Myocardial protection influences cardiac surgery results, but myocardial failure remains the leading cause of adverse postoperative events. The aim of the current study is to perform a meta-analysis concerning the effect of myocardial protection methods and cardioplegic solutions upon ischemia-reperfusion injuries in open cardiac surgery. The authors performed a systematic search for published studies on Medline database from inception to 31 January 2016 using relevant keywords. From 383 identified studies, only 7 met the inclusion and exclusion criteria. These studies and the one performed at the Cardiovascular Diseases Institute (Iasi, Romania) compared crystalloid and blood cardioplegia from myocardial protection point of view. Although the efficiency of crystalloid solutions was proved, more cardiac surgeons prefer blood cardioplegia by considering potentially prolonged ischemia, severe hemodilution and the need for blood transfusions. Blood cardioplegia was ameliorated over time by adding several myocardial protection substrates: K+, Mg2+, Ca2+, allopurinol, desferrioxamine, superoxide dismutase, mannitol, polyethylene glycol, lactobionate, histidine, aspartate, pyruvate, Q10 coenzyme, reduced glutathione, nifedipine, insulin, and procaine. Significant progress in the area of myocardial protection reduced the clinical consequences of myocardial aggression under ECC. Further reduction of morbidity and mortality requires an optimization of existent techniques, where blood cardioplegia should have a prominent place.

Keywords: hyperkalemic solutions, superoxide dismutase, isoflurane, crystalloid cardioplegia, blood cardioplegia, myocardial protection

Extracorporeal circulation (ECC) is an artificial circuit created by aspiring blood from the right atrium into a venous reservoir, circulating it over a membrane oxygenator and a thermal exchanger (hypothermia and reheating) by means of a pump and reinjecting it to the patient through an arterial cannula. ECC temporarily replaces the heart pump and pulmonary gas exchanger and involves a mechanical device connected to patient’s vascular system during open heart surgery. After ECC was discovered, open heart surgery became possible, but cardiac surgeons started facing deadly complications such as low cardiac output and postoperative acute myocardial infarction (AMI) secondary to inadequate myocardial protection against ischemia-reperfusion injuries.

Hypothermia and hyperkalemic solutions were used in the years ’50s and ’60s for myocardial protection with limited efficiency. In 1964, Bretschneider invented the first cold crystalloid cardioplegic solution that was injected in coronary arteries during surgery [1]. In 1978, Follette introduced cold blood cardioplegia [2], while in 1979 Buckberg understood the ischemia-reperfusion phenomena thus facilitating a continuous development of cold blood cardioplegia [3]. Also in 1978, Solorzano started injecting cardioplegic solutions in the coronary sinus [4] and in 2000 Gaillard proposed normothermic cardioplegia [5].

Currently, there is a trend towards hyperkalemic blood minicardioplegia with a better preservation of the contractile function of the heart. Pharmacological myocardial preconditioning with halogen-containing anaesthetics is also studied as the use of sevoflurane and isoflurane proved to reduce the risk of myocardial ischemic lesion during myocardial revascularization [6].

Myocardial protection influenced cardiac surgery results but despite its considerable progress, myocardial failure remains the leading cause of postoperative morbidity and mortality. Intraoperative myocardial preservation is essential in ensuring a sufficient energy status for normal contractile function at the end of the surgery. With inadequate myocardial protection there is a decrease in myocardial oxygenation, an increase in anaerobic glycolysis, and accumulation of metabolic waste (lactate, H+ and CO2) leading to cellular death.

Reperfusion lesions occur in response to declamping the aorta. Myocardial cells having accumulated toxic catabolites during ischemia are vulnerable, thus the restoration of circulation triggers inflammatory and oxidative reactions by inducing oxidative stress. An efficient myocardial protection method should reduce to a minimum the oxidizable substrates. The aim of the current study is to perform a meta-analysis concerning the effect of myocardial protection methods and cardioplegic solutions upon ischemia-reperfusion injuries in open cardiac surgery.

Experimental part

Material and methods

The authors performed a systematic search for published randomized trials, retrospective, prospective, and cohort studies on Medline database from inception to 31 January 2016 using the keywords myocardial protection, ischemia-reperfusion, extracorporeal circulation,
cardioplegia, crystalloid cardioplegia, blood cardioplegia, antegrade perfusion, retrograde perfusion, myocardial preconditioning.

