

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

**Ph.D. thesis**

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## ABBREVIATIONS

ACE	Angiotensin Converting Enzyme
ACT	Activated Clotting Time
ADP	Adenosine Diphosphate
ALAT	Alanin-Aminotransferase
AMI	Acute Myocardial Infarction
ARDS	Adult Respiratory Distress Syndrome
ASAT	Asparat-Aminotransferase
ATP	Adenosine Triphosphate
AVLAC	Arterio-Venous Differences in Lactate
AVR	Aortic Valve Replacement
BAL	Bronchoalveolar Lavage
CABG	Coronary Artery Bypass Grafting
COPD	Chronic Obstructive Pulmonary Disease
CPB	Cardiopulmonary Bypass
CX	Circumflex Coronary Artery
ETDA	Ethylene Diane Tetraacetic Acid
EVLW	Extravascular Lung Water
FEV <sub>1</sub>	Forced Expiratory Volume
FFP	Fresh Frozen Plasma
FiO <sub>2</sub>	Inspired oxygen fraction
FRC	Functional Residual Capacity
GSH	Reduced Glutathione
GSSG	Oxydated Glutathione
IABP	Intra Aortic Balloon Pump
INR	International Normalised Ratio
IPPV	Intermittent Positive Pressure Ventilation
LAD	Left Anterior Descending Coronary Artery
LCOS	Low Cardiac Output Syndrome
LDH	Lactate Dehydrogenase
MDA	Malondialdehyd
MIDCAB	Minimally Invasive Direct Coronary Artery Bypass
MPO	Myeloperoxidase
MVR	Mitral Valve Replacement
NADPH	Nicotine-amid Adenin Dinucleotid Phosphate
OPCAB	Off-Pump Coronary Artery Bypass
PaO <sub>2</sub>	Oxygen Pressure
PEEP	Positive End Expiratory Pressure
PMN	Polymorphonuclear Neutrophils
PVC	Pulmonary Vascular Compliance
RBC	Red Blood Cell
RC	Right Coronary Artery
ROS	Oxygen Species
SIR	Systemic Inflammatory Response
SOD	Superoxide Dismutase
V/Q	Ratio of Ventilation and Perfusion
WBC	White Blood Cell
XD	Xanthine Dehydrogenase
XO	Xanthine oxidase

# 1 BACKGROUND

## 1.1 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, which consists of an artificial blood oxygenator, blood pump(s), and other associated devices. CPB is an essential component of conventional cardiac surgery, enabling the surgeon to operate under controlled conditions on a bloodless, motionless heart or on the great vessels. Despite its complex structure, CPB needs to be absolutely safe, predictable and precise in terms of its performance.

The first concept of diverting the circulation to an extracorporeal oxygenator so that surgical procedures could be performed on the heart was started in 1885 by Frey and Gruber [1]. Subsequently, scores of laboratory studies with oxygenators and pumps were reported. Gibbon designed the first heart-lung bypass machine for the use in animals in 1937 [2]. However, it was not until the mid of the 20<sup>th</sup> century and the introduction of modern surgical and anaesthetic techniques, along with the discovery of new synthetic materials, and heparin, that the use of cardiopulmonary bypass (CPB) became a reality. In 1953, Gibbon performed the first successful use of the CPB apparatus at Massachusetts General Hospital [3]. Within two years of that, Kirklin reported a series of eight patients undergoing CPB at Mayo Clinic [4]. Since then there has been a rapid growth in the number of open heart surgical procedures performed throughout the world.

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 [5,6,7,8]. 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 [9,10]. Several equipment and techniques have been developed for ameliorating the damaging effects of CPB [11,12,13,14]. 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 [15,16,17,18]. 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 [19]

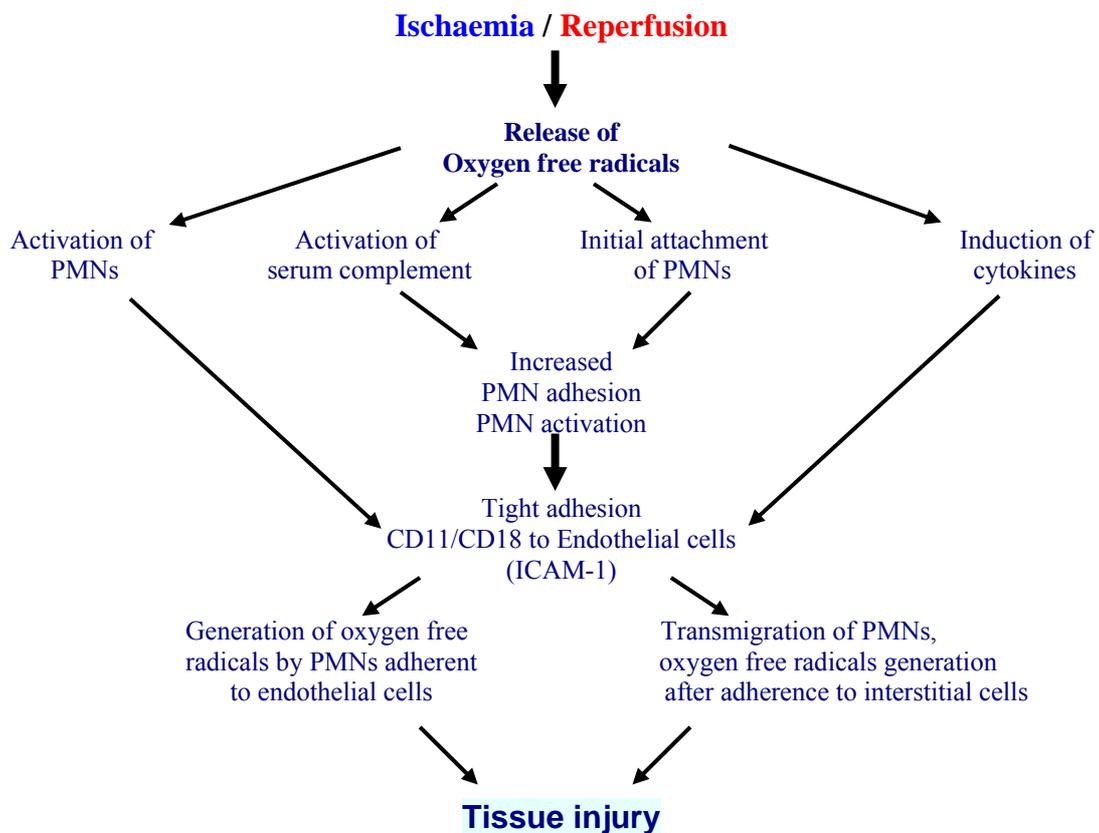
Respiratory problems are common after open heart surgery. The causes are multifactorial, but in general, the likelihood of respiratory difficulty depends directly on the patient's preoperative pulmonary function, the duration of CPB, and the cardiac performance after surgery. CPB alters the pulmonary function and morphology [20,21,22], 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.**

## **1.2 The inflammatory response to CPB**

CPB represents a unique medical condition that induces Systemic Inflammatory Response (SIR) [23] for which the human immune system has not yet evolved a specific response. Consequently, when confronted with the multiple insults of CPB, the magnitude of the immune system response is exaggerated, confusing, and complex. This response involves both humoral and cellular mediators. Concerning the latter, activated Polymorphonuclear Neutrophils (PMN) seem to play

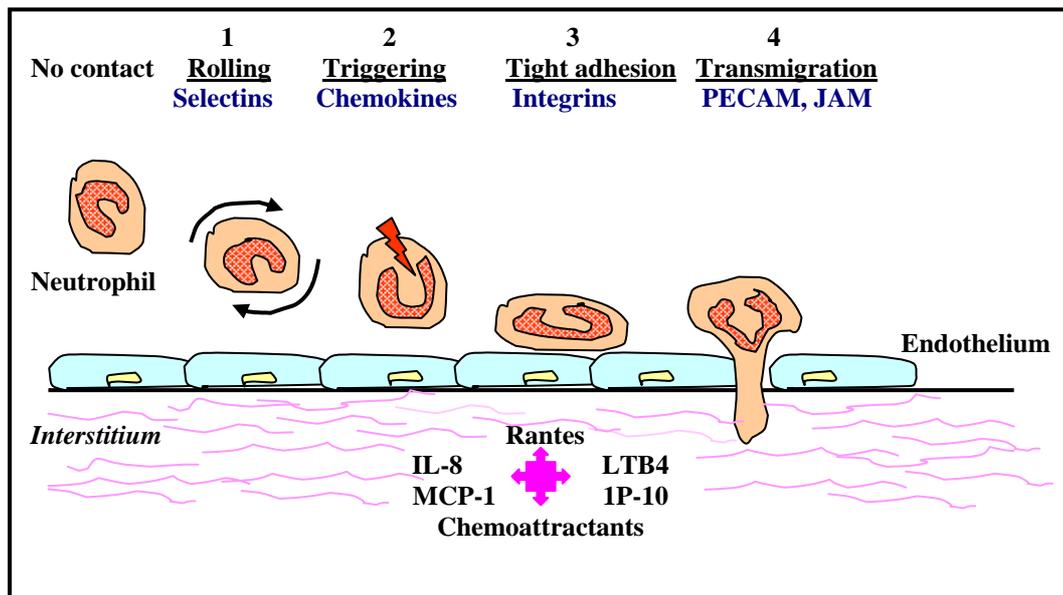
a pivotal role [24]. Their attachments to endothelial cells are linked to the release of cytotoxic proteases and free oxygen radicals, which account for a substantial part of CPB-induced inflammatory tissue damage. Hence without doubt, this extravagant reaction is responsible for a proportion of mortality and morbidity associated with cardiac surgery. How might we envision the role of the inflammatory response, given this complicated sequence of events? Based on *in vitro* and *in vivo* studies, we can suggest a paradigm describing the interactive steps that likely take place during the post-ischaemic inflammatory state after CPB. Figure 1 is a schematic representation.



**Figure 1.** A schematic representation of interactive components of the immune response that likely occurs during the postischaemic inflammatory state after CPB.

In this model, initially, oxygen free radicals are released from the endothelial cells in reperfused organs, such as the heart and the lungs, leading to alterations in endothelial cells that promote early neutrophil targeting, activation of neutrophils in transit through these organs, local activation of serum complement, and induction of cytokine synthesis. Initial neutrophil attachment to the endothelium results in the initiation of Platelet Activating Factor (PAF) synthesis. These changes lead to more

neutrophil adhesion, neutrophil activation, and neutrophil adhesion protein (CD11/CD18 integrin) expression via endothelial membrane-bound platelet activating factor [25]. Proinflammatory cytokines such as IL-1 $\beta$  and TNF $\alpha$  and chemokines such as IL-8, are newly synthesised, largely within endothelial cells, in reperfused organs within hours, and induce increased expression of adhesion molecules on endothelial cells and other tissue-specific cells (e.g., myocytes, pulmonary alveolar lining cells, glomerular or renal tubular epithelium) [26]. In turn this results in tighter neutrophil attachment via Intercellular Adhesion Molecule-1 (ICAM-1), transmigration of neutrophils into the interstitial space, and release of large amounts of free radicals. In summary the neutrophil-endothelial cell interactions involve adhesion molecules that belong to four distinct families: the selectin family, the integrin family, the cadherin family, and the immunoglobulin superfamily [27]. By their coordinate action, these molecules orchestrate the four-step sequence of events that comprise the interaction between neutrophils and the vascular wall. These four steps are known as rolling, triggering, adhesion, and transmigration (Figure 2).



**Figure 2.** Schematic representation of the interaction between neutrophil cells and the vascular wall during SIR.

## **1.3 Physiologic anatomy of the lungs**

The lungs receive blood supply from the pulmonary artery and the bronchial arteries. These two independent circulation systems which perfuse the lung are very different concerning their origin, and their function. The entire venous return of the body perfuses the pulmonary circulation, and its obstruction may result in fatal outcome. The tiny systemic bronchial arterial blood flow appears to be quite unimportant since it can even be abolished (during lung transplantation, for instance) without any obvious subsequent disorder.

### **1.3.1 The pulmonary vessels**

The pulmonary arterial branches are all very short. However, all the vessels of the arterial side, within the lung tissues have the histologic structure of that of the arterioles, resulting in having much larger vessel diameters than their counterpart systemic arteries do. This unique speciality combined with the fact that these vessels are all very thin and distensible, gives the pulmonary arterial tree a very large compliance, averaging almost 7 ml/mmHg, which is similar to that of the systemic arterial tree. This large compliance allows the pulmonary arteries to accommodate about two thirds of the stroke volume output of the right ventricle. The pulmonary veins, like the pulmonary arteries, are also short, but their distensibility characteristics are similar to those of the veins in the systemic circulation.

### **1.3.2 The bronchial vessels**

*"Small, but Vital Attributes of the Lungs"*

Why is the bronchial circulation worth for mentioning? The answer is that vital airway defences, fluid balance, and metabolic functions taking place in the lungs depend on this unobtrusive auxiliary circulation system. It is able to enlarge in response to injuries and even to take over the gas exchange function if the pulmonary circulation fails in any region of the lung [28]. The bronchial circulation is like Mother or the Red Cross; normally accepted and unsung, but capable of giving vital help when needed.



*Nature gave a vein and artery to the trachea, which would be sufficient for its life and nourishment, and somewhat removed the other large branches from the trachea to nourish the substance of the lung with greater convenience.*

**LEONARDO DA VINCI (1452-1519)**

The origin and distribution of the bronchial vasculature vary considerably among different species both at the macro- and at the microvascular level [29,30]. In humans, the larger bronchial arteries usually originate either directly from the aorta or from the intercostal arteries [31]. In the majority of people, there is at least one bronchial artery that originates from an intercostal artery. Liebow [23] found that 43% of all the right bronchial arteries were of intercostal origin, usually arising from the first intercostal artery, whereas 84% of the left bronchial artery originated directly from the aorta. The bronchial arteries enter the lung at the hilum, branching at the mainstem bronchus to supply the lower trachea, extrapulmonary airways, and the supporting structures; this fraction (about 1/3-rd) of the bronchial vasculature drains into the right heart via systemic veins. Bronchial vessels also supply the

intrapulmonary airways as far as to the level of the terminal bronchioles where they form extensive anastomoses with the pulmonary vasculature; this systemic-to-pulmonary blood drains to the left heart via the pulmonary veins. Repeated arborisation of the bronchial artery along the length of the tracheo-bronchial tree results in a vast increase of the total surface area of the vascular bed. The tracheo-bronchial vasculature consists of a continuous dense network of subepithelial capillaries that converge to form venules extending to a deeper plexus of larger venules and arterioles on the adventitial side of the smooth muscle. Innervation is under the control of vasodilatory parasympathetic nerves that release acetylcholine and vasoactive intestinal polypeptide; vasoconstrictor sympathetic nerves that release norepinephrine and neuropeptide Y; and sensory nerves that release substance P, neurokinin A, and calcitonin gene-related peptide, all of which are vasodilators. Mechanical factors such as the downstream pressure and alveolar pressure also influence the distribution of blood flow through the tracheo- bronchial vasculature.

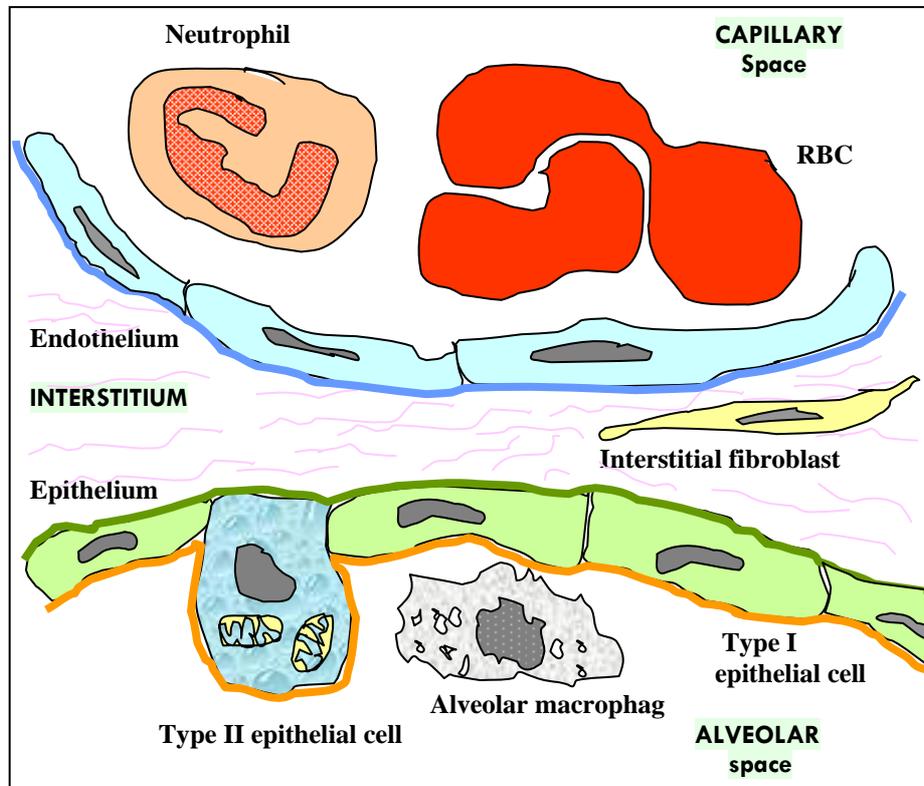
### **1.3.3 The respiratory membrane**

#### *"Ultrastructural organisation of the alveolar-capillary unit"*

The total quantity of blood in the capillaries of the lung at any given instant is 60 to 140 millilitres. The average diameter of the pulmonary capillaries is only about 5 micrometers, which means that red blood cells must actually squeeze through them. Therefore, the red blood cell membrane usually touches the capillary wall so that oxygen and carbon dioxide do not have to pass through significant amounts of plasma as they diffuse between the alveolus and the red cell. Obviously, this increases the rapidity of diffusion as well. Figure 3 illustrates the ultrastructure of the respiratory membrane. The respiratory membrane is composed of the following different layers:

1. A layer of fluid lining the alveolus and containing surfactant that reduces the surface tension of the alveolar fluid.
2. Alveolar epithelium comprised of very thin epithelial cells.
3. Epithelial basal membrane.
4. A very thin interstitial space between the alveolar epithelium and the capillary membrane.

5. Capillary basal membrane that in many places fuses with the epithelial basal membrane.
6. Capillary endothelial membrane.



**Figure 3.** Structure of the normal alveolar-capillary membrane.

Despite the large number of layers, the overall thickness of this respiratory membrane unit varies between 0.5 and 2.5  $\mu\text{m}$ . The cellular components of the unit are:

- The *epithelium*, lining the air space, and
- The *endothelium*, facing the blood compartment.

