Int J Clin Exp Pathol 2016;9(10):10796-10802
www.ijcep.com /ISSN:1936-2625/IJCEP0034938

Original Article

Extracorporeal membrane oxygenation (EMCO) is an optimal method to cure the pneumonia caused by endotoxin in mice

Qirong Du, Yong Shen, Jian Yu, Siping Huang, Shuming Pan

Department of Emergency, Xinhua Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China

Received July 3, 2016; Accepted July 18, 2016; Epub October 1, 2016; Published October 15, 2016

Abstract: Pneumonia is the biggest single cause of childhood deaths under the age of five years in developing countries. Extracorporeal membrane oxygenation (ECMO) is a valuable therapeutic option for patients with acute lung failure. In our study, ECMO and mechanical ventilation treatment was used for successful resuscitation following LPS. The results suggested that ECMO treatment was the priority selection. The results of histopathologic features, Western blot and physiological measures all suggested that ECMO treatment can effective recover the lung damage caused by LPS treatments in rats. Based on our results, we were able to show that ECMO allows for return of cardiac function and serves as the basis for subsequent investigation.

Keywords: Pneumonia, ECMO, LPS, rat

Introduction

Pneumonia is the biggest single cause of childhood deaths under the age of five years in developing countries [1]. Globally there are more than nine million deaths among the under-five population each year, of which about three million are due to pneumonia [2]. Of these deaths, 90-95% occurs in developing countries [3]. The success of the fourth United Nations Millennium Development Goal 4 (MDG 4), which aims to reduce child mortality by two-thirds by 2015, will therefore depend in no small part on a reduction of this enormous burden of child deaths from acute respiratory infection. Pneumonia is usually caused by infection with viruses or bacteria and less commonly by other microorganisms, certain medications and conditions such as autoimmune diseases. Risk factors include other lung diseases such as cystic fibrosis, COPD, and asthma, diabetes, heart failure, a history of smoking, a poor ability to cough such as following stroke, or a weak immune system. Diagnosis is often based on the symptoms and physical examination. Chest X-ray, blood tests, and culture of the sputum may help confirm the diagnosis. The disease may be classified by where it was acquired with community, hospital, or health care associated pneumonia [4].

Extracorporeal membrane oxygenation (ECMO) is a valuable therapeutic option for patients with acute lung failure [5]. Meanwhile, ECMO is also the method for supporting patients with severe adult respiratory distress syndrome (ARDS) refractory to mechanical ventilation [6, 7]. ECMO has been used in neonates and children with satisfactory outcomes [8, 9]. During the 2009 H1N1 influenza A pandemic, the use of venovenous (VV) ECMO represented a successful rescue treatment for acute respiratory distress syndrome (ARDS) in patients failing conventional ventilation techniques [10].

Currently, the decision to start ECMO is based on commonly used pulmonary scores assessing the severity of respiratory failure, such as Murray's acute lung injury score and the oxygenation index. A Murray score >3 was used for enrollment and randomization in the "Conventional ventilation versus ECMO for Severe Adult Respiratory failure" (CESAR) Trial [5], as it identifies severely hypoxemic patients failing protective mechanical ventilation with an estimated mortality risk higher than 50% in comparison to conventional treatment. Oxygenation failure, however, is rarely the direct cause of death in ECMO patients. On the contrary, a poor out-

come is more likely to be determined by the presence of complications [11]. Besides bleeding complications, most directly linked to the procedure itself, the most common causes of death are related to non-protective mechanical ventilation or to infectious or non-infectious inflammation [12], leading to various degrees of organ dysfunction. The current study represents a comprehensive evaluation of ECMO method in ARDS and will provide the necessary background information in further research on ECMO.

Method and materials

Animals

All of the animals were maintained in the Division of Animal Resources at Xinhua hospital, Shanghai jiaotong university school of medicine, an Assessment and Accreditation of Laboratory Animal Care-approved facility. All animal protocols were reviewed and approved by the Institutional Animal Care and Use Committee. Forty 250-350 g male Sprague-Dawley rats were originally sourced from Shanghai jiaotong university for use in the experiments. Rats were maintained in temperature-controlled (20°C to 22°C) cages with a 12-hour light-dark cycle, and received free access to sterilized water and standard rodent chow (Rodent diet BK002P, B & K Ltd).

