Chapter 1

Introduction

1.1 General considerations

Understanding Quality of Life is nowadays of critical importance when providing health care. Current medical practices and the expanding medical technology lay their fundamental principles on prolonging Life at all costs. Decisions on what research or treatments should invest in are closely related to their effect on patient’s Quality of Life. Conventional medical indicators of Life Quality are the rate of cure, disease-free survival, side effects, and costs. In order to build a correct diagnosis and treatment decision, the mentioned factors are taken into consideration together with indicators of the impact of patient’s personality on the disease, the level of satisfaction, and the general health condition. The work described in this thesis aims to mediate some of the today’s clinical controversies, to challenge some of the standard decisions in the current clinical practice by pointing out both weak and strong points in the algorithm of diagnosis and treatment of organ injury and dysfunction during disease.

Organ perfusion is a generic physiological term that refers to the process of oxygen and nutritive delivery of arterial blood to the capillary bed in the biological tissue of specific organs. A compromised organ perfusion might have a multifactorial etiology: low cardiac output caused by cardiogenic or hypovolemic shock\(^1\), acute respiratory
Introduction

failure\textsuperscript{2}, low blood oxygen–carrying capacity\textsuperscript{3}, macro/micro–vascular collapse\textsuperscript{4,5}, sepsis with systemic inflammatory response\textsuperscript{6,7}, hypotension and hypoxia as secondary insults to brain injury\textsuperscript{8,9}. In our approach, we investigated the organ perfusion and subsequent organ viability during acute organ support, as performed during open heart surgery with cardiac arrest and cardiopulmonary bypass, and during donor management and organ procurement prior to transplantation.

1.2 Theoretical background

\textit{Acute organ support by means of cardiopulmonary bypass}

The father of surgical cardiopulmonary bypass for humans was John H. Gibbon, Jr, MD.\textsuperscript{10}, whose interest in and determination to develop cardiopulmonary bypass arose in 1931 when he sat at the bedside of a young woman who was dying of a pulmonary embolus.

“It is only necessary to . . . withdraw blood from a vein, introduce oxygen and allow carbon dioxide to escape, and then inject the blood into a peripheral artery. It would permit, of course, operations within the heart under direct vision.”


He and his wife, Mary Gibbon, devoted the better part of their lives to the laboratory development of a usable pump and oxygenating system. On May 6, 1953, John Gibbon made surgical history by using his apparatus for the successful repair of an atrial septal defect in an 18 years old woman\textsuperscript{11}. Although he subsequently operated on 2 more patients, both died; he was so disappointed that he never again performed an open heart operation. However, by the mid–1950s, the first successful clinical procedures had been reported\textsuperscript{12}. Although it was obvious that the equipment was primitive and risks were high, the practicality of developing effective treatments for congenital and valvular heart disease had been demonstrated.

The cardiopulmonary bypass (CPB), or heart–lung machine, is an apparatus through which blood is temporarily diverted, especially during open heart surgery, to be oxygenated and pumped through the body, maintaining circulation until the heart and lungs are able to return to normal functioning\textsuperscript{13}. The heart–lung machine allows open–heart surgery to be performed on an arrested heart without the patient suffering from hypoxia. Additional to open heart surgery, extracorporeal circulation is a promising adjunct to surgical techniques in neurosurgery, thoracic aortic surgery, complex lung resections, and tumor surgery\textsuperscript{14}. Last but not least, mechanical systemic circulatory support is used successfully during bridging to transplantation, to
support life in recipient patients waiting for transplantation or to maintain organ function until organ procurement in transplant donors\textsuperscript{15}.

