CHAPTER 9

SUMMARY AND CONCLUSIONS
Activated leucocytes play a key role in the generalized inflammatory response that occurs after cardiac surgery. They have a pivotal role in reperfusion injury after ischaemia, and also interact with the vascular endothelium and the cardiopulmonary bypass circuit. This generalized inflammatory response leads to postoperative tissue injury. To minimize or even prevent postoperative tissue injury, it is thus indicated to modify the effects of leucocytes. Various anti-inflammatory strategies have been tried, such as the coating of the cardiopulmonary bypass circuit, pharmacological interventions as the use of corticosteroids and aprotinin and the use of a cell-saver for the washing of wound blood. Removal of leucocytes by means of a filter, however, seems to be the most effective treatment. Good results have been achieved with leucocyte depletion in the setting of ischaemia and reperfusion. However, not only activated leucocytes are associated with postoperative tissue injury, but also retransfused fat. Fat emboli have been demonstrated in the brain after cardiac surgery and are associated with ischaemic brain injury. Fat emboli have also been demonstrated in lung and kidney tissue.

Improved filter technology may thus include the removal of various harmful substances such as fat from the blood. Thus, the aim of this thesis as outlined in chapter 1, was to demonstrate that leucocyte and fat filtration, applied in the setting of cardiac surgery, have a beneficial effect on inflammatory markers and postoperative organ injury.

Conflicting results have been reported about the clinical effects of leucocyte depletion with an arterial line filter during the whole period of cardiopulmonary bypass. Therefore, in chapter 2 a new leucocyte depletion method is described. In a randomized prospective study in thirty patients undergoing elective cardiac surgery, we investigated whether leucocyte depletion from the residual heart-lung machine blood at the end of cardiopulmonary bypass would improve lung function and reduce the postoperative inflammatory response. In the leucocyte-depletion group all residual blood was filtered by leucocyte depletion filters before reinfusion in the patient, whereas in the control group an identical amount of residual blood was reinfused without filtration. In the leucocyte-depletion group, circulating leucocytes and granulocytes were reduced, and the postoperative arterial oxygen tension was higher one hour after arrival to the intensive care unit and after extubation. These results suggest that leucocyte depletion of the residual heart-lung machine blood improves postoperative lung gas exchange function and reduces the inflammatory response.

In chapter 3, a similar study is described in children presenting for congenital heart surgery. The inflammatory response after cardiopulmonary bypass in children is more severe than in adults. In addition, cyanotic children are also more vulnerable to oxygen radicals. We therefore expected a better clinical effect of leucocyte depletion of the residual heart-lung machine blood in children than in adults. The residual heart-lung machine blood was filtered with a leucocyte depletion filter in 25 children before reinfusion. A control group of 25 children received this blood unfiltered. We measured postoperative leucocyte counts and arterial blood oxygenation, and found that the postoperative leucocyte counts were significantly lower in the filter group than in the control group. This difference reached a maximum on the second postoperative day. However, in contrast to our study in adults, there was no difference in arterial blood oxygenation on the first postoperative day.
Chapter 4 addresses the question whether a leucocyte depletion filter removes activated granulocytes or a general leucocyte population. This has implications for the efficacy of the filtration process. After clinical use, we examined 11 filters morphologically and immunologically. In addition, β-glucuronidase was measured in 8 patients before and after the filter to determine whether leucocytes were activated during filtration. Microscopic evaluation revealed that granulocytes were trapped significantly more in the first blood contact layer of the filter material than in the other layers. A maximal CD45RO expression was measured on granulocytes trapped inside the filter material indicating that these granulocytes were activated. In contrast, the β-glucuronidase concentration did not increase after filtration, suggesting the absence of activation of granulocytes by the filtration process. These results suggest that a leucocyte depletion filter removes activated granulocytes rather than leucocytes at random and imply that a leucocyte depletion filter is suitable for use in cardiac surgical patients.

