A novel portable extra-corporeal life support system for the treatment of cardio-pulmonary failure under controlled hypothermia. Preliminary study in experimental animals*

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Summary. Purpose: Several therapeutic options are used in emergency situations, when heart and/or lung functions acutely fail. Because of the poor results of conventional treatments, the use of an extra-corporeal life support (ECLS) systems able to completely assume the heart and lung functions in emergency situations is becoming a viable alternative. We have developed a unique ECLS system for patients needing extended respiratory and/or circulatory support and controlled hypothermia. Methods: The ECLS apparatus is portable and easy to handle by incorporating a disposable centrifugal blood pump, a membrane oxygenator, a waterless heating/cooling device. A sensor’s system is integrated in the equipment for continuous monitoring/displaying of several parameters. Results: The system was tested in bench laboratory studies and in large experimental animals to check feasibility, functionality, durability, consistency and safety. Adult sheep were used, after cannulation of large vessels in the neck, either to test the apparatus in normal physiologic conditions, or under controlled mild hypothermia (33 to 34° C). Two modes of vascular access were tested: veno-arterial (V-A) and veno-venous (V-V). Vital parameters and systolic blood pressures were continuously monitored up to 48 hours. Discussion: Initial laboratory experiment of the He-Art apparatus indicated that the system was safe and able to control and stabilize the hemodynamic conditions. The device represented a second generation ECLS system, by adding the protective effect of moderate to mild hypothermia against ischemic cardiac and brain injuries. (www.actabiomedica.it)

Key words: ECMO, resuscitation, hypothermia

Introduction

Early application of complete cardiopulmonary support to patients in cardio-circulatory shock allows resuscitation and corrective therapy with an acceptable salvage rate (1). Furthermore, post-resuscitation care of cardiac arrest patients using therapeutic hypothermia increased survival and favorable neurological outcome (2). According to the 2010 American Heart Association Guidelines for Cardiopulmonary Resuscitation and Emergency Cardiovascular Care Science, unconscious adult patient with a return of spontaneous circulation (ROSC) after out-of-hospital cardiac arrest should be cooled to 32°-34° C for 12-24 hours (3). However, it is unclear which target temperature is more adequate and for how long the hypothermic

state should be carried on. For this reason, hypothermia is presently still being experimentally investigated as a method to reduce myocardial infarct size (4) and to improve brain function during hypoxia-ischemia as well (5, 6). We have developed a portable ECLS system, namely the He-Art apparatus, that was designed in order to partially replace the circulatory and pulmonary functions in cardiac and/or respiratory decompensation, or by assuming the functions of the heart and lung completely, when needed. Moreover, an heat exchanger was incorporated in the ECLS system to add the protective effect of moderate-to-mild hypothermia against ischemic cardiac and brain injuries following cardiac arrest. In order to test the feasibility and the safety of the He-Art apparatus in controlling and stabilizing the hemodynamic and respiratory conditions as an ECLS system, we performed a pilot study in healthy, large size laboratory animals. The experimental results showed the suitability of this system for all the situations where cardiac and/or respiratory assistance is needed, particularly under controlled mild-to-moderate hypothermia.

