PH.D. THESIS

THE RESPONSE OF THE LUNGS DURING CARDIAC SURGERY CARRIED OUT ON CARDIOPULMONARY BYPASS

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1 INTRODUCTION

Cardiopulmonary Bypass (CPB) is one of the major technological advances in medicine. In this “whole body perfusion” method, the functions of the heart and the lungs are replaced temporarily with an extracorporeal circuit. Despite its complex structure, CPB needs to be absolutely safe, predictable and precise in terms of its performance. Although mortality of cardiac surgery has fallen, complications and morbidity associated with the use of cardiopulmonary bypass (CPB) still persist even after nearly 50 years of research, development and practice. CPB is associated with inflammatory response, mainly caused by surgical trauma, contact of the blood with the artificial surface of the circuit, ischaemia and reperfusion injury, resulting in increased capillary permeability, respiratory distress, low cardiac output, and multiorgan failure. Several equipment and techniques have been developed for ameliorating the damaging effects of CPB. Hence in an effort to avoid the adverse effects of CPB, Coronary Artery Bypass Grafting (CABG) without CPB "off-pump method” has been gaining popularity as an alternative to the conventional "on-pump" technique for myocardial revascularization. This includes Minimally Invasive Direct Coronary Artery Bypass (MIDCAB) and full sternotomy Off-Pump Coronary Artery Bypass (OPCAB) methods. Nevertheless, these techniques still remain a subject of dispute and controversies and the decline of MIDCAB seems evident.

Respiratory problems are common after open heart surgery. The causes are multifactorial, but in general, the likelihood of respiratory difficulty depends directly on the patient's preoperative pulmonary function, the duration of CPB, and the cardiac performance after surgery. CPB alters the pulmonary function and morphology, but the exact pathogenesis of these changes is still not clear. The use of CPB predisposes some patients to acute respiratory failure, whereas in others, who already suffer from acute respiratory failure, long-term partial CPB sometimes improves lung function and therefore stands as a lifesaving modality. Theoretically based approach and investigation of the functional and structural alterations in the lungs during CPB is important for the following reasons:

1. **The heart and the lungs form an anatomical and functional unity with mutual effects on each other.**
2. **Traditional open-heart surgery with the use of CPB often results in various pulmonary complications.**
3. **Nowadays, cardiac surgeons often have to operate on patients with impaired lung function.**
4. **Lung, and heart-lung transplantation, are widely accepted treatment modalities.**

2 AIMS OF THE THESIS

The aim of this thesis has been to investigate the clinical, pathological and immune effects of cardiopulmonary bypass on the lungs during cardiac surgery.

In my thesis I have tried to find the answers to the following questions:

1. **Nearly 50 years after the first use of CPB, what kinds of pulmonary complications occur and what is their incidence, following the use of CPB?**

2. **With special emphasis on lung complications, does the dismissal of the use of CPB affect the development of postoperative complications?**

3. **What histochemical changes are induced in the lungs by CPB?**

4. **What correlations exist between the histochemical and the postoperative haemodynamical changes?**

3 MATERIALS AND METHODS IN GENERAL

Altogether 822 patients have been involved in a complex retrospective, and 81 patients in prospective study protocol that has consisted of various types of investigations. None of the involved patients has taken part in more than one kind of investigation. Only patients who had coronary artery revascularization have been involved in these studies. All but 7 patients had their operations performed on Cardiopulmonary Bypass. The clinical study protocols had been approved by the Hospital Ethics Committee. Verbal and written consent was requested from all patients before enrolling them in any investigation. All investigations complied with the rules of the Helsinki Declaration.

3.1 Notes on surgery

Median sternotomy and harvesting of the left internal mammary artery and of the saphenous vein occurred in all cases. In those CABG cases where CPB was applied, cannulation for CPB routinely involved the insertion of an arterial cannula into the ascending aorta and also the insertion of either one or two venous cannulas into the right atrium. Myocardial protection was achieved with antegrade crystalloid cardioplegia and by topical cooling with ice-sludge. In OPCAB cases the target coronary vessel was exposed, and the surrounding area was stabilised by a mechanical stabiliser. The target vessel was then snared proximal and distal to the chosen point for anastomosis. The coronary artery was then opened and the anastomosis performed. An intracoronary shunt was used only in the case of relative electrocardiographic or haemodynamic instability and excessive bleeding during the completion of the anastomosis.

3.2 Notes on anaesthesia

Anaesthesia was carried out intravenously in a similar manner with the use of midazolam, alfentanyl, propofol and pipecuronium. Priming solution of the CPB system consisted of the following: 1000 ml. Ringer lactate, 100 ml. Mannisol, 100 ml. 20% Albumin (Biotest) and 60 ml. 8.4% sodium bicarbonate. CPB was performed with the use of core cooling to 34-35 ^{O}C and pulsatile flow of 2.4 l/body m^2/minute. Coagulation was suspended with sodium-heparin in all cases. In a certain subgroup of patients, cardiac index, cardiac output, total peripheral resistance and pulmonary wedge pressure were also monitored with the use of the Swan-Ganz technique.

3.3 Statistical analysis

Because of the misleading effects of haemodilution in those operations performed on CPB, I have used the following equation for data correction:

$$\text{Corrected value} = \frac{\text{Blood sample concentration X starting haemoglobin value}}{\text{Blood sample haemoglobin value}}$$

All data were analysed with the use of a statistical software program (SPSS, 7.5.1 SPSS Inc, Chicago, Ill.). Continuous and normally distributed data are presented as mean ± SD and were analysed with the use of variance analysis (ANOVA). Not normally distributed data are presented as median (interquartile range) and were analysed with the use of the Mann-Whitney U test for comparison between groups and the Wilcoxon sign ranks for comparison within groups. A probability value of less than 0.05 was regarded as statistically significant. In case of any special implications of the methods, further remarks will be devoted to the subject.

4 ADULT RESPIRATORY DISTRESS SYNDROME FOLLOWING OPEN HEART SURGERY

In this retrospective study, I have tried to delineate those perioperative factors that might contribute to the development of ARDS following open-heart surgery.

4.1 Patients and methods

Between November 1 1994 and October 31. 1997, 837 cardiac operations on CPB were performed in the Department of Cardiac Surgery of Zala County Hospital. Those patients who did not develop ARDS after surgery have served as a control group. With retrospective, statistical analysis, we have reviewed all data concerning the past medical history and the current operation. χ^2 probe, Student *t* test and Mann-Whitney test were used for the analysis. I applied logistic regression analysis for the multivariate investigation.