LPS-induced lung injury

Forty mice were randomly divided into four subgroups, each subgroup contains ten mice: control subgroup: treated with normal saline; Model, mechanical ventilation and extracorporeal membrane oxygenation (ECMO) subgroups: mice were inoculated intraperitoneally with 1 mg/kg endotoxin (LPS) prepared from Escherichia coli 0111:B6 (Sigma, St. Louis, MO). Briefly, recipient animals were anesthetized by isoflurane inhalation. While anesthetized, endotoxin dissolved in PBS or an equal volume of PBS was injected intraperitoneally. After 24 h of the treatments, mice in Model subgroup were treated without any means, ECMO and mechanical ventilation subgroup were treated with ECMO and mechanical ventilation. Meanwhile, Respiratory rate (Number/min), Arterial pressure (mmHg) and PaO2 (mmHg) of the mice in each subgroup were measured with PiCCO Plus monitor according to the operator's manual. Animals were killed at 48 h after endotoxin. Lungs were harvested for histological analysis and determination of wet-dry ratio. This study was approved by the Institutional Animal Ethics Committee of Institute of Xinhua hospital, Shanghai jiaotong university school of medicine.

Histopathology

Haematoxylin Eosin (H&E) staining were prepared according to the previous method [22]. Samples were fixed in 10% buffered formalin, and embedded in paraffin. Three to five micrometer thick sections were stained with hematoxylin (Sigma H 3136) for 10 min and with eosin (Sigma E 4382) for 1 min to establish the diagnosis areas. The rest of all samples was immediately frozen by immersing into liquid nitrogen, and then stored at -80°C until further processing.

Western blot analysis

Total cellular protein in four different treatments was isolated by the addition of 1% PMSF and RIPA lysis buffer (50 mM Tris-HCl (pH 7.4), 150 m MNaCl, 1% NP-40, 0.1% SDS). After boiled with SDS-PAGE sample buffer for 5 min, the samples were performed for sodium dodecylsulfate-polyacrylamide gel electrophoresis. Then the proteins were transferred onto a polyvinylidene difluoride membrane (Millipore, USA). After being blocked for 1 h at room temperature, the membrane was incubated with a 1:1000 dilution of rabbit polyclonal anti-mouse p38, p-p38, JNK, p-JNK, ERK, p-ERK and GAPDH (ABGENT, USA) overnight. Before detected with an ECL chemiluminescence detection kit (Advansta, USA), proteins were incubated with the corresponding secondary antibody for 1 h at room temperature. The bands were obtained by GeneGnome 5 (Synoptics Ltd., UK).

Data analysis

Data were subjected to the Kolmogorov-Smirnov test to determine distribution. Descriptive variables are presented as means ± SD and compared with the t-test. When comparing multiple groups data was analysed by analysis of variance with Bonferroni post-test for multiple comparison of parametric data. Estimations are presented with 95% confidence intervals. Conventional levels of significance (0.05) were applied throughout. Statistical analysis was undertaken using SPSS for windows version 19