Cardiopulmonary bypass is accomplished by the use of an extracorporeal circuit and a pump. The standard CPB circuit (Fig. 1.1) consists of connective tubing, a blood reservoir, oxygenator, heat exchanger, and filter. A venous cannula is placed in the vena cava or right atrium to drain unoxygenated blood by gravity through connective tubing into a blood reservoir. The blood is then pumped to an oxygenator where oxygenation and carbon dioxide removal takes place. Blood temperature may be adjusted by the use of a heat exchanger. Generally, whole body hypothermia is induced while the patient is on CPB. Hypothermia decreases the body oxygen consumption and allows lower blood delivery rates. After the surgical correction is performed, the blood temperature is rewarmed. The blood is filtered to reduce the potential for an embolism and is pumped back to the body through an arterial cannulation. Prior to initiation of CPB, the extracorporeal pump circuit is primed with a balanced crystalloid/colloid solution (e.g. hydroxyethyl starch HES solutions). Once CPB has been initiated, the heart is allowed to beat or it may be temporarily arrested by the administration of cardioplegia solution. Cardioplegia is a cold crystalloid or blood solution that contains a high concentration of potassium. The potassium is used to induce cardiac arrest. The cold temperature of the cardioplegia solution (4\(^\circ\)C) along with other drugs in the solution reduces the oxygen requirements of the myocardium and helps to preserve the heart during the ischemic arrest period. Contact of blood components with the artificial surface of the bypass circuit, aortic cross-clamping, cardioplegic techniques, low perfusion pressures with a non-physiologic profile (continuous), hemodilution, allogenic blood transfusion, and operative trauma result in a systemic inflammatory response syndrome (SIRS) and ischemia/reperfusion.
injuries, generally acknowledged as a “post CPB syndrome”. The systemic inflammatory response in combination with the ischemia/reperfusion injuries and the multiple gaseous, lipoprotein and particulate emboli generated during CPB result in organ dysfunction affecting the heart, brain, lungs, kidneys and intestine. Special clinical attention is addressed to the pathophysiology of the systemic inflammatory response syndrome during CPB. The complex inflammatory reaction set in motion during exposure of blood to large areas of synthetic materials involve activation of complement, platelets, neutrophils, monocytes and macrophages triggering the coagulation, fibrinolytic and kallikrein cascades and increasing blood concentrations of interleukins, tumor necrosis factor, leukotriens. A subsequent increase in endothelial cell permeability allows transvascular migration of activated leukocytes into the tissues with additional vascular and parenchimal damage\textsuperscript{16–18}. Preoperative administration of corticosteroids, with methylprednisolon and dexamethasone being the two most utilized agents, has been demonstrated to inhibit the activation of the plasmatic and cellular inflammatory response\textsuperscript{16}, to decrease the pro- to anti–inflammatory interleukins ratio\textsuperscript{19}, and to minimize tissue edema\textsuperscript{20}. Over the last 50 years increased understanding of the physiology and pathophysiology of the response to CPB has led to steady improvements in circuit design and a reduction in postoperative morbidity. The development of more biocompatible polymeric materials\textsuperscript{21}, arterial in–line filters\textsuperscript{22}, membrane oxygenators\textsuperscript{23}, and centrifugal blood pumps\textsuperscript{24} have resulted in less intraoperative hemolysis, less blood activation, and decreased circulation of emboli\textsuperscript{25}. Surface modification of circuits with heparin\textsuperscript{26,27} to mimic the native circulatory system and the use of pharmacological agents such as aprotinin\textsuperscript{28} have been shown to attenuate blood activation, systemic inflammation, and organ dysfunction\textsuperscript{29,30}. Even with these developments, post–CPB inflammation, ischemia/reperfusion injury, organ injury and organ dysfunction are attenuated but not completely inhibited\textsuperscript{31–33}. The use of mechanical assistance as a bridge to transplantation or a bridge to recovery are presently the main indications for mechanical circulatory support in transplant recipients, allowing a prolonged survival with a reasonable Quality of Life. Special attention is directed to transplantation in infants and children, where the problem of organ donor shortage is even worse when compared to the situation in the field of adult heart transplantations. Newly developed pulsatile, paracorporeal ventricular assist devices designed for long–term assist in children have demonstrated their ability to provide excellent results beyond the abilities of extracorporeal membrane oxygenation and centrifugal pumps, which are still the mainstay of mechanical support in children worldwide\textsuperscript{34}. 
Pathophysiology of impaired tissue perfusion and donor management in organ transplantation

