts without giving effect to any conflict of law provision thereunder. This Agreement may be executed in counterparts, each of which shall be deemed an original, but all of which together shall be deemed to me one and the same agreement. A signed copy of this Agreement delivered by facsimile, e-mail or other means of electronic transmission shall be deemed to have the same legal effect as delivery of an original signed copy of this Agreement.

A signed copy of this document must be sent with all new submissions. Only one Agreement required per submission.

CORRESPONDING AUTHOR:

Name: DR. NODIR MADRAHIMOV

Department: DEPT. OF CARDIOTHORACIC, VASCULAR AND TRANSPLANTATION SURGERY

Institution: HANNOVER MEDICAL SCHOOL

Article Title: TECHNIQUE OF veno-venous extracorporeal membrane oxygenation in a mouse

Signature: [signature]

Date: 14.03.2018

Please submit a signed and dated copy of this license by one of the following three methods:

1) Upload a scanned copy of the document as a pfd on the JoVE submission site;
2) Fax the document to +1.866.381.2236;
3) Mail the document to JoVE / Attn: JoVE Editorial / 1 Alewife Center #200 / Cambridge, MA 02139

For questions, please email submissions@jove.com or call +1.617.945.9051

Editorial comments (in email):

1. The editor has formatted the manuscript as per the journal's style. Please retain the same. Please rephrase the title to be crisp and do not use abbreviations.

Done.

2. Please address specific comments marked in the manuscript.

Done.

3. Please do not have a paragraph of texts in the protocol section. Please have numbered steps only.

Okay. This has been changed.

4. Please specific sex, age, the strain of the animal in use.

This is already described in the first line of the "Protocol" ("male C57BL/6 mice, aged 12 weeks").

5. Once the protocol is edited, please highlight 2.75 pages of text including heading and spacing which will be used for filming purpose.

We have highlighted the relevant text the final edited Word file (58146_R2_RE_highlighted). We have also cut and paste the highlighted text into another Word file (58146_R2_RE_script).

6. Figure 2: Please use the correct degree symbol. Please leave a single space between number and units.

The degree symbol has been corrected.

Specific comments in the document:

- Please make the title more crisp. Please do not use abbreviations in the title.

This has been changed.

- Please rephrase the Short Abstract/Summary to clearly describe the protocol and its applications in complete sentences between 10-50 words: "Here, we present a protocol to …"
- Please reword this better.

- Please use American English throughout. .. this has been converted to one. Please check.

These points have been addressed/changed in the text.

- ECMO murine model?

Yes. We have added the word "murine" now.

- Due to abundance of what kind of genetically modified mice?

We would argue that this is self-explanatory and, in any case, should not be covered in the Abstract. There are hundreds of molecular pathways that could be investigated with knockout mice and dozens of disease/conditions that could investigated with geneticall altered mice. We cover these ideas briefly in the last paragraph of the Discussion.

- Please revise the Introduction to include all of the following:
  a) A clear statement of the overall goal of this method
  b) The rationale behind the development and/or use of this technique
  c) The advantages over alternative techniques with applicable references to previous studies
  d) A description of the context of the technique in the wider body of literature
  e) Information to help readers to determine whether the method is appropriate for their application

All of these aspects are included in the Introduction.

- What kind of model? Mice model?

No. We have now clarified this was in patients.

- Shouldn't this come after describing what ECMO is at the first place?

We have now added a sentence beforehand to define what ECMO is.

- The sentence before this says no mouse model is ECMO is described in literature. This one says due to the availability of huge number of genetically modified mouse strains… There seem to a link missing between the two.

We have now clarified, "Due to the availability of a huge number of genetically modified mouse strains, having a mouse ECMO model would allow the molecular mechanisms involved in ECMO-related complications to be investigated."

- We cannot have paragraphs of text in the protocol section. Please convert this to a numbered step and rewrite in imperative tense. Alternatively this detail can be moved to the introduction section as well.

Okay, but we did this in our last JOVE paper as well (Madrahimov et al 2017). We have now moved some of this text into the Introduction. Other details were requested by one reviewer but basically we just want to say that many components of the circuit are as described in our last paper (which we have now referenced the text moved to the Introduction).