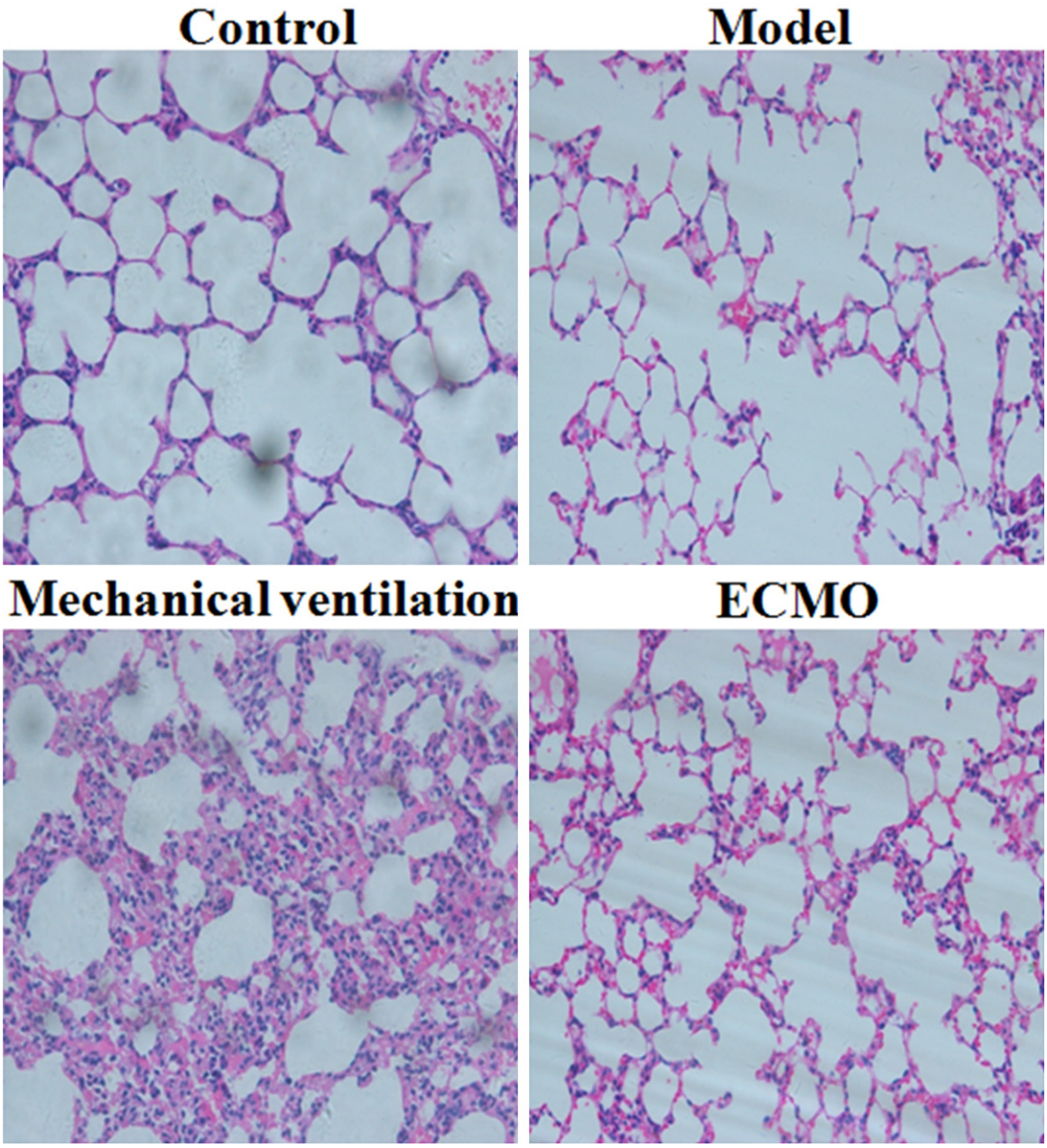

Figure 1. HE staining of rat lung tissues in four different treatments. In Model subgroup, endotoxin treatment resulted in diffusive alveolar damage represented by alveolar bleeding, atelectasis, bronchiole epithelial desquamation, and leukocyte sequestration compared with Control subgroup. In mechanical ventilation, there was prominent infiltration of neutrophils and bronchiole epithelial desquamation whereas ECMO groups had moderate changes compared with Control subgroup (HE × 200).

(Chicago, Ill) and S Plus version 6 (Seattle, Wash).

Results

General conditions of the animals

Rats were maintained in temperature-controlled (20°C to 22°C) cages with a 12-hour light-dark cycle, and received free access to sterilized water and standard rodent chow (Rodent diet BK002P, B & K Ltd). LPS infusion after 24 h, all the animals survived the whole experiment. In general, the animals were able to drink, eat and move.

Histopathologic features (in vivo)

Figure 1 showed the representative appearances of histopathological (H&E) staining of the