Transplantation has been incorporated into the treatment of patients with end-stage diseases of most major organ systems in recent years. However, organ supply is the greatest limitation to organ transplantation, and thus good management of available donors is a high priority. The majority of donors are heart beating, brain dead cadaveric donors from whom multiple organs are procured for transplantation. There are several steps on the path from potential donor to actual donor. These steps have been defined as: identification and detection of all potential donors; brain death determination; approaching potential donor families for consent; and donor medical management. Due to brain death and loss of integrated neurological function, the potential organ donor manifests major physiologic derangements that require aggressive, labor-intensive management throughout the hospitalization until organ procurement or cessation of life support in order to maintain organ function. Experimental and clinical brain death studies define as major pathophysiologic mechanisms the vascular regulation injury and metabolic cellular injury. Myocardial dysfunction and impaired systemic vascular tone trigger hemodynamic instabilities leading to impaired inotropy and chronotropy, dysrhythmias, decreased cardiac output and hypotension. Arginin–vasopressin production in the pituitary tract generally ceases after brain death, which commonly leads to diabetes insipidus. The loss of free water caused by diabetes insipidus may lead to dehydration, hypovolaemia, and blood electrolyte abnormalities. Hypokalaemia, hypocalcaemia, hypophosphataemia, and hypomagnesaemia may contribute furthermore to decrease in cardiac function and organ blood flow.

Endothelial activation in brain dead donors has gained lately considerable attention in the scientific discussion around the pathology of brain death prior to organ retrieval. The endothelium is considered to be a dynamic interface between the vascular compartment and the extravascular space regulating protein flux, local blood flow, coagulation cascade and the trafficking of the inflammatory cells from the blood into tissue. The vascular endothelial phenotype changes dramatically under pathophysiologic conditions with regards to expression of cell adhesion molecules, cytokines, and substrates that promote thrombosis and inflammation. In this respect, one of the earliest events that was demonstrated to develop after brain injury is the expression of a series of adhesion molecules in sequence by activated vascular endothelium. Additionally, an immune activation with increased endothelial cell activation and immediate early gene expression is shown to occur after brain death induction. The expression of endothelial adhesion molecules (intercellular adhesion molecule–1 and vascular cell adhesion molecule–1) and the influx of leukocytes in the kidney occurs faster and is more profound when hemodynamic instability in the brain dead donor is not corrected. In this way, the involvement of the activated vascular endothelium was linked incontestably to the progression of inflammation after brain death.
Introduction

Donor management has been considered the most neglected area of transplant medicine. In support for this affirmation stand statistical data showing that failure to provide adequate physiological support to potential donors accounts for at least 25% of lost donor organs, and that adoption of an aggressive protocol for donor management, including both intensive monitoring and therapy, has allowed the donor retrieval rate to increase by approximately 30% \textsuperscript{46–48}.

All organs may benefit from aggressive management. Current evidences regarding the evaluation and management of potential donors led to the recommendation that organ procurement organizations should use a standard protocol for donor management which should include application of pulmonary arterial catheterization \textsuperscript{49,50}.

A Critical Pathway for the Organ Donor was described, including five distinct, but often overlapping phases: Phase I, Donor Referral; Phase II, Declaration of Brain Death and Consent; Phase III, Donor Evaluation; Phase IV, Donor Management; and Phase V, Organ Recovery \textsuperscript{51}. Each phase has five subsections that the ICU staff and/or the organ procurement coordinator can use as a guide for thoroughness of evaluation and management. These five subsections are: General Management; Laboratory and diagnostic Tests; Respiratory Therapy; Treatments; Intravenous Fluids and Medications. The Critical Pathway is designed to provide the information necessary to evaluate the functional status of the kidneys, liver, pancreas, heart and lungs and to determine the management steps which need to be taken to improve and optimize the performance of each organ. In addition to an invasive monitoring, these patients need a meticulous attention to their hemodynamic variables.