Materials and Methods

Animal procedures were approved by the Institutional Animal Care and Use Committee of the University of Modena and Reggio Emilia. General animal husbandry and all the experiments were supervised by the veterinary staff of the Centralized Animal Research Center at the University Hospital in Modena. All the experiments were performed according to the National Guidelines for Animal Experimental Studies of the Italian Ministry of Health. Eight adult female sheep, averaging 50-60 kg b.w., were utilized during the period February-June 2011. Before experiments, sheep were starved for 24 hours with free access to water. The animals were tracheally intubated after general anesthesia induced by i.v. infusion of Tiletamin and Zoletilan (Zoletil®) plus Atropin. Muscle relaxation was obtained by i.v. injection of Pancuronium. Anesthesia was maintained by i.v. infusion of Fentanyl®, Propofol® and Isoflurane. The ventilator rate was adjusted to maintain the end-tidal PCO$_2$ at 30-40 mmHg. An electrocardiogram monitor was attached to the animals, and blood pressure and heart rate were continuously displayed on a screen. Blood gasses were measured with an automatic blood analyzer. Circuit’s blood pressures were monitored by membrane sensors and digital indicators (Druck Ltd, Leichester, England). Circuit blood temperatures were recorded by thermistor probes (Exacon® Medical Temperature Probe, Exacon Scientific A/S, Roskilde, Denmark) and digital thermometer (Omega HH41, Engineering Inc., Stamford, CT, USA). Blood flow rate was monitored by flow probes (Transonic System Inc., Artisan Sci. Co., Champaign, IL, USA). Monitored variables included mean artery blood pressure, ECG, blood gasses (PaO$_2$: oxygen partial pressure in the arterial blood; PvO$_2$: oxygen partial pressure in the venous blood; PaCO$_2$: carbon dioxide partial pressure in the arterial blood; FiO$_2$: measurable percent concentration of oxygen administered; SaO$_2$: hemoglobin oxygen saturation in the arterial blood, in mm Hg), hematocrit and activated clotting time (Medtronic Perfusion Systems, Minneapolis, MN, USA). Heparin (700 IU/kg b.w.) was administered before inserting the blood access cannulas and the targeted ACT value was maintained between 300-450 sec. During the experiments, Heparin was continuously infused i.v. by a pump-syringe, according to the ACT values. In the veno–arterial (V-A) perfusion mode, the right carotid artery and jugular vein were cannulated by two bypass cannulas, 23 Fr and 24 Fr, respectively (Stöckert GmbH, Bonn, Germany). In the veno-venous (V-V) mode, the right and left jugular veins were cannulated by two 24 Fr bypass cannulas; a second catheter was inserted into one jugular vein for the blood drainage from the head, and the two venous drainage catheters on the same neck side were connected with a Y shaped connector. Thermistor probes (Exacon® Medical Temperature Probe) were placed to monitor the rectal temperature. In group I, two sheep were perfused for 6 hours in the V-V mode, in normothermic condition (37.5-38°C); in group II, two sheep were perfused for 12 hours in the V-V mode in normothermic condition (37.5-38°C); in group III, two sheep were perfused for 24 hours in the V-A mode, under controlled hypothermia (33.2°C and 34,5°C); in group IV, two sheep were perfused for 48 hours in the V-A mode, including a period of time under controlled hypothermia (34,5°C). The He-Art apparatus weighted 20 kg and incorporated a complete
tube setting, including the following components: 1) a centrifugal blood pump capable to support a circuit flow rate up to 6 L/min (Bio® Pump, Medtronic™ Inc., Minneapolis, MN, USA); 2) a highly plasma resistant polymethylpentene (PMP) fiber oxygenator (Oxyplus®, Membrana Gmbh, Wuppertal, Germany), designed for extended use; 3) a heat exchanger based on the Peltier effect, with a temperature power ranging 15-39° C; 4) an arterial/venous filter with an innovative coaxial geometry and functionality that prevents the accumulation of air bubbles (Fig. 1). Complete tubing set, including the centrifugal pump, the oxygenator, the heat exchanger and the arterial-venous bubble filter takes altogether a priming volume of 760 ml. A sensor’s system for continuous monitoring/displaying of blood flow rate, pressure, temperature, pH, PaO2, PvO2, PaCO2, SaO2, Hct and Hb, is integrated in the tubing set. The part of the circuit including the pump, the arterial-venous bubble filter and the V-A line is filled in a retrograde way in order to avoid stagnation of air. The remaining parts of the circuit, comprising the heat exchanger and the oxygenator, are designed bottom-inlet/bottom-outlet in order to exploit hydraulic column for a better filling of the entire system. Microbubbles elimination is ensured by seven different entrapment levels throughout the whole extracorporeal circuit, thus enabling the system to run for several days. The venous bubble trap filter was designed by using a double platelet screen filter of 105 μm cut-off coupled with an 800 μm holder filter. The useful surface area for blood filtration was 125 cm². The internal part of the venous bubble air trap filter was designed in a bell-like chamber; in this way the filter configuration was able to collect micro- and macro-gas emboli. A level detector, activated by an alarm, was placed on a flat window around the top of the venous filter section, in order to help in recognizing a potentially dangerous volume of air collected by the system. Setting procedures and operating system are rapidly and easily handled by a touch screen display. A small 1 Kg O₂ tank and a battery pack (60 min autonomy) allows the use of the apparatus in time-critical emergency situations, after connecting the patient by venous and/or arterial cannulas (Fig. 2, 3). During experiments, the following parameters were double checked, by He-Art sensor’s system and displaying apparatus and by control devices (see above) for: a) post-oxygenator blood gasses concentration and pH, b) hemoglobin concentration, c) hematocrit, d) blood temperatures, e) arterial and venous blood pressures and f) flow rate.

