Inflammatory response and oxidative stress associated with cardiopulmonary bypass

PhD Thesis

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CONTENTS

ABREVIATIONS

AC	Adenyl cyclase
ACC	Aorta cross-clamping
AP-1	Activator protein-1
ARDS	Adult respiratory distress syndrome
ATP	Adenosine 5'-triphosphate
AU	Arbitrary unit
BHACAS	Beating heart against cardioplegic arrest studies
CABG	Coronary artery bypass grafting
CAT	Catalase
CBA	Cytometric bead array
CCS	Canadian cardiovascular society classification system
CK	Creatine-phosphokinase
CL	Chemiluminescency
CPB	Cardiopulomonary bypass
CS	Coronary sinus
DAF	Decay accelerating factor
EC	Endothelial cell
EF	Ejection fraction
EGF	Epidermal growth factor
ELISA	Enzyme linked immunosorbant assay
ESR	Electron spin resonance spectroscopy
EURO SCORE	European system for cardiac operative risk evaluation
FITC	Fluorescein-isotiocianate
GOT	Glutamine oxalate transferase
GPT	Glutamine piruvate transaminase
GPx	Glutathion peroxidase
GSH	Reduced glutation
GSSG	Oxidised glutation
HLA	Human leucocyte antigen
ICAM	Intracellular adhesion molecule
ICU	Intensive care unit
IL	Interleukin
IR	Ischaemia-reperfusion
LIMA	Left internal mammary artery
LFA	Leukocyte functional antigen
LPS	lipopolysacharide
MAPK	Mitogene activated protein kinase
MC	Monocyte
MDA	Malondialdehyde
MIDCAB	Minimal invasivedirect coronary artery bypass
MIF	Mean fluorescence intensity
MIP	macrophage inflammatory protein
MOF	Multi organ failure
MRI	Magnetic resonance imaging
NAD	Nicotinamide adenine dinucleotide

NADPH	Reduced nicotinamide adenine dinucleotide phosphate
NFKB	Nuclear factor –kappa B
NOS	Nitric oxide synthetase
iNOS	Inductible nitric oxide synthetase
NO	Nitric oxide
OP	Off-pump coronary artery bypass grafting
PACAP	Pituitary adenylate cyclase activating polypeptide
PAR	Poly (ADP-ribose)
PARP	Poly (ADP-ribose) polymerase
PBL	Peripheral blood lymphocytes
PBS	Phosphate buffered saline
PCI	Percutan coronary intervention
PE	Phycoerithrin
PG	Prostaglandin
PKA	Protein kinase A
PMA	phorbol-12 myristate-13acetate
PMN	Polymorphonuclear leucocyte
POD	Postoperative day(s)
RBC	Red blood cell
Rep	Reperfusion
ROS	Reactive oxigen species
SMART	Surgical Management of Arterial Revascularisation Therapy (trial)
SIRS	Systemic inflammatory response syndrome
SOD	Superoxid dismutase
TECAB	Off-pump totally endoscopic CABG
TI	Troponin I
TH	Helper T cell
TM7	Seven-span transmembrane protein
TNF	Tumor necrosis factor α
WBC	White blood cell

1. INTRODUCTION

1.1 Historical background

Collaborative endeavor of many researchers culminated in development of heart-lung machine, cardiopulmonary bypass (CPB), which is one of the major technological advantages in medicine. Application of CPB provides controlled operating condition thus allowing the widespread use of CPB after its development. Nowadays, CPB is routinely used around the word, nonetheless questions about the effect CPB on patients are also open now, and investigations are still active to improve our understanding about impacts of CPB on patients.

In fact, LeGallois first proposed the idea of artificial circulation in 1812, with the conception that work of heart can be replaced by arterial pump. The feasibility of total body perfusion together with removing of heart was realized by experiments of Brukhonenko but this work was ignored widely. The first instrument for extracorporeal oxygenation was constructed by Frey and Gruber [1]. For development of CPB further invention of blood groups, positive pressure ventilation, and nontoxic anticoagulant, heparin, its antagonist protamin and discovery of new synthetic materials were required. In 1934, DeBackey reported a reliable method for displacing large volume of blood by roller pump [2]. Thereafter Gibbon firstly constructed a heart lung machine and it was used in animals in 1937 [3]. In 1953, he successfully applied the CPB apparatus for closing an atrial septal defect of a young woman [4]. Till 1964 there were only sporadic cases of operation on coronary arteries, only following the systematic development of coronary angiography was it possible to standardize surgical strategies. Then there was a rapid growth in the number of procedures with CPB. In the 80s and 90s the standardized technique using CPB entered into "golden ages" following the development and improvement of oxygenators, surface of tubes and technique of myocardial preservation.

Although mortality and morbidity of open-heart surgery had decreased due to new improvements; complications and unwanted events are still associated with application of CPB. Several of these factors could be diminished by investigations. Kirklin firstly characterized the term of postperfusion syndrome for condition of unimpressionable and marked activation of inflammatory processes due to application of CPB [5], which remained the major side-effect of heart-lung machine.

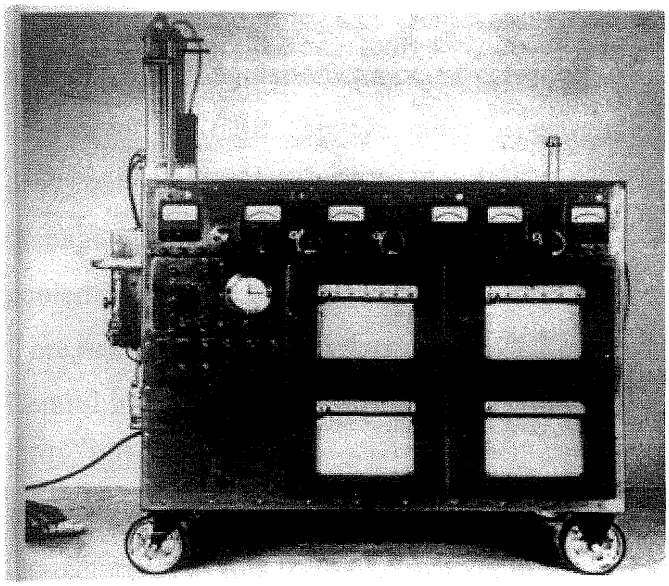


Figure 1.: The first applied heart-lung machine designed by John Gibbon (Cardiopulmonary Bypass, Ed. Mora CT, Springer –Verlag; 1995)