4.2 Results

Based on data from the past medical history and from the pulmonologist's opinion on examination, 46 patients (5.5%) had chronic obstructive, 44 (5.3%) had restrictive and 6 (1.3%) had mixed pulmonary disease preoperatively. Table 1. represents the incidence of the main postoperative pulmonary complications.

ARDS developed in 10 (1.2%) of the operated patients. One of these cases had a fatal outcome due to multiorgan failure added to ARDS. Autopsy findings justified and confirmed the clinical diagnosis in this patient. ARDS has not developed following mitral- and multiple valve replacement and repairs of congenital cardiac malformations.

ARDS has proved more prevalent in patients suffering from COPD (2/46) and in the group of combined operations (2/67) however this difference between the study and control groups has not proved statistically significant. Those factors that have shown correlation with the development of ARDS on bivariate analysis are presented in Table 2. Regarding the postoperative variables, CPB duration along with the length of ischaemia and anaesthesia has proved longer in the ARDS group.

Table 1. *Incidence of main postoperative pulmonary complications.*

Complication	Number of cases	%
ARDS	10	1,2
Pneumonia	9	1,1
Embolism	3	0,35
PTX	17	2,03
Serious pleural effusion	15	1,8

In that model where laboratory variables have also been taking in consideration (n=464), multivariate analysis has proved that pathologically elevated preoperative serum ASAT/ALAT and WBC count values were more common in the ARDS group (see the contents of Table 2.). Blood transfusion as a known risk factor has also been more prevalent in our patient population. Surprisingly enough however, significantly (p = 0,0002) more units of FFP were given to those patients who have subsequently developed ARDS. We have every reason to presume that FFP may stand as a serious independent risk factor. No similar data have been found in the literature.

Table 2. *Correlation of postoperative ARDS with perioperative variables.*

Bivariate analysis	***ARDS***	***Control***	***P***
Preoperative WBC (10^6/ml)**	9,3 ± 0,9	6,8 ± 2,0	0,01
Preoperative ASAT > 37 (U/l)*	40,0	6,4	0,003
Preoperative LDH > 450 (U/l) *	40,0	9,2	0,019
Anaesthesia time (minute)**	427 ± 104	337 ± 90	0,002
Perfusion time (minute)**	165 ± 55	122 ± 62	0,028
Ischaemic time (minute)**	101 ± 38	79 ± 34	0,049
Postoperative RCB Transfusion (unit)**	7,4 ± 4,1	4,3 ± 3,5	0,007
Postoperative FFP Transfusion	6	3	0,0002
Postoperative AMI *	40,0	6,5	0,0004
Postoperative LCOS *	50,0	5,7	0,0000

* % (χ^2 test) ** mean ± SD (T-test) *** median (Mann-Whitney test)

Table 3. *Correlation of postoperative ARDS with perioperative variables. "Logistic regression analysis"*

n = 464	B	P
Preoperative WBC (10^6/ml)	0,6355	0,0046
Preoperative ASAT > 37 (U/l)	2,4021	0,0489
Postoperative FFP transfusion (unit)	0,1662	0,0042
Postoperative AMI	4,2085	0,0035
CONSTANT	- 9,3069	0,0001
n = 833	B	P
Postoperative LCOS	2,8040	0,0000
CONSTANT	- 3,6427	0,0000

Postoperative AMI has been seen more often in those cases with ARDS. This might be partially explained by the mutual aggravating effect of LCOS and AMI. On the basis of the above results I have justified that apart from the length of anaesthesia, CPB and ischaemic time, the volume of massive blood and FFP transfusion has shown strong correlation with the incidence of ARDS. Based on the multivariate regression analysis it can be concluded that this syndrome is also correlated with LCOS following open-heart surgery.

5 OXIDATIVE STRESS IN THE LUNGS DURING CPB

5.1 Patients and methods

15 adult patients (13 males, 2 females) undergoing CABG were enrolled in this prospective study. The most important clinical data are shown in Table 4.

Table 4. *Clinical data of enrolled patients.*

Data	Mean ± SD	Range
Age (years)	56.3 ± 11.1	40-72
Body surface area (m^2)	1.84 ± 0.2	1.55-2.12
Body mass index (kg/m^2)	27.2 ± 3.9	20.4-36
Cross clamp time (min)	53.9 ± 16.6	45-106
CPB time (min)	118.5 ± 22.3	62-147
Mechanical ventilation time (hours)	8.5 ± 1.2	4-16
No. of grafts	3.1 ± 0.7	2-4

Spirometry was performed in all patients 2 days prior to surgery. All results of this investigation remained within the normal range.

5.1.1 Study protocol

The following biochemical substances were measured in blood samples taken from the radial artery and the left atrium:

- Malondialdehid (MDA)
- Reduced and Oxydated Glutathione (GSH-GSSG)

- Myeloperoxidase (MPO)
- Superoxide Dismutase (SOD)
- Absolute neutrophil count
- Stimulated radical production of isolated PMN

5.1.2 Results

Although statistically the changes were not significant but we experienced a mild decrease in the serum (starting value: 4 ± 3.4; 30th minute of ischaemia: 3.5 ± 1.5; 40th minute of ischaemia: 3.9 ± 2.3 IU/ml) and RBC (baseline value: 807 ± 322; 40th minute of ischaemia: 700 ± 300; 5 minutes of reperfusion: 636 ± 241; 30 minutes of reperfusion: 692 ± 346 IU/ml) SOD levels during ischaemia and reperfusion.

Even 24 hours after surgery the RBC SOD content proved lower than the starting value (703 ± 352 IU/ml). The concentration values of MDA originating from red blood cells did not show any statistically significant change during surgery but they proved somewhat lower compared to the baseline value (starting value: 57.7 ± 26.7; 40 minutes ischaemia: 52.3 ± 23.3; 5 minutes reperfusion: 57.4 ± 24.9 nmol/ml). However 24 hours following surgery a significant increase could be detected in MDA values (79 ± 39.6 vs. 57.7 ± 26.7 nmol/ml at the beginning of the operation, $p < 0.05$). Plasma MDA values were found continuously and significantly increased during surgery (baseline value: 1.4 ± 0.7; 40 minutes ischaemia: 2 ± 0.9; 30 minutes reperfusion: 2.3 ± 1.1; 24 hours later: 2.5 ± 1 nmol/ml).