![Figure 1](image1.png)

**Figure 1.** The He-Art as an extracorporeal life support system in the veno-arterial perfusion mode. The modular design of the apparatus comprises a sensor’s system for haemoglobin (Hb), hematocrit (Hct), O₂ saturation and blood pressure detection. A venous filter on the inlet (suction) line prevents air bubbles or clots entering the system. The centrifugal pump drives the blood to the heat exchanger and to the oxygenator. On the outlet line, an arterial filter prevents air bubbles by entering into the Patient’s systemic circulation. A sensor’s system for pressure, O₂ and CO₂ partial pressures (PO₂, PCO₂), pH and air bubbles detection, is integrated in the outlet line.
Results

The in vivo experiments were performed in eight adult sheep in which the He-Art system was connected by a V-V or a V-A perfusion mode. As a pre-clinical, safety/feasibility study, it is worth saying that in an experimental model in which the animals are not presupposed to have a picture of circulation and/or respiratory severe failure, the installation of a circuit seeking to assist the heart and/or the lung, found out a summation of effects between the pump and the normal heart, as well as between the oxygenator and the normal lung, making it hard to carry out an accurate analysis of the most part of the hemodynamic and respiratory effects established by the system. In the animals of group I and II, the V-V perfusion mode in a normothermic condition (37.5-38°C) was continued for 6 and 12 hours respectively, to preliminarily assess the blood vessel cannulation technique and to check the safety and feasibility of the whole ECLS perfusion system in vivo. In group III and IV animals, the V-A and V-V perfusion modes were tested for 24 and 48 hours to induce controlled mild hypothermia at 33/34°C; after the hypothermia period, the animals were re-warmed to the physiologic temperature. In preliminary laboratory experiments in vitro, the heat exchanger of the He-Art system showed the capacity to decrease the temperature of 5 L of physiologic saline solution by 5°C in 17 min, i.e. 0.83°C/min, from 37°C to 32°C, and to heat again the same fluid volume at 37°C in 6 min, i.e. 0.5°C/min, at a flow rate ranging 1-1.5 L/min. During the experiments in vivo, the performances of the heat exchanger were tested with acceptable results, although they were different from those obtained in vitro. In group III and IV animals, the body temperature, as recorded by rectal probes, was decreased by 4°C in 150-180 min, from 38/38.5°C to
34.5°C, i.e. 0.027°/0.022°C/min. Warming up of the animals from 34/34.5°C to 39°C was slowly achieved during a period of time ranging 4.30-7 hours, i.e. a temperature increase of 0.015-0.009°C/min (Fig. 4, A), at a flow rate ranging 2.5-2.6 L/min (Table 1). Interestingly, in the controlled hypothermia experiments the heat exchanger was activated already during the circuit priming phase, to cool the perfusion solution before connecting the ECLS system to one of the group III animal. In this way, the 760 mL priming volume was cooled at 15°C, by allowing an immediate decrease of the animal body temperature after starting of the perfusion as soon as the cold saline solution was diluted with the circulating blood. This allowed for a decrease of 2°C in 1 hour, i.e. 0.03°C/min (Fig. 4, B). This peculiarity of the apparatus may have important clinical advantage in case of using the He-Art in asystolic cardiac arrest patients, when controlled hypothermia is needed immediately after cardio-pulmonary resuscitation (see discussion). In this animal, controlled hypothermia at 33.2°C was then maintained for 6 hours and re-warming was slowly achieved during the following 17 hours (Fig. 4, B). The functions of the oxygenator, of the V-A air bubble filter and of the sensor’s system for continuous monitoring of blood flow, pressure, temperature, pH, PaO₂, PvO₂, PaCO₂, Hct and Hb were acceptable during the study periods and in all the experimental groups, by showing the maintenance of acceptable and nearly physiological values up to the end of the study periods (Table 1). Any significant difference in parameter values was recorded in comparison to second external devices of control. The performances of the oxygenator showed a decrease of the gas exchange over time, as demonstrated by a progressive decrease of the PaO₂ and PvO₂ values (Table 1). In all the perfused animals, the mean arterial pressure was lower during the hypothermia periods, in comparison to normothermia ones. In order to maintain the hematocrit at a lower level, in comparison to initial basal values, and keep the animal well hydrated as well, the animals were maintained hemodiluted by i.v. infusion of Ringer solution. The major part of the devices circuit, i.e. tubing set, blood filters, oxygenator, centrifugal pump and heat exchanger, all under evaluation in this study were designed by using DSM Somos Water-Shed® XC 11122 prototyping material, processed to be ISO 10993 and USP Class VI compliant. The mechanical strength of this material was not appropriate and strong enough to support a surface coating process and for this reason only Heparin was used. Heparin was infused as a first initial bolus of 5000 IU/mL/animal, in the systemic circulation, starting 10 min before vessel cannulation. Heparin infusion was then adjusted in order to maintain the ACT values between 200-350 sec. The dose of Heparin administration ranged 1250-1750 IU/h, and was continued by a syringe pump infusion into the carotid artery. In spite of these limitations, only very little amount of clots were detected
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at the end of the procedures and clotting was mainly addressed to the venous filter and the distal tip of the venous cannulas.