For these unwanted events associated with CPB, CABG performed without CPB gained increased attention. Off-pump coronary surgery (OP) is a quiet old technique first performed in 1964 by Kolessov [6] and was soon abolished by development of CPB. After the trials of Kolessov some working groups gathered experiences with CABG without heart-lung machine [7]. In the past decade there has been a renewed interest in performing CABG without application of CPB, thereby eliminating cannulation, aortic cross-clamping and extracorporal circulation together with their unwanted, damaging effects.

1.2 Pathophysiology of cardiopulmonary bypass

Majority of operations in cardiac surgery necessitate the applying of CPB. Despite the advances of CPB in safety and over 50 years of practice it is known to provoke complex cascades of particularly unknown physiological processes. These self-excitatory factors are responsible for generation of CPB-associated complications.

One of these factors results from opening of aorta and right atrium for cannulation. It serves as an additional invasivity and remaining sutures after emergence from CPB moreover it can also facilitate the development of air and atheroembolisms. Regardless of effective anticoagulation there is a possibility of thromboembolisms because of trombogene surfaces of

cannulas, tubes and oxygenator. Excessive anticouagulation and administration of protamin ("protamin reaction") can result in further complications, moreover disseminated intravascular coagulation can occur causing the most frequent incidence of early death during application of CPB [8]. On other aspect, injurious mechanic effect of roller pump and oxygenator takes place, while blood passes through small diameter of wires thus leading to damage of blood cells and haemolysis. Further damaging effects are produced by heater while cooled blood of extracorporeal circulation is quickly warmed up in the heater. Nonetheless there is a possibility of hypoperfusion and inadequate oxygenisation [8].

Central importance belongs to the activation and over-activation of inflammatory processes to CPB. Inflammatory response may be the most common and versatile consequence of CPB. Inflammatory-based reactions of CPB have even local and systemic aspects and several characteristic of this is less-known yet in spite of its pivotal importance.

1.2.1 Inflammatory response to cardiopulmonary bypass

The hallmark of inflammatory response is highly complex and confusing interaction of processes with various cascades and pathways involving exaggeration and over-activation of humoral and cellular immunity [5,9,10]. This condition arises mainly from the following factors during CPB [11-13].

- blood exposure to artificial surface
- contact of blood with non endothelial covered surfaces of body
- operative trauma
- excessive administration of heparin, protamin
- ischaemia-reperfusion injury after global ischemia of heart
- damage of lung arising from hypoperfusion
- damage of barrier of intestinal mucosa
- endotoxaemia
- systemic embolisms
- abnormal blood gas interfaces

As an initial step exposure of blood to non-endothelial surfaces activates the complement system [9]. Both alternative and classic pathways are involved in complement activation. Contact of blood with extracorporeal circuit triggers formation of C3a, C5a whereas C4a and

C2 is activated by heparine and protamin administration further rising the level of C3a. Both pathways are activated by endotoxin release [12]. Vasoconstriction, elevated vascular permeability, activation of neutrophil leukocytes and mast cells, aggregation of platelets, chemotaxis are facilitated by C3a and C5a proteins arising from lysis of complement proteins [14]. Complement receptors are expressed on WBC regulating their activation. On the other hand it also serves as natural inhibitor of complement activation [9]. Accumulating evidences show the activation of complement system and appearance of complement anaphylotoxins in patients plasma during and following CPB [11,12,15]. Complement degradation products peaks at the end of CPB till the end of 1st postoperative day (POD). Kirklin and colleagues demonstrated direct correlation between CPB time and C3a concentration moreover association between organ dysfunction and elevated complement levels were established [11].

Complement products interacts with their high affinity receptors of WBC. Thus WBC convert to activated state leading to changes in gene expression and function. Activation of different subsets of WBC is a pivotal part of inflammatory response to CPB with further important influences. Ischemia –reperfusion (IR) is a crucial activator of WBC in course of CPB. Typically, application of CPB is associated with aortic cross clamping (ACC) thus causing global ischaemia of the heart and discontinuing pulmonary perfusion through pulmonary artery. Various factors have been implicated as mediators of leukocyte activation in IR (reactive oxygen species, inflammatory mediators, cytokines, nitric oxide) [16].

As a general consequence of CPB, adhesion molecules are presented on WBC leading to interaction between the endothelial cells (EC) thereby extending tissue damage [17]. Upon expressing adhesion molecules WBC can attach to endothelial cells and transmigrate through vessel wall trough four-steps process: firstly, WBC become less deformable and roll along the endothelial cells, secondly, leukocytes join to EC. During third period of extravasation WBC are firmly attached to EC and finally they migrate through the gaps of endothelial layer. Subsequent to extravasation, WBC enter into interstitial space and bind to interstitial cell or extracellular matrix [16,17]. Four families of adhesion molecules regulate the interaction between endothelial cells and WBC and the migration of leukocytes across the capillaries: selectin, integrin, immunglobulin and kadherin families [17]. The integrins have the most important role during the phase of tight binding between WBC and EC. The leukocyte functional antigen (LFA-1) belongs to the integrin family, which is made up of the complex of CD11a and CD18 molecules. The LFA-1 appears to be the ligand of intercellular adhesion

molecule-1 (ICAM-1) produced by the endothelial cells. Several papers have noted the elevation in adhesion molecule expression as a consequence of CPB [18-19].