The epithelium is composed of two types of cells:

- 1) Type I, broad, squamous, highly branched cells occupying approximately 97% of the total alveolar surface and based on the thin basal membrane. This cell type has in its most frequent form an oval elongated nucleus with one or two large nucleoli. The organelles are presented by a few scattered mitochondria and profiles of the granular endoplasmic reticulum, numerous ribosomes and vesicles. These cells mostly seem to be involved in gas exchange.

- 2) Type II, cubicle cells containing characteristic osmiophilic lamellar bodies in their cytoplasm. These cells are fitted among type I cells.

Type II cells produce the active material, the surfactant, which covers the epithelium of the alveoli and the alveolar ducts with a thin layer toward the air space. This cell type contains, in its differentiated form, a varying amount of osmiophilic lamellated inclusions. The mitochondrial apparatus is rich and the mitochondria are substantially larger and the matrix much denser in comparison to type I cells. The cytoplasm contains numerous polyribosomes, and the granular endoplasmic reticulum is well developed. Surfactant is a mixture of approximately 75% of lipids (dipalmitoyl-phosphatidylcholine) and proteins, and it is responsible for the reduction of surface tension. If serious alveolar capillary membrane injury occurs, type II cells start to proliferate and they most likely have their own share in the process of healing as well. In the capillary-, and vascular endothelium, the existence of rich plasmalemmal vesicular structures related to the pinocytotic activity should be emphasised. In the juxtranuclear position numerous dense bodies, probably lysosomes, are present. The endothelial cells are seated on a distinct basal lamina and linked together by tight junctions. Morphometric studies estimated that in healthy human adults the capillary surface area is approximately 120 m<sup>2</sup> and the alveolar surface area ranges between 40 and 120 m<sup>2</sup>.

## **1.4 Function of the lungs**

As a result of their special position in the circulatory system the lungs can screen and monitor the composition of the blood, which comes from and is returned to all the tissues. This function, together with gas exchange, takes place at the level of the alveolar-capillary unit. The primary engagement of the lungs is to filter out the CO<sub>2</sub> content of the blood arriving from the right ventricle before this blood would get back to the left atrium of the heart. There are great differences in gas exchange volumes. In case of heavy activity the change of O<sub>2</sub> or CO<sub>2</sub> content may rise from the basic 3-4 ml/kg/min value up to 60 ml/kg/min. In order to ensure that the gas exchange occurs in a short period of time, blood and gas of satisfactory amount must meet on a surface of satisfactory size.

Beyond the most important task of the lungs -the maintenance of gas exchange- they have their own role in phagocytosis, endocrine secretion and they serve as a terminal for certain emboli. The epithelial surface of the lungs –alike skin- is open to outer space therefore the effect of environmental damaging agents often appears in the form of airway diseases. The lungs play a vital role in the regulation of blood pH, and together with the upper airways they warm up and vaporise the inhaled air. Obviously loss of heat and fluid goes together with the work of lungs but any disease or injury affecting the lungs might influence all the above functions.

The lungs are metabolically active organs where anabolism and catabolism of pharmacologically active substances and synthesis of lipids take place and a proper balance of blood homeostasis is maintained. Under physiologic conditions the lungs also take part in the metabolism of arachidonic acid by means of both the lipoxygenase and the cyclooxygenase enzymes. Air embolism and mechanical hyperventilation stimulate the lungs to generate more prostacyclin, which may lead to severe haemodynamic instability and death.

Some of the metabolic functions ascribed to the lungs have been localised to cellular components. Phospholipids needed for the constantly renewed surfactant are synthesised in type II epithelial cells. Angiotensin Converting Enzyme (ACE) is associated with the endothelial cell membrane and vesicles opening to the blood front. There are signs suggesting that pulmonary cells also intervene in the metabolism of circulating vasoactive substances which -during their passage through the lungs- can be activated (prostaglandins, angiotensin I → angiotensin II), inactivated (serotonin, bradykinin) or removed from the circulation (norepinephrine, 5-hydroxytryptamine).

## **1.5 Biochemistry of the lungs**

### **1.5.1 Carbohydrate metabolism**

Glucose is used by the lungs both for energy supply and for the synthesis of glycogen and lipids. Their glucose transport system that can be activated by insulin ensures increased glucose uptake. At 36 °C, healthy adult human lungs consume about 11 ml O<sub>2</sub>/min (5% of whole body oxygen uptake) [33]. This means that the lungs are characterised by low oxidative capacity therefore anaerobic processes are

of primary importance. Thus, more than half of the glucose (app. 60%) is transformed to lactate and only 10 % enters the citrate cycle (and the respiratory cycle) whilst another 4% is getting transformed in the hexose-monophosphate shunt. 4 to 10% of the glucose taken up by the lungs are transformed in the hexose-monophosphate shunt and in the citrate cycle respectively.

### **1.5.2 Lipid metabolism**

Under physiologic conditions the lungs utilise a small amount of fatty acids and keton bodies. Starving may result in a 2 to5-fold increase in the utilisation of nutritiants. NADPH produced in the hexose-monophosphate shunt is used for fatty acid synthesis. These fatty acids take part in the build-up of the surfactant and of those lipids protecting the lung tissue from oxidation. The majority of phospholipids are either saturated or unsaturated phosphatidil-choline (in other term lecithin).

## **1.6 The lungs during CPB**

During total CPB, the heart and the lungs are excluded from circulation. Having said that, blood continues to enter the pulmonary circulation from several sources. Blood from the coronary sinus and the Thebesian veins passes through the right side of the heart into the pulmonary artery. In some patients with cyanotic heart disease and reduced pulmonary flow, bronchial arteries empty 10 to 20 percent of the cardiac output into the pulmonary veins instead of the normal 1 to 2 percent. A patent ductus arteriosus, aorto-pulmonary window, or previously constructed shunt between the systemic and pulmonary arteries all cause torrential pulmonary blood flow during bypass; these connections must be interrupted as soon as cardiopulmonary bypass commences. Residual pulmonary blood flow during bypass causes left ventricular distension and acute pulmonary venous hypertension. Usually, pulmonary blood flow during bypass is only a small fraction of the normal flow. However, when the flow is abnormally high, the left side of the heart must be protected by either venting or by permitting the heart to contract.

Usually, pulmonary arterial pressure during bypass is about 2 torr; and if the left side of the heart is vented, the pulmonary venous pressure is near zero. Ischaemic injuries to the lung parenchyma do not occur if the lungs remain inflated, but atelectatic portions of the lungs may become haemorrhagic.

CPB alters at least two factors in the Starling-equation for fluid exchange across capillaries [34,35]. Both pulmonary capillary hydrostatic pressure and plasma colloid osmotic pressure is reduced. Pulmonary capillary hydrostatic pressure approaches zero during bypass if the left ventricle is adequately decompressed. Also, dilution of the perfusate with crystalloids reduces colloid osmotic pressure to 11 to 14 torr. The net effect of these changes is to reduce the forces driving fluid into the interstitial space.

The extent to which interstitial components of the Starling-equation are affected is unknown, since they are not available for direct measurement. However, indirect results indicate an increased gradient in pulmonary endothelial permeability after CPB if bubble oxygenator was used. In this study no statistical correlation between the increase of Extravascular Lung Water (EVLW) and the plasma colloid oncotic pressure, pulmonary artery wedge pressure gradient, CPB time or intraoperative fluid balance has been observed [36]. Despite these results, changes of colloid osmotic pressure in the plasma and in the interstitial space, seem either to cancel each other or to favour a decrease in EVLW. Patchy accumulation of interstitial and alveolar fluid that occurs after bypass is best explained by the local increase of capillary (microcirculatory) permeability [37]. Extracorporeal circulation exposes blood to foreign surfaces and haemodynamic stress that damage blood elements. Red blood cells are hemolyzed, white blood cells are injured, and platelets aggregate and adhere to foreign surfaces. Adenosine Diphosphate (ADP), a vasodilator, is released from hemolyzed red cells. Injured white cells probably release lysosomal enzymes. In addition, proteins are denatured, and lipids, lipoproteins, cholesterol, and phospholipids are altered. The concentration of catecholamines rises at the start of bypass but returns to normal before bypass stops. Because the lungs are excluded from the circulation during bypass, and because residual pulmonary blood flow is usually small, any vasoactive materials released into the circulation probably affect the systemic vessels in a greater extent than they do with the pulmonary vessels. There is no evidence to support the hypothesis, that there is any one certain circulatory chemical substance produced during bypass that solely and selectively injures the pulmonary capillaries. In summary, it seems that gross molecular and cellular mechanisms are involved in the phenomenon of the pulmonary dysfunction after CPB.

## **1.7 Effects of cardiac surgery and CPB on the respiratory system**

Patients undergoing open heart surgery are exposed to the risk of considerable pulmonary morbidity requiring aggressive postoperative management [38,39]. Prolonged mechanical ventilation, infection, and immune alterations, atelectasis, permeability oedema, bronchospasm, pleural effusions, and thromboembolic disease may all complicate the patient's postoperative course. The Adult Respiratory Distress Syndrome (ARDS) following CPB is a serious pulmonary complication, which will be discussed in chapter three. The rest of the pulmonary complications are going to be briefly reviewed in this chapter. However no such discussion would be complete without an in-depth analysis of the preoperative evaluation of this surgical patient population.

### **1.7.1 Preoperative evaluation**

The number of patients undergoing cardiac surgery has increased dramatically in recent years. A large percentage of these patients have concomitant respiratory disease. Respiratory complications are second only to cardiac problems as a cause of postoperative morbidity and prolonged intensive care unit stay. Factors shown to increase the risk of postoperative pulmonary complications include chronic obstructive pulmonary disease (COPD), obesity, advanced age, smoking history, and persistent cough [40]. Patients undergoing cardiac surgery require thorough preoperative pulmonary assessment. With the preoperative recognition and appropriate treatment of pulmonary disorders, combined with meticulous postoperative care, this large group of patients can benefit from surgery with low morbidity.

### **1.7.2 Expected respiratory changes after cardiac surgery**

The various components of the respiratory system - airways, lungs, chest wall, intercostal muscles, diaphragm, and neural pathways to and from these various components- are subject to damage caused by a variety of processes associated with cardiac surgery and CPB. Cardiac surgery, through either sternotomy or thoracotomy, has deleterious effects on the efficacy of the muscle pump and the

chest wall. Additionally, phrenic nerve damage and/or diaphragm dysfunction, resulting from cold topical solutions applied inside the pericardium, may cause mechanical problems. Physical handling of the lungs, fluid collections, and pain, due to both the operation and the presence of chest drains, may all interfere with normal respiratory function. Left-sided cardiac distension or elevated pressures may cause alveolar oedema, and transfusion reactions or allergic reactions to drugs (e.g. Protamine) may increase capillary permeability, leading to alveolar flooding. Table 1 summarises the changes in the pulmonary function found after cardiac surgery and CPB.

**Table 1.** *Changes in pulmonary functions after CPB.*

◆ Alveolar-arterial oxygen difference	↑
◆ Physiologic shunt	↑
◆ Respiratory rate	↑
◆ Tidal volume	↓
◆ Minute ventilation	→
◆ Alveolar ventilation	↓
◆ Alveolar volume	↓
◆ Static compliance	↓
◆ Airway resistance	↑
◆ Breathing work	↑
◆ Oxygen consumption	↑
◆ Respiratory quotient	→
◆ Physiologic dead space	↑
◆ Dead space/tidal volume (VD/VT)	→
◆ Pulmonary vascular resistance	↑

↑ = increase

↓ = decrease

→ = no change

### 1.7.3 Mechanics

A number of investigators have documented changes in pulmonary mechanics and gas exchange after thoracotomy and cardiac surgery, with or without CPB. In many respects, these changes are parallel with those occurring after upper abdominal procedures, and appear to be secondary to surgical factors other than CPB. The mechanical properties of the respiratory system are usually referred to in terms of **compliance** (pressure changes for a given volume change, either static or dynamic), **elastance** (the reciprocal of compliance), and **resistance** (pressure change related to gas flow rate). After thoracotomy, either lateral or midline (i.e., sternotomy), both the lung and chest wall compliance decrease significantly [41]. The maximum decrease (30%) occurs at 3 days, although a significant decrease in compliance (approximately 25%) is still present 6 days after sternotomy and myocardial revascularisation [42]. Although chest wall resistance increases significantly, airway resistance is unaltered after uncomplicated coronary revascularisation procedures [43]. However, airway resistance is reduced after mitral valve replacement for valvular stenosis. Associated with these changes, a significant fall of lung volumes and flow rates can be observed. Forced Expiratory Volume (FEV<sub>1</sub>) decreases immediately after coronary bypass surgery [44]. These changes persist in the first 6 postoperative weeks [45,46]. Functional Residual Capacity (FRC) is reduced by 40% to 50% immediately after extubation, with only a modest recovery (5% to 25%) in the next 72 hours [47]. Opening of the pleural space does not affect the postoperative change in respiratory volumes. These restrictive changes in volumes and flow rates are probably due to alterations in chest wall mechanics, since similar changes are also seen after upper abdominal surgery [48,49]. In addition to the changes in flows and volumes, both reduced inspiratory strength [50] and reduced or uncoordinated rib cage expansion occur [51]. These alterations lead to a rise in respiratory rate and to a fall in tidal volume, to decreased respiratory rate "efficacy" and to increased oxygen cost of breathing [52]. Indeed, the percentage of total body oxygen consumption attributed to the respiratory system after coronary bypass surgery (approximately 20%) is similar to that seen in medical patients recovering from respiratory failure [53]. Factors leading to the reduced efficacy of breathing efforts after cardiac surgery are summarised in Table 2.

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**Table 2.** *Factors leading to reduced efficacy of breathing efforts after cardiac surgery and CPB.*

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◆ **Chest wall motion**

The majority of patients show discoordination between airflow and rib cage motion at 1 week, improving at 3 months [51].

◆ **Maximum inspiratory pressure**

30% decrease, 10 days postoperatively [50]

◆ **Breathing pattern**

Reduced tidal volume and increased respiratory rate

◆ **Breathing work**

Significantly increased [41]

Oxygen cost of breathing 20% of whole body  $VO_2$  [52]

◆ **Lung volumes**

FRC 15% decreased at chest closure

40-50% decrease immediately after extubation

Still down by 25-40% at 72 hours [47]

Still down by 20% at 2 weeks [46]

FEV<sub>1</sub> 50% decrease in the first 3 days [44]

At 6-8 weeks, recovered to 75% of the preoperative values[46]

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#### **1.7.4 Phrenic nerve damage**

Phrenic nerve damage or dysfunction secondary to surgical trauma or extreme cold (i.e., exposure to topical ice slush) may result in significant postoperative loss of lung volume. A direct injury of the phrenic nerve induced by hypothermia would explain the predominant localisation of postoperative atelectasis to the left lower lobe. It has been well established that cold may result in functional and structural abnormalities of peripheral nerves. A progressive decline in conduction velocity occurs when a nerve is cooled to below 17 °C, culminating in a complete blockade below 5 °C. Nerve conduction recovers rapidly upon rewarming. However, pathologic changes may occur at a later period, depending on both the temperature and the duration of exposure.

### 1.7.5 Atelectasis

Atelectasis remains the most frequent reason for postoperative hypoxaemia after CPB, with an overall incidence of between 60% and 84% [54,55,56]. The major mechanisms of atelectasis are outlined in Table 3. The physiologic effects of atelectasis include the development of hypoxaemia as a result of low V/Q ratios and frank intrapulmonary shunting [57]. The lack of lung-inflation during bypass may contribute to this problem [58]. The treatment of postoperative atelectasis in most cases is the application of routine physical therapy manoeuvres, most importantly postural drainage and aggressive suctioning. Occasionally fiberoptic bronchoscopy may be required; with the instillation of acetylsysteine in order to mobilise large and stubborn mucus plugs. Early mobilisation, as well as the encouragement of coughing, is essential in any treatment routine.

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**Table 3.** *Mechanisms of postoperative atelectasis after CPB*

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- Retained secretions [59]
  - Suppression of ciliary activity [59]
  - General anaesthesia [60]
  - Sedation [54]
  - Surfactant alterations [58]
  - Mechanical factors [54]
  - Phrenic nerve injury [61]
- 

### 1.7.5 Pulmonary embolism following CPB

The incidence of clinically significant pulmonary emboli following CPB is extremely low, and it is very rare in the first 48 hours after surgery; however if this complication occurs, it usually means a chain of catastrophic events [62,63]. The approach to the diagnosis, treatment, and prophylaxis of pulmonary emboli on patients after CPB means a serious and difficult challenge to the physician. Dyspnea, hypoxaemia and chest x-ray abnormalities are common disturbances following open heart surgery, and may be attributed to numerous predisposing factors. Thus, in this situation ruling out pulmonary emboli can be difficult. Early ambulation, with

vigorous exercise, and support of the lower extremities still remain the best prophylactic measures in this patient population. Once the patient has passed the initial perioperative period and has had all chest tubes removed, judicious use of low molecular weight heparin should be considered. By adopting these measures, the incidence of pulmonary embolism following CPB can be substantially reduced.

#### **1.7.6 Pneumonia**

Postoperative bacterial pneumonia remains a difficult diagnosis that can rarely be made with certainty [64]. Those clinical features characteristic of pneumonia -fever, purulent sputum, pulmonary infiltrates, and hypoxaemia- are non-specific in the postoperative patient, especially in one who is still intubated. Local tracheitis from the endotracheal tube can produce purulent sputum. Colonisation of the respiratory tree with nosocomial bacteria very commonly occurs and reduces the value of sputum Gram's stain and culture. Recent interest in better diagnostic techniques has centred the attention upon the use of specimens obtained by the use of a bronchoscope protected brush catheter, which is designed to minimise the contamination of lung specimens by upper airway organisms [65,66].

Other respiratory system complications, following CPB, like diaphragm dysfunction, and post pericardiotomy syndrome are not discussed.

## **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. This has been so because of the subsequent type of these studies and also due to the burden put on those patients who have volunteered to get enrolled. In order to form a homogenous cardiac surgical patient population, and also to avoid possible differences originating directly from various types of surgical procedures, only patients who had coronary artery revascularization have been involved in these studies. All but 7 patients had their operations performed on Cardiopulmonary Bypass. All clinical investigations included in this study have been performed on the basis of the following policy:

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. The Exclusion Criteria were as follows:

- Emergency operation
- Acute myocardial infarction within 3 months
- Diseases of the immune system
- Renal and hepatic failure
- Malignant diseases
- Steroid treatment within 1 month prior to surgery
- Coagulation disorders
- Repeated cardiac surgery

All patients had full cardiac studies - including chest x-ray, resting ECG, treadmill, echocardiography, coronarography, duplex scanning of the carotid arteries and abdominal ultrasonic assessment- performed prior to admission. On admission, full routine laboratory tests were carried out (serum electrolytes, serum protein, lipid and glucose, total blood count, hepatic and renal function tests, INR, coagulation and bleeding time, cardiac enzymes, urine laboratory tests).

