Techniques to improve neurological outcome after cardiac surgery
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Chapter 9

Summary and discussion
Summary

The aim of this thesis was to investigate and improve therapeutic interventions, which are currently used in cardiac surgery with the ultimate goal to improve postoperative outcome.

Part A of this thesis focuses on the results of the TO-CABG study, investigating intraoperative tissue oxygenation in patients randomized to undergo elective coronary artery bypass grafting surgery, either on-pump or off-pump. Near infra-red spectroscopy (NIRS) was used to evaluate cerebral, renal and peripheral tissue oxygenation. Further, standardized blood and urine samples were taken and cognitive function was assessed before and after the surgical procedures.

Chapter 2 is the first published article originating from the TO-CABG study, which investigated the effect of surgical technique (on-pump or off-pump) during CABG procedures on the non-invasive intra-operative cerebral tissue oxygenation measurements in relation to development of postoperative cognitive dysfunction (POCD). We found that significant cerebral desaturation was uncommon among our population of patients undergoing CABG procedures, and that the incidence of desaturation, even when using different threshold criteria, was not different among patients randomized for on-pump or off-pump cardiac surgery. However, we did find a significant reduction in the incidence of cognitive dysfunction after 3 months in patients who underwent off-pump CABG. Hence, investigation of the effects of cardiopulmonary bypass (CPB) during cardiac procedures on neurological outcome may render new insights on how to prevent neurological complications and improve postoperative outcome.

In chapter 3, we examined the effects of CPB on biomarkers of neuronal damage and how this associates with long-term postoperative cognitive dysfunction and survival. Classical neuronal injury-related biomarkers did not have a clear prognostic value in our study population. However, free plasma haemoglobin (PfHb) at sternal closure and High-Sensitive Troponin T(hs-TnT) measured within one day after surgery as a measure of myocardial injury were significantly associated with long-term cognitive dysfunction. Additionally, we found significantly higher levels of brain fatty acid binding protein (BFABP) in the pre-operative blood samples of patients who had symptoms of POCD at 15
months after surgery. Further, cognitive dysfunction established at 3 months after CABG, beyond the acute phase, serves as a valuable prognostic factor both for ongoing cognitive decline a year later and for all-cause postoperative mortality. Further research is necessary to investigate the value of alternative neuronal injury-related biomarkers. Our data also suggest that POCD might be considered as an interesting option to function as a surrogate marker for postoperative mortality. Previous studies established that postoperative mortality is closely related to acute kidney injury. Therefore, we evaluated the ability of renal tissue oxygenation measurements to predict postoperative renal function change in adult patients, in comparison with renal injury biomarkers neutrophil gelatinase-associated lipocalin (NGAL), and kidney injury molecule-1 (KIM-1) in Chapter 4. In our analysis, renal tissue specific deoxygenation was associated with increased postoperative sCr in patients undergoing off-pump CABG, thus confirming our hypothesis that renal specific deoxygenation might predict adverse renal outcome. However, in on-pump CABG surgery we found an inverse association between renal specific deoxygenation and renal function, as deoxygenation was paradoxically associated with better renal outcome in a multivariate regression analysis. Hence, we analyzed whether loss of autoregulation renders a logical solution for these paradoxical association by evaluating the relation between intra-operative MAP and SrtO₂. We found a strong positive correlation between MAP and SrtO₂ in the last phase of on-pump CABG, whereas such relation was absent in off-pump procedures. Likely, the positive correlation at the end of on-pump procedures is an indicator of loss of autoregulation of renal blood flow in this population. Our data indicate that the intra-operative loss of renal autoregulation in CABG surgery patients may underlie the opposite relation between intra-operative renal tissue specific deoxygenation and postoperative impaired renal function in on-pump procedures.