**Discussion**

Therapeutic hypothermia and treatment of the underlying cause of cardiac arrest impact survival and neurological outcomes (3). Mild-to-moderate hypothermia after resuscitation from cardiac arrest not only is neuroprotective, but also acts on the cardiovascular system with evidence of a decrease in heart rate and increase in systemic vascular resistance (7). It has been unclear which target temperature is more adequate and for how long the hypothermic state should be carried on in cardiac arrest patients after ROSC. Previous laboratory animal studies indicated that survival in an experimental pig model of prolonged electrically induced cardiac arrest was possible by deep hypothermia with cold saline aortic flush at 16.7°C (8). A study in pigs with ventricular fibrillation showed that none of the 8 animals in the normothermia group achieved ROSC, compared to 3 of the 8 pigs in mild hypothermia (35°C), to 7 of the 8 pigs in moderate hypothermia (33°C; \( p < 0.001 \)) and to 5 of 8 animals in severe hypothermia (30°C; \( p < 0.03 \)), by indicating that when cardiac arrest was induced in the setting of moderate hypothermia, resuscitative measures were facilitated with significantly improved defibrillation success and resuscitation outcome (9). More recently, an ECLS system and a cooling apparatus to induce hypothermia at 30°, 24° and 18°C was tested in pigs after cardiac arrest, to identify the optimal level of hypothermia following defibrillation and intensive care, by showing a trend to better survival in the animals with the least temperature reduction (10). In a prospective study in unconscious adult patients with ROSC after out-of-hospital cardiac arrest, severe hypotension significantly increased when the target temperature reached 32°C, by indicating that the target temperature would be set to 33° or 34°C (11). A clinical paper describing the outcome of 171 out-of-hospital cardiac arrest patients who failed to respond to conventional cardio-pulmonary resuscitation, reported that when hypothermia at 34°C was induced, the rate of favorable neurological outcome was only in function of the time interval between collapse and or cardiopulmonary bypass (12). The traditional devices currently utilized as an heat exchanger in cardiac surgery, ECLS and extracorporeal membrane oxygenator (ECMO) systems, are generally based on external water bath, integrating a refrigerator. This arrangement could not be easily adopted in a compact fully-portable ECLS system. For this reason, a thermoelectric cooling system based on a Peltier cell module, was designed to be integrated in our device. The Peltier effect was discovered in 1834 and was established theoretically in the early 1900’s, but due to the fact that metallic materials were used, the efficiency was limited and not suited to practical applications. It was first applied to electronic cooling in the 1960’s when semiconductor materials became available and electronic cooling elements with a decent level of efficiency could be produced. Compared to standard design using refrigeration cycles with compressors and cooling mediums, the thermoelectric