Responding to CPB polymorhonuclear leucocytes (PMN) release oxidants and proteases and further inflammation- amplifying products resulting in tissue damage [19]. Monocytes (MC) also play a pivotal role in regulation of inflammatory processes and the exact change in phenotype of monocytes during CPB is not well determined [20,21]. The crucial role of lymphocytes (peripheral blood lymphocytes, PBL) in the pathophisiology of reperfusion and inflammation is now well established, nevertheless the change in activation and the modification of lymphocytes phenotype under CPB appear to be ambiguous [22]. Mast cells and basophils are also involved in inflammatory response in cardiac surgical patients. Product of above mentioned cells can affect other inflammatory cells, EC and vascular smooth muscle cells moreover these products have multiple pro-inflammatory and coagulation-influencing effects [13]. Similarly to leukocytes, platelet exhibit change in shape, presentation of adhesion molecules and activation responding to CPB and ACC. Upon platelet activation WBC-platelet conjugates can be formulated leading to intravascular obstruction. Even though, release of intracellular contents can lead to tissue destruction and disturbances in coagulation [23].

Alteration in cascade of coagulation during and following CPB worth mentioning here. Process of inflammation and coagulation correlate considerably and according to induction of coagulation both local and systemic inflammatory response is activated [23]. Tissue factor appears on mononuclear cells due to cytokines. Throughout VIIa factor trombin is generated playing pivotal role in coagulation disturbances during CPB. Production of thrombin is known to amplify inflammatory response further.

Essential consequence of inflammatory-cell activation is the release of different cytokines. Cytokines might be the most crucial and central contributor of inflammatory processes thus prolonging and enhancing or even blunting the inflammatory reactions. Central importance belongs to production of cytokines regarding these mediators orchestrate the inflammatory processes, cellular activation, and leukocyte migration. At normal, healthy condition cytokines are almost undetectable in peripheral blood, nonetheless these proteins are necessary for optimal function of immune system. Production of cytokines can be stimulated by wide variety of insults such as ischaemia, endotoxin release, surgical trauma or infections both locally and systematically [12]. Although cytokines at appropriate dose act as

an important regulator of normal defence and repair processes. Moreover in complicated cases the sensitive balance between different cytokines can be blunted implicating in development of impaired healing processes and complications. Some cytokines in extremely elevated concentration can modulate the function of organs locally and even at distant location from involved organ. Dominant anti-inflammatory effect however can blunt adequate immune response aggravating defensive mechanisms and healing processes. The most studied pro-inflammatory cytokines include interleukin-6, interleukin –8 (IL-6, IL-8) and tumor necrosis factor α (TNF) [24]. There is no doubt in the literature that CPB is associated with increased release of different cytokines [24].

Further suggestion on damaging effect of WBC activation comes from studies reporting protease release to the circulation following CPB. These products of WBC break down collagen, elastin and fibronectin thus destroying extracellular structures moreover it contribute to capillary leakage that leads to electrolyte imbalance and fluid extravasation [34]. On other respect these proteases augment complement activation and systemic inflammatory response and evidences show the correlation between the degranulation products and multiple system organ failure [34].

Release of arachidonic acid from various sources leads to formation of tromboxanes, leukotrienes and prostanoids. Tromboxane A2 is a metabolite of vasoconstrictor effect and also promotes platelet aggregation. Leukotrienes are potent chemoatractants and exhibit increasing of vascular permeability. One the other hand, prostaglandins (PG), such as PGE1,PGE2, PGI2 has platlet antiaggregant function and protective role on the heart and lungs has also been described[12, 35].

Cascades of inflammatory processes cause alteration in functional state of endothelial cells. EC activation during and after application of CPB exhibits wide variations. Like leukocytes endothelial cells are known to produce adhesion molecules upon CPB referring to their activation. Several paper stated endothelial activation on the basis of examination of selectins and nitric oxide (NO) [18, 26]. It is proved that proinflammatory cytokines and endotoxin can provoke NO release through inducible form of nitric oxide synthase (iNOS) [27]. Disproportionate amount of iNOS mediated NO release results in vasodilatation and increased vascular permeability [27, 28]. However EC are the target of harmful effects of inflammatory processes so that endothelial damage and dysfunction is also widely reported

[28]. On this reason expression of NO is impeded and G- protein dysfunction occurs thus provoking coronary vasospasm or even pulmonary hypertension and mesenteric artery spasm [29,30]. With regard of endothelins, significant release of this peptide has been shown in patients receiving CPB [12].

Zu and colleagues supposed correlation between endothelin-1 level and renal dysfunction after CPB [31]. Additionally, association between endothelin-1 concentration and endotoxin level were also established [32].

Vasomotor and EC dysfunction to CPB can be summarized as follows: decreased basal tone and impaired vasoconstriction of skeletal muscle vessels, initially and decreased coronary tone during cross-clamping and amplified vascular tone with increased propensity to spasm, vasocontractile response in vasculature of lungs, intestine and brain thereafter [33]. Vasoconstrictor effect of endothelin may aggravate intestinal hypoperfusion during CPB which can damage the intestinal barrier thus enhancing the invading of endotoxin in the blood stream.

Indeed, endotoxin release is one of the major factors for maintaining and augmenting inflammatory response in course of CPB. Lipopolysaccharide (LPS) is produced by gram-negative bacteria. LPS anchorages to CD14 molecule of macrophages through its complex with LPS-binding protein. Consequently, macrophage cell increase their production of TNF and other pro-inflammatory cytokines, thereby generating inflammatory response to endotoxin. Elevation of endotoxin level during application of CPB was demonstrated in numerous studies [12].

1.2.2 Role of oxidative injury in the pathomechanism of cardiopulmonary bypass

One of the most important consequence of the CPB-mediated inflammation is excessive generation of reactive oxygen species (ROS).The impact of ROS is widely accepted in aging, reperfusion, atherosclerosis, heart failure, diabetes, several chronic and autoimmune diseases [36,37]. Open heart surgery is associated with markedly increased generation of ROS partly because of IR of myocardium and other organs, partly due to application of extracorporeal circulation alone [38, 39]. ROS are implicated essentially in the generation of complications, increasing the incidence of postoperative morbidity and worsening recovery after CPB [40].