The GSH/GSSG ratio derived from the results of measurements in red blood cells has shown a contrasting pattern in the period of ischaemia and reperfusion. This ratio has decreased in the period of ischaemia (from 24 ± 6.3 to 21.5 ± 11.3), indicating the presence of oxidative stress. These unfavourable changes have only come to an end by the conclusion of reperfusion, whilst 24 hours after surgery the GSH/GSSG ratio has exceeded the baseline value (29.4 ± 9.1). While the absolute value of neutrophil count measured in the pulmonary artery has shown a two-fold rise (baseline value: 3.1 ± 1.1 G/L; 40 minutes ischaemia: 6.1 ± 3.3 G/L) it has been first found decreased in the samples taken from the left atrium (baseline value: 3.1 ± 1.1 G/L, 30 minutes ischaemia: 2.5 ± 1.8 G/L), then in the early reperfusion period it has been found suddenly increased again (20 minutes reperfusion: 5.3 ± 3.1 G/L). During ischaemia and reperfusion there has been a significant difference in the pre- and postpulmonary absolute neutrophil count in each case. The superoxide anion producing capacity of PMN cells has increased during the time of ischaemia, then, after the release of the aortic cross clamping it has been found drastically decreased (at the top of ischaemia:

18.7 ± 4.9 early reperfusion: 3 ± 1,3 nmol O^-_2/min/1,5xl0^5 PMN). The changes have proved significant in both cases ($p < 0.001$).

By the end of reperfusion the free radical producing capacity of PMN cells has returned to the values measured preoperatively, then it has risen again 24 hours after surgery. At the same time MPO activity values have shown a 1,5-time rise compared to the baseline values at the end of ischaemia and during early reperfusion and these changes have proved statistically significant. No postoperative pulmonary complication has been experienced in any of the patients.

5.1.3 Discussion

The occurrence of oxidative stress after lung ischaemia and reperfusion has already been shown in animal experiments, but it is not yet well documented in humans during CPB condition.

In this study, I have found neutrophil gradient through the lungs in the prepulmonary (pulmonary artery) and postpulmonary (left atrium) blood samples in the ischaemic and in the early reperfusion period. This phenomenon has indicated that a considerable amount of neutrophil cells has migrated and trapped into the lung tissues due to ischaemia. The superoxide anion producing capacity of PMN cells has clearly demonstrated the ischaemic and reperfusion injury -affecting the lungs as well- taking place during surgery. It has been notable that at the early stages of reperfusion the superoxide producing capacity of PMN cells has come back to normal values for a short term. I assume that this is due to the „wash out" phenomenon. The MPO enzyme that can be found in large quantities in neutrophil granulocytes and which facilitates reactions producing various reactive intermediates appears in the serum under pathologic conditions. Thus a rise in the level of MPO has been found suggestive of PMN activation and of an increase of the free radical reaction as well. Summing up our results I may draw the following conclusions: A large quantity of neutrophil cells migrate into the lungs during heart surgery on CPB due to ischaemia. I have justified the activity of these neutrophils by the detection of the increased superoxide radical producing capacity and of the increased release of MPO. Based upon what stated above one can claim that increased cell activation and pathologic free radical reactions in the lungs should be taken in account in cardiac operations on CPB. Changes of malondialdehid, reduced and oxydated glutathion levels confirm the above statement. In our patient population no serious clinical pulmonary complication has occurred postoperatively, but this fact must not refute our assumption that it is the free radical reactions that serve as a basis for the pulmonary complications occurring in the postoperative period.

6 LACTATE PRODUCTION BY THE LUNGS DURING CPB

Theoretically, the detection of excessive pulmonary lactate production could help grade the severity of lung disease or injury, but these measurements are fraught with technical difficulties. During CPB, pulmonary artery blood flow is either completely or partially shut off and the lungs are mostly perfused by the bronchial flow. This potentially leads to lung ischaemia that depletes the energy stores of the lung tissues.

In the following prospective study I sought to determine whether the lungs release lactate in humans during CPB.

6.1 Patients and methods

In this study I measured lactate concentrations across the lungs in 23 patients who underwent CABG surgery with the use of partial, normothermic CPB. The main data of the patients are summarised in Table 5.

Table 5. *Enrolled Patient's data.*

N:	23
Male/Female:	17/6
Mean age:	60 ± 8.8 years
Mean aortic cross clamp time:	58 ± 9 min.
Mean CPB time:	89 ± 15 min.
Mean graft number:	3.11 ± 0.9
Mean ejection fraction (EF)	48.7 ± 11.3 %
AMI:	20/23
COPD:	9/23

Cardiopulmonary functions were measured by thermodilution immediately before CPB, 10 minutes, 2 and 24 hours after CPB cessation.

6.1.1 Results

I have found that lactate concentration in the arterial (left atrium), and in the mixed venous blood (pulmonary artery) was significantly increased 5 minutes after aortic cross clamping. In the arterial blood it was raised from 1.18 (0.75-2.11) to 3.31 (1.62-5.03) mmol/l ($p < 0.001$) and in the mixed venous blood from 1.21 (0.92-1.99) to 3.07 (1.98-5.12) mmol/l ($p< 0.001$). However, lactate concentration

in the arterial blood slightly exceeded those values found in the mixed venous blood. Lactate ratio figures were constantly increased during the ischaemic period and they were significantly higher compared to the value of baseline ratio. Ten minutes after the release of the aortic cross clamping the ratio returned to the baseline value. Two hours after the cessation of CPB lactate concentration reached another peak in both of the arterial and venous blood samples, 3.21 (1.29-6.68) mmol/l and 3.04 (1.11-6.96) mmol/l consecutively, but the ratio remained unchanged.

Table 6. *Median values of lactate concentrations*

Sample	Postpulmonary blood samples Median (range)	Prepulmonary blood samples Median (range)	*P*
S1	1,18 (0,75 – 2,11)	1,21 (0,92 – 1,99)	Ns
S2	3,31 (1,62 – 5,03)	3,07 (1,98 – 5,12)	Ns
S3	3,02 (1,82 – 4,83)	2,66 (1,47 – 5,02)	**< 0,006**
S4	3,13 (1,66 – 4,58)	2,16 (1,31 – 3,51)	**< 0,003**
S5	2,96 (1,60 – 4,81)	2,18 (1,45 – 4,22)	**< 0,019**
S6	2,37 (1,41 – 4,34)	2,13 (1,21 – 4,96)	Ns
S7	1,92 (1,19 – 2,94)	1,88 (1,16 – 2,50)	Ns
S8	3,21 (1,29 – 6,68)	3,04 (1,11 – 6,96)	Ns
S9	1,99 (1,59 – 7,38)	2,11 (1,43 – 7,16)	Ns

I have found a correlation between body weight and the maximal value of lactate ratio (S4) (0,627 p=0.039). The lactate ratio of those patients with COPD has proved higher after the release of aortic cross clamping compared to the rest of the patients 1,56 vs. 1,15; however the difference has not proved statistically significant (p = 0,055). Lactate values in blood samples taken from the pulmonary artery following aortic cross clamping (S2, S3) have shown positive correlation with the amount of blood transfusion given during the operation (0,56 p= 0,047 in both cases). SV and CI measured 2 hours after the cessation of CPB have shown a strong and negative correlation with the lactate values measured during the aortic cross-clamping period.