- Please do not use commercial terms in the manuscript. Please refer to the commercial term in the table of materials and use generic term instead.

We only added the commercial names of the solution at the request of one of the reviewers. We'll take it out again.

- How do you check this – by toes pinch? Please mention the same.

We already mentioned this is the text ("check pedal withdrawal and pain reflexes") but we have now moved these details into the same sentence so you can't miss it.

- Please mention this step right where the step is happening. Please write the protocol in the way you are performing the experiment.

Okay, the steps have now been rearranged.

- As we are a methods journal, please revise the Discussion to explicitly cover the following in detail in 3-6 paragraphs with citations:
  a) Critical steps within the protocol
  b) Any modifications and troubleshooting of the technique
  c) Any limitations of the technique
  d) The significance with respect to existing methods
  e) Any future applications of the technique

Yes, we have covered all of these topics. The Discussion was originally 6 paragraphs but due to the comments of the scientific reviewers, a new paragraph was added to address their comments.

TITLE:

Technique of veno-venous extracorporeal membrane oxygenation in a mouse

AUTHORS & AFFILIATIONS: (Instructions)

Nodir Madrahimov, Abdurasul Khalikov, Erin C. Boyle, Ruslan Natanov, Ann-Kathrin Knöfel, Thierry Siemeni, Klaus Höffler, Axel Haverich, Ulrich Maus*, Christian Kühn*

* equal contribution

Nodir Madrahimov
Department of Cardiothoracic, Transplantation, and Vascular Surgery
Hannover Medical School
Hannover, Germany
Madrahimov.Nodir@mh-hannover.de

Abdurasul Khalikov
Department of Cardiothoracic, Transplantation, and Vascular Surgery
Hannover Medical School
Hannover, Germany
Khalikov.Abdurasul@mh-hannover.de

Erin C. Boyle
Department of Cardiothoracic, Transplantation, and Vascular Surgery
Hannover Medical School
Hannover, Germany
Boyle.Colleen@mh-hannover.de

Ruslan Natanov
Department of Cardiothoracic, Transplantation, and Vascular Surgery
Hannover Medical School
Hannover, Germany
Natanov.Ruslan@mh-hannover.de

Ann-Kathrin Knöfel
Department of Cardiothoracic, Transplantation, and Vascular Surgery
Hannover Medical School
Hannover, Germany
Knoefel.Ann-kathrin@mh-hannover.de

Thierry Siemeni
Department of Cardiothoracic, Transplantation, and Vascular Surgery
Hannover Medical School
Hannover, Germany

Siemeni.Thierry@mh-hannover.de

Klaus Höffler
Department of Cardiothoracic, Transplantation, and Vascular Surgery
Hannover Medical School
Hannover, Germany
Hoeffler.Klaus@mh-hannover.de

Axel Haverich
Department of Cardiothoracic, Transplantation, and Vascular Surgery
Hannover Medical School
Hannover, Germany
Haverich.Axel@mh-hannover.de

Ulrich Maus
Department of Pneumology
Hannover Medical School
Hannover, Germany
Maus.Ulrich@mh-hannover.de

Christian Kühn
Department of Cardiothoracic, Transplantation, and Vascular Surgery
Hannover Medical School
Hannover, Germany
Kuehn.Christian@mh-hannover.de

CORRESPONDING AUTHOR:
Nodir Madrahimov, MD

KEYWORDS:
Extracorporeal membrane oxygenation, extracorporal circulation, animal model, mouse, organ damage, surgery

SHORT ABSTRACT:
Here we present a protocol describing the technique of veno-venous extracorporeal membrane oxygenation in a non-intubated, spontaneously breathing mouse. This murine model of ECMO can be effectively implemented in experimental studies of acute and end-stage lung diseases.