In chapter 5 three major leucocyte filtration strategies were compared in order to define optimal duration of the filtration procedure as well as flow and pressure conditions in the filter. These filtration strategies were: filtration of arterial blood throughout cardiopulmonary bypass (associated with high flow and pressure gradients), filtration of a part of the venous return blood in the rewarming phase during cardiopulmonary bypass (associated with intermediate flow, but high pressure), filtration of residual heart-lung machine blood during transfusion into the patient after cardiopulmonary bypass (associated with low flow and low pressure), and a control group without filtration. We measured circulating leucocyte counts, plasma elastase levels and arterial blood oxygenation, and examined filters postoperatively by scanning electronmicroscopy. Although we could not demonstrate a clinical difference among the three leucocyte depletion strategies, the laboratory results suggested that leucocyte filtration at low flow and pressure conditions is associated with less leucocyte damage and less release of elastase.

Chapter 6 gives an introduction into the concept of fat filtration. Recently, fat microemboli have been demonstrated in brain tissue after cardiopulmonary bypass. These were related to retransfusion of cardiotomy suction blood and associated with postoperative neurocognitive dysfunction. Therefore, attention is again focused on the adverse effects of retransfusion of cardiotomy suction blood during cardiac surgery. In addition, the role of fat on organ injury may have been underestimated, because fat microemboli have not only been demonstrated in brain tissue after cardiopulmonary bypass, but also in lung and renal tissue.

In chapter 7 the use of a fat removal filter for surgical wound suction blood was examined with emphasis on the efficacy of the filter in a clinical setting. We choose wound suction blood during cardiac surgery as this blood contains a considerable quantity of fat and particulate microemboli. Coronary artery bypass patients were randomly divided into two groups. In one group cardiotomy suction blood was filtered with a fat removal filter, in the other group this blood was retransfused without filtration. Filter efficacy was evaluated using biochemical assays and thin layer chromatography of blood samples taken simultaneously before and after the filter. In addition, clinical and biochemical markers for organ injury were determined in both groups. The fat filter removed 40% of fat, leucocytes and platelets from cardiotomy suction blood. Chromatography showed a significant reduction in free fatty acids,
phospholipids and triglycerides. Clinically, leucocyte counts were similar, but platelet counts were higher in the filter group on the first postoperative day. Although from this small scale study fat filtration appeared promising, a larger study is necessary to determine the clinical effects on organ injury.

In chapter 8 the fat filter was used for the cardiotomy suction blood during cardiac surgery, but emphasis was put on cerebral effects and on the inflammatory response. In one group of coronary artery bypass patients the cardiotomy suction blood was filtered with a fat removal filter and retransfused, in the other group the cardiotomy suction blood was completely discarded. We measured triglyceride and glycerol, and neuron-specific enolase and S-100\(\beta\) as brain injury markers, and circulating total white blood cell and granulocyte counts and interleukin-6 as inflammatory markers. Apart from a transient increase in S-100\(\beta\) and neuron specific enolase values in the filter group, there was no difference between the groups on the first postoperative day. Triglyceride levels on the first postoperative day were similar. Total white blood cell and granulocyte counts were higher in the filter group.

The filtration related transient increase in brain markers and the higher white blood cell and granulocyte counts in the filter group suggested that the filter efficacy should be improved.

**Conclusions**

In the clinical studies presented in this thesis leucocytes and fat particles were filtered during and after cardiac surgery. The results of these studies suggest a beneficial effect of perioperative leucocyte and fat filtration on postoperative tissue injury. The best clinical results were achieved with the filtration of the residual heart-lung machine blood. Filtration techniques for cardiotomy suction blood are promising. However, it should be kept in mind that the fat filter we used was not very effective. Therefore, with an improved filter better clinical results might be obtained.

There is evidence that leucocytes, fat and particulate all contribute to postoperative tissue injury. The results of this thesis suggest that leucocyte, particulate and fat filtration may be considered as one entity from an inflammatory point of view. Therefore, from a clinical point of view, one filter suitable for the cardiotomy suction blood as well as the residual heart-lung machine blood should be developed.