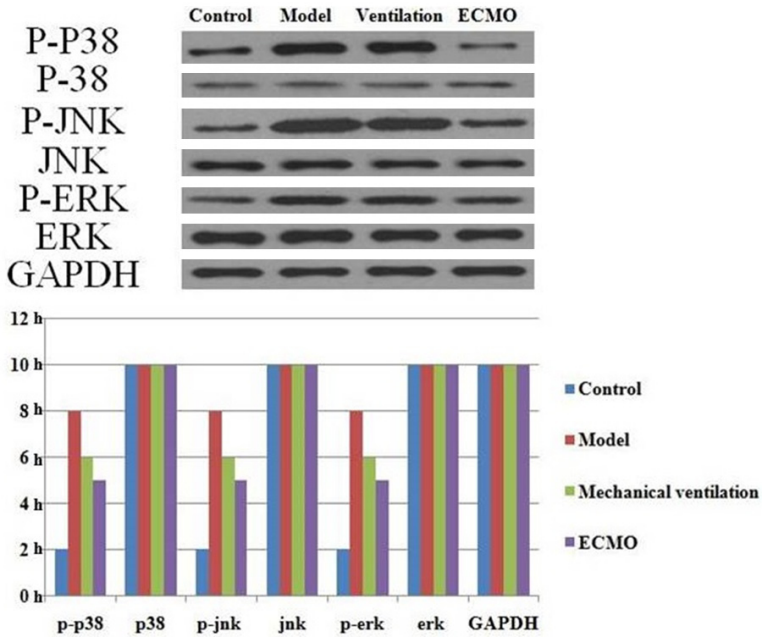

Figure 2. Western blot was conducted to detect the expression levels of p38, p-p38, JNK, p-JNK, ERK, p-ERK and GAPDH in lung tissues of four different subgroups.

mice lung tissue with four different treatments. Haematoxylin Eosin (H&E) staining was used to assess the damage status of pulmonary alveoli in four different subgroups (**Figure 1**). There were no significant differences in relative density of collagen and elastic fiber staining of the lung sections among all the groups. At 24 h, animals in Model, Mechanical ventilation and EMCO subgroups infused with LPS were sacrificed. Their lungs showed diffusive alveolar damage represented by alveolar bleeding, atelectasis, leukocyte sequestration, bronchiole epithelial desquamation, and perivascular edema. However, Model subgroup had the most serious lung damage compared with the Control subgroup. Mechanical ventilation owed intermediate to severe neutrophil infiltration compared with Control subgroup. In contrast, animal lungs in EMCO subgroups had moderate changes. The results indicated that LPS treatments can effective cause harm to pulmonary alveoli. Mechanical ventilation and EMCO treatments can recover the damage that LPS caused. However, EMCO treatments were more effective method to recover the damage that LPS caused than Mechanical ventilation treatments.

West blot analysis of key factors of lung damage

Three key factors of lung damage (p38, p-p38, JNK, p-JNK, ERK, p-ERK and GAPDH) were selected to study the status of their expressions in four different treatments [13]. Cell extracts were analyzed with Western blotting using phosphorylated p38 (p-p38), total p38; phosphorylated JNK (p-JNK), total JNK; phosphorylated ERK (p-ERK) and total ERK. GAPDH served as the loading control. Western blot analysis showed that LPS treatment caused a significant up-regulated protein expression of the activated key factors with lung damage (p-p38, p-JNK and p-ERK) compared with Control group (**Figure 2**). In summary, LPS treatment had none of influence on the protein expression of the total p38, total JNK and total ERK. However, LPS treatment could lead to the significantly up-regulated of the protein expression of p-p38, p-JNK and p-ERK, which were practically useful in biological function. Meanwhile, the expression patterns of p-p38, p-JNK and p-ERK with LPS treatments were similar. For example, the expression level of p-p38 in Model subgroup was the highest and the expression level of p-p38 in ECMO subgroup was the lowest. The results suggested ECMO treatment can effective resist the high expression of p-p38.

Physiological measures of four subgroups

Figure 3 showed the physiological measures of four subgroups. **Figure 3A** indicated that respiratory rate of Model subgroup was significant up-regulated, and the respiratory rate of mechanical ventilation subgroup was significant down-regulated compared with Control subgroup. However, the respiratory rate of ECMO subgroup was similar with Control subgroup. **Figure 3B** indicated that arterial pressure of Model subgroup and mechanical ventilation subgroup was significant down-regulated compared with Control subgroup. Moreover, the arterial pres-