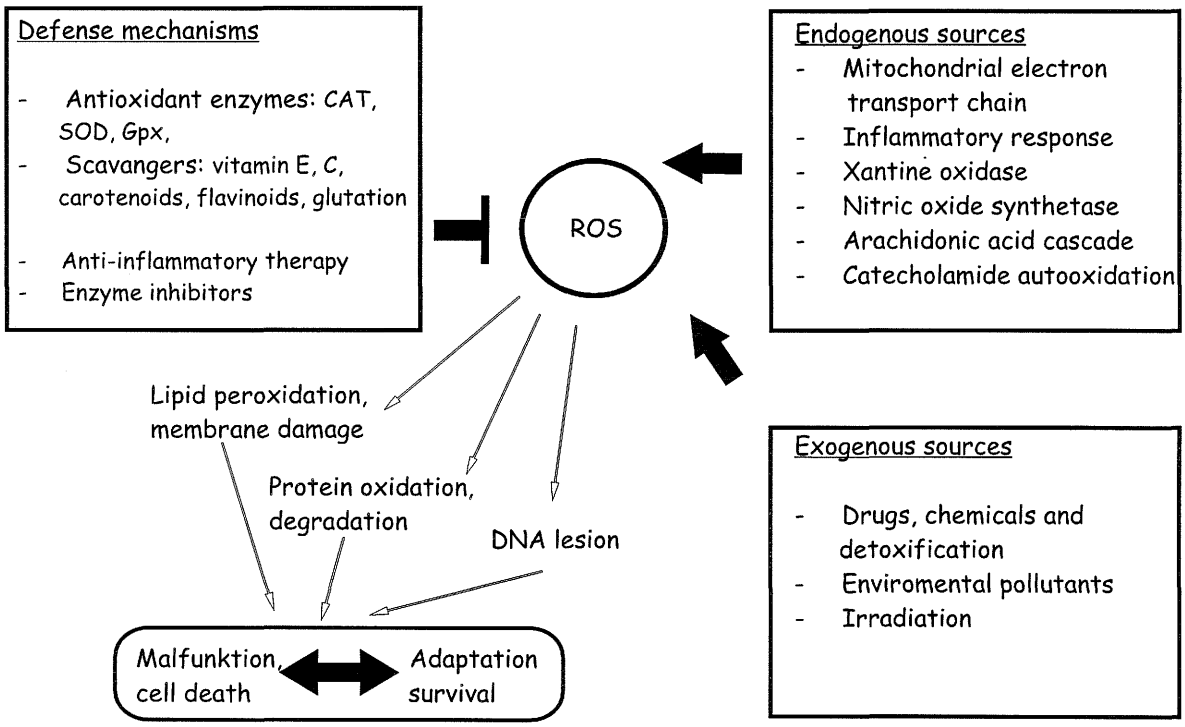


Figure 2a.: The generation, deactivation, and role of reactive oxigen species (ROS).