To gain further insight into mechanistic effects of a major systemic surgical stimulus on neurological outcome, we decided to investigate the neuroinflammatory response in an experimental model of major surgery. We expected that microglial activation would be triggered more strongly and for a longer time in aged subjects, compared to adults. Therefore, in Chapter 5, both reactive and priming aspects of neuroinflammation after systemic inflammation
in an experimental model of major surgery without sepsis were studied. In this chapter we showed that aged mice expressed higher levels of pro-inflammatory cytokines both in operated and control animals compared to adult mice, whereas they had lower levels of brain derived neurotrophic factor. The activation of pro-inflammatory pathways was increased in aged mice at 4h post-surgery compared to control, yet decreased significantly within 24h, as was seen in adult mice. Thus, we conclude that major surgery on its own does not trigger a prolonged and exaggerated neuroinflammatory response in aged mice.

Part B of this thesis examined the therapeutic benefits of hypothermia or targeted temperature management (TTM) in models of ischemia/reperfusion injury, as this might occur during cardiac surgery. In chapter 6, we review the mechanisms by which TTM conveys a protective effect against ischemia/reperfusion (I/R) injury in the brain. TTM reduces glutamate release and oxidative stress and inhibits the release of pro-inflammatory factors. Hence, TTM counteracts mitochondrial induced apoptosis, neuronal excitotoxicity, and neuroinflammation. Recent literature points out that TTM reduces the unfolded protein response (UPR), by reducing activation of the PERK-eIF2α-ATF4 pathway, and induces SUMOylation and the production of cold shock protein RBM3. Further study of TTM is necessary to refine its utilization and to search for pharmacological agents mimicking or enhancing the cellular effects of TTM. Improving the application of TTM may decrease disease burden of patients suffering from cardiac arrest, stroke, or other post-cardiac surgery complications.

The effects of TTM on long-term postoperative survival in a large population of patients who underwent cardiac surgery were investigated in chapter 7. We identified mild hypothermia (32-35°C) in CABG surgery and mild to moderate hypothermia (30-35°C) in valve procedures to be associated with superior long-term postoperative survival. Interestingly, we also found a large variation in the rate of adaptation of the patient’s core body temperature with the forced cooling by the cardiopulmonary bypass. Consequently, patients may show either high compliance or low compliance with cooling. In both CABG and valve surgery, low compliance with forced cooling was associated with a significant better postoperative survival, independent of targeted temperature. Hence, we established the ideal target temperature for the two most common types of
cardiac surgery and identified a novel temperature related patient factor associated with postoperative survival.

The effect of mild and deep hypothermia on the neuroinflammatory response in a model of cardiopulmonary bypass in the rat is described in Chapter 8. In this chapter, we examined neuroinflammation in rats that underwent CPB procedure under hypothermic conditions either at 33°C or 18°C at 1 day or 7 days of follow-up by comparing standard uptake values (SUV) of [¹¹C]-PBR28 tracer in PET-scans. We found significantly increased SUV in hippocampus and amygdala of rats who underwent CPB at 18°C at 7 days after the procedure, whereas no difference was observed at day 1. Furthermore, we found increased cold shock protein activation in cortex at 1 day and in hippocampus at 7 days of rats who underwent CPB procedure at 33°C. We therefore conclude that deep hypothermia increased the neuroinflammatory response in amygdala and hippocampus compared to mild hypothermia in rats that underwent cardiopulmonary bypass procedure. Additionally, mild hypothermia induced increased expression of TrkB and RBM3 in cortex and hippocampus of rats on CPB compared to deep hypothermia. Together, these data indicate that neuroinflammation is alleviated in mild hypothermia, possibly by recruiting protective mechanisms through cold shock protein induction.
General discussion

Here we will discuss the role of tissue oxygenation during cardiopulmonary bypass in cardiac surgery, in relation to organ function. In addition, we summarize how treatment of elderly patients, and their high risk of developing adverse neurological outcome, is currently being investigated in cardiac surgery. To elaborate on therapeutic options, we will discuss the possibility to turn anaesthesia into an advantage and the benefits of hypothermia in targeted temperature management. Finally, we will discuss our vision on the role of neuroinflammation in postoperative cognitive decline.