Platelet-aggregation inhibiting drugs were stopped 7 days prior to surgery.

### **3.1 Notes on surgery**

Median sternotomy and harvesting of the left internal mammary artery and of the saphenous vein occurred in all cases. No other kinds of grafts – either autologous or heterologous- were used. Side branches of the mammary artery were closed by metal clips whilst vein harvesting was carried out by the traditional open method, using 6/0, 7/0 monofilament polypropylene sutures for the side branches. 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. After aortic cross-clamping myocardial protection was achieved with cold (+4 °C) antegrade crystalloid cardioplegia (Bretschneider solution) and by topical cooling with ice-sludge. A suction line was inserted in the ascending aorta proximal to the aortic cross clamping site in order to decompress the heart and also to ensure a bloodless surgical field. In OPCAB cases the target coronary vessel was exposed and the surrounding area was stabilised by a mechanical stabiliser (Origin Medsystems, USA). The target vessel was then snared proximal and distal to the chosen point for anastomosis by 4/0 monofilament polypropylene suture in order to provide bloodless surgical field, using soft teflon pledgets to prevent coronary injury. The coronary artery was then opened and the anastomosis performed. An intracoronary shunt (Flo-Thru, Bio-Vascular, Inc., USA) was used only in the case of relative electrocardiographic or haemodynamic instability and excessive bleeding during the completion of the anastomosis. All distal anastomoses (graft to coronary) were completed with 7/0, 8/0 monofilament polypropylene continuous sutures whilst 6/0 continuous sutures were used for the central anastomoses (graft to ascending aorta). No glue was used in any form or on any location.

### **3.2 Notes on anaesthesia**

Anaesthesia was carried out intravenously in a similar manner with the use of midazolam, alfentanil, propofol and pipecuronium. Cobe-Stöckert (Cobe International Ltd, Belgium) roller pump and membrane oxygenators were used in all operations. Priming solution of the CPB system consisted of the following: 1000 ml. Ringer lactate, 100 ml. Mannitol, 100 ml. 20% Albumin (Biotest) and 60 ml. 8.4% sodium bicarbonate. Immediately before starting CPB, and depending on the

haemodynamic status, 400-500 ml. blood was collected for immediate post-CPB autotransfusion. CPB was performed with the use of core cooling to 34-35 °C and pulsatile flow of 2.4 l/body m<sup>2</sup>/minute. Coagulation was suspended with sodium-heparin in all cases. The coagulation status was regarded optimal if the Activated Clotting Time (ACT) proved to be longer than 400 seconds. ACT was determined in every 30 minutes together with blood gas and serum electrolyte analysis, and haemoglobin count. The effect of heparin was antagonised with protamine-sulphate in 1:1 ratio. No patient was given aprotinin in the perioperative period and no ultrafiltration was carried out during or after surgery. During the whole course of surgery ECG, heart rate, arterial blood pressure, central venous pressure, urine output, rectal and oesophageal temperatures were continuously and simultaneously monitored in each case. 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**

Adult Respiratory Distress Syndrome (ARDS) is a non-specific inflammatory reaction with a varying degree of severity affecting both lungs and resulting in acute respiratory failure. The pathogenic causes evoking this syndrome may also be direct and indirect forms of lung injury [67,68]. The site of events initiating the inflammatory reaction is the alveolo-capillary membrane. The exact definition of ARDS has still been somewhat unclear in the literature and numerous different names have been used for the same disease. The characteristic clinical and pathological observations were first presented by Ashbaugh and Petty in 1967 [69]. After the infant hyaline membrane disease (idiopathic respiratory distress syndrome in the new born), they named the syndrome adult respiratory distress syndrome. Since the first record of the disease, our knowledge has grown rapidly and significantly, however this syndrome still results in high morbidity and mortality rates. ARDS is characterised by the following clinical patterns:

- 1) Untreatable hypoxaemia
- 2) Progressive respiratory failure
- 3) Bilateral disseminated pulmonary infiltration on X-ray

The syndrome stands as a non-specific reaction to a number of different underlying diseases and pathologic effects. Regardless to the provoking factor, injury of type II alveolar cells producing surfactant plays a central role [70,71]. The lack of surfactant results in two basic disorders: atelectasis and pulmonary oedema. In non-cardiac pulmonary oedema that is characteristic to ARDS, protein-rich effusion accumulates in the interstitial space and in the alveoli [72]. In addition to the leakage of water and protein to the interstitial space, corpuscular bodies also follow this route resulting in atelectasis that leads to the reduction of pulmonary compliance.

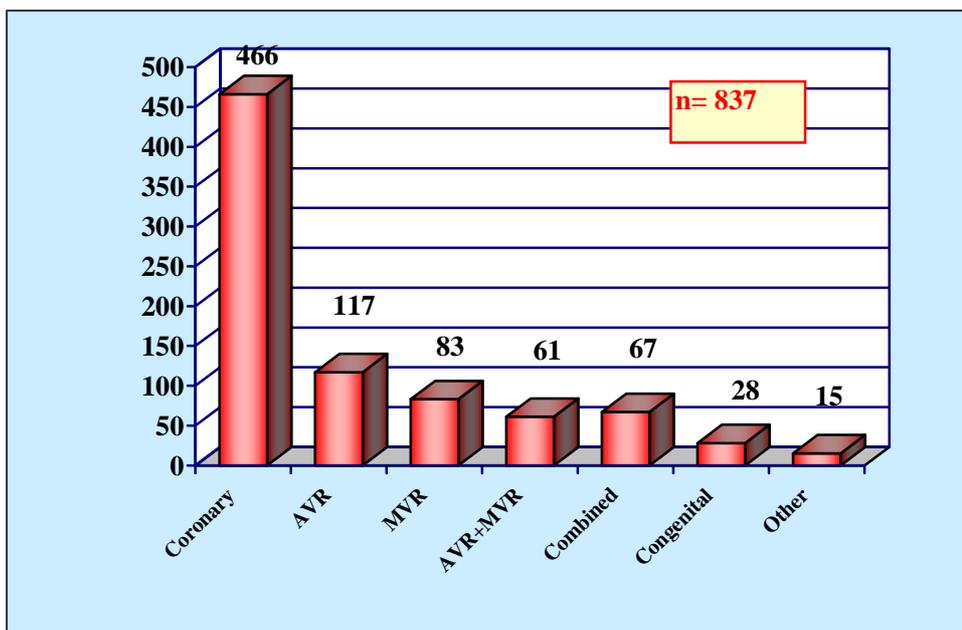
As the air spaces are filled up with fluid, gas exchange and the mechanical habits of the lungs deteriorate. The global respiratory failure cannot be properly controlled with ventilation therapy resulting in fatal outcome due to hypoxaemic cardiac failure. In addition, more than one humoral mediator system and endotoxin [73], activated neutrophil granulocytes, alveolar macrophages and the microvascular

endothelial cells of the lungs also play an important role in the pathomechanism of ARDS [74,75,76,77]. The individual role of the humoral and cellular mediator system has not been satisfactorily cleared yet, why it is often difficult to decide whether the presence of a certain substance is either the matter or the result of pulmonary injury.

Since the 1950s, following the milestone discovery of Gibbon, the use of Cardiopulmonary Bypass (CPB) has gained widespread acceptance in the field of cardiac surgery. Unfortunately however, in the past two decades we have gradually been forced to face the dangerous side effects of CPB. By today, we know that CPB activates the coagulation cascade, the complement system, and the leukocytes and inflicts platelet function disorders and free radical production [78,79]. In spite of all the above few studies have been devoted to the clinical correlations between CPB and ARDS.

#### 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. The types of operations are shown on Figure 4.



**Figure 4.** Types of operations.

AVR: Aortic Valve Replacement

MVR: Mitral Valve Replacement

Data of the group “Other” have been excluded from the statistical analysis due to the inhomogeneity of the group and to the small number of cases. Data of the remaining 822 patients have been analysed. Those patients who did not develop ARDS after surgery have served as a control group. In the above period the technique of operation and anaesthesia along with the postoperative treatment has been based on the same unified standards. Patients’ data are presented in Table 4.

---

**Table 4.** *Enrolled patients’ data.*

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N:	822
Male:	574
Mean age:	56,5 ± 11,2 years (18-80)
> 69 years:	133 (9,8%)
Mean aortic cross clamp time:	76,9 ± 33,2 minutes
Mean CPB time:	115,7 ± 53,3 minutes
Mortality (within 30 days):	2,03 %

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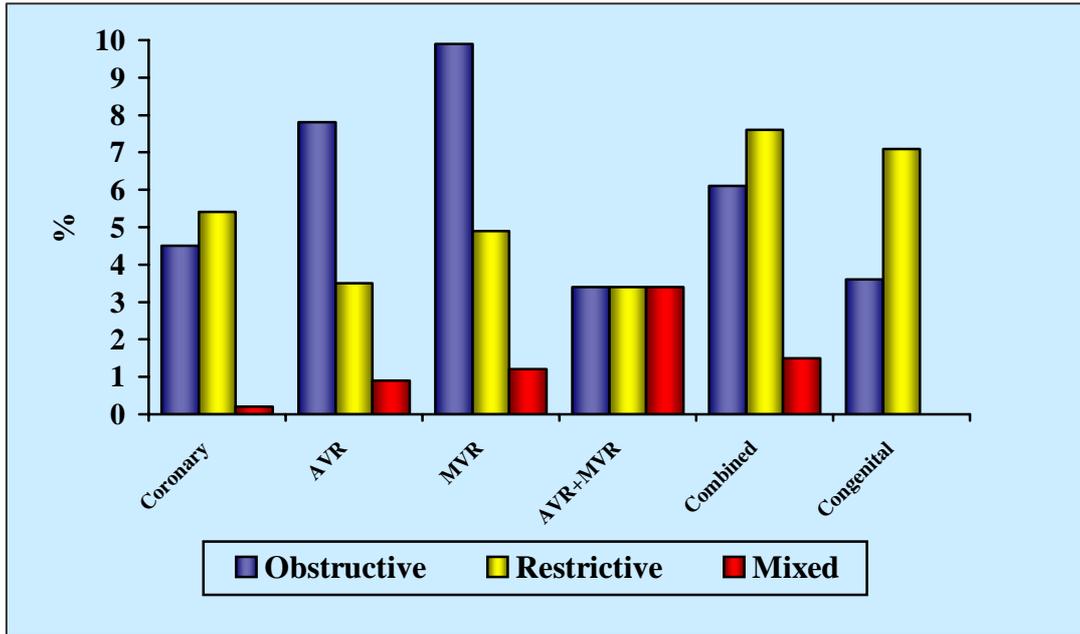
With retrospective, statistical analysis, we have reviewed all data concerning the past medical history and the current operation.  $\chi^2$  probe, Student *t* test and Mann-Whitney test were used for the analysis. I applied logistic regression analysis for the multivariate investigation. The outcome of ARDS has been calculated by the Murray scoring system. ARDS has been diagnosed if all of the following criteria could be detected after open heart surgery:

- ◆ Bilateral diffuse infiltration on chest x-ray
- ◆ Left atrial/Pulmonary wedge pressure >15 mmHg
- ◆ PaO<sub>2</sub> ≤ 60 mmHg (with 100%-os inhaled O<sub>2</sub>)
- ◆ Reduced pulmonary compliance requiring IPPV and PEEP
- ◆ PaO<sub>2</sub>/FiO<sub>2</sub> ratio ≤ 150 without the use of PEEP or;
- ◆ PaO<sub>2</sub>/FiO<sub>2</sub> ratio ≤ 200 with the use of PEEP.

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

## 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. The correlation between the occurrence of preoperative pulmonary disease and the types of surgery is presented in Figure 5.



**Figure 5.** Incidence of preoperative pulmonary disease correlated to types of surgery.

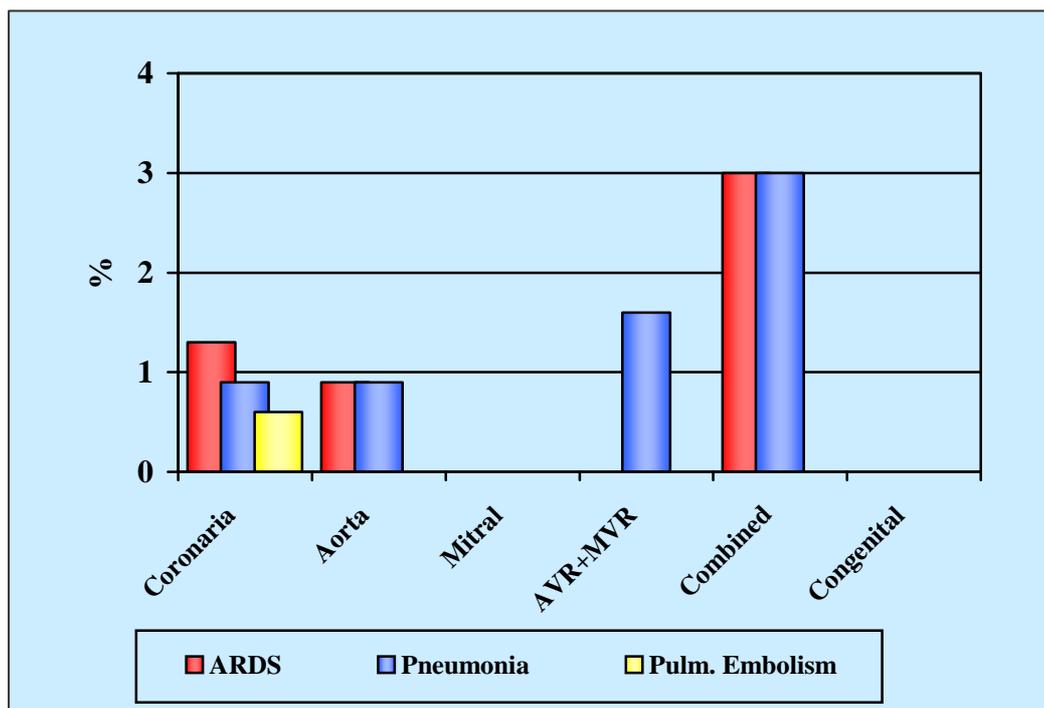
ARDS developed in 10 (1.2%) of the operated patients. One of these cases had a fatal outcome due to multiorgan failure added to ARDS. Autopsy findings justified and confirmed the clinical diagnosis in this patient. ARDS has not developed following mitral- and multiple valve replacement and repairs of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

The occurrence of ARDS and other types of pulmonary complications after cardiac operations are shown in Table 5 and Figure 6. 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 6. Regarding

the postoperative variables, CPB duration along with the length of ischaemia and anaesthesia has proved longer in the ARDS group.

**Table 5.** 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



**Figure 6.** Incidence of postoperative pulmonary complications following open heart surgery

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 6.). 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. Concerning the whole patient population (where no laboratory variables have been studied) multivariate regression analysis has only justified the role of LCOS. These data are presented in Table 7.

**Table 6.** Correlation of postoperative ARDS with perioperative variables.

<i>Bivariate analysis</i>	<i>ARDS</i>	<i>Control</i>	<i>P</i>
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 (unit) ***	6	3	0,0002
Postoperative AMI *	40,0	6,5	0,0004
Postoperative LCOS *	50,0	5,7	0,0000

\* % ( $\chi^2$  test)    \*\* mean ± SD (T-test)    \*\*\* median (Mann-Whitney test)

**Table 7.** Correlation of postoperative ARDS with perioperative variables.  
"Logistic regression analysis"

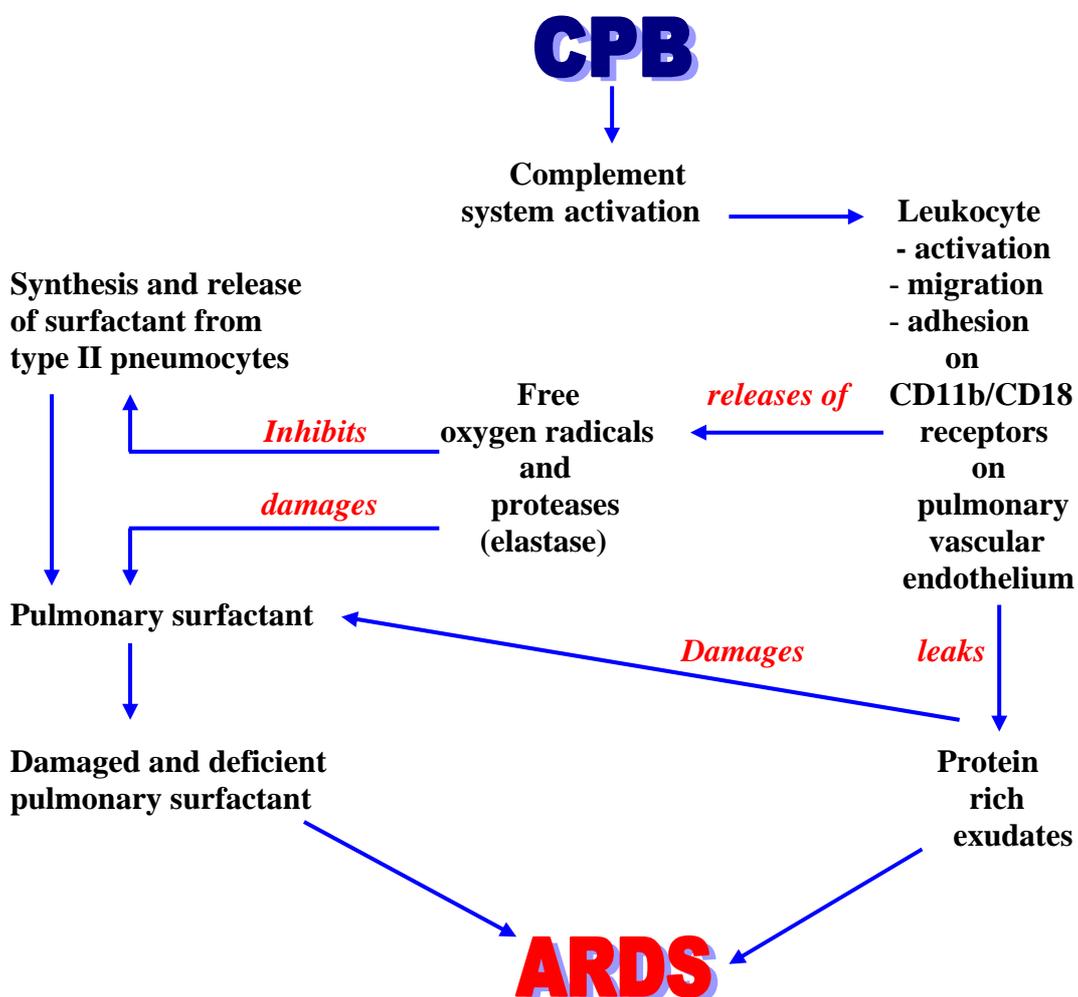
<b>n = 464</b>	<b>B</b>	<b>P</b>
Preoperative WBC ( $10^6/ml$ )	0,6355	0,0046
Preoperative ASAT > 37 (U/l)	2,4021	0,0489
Postoperative FFP transfusion (unit)	0,1662	0,0042
Postoperative AMI	4,2085	0,0035
CONSTANT	- 9,3069	0,0001
<b>n = 833</b>	<b>B</b>	<b>P</b>
Postoperative LCOS	2,8040	0,0000
CONSTANT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

### **4.3 Discussion**

Pulmonary complications following open-heart surgery have a negative impact on the postoperative course. CPB is responsible for about 15%-of all ARDS cases [80,81]. The incidence of ARDS following the use of CPB is around 0,25% - 2,5% [70,82,83]. According to the available, present international clinical data the syndrome results in a 35-75% mortality rate [71,84,85]. The figures of incidence and mortality clearly correlate with the types of the applied criteria. In the majority of cases with a fatal outcome multiorgan failure is added to ARDS [86]. Oxidative stress has been demonstrated in patients with ARDS by the presence of Myeloperoxidase (MPO) and oxidised alpha-1-antiproteinase in Bronchoalveolar Lavage (BAL) fluid [87], and the presence of hydrogen peroxide in expired air [88]. Both primary, [89] and secondary [90,91] plasma antioxidants are depleted in patients with ARDS, and there is frequent evidence for molecular damage to proteins [92,93]. The pathomechanism of ARDS developing after the use of CPB is shown on Figure 7. Numerous factors evolve during cardiac operations that may activate the complement system. The interaction between the blood, and the exchangeable parts used in the CPB device, (oxygenator, filters, tubing etc.) can be regarded as the most important factor, [94] therefore following the initiation of CPB, intermediate products of the complement system start to appear, as the first-wave mediators of general inflammatory response. The complement system is activated by two –the classic and the alternative- ways that are totally independent of each other. The alternative way is the most common response during CPB. The alternative way is initiated by complex polysaccharide and/or lipopolisaccharide molecules released from injured cells, however certain antibodies (e.g. aggregated IgA) are also capable

of the same effect. The final products of the complement cascade activate the neutrophil cells. Meanwhile cytokines activate the endothelial cells even before these would throw themselves into the process of inflammatory response [95]. Following their adhesion to the endothelial cells the activated neutrophil cells give off cytotoxic protease derivatives and oxygen free radicals. The emerging protease derivatives take down the elastin, collagen and fibronectin and destroy the extracellular structure; moreover they also play a certain role in the development of capillary membrane injury.