LONG ABSTRACT:
The use of extracorporeal membrane oxygenation (ECMO) has increased substantially in recent years. ECMO has become a reliable and effective therapy for acute as well as end-stage lung diseases. With the increase in clinical demand and prolonged use of ECMO, procedural optimization and prevention of multi-organ damage is of critical importance. The aim of this

protocol is to present a detailed technique of veno-venous ECMO in a non-intubated, spontaneously breathing mouse. This protocol demonstrates the technical design of the ECMO and surgical steps. This murine ECMO model will facilitate the study of the pathophysiology related to ECMO (e.g., inflammation, bleeding and thromboembolic events). Due to the abundance of genetically modified mice, the molecular mechanisms involved ECMO-related complications can also be dissected.

INTRODUCTION:

Extracorporeal membrane oxygenation (ECMO) is a temporarily life support system that takes over the function of the lungs and heart to allow adequate gas exchange and perfusion. Hill et al[1] described the first use of ECMO in patients in 1972, however, it only became widely used after its successful application during the H1N1 influenza pandemic in 2009[2]. Today, ECMO is routinely used as a lifesaving procedure in end-stage heart and lung diseases[3]. Veno-venous ECMO is increasingly employed as an alternative to invasive mechanical ventilation in awake, non-intubated, spontaneously breathing patients with refractory respiratory failure[4].

Despite its widespread adoption, diverse complications have been reported for ECMO[5,6,7]. Complications that can be experienced by patients on ECMO include bleeding, thrombosis, sepsis, thrombocytopenia, as well as device-related malfunction and air embolism. Moreover, a systemic inflammatory response syndrome (SIRS) resulting in multi-organ damage is well described both clinically and in experimental studies[8,9]. Neurological complications such as brain infarction are also frequently reported in patients undergoing long-term ECMO therapy. To confuse matters, it is often difficult to distinguish whether complications are caused by ECMO itself or arise from the underlying disorders accompanying acute or end-stage diseases.

To specifically study the effects of ECMO on a healthy organism, a reliable experimental animal model must be established. There are only very few reports regarding performing ECMO on small animals and they all are limited to rats. To date, no mouse model of ECMO has been described in the literature. Due to the availability of a huge number of genetically modified mouse strains, having a mouse ECMO models would allow the molecular mechanisms involved in ECMO-related complications to be investigated[10,11].

Based on our previously described murine model of cardiopulmonary bypass (CPB)[12], we have developed a stable method of veno-venous ECMO in non-intubated, spontaneously breathing mice. The ECMO circuit (**Figure 1**) containing outflow and inflow cannulas, a peristaltic pump, oxygenator, and air-trapping reservoir is similar to our previously described model of murine CPB[12] with the exception of having a smaller priming volume (0.5 mL). This protocol demonstrates the detailed techniques, physiological monitoring, and blood gas analysis involved in a successful ECMO procedure.

PROTOCOL: (Instructions)

Experiments were performed on male C57BL/6 mice, aged 12 weeks. This study was conducted

in compliance with guidelines of the German Animal Law under Protocol TSA 16/2250.

1. Materials preparation

All steps are performed under clean, non-sterile conditions.

1.1. Introduce 3 fenestrations into a 2 Fr polyurethane tube using a surgical blade under a microscope using 16x magnification.

Note: All fenestrations must be located in distal third of cannula to ensure optimal blood drainage.

1.2. Prepare the priming solution (**Table 1**). Include 30 IU/mL heparin and 2.5% v/v of an 8.4% solution of $NaHCO_3$. Refrigerate this solution at 4 ºC until ready to use. Prime the circuit with 500 uL of priming solution.

1.3. Place the outflow cannula into the priming solution and fill the ECMO machine by switching on the peristaltic pump. Continue to circulate the priming solution through the machine for the next 30 min at a flow rate of 1 mL/min.

1.4. Give 0.5 L/min of 100% oxygen to the oxygenator.

2. Anesthesia

2.1. Place the animal in an induction chamber filled with 2.5% v/v isoflurane/oxygen mixture. Provide 0.5 L/min of 100% oxygen to the vaporizer. Before surgery, check that full anesthesia is achieved by testing pedal withdrawal and pain reflexes. Apply eye gel to prevent drying damage.

2.2. Use a warming pad to maintain the body temperature at 37 ^{o}C.

2.3. Perform inhalation mask anesthesia using an isoflurane vaporizer and inject 5 mg/kg carprofen subcutaneously.

2.4. Regularly observe the spontaneous breathing and adjust the concentration of isoflurane so that it is between 1.3 to 2.5%.