1. Cardiopulmonary bypass and tissue oxygenation

1.1 Tissue oxygenation and cardiopulmonary bypass

POCD initially became known as “pump-head”, which referred to cognitive decline observed in patients operated on cardiopulmonary bypass, although a clear etiological relation was not established. As we described in Chapter 2, we found that patients who underwent CABG with CPB displayed a higher incidence of POCD than patients operated off-pump. These findings contribute to the ongoing debate in the field of on-pump vs. off-pump CABG research. Although many papers report similar findings, i.e. increased incidence of POCD following CPB, meta-analyses tend to prove the opposite (Kennedy et al., 2013). Probably, a more dynamic and nuanced picture of interactions between underlying patient-related and perioperative risk factors is more appropriate (Patel et al., 2015). For this reason, we investigated cerebral hypoxia as a mechanism of potential cerebral damage, by applying NIRS monitors to evaluate intra-operative cerebral tissue oxygenation. Although POCD was found in 40% of our on-pump operated patients at 3 months follow-up, very few of them showed a decrease in cerebral tissue oxygenation of the magnitude we expected based on results from previous studies. Apparently, intra-operative cerebral oxygenation during CABG is not a critical factor in the development of POCD.

However, this does not preclude the involvement of vascular dysfunction in the induction of post-CPB organ damage. It might also be possible that the serious damage occurred not during but after utilizing CPB. As we reported in Chapter
4, renal tissue deoxygenation was related to renal dysfunction in off-pump operated patients, but the opposite was true in on-pump operated patients. Thus, we investigated the relation with mean arterial pressure. This revealed a correlation between MAP and renal tissue oxygenation in the last phase of CPB-utilizing CABG being predictive for renal function loss. In cerebral tissue similar autoregulatory failure may underly later cerebral hypoxia during the postoperative phase in on-pump operated patients. We expect common pathways with respect to decreased autoregulatory function in brain as found in kidney after on-pump procedure. Thus, investigating autoregulation of the cerebral vasculature following on-pump and off-pump cardiac surgery would be a logical next step.

1.2 Improving organ function during cardiac surgery

As our studies regarding tissue oxygenation pointed out, preserving organ function is not just a matter of providing adequate oxygen delivery. More important is how the tissues deal with loss of autoregulation and other damaging factors. Recent research has been focusing on the production of damage associated molecular patterns (DAMPs) during CPB. One of those DAMP’s, mitochondrial DNA (mtDNA), is released into the circulation after injury and is associated with the release of reactive oxygen species. For instance, MtDNA increases 9-fold at 12h after CPB utilizing surgery compared to pre-operative levels (Qin et al., 2016). Consequently, new therapeutic strategies to scavenge DAMPs might improve cerebral and renal function.

2. Prevention of neurological injury in the elderly

2.1 Risk assessment prior to surgical treatment

Part A of this thesis focuses on prediction and improvement of postoperative neurological outcome after cardiac surgery. Coronary artery bypass grafting is performed more and more often in an ageing population, which increases the risk of postoperative complications and adverse events. POCD has the greatest impact on the lives of patients when they are back in their homes and specifically not in a hospital environment. Hence, clinicians should be aware of what they do not know. This seems even more warranted, because of the
additional association of POCD at 3 months after surgery and postoperative mortality we observed in our cohort of 49 patients in Chapter 3. Focussing on POCD would thus relay the focus of medical support from medicalization towards quality of life.