**Figure 7.** The pathophysiologic mechanism of the development of ARDS.

Due to the increased capillary permeability the extracellular fluid load is increased and electrolyte imbalance occurs in the postoperative period. Mainly these enumerated agents are responsible for the pulmonary and other organ injuries developing after cardiac operations.

An earlier study [96] verified the correlation of ARDS following cardiac surgery with age (>60 years), dialysis, the use of Intraaortic Balloon Pump (IABP) and with the amount of the filling fluid used in the CPB device (>3 litres). Since the use of IABP is justified by the LCOS, it is absolutely clear-cut that the two studies confirm and support each other. In our patient population we have been unsuccessful to justify the role of age and CPB filling volume.

The mortality rate of ARDS has still remained high in the past two decades. Apparently those conventional methods (IPPV, PEEP, higher inhaled O<sub>2</sub> concentration and diuretics) aiming the support of pulmonary ventilation and function have failed to improve the outcome of the syndrome. The main cause of death is multiorgan failure originating from tissue hypoxaemia. The challenge is increased by the fact that there is still no specific therapy for the treatment of ARDS. Having said that treatment strategy must include the following most important aspects:

- 1) Early diagnosis.
- 2) Improvement of oxygenation.
- 3) Reduction of oedema forming.
- 4) Prevention of secondary infections and other complications.

In order to warrant good survival chances for the patients, properly planned perioperative pulmonary prophylaxis and treatment are utterly important. To achieve that however exploration, delineation and understanding of the pathophysiology of ARDS and that of the predisposing factors are certainly required.

## 5 OXIDATIVE STRESS IN THE LUNGS DURING CPB

### 5.1 What is oxidative stress?

To answer this question it is necessary to define the concept of free radicals. A free radical can be defined as: *any species capable of independent existence that contains one or more unpaired electrons occupying an atomic orbital by itself* [97,98]. This situation is energetically unstable, often making such species highly reactive and short-living [97,98]. Stability is achieved by the removal of electrons from (i.e. oxidation of) surrounding molecules to produce an electron pair. However, the remainder of the attacked molecule then possesses an unpaired electron and has therefore become a free radical. Subsequent events depend on the reactivity of the target radical. Physiological metals such as iron or copper acting as catalyst agents are strongly associated with free radical chemistry [99,100]. Free radicals can disturb biological systems by damaging a variety of their constituent molecules. Lipids, proteins, carbohydrates and DNA are all potential targets for the chaotic oxidative attack of radicals produced in their vicinity [101,102,103,104]. Most of the important free radicals in human biology are derived from oxygen [105,106,107,108]. As the terminal electron acceptor for oxidative phosphorylation, molecular oxygen plays a key role in many of the metabolic processes associated with aerobic existence. Such strong affinity for electrons, however, also leads to the formation of Reactive Oxygen Species (ROS): oxygen intermediates that have either unpaired electrons (i.e. *superoxide anion* ( $O_2^-$ ), *hydroxyl radical* ( $\cdot OH$ ), *peroxyl and alkoxyl radicals*, or the ability to attract electrons from other molecules (i.e. *hydrogen peroxide* ( $H_2O_2$ ), *singlet oxygen*, *hypohalous acids* (HOC) [109]. The paradoxical need for an ultimately toxic oxygen species must have presented a major hurdle during the earliest stages of aerobic evolution. Under healthy conditions, the human body is continuously submitted to ROS but a large battery of antioxidants perfectly regulates the likely harm [110]. Indeed, ROS may play an important physiological role as illustrated with the killing of bacteria by granulocytes and macrophages [111], the regulation of blood arterial pressure by  $NO\cdot$  [112], or as recently linked to fertilisation [113]. However, if free radical activity suddenly increases as the consequence of

either a primary (*e.g. excess radiation exposure*) or a secondary (*e.g. tissue damage by trauma*) event, the antioxidant defence will be weakened, and in case of too prolonged ROS production, rapidly overwhelmed. Hence a useful definition of 'oxidative stress' would be ***a disturbance in the prooxidant-antioxidant balance in favour of the former, leading to potential damage***. Such a definition would incorporate damage products as indicators of oxidative stress [114].

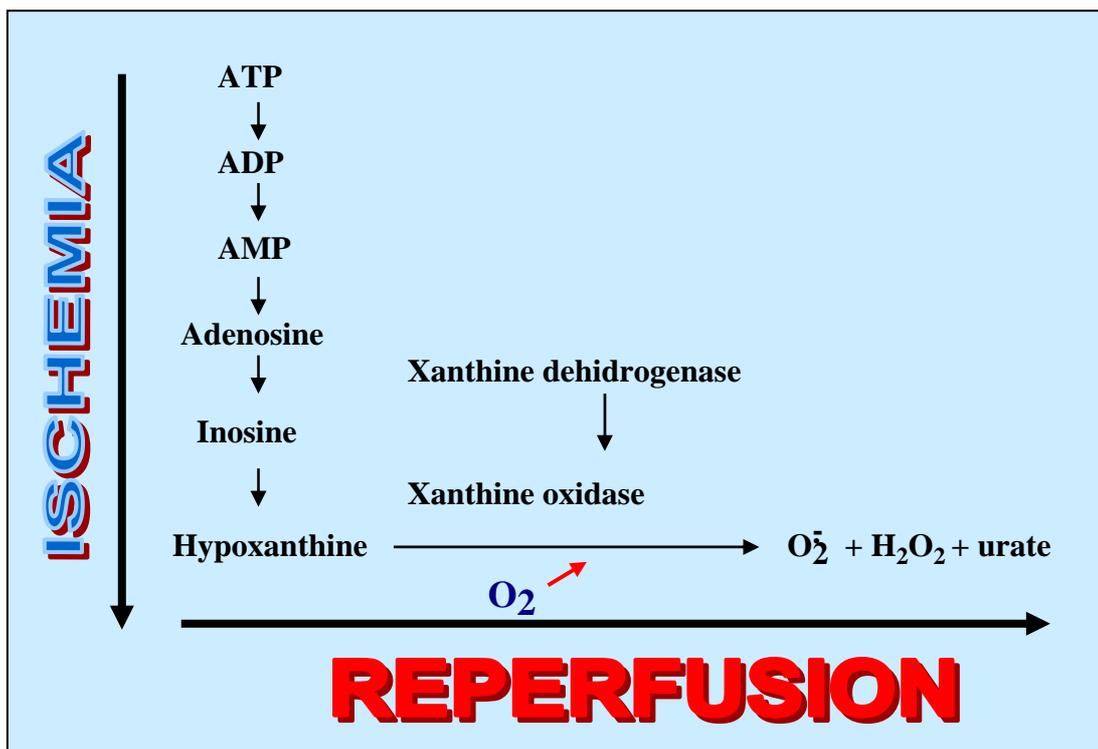
In order to exploit O<sub>2</sub> as a terminal electron acceptor for respiratory energy production, early life forms had to simultaneously develop an effective defence system to cope with unwanted and toxic oxygen species. This latter requirement took the form of a group of antioxidants that both scavenge and detoxify. Consequently, aerobic existence is accompanied by a persistent state of oxidative 'siege', where the survival of a given cell is determined by its balance of ROS and antioxidants.

Reperfusion of a tissue exposed to prior ischaemia results in increased free radical production [115,116]. Free radicals play an important role in organ dysfunction [117,118,119]. The involvement of various organs and tissues in the process of oxidative stress during CPB is still less known.

The lungs, exposed to high concentration of molecular oxygen, appear to be a common site for both the formation of free radicals, and also for serving as a target for oxygen radical-mediated processes. The spectrum of pulmonary diseases encompassed by this common mechanism of injury is extremely broad, reflecting the multiple cellular and subcellular sources and targets for reactive oxygen metabolites [120]. The lungs contain specific mechanisms for the inactivation of oxygen radicals that are found both intracellularly and extracellularly. Within mitochondria, cytochrome oxidase reduces oxygen to water and acts as a sink for free radicals. Superoxide Dismutase (SOD), catalase, and glutathione peroxidase act together to minimise the concentrations of toxic oxygen products. Antioxidant activity is also found in vitamins A, E and C.

Several lines of evidence indicate that reperfusion of the ischaemic lung results in some free radical generation [121]. Koyama et al [122] demonstrated that reperfusion of an isolated perfused dog lung lobe subjected to 6 hours of ischaemia results in progressive injury, assessed by an increase in lung weight. This injury was markedly attenuated by SOD, indicating a free radical mechanism [122]. Several sources of oxy-radical production in the lungs have been implicated. One important

source seems to be Xanthine Oxidase (XO) [123]. The presence of XO activity has been demonstrated in the lungs of several animal species, including dogs and rats [121,124,125]. The slow conversion of Xanthine Dehydrogenase (XD) to XO in the lungs raised some questions about the amount of damage caused by this source of free radicals. However, such conversion might not be necessarily required, since the initial XO content itself is high and may contribute to oxidative stress. Simply stating, tissue ischaemia appears to irreversibly convert XD to XO. XD, normally the predominant form of tissue xanthine/uric acid oxidoreductase activity, does not produce  $O_2$  metabolite products whereas XO does. Concomitantly, tissue ischaemia causes the catabolism of ATP to hypoxanthine, a substrate for both XD and XO. Hence, reperfusion supplies molecular oxygen that drives the reaction of XO and hypoxanthine forward to ROS (Figure 8).



**Figure 8.** Mechanism of tissue injury from ischaemia-reperfusion, as proposed by McCord [126].

Another source of free radicals is the mitochondrion. Lung mitochondria have been shown to increase their hydrogen peroxide production dramatically as oxygen tension rises [127]. A third potential source of free radicals in the lungs is neutrophil

cells. A number of reports support the involvement of neutrophils in the pathogenesis of lung injury [128,129].

When CPB is used, ventilation of the lungs is suspended in the ischaemic period and blood supply in this interval is only ensured through the bronchial arteries. Therefore, it is reasonable to assume that lung tissues may suffer an ischaemic-reperfusion injury during open-heart surgery. Type I cells are the most vulnerable for oxidant damage in the alveolus because of their large surface area and the possibility of a reduced antioxidant capacity compared to type II alveolar epithelium [130]. One of the best ways to prove these damages is to justify free radical reactions. The objective of the present study has been the investigation of the presence and the extent of oxidative stress in the lungs during heart operations requiring CPB.

## 5.2 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 8.

**Table 8.** *Clinical data of enrolled patients.*

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

### 5.2.1 Study protocol

Blood samples were taken to cuvettes containing EDTA for laboratory analysis from the pulmonary and radial artery and from the left atrium. The samples were analysed immediately. Sampling protocol is shown in Table 9.

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

- Malondialdehyd (MDA)
- Reduced and Oxydated Glutathione (GSH-GSSG)
- Myeloperoxidase (MPO)
- Superoxide Dismutase (SOD)
- Absolute neutrophil count
- Stimulated radical production of isolated PMN

**Table 9.** *Blood sampling protocol.*

Sample	Time of blood sampling	Blood sampling site
S1	Immediately after the conduction of anaesthesia	PA, RA
S2	Immediately before aortic cross clamping	PA, LA
S3	30 minutes after aortic cross clamping	PA, LA
S4	40 minutes after aortic cross clamping	PA, LA
S5	5 minutes after the release of aortic cross clamping	PA, LA
S6	30 minutes after the release of aortic cross clamping	PA, LA
S7	24 hours after surgery	PA, RA

*PA: Pulmonary Artery*

*RA: Radial Artery*

*LA: Right Atrium*

*N.B.: Blood samples taken from the pulmonary artery (Swan-Ganz catheter) served only for quantitative and qualitative leukocyte analysis.*

The activity of SOD was calculated by the inhibition of the adrenalin-adrenochrom transformation induced by the superoxide anion [131], and the results are given in the form of IU/ml. Malondialdehyd concentration was determined by spectrophotometry with the use of Placer's method [132] whilst Ohakawa's method was used to determine the MDA content of blood plasma [133]. GSH and GSSG levels were assessed both in the plasma and in the RBC with Tietze's enzymatic method, using spectrophotometric measurements [134]. The superoxide radical producing capacity of PMN was determined with Guarnieri's cytochrom-C reduction method [135], whilst, MPO released by the PMN was detected by the Schultz technique [136]. Blood plasma MPO levels are displayed in the form of optical

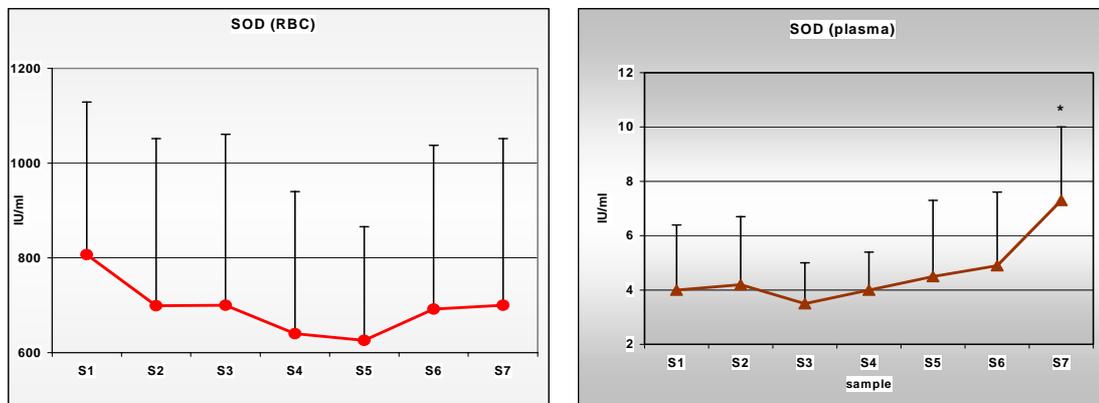
density (OD/ml). The absolute neutrophil count was determined in all blood samples using an automatic laboratory device (Cell-Dyn 3500SL, Abbott Laboratories).

### 5.2.2 Statistical analysis

All values are given as mean  $\pm$  SD. Ratio of GSH and GSSG concentrations were calculated (Ratio = GSH concentration/GSSG concentration). Statistical comparisons were performed using paired and unpaired Student's *t*-test. Values of *p* < 0.05 were considered to be of statistical significance.