3. Surgery

3.1. Expose the left jugular vein by using a lateral skin incision of 4 mm with the help of fine scissors on the left side of the neck. Together with sharp and blunt preparation using micro-forceps as well as cotton swabs, use bipolar coagulation of the small vessels.

3.2. Once the left jugular vein is exposed, ligate the distal part using an 8-0 silk suture with the

help of micro-forceps.

3.3. Place a slip knot at the proximal end of the vein. Incise the anterior wall of the vein using micro-scissors.

3.4. To achieve full heparinization, inject 2.5 IU/g heparin into the jugular vein via 26 G braunula.

3.5. Raise the head side of the animal pad by 30° to avoid excessive blood loss from the vein during insertion of the cannula.

3.6. Insert a 2 Fr polyurethane (PU) cannula into the proximal part of the jugular vein, rotating it slightly while pushing it to a depth of 4 cm. In doing so, the iliac bifurcation of inferior vena cava (IVC) will be reached.

3.7. Secure the cannula with 8-0 silk knots using microforceps.

3.8. Now expose the right jugular vein using the steps described in 3.1, 3.2, and 3.3.

3.9. Cannulate the right jugular vein with a 1 Fr PU cannula and gently move it 5 mm in the direction of right atrium.

3.10. Repeat step 3.7.

3.11. Catheterize the left femoral artery with another 1 Fr PU cannula and use it for invasive pressure monitoring as well as blood sampling for blood gas analysis (BGA).

3.12. Insert electrocardiogram (ECG) needles connected to a data acquisition device subcutaneously into both forelimbs and into the left thoracic wall.

3.13. Insert a rectal thermometer connected to a data acquisition device.

4. Veno-venous extracorporeal membrane oxygenation and blood gas analysis

Note: For a schematic of the complete ECMO circuit, see **Figure 1**.

4.1. Initiate ECMO on the animal by turning on the pump with an initial flow rate of 0.1 mL/min. Adjust the flow rate of the pump within the next 2 min to 3 – 5 mL/min.

4.2. In case of air-suction in the outflow cannula via the cannulation site, reduce the flow and add 0.1 mL of priming solution to the circuit via air-trapping reservoir.

4.3. Under stable flow, continue to monitor in real-time mode all vital parameters via the data acquisition device.

4.4. Constantly observe backflow from the venous drainage and monitor the level of the blood in the air-trapper reservoir.

4.5. Collect any blood leaking from wounds into a 1 cc syringe with the tip of a 24 G brannula and return it to the ECMO circuit via the air-trapping reservoir.

4.6. For BGA, use a blood sampling cartridge to collect approximately 75 µL of arterial blood at the following time points and from the following locations:
i) 10 min after the initiation of ECMO, collect blood from the IVC before the oxygenator, directly after oxygenator (control), and from the femoral artery
ii) 30 min after the initiation of ECMO, collect blood from the femoral artery

4.7. Give an extra 0.1 mL of priming solution to compensate for intravasal liquid loss every 45 min via the air-trapper or femoral artery catheter or by sucking the air bubbles through the blood draining cannula.

4.8. For BGA, use a blood sampling cartridge to collect approximately 75 µL of arterial blood:
i) 1 h after the initiation of ECMO from the femoral artery
ii) 2 h after the initiation of ECMO from the oxygenator, IVC, and the femoral artery

4.9. After 2 h, reduce the flow rate on the pump gradually (over the course of 5 min), thereby stopping ECMO.

4.9. Continue to record vital parameters for a further 10 min.

4.10. Finish the experiment by exsanguinating the animal and harvest blood and organs.

REPRESENTATIVE RESULTS:

This protocol describes the method of veno-venous ECMO in a mouse. This model is reliable and reproducible, and in comparison to our previously-described model of CPB with respiratory and circulatory arrest[12,13], was less technically demanding to establish.