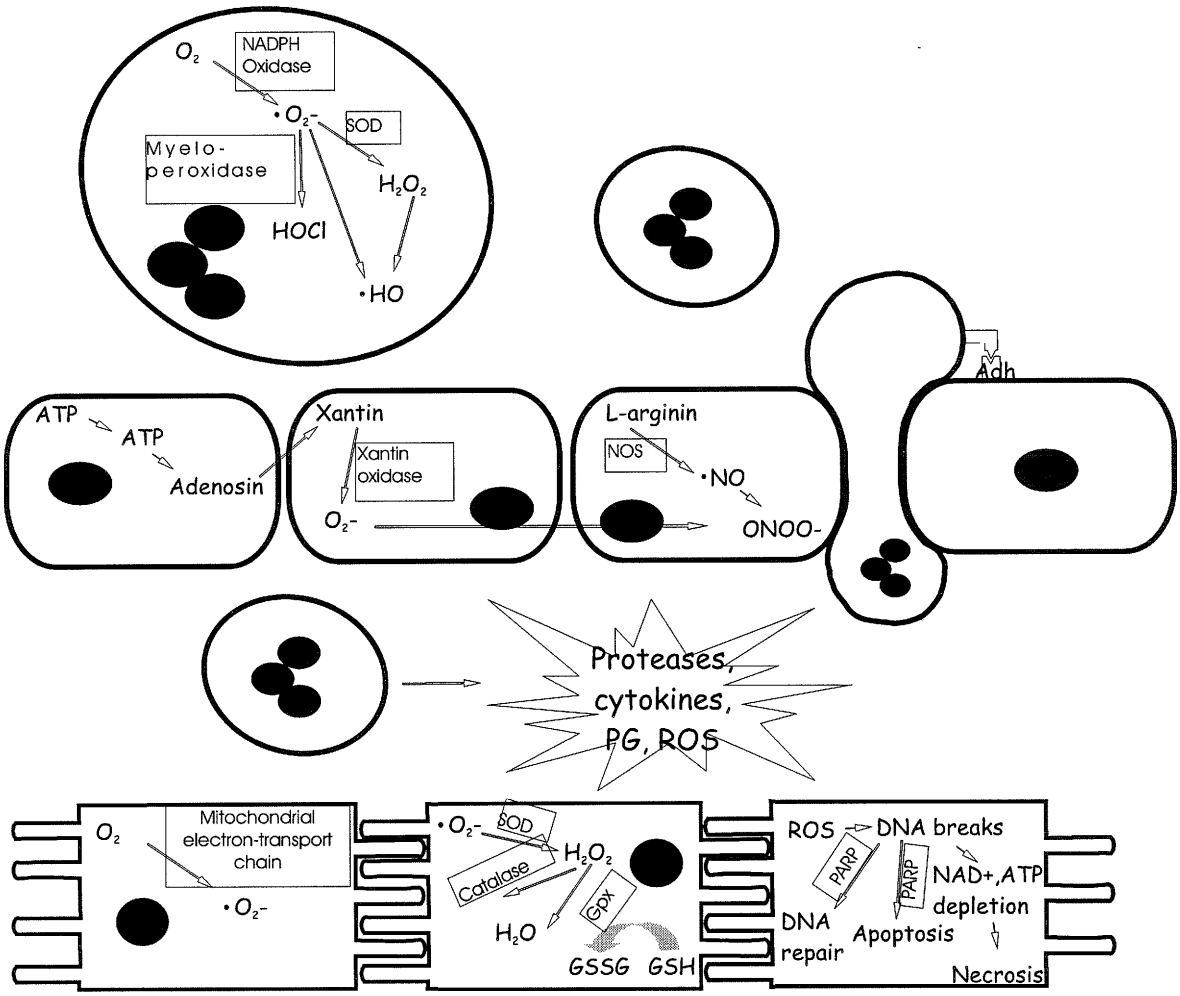


Figure 2b.: Schematic representation of oxidative stress. Under several pathological circumstances leukocytes are activated including polymorfonuclear leukocytes(PMN) which results in ROS production. During its activation PMN go through capillaries by binding its adhesion molecule(Adh) to adequate molecule of endothelial cells (EC). EC cell also contribute to production of ROS. Leukocyte enter tissues and bind to extracellular matrix thus causing oxidative injury and create inflammatory-enchancing products. Tissue cells generate increased amount of ROS endogenously, and antioxidant processes are activated.

ROS: reactive oxygen species; SOD:superoxid dismutase; NOS:nitric oxide synthethase ;Gpx:glutation peroxidase; PARP:poly (ADP-ribose) polymerase;

Main chemical hallmark of the production of ROS is the gaining of molecular oxygen by electrons formulating highly reactive intermediate of short live-time, this event generate free radical known as superoxide anion (O_2-·). Further reactive products are generated by catalytic reactions (Haber-Weiss, Fenton reactions, dismutation), such as hydroxil radical (HO·), hydrogen-peroxide (H_2O_2), singlet oxygen ($^1O_2$) and hypochlorous acid (HOCl) (Fig 3.). Superoxide can further react with nitric oxide generating more reactive radical, peroxinitrit (ONOO-) [41]. Although these radicals are continuously produced by healthy body, in pathologic condition the generation of ROS exceeds the batteries of counter-regulators and cellular damage occurs. ROS can damage cells by reacting with their constituent molecules. All important components, macromolecules are susceptible to ROS attack [41]. In proteins primary sulphur-containing amino acids are targeted to reaction with ROS thus deteriorating function of transcription factors, Na-Ca exchangers, and proteins required in energy metabolism. Lipids, membranes are highly vulnerable to oxidation trough chain reaction, known as lipid-peroxidation. Attack against nucleotides leads to DNA damage, resulting in impaired protein expression, cell death or mutagenesis. Reaction with thiols can cause activation ROS-associated signal transduction disturbing cell functions.

The most general source of ROS is the mitochondrion. In certain condition such as reperfusion this endogenously formed ROS increase considerably, disproportional to endogen defense mechanisms. Inflammatory cells, however, are the most important source of ROS during disturbances producing huge quantities of radicals by nicotine-adenin dinucleotide phosphate (NADPH)- linked oxidase (Fig. 3.) [41]. The abovementioned process of leukocyte-mediated ROS generation occurs in the surroundings of activated leukocyte. If these activated phagocytotic cells are in extracellular localization they can inflict tissue injury through this way. Even though, NADPH oxidase is presented by EC, vascular smooth muscle, myocardial cells, which is a major source of ROS in pathological conditions.

All of cells evolve a defence mechanism to prevent harmful effect of free radicals. Enzymatic antioxidant includes superoxide dismutase (coverts superoxide anion to hydrogen peroxide), catalase (degrades hydrogen peroxide to water), peroxidases such as gluthation peroxidase (destroy hydrogen peroxide). Glutation, vitamin E, ascorbic acid, uric acid belong to non-enzymatic antioxidants which can directly scavenge free radicals. Specific defence mechanism functions against ROS-mediate DNA damage. The function of poly (ADP-ribose) polymerase (PARP) contributes to DNA repair and maintenance of genomic stability by forming ADP-ribose polymers (PAR) [42]. Formation of PAR (ribosilation) also regulates the

function of transcription factors and expression of various proteins thus influencing the cellular response to injury of ROS (Fig. 3.). Nonetheless, excessive generation of ROS causes overactivation of PARP which consumes NAD+ and ATP leading to cellular death and/or enhancing inflammatory processes.

ROS takes important place in regulation of cellular signal transduction [37]. Most of signal pathways are known to be redox-sensitive [43]. Mitogen activated protein kinases (MAPK) and nuclear factor kappa B (NFKB) are the well studied ROS-associated pathways. The activation of different pathways is highly dependent on dose and durability of oxidative stress. Signalling of proliferation, cellular damage, impaired function and type of cell death is regulated through these. NFKB is activated by oxidative injury and by pro-inflammatory cytokines like IL-1 and TNF. Anti-inflammatory mechanisms like expression of IL-10 are known to block NFKB [9]. Activated NFKB plays key role in regulation of pro-inflammatory cytokines, adhesion molecules and activation of inflammatory and endothelial cells. Drugs of antioxidant effect act as influencing of signalling pathways of oxidative stress.

Certain neurogen peptides, have well-known effect influencing the consequences of oxidative stress. Pituitary adenylate cyclase activating polypeptide (PACAP) was first isolated from the hypothalamus and it takes an effect on stimulating adenylate cyclase (AC). Through AC activation, it elevates cAMP, and activates protein kinase A (PKA), which can further influence signalling pathways involved in cytoprotection against oxidative stress. PACAP has potent anti-apoptotic effect in various cell types. On the other hand, neurogen peptides, like PACAP provide significant anti-inflammatory effect.

