Recent improvements in the clinical care of patients have their roots in two distinct fields of modern medicine: biomedical research and clinical ethics. In order to improve the ability of clinical medicine to apply successfully and ethically the new developments in medical science, research must be undertaken to understand the full effects of medical treatments and the proper threshold for medical intervention. Moreover, it is of critical importance how physicians understand the risks and benefits of treatment and how to guide the decision making for individual patients. The ethical aspects of treatment decisions are of equal importance, with emphasis on patients expectations when they consent to manipulation involving risk factors and on their participation in a treatment decision. Only by investing time and effort in both medical education and research, the ethical ideals that underlie the physician–patient relationship can be fulfilled.

The present thesis describes a generally recognized pathophysiologic mechanism: impairment of organ perfusion with its diagnostic and therapeutic challenges. Out of the multitude of possible etiologic factors for organ perfusion impairment, we chose to investigate two extreme situations of acute organ support: (1) organ perfusion during cardiopulmonary bypass with cardiac arrest and (2) organ perfusion during
organ donation and procurement prior to transplantation. Even if these two clinical fields seemed to be segregated at a first approach, our results conclusively showed a parallelism in etiology, pathologic mechanisms, and therapeutic approaches.

Using this original approach, we investigated several issues of concern for both cardiac surgery and organ transplantation: use of artificial colloids, use of prophylactic corticosteroids, diagnostic value of organ injury markers, consequences of hemodilution, hypothermia induced injury, and vascular endothelial activation.

**Effects of hydroxyethyl starches (HES) on red blood cell aggregation**

The use of HES solutions as priming and plasma substitution fluids in patient undergoing cardiopulmonary bypass results in altered red blood cell aggregation. In parallel with the decrease in red blood cell aggregation, blood viscosity declines also. The subsequent variations in blood rheology activate the vascular endothelium with pro-inflamatory and pro-thrombotic effects. A distinct effect of different molecular weight starches was evident post-bypass. While the markers of endothelial activation went down to baseline levels in HES 200/0.5 group, HES 130/0.4 was associated on the first postoperative day with sustained endothelial activation.

In organ transplantation, the use of high molecular hydroxyethyl starches (HES 450/0.7 and higher) as components of the University of Wisconsin preservation solution accounts for accelerated and augmented red blood cell aggregation. The aggregates are of large size and their resistance to dissociation by flow induced shear stress is high. These data suggest that gravity-induced hydrostatic perfusion pressures presently used in procurement can not easily dissociate the abnormal red blood cell aggregates. In addition, the small vessel diameter in marginal areas compared to the aggregates size make these vessel category prone to mechanical obstruction during organ procurement. The presence of remaining host erythrocyte aggregates, trapped in the microvasculature after the initial wash-out of the donor organ could contribute to an inadequate microvascular perfusion with preservation solution and therefore to a poor maintenance of graft viability during ischemic storage. The areas of the respective organs that are only marginally equilibrated with University of Wisconsin solution are expected to be less protected during the subsequent ischemic storage period, thus contributing to an overall reduced structural and functional integrity of the organ. Low molecular weight HES treatment of blood yields a decline of blood viscosity values, decreased the red blood cell aggregability and slows the process in time.

Our results documenting the effect of hydroxyethyl starches on red blood cell aggregation suggest the necessity of a more careful selection of HES solutions when
considering a therapeutic strategy. In cardiac surgery, hypertensive and atherosclerotic patients who have already a high basal levels of circulating von Willebrand factor might benefit from HES 200/0.5. HES 130/0.4 could represent a first choice for patients with bleeding tendencies and patients with acquired von Willebrand syndrome after aortic stenosis. In organ preservation prior to transplantation, the exclusive use of low molecular weight HES will improve the quality of the University of Wisconsin solution by preventing intravascular red blood cell aggregation. By preventing mechanical obstruction during wash-out, use of HES 130/0.4 might have a beneficial effect on organ preservation and possibly reduce the chance of postreperfusion primary nonfunction and posttransplant biliary lesions with delayed recovery in organ transplantation.