ECMO flow in the venous system was maintained between 1.5 – 5 mL/min. The mean arterial pressure was kept between 70 and 85 mmHg by adding extra priming solution into the ECMO circuit. Usually, the adding of 0.1 mL of priming solution to the circuit during ECMO allows substituting of blood volume. All volume substituting or buffering solutions were given via the femoral artery or air-trapping reservoir.

Physiological parameters were recorded every 10 min and data from a representative ECMO experiment are presented in **Figure 2**. BGA data from a successful ECMO are shown in **Table 1**.

Hematological parameters showed relevant hemodilution during ECMO (**Table 1**). However, no

blood transfusion was necessary to compensate for moderate anemia (**Table 1**). Oxygenation parameters from BGA demonstrated proper performance of the oxygenator at an oxygen/air mixture at FiO_2 1.0 (**Table 1**).

Metabolic changes during ECMO showed respiratory alkalosis (**Table 1**) at the start and moderate acidosis at the end of the experiment (**Table 1**). No extra buffering of the blood was performed.

FIGURE AND TABLE LEGENDS:

Figure 1: ECMO layout in a mouse. Blood is drained from the inferior vena cava (IVC) via the left jugular vein and oxygenated blood is pumped into the superior vena cava (SVC) via the right jugular vein.

Figure 2: Physiological parameters measured during 2 h of ECMO. A - Heart rate, B - Mean arterial pressure (VS – volume substitution) and C - Rectal temperature.

Table 1: BGA results over the course of the experiment. O - oxygenator, FA - femoral artery, IVC – inferior vena cava.

DISCUSSION:

Previously, we described a successful model of CPB in a mouse[12,13]. To implement such a model for acute or end-stage lung disorders we developed an easy to use veno-venous ECMO circuit for mice. Different to the CPB model, veno-venous ECMO does not require complicated surgical procedures such as sternotomy and clamping of the aorta, thus reducing the risk of wound bleeding in a fully heparinized animal. To avoid embolization of the oxygenator with blood clots, 2.5 IU of heparin/kg is given to each animal. This dose was based on previous measurements of the activated clotting time (ACT) that showed full anticoagulation of the blood (ACT > 800 sec). Due to the absence of heparin coating in the micro-oxygenator, our anticoagulation protocol was kept similar to our CPB procedure.

In comparison to the CPB circuit, we could reduce the overall priming volume to 0.5 mL by reducing the volume of the air-trapper and micro-oxygenator. Moreover, a slower flow was necessary to keep adequate oxygenation of the animal. Intravasal loss of the blood volume resulted in a gradual drop of mean arterial pressure. Adding an extra 0.1 mL of priming volume to the animal lead to an increase in blood pressure over 20 mmHg but a small linear reduction in arterial pressure over the next 30 min was always present. Volume substitution was called for if air was sucked through drainage cannula or there was a drop in blood pressure below 75 mmHg.

The most difficult challenge in the surgical procedure for mouse ECMO model is the placement of the cannula via the left jugular vein into the IVC. To establish this method, different types of cannulas were tested and a laparotomy was performed in mouse cadavers to perfect

positioning of the tip of the cannula in the IVC just before the iliac bifurcation. Sometimes, in bigger animals, placement of the cannula can lead to dislocation of the cannula into the right kidney vein. Nevertheless, the whole blood from all segments of the IVC could be well drained due to side fenestrations of the cannula.

In preliminary trials, we performed cannulation via the femoral vein. Unfortunately, only a 1 Fr cannula can be placed into the femoral vein which results in inadequate blood flow (≤1 mL/min). 1 Fr catheters pushed into the IVC all displayed insufficient backflow. To achieve substantial backflow, both femoral veins would need to be cannulated. Therefore, we abandoned this procedure and achieved adequate draining via a 2 Fr cannula placed in the IVC via the jugular vein. Blood loss during placement of the cannula into jugular vein is very typical. Therefore, before placement, the head end of the animal pad is raised 30-40°. Thus, backflow from the vein is significantly reduced.

A gradual reduction in hemoglobin and hematocrit is explained by hemolysis and repetitive blood samplings taken to demonstrate the performance of the device. For survival experiments, to avoid blood transfusions, blood sampling should be extremely limited or even avoided. Moreover, at the end of the experiment, the blood from the ECMO circuit should be returned into the animal. However, survivability of the model has to be studied in a separate project using a less invasive protocol.