Inclusion criteria included studies reporting at least one result relevant for the topic, no repetitive data, coronary artery bypass grafting or valve surgery for adult patients and transplantation of great arteries for paediatric patients. Exclusion criteria included heart transplantation, patients receiving both cardioplegia types (crystalloid and blood), exclusive retrograde cardioplegia, emergency surgery, cannulation issues. Three authors independently assessed eligibility based on inclusion and exclusion criteria. Methodological quality was verified using the STROBE (Strengthening the Reporting of Observation-al Studies in Epidemiology) checklist version 4 available online [7].

The meta-analysis was carried out using random effects model and fixed effects Mantel-Haenszel model (Review Manager software). Heterogeneity between trials was tested with Cochran’s Q and I² statistics. Meta-regression was performed using SPSS 22.0.

Relative risk for each clinical event was considered statistically significant at a 5% level (p ≤ 0.05).

Results and discussions
Crystalloid cardioplegic solutions

The search returned a total of 383 studies, but only 7 met the inclusion and exclusion criteria. The characteristics of these studies are presented in Table 1.

Several studies compared crystalloid and blood cardioplegic solutions from myocardial protection point of view. Ibrahim et al. [9] studied 50 patients undergoing coronary artery bypass grafting (CABG) and compared myocardial protection offered by blood cardioplegia and crystalloid cardioplegia demonstrating a clear superiority of blood cardioplegia on several levels: metabolic (significant increase in creatine phosphate), myocardial function amelioration, spontaneous sinus rhythm decreased rate of supraventricular arrhythmias. The results were confirmed by Rousou [8] and Ferreira [10]. Guru et al. [11] performed a meta-analysis on 34 randomized studies (5044 patients) revealing a decreased rate of low cardiac output syndrome with the use of blood cardioplegia (odds ratio 0.54, 95% CI 0.34-0.84) and an increased liberation of creatine kinase muscle brain (CK-MB) at 24 h postoperatively with the use of crystalloid solutions (mean difference 5.7 U/L, 95% CI 1.6-10.2). No difference was noted in case of AMI and mortality rates.

Another meta-analysis was carried out in China by Zeng et al. [14] and included 2866 patients (12 studies), 1357 having received crystalloid cardioplegia and 1509 cold blood cardioplegia. 2053 patients (71.63%) were in sinus rhythm after declamping aorta with no significant difference between the two groups. The authors calculated a relative risk (RR) of 0.92 for the global effect, with a heterogeneity p = 0.006 and I² = 77%. 11 studies reported postoperative AMI rates, 32 patients from the crystalloid cardioplegia group (2.44%) and 17 from the cold blood cardioplegia group (1.19%) having such a complication. RR for AMI was calculated at 2.30 in case of crystalloid cardioplegia (95% CI 1.33-3.98, p = 0.003). Mortality rate was signalled in 11 studies (2804 patients), 1.5% (20 patients) in crystalloid cardioplegia group versus 1.63% (24

<table>
<thead>
<tr>
<th>Study</th>
<th>No. of subjects</th>
<th>Type of solution</th>
<th>Type of surgery</th>
<th>Parameters assessed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rousou et al., 1985 [8]</td>
<td>262</td>
<td>Blood cardioplegia – 50 cases, low-volume</td>
<td>CABG</td>
<td>Postoperative arrhythmias, conduction disturbances</td>
</tr>
<tr>
<td>Ibrahim et al., 1999 [9]</td>
<td>50</td>
<td>Crystalloid St. Thomas No. 1 cardioplegia – 25 cases,</td>
<td>CABG</td>
<td>Postoperative arrhythmias, haemodynamic data, high-energy phosphate compounds,</td>
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<td>blood cardioplegia – 25 cases</td>
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<tr>
<td>Ferreira et al., 2003 [10]</td>
<td>17</td>
<td>Crystallloid cardioplegia – 9 cases, warm blood</td>
<td>CABG</td>
<td>a-tocopherol, b-carotene, ubiquinol, and thiobarbituric acid reactive substances</td>
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<tr>
<td></td>
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<td>cardioplegia – 8 cases</td>
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<td>cardioplegia – 2462 cases</td>
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<tr>
<td>Sa et al., 2012 [13]</td>
<td>5576</td>
<td>Blood cardioplegia – 2834 cases, crystalloid</td>
<td>Aortic valve</td>
<td>Mortality, myocardial infarction, low output syndrome</td>
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<td></td>
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<td>cardioplegia – 2742 cases</td>
<td>surgery ±</td>
<td></td>
</tr>
<tr>
<td>Zeng et al., 2014 [14]</td>
<td>2866</td>
<td>Crystallloid cardioplegia – 1357 cases, blood</td>
<td>CABG</td>
<td>Spontaneous sinus rhythm, perioperative myocardial infarction, stroke, postoperative atrial fibrillation, within 30 days mortality</td>
</tr>
<tr>
<td></td>
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<td>cardioplegia – 1599 cases</td>
<td></td>
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<tr>
<td>Tinca et al., 2016</td>
<td>1131</td>
<td>Crystallloid cardioplegia single dose or every 20</td>
<td>CABG</td>
<td>Spontaneous sinus rhythm, perioperative myocardial infarction, stroke, postoperative arrhythmias, within 30 days mortality</td>
</tr>
<tr>
<td></td>
<td></td>
<td>minutes – 654 cases, blood cardioplegia – 477 cases</td>
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</tbody>
</table>