### 5.2.3 Results

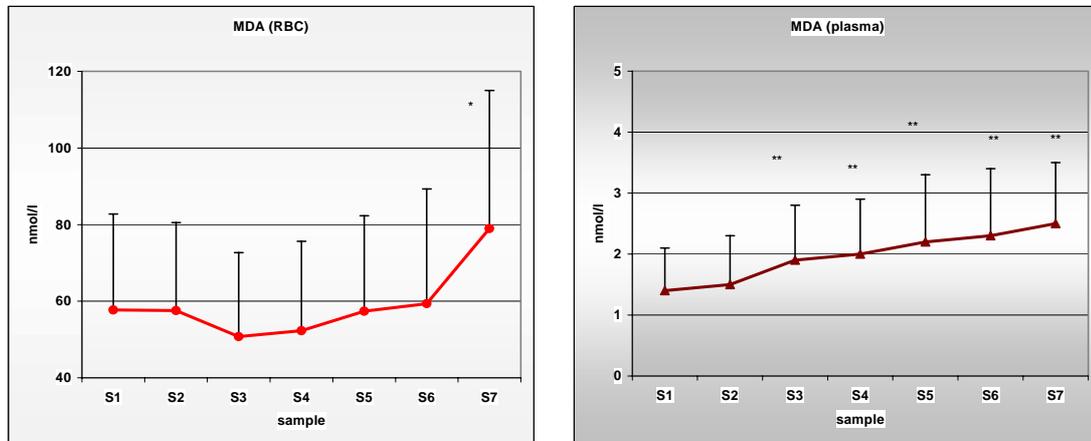
Although statistically the changes were not significant but we experienced a mild decrease in the serum (starting value:  $4 \pm 3.4$ ; 30<sup>th</sup> minute of ischaemia:  $3.5 \pm 1.5$ ; 40<sup>th</sup> minute of ischaemia:  $3.9 \pm 2.3$  IU/ml) and RBC (baseline value:  $807 \pm 322$ ; 40<sup>th</sup> minute of ischaemia:  $700 \pm 300$ ; 5 minutes of reperfusion:  $636 \pm 241$ ; 30 minutes of reperfusion:  $692 \pm 346$  IU/ml) SOD levels during ischaemia and reperfusion.(Figure 9)



**Figure 9.** RBC and plasma SOD concentrations.

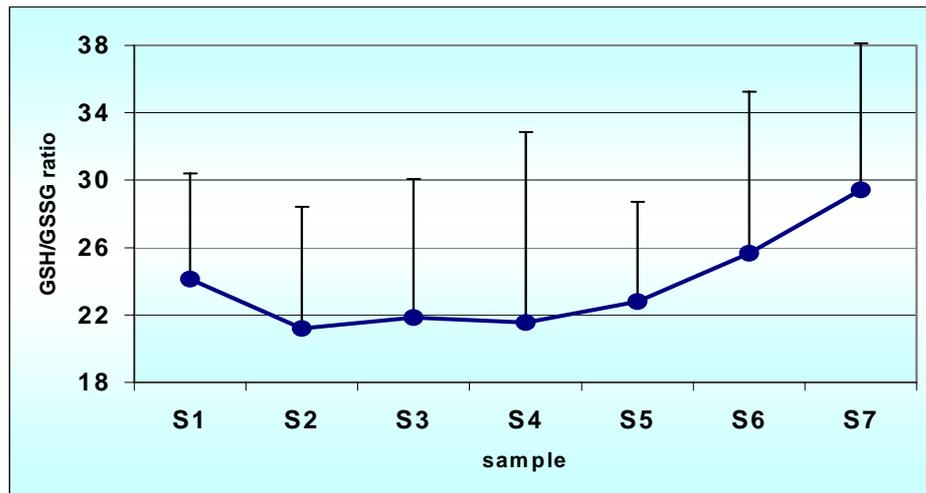
Even 24 hours after surgery the RBC SOD content proved lower than the starting value ( $703 \pm 352$  IU/ml). The concentration values of MDA originating from red blood cells did not show any statistically significant change during surgery but they proved somewhat lower compared to the baseline value (starting value:  $57.7 \pm 26.7$ ; 40 minutes ischaemia:  $52.3 \pm 23.3$ ; 5 minutes reperfusion:  $57.4 \pm 24.9$  nmol/ml). However 24 hours following surgery a significant increase could be detected in MDA values ( $79 \pm 39.6$  vs.  $57.7 \pm 26.7$  nmol/ml at the beginning of the operation, *p*

< 0.05). Plasma MDA values were found continuously and significantly increased during surgery (baseline value:  $1.4 \pm 0.7$ ; 40 minutes ischaemia:  $2 \pm 0.9$ ; 30 minutes reperfusion:  $2.3 \pm 1.1$ ; 24 hours later:  $2.5 \pm 1$  nmol/ml) (Figure 10)

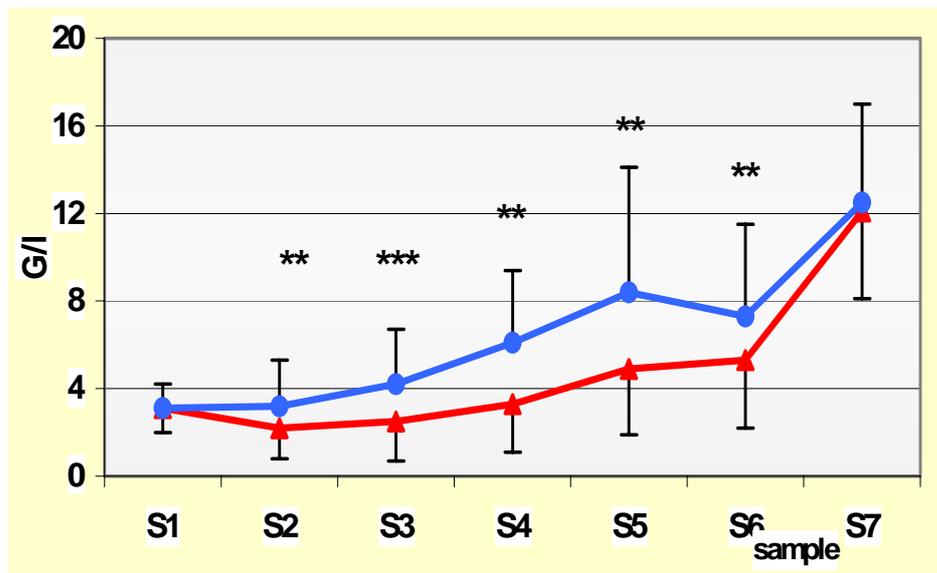


**Figure 10.** RBC and plasma MDA Concentrations.  
\*  $p < 0.05$  \*\*  $p < 0.01$

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  $24 \pm 6.3$  to  $21.5 \pm 11.3$ ), 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 ( $29.4 \pm 9.1$ ) (Figure 11). While the absolute value of neutrophil count measured in the pulmonary artery has shown a two-fold rise (baseline value:  $3.1 \pm 1.1$  G/L; 40 minutes ischaemia:  $6.1 \pm 3.3$  G/L) it has been first found decreased in the samples taken from the left atrium (baseline value:  $3.1 \pm 1.1$  G/L, 30 minutes ischaemia:  $2.5 \pm 1.8$  G/L), then in the early reperfusion period it has been found suddenly increased again (20 minutes reperfusion:  $5.3 \pm 3.1$  G/L). During ischaemia and reperfusion there has been a significant difference in the pre- and postpulmonary absolute neutrophil count in each case. (Figure 12) 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:  $18.7 \pm 4.9$  early reperfusion:  $3 \pm 1.3$  nmol  $O_2^-$ /min/ $1.5 \times 10^5$  PMN). The changes have proved significant in both cases ( $p < 0.001$ ).



**Figure 11.** The kinetics of GSH/GSSG ratio



**Figure 12.** Average leukocyte count during surgery

● prepulmonary samples (pulmonary artery)

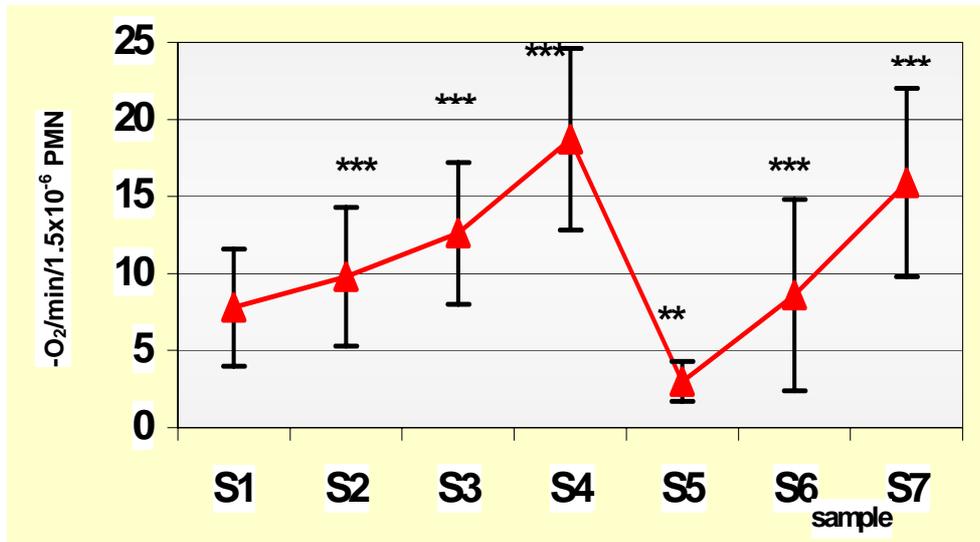
◻ postpulmonary samples (left atrium)

\*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$

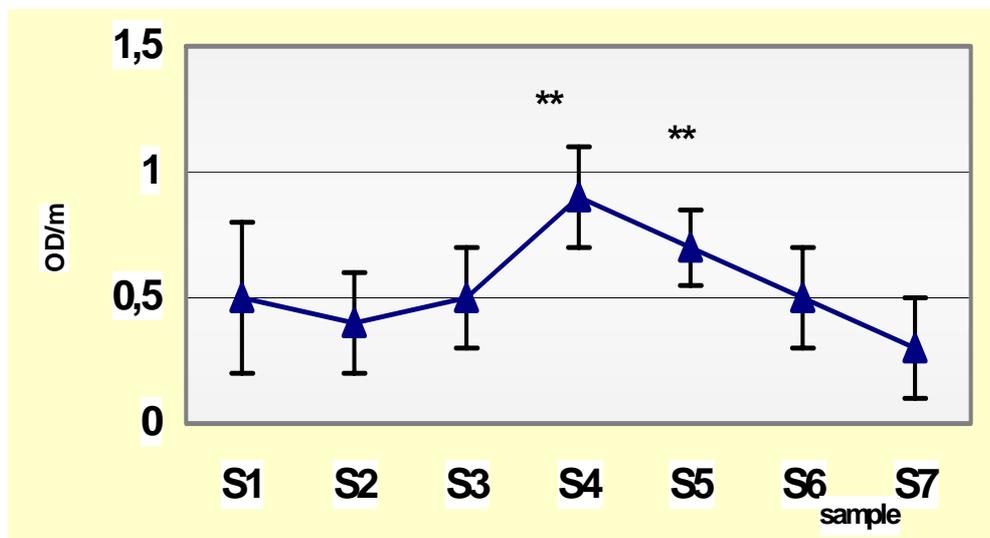
Differences were calculated between the samples taken at the same time

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 (Figure 13). 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 (Figure 14). No postoperative pulmonary complication has been experienced in any of the patients.



**Figure 13.** Changes of free radical producing capacity of PMN cells  
 \*\*\*  $p < 0.001$  Normal value:  $8.5 \pm 1.8$  nmol  $^{-}O_2$ /min/1.5x10<sup>-6</sup> PMN



**Figure 14.** Average values of myeloperoxidase produced by PMN  
 \*  $p < 0.05$  \*\*  $p < 0.01$

#### 5.2.4 Discussion

The occurrence of oxidative stress after lung ischaemia and reperfusion has already been shown in animal experiments [121,122], but it is not yet well documented in humans during CPB condition. From a biochemical point of view, the

major feature of this reaction is that molecular oxygen gains electrons in a sequential fashion, each gain leading to the generation of a reactive intermediate. The main final products are  $O_2^-$ ,  $H_2O_2$ , and its hydrogen radicals. All these molecules can initiate the peroxidase decomposition of cellular phospholipid membranes and alternatively damage the mitochondrial de-energization; similarly, both events lead to the loss of cell viability [137,138]. Under normal conditions, free radicals are produced in very small quantities within the cell. However, rapid scavenging of  $H_2O_2$  and  $O_2^-$  radicals by superoxide dismutase, catalase and glutathione peroxidase, prevents these reactive products from attacking other molecules in the cells. Because the free radicals themselves are highly reactive, with life spans of the order of microseconds or shorter, they can only be measured directly in tissues and in cell-free systems by electron spin resonance spectroscopy (electron paramagnetic resonance). Thus to prove ROS production in clinical conditions, the first important problem to face is the determination of techniques. Direct visualisation by electron spin resonance is not yet available in clinical practice [139,140], and for this reason I chose indirect methods.

GSH, a ubiquitous tripeptide thiol, is a vital intra- and extracellular protective antioxidant, which plays a key role in the regulation oxidant induced lung epithelial cell function and also in the control of pro-inflammatory processes in the lungs [141]. After oxidative ischaemia-reperfusion events this enzyme is converted to its dimeric oxidised form GSSG which is then re-reduced by glutathione reductase utilising NADPH [142]. As GSSG is toxic to cells and is extruded through an active mechanism dependent on intracellular GSH levels, substance appears in plasma. The rate limiting enzyme in GSH synthesis is gamma-glutamylcysteine synthetase ( $\delta$ GCS). Both GSH and  $\delta$ GCS expression are modulated by oxidants, phenolic antioxidants, inflammatory, and anti-inflammatory agents in lung cells.

When the lungs are reperfused after ischaemia by oxygenated blood during CPB, a probably an increased 'leakage' of electrons from activated neutrophils and disrupted mitochondria leads to a extensive production of  $O_2^-$  or  $H_2O_2$ . Meanwhile, GSH is oxidised to GSSG [142]. In this way, any increment in intracellular  $O_2^-$  is evidenced by the further increase in GSSG and a decrease in GSH/GSSG ratio. Ferrari and associates have demonstrated that serum GSSG increases in human heart

during ischaemia. Erythrocytes would theoretically represent the most efficient site for GSH cycle activation during ischaemia, and on the basis of this possibility; I monitored the variations in total GSH within the RBC.

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 malondialdehyd, 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

All organs are able to release lactate in both physiologic and pathophysiologic conditions. The organs producing the largest amounts of lactate are the skin, erythrocytes, skeletal muscles, and leukocytes (20, 16, 12 and 11 mg/kg\*min, respectively) [143].

Lactate is often used clinically as a marker of anaerobic metabolism and thus is assumed to represent inadequate tissue perfusion [144]. This hypothesis is supported by the high mortality seen in patients with hyperlactatemia [145] and by the disturbed oxygen transport exhibited by patients with sepsis [146]. Many clinicians routinely use therapies to improve global oxygen transport on the assumption that such treatment will reverse tissue hypoxia and ameliorate hyperlactatemia [147,148].

In normal human beings, the concentration of lactate in arterial blood varies between 0.1 and 1 mmol/l. This concentration reflects the balance between the rate of lactate production and removal [149,150]. Since lactate is freely diffusible in body fluids, the concentration gradient between cells and the plasma will determine whether a particular organ is a consumer or a producer. It has long been recognised that approximately 50% of the glucose consumed by the lungs is converted to lactate and appears in the pulmonary venous outflow [151]. In contrast, less than 25% of glucose consumed in other tissues (e.g. the heart) is converted to lactate. This level of lactate production by the lungs is puzzling since they are exposed to the highest oxygen tensions of any internal organ. However, the lungs do not release much lactate, so that the arterio-venous differences in lactate (AVLAC) across the lungs are small and close to zero under physiologic conditions [152,153,154].

Pulmonary lactate production can be attributed to the absence of mitochondria in the attenuated portions of type I. pneumocytes and there is little room for mitochondria in the adjacent endothelium [155]. Extrapolating from animal studies, one can estimate that normal human lungs, weighing a total of 1000 g, produce 11 mmol of lactate each hour [151]. It is certainly conceivable that injured lungs containing large numbers of leukocytes, which also produce lactate, may release significantly more lactate into the circulation. In critically ill patients, Weil

and colleagues [156] observed that venous blood samples from a pulmonary artery catheter yielded lactate concentrations equivalent to those in arterial blood. Similar results were obtained by Nimmo and associates in patients with sepsis or ARDS [157]. However, Brown and co-workers [158] reported in 19 patients that lactate production by the lungs could increase in patients with sepsis and ARDS, and lung lactate production was proportional to the degree of respiratory failure. Similarly, Douzinas and colleagues [159] reported lactate production by the lungs in 14 patients with multiple organ failure including ARDS. One can wonder if lactate production by the lungs is specific to ARDS or it could also be observed in other forms of lung injury, such as the injury occurring after CPB. In total CPB without aortic cross clamping and normothermic conditions there was an increase of lactate content in piglet lung tissue at the end of CPB [160]. 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. Whether the lungs can generate as much as 200 mmol/h remains to be confirmed. Even if the lungs can produce this much lactate, it cannot be concluded that they are the principal culprits responsible for lactate accumulation, because other organs that metabolise lactate should be able to accommodate this additional metabolic load. It can be estimated that a normal liver can consume a maximum of 140 mmol of lactate each hour and even more can be metabolised by other tissues, particularly skeletal muscle [161]. Liver abnormalities are common in septic patients [162], and decreased extrapulmonary lactate consumption may play a more important role in the development of lactic acidosis than abnormal pulmonary metabolism.

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 10.

---

**Table 10.** *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

---

Membrane oxygenator (Biocor 200 IHS, Minntech Corporation USA) was used in all cases. In addition to general patient exclusion clauses shown before, further exclusion criteria were as follows: patients with diabetes mellitus and those with a history of lung surgery. Simultaneous blood samples were obtained from left atrial and pulmonary arterial (Swan-Ganz) catheters before, during and after CPB (table 11)

---

**Table 11.** *Blood sampling protocol.*

---

S1	before CPB
S2	5 minutes after aortic cross clamping
S3	20 minutes after aortic cross clamping
S4	40 minutes after aortic cross clamping
S5	1 minute after aortic cross clamp release
S6	10 minutes after aortic cross clamp release
S7	10 minutes after CPB cessation
S8	2 hours after CPB cessation
S9	24 hours after CPB cessation

---

*N.B.: S1, S8, S9 for postpulmonary blood were obtained from the radial artery.*

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

### 6.1.1 Measurements and calculations

Blood gases, pH, haemoglobin, haematocrit and serum blood lactate levels were measured using a blood analyser (Radiometer ABL-625; Copenhagen, Denmark). The transpulmonary lactate gradient was determined as the ratio of the lactate concentration measured after and before the lungs.

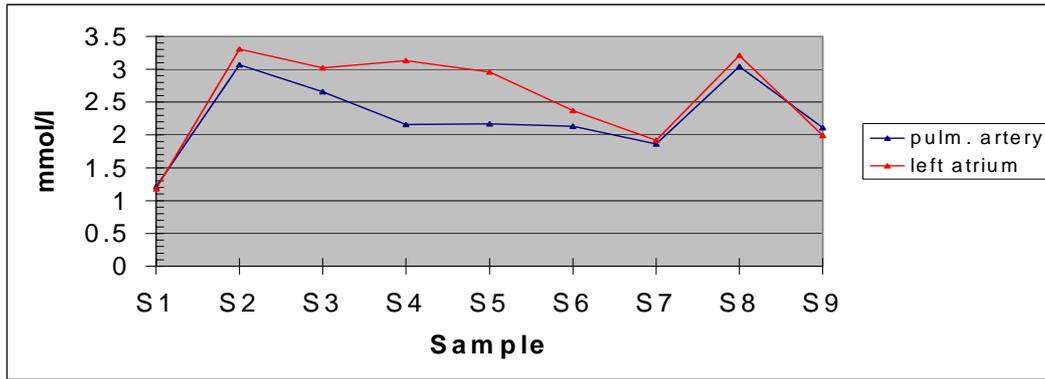
$$\text{Lactate ratio} = \frac{\text{Lactate concentration measured in the postpulmonary blood}}{\text{Lactate concentration measured in the prepulmonary blood}}$$

### 6.1.2 Statistical analysis:

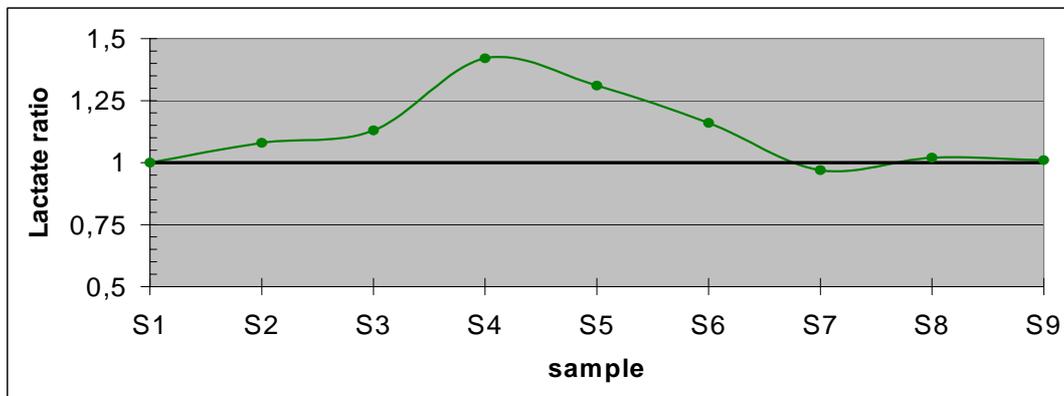
Data are given in the form of median and range. Wilcoxon and Mann-Whitney tests were applied. Spearman's rank correlation coefficient was used to assess the linear association of measurements.