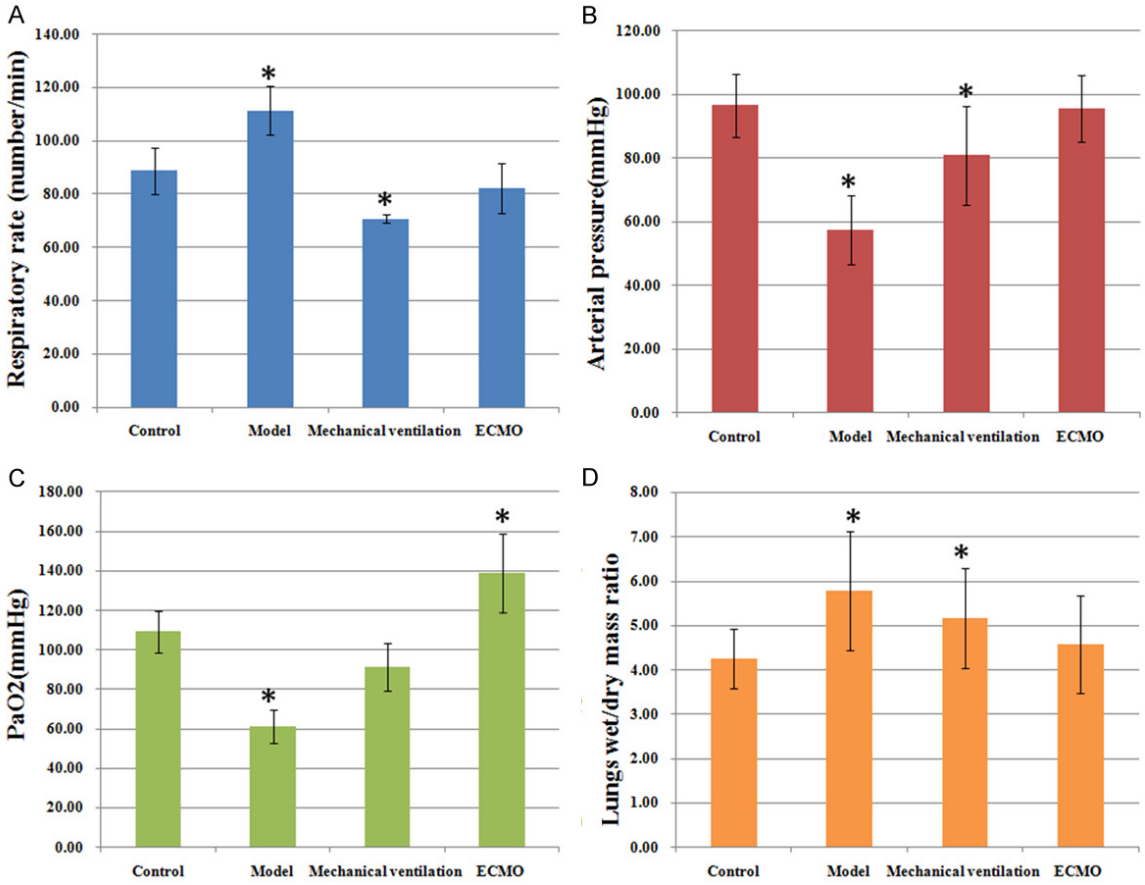

Figure 3. Respiratory rate, arterial pressure, PaO2 and lung W/D mass ration of four subgroups after 24 h treatment. A: Respiratory rate; B: Arterial pressure; C: PaO2; D: Lung W/D mass ration. Values are means ± SD. Statistical significance: A *P* Value of less than 0.05 was considered significant for both tests.

sure of ECMO subgroup was similar with Control subgroup. **Figure 3C** indicated that PaO2 of Model subgroup was significant down-regulated, and PaO2 of ECMO subgroup was significant up-regulated compared with Control subgroup. The PaO2 of mechanical ventilation subgroup was similar with Control subgroup. **Figure 3D** indicated that lung W/D mass ration of Model subgroup and mechanical ventilation subgroup was significant up-regulated compared with Control subgroup. The lung W/D mass ration of ECMO subgroup was similar with Control subgroup. The results mentioned above indicated that physiological measures in ECMO subgroup were similar with Control subgroup, which suggested the ECMO can obtain a good effect after LPS treatments. Mechanical ventilation subgroup can also provide improvements in physiological measures after LPS treatment. However, Mechanical ventilation subgroup was more inferior than ECMO treatment.