Oxidative stress, damaging effect of ROS is manifested if generation of free radicals is excessive in relation to antioxidant defences. Cells can adapt to oxidative stress if it is moderate and occurs slowly. Overwhelming oxidative injury however leads to cellular death or impaired cellular function.

Depending on severity, damage of oxidative stress can either cause irreversible dysfunction or cellular death. Reversible injury in the heart is referred to as myocardial stunning, which is widely documented following short-term restoration of coronary flow, leading to ischemia less than 15-20 minutes in duration. This condition results in prolonged depression in cardiac contractile function after reperfusion [44]. The incidence of myocardial stunning in patients after conventional CABG ranges from 20% to 80%. In patients with poor preoperative results, stunning may lead to profound reduction of contractile function [44, 63]. Another cause of loss of cardiac function following IR or CPB is the programmed cell death, apoptosis. Attack of ROS generates the signalling pathways of apoptosis. Increasing body of

evidences suggest that apoptosis play important role in pathomechanism of CPB-mediated myocardial damage. More severe injury of oxidative stress leads to necrotic cell death, which is the less desirable form of cellular damage. Removing of cellular components is impeded and local inflammatory reactions are generated by necrotic cell death. Mentioned function of ROS to influence cellular signalling is responsible for decision of apoptosis or necrosis.

1.2.3 Inflammatory events at the organ level: Systemic inflammatory response syndrome

CPB is often associated with pathophysiological changes involving systemic activation of inflammation together with clinically manifested symptoms. This condition is similar to circumstance which occurs in sepsis or shock and is known as systemic inflammatory response syndrome (SIRS). Activation of cellular and humoral cascades of inflammation can be exaggerated in certain cases and its cellular accumulation is believed to play a major role in the pathophysiological events leading to organ dysfunction or multiple organ failure (MOF) [23].

Myocardial dysfunction:

The heart receives significant stress during conventional cardiac operation resulting in myocardial dysfunction. The damage of heart is a consequence of ischemia-reperfusion injury, mechanical effects (surgical maneuvers, moving, positioning of the heart, and opening of cavities of the hearts), cardioplegia, applying of cardioplegic solutions and chilling of heart [23]. Using of CPB is associated with enormous postoperative release of markers of myocardial damage [44, 45]. Accumulating evidence indicates correlation between inflammatory markers and development of myocardial functional impairment following CPB.

Pulmonary dysfunction, "pump-lung":

Clinical and experimental studies suggest that lungs are at greatest risk during application of CPB. In setting of CPB lung are excluded from circulation and are nourished only through bronchial vessels. The pulmonary dysfunction following CPB - also know as "pump lung" - is manifested by increased vascular resistance, interstitial leakage of fluid, decreased pulmonary compliance [23],. The subsequent inactivation of surfactant causes atelectases and increase in intrapulmonary shunting [23, 44]. Although this effect tends to be subclinical in majority of cases, 10 % of patients develop marked respiratory failure including 0,25 to 2,5% of cases developing adult respiratory distress syndrome (ARDS) [46]. Recent

studies suggest that systemic and pulmonary accumulation of inflammatory mediators are primary responsible for clinical signs of respiratory failure after CPB [9, 23, 46].

Renal dysfunction

Moderate to severe renal failure can occur in 7% of patients. An additional 20% of patients receiving CPB develop with transient azothemia [47]. Duration of CPB, preoperative renal function are reported to be significant risk factors for acute renal failure [47]. Manifest renal failure after CPB is related to extremely high mortality rate above 50% [48]. Correlation was observed between plasma endothelin and elastase level, and renal tubular damage [48]. PMN accumulation shows a relationship with renal damage following CPB. On the other hand it is evidenced that high concentration of TNF increase glomerular fibrin deposition and causes vasoconstriction with decreased glomerular filtration leading to kidney dysfunction [9]. Apart from activation of inflammatory events hypoperfusion, non-pulsatile flow and hypothermia are thought to contribute to acute renal failure [48].

Cerebral dysfunction

The development of neurological dysfunction occurs in 7% to 25% of patients undergoing CPB, with smaller incidence of permanent or local defects [47]. Nonetheless, majority of CPB-associated neurological defects are proved to be reversible resolving over a 3- to 6-mounth period [47]. However Garner reported a persistent decline in cognitive function in 10 % of patients 1 year after cardiac surgery [47]. Pathophysiological changes causing neurological complication are not fully elucidated but resulted from decreased cerebral blood flow, embolic, infarct events. Central contributor of this dysfunction is the release of inflammatory mediators [23]. Blood brain barrier is seems to be intact following an usual CPB suggesting that cerebral dysfunction is not directly caused by accumulation of inflammatory cells, rather systematically released mediators [49].

1.2.4 Therapeutic, anti-inflammatory strategies

Based on improving understanding about pathomechanism of CPB therapeutic measures have been tried to diminish damaging effects.

Pharmacological strategies

Glucocorticoids. Corticosteroids act to reduce pro-inflammatory processes such as, oedema formation, complement activation, leukocyte migration, release of pro-inflammatory

cytokines, collagen deposition [9]. Steroids have been used in studies during open heart surgery for 30 years [9]. Administration of glucocorticoids caused significantly less vasoconstriction, improved perfusion flow and postoperative cardiac index [12]. Although some studies confirmed the superiority of steroid pre-treatment without negative outcome, controlled trials have not demonstrated efficacy in reduction of complications and prolonged recovery [9, 12]. Well-known side effects and adverse outcomes are resulted from steroid treatment which can explain why administration of steroid has not been spread in the clinical routine [12].