### 6.1.3 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 (Figure 15). Lactate ratio figures were constantly increased during the ischaemic period and they were significantly higher compared to the value of baseline ratio (Figure 16). 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. The exact values are shown in Table 12.



**Figure 15.** Median lactate concentration values during and after CPB in the pre-, and postpulmonary blood samples.



**Figure 16.** Lactate ratio figures during and after CPB

**Table 12.** Median values of lactate concentrations

Sample	Postpulmonary blood samples	Prepulmonary blood samples	<i>P</i>
	Median (range)	Median (range)	
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)	<b>&lt; 0,006</b>
S4	3,13 (1,66 – 4,58)	2,16 (1,31 – 3,51)	<b>&lt; 0,003</b>
S5	2,96 (1,60 – 4,81)	2,18 (1,45 – 4,22)	<b>&lt; 0,019</b>
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.

#### **6.1.4 Discussion**

Notable lactate production occurs during and after CPB. Two peaks of lactate values could be observed: one in the ischaemic period, the other one in the 2<sup>nd</sup> hour of reperfusion. The elevation of lactate values in the ischaemic period has proved more significant in the postpulmonary (left atrial) blood samples. Based on the lactate concentrations from pre-and postpulmonary blood samples and the ratio derived from these values, one can speculate that it is the lungs that should mostly be responsible for the above phenomenon. The ethiology of lung lactate release in this condition unclear. However, it is possible that lung hypoxia had a role. Alternatively, the lungs have the largest vascular endothelial surface in human body and injury to the endothelial cells in this vasculature may result in lactate release from either aerobic or anaerobic metabolism or from the inhibition of pyruvate dehydrogenase. Furthermore, neutrophils are sequestered in the pulmonary circulation during CPB and they may also serve as source of lactate release. With referral to these results it is vitally important to take in consideration that if in any case lactate concentrations are measured during CPB, there is a difference of theoretical significance between those lactate values measured in the arterial and venous blood. Blood transfusion given during CPB may influence the lactate values measured in the ischaemic period. This phenomenon can be explained by the low oxygen delivery capacity. I can suggest that, effects from other organs, primarily the skin should result in the second peak.

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

### **7.1 Inflammatory response and the lungs**

There is no doubt that the lungs are in a unique position and therefore may be particularly susceptible to different mechanisms of injury. It is one of the three sites in the body where inside meets the outside. It is obvious therefore that the lungs contain a number of special defence mechanisms and also special cell types that may be inappropriately triggered. Together with the skin and gut, the lungs are a rich site for mast cells. In addition, there is a large population of macrophages, which are mobile and scavenge the alveoli and small respiratory bronchioles. If activated, both of these cell types are able to produce deleterious effects that may be categorised as lung inflammation leading to abnormal lung function. An example of this activation of lung inflammation after nonpulmonary trauma comes from studies of peripheral ischaemia and reperfusion. These showed that reperfusion of the lower limbs after a period of ischaemia was associated with an abnormal and injurious response within the lungs [163]. This was shown as an increase of protein flux into the lungs together with an increase in lung water content [164,165]. In addition to having a variety of different cell types in the lungs, the pulmonary circulation and its architecture are different from that found in the skin or in other microvascular beds. Whether the human pulmonary vascular endothelium behaves differently from the systemic endothelium when stimulated, is yet not known.

The influence of cytokines on the inflammatory response has been the subject of investigations in cardiac surgery recently. The results of these investigations during CPB suggest alterations in both the pro- and in the anti-inflammatory cytokines [166,167,168,169]. Few efforts have been devoted to the assessment of the role of the lungs in this process, and on the other hand, the existing data are controversial.

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.

## **7.2 Cytokines**

Cytokines are a large and rapidly expanding group of polypeptides or glycopeptides with molecular weights from 5 to 70 kilodalton (kDa). They are produced by many different cell types; the major site of synthesis, however, appears to be cells of the macrophage and monocyte series [170]. Cytokines can be either protective or damaging. Most cytokines act locally at extremely low concentrations (picomolar or femtomolar levels) in the vicinity of the production site, and under physiological conditions cytokines are undetectable or only found at low concentrations in peripheral blood. At low levels, cytokines seem to be essential for the optimal function of the defence and repair system of the body. The release of cytokines can be stimulated by a number of factors including ischaemia-reperfusion, complement activation, endotoxin release and the effect of other cytokines [171]. Few cytokines augment or inhibit their own production and secretion [172]. Some of the clinically most important cytokines also act systemically as pleiotropic hormones that modulate functions of cells at distant locations via blood and lymphatic circulation. These cytokines seem to be potentially harmful mediators under pathological conditions such as major trauma, sepsis and shock, where significant plasma levels may be reached. Cytokines profoundly influence vascular endothelium [172] and they affect the vasomotor tonus in small arteries by inducing the inducible nitric oxide synthesis (iNOS), which stimulates the synthesis of the vasodilatory nitric oxide radical [173,174]. It appears from both human and animal studies that the cytokine effect is dose dependent. CPB has been associated with the production of the following cytokines; tumour necrosis factor (TNF- $\alpha$ ) and interleukins (IL) 1, 6, 8, 10 [171,175].

## **7.3 Cytokine networks in the lung**

The lungs are constantly exposed to inhaled antigens and particulates. The majority is cleared by normal pulmonary defences without the development of inflammatory response. Cytokines regulate the same range of biologic events in the

lungs as in other organs. However, the cytokine network in the lungs has a number of special features that appear to allow for the specialised regulation that normal lungs require. To understand cytokine networking in the lungs, I have reviewed the cytokine profiles of a number of the major cells in the lungs.

### **7.3.1 Tumour Necrosis Factor (TNF- $\alpha$ )**

Tumour necrosis factor alpha (TNF- $\alpha$ ) [176,177], also known as *cachectin*, is produced by various cells of reticular endothelial cell system, neutrophils, activated T and B lymphocytes, natural killer cells, astrocytes, endothelial cells, smooth muscle cells, and some transformed cells [178,179,180]. The expression of TNF- $\alpha$  is tightly controlled both at transcriptional and translational levels. The properties and activities of the TNF- $\alpha$  has been the subject of numerous reviews [181,182,183]. The half-life of circulating TNF- $\alpha$  is short, 14-18 minutes, and it is degraded in several organs including the liver, skin, gastrointestinal tract and the kidneys [184]. Experimental data suggest that ischemia and reperfusion in isolated hearts cause liberation of TNF- $\alpha$  [183]. TNF- $\alpha$  is an extremely pleiotropic factor, and it is capable of producing a wide variety of effects due to the ubiquity of its receptors, to its ability to activate multiple signal transduction pathways, and to its ability to induce or suppress the expression of a vast number of genes, including those for growth factors and cytokines, transcription factors, receptors, inflammatory mediators and acute phase proteins, etc. [178, 185].

### **7.3.2 Interleukin-2 (IL-2)**

Interleukin 2 (IL-2) is a pleiotropic cytokine produced primarily by mitogen- or antigen -activated T helper (Th1) lymphocytes [186]. It has been found to play an important role in anti tumour responses [187]. In addition, IL-2 has also been shown to mediate multiple immune responses on a variety of cell types. It stimulates the proliferation of thymocytes; stimulates the proliferation and differentiation of activated B cells; promotes growth, differentiation and cytotoxic activity of monocytes; and induces the proliferation and differentiation of oligodendrocytes. IL-2 induced microvascular lung injury is an experimental paradigm commonly used to investigate the pathogenesis of the ARDS. Rabinovici et al [188] have proved that locally produced TNF- $\alpha$  mediates IL-2 induced lung inflammation and tissue injury.

### **7.3.3 Interleukin-6 (IL-6)**

IL-6 is a cytokine with pleiotropic biological activity [189]. It regulates immune responses and haemopoiesis, and several studies have confirmed that it initiates and regulates the synthesis of acute phase reactants such as fibrinogen, haptoglobin, serum amyloid-P (SAP) and C-reactive protein (CRP) [190,191]. IL-6 is the main cytokine released after different types of surgery. In relation to cardiac surgery, a marked increase in IL-6 levels appears during and immediately after CPB [192,193,194]. Peak concentration occurs a few hours after the end of CPB with a gradual decrease towards preoperative levels in the following 24 h [195]. In only a few studies, however, have patients been tested for cytokines for a prolonged postoperative period. The characteristic IL-6 response to CPB has been demonstrated after hypothermic as well as normothermic CPB [196], and after CPB performed with heparin-coated CPB circuits [195]. In a study of patients undergoing cardiac surgery pretreatment with methylprednisolone (30 mg/kg) suppressed the CPB-induced increase of IL-6 [197], while methylprednisolone given in the same dose did not modify the IL-6 levels in patients undergoing lung surgery [198]. It has not yet been clarified whether the plasma IL-6 concentration simply reflects the degree of tissue damage or plays a more active part in the induction of, or defence against, postoperative complications. In contrast to TNF and IL-1, IL-6 does not produce tissue damage or haemodynamic instability in vivo. This suggests that IL-6 is a marker rather than a mediator [199]. IL-6 is the cytokine whose levels best predict patient outcome [200]. The higher is the level of IL-6, the higher is the incidence of death. Clinical study on adult patients has quantitatively shown that IL-6, a potent neutrophil stimulator [201].

### **7.3.4 Interleukin-8 (IL-8)**

The molecular weight of IL-8 is 8-10 kilodaltons. IL-8 is suspected to be a trigger of neutrophil-induced endothelial injury, and is the first known chemoattractant for neutrophils [202]. IL-8 is produced in vitro by a variety of cells in response to stimulation by IL-1 and TNF [202]. A local production of TNF- $\alpha$  and IL-1 $\beta$ , even if undetectable in the circulation, can induce IL-8 synthesis and secretion. The production is increased in the lungs following hypoxia-hyperoxia

[203] and in the setting of neutrophilic alveolitis [204]. IL-8 has been shown to prime human neutrophils for enhanced superoxide production [205], and its levels rise markedly in humans after the intravenous administration of small doses of endotoxin [204]. High IL-8 concentrations have recently been demonstrated in septic shock patients [206]. Increased plasma/serum IL-8 levels have been shown in relation to major surgery [196, 207], and the IL-8 response seems to parallel that of IL-6 in time course. It has been suggested that IL-8 might play a major role in reperfusion injury described after cardiac surgery [196]. The cells involved in the production of IL-8 after cardiac surgery have not been identified, but alveolar macrophages might be the potential source. This is supported by the detection of high levels of IL-8 in the BAL fluid in patients who had previously undergone CPB [208,209].

### **7.3.5 Interleukin-10 (IL-10)**

In contrast to proinflammatory cytokines IL-1 $\alpha/\beta$ , IL-6, IL-8 and TNF $\alpha/\beta$ , recent data indicate that IL-10 is an anti-inflammatory cytokine, suppressing immunological and inflammatory reactions. IL-10 is produced by a number of cells: Th2 lymphocytes, monocytes, neoplastic B cells [210] and Kupffer cells [211]. IL-10 has a regulatory function on the blood mononuclear cells and connective tissue cells; it stimulates the IL-1 receptor antagonist (IL-1ra), and suppresses the IL-8 [212] and IL-6 synthesis by T-cells [213].

In order to suppress the lipopolysaccharide induced production of proinflammatory cytokines; IL-10 was administered to humans. It was found that IL-10 suppressed the TNF- $\alpha$  and IL-1 $\beta$  production, and that IL-10 medication was safe and without major side-effects [214].

The role of IL-10 as part of the cytokine response to surgery and sepsis still remains to be clarified. It has been suggested that IL-10 is essential for the control of the inflammatory response induced by bacterial infection [215]. In animals subjected to surgery, IL-10 administration minimised postoperative intraperitoneal adhesions [216]. It has also been demonstrated that the concentration of IL-10 in septic patients correlates with the severity of disease [217].

Interleukin-10 is a potent inhibitor of the production of TNF- $\alpha$ , IL-1 $\beta$ , IL-6 and IL-8. This cytokine exerts a protective role.

## 7.4 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

After sternotomy, a catheter (8 F) was inserted in the left atrium through the right upper pulmonary vein to collect postpulmonary blood samples.

Pulmonary arterial and left atrial blood samples were obtained simultaneously. Blood sampling times were as follows:

- S1:** Before CPB.
- S2, S3, S4:** 5, 20, 40 minutes after aortic cross clamping.
- S5, S6:** 1 and 10 minutes after aortic cross clamp release.
- S7, S8, S9:** 10 minutes, 2 and 24 hours after CPB cessation.

S1, S8 and S9 postpulmonary blood samples were obtained from the radial artery catheter.

Although no complications were associated with the catheterisation of the left atrium, the inserted catheter was withdrawn under visual control before the closure of the chest to minimise any potential risk. All blood samples were anticoagulated with ethylenediaminetetraacetic acid and immediately centrifuged in a cooled device, (4000 revs/min for 20 minutes at 4 °C). The plasma was transferred to a sterile polypropylene test tube and stored at -20 °C until the laboratory investigations took

place. The samples were analysed within 4 weeks. TNF- $\alpha$ , 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, R&D systems, Inc. Minneapolis, USA). The sensitivity was less than 4.4 pg/ml for TNF- $\alpha$ , less than 7 pg/ml for IL-2, less than 0.7 pg/ml for IL-6, less than 10 pg/ml for IL-8 and less than 3.9 pg/ml for IL-10. Different types of leukocyte counts as well as the transpulmonary cytokine gradient were measured. The cytokine gradient was calculated as the ratio of cytokine concentration measured after and before the lungs.

$$\text{Cytokine ratio} = \frac{\text{Cytokine concentration measured in the postpulmonary blood}}{\text{Cytokine concentration measured in the prepulmonary blood}}$$

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.

## 7.5 Results

Morphometric and demographic characteristics, preoperative cardiopulmonary function, and the duration of surgery are shown in Table 13. None of the patients had any difficulty during weaning from CPB without inotropic agents. Once extubated, none of the patients experienced subsequent serious respiratory complications or required reintubation. PaO<sub>2</sub> decreased, and the lung injury score increased significantly. Although pH and PaO<sub>2</sub> decreased at the end of surgery, the other hemodynamic and respiratory measurements did not differ significantly over time (Table 14). 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 15).

**Table 13.** *Main clinical data.*

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

**Table 14.** *Intraoperative data.*

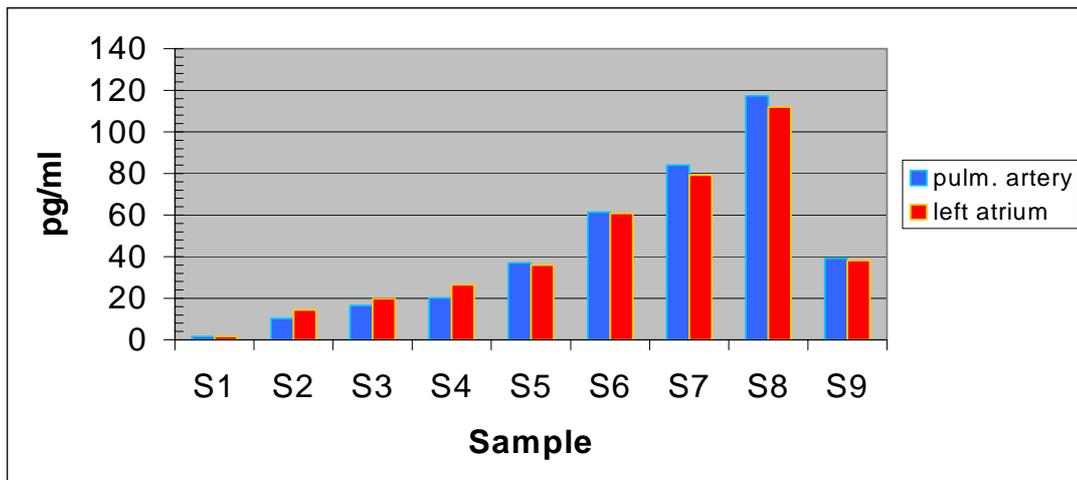
	Beginning	End
Mean arterial pressure (mmHg)	92 $\pm$ 9	90 $\pm$ 11
Heart rate (bpm)	77 $\pm$ 9	86 $\pm$ 9
Central venous pressure (cm H <sub>2</sub> O)	7.9 $\pm$ 2.3	7.8 $\pm$ 2.7
Cardiac index (L*min <sup>-1</sup> *m <sup>-2</sup> )	2.6 $\pm$ 0.2	2.8 $\pm$ 0.4
Oesophageal temperature (°C)	36.5 $\pm$ 0.3	36.3 $\pm$ 0.5
Arterial pH	7.41 $\pm$ 0.04	7.38 $\pm$ 0.04
Arterial PCO <sub>2</sub> (mmHg)	39 $\pm$ 3	40 $\pm$ 2
Arterial PO <sub>2</sub> (mmHg)	475 $\pm$ 35	328 $\pm$ 88 *
Activated clotting time (s)	133 $\pm$ 24	144 $\pm$ 32

**Table 15.** *Plasma leukocytes.*

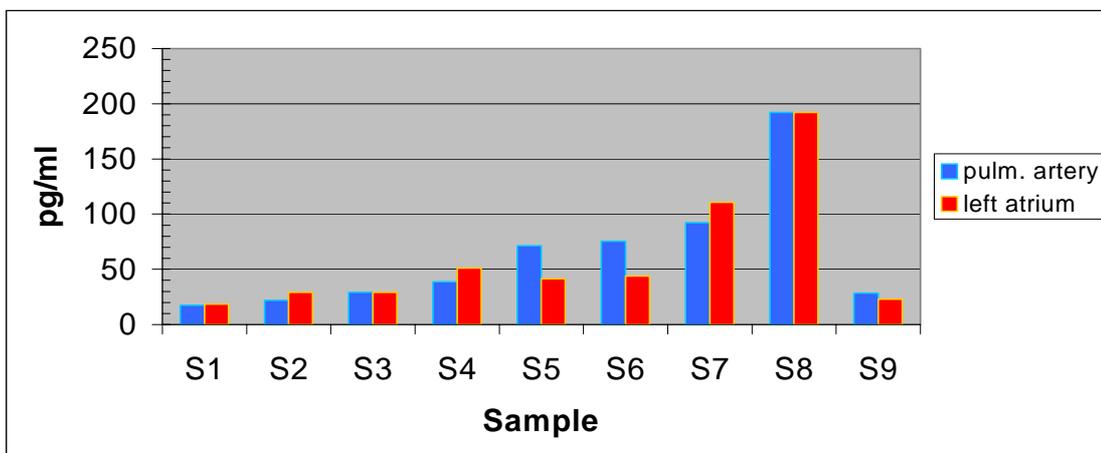
	Beginning	End
Leukocytes (x 10 <sup>9</sup> / L)	6.5 $\pm$ 1.5	11.8 $\pm$ 2.8 *
Neutrophils (%)	58 $\pm$ 9	66 $\pm$ 7 *
Neutrophils (x 10 <sup>9</sup> / L)	3.7 $\pm$ 0.8	7.6 $\pm$ 2.1 *
Lymphocytes (%)	32 $\pm$ 9	23 $\pm$ 5 *
Lymphocytes (x 10 <sup>9</sup> / L)	2.2 $\pm$ 0.9	2.7 $\pm$ 0.8 *
Monocytes (%)	6 $\pm$ 4	7 $\pm$ 3 *
Monocytes (x 10 <sup>9</sup> / L)	0.4 $\pm$ 0.2	1.1 $\pm$ 0.4 *
Other cells (%)	4 $\pm$ 2	2 $\pm$ 1 *
Other cells (x 10 <sup>9</sup> / L)	0.2 $\pm$ 0.1	0.4 $\pm$ 0.3 *

\* Statistically significant differences from induction.