The early administration of desmopressin to treat diabetes insipidus, differentiated use of fluid resuscitation and distinct catecholamine support are special features of an appropriate basic treatment. Hormonal resuscitation (thyroxin, vasopressin, insulin) has been reported to stabilize and improve cardiac function in brain dead donors. The administration of corticoids has to be considered if a sufficient circulation can not be regained \textsuperscript{52}.

As the management of organ donor patients becomes more complex, recovery coordinators often have to change their thinking and resort to extra, nonconventional means of diagnosis and management. The standard laboratory diagnostic tests are not always early, specific and sensitive enough, so addition of new biomarkers to diagnose organ injury is needed. Furthermore, instead of aggressive pharmacologic interventions often implemented in an attempt to stabilize donor hemodynamics, the addition of an extracorporeal circulatory assist device might prove beneficial in optimizing organ perfusion. There are reports in literature showing that cardiopulmonary bypass and profound hypothermic circulatory arrest may be easily combined with traditional procurement flushing techniques, providing excellent organ preservation for subsequent transplantation. This approach could optimize organ recovery from hemodynamically unstable donors, increasing the number of organs available for transplantation \textsuperscript{53}.  

14
Organ procurement: wash–out and preservation procedures prior to transplantation

In the 1930s, the classical experiments of Carrel and Lindbergh included perfusion techniques to preserve organs for transplantation. They established ground rules for organ preservation by continuous ex–vivo perfusion; expert technology, perfect asepsis, and controlled biological conditions. The Spanish Civil War marked the advent of blood banks and clinical application of tissue storage techniques. Cold storage was used to diminish metabolic demand. Fortunately blood, the first widely preserved and transplanted biological substance, lent itself well to extended storage. Refrigerated storage was possible for 3 weeks.

The discovery of cryoprotectants by Polge and coworkers in 1949 ushered in a major extension of preservation times for a variety of simple cells and tissues. Blood cells and gametes (even embryos) could be stored after freezing. Weeks or months later, they could be thawed and successfully reimplanted as autografts or allografts. Such freezing was only successful for isolated cells or undifferentiated multicellular embryos: complex and heterogeneous organs were highly sensitive to freezing damage. The biophysical problems of freezing large organs were subsequently defined by Pegg and other workers. To date no consistent success has been obtained with the use of freezing to preserve organs such as kidneys, liver, or hearts. Concepts of frozen humans waiting revival at an appropriate future time on another planet or in an after–life remain science fiction

Nowadays, standard procedures for organ procurement and preservation require each organ to be flushed free of blood with a specially prepared ice–cold preservation solution, just prior to being removed from the donor. The organs are then placed in sterile containers, packaged in wet ice, and transported to the recipient’s transplant center.

In order to allow inclusion of additional less–optimal donor categories, the organ procurement and initial perfusion technique are key factors toward an improved outcome in organ transplantation.

Good organ preservation starts with an effective blood washout of the donor organ. Major determinants to maintain graft viability, irrespective of the chosen preservation solution or method, are a rapid decrease in core temperature and an equilibration between the intravascular preservation solution and the parenchyma. Following the wash–out procedure, organ preservation is performed, using either static cold storage or continuous machine perfusion. Using simple cold storage methods, it is easy to transport organs for transplantation. However, despite the complexity and difficulty of organ transportation, continuous machine perfusion has some advantages by opening opportunities for viability assay and pretreatment of organs before transplantation. Preservation solutions have been designed to ameliorate the adverse physiological and biochemical effects of ischemia under hypothermic conditions. Three principles are important in effective cold storage. First, the vascular wash–out during harvest should
Introduction