7 ROLE OF THE LUNGS ON THE ALTERATIONS OF CYTOKINES DURING CPB

The purpose of this study has been to investigate the fluctuation in cytokine production, during and after CPB and to define whether the lungs produce or consume inflammatory mediators under this clinical condition. The other goal has been to assess the influence of these mediators on the postoperative haemodynamic status.

7.1 Patients and methods

13 consecutive patients (10 males, 3 females) undergoing CPB for elective CABG were prospectively entered into this study. Exclusion criteria were as follows: patients with renal failure, patients with a history of, either obstructive or restrictive pulmonary disease and patients with any kinds of immune disease.

Before the induction of anaesthesia, a Swan-Ganz thermodilution catheter (Corodyn TD-I Touch-Free 7.5 F; B. Braun Medical Inc., Bethlehem PA USA) and an 18 G radial artery catheter were inserted in order to obtain haemodynamic and oxygen transport parameters. The haemodynamic parameters were studied in the following five time intervals:

M1: before anaesthesia induction
M2: before start of CPB
M3: 10 minutes after CPB cessation
M4: 2 hours after CPB cessation
M5: 24 hours after CPB cessation

Pulmonary arterial and left atrial blood samples were obtained simultaneously.

TNF-α, IL-2, IL-6, IL-8 and IL-10 levels in the plasma were determined in the collected samples by means of commercially available enzyme linked immunosorbent assays (ELISA). Different types of leukocyte counts as well as the transpulmonary cytokine gradient were measured.

Because comparisons were made between paired samples, with each patient serving as his or her own control, and because the data were not normally distributed, the concentration figures of cytokines were presented in median and were compared to the baseline values with the Wilcoxon signed-rank test. Data of the pre- and postpulmonary blood samples were compared with the Mann-Whitney U test. Correlation between peak values, and different parameters were assessed by Spearman's rank correlation coefficient.

7.2 Results

Morphometric and demographic characteristics, preoperative cardiopulmonary function, and the duration of surgery are shown in Table 7. Although pH and PaO_2 decreased at the end of surgery, the other hemodynamic and respiratory measurements did not differ significantly over time (Table 8). In both the arterial and venous blood samples the number of plasma leukocytes increased significantly, with the percentage of neutrophil and monocytes increasing and the percentage of lymphocytes decreasing significantly over time.

Table 7. *Main clinical data.*

	Mean ± SD	Range
Age (years)	61.6 ± 8.5	46 – 75
Body surface area (m^2)	1.87 ± 0.2	1.48 - 2.15
Body mass index (kg/m^2)	27.2 ± 3.9	19.6 - 35.3
FVC (% predicted)	98 ± 12	67 - 128
FEV1 (% predicted)	82 ± 6	70 - 95
Cross clamp time (min)	68.8 ± 15.8	41- 102
CPB time (min)	124.5 ± 29.3	60 -167
Mechanical ventilation time (hours)	9.2 ± 4	5 - 17
No. of grafts	3.4 ± 0.8	2 - 5

Table 8. *Intraoperative data.*

	Beginning	End
Mean arterial pressure (mmHg)	92 ± 9	90 ± 11
Heart rate (bpm)	77 ± 9	86 ± 9
Central venous pressure (cm H_2O)	7.9 ± 2.3	7.8 ± 2.7
Cardiac index ($L*min^{-1}*m^{-2}$	2.6 ± 0.2	2.8 ± 0.4
Oesophageal temperature (^oC)	36.5 ± 0.3	36.3 ± 0.5
Arterial pH	7.41 ± 0.04	7.38 ± 0.04
Arterial PCO_2 (mmHg)	39 ± 3	40 ± 2
Arterial PO_2 (mmHg)	475 ± 35	328 ± 88 *
Activated clotting time (s)	133 ± 24	144 ± 32

TNF-α and IL-2 could only be detected in six patients at all. All IL-2 values have remained within the normal range. TNF-α levels did not show any significant variation in the pulmonary or systemic circulation, values above the normal range could only be detected in two patients, however these elevated values did not show any correlation with the patients' clinical condition. In both the arterial and venous blood samples the concentrations of IL-6, IL-8 and IL-10 increased significantly during the investigation period and reached a peak 2 hours after the cessation of CPB.

The concentration of IL-6 has proved somewhat higher in the left atrium in samples S2, S3 and S4 compared to those values measured in the pulmonary arterial samples, however this difference has not been statistically significant. (Table 9)

Table 9. *Values of IL-6.*

Sample	Postpulmonary blood Median (range)	Prepulmonary blood Median (range)	*P*
S1	1.7 (0.4-13.5)	1.6 (0.4-12)	0.84
S2	14.3 (1.4-52.8)	10.4 (1-56.9)	0.38
S3	19.7 (2.5-71.5)	16.6 (3.1-75.2)	0.59
S4	26.5 (2.4-91.1)	20.2 (3.3-88.5)	0.72
S5	35.9 (8.5-309.5)	37 (10-286.4)	0.49
S6	60.8 (18.3-271.3)	61.5 (16.1-300)	0.36
S7	79.2 (14.6-160.4)	84 (17.6-155.2)	0.31
S8	112 (25.3-216.9)	117.3 (22.7-195.5)	0.22
S9	38.2 (16.8-177.6)	39.1 (15.7-182)	0.55

24 hours after the operation the level of IL-8 returned to the baseline. The ratio of IL-8 concentration showed a biphasic pattern (figure 6). In the ischaemic and in the early reperfusion period (time course between 3rd and 7th blood samples) the ratio equalled less than 1,0. Following CPB the ratio equalled more than 1,0. However only 10 minutes after the release of the aortic cross clamp the concentration of IL-8 in the venous blood 71.21 (9.2-411.2 pg/ml), was significantly higher than the concentration in the arterial blood 41.4 (8-127.8 pg/ml).