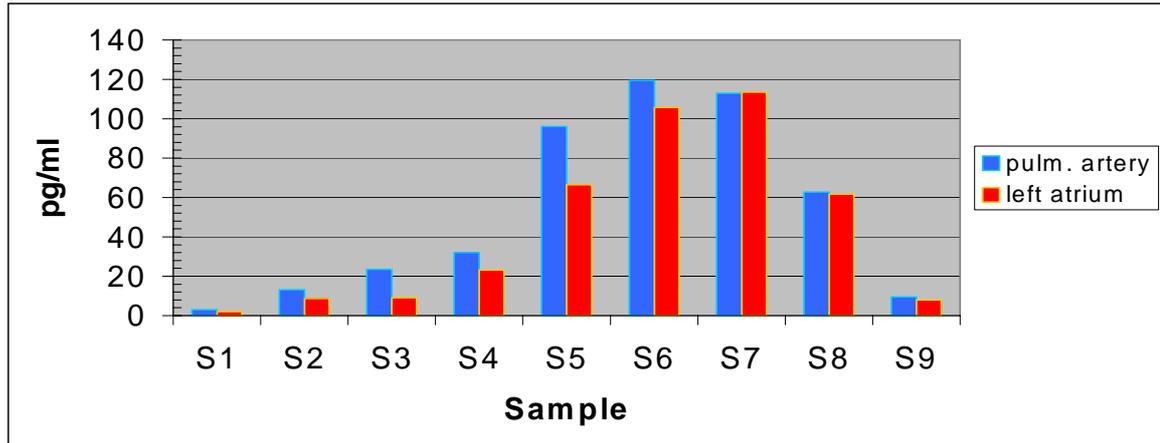
TNF- $\alpha$  and IL-2 could only be detected in six patients at all. All IL-2 values have remained within the normal range. TNF- $\alpha$  levels did not show any significant variation in the pulmonary or systemic circulation, values above the normal range could only be detected in two patients, however 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 (Figure 17, 18, 19)



**Figure 17.** Median IL-6 concentration values during and after CPB in the pre-, and postpulmonary blood samples.



**Figure 18.** Median IL-8 concentration values during and after CPB in the pre-, and postpulmonary blood samples.



**Figure 19.** Median IL-10 concentration values during and after CPB in the pre-, and postpulmonary blood samples.

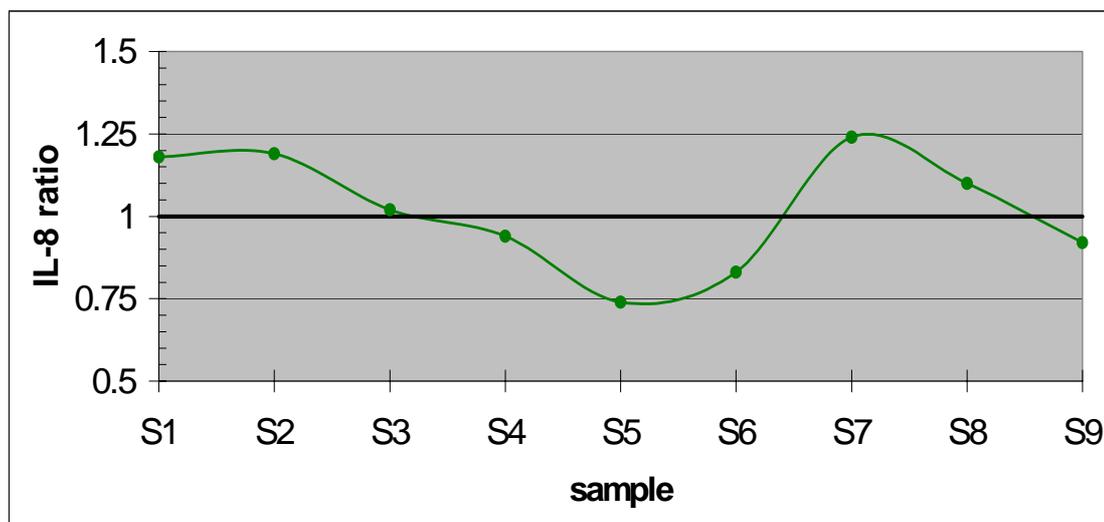
The concentration of IL-6 has proved somewhat higher in the left atrium in samples S2, S3 and S4 compared to those values measured in the pulmonary arterial samples, however this difference has not been statistically significant. (Table 16)

**Table 16.** Values of IL-6.

Sample	Postpulmonary blood Median (range)	Prepulmonary blood Median (range)	<i>P</i>
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 3<sup>rd</sup> and 7<sup>th</sup> 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).



**Figure 20.** IL-8 ratio figures during and after CPB.

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 17)

**Table 17.** Values of IL-10.

Sample	Postpulmonary blood Median (range)	Prepulmonary blood Median (range)	<i>P</i>
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)	<b>0.041</b>
S3	9 (0.8-118.1)	23.5 (1.4-127.5)	<b>0.007</b>
S4	23.1 (3.2-141.9)	32.1 (3.7-222.2)	<b>0.003</b>
S5	66.5 (11.9-314.9)	96.1 (17.2-402.5)	<b>0.01</b>
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 2<sup>nd</sup> 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 2<sup>nd</sup> 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.

## 7.6 Discussion

Numerous clinical studies have shown significant elevation of blood cytokine levels during and after CPB [218,219,220]. Such a phenomenon can be induced by several factors, including ischaemia-reperfusion [221], complement activation, and release of endotoxin [222]. The release of proinflammatory cytokines may be important because such a release seems to be implicated in the development of postoperative complications [221,223]. 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 [224]. Anti-TNF antiserum may reduce pulmonary and hepatic injury caused by hepatic ischaemia-reperfusion [225]. Removal of TNF- $\alpha$  and IL-6 by hemofiltration has been shown to have beneficial effects in children undergoing CPB [226]. Administration of steroids before CPB not only reduces significantly the release of proinflammatory cytokines [227] but also increases the release of IL-10 [228].

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- $\alpha$  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 [229], but also because it may trigger the release of other important cytokines, such as IL-8 [230], and IL-10 [231]. Although many have already justified the role of TNF- $\alpha$  in the inflammatory response, I have not been able to obtain a clear-cut view on the kinetics of TNF- $\alpha$ . Presumably, this could happen due to the short half-life and to the locally acting nature of the TNF- $\alpha$ . It would have been much more efficient to measure the TNF- $\alpha$  levels in the lung tissues. The investigation of the soluble receptors of TNF- $\alpha$  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, [232,233,234]. Those studies indicated that the restoration of IL-2 production was not complete 48 hours postoperatively. Deng and associates [235] reported that the decrease of IL-2 was strictly limited to the CPB period. 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 [236,237]. IL-8 is a crucial mediator in ischaemia-reperfusion injury in patients undergoing CPB [238,239]. IL-8 release is induced only after reperfusion of the ischaemic myocardium in animals [239,240] as well as in human beings [241].

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

Cell surface adhesion molecules play vital roles in numerous cellular processes. Some of these include: cell growth, differentiation, embryogenesis, immune cell transmigration and response, and cancer metastasis. Adhesion molecules are also capable of transmitting information from the extracellular matrix to the cell. There are four main groups of adhesion molecules: **selectins**, **integrins**, **cadherins** and the **immunoglobulin (Ig) superfamily cell adhesion molecules (CAMs)** [27]. Certain proofs exist concerning the importance of the adhesion of neutrophils and monocytes to the capillary endothel in the inflammatory process and reperfusion injury [242]. Due to the actions of activated neutrophil cells, free radicals, proteolytic enzymes and arachidonic acid metabolites will be released that further increase the tissue injury of various organs. During the inflammatory response, triggered by CPB, interaction between activated leukocytes, platelets, and endothelial cells is mediated through the expression of adhesion molecules.

The selectins, which mediate the initial rolling of the leukocyte on the endothelium, are divided in three subgroups [243]: **L-selectin** is expressed on all three leukocyte types, **P-selectin** is expressed on platelets and endothelial cells, and **E-selectin** is only expressed on endothelial cells. The role of the selectins, unlike most other adhesion molecules, is restricted to directing leukocyte interaction with vascular endothelium. The role makes these molecules extremely attractive targets for therapeutic agents designed for treating conditions such as ischemia reperfusion injury, allograft rejection, inflammation, etc. [244].

Integrins can be found on most cell types, consist of an alpha and beta subunit and mediate firm adhesion of the leukocyte and migration into the tissues. They are classified into subgroups according to the type of their beta subunit. The neutrophil displays three primary surface adhesive integrins that share a common (CD18) beta subunit. These integrins are identified as **CD11a**, **CD11b** (*Mac-1*), or **CD11c** [245]. The CD11b integrin is primarily responsible for endothelial binding [245]. Surface expression of this integrin is rapidly (2 to 4 minutes) [246], permanently [247], and preferentially [248] expressed on exposure to cytokines [246] including TNF and IL-

1, and to LPS [249]. The CD11b integrin has an endothelial receptor (ligand), Intercellular Adhesion Molecule-1 (ICAM-1) [245] that allows the neutrophil to adhere to the endothelium.

Immunoglobulins such as **ICAM-1** and **VCAM-1** are expressed mainly on endothelium and act as ligands for certain integrins. Certainly deeper understanding of the behaviour and the role of adhesion molecules during CPB bypass may facilitate effective intervention in the inflammatory response process and suppression of its adverse effects.

## **8.1 ICAM-1**

The molecular weight of ICAM-1 is 90-115 kDa. ICAM-1 expression is weak on leukocytes, epithelial and resting endothelial cells, as well as some other cell types, but expression can be stimulated by IFN- $\gamma$ , TNF- $\alpha$ , IL-1  $\beta$ , and LPS [250]. ICAM-1 is found in a biologically active form in serum probably as a result of proteolytic cleavage from the cell surface, and is elevated in patients with various inflammatory syndromes such as septic shock, cancer and transplantation [251,252].

## **8.2 E-selectin**

E-selectin (CD62E, ELAM-1), molecular weight 95-115 kDa (different glycosylated forms), is expressed by cytokine-activated endothelial cells [253]. In vitro, E-selectin expression is protein synthesis-dependent, peaks after 4-6 hours of cytokine stimulation, and then subsequently declines to basal levels within 24 hours [254]. E-selectin mediates neutrophil, monocyte and some memory T cell adhesion to vascular endothelium, and may function as a tissue specific homing receptor for T cell subsets [255,256]. E-selectin is broadly expressed within the vasculature at sites of inflammation [244].

A host of glycoprotein ligands unique to E- and P-selectin have been identified. However, it is likely that only a few high affinity ligands mediate physiological function. A 150 kDa ligand for E-selectin, found on neutrophils, is called ESL-1 (E-selectin ligand-1) [244]. E-selectin can also bind PSGL-1 (P-selectin Glycoprotein Ligand-1), expressed by all blood neutrophils, monocytes and

lymphocytes, but specific glycosylation is required for ligand function [244]. E-selectin is found in a biologically active form in serum, probably as a result of proteolytic cleavage from the cell surface, and is elevated in-patients with various inflammatory syndromes [252]. However changes of circulating soluble adhesion molecules have only rarely been documented in adults undergoing cardiac surgery. *Boldt et al.* reported unchanged levels of soluble adhesion molecules sELAM1, and sVCAM1 in children undergoing open-heart surgery[257].

In the following study, I sought to analyse the expression dynamics of ICAM-1 and E-selectin adhesion molecules in clinical settings, during coronary artery operations with and without CPB.

### 8.3 Patients and methods

12 adult patients (mean age:  $59.4 \pm 7.2$  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:  $59.1 \pm 11.2$  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 18.

**Table 18.** *Patients' clinical data*

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

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. The timing of blood sampling is shown in Table 19.

**Table 19.** *Blood sampling times.*

Sample	Group A	Group B
S1	After induction of anaesthesia	After induction of anaesthesia
S2	5 minutes after aortic cross clamping	N.A.
S3	20 minutes after aortic cross clamping	During completion of the distal anastomosis
S4	1 minute after the release of aortic cross clamping	N.A.
S5	20 minutes after the release of aortic cross clamping	During completion of the distal anastomosis
S6	10 minutes after the cessation of CPB	N.A.
S7	2 hours after the cessation of CPB	2 hours after surgery
S8	24 hours after the cessation of CPB	24 hours after surgery
S9	48 hours after the cessation of CPB	48 hours after surgery
S10	72 hours after the cessation of CPB	72 hours after surgery

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  $-20^{\circ}\text{C}$  temperature until the analysis. The blood samples were analysed within 4 weeks for soluble ICAM-1 (normal average value: 211 ng/ml,  $\pm$  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)

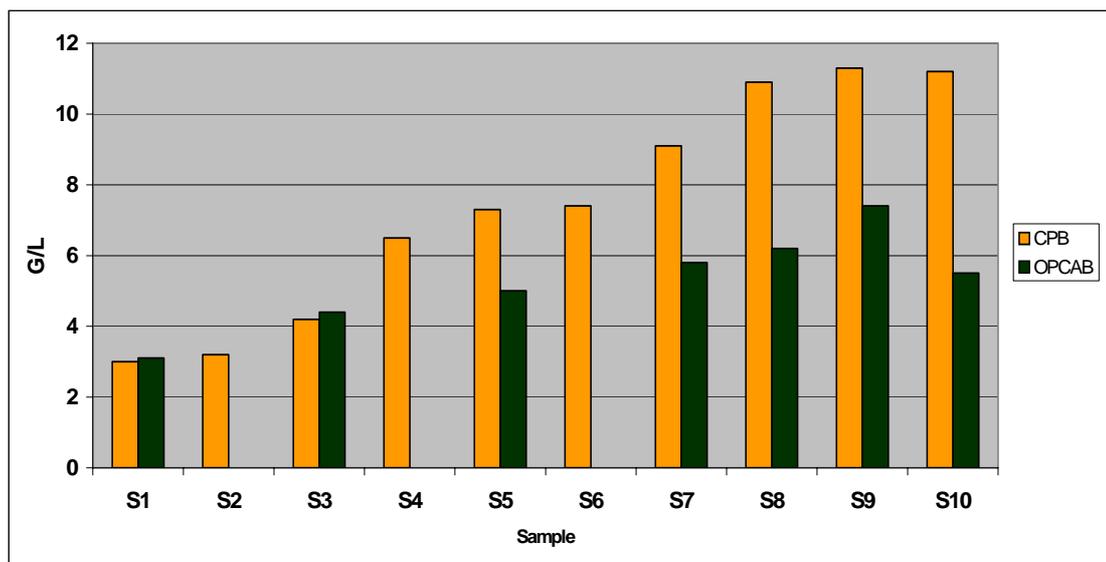
The principle of the measurement is the reaction of the adhesion molecules with two monoclonal antibodies directed against the various epitops on the surface of the adhesion molecules. All measurements were standardised by reactions against either purified recombinant ICAM-1 or E-selectin. The sensitivity (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  $\pm$  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  $<0.05$ .

## 8.4 Results

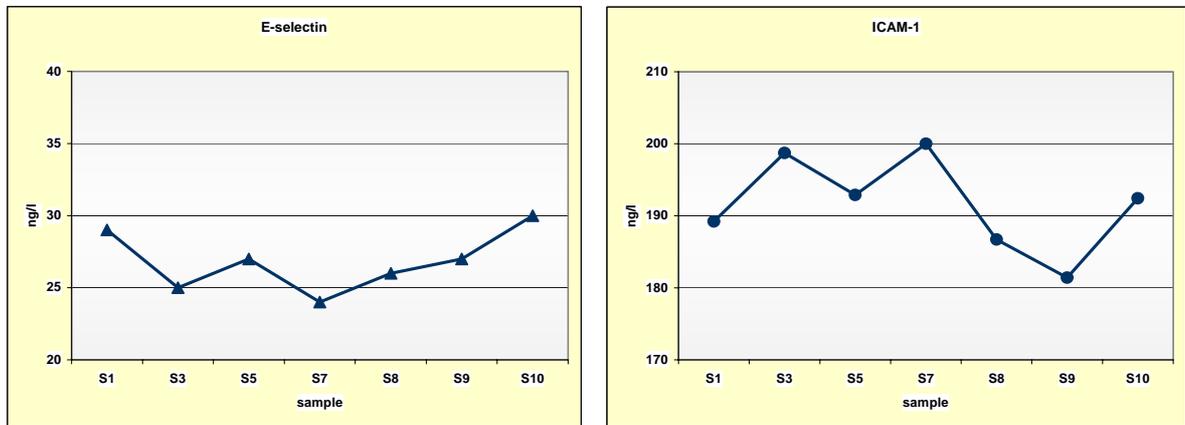
No complications occurred and no reoperations were necessary in those patients enrolled in the study. The mean length of ICU stay has proved less than 48 hours similarly in both groups.