*CABG – coronary artery bypass grafting
of 1043 patients) and 35.10% (365 of 1040 patients) in an AF rate of 31.54% in crystalloid cardioplegia group (329 postoperative atrial fibrillation (AF), 6 studies have reported p = 0.19, heterogeneity p = 0.02, I² = 69%). Concerning cardioplegia group. The authors calculated a RR = 2.18 cardiochimia (Bucharest) ♦

Cardioplegia in CABG, 14% warm blood cardioplegia, 14% showed that 56% of cardiac surgeons use cold blood cardioplegia in adult cardiac surgery but despite this conclusion, a study performed in the UK [15] in 2004 being statistically significant.

A third meta-analysis was performed in 2012 by Sa et al. [13] on 5576 patients from 36 randomized trials, 2834 having received blood cardioplegia and 2742 crystalloid cardioplegia. This meta-analysis proved no statistically significant difference concerning mortality rates (RR = 0.951, 95% CI 0.951-1.514, p = 0.828), AMI rates (RR = 0.795, 95% CI 0.547-1.188, p = 0.164), and low cardiac output syndrome (RR = 0.765, 95% CI 0.480-1.042, p = 0.072) between the two study groups.

A single retrospective study [96] performed by Angeli E. [12] on a group of 30 infants with transposition of great arteries undergoing an arterial switch intervention analysed the effects of crystalloid cardioplegia in paediatric patients. In this study group (mean age 10.9 days, mean weight 3.05 kg) a single dose of crystalloid cardioplegia (50 mL/kg) was administered in the aortic root for 7 minutes with a constant perfusion pressure < 50 mmHg. Electrolytes were monitored continuously during ECC. Troponin-I (TnI) level (a marker of myocardial ischemia) was determined preoperatively, 30 min after aorta declamping, and at 6, 12, 18 and 24 h postoperatively. Mean ECC time was of 148 ± 29 min and mean aortic cross-clamp time was of 98 ± 14 min. After declamping, heart resumed spontaneously sinus rhythm in all cases and haemotocrit was stable at 35% despite continuous blood filtering during ECC. Mean intensive care unit (ICU) stay was of 2.1 days and inotropic support was necessary in all cases (dopamine, dobutamine and adrenalin). TnI level increased up to a maximum of 3.1 mg/mL in the first 6h after declamping and diminished to less than 2 mg/mL after 24 h. Basal level was not obtained even after 48 h. Postoperative ecocardiographic evaluation showed a good biventricular systolic function without segmental contractility alterations. Early and late mortality was 0% in this study.

In new-borns myocardial metabolism is different compared to adults thus explaining the results obtained with crystalloid solutions. Important glycogen reserves allow sufficient anaerobic metabolism and a reduction of energetic substrate of myocardial contraction. Myocardium is unable to develop oxygen debt compared to other tissues. Restoring blood flow to ischemic tissues may lead to lesion aggravation depending on ischemia’s duration and severity. Several mechanisms explain these lesions, like free radicals liberation, neutrophil-endothelium interaction, apoptosis and hyper contractility secondary to calcium overload of myocardial cells. Superoxide is generated by mitochondrial autoxidation as a result of the action of xanthine oxidase [16, 17].

The superoxide may be spontaneously inactivated or by superoxide dismutase (SOD).

H₂O₂ may be produced from superoxide molecules or under the action of peroxynosomal oxidases.