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Time</th>
<th>( \text{PaO}_2 )</th>
<th>( \text{PvO}_2 )</th>
<th>( \text{PaCO}_2 )</th>
<th>Hct</th>
<th>Hb</th>
<th>pH</th>
<th>SatO2</th>
<th>Blood flow (L/min)</th>
</tr>
</thead>
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<tr>
<td>group I</td>
<td>6 h</td>
<td>575.1</td>
<td>58.0</td>
<td>59.0</td>
<td>29</td>
<td>10.3</td>
<td>7.109</td>
<td>100</td>
<td>2.89</td>
</tr>
<tr>
<td>group II</td>
<td>12 h</td>
<td>557.6</td>
<td>54.5</td>
<td>33.9</td>
<td>21</td>
<td>7.3</td>
<td>7.342</td>
<td>100</td>
<td>2.05</td>
</tr>
<tr>
<td>group III</td>
<td>24 h</td>
<td>539.8</td>
<td>91.4</td>
<td>24.5</td>
<td>20.5</td>
<td>6.8</td>
<td>7.370</td>
<td>100</td>
<td>1.85</td>
</tr>
<tr>
<td>group IV</td>
<td>48 h</td>
<td>390.4</td>
<td>82.4</td>
<td>24.3</td>
<td>19.5</td>
<td>6.5</td>
<td>7.340</td>
<td>100</td>
<td>2.35</td>
</tr>
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cooling shows several advantages including an environment-friendly impact since no cooling medium is used, a compact configuration allowing switching to heating or cooling functions by simply changing the current polarity, a short response time and quickly heats or cools, with no vibrations or noise because of the absence of moving parts, a long-lasting and reliable cooling method because no fatiguing or breaking machine parts are present, and finally an easy maintenance because no worry of coolant gas or corrosive gas leakages (13). A thermoelectric cooler structure can achieve the maximum heat pumping performance. Advantages of Thermoelectric Coolers Thermoelectric coolers have limited amount of moving parts. For that reason, they offer high reliability and low maintenance over other types of cooling or heating devices. Thermoelectric coolers offer low noise performance and are ideal for use in medical offices, laboratories and all other places where noise reduction is a factor. Thermoelectric coolers are ecologically clean - no CFC, HCFC or even HFC types of refrigerants are involved in the process. Applications medical chillers are completely self contained devices, able to serve the purpose of removing heat from the human body. Medical chillers are used: for cooling/heating thermal pads for hypothermia procedures, for cooling human skin during laser surgery, in place of an ice pack anytime chilling is required for a medical procedure, for treating for hypothermia, in burn room to comfort medical personnel, in sports medicine for orthopedic rehabilitation, in many other medical chiller applications, for hair and tattoo removal, for vein treatments, in laser eye surgery. Among the potential clinical uses in an emergency setting, the He-Art apparatus integrating an heat exchanger and an extra-corporeal membrane oxygenator, could also be used to rapidly rewarming of severe hypothermic patients, as those victims of avalanche, drowning, long term exposure to cold, or falling into a crevasse, as it was recently reported in adult (14) and pediatric patients (15). The performances of the oxygenator showed a decrease of the gas exchange over time and this behavior should be put in relation with the condensation of water on the gas side of the oxygenator membrane. The water film on the internal surface of the membrane is affecting the gas transfer and thus influencing the PaO₂ values in the arterial blood. For this reason, during treatment it is advisable to increase the gas flow up to 10 L/minute for few seconds, and to repeat this manoeuvre several time during treatment, in order to flush the gas side of the membrane and to reduce the water film. The main characteristics that were taken into consideration in designing the fiber oxygenator were to generate a gas module without the standard integration of an heat exchanger based on the use of heated or cooled water. In order to simplify the priming phase and to enhance a safe use of the device, a blood pathway design -type bottom inlet/bottom outlet- was preferred by creating a brand new design, and by mixing the two main blood flow motions inside the fiber bundle and the pure cross-flow motion, with a wave like motion flow, by coupling the membrane with special plastic inserts placed inside the fiber bundle. This was helping to have a constant hydraulic section along the blood pathway and to maintain a low pressure drops, with the beneficial effect to have the upper side of the gas module as a big air bubble trap. By using the polyethylpentene membrane, the total useful surface needed to cover the requested gas exchange performances, was set to 1,81 m² (Figure 5). The priming volume of the gas module was about 180 mL while that of the heat exchanger module was 80 ml. The adoption of the Peltier cells technology for the heating/cooling device, allowed us to set the useful exchange area for the disposable components at 225 cm² only, and this was realized by a thin interface of stainless steel plate. In the last decade a wide number of application in extra-corporeal circulation devices showed an unquestionable benefit in the use of functional coating. Coating resulted of utmost importance in improving platelet preservation and in reducing platelet foreign surface adhesion. Adsorption onto surfaces may results in protein denaturation, ultimately leading to activation of the plasma proteolytic system. By thinking to a system that could be used for more than few hours, manufacturing of disposable devices have to take in consideration that all these reactions on polymer surfaces are strictly associated with the immunitary defense system which may affect heart, lungs, brain and other organs. These immunitary reactions are causing dangerous conditions that have been described as the “systemic inflammatory response syndrome”. Products