Neutrophil count has raised significantly in both groups shortly after the initiation of CPB. 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 (Figure 21). A significant difference could be detected between the two groups in samples 5, 7, 8, 9 and 10.



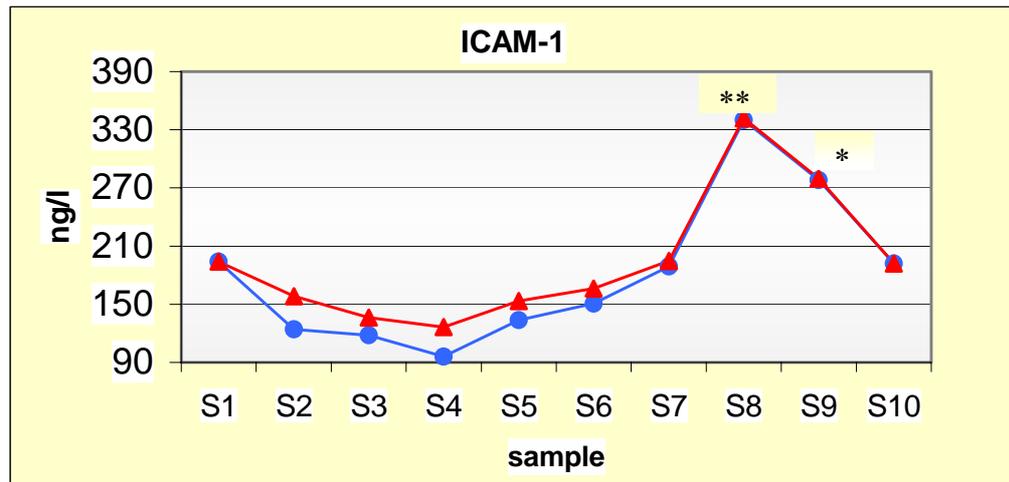
**Figure 21.** Changes in neutrophil count during and after operations with and without CPB

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 (Figure 22).



**Figure 22.** Changes of ICAM-1 and E-selectin during and after off-pump operations

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. ( $p < 0.003$ ) (Figure 24) The comparison of the measurements made at similar points of time in the two groups is shown in Table 20.



**Figure 23.** Changes of ICAM-1 during and after operations on CPB

- uncorrected values
- ◻ values corrected to haemodilution

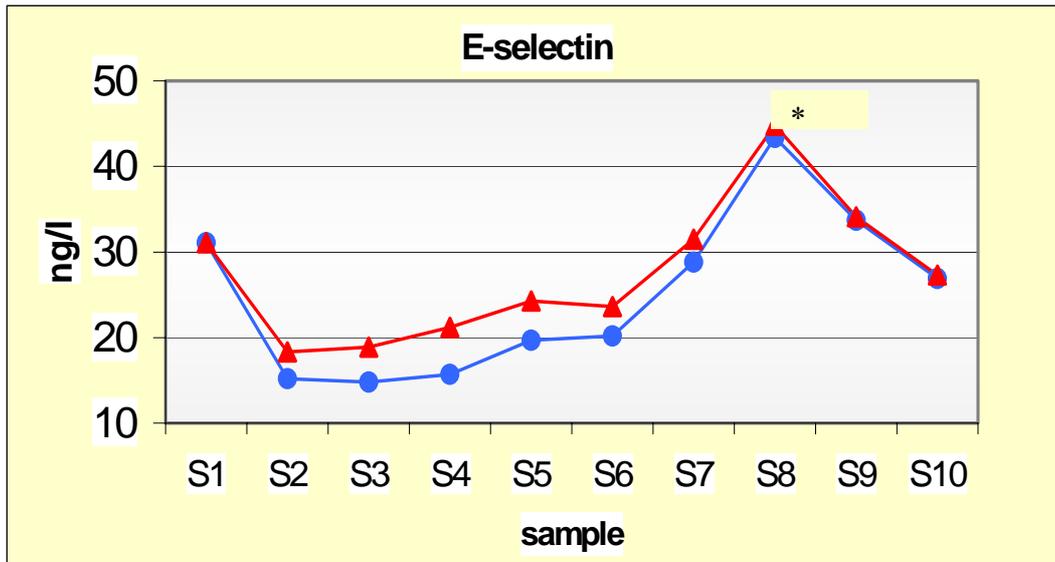
**Table 20.** Median values of E-selectin and ICAM-1 in the two groups in nearly coinciding times.

Sample	E-selectin			ICAM-1		
	Group A	Group B	P	Group A	Group B	P
1	<b>31.1</b> (20.9-53.3)	<b>29.2</b> (26.1-39.7)	Ns	<b>193.8</b> (148.7-254.3)	<b>189.2</b> (168.5-219.4)	Ns
3	<b>18.9</b> (12.7-31.4)	<b>25.3</b> (22.3-36.3)	*	<b>136.1</b> (115.4-197.3)	<b>198.7</b> (171.9-212.8)	**
5	<b>24.3</b> (13.9-40.2)	<b>27.1</b> (22.6-35.2)	Ns	<b>153.5</b> (121.8-213.6)	<b>192.9</b> (183.7-214.2)	*
7	<b>31.5</b> (17.8-49.5)	<b>24.2</b> (23.1-36.8)	Ns	<b>194.7</b> (147.2-222.3)	<b>200</b> (179.2-232.6)	Ns
8	<b>44.8</b> (30.9-58.8)	<b>26.7</b> (23.2-33.6)	**	<b>341.9</b> (239.1-404)	<b>186.7</b> (165.7-218.3)	**
9	<b>34.1</b> (20.9-51.7)	<b>27.5</b> (24.2-31.8)	Ns	<b>279.8</b> (208.8-382.4)	<b>181.4</b> (164.5-221)	**
10	<b>27.7</b> (20.4-56.8)	<b>30.5</b> (25.1-32.4)	Ns	<b>192</b> (156.4-242)	<b>192.4</b> (154.7-234)	Ns

\*: p < 0.05

\*\* : p < 0.001

\*\*\*: p < 0.0001



**Figure 24.** Changes of E-selectin during and after operations on CPB

- uncorrected values
- ◻ values corrected to haemodilution

## 8.5 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 sequestered 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.**

Cardiac surgery performed 30-40 years ago was associated with histological structural damage of the terminal part of the respiratory system [258,259]. This was due to the adverse effects related to the use of disc or bubble oxygenators and to long CPB times. It is evident that severity of respiratory dysfunction after CPB is directly related to preoperative pulmonary disease, intraoperative events, duration of CPB, and postoperative cardiac function.

In the 1970s Ratliff performed electron microscopic investigations on biopsy specimens from lungs taken after the use of CPB with bubble oxygenator. He experienced serious detrimental alveolar changes in the specimens similar to alterations reported in human and animal lungs following various forms of shock and trauma. Probably the continuous development of CPB devices along with the more sophisticated surgical approach have all reduced the severity of these alterations. Since Ratliff's pioneering reference few similar clinical studies have been devoted to the subject. It has been unclear whether the new techniques still result in the same histologic structural alterations seen by the original investigator or not. Unfortunately Ratliff did not clearly indicate the preoperative lung condition of those patients whose biopsy specimens he investigated. No data were available whether any underlying pulmonary disease was or was not in correlation with those changes he found in his studies. Schlensak and associates [260] reported their observations on ischaemia-reperfusion lung injury in pigs. They proved increased thickening of the alveolar septum and reduction of the overall alveolar surface area and they found that these structural changes of the lung parenchyma were associated with a reduced capacity to oxygenate blood. Their research modalities were outstanding for finding the answers to those questions they were searching. However the animals used in the project all had healthy lungs. In his study on rabbit pulmonary tissue biopsies Premaratne [261] suggested that damage due to ischaemia alone might be reversible. Initial recovery is due to the re-establishment of circulation.

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  $58 \pm 14$  and  $101 \pm 13$  minutes respectively. After sternotomy, lung biopsy was taken from the 4<sup>th</sup> 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  $75 \text{ mm}^3$ . 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  $4 \mu$  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**

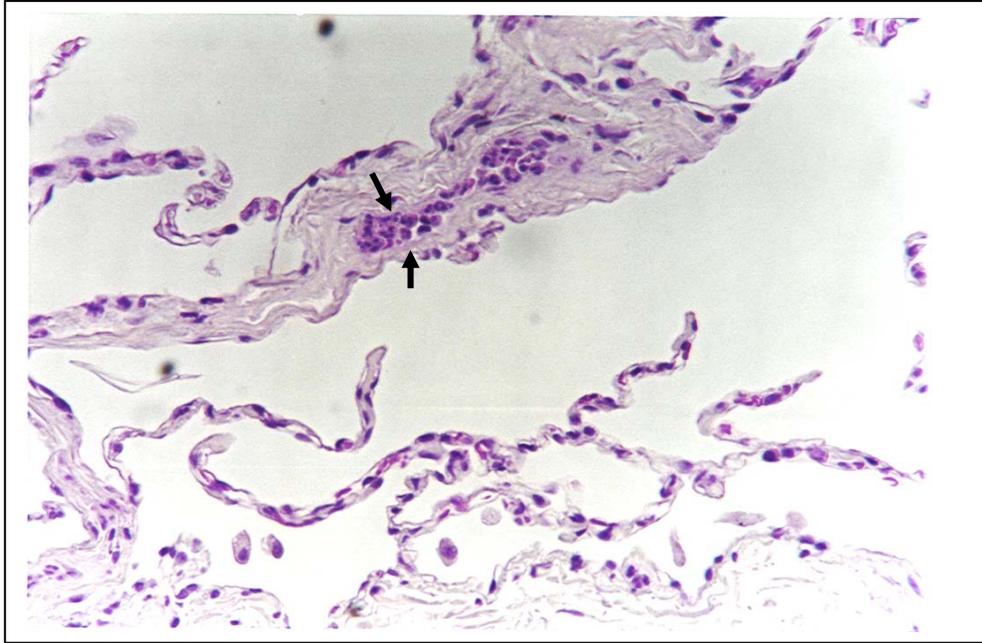
(6 cases, 3 of them with signs of centroacinar type)

- 3. Interstitial fibrosis**

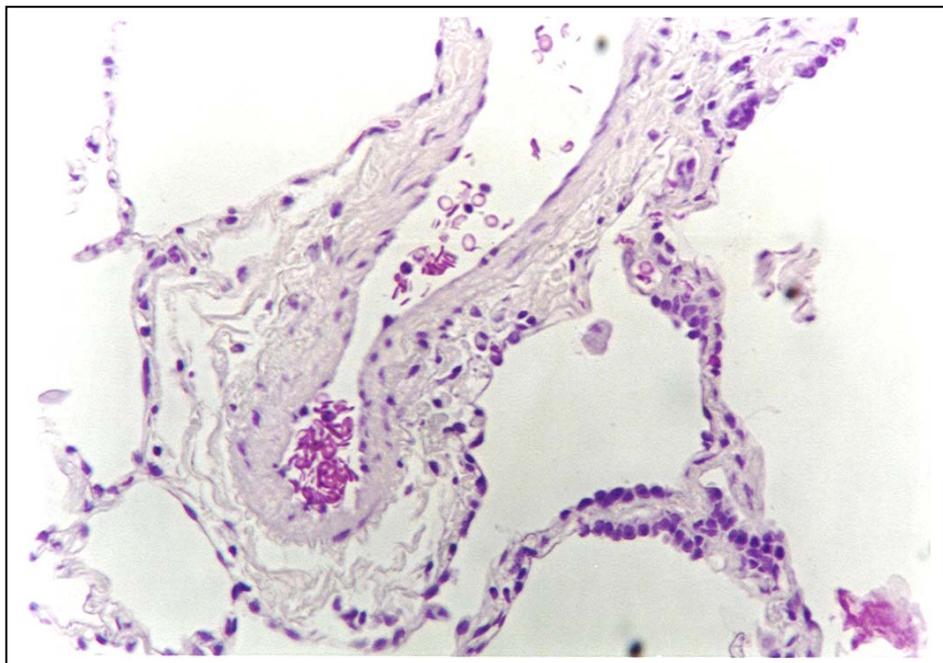
(8 cases, 3 of those with signs of severe disease)

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 (Figure 25). The accumulation of polymorphonuclear leukocytes was

associated with the swelling of capillary endothelial cells and with the proliferation of type II granular pneumocytes (Figure 26). Reperfusion injury following ischaemia together with moderate passive hyperaemia and extravasated erythrocytes and neutrophils could be primarily detected.



**Figure 25.** Lung biopsy specimen following the cessation of CPB. Increased number of PMN is seen (hematoxylin-eosin, original magnification x200)



**Figure 26.** Lung biopsy following the cessation of CPB. Type II granular Pneumocyte proliferation, and capillary endothelial swelling is observed (hematoxylin-eosin, original magnification x200)

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, however signs of fibrous pleuritis were evident in one of them.

The basic histopathological findings in "specimen A" biopsies and the alterations found in "specimen B" biopsies are summarised in Table 21.

**Table 21.** *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

Alveolar thickness measured in the control group has proved 0.5-3.0  $\mu\text{m}$  (Textbook data: 0.5-2.5  $\mu\text{m}$ ). For suitable understanding I use abbreviations to determine the followings:

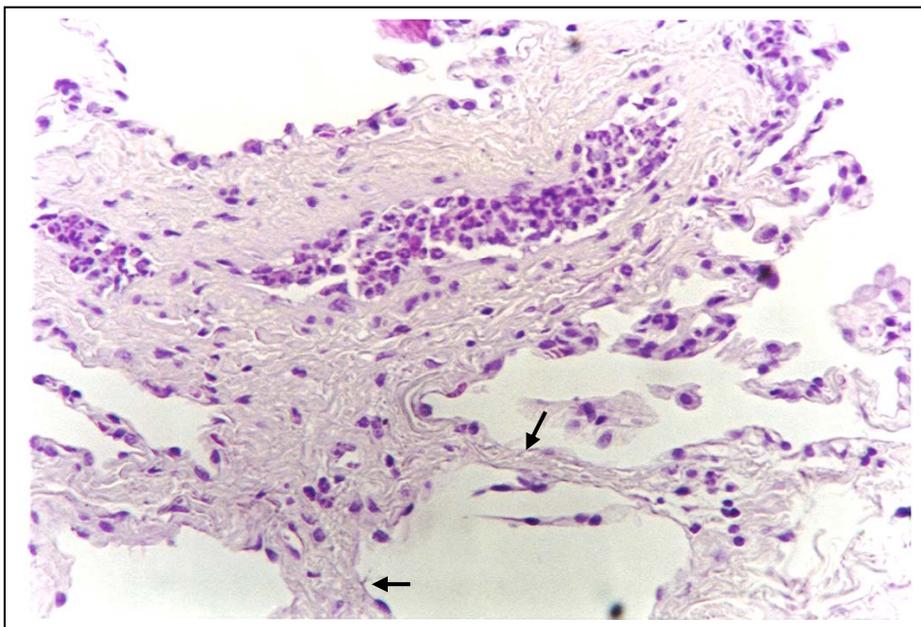
**D1:** mean alveolar thickness of biopsies of "specimen A".

**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 (Figure 27) (**D1:  $5.32 \pm 1.51 \mu\text{m}$  vs. D2:  $5.88 \pm 1.33 \mu\text{m}$ ,  $p = 0.04$** ). RD of the alveolar septum has proved 1.14-fold ( $SD: 0.23$ ). This equals an average increase (DD) of 0.55  $\mu\text{m}$  ( $SD: 0.98$ ). There has been no correlation between age and either the figure of D1, D2, DD and RD.

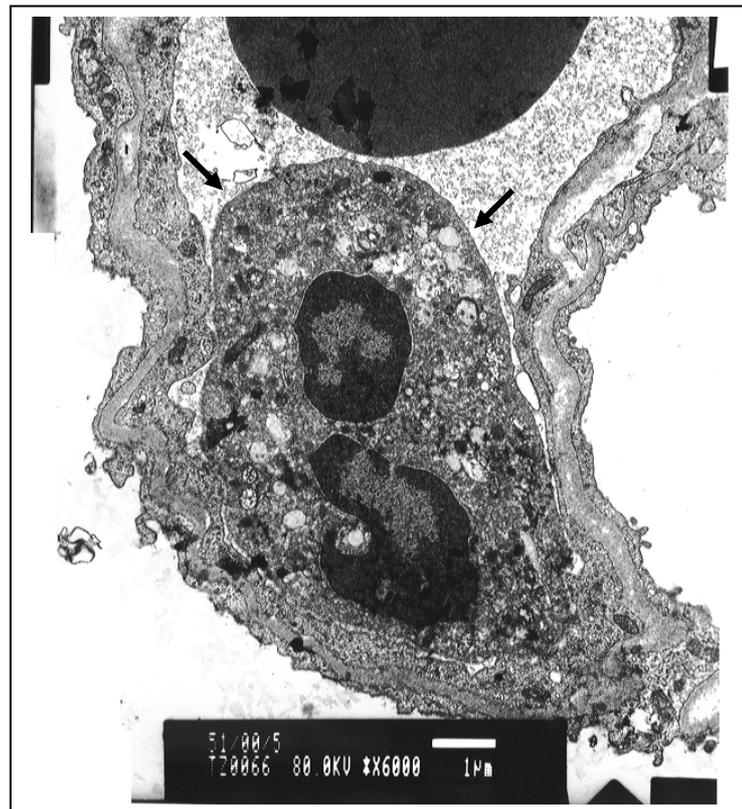


**Figure 27.** Intensive reperfusion injury of the lungs after the cessation of CPB. Significant thickening of the alveolar septum can be observed (hematoxylin-eosin, original magnification x200)

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 ( $R = 0.755$ ,  $p = 0.001$ )

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 ( $R = -0.598, p = 0.014$ ) 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 (Figure 28).



**Figure 28.** Electron micrograph of lung biopsy specimen following CPB. Neutrophil (arrow) is filling and adhering to the capillary wall. Note the absence of identifiable granules and marked swelling of the endoplasmic reticulum and mitochondria. (original magnification x6000)

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 (Figure 29). 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.



**Figure 29.** Marked endothelial alteration with swelling of cytoplasm, endoplasmic reticulum, and mitochondria. The basal membrane is thick and signs of swelling are present. (original magnification x6000)

### 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 [41]. 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 [56], 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 1<sup>st</sup> 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 2<sup>nd</sup> 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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