Protease inhibitors. Aprotinin administration improves haemostasis after CPB thus reducing transfusion requirement. Aprotinin reduces TNF release and neutrophil integrin expression [51]. High dose of aprotinin treatment inhibits complement activation, neutrophil elastase release and kallikrein production [51]. Recent publications emphasized that protease inhibitors act to prevent the transcription factor NFKB consequently reducing the expression of several inflammatory mediators [50]. Wendel and associates demonstrated significant reduction of postoperative troponin T, CK-MB values in patient following CABG [176]. Treatment with aprotinin also decreases blood loss following surgery and reduces mortality to almost two fold, furthermore decreases the frequency of surgical re-exploration and the length of hospital stay [9, 51]. Despite the evidences further studies are needed to evaluate the clinical use of aprotinin as an agent of anti-inflammatory effect [9]. Recent trials conclude contradictions about aprotinin administration during cardiac surgery [177]. Aprotinin treatment may associated with increased risk of serious end-organ damage.

Antioxidants. Release of ROS is a crucial event of pathophysiology of CPB, so there is a place of antioxidant administration. Application of CPB lead to marked depletion of endogenous α–tocopherol and ascorbic acid [52]. Some papers reported beneficial effect of vitamin C or E treatment, others established difficulties to demonstrate clinical significant benefits [9, 12].

Phosphodiesterase inhibitors. These drugs increase intracellular cAMP and Ca levels increasing myocardial inotropy and decreasing vascular resistance. Preliminary results confirmed encouraging results [53].

Complement inhibitors. Application of antibody that binds to complement C5 has been shown to be safe, well tolerated and effective. Treatment with this antibody reduces C5a and C5-C9 levels and decreases CK-MB concentration following CPB [54]. Despite complement activation appears as an initial event of inflammatory process blocking of complement system may be unable to inhibit inflammation due to its multiple pathways [9].

Sodium nitroprusside. Administration of NO donors can prevent pro-inflammatory effects by inhibiting inducible NOS and can block adhesion molecules [55]. Seghaye and coworkers and Massoudy et al. documented anti-inflammatory properties of nitroprusside in CPB-patients [55].

Modification of technique of CPB or mechanical devices

Heparine-coated circuits. A thin layer of heparin molecules on the surface of circuit and oxygenator act as glucoseaminoglycans on EC surface and are proved to reduce contact of blood with foreign materials [9]. Complement activation, leukocyte priming, cytokine release and kallikrein activation are decreased using heparin-coated surfaces [56, 57]. Ovrum and co-workers reported that low dose heparin is as safe as usual high dose during application of heparin coated circuit thus several heparin-associated side effects can be eliminated [57]. Some experimental and clinical studies demonstrated advantageous effect of heparin –coated circuits on pulmonary or myocardial function [56, 57].

Leukocyte depletion. Since leukocyte activation is a crucial part of inflammatory response to CPB, there is a reality of leukocyte depletion using specific filters. Although this technique is effective of reducing CK-MB release and improving pulmonary function routine application of leukocyte filters remains to be demonstrated [9].

Ultrafiltration. This effort was firstly tried to remove excessive water thereby improving haemodynamic parameters [58]. Certain low molecular weight inflammatory markers are also removed leading to reduction of cytokines and adhesion molecules, nevertheless ultrafiltration has not been associated with clinical advantages [58].

CPB flow, pumps and oxygenator. Pulsatile flow leads to reduction of endotoxin and other pro-inflammatory mediators [59]. Application of centrifugal pumps induces controversial results regarding inflammatory response [9]. Type of oxygenator can also influence inflammation and clinical outcomes. Usage of membrane oxygenators of "ancient ages" can result in deteriorated inflammatory parameters [60].

Preconditioning and heat shock. It is widely known that preservation of an organ can be stimulated by intermittent, short periods of ischemia and reperfusion [61]. Even though, Yellon and associates reported some extent of protection during CABG [62], most of later studies established adverse or non beneficial effect of preconditioning before aorta cross-clamping [12, 62]. Liu and colleagues described that 42 °C blood cardioplegia for 15 minutes resulted in augmented heat shock protein expression causing improved postischemic ventricular function [63]. Further approaches for preconditioning have been performed by

pharmacological agents such as adenosine, potassium channel opener, low dose IL-1α, etc. [12].

Postconditioning. Postconditioning is a series of brief interruptions of reperfusion following applied at the very onset of reperfusion [64]. This algorithm lasts only from 1 to 3 minutes. Postconditioning has been observed to reduce infarct size and apoptosis in myocardial reperfusion. This algorithm was observed to reduce endothelial and neutrophil cell activation and release of inflammatory mediators [64]. This new approach may hold some promise in clinical application, especially following aorta de-clamping.

Modification of surgical technique

Off-pump CABG (OP), "the most popular technique to eliminate side-effects of CPB". CABG on the "beating heart" through standard median sternotomy is now an accepted technique of coronary revascularization. This technique utilizes one of several available cardiac stabilizer devices for reducing free wall-motion. Formerly it was applied to revascularization of LAD and diagonal branches since these branches are the most easily approached with OP technique. Since then it was safely applicable for grafting technique of all coronary arteries [47].

Minimal invasive direct CABG (MIDCAB). MIDCAB is a surgical technique without CPB, through minithoracotomy which is for performing revascularization of the left anterior descendent artery by left internal mammary artery. This approach is aimed to minimize surgical trauma together with neglecting CPB. Although MIDCAB is performed on patients with one vessel disease and are not comparable to other techniques, it indicates encouraging results. This technique reduced both necrosis enzyme levels and pro-inflammatory cytokine concentrations following surgery also related to OP technique [65].

"Heartport" technique. This approach is performed through limited anterior thoracotomy and utilizes femoral arterial and venous cannulae and intraaortic balloon occluder for cardioplegia. Multiple anastomoses can be performed using this technique and patency rates are similar to conventional technique [47].

Off-pump totally endoscopic CABG (TECAB). The concept of thoracoscopic revascularization started in 1996. In 2001 a special stabilizer was developed which can be applied endoscopically. Consequently, total endoscopic CABG can be carried out on the beating heart, thus reducing surgical invasivity and eliminating CPB [66]. This approach , however elongates operation time significantly which might be the major counter-argument for this technique.