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Currently present on the market are divided in two big families, which are mainly related to two different surface coating strategies: 1) coating with molecules like the phosphorylcholine; 2) manufacturing with heparin-based coating. In the first case, thanks to the presence of polar groups in the main polymer, there is a phospholipid-like structure that creates a sort of biomembrane on the surface when they come in contact with the blood cell membrane phospholipids. The polymer's physical structure consists in the presence of hydrophilic and hydrophobic parts and in the balanced presence of positive and negative charges. This configuration consents an optimum interface between the polymeric surfaces and the blood. Heparin-based coating consists in more complex procedures that bring to higher costs, and severe requisite for material durability since these procedures are much more aggressive when compared with the previously mentioned kind of coating. Nevertheless, heparin-coated devices show good evidences as less blood product needing (16, 17), less peri-operative blood loss (18), shorter ventilator time (19), shorter hospital staying (16, 17, 18), less postoperative body temperature rise (20), a significantly greater urine output during the extra-corporeal circulation (21) and lower total costs, mainly related to an improvement in the clinical outcome. For these reasons, heparin-coating should be considered as the preferable manufacturing strategy and a basic requisite in case a new system is addressing an extensive use. At this stage of the He-Art project it was not possible to implement an antithrombotic heparin-coating, since the mechanical and physical characteristics of the basic material used in manufacturing the different disposable components of the devices did not permit to apply this technology with sufficient stability. In spite of high levels of ACT we found few clots and fibrin strings in venous filter and in the venous cannulae. For the first time in the field of extra-corporeal circulation, the management of the air embolism in the venous and arterial line, was thus managed by displacing a single device in the A/V lines. The design concept was mainly addressed to simplify the use of this device, named integrated air removal device (IARD) and intended to place all the vented ports in a dedicated portion area of the circuit, in order to be easily managed. The arterial filter was designed with a double platelet screen filter of 40 μm cut-off, coupled with an 800 μm holder filter and with a useful filtration surface area of about 450 cm². In particular, the use of a new high performance fabric with an open area of 40% and an unaffected mesh opening of 40 μm, increased the benefits of the arterial filter. This choice helped us in decreasing the dimension of the device due to the limitation of previously available standards. The unique design obtained by coupling the two different filtration devices helped in saving volume, by obtaining a final total priming volume for the entire device, of only 320 ml. A new, more advanced device configuration is under evaluation in our laboratory, leading to an additional integration of the IARD.

Figure 5. Chart showing the ability of the oxygenator to minimizing the transmembrane pressure drop and showing oxygenator CO₂ and O₂ transfer rate.
with the centrifuge pump. In this case, in order to simplify the set-up of the disposable circuit, the pump will maintain the ability to rotate just in front of the filter connection. The use of the ECLS/ECMO and milder-to-moderate therapeutic hypothermia are gaining acceptance within the emergency medical services community as a standard treatment to improve neurological recovery in out-of-hospital cardiac arrest survivors. A systematic review of the current medical literature indicated that therapeutic hypothermia can be efficiently induced in the pre-hospital environment (22). The unique peculiarities of the new He-Art apparatus, namely lightness, transportability, easy handling, rapid set up and efficient heating/cooling, as it was experimentally tested in this preliminary study, together with further experimental and clinical researches in this area, may help clinicians in handling and solving the many problems that are still related to effectiveness and timing of early therapeutic hypothermia in the out-of-hospital emergency care of cardiac arrest patients. We are aware that our study was only a preliminary experiment and we are not able to get firm conclusion. Nevertheless, our study suggests the potential usefulness of the He-Art system although it is necessary to conduct further studies.

References


Received: 22 April 2015
Accepted: 18 May 2015
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