Prophylactic corticosteroid treatment

The assumption that prophylactic corticosteroid therapy, by its virtue to inhibit the inflammatory response, would also transfer a protective effect of organ injury associated with cardiopulmonary bypass, was rejected by the results presented in this thesis. Dexamethasone treatment offered no protection against transient, perioperative renal, intestinal and hepatic injury in patients undergoing on–pump coronary artery bypass grafting. In fact, dexamethasone treatment seemed to be detrimental, resulting in a pronounced postoperative pulmonary dysfunction, prolonged time to tracheal extubation and by initiating postoperative hyperglycaemia. An important observation was the strong positive correlation found between high blood glucose level, as side effect of dexamethasone, and end organ injury. The necessity of a stricter management of serum glucose emerged, suggesting insulin therapy at serum glucose lower than 10 mmol.L$^{-1}$ (as standard) in order to avoid kidney and intestinal injury. This message is also important for the clinicians responsible for the management of the brain dead organ donors, confronted as well with the use of corticosteroid therapy and glucose management. An early institution of insulin therapy might decrease brain death–related donor organ damage.

Diagnostic value of organ injury markers

With the goal of diagnosing impairment in organ perfusion and subsequent organ injury, the present work investigated in a multitude of clinical settings the use of both standard and newly available organ injury biomarkers. Most of the standard laboratory investigations proved to require long assay times, to lack sufficient specificity and/or sensitivity. In contrary, using newly available, sensitive and specific organ injury biomarkers we were able to document transient, subclinical cardiac, renal, intestinal and hepatic tissue injury even in low risk patients undergoing cardiopulmonary bypass. Similar, these new markers proved to be useful when investigating
Early brain death–related donor organ damage.

Fatty acid binding proteins (FABP) are cytosolic proteins with various tissue specific isotypes, released in circulation and subsequently in urine in case of cellular damage. We investigated the use of heart (H), intestinal (I), and liver (L) type fatty acid binding proteins. In cardiac patients undergoing cardiopulmonary bypass, plasma H–FABP correlated with other cardiac injury markers (cardiac Troponin I and creatine kinase MB). The advantage of including H–FABP in the diagnosis of myocardial injury is the early peak arising already one and a half hour after reperfusion, which was significantly earlier than the peaks of cardiac Troponin I (fourteen hours) and creatine kinase MB (sixteen hours). Urinary concentration of H–FABP proved to be a better indication of kidney damage than of myocardial damage, explained possibly by a primary release of H–FABP in urine from the damaged distal renal tubules. In our study on the patients undergoing cardiac surgery, the urinary peak of H–FABP did not correlate with the others cardiac markers but correlated strongly and significantly with the urinary peak of N-acetyl-glucosaminidase (NAG, proximal tubules injury) and peak microalbuminuria (glomerular injury). Similar, in brain dead rats donors, H–FABP and NAG urine concentration reached significantly higher values as early as half hour and one hour, respectively, after brain death induction, as compared with sham operated animals. A highly positive correlation was documented between the two renal tubules markers, consolidating the diagnose of renal tubular damage during brain death.

I/L–FABP are cytosolic proteins readily released into the circulation following enterocytes damage, with a 40-fold higher content of L–FABP, reported as useful urine markers for the detection of intestinal injury. Both urinary I–FABP and L–FABP increased significantly during CPB, reaching peak values in the urine collected during the first two hours and six hours postoperative, respectively. Urine I–FABP correlated significantly with urine L–FABP. The increased values of I–FABP and L–FABP during CPB reported in this study verify the indirect evidence of mucosal integrity loss during CPB reported previously as perioperative reduction in intramucosal pH, increase in gut permeability and endogenous endotoxemia. Using the test of I–FABP concentration in the urine of brain dead rats, we were able to detect intestinal injury as early as two hours after brain death (data not shown).

Additional to fatty acid binding proteins, we would like to emphasize the utility of N–acetyl–glucosaminidase in diagnosing proximal tubules injury, and of α-Glutathione S–transferase in diagnosing hepatic injury.

Consequences of hemodilution

Using a complex operative strategy in patients undergoing on-pump coronary artery bypass grafting, we showed an important attenuation of the transient renal and intesti-
nal postoperative injury achieved by means of limiting intra–operative hemodilution and blood transfusion requirements. Variation in hematocrit explained more than a third of the variation of both postoperative NAG and I–FABP. A decrease with one unit (1%) in hematocrit predicted significantly an increase with a quarter of the peak postoperative NAG values. The same decrease with one unit (1%) in hematocrit predicted significantly an increase with a tenth of the peak postoperative I–FABP values in patients undergoing on–pump cardiac surgery. Hemodilution, besides lowering the oxygen carrying capacity of blood, alters as well blood rheology with possible pathological consequences.