Blood flow during our ECMO runs was between 3 – 5 mL/min. Normal mouse cardiac output is reported to be between 6 to 9 ml/min. Therefore, on average, we were able to achieve an ECMO flow of 54% of the mouse's cardiac output. Usually, veno-venous ECMO requires lower blood flow in comparison to veno-arterial ECMO as overperfusion of the right atrium can lead to right ventricular overload and consequently, heart failure. Clinically, to achieve adequate oxygenation, a veno-venous ECMO flow of 50 to 75% of patients' cardiac output is enough to provide sufficient oxygenation in ventilated or spontaneously breathing patients. Unnecessarily increasing the ECMO flow could lead to more damage caused by SIRS and hemolysis and useless recirculation of the major part of the venous blood between the IVC and SVC. Moreover, we have observed that by increasing of the flow in the veno-venous ECMO, excessive negative pressure leads to air suction at the site of cannulation. Moreover, our animals received 100% oxygen under isoflurane anesthesia, and therefore, with the help of veno-venous ECMO, were hyper-oxygenated. In our model we have tried to reproduce conditions of "awake ECMO"[4] having less damage to lungs.

The molecular mechanisms involved in ECMO-related complications can now be investigated due to the plethora of genetically modified mouse strains available. There are also over eighty strains of mice having lung disorders that would simulate ECMO in the context of these underlying diseases. Therefore, we believe that our veno-venous ECMO mouse model could be implemented in multiple synergistic projects.

ACKNOWLEDGMENTS:

This project was supported by KFO 311 Grant from Deutsche Forschungsgemeinschaft.

DISCLOSURES:

The authors have nothing to disclose.

REFERENCES: (Instructions)

1. Hill, J. D. *et al.* Prolonged Extracorporeal Oxygenation for Acute Post-Traumatic Respiratory Failure (Shock-Lung Syndrome). *New England Journal of Medicine.* **286**(12), 629–634 (1972).
2. Noah, M. A. *et al.* Referral to an Extracorporeal Membrane Oxygenation Center and Mortality Among Patients With Severe 2009 Influenza A(H1N1). *Journal of the American Medical Association*. **306**(15), 1659 (2011).
3. Maslach-Hubbard, A. & Bratton, S. L. Extracorporeal membrane oxygenation for pediatric respiratory failure: History, development and current status. *World Journal of Critical. Care Medicine.* **2**(4), 29–39 (2013).
4. Langer, T. *et al.* "Awake" extracorporeal membrane oxygenation (ECMO): pathophysiology, technical considerations, and clinical pioneering. *Critical Care*. **20**(1), 150 (2016).
5. Esper, S. A. Extracorporeal Membrane Oxygenation. *Advances in Anesthesia.* **35**(1), 119–143 (2017).
6. Millar, J. E., Fanning, J. P., McDonald, C. I., McAuley, D. F. & Fraser, J. F. The inflammatory response to extracorporeal membrane oxygenation (ECMO): a review of the pathophysiology. *Critical Care.* **20**(1), 387 (2016).
7. Lubnow, M. *et al.* Technical complications during veno-venous extracorporeal membrane oxygenation and their relevance predicting a system-exchange--retrospective analysis of 265 cases. *PLoS One.* **9**(12), e112316 (2014).
8. Passmore, M. R. *et al.* Inflammation and lung injury in an ovine model of extracorporeal membrane oxygenation support. *American Journal of Physiology - Lung Cellular and Molecular Physiology.* **311**(6):L1202-L1212 (2016).
9. Vaquer, S., de Haro, C., Peruga, P., Oliva, J. C. & Artigas, A. Systematic review and meta-analysis of complications and mortality of veno-venous extracorporeal membrane oxygenation for refractory acute respiratory distress syndrome. *Annals of Intensive Care.* **7**(1), 51 (2017).
10. Houser, S. R. *et al.* Animal Models of Heart Failure A Scientific Statement From the American Heart Association. *Circulation Research.* **111**(1), 131–150 (2012).
11. Russell, J. C. & Proctor, S. D. Small animal models of cardiovascular disease: tools for the study of the roles of metabolic syndrome, dyslipidemia, and atherosclerosis. *Cardiovascular Pathology.* **15**(6), 318–330 (2006).
12. Madrahimov, N. *et al.* Novel mouse model of cardiopulmonary bypass. *European Journal of Cardio-thoracic Surgery.* **53**(1):186-193 (2017).
13. Madrahimov, N. *et al.* Cardiopulmonary Bypass in a Mouse Model: A Novel Approach *J. Journal of Visualized Experiments.* **127**, doi: 10.3791/56017 (2017).