Hydroxyl radicals are generated by [17]:
- Hydrolysis, H₂O₂ → H₂ + HO²⁻.
- Metallic ion interaction, Fe²⁺ + H₂O₂ → Fe³⁺ +OH + HO²⁻.
- Haber-Weiss reaction, H₂O₂ + O²⁻ → OH + HO⁻ + O₂.
- Nitric oxide (NO) is a chemical mediator that interacts with O₂ free radicals to produce peroxynitrite anions with increased reactivity: NO + O₂ → ONOO⁻ + H⁺. Peroxynitrites may interact with CO₂ to generate extremely toxic free radicals like NOCO₂⁻ and CO₃²⁻.

O₂ free radicals are extremely unstable and may interact with cell membrane molecules and nucleic acids (lipid peroxidation, protein denaturation, DNA fragmentation) and initiate an autocalytic reaction. Endothelial lesions are caused by a imbalance between NO- production and •O₂, free radicals [16].

O₂ free radicals production during ischemia/reperfusion was demonstrated using electron paramagnetic resonance spectroscopy and chemiluminescence [16]. Effective cardioplegia avoids this cascade of events by protecting the myocardium against ischemia and reperfusion lesions. Another condition that has to be met by cardioplegic solutions, except myocardial protection, is offering an adequate surgical view.

Introduction of cardioplegic solutions revolutionized myocardial protection and open heart surgery as they allowed:
- preservation of any mechanical or electrical energy consumer activity of the heart;
- maintaining an efficient hypothermia;
- buffering to avoid acidification;
- cCellular membrane protection;
- an anti-edematous effect.

Currently there are used crystalloid (intra and extracellular) solutions and blood cardioplegia. First crystalloid cardioplegic solutions assumed injecting several litres of cold physiological serum or Ringer lactate into the coronary arteries. With the use of these solutions, an efficient heart paralysis was not obtained with cardiac
arrest, and there was an increased risk of hemodilution and arrhythmia (ionic disequilibrium). Due to these adverse effects, the first generation of crystalloid cardioplegic solutions was abandoned and a new generation appeared in the years ’70s [18] when histidine, ketoglutarate and mannitol were added. Buffering was then possible together with amelioration of energy production, cell membrane stabilisation, membrane osmotic regulation, heart arrest up to 180 min with a single dose, simple preparation and utilisation.

Most crystalloid cardioplegic solutions are divided into two groups:
- purely extracellular composition (Saint-Thomas, Celsior) similar to that of plasma – heart arrest is induced by a moderate K+ dose (30mEq/L) or by a balanced concentration of K+ (15-25 mEq/L) and Mg2+;
- purely intracellular composition (Brestrchneider, Belzer) – decreased Na+ and Ca2+ and increased K+ concentrations.

Other solutions like Standford and Custodiol cannot be included in one of the above categories.

Nowadays only Pleegisol (modified Saint-Thomas solution or STH2), an extracellular solution, and Custodiol are approved for use in ECC. Other solutions are used for organ preservation.

Extracellular solutions carry several advantages over intracellular solutions:
- easy to obtain equilibrium with living tissues;
- normal or decreased Ca2+ concentration associated with increased Mg2+ avoids calcium paradox (hypomagnesemia contributes to reducing ionized Ca2+ that triggers hyperkalemia).

Intracellular solutions may destroy cell membranes through the calcium paradox.

In the clinical setting, crystalloid solutions proved their efficiency and safety for heart arrest of up to 3 h. Beyond this period, the buffer system becomes inefficient, myocardial oedema occurs together with ischemic debt and cytotoxicity (K+ has a toxic effect and a rapid acidification occurs).

Although the efficiency of crystalloid solutions was proved, more cardiac surgeons prefer blood cardioplegia by taking into consideration potentially prolonged ischemia, severe hemodilution during ECC despite filtration (the haematocrit was 35% in the study of Angeli detailed above) and the number of necessary blood transfusions.

**Blood cardioplegia**

Blood cardioplegia was developed and promoted by the team of Buckberg et al. [19] at the end of years ’70s. The original technique involved induction of cardiac arrest and myocardial depolarisation with a cold blood hyperkalemic solution followed by a new reinjection of the same solution every 30 min. Before aorta declamping, a warm blood solution (reperfusion) was injected followed by a warm induction of myocardial function recovery.

This blood solution is diluted 1/4-1/5 with crystalloid solutions and offers better myocardial protection and left ventricular function compared with crystalloid solutions alone. Myocardial compliance is preserved and a lower elevation of myocardial enzymes was noted postoperatively. Compared to plasma, blood cardioplegia solution is enriched with K+, Mg2+, anti-oxygen free radical’s agents and procaine. Compared to simple crystalloid cardioplegia, blood cardioplegia offers several advantages.
- Endothelial and myocardial nutrition;
- efficient oxygen and nutrient transportation;
- increased buffer capabilities;
- limited onotic and osmotic variations thus diminishing cellular lesions;
- Erythrocytes can eliminate oxygen free radicals.