Recent evaluation of the literature revealed that there is a general shortcoming in evidence to guide clinical decision making among elderly patients who require cardiac surgery (Rich et al., 2016). Additionally, there is a paucity of data on the impact of diagnostic and therapeutic cardiac interventions on key outcomes that are particularly important to older patients, such as quality of life, physical function, and maintenance of independence. To evaluate individual risk of patients prior to cardiac surgery, many hospitals apply the Society of Thoracic Surgeons Predicted Risk of Mortality or Major Morbidity (STS-PROMM) or the EuroSCORE. Regarding elderly patients, the STS-PROMM score incorporates gait speed as a surrogate for frailty. The EuroSCORE includes an assessment of mobility, indicating that the presence of “severe impairment of mobility secondary to musculoskeletal or neurological dysfunction” contributes to mortality. However, both instruments do not adjust for functional capacity in daily life, cognitive impairment or signs of dementia, thus limiting their utility in patients above 75 years of age (Rich et al., 2016). Of note, the TO-CABG study used EuroSCOREs to analyze pre-operative risk as described in Chapter 2. Although EuroSCOREs indicated that the patients in our population would have a low risk of adverse events, we diagnosed 1 in 4 patients with long-term POCD. Hence, more research on cardiac procedures is necessary in the light of long-term follow-up, focussing on general health status, neurocognitive outcome, quality of life, activities of daily living, and maintenance of independence in elderly patients.

Medical professionals should balance the needs of the individual with pressures to protocolize care. Therefore, we should try and reach the situation in which patients and relatives are able to represent themselves in this discussion as the major beneficiaries of a good decision. Time has come for the debate on whether we should provide more funds to the improvement of cardiopulmonary bypass management to serve the frailest patients or whether we should focus further research on the assessment of long-term outcome and be able to inform frail patients about the consequences of cardiac surgery. Initiatives like the
“Choosing wisely” campaign, by the American Board of Internal Medicine (ABIM) foundation have started to raise awareness about the necessity of medical tests and treatments (choosingwisely.org). The goal of the initiative is to advance a dialogue between patients and clinicians on avoiding wasteful or unnecessary medical tests, treatments and procedures. Until now, recommendations of the Society of Thoracic Surgeons mainly encompass advice about unnecessary pre-operative diagnostic tests. On the other hand, the American Geriatrics Society emphasizes that for elderly patients they do not recommend screening for breast, colorectal, prostate or lung cancer without considering life expectancy and the risks of testing, overdiagnosis and overtreatment. Moreover, abundant screening and pre-operative diagnostics might be too much to handle in frail elderly patients (Clarfield, 2010). These social initiatives can only exist when sufficient evidence is gathered. Close collaboration between government and medical professionals, combined with financial support may aid the process of national discussion about when to treat and when not to treat. Eventually, this will improve quality of life, aid maintenance of independence in elderly patients, and release some of the financial and caretaking burden on our health care system.

2.2 Prediction of neurological deficits

Section 2.1 highlighted the advantages of pre-operative assessment of the individual’s vulnerability to adverse effects, and the necessity for accurate parameters. In Chapter 3, we investigated patients with POCD with 3 months short-term and 15 months long-term follow-up. We found that patients with POCD had significantly higher pre-operative levels of brain-specific fatty acid-binding protein (BFABP), compared to patients without POCD. Healthy elderly individuals usually have BFABP plasma levels <5 pg.ml⁻¹, whereas BFABP in patients with Alzheimer’s disease, Parkinson’s disease or other neurodegenerative disorders usually ranges between 10-20 pg.ml⁻¹ (Teunissen et al., 2011). In our patients, we found pre-operative BFABP levels of 22.8 (8.3-33.0) pg.ml⁻¹ in patients who developed POCD at 3 months after CABG, compared to 9.7 (3.9-17.3) pg.ml⁻¹ in patients who did not develop POCD. Therefore, we suggest the assessment of serum BFABP levels prior to surgery in relation to POCD development in a larger cohort of patients to analyze its predictive value.
3. Turning anaesthesia into an advantage

3.1 Anaesthesia as a constant in surgery

Cardiac surgery is almost always performed under general anaesthesia. In the light of neurological outcome, the effects of intra-operative anaesthetic management and its postoperative consequences should be discussed. All regularly used anaesthetic agents, such as ketamine (Yan & Jiang, 2014), isoflurane (Wei et al., 2007), and propofol (Ulbrich et al., 2016), have been shown to induce neurotoxicity in some in vitro settings, but also to have neuroprotective properties in other settings. Most anaesthetics alter the processes of neuronal survival and death.