Discussion

ECMO has been considered an effective means of therapy for the severe ARDS patients, and the efficiency rate has been between 53 and 76% [14]. Well-functioning rat models of cardiopulmonary bypass have been established, including recent examples that do not require priming with blood [15]. While these models are helpful, ECMO is fundamentally different than cardiopulmonary bypass in its clinical applications. The full bypass circuit is not applicable to the patient who needs long-term cardiopulmonary support. ECMO is applicable to a broad range of clinical scenarios, including bedside cannulation for support during instances of hypoxic cardiac arrest [16]. A rabbit model of ECMO has recently been described to test these applications [17]. Similar to the rabbit model, rats have a nearly identical anatomy to humans with the advantage of being smaller, less expensive, and easier to handle than larg-

er animal models. A small animal model for ECMO is necessary because it provides an efficient, economical, and accurate model for the study of physiologic changes during ECMO. The estimated cost of equipping a laboratory with a basic micro-surgical setup such as dissecting microscope, small animal ventilator, and recover instruments is approximately $3000. The costs specifically related to the ECMO circuit include micro-oxygenators ($250 each/4-6 uses), Tygon tubing ($200), intravenous cannula ($200) and the micro-peristaltic pump ($2400). In our model, nearly all equipment is reusable and the cost incurred per experiment is largely related to the purchase and boarding costs of the rat.

Moreover, some authors have reported high-complication rates because of ARDS and multi-organ failure after chest injuries [18]. Mechanical ventilation is required for adequate tissue oxygenation but most likely may increase lung damage by over-distending and rupturing alveoli and by triggering a secondary inflammatory response syndrome that intensifies lung injury [19]. In patients who were unresponsive to treatment strategies, extracorporeal membrane oxygenation (ECMO) could be an option for salvage therapy. Criteria for ECMO treatment are severe hypoxaemia, reduced total thoracic compliance, and bilateral infiltrates on chest radiographs. The Murray lung injury score could be helpful in detecting ARDS. Extracorporeal membrane oxygenation (ECMO) or extracorporeal lung assist can be used for ARDS treatment [20]. Medical technology has changed in the last 10 to 15 years, and new therapy options are being developed. In ARDS cases that are refractory to ECMO therapy, the additive use of high-frequency oscillation ventilation (HFOV) could be a possible treatment option to improve ARDS patients survival rates. High-frequency oscillation ventilation is an alternative type of ventilation used to maintain small tidal volumes that are delivered at high frequencies (3-15 Hz) with an oscillation pump. This form of ventilation satisfies the strategic goal of protective lung ventilation with extremely small tidal volumes (1-4 ml/kg) and constant lung recruitment [21].

In our study, ECMO and mechanical ventilation treatment was used for successful resuscitation following LPS. However, ECMO treatment was the priority selection. The results of histopathologic features, West blot analysis and physiological measures all suggested that ECMO treatment can effective recover the lung damage caused by LPS treatments in rats. Based on our results, we were able to show that ECMO allows for return of cardiac function and serves as the basis for subsequent investigation.

Conclusion

Pneumonia is the biggest single cause of childhood deaths under the age of five years in developing countries. Histopathologic features, West blot analysis and physiological measures showed that ECMO treatments can effective recover the lung damage caused by LPS treatments in rats. The study results may provide a basis for the development of treatment strategies for pneumonia during and/or ECMO therapy in clinical practice.

Acknowledgements

We would like to thank International Trauma Life Support of Children Foundation (No. 2013-SY038) for financial support.

Disclosure of conflict of interest

None.

Address correspondence to: Shuming Pan, Department of Emergency, Xinhua Hospital, Shanghai Jiaotong University School of Medicine, No. 1665 Kongjiang Road, Shanghai 200092, China. Tel: (+86)-021-25076700; E-mail: shumingpan_1@sina.com

References

[1] UNICEF & World Health Organization Pneumonia: the forgotten killer of children. New York: UNICEF/WHO; 2006.

[2] Black RE, Morris SS, Bryce J. Where and why are 10 million children dying every year? Lancet 2003; 361: 2226-2234.

[3] Mulholland K. Global burden of acute respiratory infections in children: implications for interventions. Pediatrics Pulmonology 2003; 36: 469-474.

[4] Torres A, Peetermans WE, Viegi G, Blasi F. Risk factors for community-acquired pneumonia in adults in Europe: a literature review. Thorax 2013; 68: 1057-65.