rapidly cool the organs, remove the blood and allow balance between the cold storage solution and the tissue. Second, the cold storage solution should prevent cell swelling and interstitial edema formation by including substances that are osmotically active and impermeable to the cell. Impermeants and saccharides achieve homeostasis of the intracellular water content. Homeostasis of the interstitial compartments is achieved by counteracting a hydrostatic force during the initial wash–out using colloids. The intravascular fluid compartment does not need an effective component in static cold–storage. Third, the cold storage solutions should prevent excessive cellular acidosis by containing sufficient concentration of hydrogen–ion buffer, histidine or citrate\textsuperscript{56,58}. Since its introduction by Belzer et al. in the late eighties, the University of Wisconsin (UW) solution has become the standard solution for the preservation of most organs in transplantation. Despite the fact that UW solution made extended cold preservation feasible, some studies have demonstrated that prolonged cold ischemic time of hepatic allografts enhance bacterial infection\textsuperscript{59}, cause biliary and hepatic artery complications\textsuperscript{60,61} and increase the frequency of primary non function posttransplant\textsuperscript{62}. The inclusion and importance of the colloid hydroxyethyl starch (HES) as one of the components of the UW solution has been both advocated and denied. HES prevents interstitial edema and has a beneficial effect on matrix metalo–proteinases\textsuperscript{63} but at the price of a higher solution viscosity. Due to the presence of HES, the viscosity of UW solution at 4°C increased by a factor of 2.5 when compared with the viscosity of the same solution at 37°C\textsuperscript{64}.

“Folkert O. Belzer, the “father” of the University of Wisconsin preservation solution, was an outstanding practitioner of transplantation medicine, a brilliant, technically beautiful surgeon, and a superb educator–trainer of surgeons. However, his methods in the laboratory might be considered unorthodox. His goal was simple to improve organ preservation so he could offer his patients a better organ for a long and healthy life. In the early 1980s, Bob Hoffmann and I were trying to extend kidney preservation beyond 3 days; we believed that one of the problems was the lack of oxidizable substrates (fatty acids) in the perfusion fluid. We had trouble dissolving these fatty acids because we were no longer using serum albumin as a colloid but rather hydroxyethyl starch that did not bind fatty acids. One afternoon, Dr Belzer was drinking coffee and smoking his pipe in the laboratory, a pastime that consumed his off hours, led to great discussion between us, and catalyzed his mind. While reading the label on the coffee creamer carton, he noticed a list of monoglycerides and diglycerides and asked me about their purpose. I responded that these agents served as emulsifiers to help keep the lipid materials in solution. It was immediately obvious to me that the lights went on in his brain because he responded with, “Why not use this coffee creamer to solubilize fats in the perfusion solution?” I had no good explanation; hence, we tried the kidney perfusion with the addition of coffee creamer. (By the way, it did not work!) This is only one of the myriad “esoteric” compounds and chemicals we tried.” Southard JH.\textsuperscript{65}
1.3 Aim of research

The aim of this work was to investigate the efficiency of organ perfusion during acute organ support, as performed during extracorporeal mechanical blood circulation in cardiac patients, and during donor management, organ procurement and organ preservation prior to transplantation. The investigations were conducted in clinical studies, animal studies and in–vitro experimental settings. The efforts were concentrated on testing the diagnostic value of new, specific and sensitive biomarkers for organ injury, in order to help an early and effective therapeutic strategy. Given the complexity of this subject and the large diversity of clinical problems associated with impairment of organ perfusion, we approached only main points of debate in the current clinical world. The questions we addressed in the context of cardiopulmonary bypass associated morbidity concern the prophylactic use of corticosteroids, the isovolemic hemodilution, the therapeutic choice for plasma expanders, the myocardial protection and the corporeal temperature during extracorporeal circulation. In organ donation and transplantation, we investigated the consequences of cerebral injury in brain dead donors on organ viability. Impairment of perfusion during organ procurement and organ preservation was investigated in relation with the initial wash–out procedure. Beside clinical questions, the attention was also distributed towards the pathophysiologic mechanisms involved; vascular endothelial function and red blood cell function were especially addressed.