Several alternatives to manage the effect of anaesthetics in experimental study design have been suggested. For instance, procedures aimed at postoperative survival should include sufficient sham operated groups, receiving similar anaesthetic treatment but not the surgical intervention. Also, it can be informative to include another anaesthetic agent, with a different mechanism of action from the primary anaesthetic used. Survival studies should follow-up at least 7 days after the procedure (Karmarkar et al., 2010). In the study described in chapter 8, we initially planned to perform CPB under propofol and fentanyl anaesthesia. However, due to unknown causes, the animals did not survive the first 24 h after the procedure. A subsequent switch to ketamine anaesthesia allowed us to continue with the study without these untimely losses. Sufficient sham groups were included under ketamine anaesthesia and follow-up lasted up to 7 days. A major benefit of in vivo PET-scans is that animals are not sacrificed immediately, but neuroinflammatory processes can be followed in time, thus allowing the monitoring of potential neurotoxic or neuroprotective processes. However, the rats were anaesthetized with volatile anaesthetics while undergoing PET-scans. This is a limitation, which can be avoided by performing human studies.
3.2 Anaesthetic pre-conditioning

It might be worthwhile to address the neuroprotective properties of anaesthetics to prevent secondary neurotoxic effects. Several studies on pre-conditioning point out the neuroprotective features. Preconditioning with isoflurane can inhibit later isoflurane-induced neurotoxicity (Wei et al., 2007). Ketamine anaesthesia as a neuroprotective agent is frequently investigated in animal and cellular studies, which demonstrate the neuroprotective effect in various settings. However, virtually no clinical trial data are available (Himmelseher & Durieux, 2005).

A recent study on the expression of brain-derived neurotrophic factor (BDNF) in hippocampal sections associated aging with a decrease in NMDAR-CaMKII activity, which leads to decreased BDNF levels (Palomer et al., 2016). As ketamine antagonizes the NMDAR, one would not consider this as a prime anaesthetic agent to induce neuroprotection in aged subjects. However, as we found Bdnf expression to increase significantly only in ketamine anaesthetized aged mice at 4h after i.v. ketamine administration (chapter 5), it might serve as a valuable inducer specifically in aged brain. We would therefore suggest studies designed to investigate the effect of preconditioning with ketamine in low dose prior to cardiac surgery in two age groups. Indeed, ketamine causes dose dependent direct stimulation of the CNS that leads to activation of the sympathetic nervous system. Consequently, ketamine is associated with increased blood pressure, heart rate, cardiac output, and myocardial oxygen requirement. However, critically ill patients may unexpectedly respond to ketamine with decreases in blood pressure and cardiac output. This represents depletion of endogenous catecholamine stores and exhaustion of sympathetic compensatory mechanisms (Erstad et al., 2016). In a cardiac surgery population, initial studies should therefore only include patients eligible for elective procedures, with a relatively low pre-operative risk. Moreover, monitoring of these patients while on preconditioning therapy with continuous ECG, and adequate dosing are highly important.
4. Benefits of hypothermia

4.1 Targeted temperature to improve survival

We identified 32°C as the target temperature associated with the best postoperative survival in Chapter 7. Over the years, there has been a tendency to abandon deep cooling (below 30°C) during cardiac surgery and to adopt more tepid temperatures (above 34°C) in standard procedures such as CPB assisted CABG and valve surgery. Mild hypothermia was associated with the highest survival rates, as is shown in Figure 1, during a follow-up of 5 years after CABG and valve surgery. Had TTM been applied with a temperature target of 32-33°C, survival percentages might have been higher in both the mild hypothermic category of which patients were cooled to 34°C and 35°C, as well as in all other temperature categories.

To illustrate the impact of proper TTM, we calculated the number of patients in whom survival was extended beyond 5 years for all temperature categories should they have been cooled to 32-33°C during surgery (Table 1). The temperature adjusted mortality was then 525 patients in CABG surgery and 436 patients in valve surgery. Mortality rates then dropped with 174/699 = 25% for CABG and with 111/547 = 20% for valve procedures by evadable deaths. Thus, there is a considerable theoretical advantage of targeted temperature management to 32-33°C during cardiac surgery.