Concentrations of IL-10 in the venous blood samples were higher than the concentration in the arterial blood samples and this difference was significant in samples S2, S3, S4, S5 (see table 10)

Table 10. *Values of IL-10.*

Sample	Postpulmonary blood Median (range)	Prepulmonary blood Median (range)	*P*
S1	2.1 (0.8-8.3)	2.9 (1.3-10.8)	0.074
S2	8.7 (0.7-104.6)	13.22 (1.8-102.1)	**0.041**
S3	9 (0.8-118.1)	23.5 (1.4-127.5)	**0.007**
S4	23.1 (3.2-141.9)	32.1 (3.7-222.2)	**0.003**
S5	66.5 (11.9-314.9)	96.1 (17.2-402.5)	**0.01**
S6	105.8 (18.5-545.8)	119.5 (18.3-574.6)	0.055
S7	113.6 (15.9-533.5)	114.9 (15.9-516)	0.42
S8	61.7 (20.4-227.9)	62.8 (19.2-330)	0.51
S9	8 (3.1-40.9)	9.5 (4-59.1)	0.13

I have found a correlation between the peak values of IL-6, IL-8, IL-10 and the duration of CPB (0.69 $p = 0.02$; 0.71 $p = 0.001$; 0.72 $p = 0.028$ respectively), but not with the aortic cross clamp time.

The value of pulmonary vascular resistance (PVR) measured in the 2nd hour (M4) following the cessation of CPB has shown a negative correlation with the IL-6 value measured in the pulmonary artery (S6) after the cessation of CPB: (-0.616; $p = 0.033$).

The value of left ventricular stroke work index (LVSWI) measured in the 2nd hour (M4) following the cessation of CPB has shown a correlation with the IL-10 values measured in samples (S8) from the radial and from the pulmonary artery. Correlation values were: 0.65; $p = 0.022$ and 0.64; $p = 0.03$ respectively. Values of S7 from the left atrium and from the pulmonary artery have shown a negative correlation with the mechanical ventilation length: - 0.71 $p = 0.007$ and – 0.65 $p = 0.02$ respectively.

7.3 Discussion

Numerous clinical studies have shown significant elevation of blood cytokine levels during and after CPB. Such a phenomenon can be induced by several factors, including ischaemia-reperfusion, complement activation, and release of endotoxin. The release of proinflammatory cytokines may be important because such a release seems to be implicated in the development of postoperative complications. Advances in knowledge about the interactions of cytokines involved in the response to CPB may lead to new therapeutic implications which could improve the outcome of patients undergoing cardiac surgery. Administration

of anti-IL-8 antibodies prevents lung ischaemia-reperfusion injury in rabbits. Anti-TNF antiserum may reduce pulmonary and hepatic injury caused by hepatic ischaemia-reperfusion. Removal of TNF-α and IL-6 by hemofiltration has been shown to have beneficial effects in children undergoing CPB. Administration of steroids before CPB not only reduces significantly the release of proinflammatory cytokines but also increases the release of IL-10.

For better insight into the pathophysiology involved, it is important to identify the primary source of these cytokines. There are, however few reported data indicating the resource organ of these cytokines in patients undergoing CPB. To study lung contribution to the release of cytokines I compared lung specific cytokine concentrations in plasma samples taken from blood space immediately before, and after the lungs.

TNF-α may play an important role in the inflammatory response after CPB, not only because it may directly induce some symptoms, such as fever, tachycardia, and hypotension, but also because it may trigger the release of other important cytokines, such as IL-8, and IL-10. Although many have already justified the role of TNF-α in the inflammatory response, I have not been able to obtain a clear-cut view on the kinetics of TNF-α. Presumably, this could happen due to the short half-life and to the locally acting nature of the TNF-α. It would have been much more efficient to measure the TNF-α levels in the lung tissues. The investigation of the soluble receptors of TNF-α seems more promising since these mediators offer a more sophisticated picture on the inflammatory response. These receptors are still to be studied in further investigations in my department.

With respect to IL-2, this study could not prove any serious changes in IL-2 concentrations during CPB. Previous studies reported a decrease of IL-2 and IL-2 receptor expression directly after the start of CPB. However it seems that IL-2 does not have a leading role in the inflammatory response during CPB.

IL-6 is a good marker of injury severity even though it does not have toxic effects itself. IL-8 is a crucial mediator in ischaemia-reperfusion injury in patients undergoing CPB. IL-8 release is induced only after reperfusion of the ischaemic myocardium in animals as well as in human beings.

I found that the plasma levels of IL-6, IL-8 and IL-10 increase significantly in the first hours after surgery. I could prove a positive correlation between the magnitude of the examined cytokines response to CPB and the duration time of the extracorporeal circulation, but not the duration time of the aortic cross-clamp. Moreover my observed data indicate that the lungs are not predominant sources of IL-6 and they may rather consume than release IL-8 and IL-10 during CPB.

The tissue source of the antiinflammatory cytokine IL-10 under CPB still needs to be determined. In this study IL-10 shows a positive correlation with

favourable haemodynamic variables. The experienced phenomenon perhaps verifies the protecting effect of IL-10. My data do not exclude significant cytokine release by other organs. In fact, other organs also have inadequate blood supply during CPB and could similarly be important sources of mediators. Furthermore, the release of endotoxin frequently observed during CPB, as well as complement activation, may trigger the release of cytokines.

8 ADHESION MOLECULES IN PATIENTS UNDERGOING CORONARY ARTERY REVASCULARIZATION

8.1 Patients and methods

12 adult patients (mean age: 59.4 ± 7.2 years) undergoing coronary revascularization surgery requiring the use of CPB have been enrolled in this study (group A). There have been 5 patients (mean age: 59.1 ± 11.2 years) in the control group whose coronary surgery did not require the use of CPB (group B). 3 grafts were carried out in 1 patient (to LAD, CX, RC coronaries), 2 grafts in 3 patients (to LAD, CX or RC coronaries), and 1 graft in 1 patient (to LAD coronary artery) during off pump surgery. The main clinical data are shown in Table 11.

Table 11. *Patients' clinical data*

	Group A	Group B
Case No.	12	5
Gender (male/female)	9/3	4/1
Mean age (years)	59.4 ± 7.2	59.1 ± 8.6
Mean aortic cross clamp time (minutes)	52 ± 6	0
Mean CPB time (minutes)	89.7 ± 18.3	0
Mean mechanical ventilation duration (hours)	7.8 ± 4	6.3 ± 1.5
Mean ICU stay (hours)	43.5 ± 5	40 ± 6
Mean number of grafts	3.23 ± 0.7	2 ± 0.8

Serial blood samples were taken from the central venous line. Since the method of surgery differed in the two groups, we made our best efforts so that the blood samples be taken at the same times in both groups.