**DEXAMETHASONE: BENEFIT AND PREJUDICE FOR PATIENTS UNDERGOING ON–PUMP CORONARY ARTERY BYPASS GRAFTING – A study on myocardial, pulmonary, renal, intestinal, and hepatic injury. (Chapter 2)**

Administration of corticosteroids to patients undergoing on–pump cardiac surgery was demonstrated to inhibit the activation of the plasmatic and cellular inflammatory response, to decrease the pro– to anti–inflammatory interleukins ratio, to minimize tissue edema and to optimize the intravascular/extravascular fluid balance. Based on this constellation of findings, a routine prophylactic administration of corticosteroids was instituted in a multitude of clinical centers when performing on–pump heart surgery, assuming that inhibition of the systemic inflammatory response is automatically associated with clinical benefits. However, only a few clinical trials have been conducted to extend these results and to investigate the effect on clinical outcome in patients receiving corticosteroids.

As an original contribution to the issue of CPB related inflammatory response and organ injury, we document the effect of dexamethasone on perioperative myocardial, pulmonary, renal, intestinal and hepatic injury, as assessed by newly available specific and sensitive (bio)markers. Furthermore, to describe the effects of corticosteroids on the systemic inflammatory response, we measured cytokine response and systemic tryptase release as a marker of mast cells activation. Finally, a new hypothesis relating
tryptase to the attenuation of perioperative organ injury is discussed.

**COMBINED STRATEGY TO LIMIT PERIOPERATIVE MYOCARDIAL, RENAL AND INTESTINAL TISSUE INJURY IN PATIENTS UNDERGOING ON–PUMP CORONARY ARTERY BYPASS GRAFTING (Chapter 3)**

The available scientific data concerning the effectiveness and safety of cardiopulmonary bypass (CPB) for patients undergoing coronary artery bypass grafting (CABG) brings to attention several key principles to serve as basis for practical guidelines. Encouraged and generally accepted techniques are cold crystalloid cardioplegia, mild corporeal hypothermia and hemodilution to a hematocrit as low as 20%. Our therapeutic strategy aimed to explore, advance and optimize simultaneously more than one method of protection by eliminating several potential stress factors that might lead to injury and dysfunction during CPB: inadequate hypothermic cardioplegia, excessive hemodilution with subsequent need for perioperative blood transfusion, and corporeal hypothermia.

An experimental operative protocol was developed to meet multiple objectives: (1) homogeneous cooling of the myocardium by combining cold crystalloid cardioplegia technique with an additional intracavitary cooling of the heart; (2) prevention of excessive hemodilution by autologous priming of the extracorporeal circuit and partial recovery of the cardioplegic fluid; (3) corporeal normothermia.

The consequences on the postoperative organ injury and clinical outcome of the patients were investigated.

**RED BLOOD CELL AGGREGATION DURING CARDIOPULMONARY BYPASS: A PATHOGENIC COFACTOR IN ENDOTHELIAL CELL ACTIVATION? (Chapter 4)**

The relations between a low hematocrit and the adverse outcomes in patients undergoing CPB is extensively discussed in the literature. There are also reports addressing the mechanical trauma of red blood cells and the decrease in red blood cell deformability during extracorporeal circulation. In our opinion, a complete chapter has been excluded from the discussion around the pathogenesis of the “post–CPB syndrome”: modifications induced in red blood cell aggregation and potential consequences on microcirculation. The present study aimed to test the potential effect of priming solutions and the extracorporeal circulation on red blood cells aggregability and endothelial cell activation. We document the effects induced by two different prime solutions often used in the clinical practice, HAES–steril 6% and Voluven 6%. The clinical relevance and possible correlation between the pathophysiological mechanisms implicated are discussed.
The essential role of erythrocytes as oxygen carriers is historically well established, however, their function to aggregate with consequences on homeostasis is strongly under debate. The aggregation property of red blood cells is mainly considered to be pathophysiologic, since aggregation is elevated in many disease states such as diabetes mellitus and hypertension.

To date and rather remarkable, the scientific approach unravelling this subject has completely ignored the pathogenic potential of low erythrocyte aggregation states. Some authors have postulated the possibility that normal levels of aggregation may serve homeostasis, having functional significance for normal physiology, since red cell aggregation is normally present in humans and other “athletic” species. This hypothesis, however, has never been investigated before, and also, never been placed in a clinical relevant context.