Figure 1. Postoperative survival in CABG (Panel A) and valve surgery (Panel C) per category of hypothermia. Mild hypothermia (green) has the highest survival rates in both types of surgery, whereas normothermia (blue) in CABG and severe hypothermia (black) in valve surgery have the lowest survival (75% after 5 years).
### Table 1. Current survival rates at 5-year follow-up and survival adjusted for TTM at 32-33°C in CABG and valve surgery

<table>
<thead>
<tr>
<th>CABG</th>
<th>Patients in category</th>
<th>Survival at 5 year</th>
<th>Mortality at 5 year</th>
<th>Adjusted TTM mortality</th>
<th>Lives saved</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normothermia 35-37°C</td>
<td>196</td>
<td>156</td>
<td>40</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Mild hypothermia 32-35°C</td>
<td>1694</td>
<td>1506</td>
<td>188</td>
<td>176</td>
<td>12</td>
</tr>
<tr>
<td>Mild/Moderate 30-32°C</td>
<td>2633</td>
<td>2263</td>
<td>370</td>
<td>274</td>
<td>96</td>
</tr>
<tr>
<td>Moderate/Severe 28-30°C</td>
<td>393</td>
<td>310</td>
<td>83</td>
<td>41</td>
<td>42</td>
</tr>
<tr>
<td>Severe hypothermia &lt;28°C</td>
<td>134</td>
<td>116</td>
<td>18</td>
<td>14</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>5050</td>
<td>4351</td>
<td>699</td>
<td>525</td>
<td>174</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Valve</th>
<th>Patients in category</th>
<th>Survival at 5 year</th>
<th>Mortality at 5 year</th>
<th>Adjusted TTM mortality</th>
<th>Lives saved</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normothermia 35-37°C</td>
<td>112</td>
<td>93</td>
<td>19</td>
<td>15</td>
<td>4</td>
</tr>
<tr>
<td>Mild hypothermia 32-35°C</td>
<td>1016</td>
<td>879</td>
<td>137</td>
<td>137</td>
<td>0</td>
</tr>
<tr>
<td>Mild/Moderate 30-32°C</td>
<td>1436</td>
<td>1177</td>
<td>259</td>
<td>194</td>
<td>65</td>
</tr>
<tr>
<td>Moderate/Severe 28-30°C</td>
<td>411</td>
<td>349</td>
<td>62</td>
<td>55</td>
<td>7</td>
</tr>
<tr>
<td>Severe hypothermia &lt;28°C</td>
<td>258</td>
<td>188</td>
<td>70</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Total</td>
<td>3233</td>
<td>2686</td>
<td>547</td>
<td>436</td>
<td>111</td>
</tr>
</tbody>
</table>

4.2 Analysis of patient temperature compliance

In this thesis, we identified patient compliance with forced cooling as a novel variable that was independently associated with postoperative survival. The cause of the large variation in how patients respond to forced temperature changes is still unknown. Furthermore, we will have to speculate on why it is independently associated with survival.

Further research on patient temperature compliance with forced cooling should target achievement of three major aims:

1. To test theories that might explain the independent relation of temperature compliance and postoperative survival.
2. To develop a theoretical model to predict the temperature compliance response.
3. To investigate methods to alter compliance in the individual patient and investigate its effects on surrogate markers for outcome.

As we found patient compliance to be a determining factor for long-term postoperative survival, it might be valuable to assess the possibilities to determine patient compliance prior to cardiac surgery. By doing so, we might improve individual risk stratification of patients and investigate possibilities to alter patient compliance to forced cooling. Thusfar, we have analyzed patient compliance while patients were under anaesthesia, had undergone thoracotomy and had the cardiopulmonary bypass machine connected to induce the forced cooling. At this stage, additional monitoring may be applied to analyze central and peripheral vasodynamics. In addition, blood samples can be taken to analyze the presence of vaso-active substances, cold shock proteins, and proteins related to mitochondrial uncoupling. Future studies are necessary to assess the possibility to establish compliance prior to cardiac surgery, for instance by analyzing the effect of isolated limb cooling on vasoreactivity with Doppler ultrasound and NIRS monitors.