With the aim set on investigating variation in blood rheology during isovolemic hemodilution and subsequent effects on vascular endothelial activation, we designed an animal study to answer previously formulated hypothesis in clinical studies. To bring relevance, the study addressed two different hydroxyethyl starch (HES) solutions commonly used in the clinical practice as priming solutions for the heart–lung machine and as plasma expanders. An important observation was that hemodilution up to 50% resulted in negligible hypoxia/reperfusion injury, as quantified by the reactive oxygen species production measured in the vital organs. Low red blood cell aggregation, as documented in this model of acute isovolemic hemodilution, was associated with activation of vascular endothelium, especially in lungs and small intestine. Translation of these data in clinical terms suggests that acute hemodilution may lead to inflammatory stress of pulmonary capillaries. Subsequent diffusion limitation may be expected. Similar, an increased inflammatory response in the small intestine associated with acute hemodilution, might contribute to a loss in barrier function of the intestinal mucosa with subsequent translocation of endotoxins and/or bacteria. Additionally, the data presented in this study suggest a new pathway for the erythrocyte involvement in clot formation: due to their function to aggregate, erythrocytes can modulate endothelial activation with von Willebrand factor release, with a subsequent pro–thrombotic effect. The investigations on acute isovolemic hemodilution might be clinically relevant for the patients undergoing on–pump cardiopulmonary bypass, the patients in traumatic–hemorrhagic shock with sustained fluid resuscitation, or the brain dead organ donors with large volume of fluid infusion to correct for hypotension. Based on the results demonstrating increased endothelial activation, we hypothesize that lower incidence of thrombotic events and decreased inflammatory reactions could be achieved by avoiding excessive hemodilution.

**Hypothermia–related injury**

Contrary to conventional thinking about the benefits of corporeal hypothermia on systemic protection against global ischemic injury during extracorporeal circulation, an increasing number of clinical studies support corporeal normothermia. The re-
sults of our clinical investigation comparing normothermia with hypothermia showed a negative correlation between body temperature during cardiopulmonary bypass and postoperative I–FABP urine concentrations. In other words, lower body temperatures during CPB were associated with higher intestinal damage. These findings confirm at a different level, the studies performed in organ transplantation that document a higher organ damage when cold ischemic preservation time is extended. In the clinical setting of organ transplantation, cooling down the organ followed by rewarming is a generally recognized trigger of injury.

Vascular endothelial activation

Pro–inflammatory and pro–coagulatory vascular endothelial activation was demonstrated to be a central pathological finding in both cardiac surgery and brain death organ donation. In cardiac surgery with cardiopulmonary bypass, endothelial activation was demonstrated to arise in the first hours after myocardial reperfusion, as documented by elevated plasma concentrations of von Willebrand factor, thrombomodulin, tissue Plasminogen Activator and E–Selectin. At gene regulation level, endothelial activation is shown in our pig experimental study to arise already three hours after induction of isovolemic hemodilution. In brain dead donor rats, endothelial activation was documented as early as half hour after brain death induction.

The etiology of endothelial activation is multifactorial: systemic inflammatory response, surgical stress, and systemic mobilization of wound–release factors. As original contribution, the present thesis introduces a new etiologic factor: decreased red blood cell aggregation as a trigger of impaired blood rheology and thus mechanical endothelial activation. We hypothesize that the drop in RBC aggregation added to plasma viscosity reduction during hemodilution alone, or even more during extracorporeal circulation, are important factors contributing to variation in shear stress at the vascular endothelial wall. The variation in shear is known to lead to a complex signaling response eventuating in pro-inflammatory and pro–coagulatory vascular endothelial activation.

In conclusion, the work described here aims to add a new foundation stone on the scientific basis for diagnosing and treatment, by contributing to current clinical debates and suggesting new directions for clinical and fundamental research. Additionally, the results included in this thesis emphasize the need of collaborative decision making between physicians with different expertise, and between physicians and researchers.
Perspectives

Development and validation of new extracorporeal assist devices are highly desirable when performing artificial organ support. Pulsatile perfusion remains a challenging therapeutic choice. Either used as bridge to transplantation, bridge to recovery, or during coronary artery bypass grafting with cardiac arrest, an effective pulsatile perfusion might improve clinical outcomes.

In the same line of research, hypothermic machine perfusion providing a pulsatile blood flow is known to offer better protection against cold ischemic injury when compared with cold storage in marginal donor organs. Special effort has to be invested in testing in both experimental and clinical settings the benefits on graft viability when perfused with this newly available hypothermic, pulsatile machine preservation systems.

Last but not least, special scientific attention has to address the pathophysiology of disease and placing it in a clinical relevant context. In this respect, our efforts in documenting new mechanisms of endothelial activation related to variation in blood rheology parameters, the potential consequences of red blood cell aggregation on (micro)circulation might prove to be valuable in managing complications in both cardiac and transplant patients.