At the same time samples added to EDTA were taken for haemoglobin, haematocrit and WBC count. Those blood samples for the analysis of serum levels

of soluble molecules were immediately centrifuged in a cooled centrifuge system. (20 min., 4000/minute revs.) After this, the samples were stored on –20°C temperature until the analysis. The blood samples were analysed within 4 weeks for soluble ICAM-1 (normal average value: 211 ng/ml, ± 2 SD, reference: 115-306 ng/ml) and for soluble E-selectin (normal average value: 46 ng/ml SD, reference: 29-63 ng/ml). The analysis was carried out with the ELISA method (R&D Systems Inc. Minneapolis, USA)

The principle of the measurement is the reaction of the adhesion molecules with two monoclonal antibodies directed against the various epitops on the surface of the adhesion molecules. All measurements were standardised by reactions against either purified recombinant ICAM-1 or E-selectin. The sensitivity (minimal detectable dose) to E selectin and ICAM-1 proved <1.0 ng/ml and <0.35 ng/l respectively. All results are calculated from the average of two different measurements. The serum levels of circulating adhesion molecules were calculated by the use of simultaneous standard charts. In those operations requiring CPB, errors due to haemodilution were corrected as mentioned previously. Normally distributed data are presented as mean ± SD and were analysed with the use of variance analysis (ANOVA). Not normally distributed data are presented as median (interquartile range) and were analysed with the use of the Mann-Whitney U test for comparison between groups and the Wilcoxon sign ranks for comparison within groups. Differences have been regarded significant if the value of p has proved <0.05.

8.2 Results

No complications occurred and no reoperations were necessary in those patients enrolled in the study. The mean length of ICU stay has proved less than 48 hours similarly in both groups.

Neutrophil count has raised significantly in both groups. However, this elevation proved more pronounced in group *A*. A four-fold raise could be detected in group *A* 24 hours following surgery and the figures remained the same throughout the next two days. A significant difference could be detected between the two groups in samples 5, 7, 8, 9 and 10.

There has been no explicit change in the level of serum ICAM-1 and E selectin in those operations without CPB neither during nor after surgery. The figures of serum ICAM-1 and E selectin in blood samples taken in different points of time have remained within the normal range from first to last.

In group *A* however I have found a significant fall in the serum levels of both the ICAM-1 and E-selectin during the cross clamp period. Following the correction of the effects of haemodilution the decrease could still be seen but has already not proved statistically significant. 24 hours after CPB the measured serum

level of ICAM-1 has increased by 76% compared to the starting figure, from 193.8 (148.7-254.3) ng/ml to 340.9 (239.1-404) ng/ml, (p < 0.002). 48 hours after surgery the average value of ICAM-1 has been found slightly decreased but it has still remained significantly higher compared to the starting figure; 193.8 (148.7-254.3) vs. 279.8 (208.8-382.4) ng/ml, (p < 0.002). 72 hours after surgery the serum level of ICAM-1 has come down close to its original starting value 192 (156.4-242) (Figure 23) The serum levels of E selectin have shown a trend similar to that of ICAM-1. The baseline median value of E-selectin was 31.1 (20.9-53.3) ng/ml and it have reached its peak 24 hours after CPB 44.8 (30.9-58.8) ng/ml. This change has proved statistically significant to the baseline value. (p<0.003). The comparison of the measurements made at similar points of time in the two groups is shown in Table 12.

Table 12. *Median values of E-selectin and ICAM-1 in the two groups in nearly coinciding times.*

Sample	E-selectin			ICAM-1		
	Group A	Group B	*P*	Group A	Group B	*P*
1	**31.1** (20.9-53.3)	**29.2** (26.1-39.7)	Ns	**193.8** (148.7-254.3)	**189.2** (168.5-219.4)	Ns
3	**18.9** (12.7-31.4)	**25.3** (22.3-36.3)	*	**136.1** (115.4-197.3)	**198,7** (171.9-212.8)	**
5	**24.3** (13.9-40.2)	**27.1** (22.6-35.2)	Ns	**153.5** (121.8-213.6)	**192,9** (183.7-214.2)	*
7	**31.5** (17.8-49.5)	**24.2** (23.1-36.8)	Ns	**194.7** (147.2-222.3)	**200** (179.2-232.6)	Ns
8	**44,8** (30.9-58.8)	**26.7** (23.2-33.6)	**	**341.9** (239.1-404)	**186,7** (165.7-218.3)	**
9	**34.1** (20.9-51.7)	**27.5** (24.2-31.8)	Ns	**279.8** (208.8-382.4)	**181,4** (164.5-221)	**
10	**27.7** (20.4-56.8)	**30.5** (25.1-32.4)	Ns	**192** (156.4-242)	**192.4** (154.7-234)	Ns

*: p < 0.05 **: p < 0.001 ***: p < 0.0001

8.3 Discussion

Although I have experienced neutrophilia in both groups, this phenomenon has proved more explicit in group *A*. This makes it clear that apart from the

surgical trauma, which has been present in both groups, the effects of CPB resulting in an inflammatory response must also stand out.

In this study the data have proved that the serum level of soluble adhesion molecules decreases in the ischaemic period of CPB. I have found no significant difference in the level of adhesion molecules in the control patients. I believe that due to the nature of the control group I have been successful in offering a straight and clear-cut view on the characteristics of soluble E-selectin and ICAM-1 actions during CPB and in the early postoperative period.

My data confirm the theory that the artificial surface of CPB itself is also responsible for the inflammatory reaction following the use of CPB. Having said that a question still arises: why has the serum level of ICAM-1 and E selectin been decreased during CPB even in the corrected figures, too? I suppose that during the initial period of CPB the adhesion molecules are sequestrated either in the tissues of the lungs and liver or in the components of the artificial circulation (e.g. oxygenator membrane) It is also possible that the mentioned phenomenon is the result of increased adhesion of the adhesion molecules to activated neutrophils.

The serum levels of soluble E-selectin 24 and 48 hours after CPB have reached and exceeded the level recorded before CPB but it has increased beyond the assumed values of the normal population in only two samples. I claim that the expression of adhesion molecules is a particular occurrence of CPB used during cardiac operations. Off pump surgery seems to be confirming this hypothesis.

9 PULMONARY ALVEOLAR DAMAGE DURING CORONARY ARTERY GRAFTING WITH THE USE OF CPB.

The aim of this study was to evaluate the microstructural effects of the use of contemporary CPB on the lungs and at the same time to assess the potential benefits of surgery without CPB.