Hypothesizing on the underlying mechanism of temperature compliance to forced cooling, we consider two explanations: altered vasoreactivity and metabolism related temperature induction. Vasoreactivity was previously investigated in a swine model of cardiopulmonary bypass, where continuous cold cerebral perfusion caused a temporary cerebral arteriolar constriction. However, when combined with circulatory cardiac arrest, cold cerebral perfusion was associated with endothelium-dependent vasospasm (Schmoker et al., 2009). Nitric oxide (NO), a potent vasodilator, was investigated for its effect on systemic vascular resistance during TTM in CPB-assisted valvular surgery. Serum NO levels were higher in mild hypothermic (34°C) CPB, when compared to deep hypothermic (28°C) procedures (Ohata et al., 2000). However, our data from the cohort described in Chapter 7 suggest that these differences in NO are absent or that they do not influence patient temperature compliance, as we did not find an association between patient temperature compliance and targeted temperature.

Another possible explanation for the differences in temperature compliance might be from metabolic origin, such as mitochondrial uncoupling in response
Summary and discussion

to TTM. Cold exposure activates and recruits brown adipose tissue (BAT), which has high metabolic activity. BAT recruitment is associated with increased energy expenditure and decreased fat percentage (Saito et al., 2016). Possibly low compliant patients had more BAT activity and uncoupling activation in response to forced cooling than medium and high compliant patients. After cold exposure, skin temperature of healthy volunteers in the supraclavicular region close to BAT deposits dropped by 0.14°C when high BAT activity was found by $[^{18}F]$-fluorodeoxyglucose (FDG)-PET scan, whereas it dropped significantly lower by 0.60 °C in the BAT-negative group (Yoneshiro et al., 2011). These findings were independent of patients BMI or body fat index and possibly explains why an association between body temperature compliance and BMI was absent in our cohort. BAT can produce body warmth through uncoupling protein-1 (UCP-1), which uncouples the respiratory chain from ATP production. Uncoupling then induces non-shivering thermogenesis. Additionally, UCP1 decreases the production of free radicals during cooling, hence underlining the role of metabolic thermogenesis in preventing organ damage during hypothermia (Stier et al., 2014).

Hence, it might be worthwhile to investigate compliance in a pre-operative setting. As we consider temperature compliance to be a systemic response that involves the adjustment of core body temperature, the process itself is not easily simulated. However, the response of the vascular system to regionally applied hypothermia can be assessed in relation to peripheral vasoconstriction and oxygenation. In order to achieve aim 2, we need strategies to investigate patient temperature compliance in a pre-operative setting. For instance, it might be worthwhile to assess in cardiac patients whether limb cooling or a brief drop in core body temperature induce a vasoreactive response and the activation of BAT and UCP1 expression. Thereafter, these findings can be related to intra-operative findings on temperature compliance.

4.3 Cold shock pre-conditioning

As a third aim in investigating patient temperature compliance, it might prove valuable to induce a low compliance response to cooling in the individual patient. Based on the results we found in Chapter 8, we should intend to induce cold shock as a form of pre-conditioning. Cooling induces the production of
cold shock protein RBM3. Increased levels of RBM3 are associated with regeneration of synapses and improved neurobehavioural outcome in two models of neurodegeneration (Peretti et al., 2015). Hence, it would be interesting to investigate patient temperature compliance in relation to endogenously produced cold shock proteins. Hypothetically, we might already achieve such a cold shock response in the brain by applying systemic hypothermia for a relatively short time period, preferably in an anaesthetized patient to combine it with the positive effects of anaesthetic pre-conditioning and to prevent shivering which might prevent a decrease of core body temperature to the targeted temperature.