Additional parameters like solution haematocrit (has to be balanced with hypothermia level), temperature (4-36°C according to various teams) and intervals between reinjections (10-40 min upon temperature) have to be controlled with blood cardioplegia. Cold blood cardioplegia (4-10°C) protects myocardial cells against apoptosis compared to warm blood cardioplegia (36°C) and crystalloid solutions.

Blood cardioplegia can be combined with myocardial preconditioning with halogen-containing anaesthetics. Cope et al. [20] showed that the use of halogens like halothane, enflurane and isoflurane diminishes the size of myocardial infarction compared with intravenous anaesthetic drugs (pentobarbital, propofol).

One additional advantage of blood cardioplegia is the provision of the buffering capacity of blood proteins, especially histidine-imidazole.

Although it carries many advantages, blood cardioplegia also comprises some risks:
- impaired microcirculation perfusion secondary to rheologic perturbations caused by solution temperature and no pulsating flow. This can lead to non-perfused uncooled areas at high risk of ischemia and necrosis [21]. This risk is highly increased in case of hypertrophic cardiomyopathy and coronary arteries disease necessitating both antegrade and retrograde perfusion.

Oxygen delivery depends on even distribution of cardioplegia solution, haematocrit and hypothermia level (oxygen release by erythrocytes is severely reduced with severe hypothermia), and tissue acidosis can offset this effect by improving oxygen delivery;
- the presence of leucocytes in cardioplegia solution may trigger a significant activation of reperfusion injury [22] by the presence of effector elements of this reaction. Many authors have proposed the use of filters to limit the burden of neutrophils present during reperfusion [22] as well as anti-oxidant agents;
- hyperkalemic blood cardioplegia does not eliminate the risk of Na+ and Ca2+ cell overload;
- the need of repeated injections may impair certain surgical procedures or rend them more difficult;
- blood cardioplegia needs adding a complementary circuit to the ECC machine thus inducing an additional priming, a timing for solution passage and a dose calculator.

Blood cardioplegia solution was ameliorated over the time by adding several substances to compensate its flaws and to offer maximum security in case of myocardial protection: K+ (most powerful cardioplegic agent), Mg2+ (potentiates K+ effect thus reducing its dose), Ca2+ (essential to ensure the integrity of the membrane lipid bilayer), allopurinol (competitive inhibitor of xanthine oxidase) [23], desferoxamine (iron chelator) [24], superoxide dismutase [25], mannitol, polyethylene glycol and lactobionate (free radicals scavengers) [26], histidine, aspartate and pyruvate (buffer capabilities) [27], Q10 coenzyme (strong antioxidant) [28], reduced glutathione (free radicals chelator) [29], nifedipine (significantly decreases calcium entry to the myocardial cell) [30], energetic substrates (glucose, aminoacids, high energy phosphates, aspartate, glutamate), insulin, procaine (anti-arrhythmic) [31, 32].

In order to ameliorate coronary perfusion in high risk patients with left ventricular dysfunction, both antegrade and retrograde cardioplegia was proposed by Patch [33] in 2000 and nowadays is widely used. Patch showed a reduction in postoperative morbidity rate with the use of this double approach and no benefit for within 30 days survival rate.
Several others myocardial protection strategies have been proposed [21]. Injection of air bubbles can be avoided by using filters, increasing septal temperature beyond 20°C or appearance of ventricular fibrillation on the ECG imposes a new cardioplegia injection, continuous pH monitoring allows an accurate adjustment of cardioplegia.

Conclusions
Significant progress in the area of myocardial protection in recent years has reduced the clinical consequences of myocardial aggression under ECC. Further reduction of morbidity and mortality requires an “optimization” of existent techniques, where blood cardioplegia should have a prominent place. The use of blood cardioplegia can be troublesome for the surgeon, but several studies have demonstrated its superiority over crystalloid cardioplegia on improving left ventricular function, decreasing the need for inotropic support and reduction of transfusion requirements (diminished hemodilution). Solutes currently used as cardioplegic fluids differ from one hospital to another by their composition (crystalloid, blood), the route of administration (antegrade, retrograde), mechanism of action (depolarizing, polarization) or the temperature (cold, warm). After analysing literature data to determine the selection criteria for these products, it appears that there is no standard solution but the choice depends mainly on the practical and clinical experience of surgeons.

References