9.1 Patients and methods

The study included 18 (15 males, 3 females) patients, aged 45-72 years, who underwent coronary artery bypass grafting. None of them had been previously treated for pulmonary disease. Two of the patients underwent OPCAB operation. Otherwise anaesthesia and surgical procedures were uniform, as I have previously described. In CPB procedures, the average time of aortic cross- clamping and bypass time was 58±14 and 101±13 minutes respectively. After sternotomy, lung biopsy was taken from the 4th segment of the left lung (specimen “A”) prior to the initiation of CPB and prior to performing the first distal coronary anastomoses in

the OPCAB group. These biopsies served as control specimens of the basic histopathological findings before surgery. Immediately after the cessation of CPB and after the last coronary bypass was established in the OPCAB group, but before the chest was closed, another lung biopsy was obtained, proximate to the site of the first one (specimen "B"). The average size of biopsy specimens was approximately 75 mm^3. A control group of lung tissue specimens from 5 victims of traffic accidents who had no prior and/or underlying pulmonary disease was set up to determine the normal alveolar cellular constitution and the alveolar septum thickness values.

9.1.1 Histologic processing:

Biopsy specimens were fixed at room temperature in 4% formaline for 12 to 16 hours. Citadel 2000 Shandon automated device was used for processing. Slices of 4μ thickness were stained by HE basic staining with Sakura Tissue TEK DRS device. Nikon Eclipse 800 microscope with built in; factory-fitted, standardised ocular micrometer was used for the microscopic measurement of the alveolar septum.

In each HE stained slices we performed the measurement of 10 randomly chosen alveolar septum with 600x magnifying rate.

Whenever the confirmation of the diagnosis was required, further special staining was applied. (Berlin blue, Gömöri silver, van-Gieson-Picosyrius). Occasionally immune-histochemical methods were also used to detect CD34, CD64, smooth muscle actin and vimentin. Further examinations were carried out with electron microscope on specimens taken from two randomly selected patients.

9.2 Results:

No serious pulmonary complication has developed in the postoperative period. All specimens have proved suitable for histologic evaluation. From biopsies taken before the initiation of CPB (specimen “A”) the diagnosis of the basic pulmonary histological status could be established and the findings could be classified into three main different diagnostic groups allowing intersection between the groups:

1. **Secondary pulmonary hypertension**
   (12 cases, in 5 of those signs of severe disease)
2. **Emphysema**
   (6 cases, 3 of them with signs of centroacinaer type)
3. **Interstitial fibrosis**
   (8 cases, 3 of those with signs of severe disease)

In 3 cases however histologic examination has found intact pulmonary tissue structure. Concerning specimens taken after the initiation of CPB the basic changes have naturally remained the same, however additional superimposed alterations could be observed. Light microscopic observations revealed only a few alterations in the structure of lung alveoli. Oedema as well as extravasated erythrocytes and neutrophils were present in specimens of six patients. Swelling of endothelial cells, of membranous pneumocytes, and of mitochondria in granular pneumocytes; interstitial haemorrhage (predominantly perivascular); engorgement of the pulmonary vascular bed; and miliary atelectasis are the histological features of pulmonary injury. These features were observed in some of the specimens. Frank oedema was present in some alveoli. In 12 patients who were operated with the use of CPB polymorphonuclear leukocytes accumulated within pulmonary capillaries during bypass. The accumulation of polymorphonuclear leukocytes was associated with the swelling of capillary endothelial cells and with the proliferation of type II granular pneumocytes. Reperfusion injury following ischaemia together with moderate passive hyperaemia and extravasated erythrocytes and neutrophils could be primarily detected.

These alterations were accompanied by scarce appearance of proteins in the alveoli. Surprisingly enough the signs of ischaemia/reperfusion injury proved the most severe in 2 of those 3 patients who had intact pulmonary tissue structure before the starting of CPB. In those patients who underwent OPCAB surgery, none of the detrimental alterations mentioned above could be observed.

The basic histopathological findings in "specimen A" biopsies and the alterations found in "specimen B" biopsies are summarised in Table 13.

Alveolar thickness measured in the control group has proved 0.5-3.0 μm (Textbook data: 0.5-2.5 μm). For suitable understanding I use abbreviations to determine the followings:

D1: mean alveolar thickness of biopsies of "specimen A".

D2: mean alveolar thickness of biopsies of "specimen B"

RD: average figure for the thickening.

RD: average septum thickness increase.

Light microscopy has revealed significant alveolar septum thickening (**D1: 5.32 ± 1.51 μm vs. D2: 5.88 ± 1.33 μm, *p = 0.04***). RD of the alveolar septum has proved 1.14-fold (*SD: 0.23*). This equals an average increase (DD) of 0.55 μm (*SD: 0.98*). There has been no correlation between age and either the figure of D1, D2, DD and RD.

Strong correlation could be observed however, between the figures of aortic cross clamp time and the degree of RD (R = ***0.837, p < 0.001***) and DD (R = ***0.833, p < 0.001***). CPB duration showed another similar strong correlations with the degree of RD (R = ***0.745, p = 0.001***) and DD (***R = 0.755, p = 0.001***)

Table 13. *Basic histopathological findings in the lung before CPB and alternations after CPB secession*

Case No	Specimen A				Specimen B	
	Normal	**PH**	**Emphysema**	**IF**	**I/R**	**Oedema**
1.	+				++	
2.		++	+			
3.		++			++	
4.		+				
5.		+		+	+	
6.	+					
7.		++				
8.		+	++			+
9.		++	+	++		
10.		+		+		
11.				++		+
12.	+				++	
13.				+		+
14.		+	++			+
15.		+	+	+		++
16.		+		++		++
17.				+		
18.		++	++			

PH: Pulmonary Hypertension IF: Interstitial fibrosis I/R: Ischaemia/Reperfusion
+: mild ++: severe

Investigating the basic histologic findings (results of biopsy specimens "A") with the use of paired *t* probe we have found the followings: the presence or the absence of intact septum, pulmonary hypertension and interstitial fibrosis and the degree of these categories has not affected the figures of D1, D2, RD and DD. Otherwise the severity of emphysema has showed correlation with the figures of D1 (***R = -0.537, p = 0.032***) and D2 (***R = -598, p = 0.014***) but not with those of RD and DD.

Electron microscopic findings confirmed the results of light microscopy. Moreover, in many alveoli, extensive injury to basal membrane of air-blood barrier

was observed. Intravascular PMN's were found in increased numbers in the post CPB biopsy as compared to the pre-CPB biopsy. Intravascular PMN's were usually found in capillaries, where they appeared to fill the vessel lumen.