The pathogenicity of low erythrocyte aggregation could have major implications for hemodiluted patients. This situation routinely occurs in cardiac patients undergoing on-pump cardiopulmonary bypass who are severely hemodiluted due to therapeutic preoperative isovolemic hemodilution, priming of the extracorporeal circuit and large fluid infusions perioperatively. Excessive hemodilution prevails also during sustained fluid resuscitation in traumatic-hemorrhagic shock patients. In addition to the consequences of hypoxic stress, the implications of low erythrocyte aggregation during acute hemodilution might prove to be essential for a good understanding of microcirculation impairment and deteriorated tissue perfusion in these patients. Considering the sensitivity of vascular endothelium to variations in blood rheology, we hypothesized that low erythrocyte aggregation will be responsible for activation of vascular endothelium during acute isovolemic hemodilution.

**CONSEQUENCES OF CEREBRAL INJURY IN BRAIN DEAD DONORS ON ORGAN VIABILITY: PROGRESS OF PRO-COAGULATORY AND PRO-INFLAMMATORY ENDOTHELIAL ACTIVATION (Chapter 6)**

Endothelial activation in brain dead donors has gained lately considerably attention in the discussion concerning the pathology effects of brain death on donor organ quality prior to retrieval. In this respect, previous studies conducted in our laboratory suggest that an immune activation with increased endothelial cell activation and immediate early gene expression occurs after brain death induction. Moreover, the expression of endothelial adhesion molecules (intercellular adhesion molecule-1 and vascular cell adhesion molecule-1) and the influx of leukocytes in the kidney occurs faster and is more profound when the hemodynamic instability in the brain dead donor is not
corrected\textsuperscript{45}. In this way, the involvement of the activated vascular endothelium was linked incontestably to the progression of inflammation after brain death. As an original contribution, this study aimed to analyze and document the time sequence for the progression of pro–inflammatory and pro–coagulatory endothelium activation, oxidative stress and organ viability in brain dead rat donors. Furthermore, this study investigated for the first time to our knowledge, extrahepatic fibrinogen synthesis in brain dead donors. We hypothesized that activated endothelium in the brain dead donor expresses and releases pro–inflammatory and pro–coagulatory factors into the circulation that will mediate inflammation, platelet adhesion and possibly promote microthrombosis. In addition, we expected that due to activation of endothelium and hypoxic stress, the oxidative stress and brain death related organ dysfunction are enhanced. This investigation could pinpoint the time points necessary for anti–inflammatory and anti–coagulatory therapeutic interventions, that could effectively reduce endothelial activation, prevent platelet adhesion and leukocyte infiltration, and possibly slow down an ongoing organ deterioration during brain death.

**HYPERAGGREGATING EFFECT OF HYDROXYETHYL STARCH COMPONENTS AND UNIVERSITY OF WISCONSIN SOLUTION ON HUMAN RED BLOOD CELLS: A RISK OF IMPAIRED GRAFT PERFUSION IN ORGAN PROCUREMENT? (Chapter 7)**

The viability of organ grafts depends on several factors such as cold ischemia time, the perfusion procedure, preservation methods and reperfusion quality. The efficacy of perfusion during the initial wash–out procedure, however, has to be also considered a major determinant of functional recovery after transplantation\textsuperscript{55,73}. Preservation solutions have been designed to ameliorate the adverse physiological and biochemical effects of ischemia under hypothermic conditions. The inclusion and importance of the colloid hydroxyethyl starch (HES) as one of the components of the University of Wisconsin (UW) solution has been both advocated and denied. In an experimental setting analyzing the effect of HES on the rheological properties of blood, Corry and collaborators have drawn the attention to the aggregating effect of HES on erythrocytes\textsuperscript{74}.

The present study aimed to test the effect of HES and UW solution on red blood cell aggregability and to correlate aggregation parameters with HES molecular weight. In addition, the study aimed to detail the extent and kinetics of HES–induced human RBC aggregation, as well as the morphological characterization of these aggregates. It could be possible that by identifying the RBC hyperaggregating effect of UW solution as an etiology–related factor for these complications immediate function, patient and graft survival would improve.
References