397

Movie script for: **Technique of veno-venous extracorporeal membrane oxygenation in a mouse**

Here we present a protocol describing the technique of veno-venous extracorporeal membrane oxygenation in a non-intubated, spontaneously breathing mouse. This murine model of ECMO can be effectively implemented in experimental studies of acute and end-stage lung diseases.

1. Preparation

Prepare the priming solution (**Table 1**). Include 30 IU/mL heparin and 2.5% v/v of an 8.4% solution of $NaHCO_3$. Refrigerate this solution at 4 ºC until ready to use. Prime the circuit with 500 uL of priming solution.

Place the outflow cannula into the priming solution and fill the ECMO machine by switching on the peristaltic pump. Continue to circulate the priming solution through the machine for the next 30 min at a flow rate of 1 mL/min.

Give 0.5 L/min of 100% oxygen to the oxygenator.

2. Anesthesia

Place the animal in an induction chamber filled with 2.5% v/v isoflurane/oxygen mixture. Provide 0.5 L/min of 100% oxygen to the vaporizer.

Use a warming pad to maintain the body temperature at 37 ºC.

Perform inhalation mask anesthesia using an isoflurane vaporizer and inject 5 mg/kg carprofen subcutaneously.

Regularly observe the spontaneous breathing and adjust the concentration of isoflurane so that it is between 1.3 to 2.5%.

3. Surgery

Expose the left jugular vein.

Once the left jugular vein is exposed, ligate the distal part using an 8-0 silk suture with the help of micro-forceps.

Insert a 2 Fr polyurethane (PU) cannula into the proximal part of the jugular vein, rotating it slightly while pushing it to a depth of 4 cm. In doing so, the iliac bifurcation of inferior vena cava (IVC) will be reached.

Secure the cannula with 8-0 silk knots using microforceps.

Now expose the right jugular vein.

Cannulate the right jugular vein with a 1 Fr PU cannula and gently move it 5 mm in the direction of right atrium.

Catheterize the left femoral artery with another 1 Fr PU cannula and use it for invasive pressure monitoring as well as blood sampling for blood gas analysis.

Insert electrocardiogram needles connected to a data acquisition device subcutaneously into both forelimbs and into the left thoracic wall.

Insert a rectal thermometer connected to a data acquisition device.

4. Veno-venous extracorporeal membrane oxygenation and blood gas analysis

Initiate ECMO on the animal by turning on the pump with an initial flow rate of 0.1 mL/min. Adjust the flow rate of the pump within the next 2 min to 3 – 5 mL/min.

Under stable flow, continue to monitor in real-time mode all vital parameters via the data acquisition device.

Collect any blood leaking from wounds and return it to the ECMO circuit via the air-trapping reservoir.

For BGA, use a blood sampling cartridge to collect approximately 75 µL of arterial blood at the following time points and from the following locations:

- 10 min after the initiation of ECMO, collect blood from the IVC before the oxygenator, directly after oxygenator (control), and from the femoral artery
- 30 min after the initiation of ECMO, collect blood from the femoral artery
- 1 h after the initiation of ECMO from the femoral artery
- 2 h after the initiation of ECMO from the oxygenator, IVC, and the femoral artery

After 2 h, reduce the flow rate on the pump gradually (over the course of 5 min), thereby stopping ECMO.

Physiological parameters were recorded every 10 min and data from a representative ECMO experiment are presented in **Figure 2**. BGA data from a successful ECMO are shown in **Table 1**.