First, it might prove efficient to analyze the optimum time at which the highest levels of RBM3 are present in specific brain regions. In Chapter 8, we found highest expression levels of RBM3 and TrkB receptor in rat cortex cooled at 33°C at 1 day after cooling, compared to 7 days after cooling. We saw a similar response in CPB treated and sham animals, hence the effect was likely related to TTM rather than to the procedure. Moreover, other time frames need to be tested prior to application in clinical studies. Ideally, the initiation of RBM3 RNA translation takes place within minutes after the ideal cooling temperature is reached. Then, cooling pre-conditioning does not need a separate intervention other than the usual cardiopulmonary bypass initiated procedure. If highest levels are found at one to several days after cooling, it might be worthwhile to investigate further. Our CPB study applied hypothermia to 33°C for 1 hour. Other studies need to investigate whether longer application of cold induces higher increases in RBM3 and TrkB receptor expression.
5. Neuroinflammation and cognitive decline

5.1 Neuroinflammation in our studies

We investigated the neuroinflammatory response in two experimental studies. When looking at the response generated after a major surgical procedure in aged and adult mice, described in Chapter 5, we did not find a strong, longlasting or primed neuroimmune response. The other model of the cardiopulmonary bypass did show a significant neuroinflammatory response in hippocampus and amygdala, but only at 7 days after the procedure and only in rats cooled to 18°C (Chapter 8). The application of CPB at 33°C did not induce a significant neuroinflammatory response, compared to pre-operative levels or sham procedure. From this, we conclude that the prevalence of neuroinflammation after surgery in neurologically healthy adult and aged patients is overestimated. It is known that the initiation of neuroinflammatory responses is sensitive to the accumulation of stimuli, which by themselves just induce a primed status of the microglia (Perry et al., 2007). The situation might be different when patients have underlying neurocognitive dysfunction (Holmes et al., 2009). Combining the results from our experimental studies with the incidence of POCD in Chapter 2 and Chapter 3 in patients with a low risk EuroSCORE profile, an association between neuroinflammation and POCD is unlikely.

5.2 Models inducing neuroinflammation

We therefore suggest that neuroinflammation is generally overestimated in experimental surgical models. This view is endorsed by the fact that many studies on neuroinflammation and postoperative cognitive outcome use a septic model or lipopolysaccharide (LPS) to help induce the neuroinflammatory response. More research needs to be done on the effects of surgery on the neurological processes and pathways without sepsis induction. Obviously, after cardiopulmonary bypass assisted cardiac surgery, the magnitude of potential DAMP expression is larger than after non-cardiac surgery or off-pump cardiac surgery.

Microglial activation is assumed to be initiated in response to surgical interventions, for instance by peripheral inflammatory processes or circulating
pathogen associated molecular patterns (PAMPs) such as lipopolysaccharide (LPS) in the case of bacterial infections and sepsis. However, most animal studies using LPS describe the administration of supraphysiological dosages, when taking the LPS content of gram-negative bacteria into account (Hoogland et al., 2015). Consequently, the microglial response of triggering inflammation with alive or heat-killed bacteria is less profound compared to neuroinflammation found in the experiments using a challenge with these large LPS doses. Because of the differences in neuroinflammatory response between a peripheral challenge with LPS or live bacteria, the clinical relevance of using LPS as a model for surgery is questionable (Hoogland et al., 2015).

To tackle the issue, it would be very interesting to apply the $^{11}$C-PBR28 PET-scans in patients with postoperative delirium and POCD in acute phase and at 3 months follow-up after cardiac surgery. However, these study designs may be considered unethical, as they are exhausting and invasive procedures in an already vulnerable patient population. Meanwhile, it is necessary to focus on better risk assessment in elderly patients, in addition to high quality research after cardiac surgery with long-term follow-up, focusing on general health status, neurocognitive outcome, quality of life, activities of daily living, and maintenance of independence in elderly patients.