[5] Peek GJ, Clemens F, Elbourne D, Firmin R, Hardy P, Hibbert C, Killer H, Mugford M, Thalanany M, Tiruvoipati R, Truesdale A, Wilson A. CESAR: conventional ventilator support vs ex-

tracorporeal membrane oxygenation for severe adult respiratory failure. BMC Health Serv Res 2006; 6: 163.
[6] Peek GJ, Moore HM, Moore N, Sosnowski AW, Firmin RK. Extracorporeal membrane oxygenation for adult respiratory failure. Chest 1997; 112: 759-764.
[7] Hemmila MR, Rowe SA, Boules TN, Miskulin J, McGillicuddy JW, Schuerer DJ, Haft JW, Swaniker F, Arbabi S, Hirschl RB, Bartlett RH. Extracorporeal life support for severe acute respiratory distress syndrome in adults. Ann Surg 2004; 240: 595-605.
[8] Campbell BT, Braun TM, Schumacher RE, Bartlett RH, Hirschl RB. Impact of ECMO on neonatal mortality in Michigan (1980-1999). J Pediatr Surg 2003; 38: 290-295.
[9] Carey WA, Colby CE. Extracorporeal membrane oxygenation for the treatment of neonatal respiratory failure. Sem Cardiothorac Vasc Anesth 2009; 13: 192-197.
[10] Davies A, Jones D, Balley M, et al. The Australia New Zealand Extracorporeal Membrane Oxygenation (ANZ ECMO) Influenza Investigators (2009) Extracorporeal Membrane Oxygenation for 2009 Influenza A (H1N1) Acute respiratory distress syndrome. JAMA 2009; 302: 1888-1895.
[11] Brogan TV, Thiagarajan RR, Rycus PT, Bartlett RH, Bratton SL. Extracorporeal membrane oxygenation in adults with severe respiratory failure: a multi-center database. Intensive Care Med 2009; 35: 2105-2114.
[12] Beutel G, Wiesner O, Eder M, Hafer C, Schneider AS, Kielstein JT, Kühn C, Heim A, Ganzenmüller T, Kreipe HH, Haverich A, Tecklenburg A, Ganser A,Welte T, Hoeper MM. Virus-associated hemophagocytic syndrome as a major contributor to death in patients with 2009 influenza A (H1N1) infection. Crit Care 2011; 15: R80.
[13] Davidson B, Konstantinovsky S, Kleinberg L, Nguyen MT, Bassarova A, Kvalheim G, Nesland JM, Reich R. The mitogen-activated protein kinases (MAPK) p38 and JNK are markers of tumor progression in breast carcinoma. Gynecol Oncol 2006; 102: 453-461.
[14] Peek GJ, Mugford M, Tiruvoipati R, Wilson A, Allen E, Thalanany MM, Hibbert CL, Truesdale A, Clemens F, Cooper N, Firmin RK, Elbourne D; CESAR trial collaboration. Efficacy and economic assessment of conventional ventilatory support versus extracorporeal membrane oxygenation for severe adult respiratory failure (CESAR): a multicentre randomised controlled trial. Lancet 2009; 374: 1351-63.
[15] Zhu X, Ji B, Liu J, Sun Y, Wu S, Zheng Z, Long C, Tang Y. Establishment of a novel rat model without blood priming during normothermic cardiopulmonary bypass. Perfusion 2013; 29: 63-69.
[16] Mair P, Hoermann C, Moertl M, Bonatti J, Falbesoner C, Balogh D. Percutaneous venoarterial extracorporeal membrane oxygenation for emergency mechanical circulatory support. Resuscitation 1996; 33: 29-34.
[17] Lu S, Pan S, Wang C, Hu K, Hong T. Establishment of an animal model of extracorporeal membrane oxygenation in rabbits. Perfusion 2012; 27: 414–418.
[18] Johnson JA, Cogbill TH, Winga ER. Determinants of outcome after pulmonary contusion. J Trauma 1986; 26: 695-697.
[19] Dreyfuss D, Saumon G. Experimental changes in the alveolocapillary barrier induced by artificial ventilation. Schweiz Med Wochenschr 1997; 127: 1023-9.
[20] Michaels AJ, Schriener RJ, Kolla S, Awad SS, Rich PB, Reickert C, Younger J, Hirschl RB, Bartlett RH. Extracorporeal life support in pulmonary failure after trauma. J Trauma 1999; 46: 638-45.
[21] Rimensberger PC. ICU cornerstone: high frequency ventilation is here to stay. Crit Care 2003; 7: 342-4.
[22] Chan JK. The wonderful colors of the hematoxylin-eosin stain in diagnostic surgical pathology. Int J Surg Pathol 2014; 22: 12-32.