Cytoplasmic swelling, mitochondrial swelling and swelling of the endoplasmic reticulum of PMN were not often seen. Nuclear abnormalities were not observed. PMN's were observed to be disintegrating intravascularly with release of PMN granules into the capillary lumen. Platelets were rarely seen in either the pre- or the post CPB biopsies. Endothelial and type I cell swelling was observed in some cells. Swelling of the endoplasmic reticulum and of the matrix compartment of mitochondria in type II granular pneumocytes was present. In many alveoli, surfactant was not homogeneously widespread and the cells producing it were swollen. Structures of pulmonary surfactant were present in the lumen of alveolar capillaries.

9.3 Discussion

The results of this investigation confirm, that even in the present era of cardiac surgery, cardiac operations with the use of all highly sophisticated technical equipment, myocardial protection and surgical approach (membrane oxygenator, normothermic surgery), CPB are still associated with positive signs of micro-structural damage and a degree of lung injury. In comparison with those structural damages found to occur in the early era of CPB, the degree of these alterations has certainly decreased. On the other hand this injury seems to be temporary because none of the investigated patients had any serious pulmonary complication in the postoperative course.

My results suggest that the air-blood barrier becomes leaky after such procedures or even gets injured. Alveolar septum thickening seen in lung specimens taken after the ischaemic period might be explained with the accumulation of fluid and protein. The postulated mechanisms of such injury include neutrophil and complement activation, oxidative stress, lipid peroxidation or ischaemia-reperfusion injury.

It is not surprising that I have been successful to statistically verify the significant correlation between the severity of lung injury and CPB duration. The reduction in the compliance of the lungs after CPB is well established. The observed interstitial oedema and haemorrhage as well as vascular congestion probably all contribute to this increase in stiffness by disturbing the fine balance between elasticity and structural rigidity of the lungs. The development of atelectasis after CPB is well documented, but the ethiology still remains controversial. Maybe that the observed injury to granular pneumocytes and the loss of surfactant activity contribute to the development of post CPB atelectasis. Although in this patient population there were only two cases operated without the use of CPB, findings from these cases clearly justify that no pulmonary

ultrastructural injury has occurred during cardiac surgery without CPB. Surprisingly enough, my results indicate that the signs of ischaemia/reperfusion injury have proved more severe in 2 of those 3 patients who had intact pulmonary tissue structure before the initiation of CPB.

A much clearer view could have been gained on the subject if specimens had been available e.g. on the 1st postoperative day or from patients who developed pulmonary complications in the postoperative period. Having said that certain ethical and technical considerations and limitations must be respected.

The above observations provide strong evidence that CPB causes localised injuries to alveolar capillaries by damaging circulating polymorphonuclear leukocytes. Whether similar ultrastructural changes occur in systemic organs is not yet known. To assess the degree of lung injury, I suggest that, additional, quantitative studies are necessary.

10 SUMMARY AND NEW RESULTS

CPB is an invaluable tool for cardiac surgery ever since the heart has been operated on. Through the long years of its use certain side effects and drawbacks of the concept and the ever-developing device have come into the limelight. By today there are two alternatives for the reduction of these negative attributes of CPB: either to improve the biocompatibility of the device and its accessories or to give up its use completely. Recently Cardiac Surgery has entered a new era and great emphasis seems to be taken on operating techniques without the use of CPB. After all, what future is to be expected for CPB? It is difficult to give a straight answer to this question, especially if the appearance of gene research and technique that might induce revolutionary changes in the field of Cardiac Surgery overall, is taken in consideration. Having said that, in my view, the use of CPB still remains on the cardiac surgical palette in the next future. Thus far it is only coronary surgery where CPB seems to be dispensable at all, while in other cardiac diseases there are no alternative techniques other than the use of CPB if surgery is to be performed.

Immediately after the start of CPB, lung ischaemia occurs. This phenomenon has been justified with transpulmonary lactate ratio disturbances. During the ischaemic period, there is a considerable increase in activated neutrophil count in the lung tissue that could be verified with the measurement of the free radical production capacity of neutrophil cells. Histologic assessment of tissue specimens from the lungs after CPB has revealed gross neutrophil sequestration and alveolar basal membrane injury.

My results have shown the kinetics of some elementary pro- and anti-inflammatory cytokines during CPB. The role of the lungs in these changes has also been revealed. Certainly, the drawback of the above investigations is the relatively small

patient population. The only reason for that, is the financial burden associated with studies of this kind. In spite of this drawback, I am convinced that these results are suitable both for further comparative studies and for the investigation and assessment of the biocompatibility of newly developed oxygenators and CPB circuits. Deeper understanding of the, behaviour of and the role of cytokines and adhesion molecules during CPB may facilitate effective intervention in the inflammatory response process and suppression of its adverse effects and may can help in monitoring the efficacy of new therapeutic strategies.

On the basis, of my results it has come clear that the lungs play an important role in the inflammatory response following operations on CPB. The lungs could be probably regarded as a “primary filter” or “primary guarding line” against those various jeopardising effects.

In spite of the fact, that no thorough clinical investigation has been carried out to justify the difference between coronary artery bypass grafting operations with or without CPB, my study that consisted of a relatively small patient population has verified that concerning the inflammatory response there is a significant difference between the two groups. Investigation on the beneficial effects and outcome of “off-pump” surgery is still under way in my Department.

All my results and primarily new observations are summarised below:

1. I could prove that ARDS after CPB correlates with the duration of ischaemia and CPB. Blood and FFP transfusion are independent risk factors.

2. I could show that, in spite of the use of partial CPB perfusion lung ischaemia has occurred and resulted in reperfusion injury.

3. I have justified that notable lactate production occurs in the lungs during CPB. Two peaks of lactate values could be observed during and after CPB; one in the ischaemic period, the other one in the 2nd hour of reperfusion.

4. I could certify that there is a transpulmonary neutrophil gradient and neutrophil sequestration in the lungs during CPB.

5. Superoxide anion producing capacity of PMN cells during cardiac surgery with CPB is significantly increased in post-pulmonary blood samples, hence I can suggest oxidative stress in the lungs during CPB.

6. My observations support the idea, that there are significant fluctuations in the levels of serum IL-6, IL-8, and IL-10 occurring during CPB.

7. Concerning alterations of IL-8 and IL-10, I suggest that, the lungs consume rather than, produce these substances and IL-10 changes show a positive correlation with favourable haemodynamic variables.

8. I could not justify any role for IL-2 during CPB although this interleukin is held responsible for certain lung diseases.

9. I have verified that expression of E-selectin and ICAM-1 is a particular occurrence of CPB. This observation has failed in OPCAB surgery.

10. Light and electron microscopic histology observations of the lung tissue after CPB suggest relevant cellular damage. I have revealed that there is an alveolar septum thickness increase after cardiac surgery carried out on CPB and this increase strongly correlates with the duration of ischaemia and CPB.

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