Minimal invasive conduit harvesting. The incision for vein harvesting can be the longest incision performed on the human body during routine surgery [66]. Development of minimal invasive vein harvesting techniques indicates the reduction of surgical trauma, improving cosmetic results and decreasing morbidity and inflammatory process [66].

1.4. "Off-pump" coronary artery bypass grafting

Off pump technique is the most popular and obvious measure to diminish CPB together with all of its side-effects, in course of CABG. Most sections of this thesis try to assess the different effects of CPB dealing with the comparisons between conventional and OP surgeries. The OP operation should be lined out in detail.

Because its advantageous effects in inflammatory response and early and mid-term patient outcome, OP has been gaining increasing popularity in many centers. Due to the introduction of second generation stabilizers, the applicability of off-pump is growing and in certain centers it reached 70 to 90% [68]. A survey to determine the applicability of OP surgery described that only 6% of the respondents did not perform any OPCAB currently and 5% did not plan to do any in the following year. Approximately 34% of the surgeons did OPCAB in at least 50% of their coronary bypasses and 15% of all the surgeons performed at least 90% of their coronary revascularization cases off-pump. The results of the survey also showed that more surgeons planned to increase their percentage of off-pump cases [69]. Other questionnaire stated that the respondents performed OP procedures within the same institutions ranged from 20 and 375 cases per year. The per cent of CABG performed as OPCAB in these institutions ranged from 3% to 94% [67-69]. In Hungary, the applicability of OP operations was 34,8 % in 2002 [70, 71].

Increasing evidences is shown that OP decreased the incidence of SIRS and reduced the postoperative rise of inflammatory markers, such as certain cytokines, complement system [15, 72-74]. With regard patient outcome, results are confusing and include relatively small number of patients despite there are up to 15 prospective controlled studies. Taken these together, OP seems to be more that just theoretically superior to conventional CABG [48, 74].

Nevertheless, there are serious concerns with this surgical technique. Disadvantages of OP surgery can be described briefly as follows: During performing OP procedure surgeons and anaesthesiologists are faced with tree major problems: firstly, it is frequently difficult to obtain an adequate exposure of diseased vessel; secondly, hemodynamic changes occur

during moving of the heart; third, myocardium receives ischaemic insults without protection [74, 75]. Manipulation of the heart to expose the target vessel results in decrease of stokes volume, mean arterial pressure, increase of atrial pressures, ventricular end-diastolic pressure. Haemodinamic changes are responsible for catastrophic events during OP most frequently and it is the commonest cause of conversion to open-heart operation. Vertical position increases atrial pressure, and induces distortions of both atrioventricular valves. Stabilizer restricts local motion of ventricles. Local ischemic events of 16 minutes in average occur during performing each anastomoses in course of OP surgery.

Pharmacological profilaxis, intracoronary shunting, positioning of the heart using apical succion device, meticulous monitorisation are the main measures to eliminate these effects [73, 74].

1.4 Conclusion

In conclusion, CPB is an indispensable tool of cardiac surgical armamentarium and it is used in the majority of cardiac operation moreover it is used recently in high risk patients and elderly. Nonetheless, CPB constitute a double –edged weapon that causes cascades of inflammatory reactions. Although more and more contributors of CPB- associated disturbances are elucidated, several events of inflammatory processes are unclear yet. Majority of therapeutic measures failed to improve clinical outcomes. Better therapeutic strategies are based only on solid understanding of mechanisms involved. Regarding the recent observations, CPB-mediated changes seem to be a blunting of inflammatory mediators. It can mean, increasing and also decreasing of certain mediators to pro or anti-inflammatory processes. Studies analyzing the relationship between mediators, cascades and clinical course of patients demonstrate the importance of investigation of mechanism associated with CPB. On the other hand, individual response to CPB varies significantly from person to person since many patients recover from CPB despite the massive and excruciating inflammation. It proves the physiologic reserve of body preventing multiple organ damage. However, increasing number of patients has limited physiological reserve (elderly, high risk patients, infants). It is hoped that better understanding of inflammatory reaction after CPB may provide new prospects for interventions to diminish its damaging effect. Such therapies may improve patients outcome following open-heart surgery.

2. AIMS AND HYPOTHESIS

The aim of the thesis was to investigate clinical, pathophysiological and biochemical aspects of inflammatory response associated with CPB and accordingly try to find modalities, agents which might be able to diminish the deleterious effects of CPB.

It was aimed to measure the following aspects of inflammatory process in patients:

-The balance between pro and anti-inflammatory batteries in course of CABG with or without CPB, and temporally changes of this balance during a longer postoperative period.

- The contribution of myocardial tissue in pro-inflammatory processes.

- Release of IL-12 as a marker of cell mediated immunity

- Influence of CPB on the expression of adhesion molecules on leukocytes.

- Activation of different subsets of leukocytes as a result of application of CPB or OP surgery.

- The extent of oxidative injury during and following CPB or OP technique.

- Change in activity of antioxidant enzymes during CABG with or without CPB.

- Do PARP systematically activated in human beings following open-heart surgery?

3. CLINICAL OUTCOMES AND MYOCARDIAL DAMAGE FOLLOWING CORONARY SURGERY: ON-PUMP VERSUS OFF-PUMP

(Several parts of this section has been published in paper number 1 and 2 as mentioned in section 11, List of publications, Manuscript related to thesis)

3.1 Introduction

Coronary revascularization through CPB is the "gold standard" of CABG surgery, nonetheless it initiates complex cascades of inflammatory and coagulopathic pathways. Ischemia of myocardium due to cross-clamping and cardioplegic arrest further magnifies the derangements directly on the heart itself. Keeping the heart beating during the surgical procedure theoretically eliminates these effects during the CABG. In addition, advantages have been realized with the avoidance of extracorporeal circulation in coronary revascularization, known as OP operation. Initial studies reported excellent results primary early postoperative outcomes associated with OP technique. Even though, it remains uncertain whether OP indicates distinct advantages and reduction of complications. Various studies, meta-analyses, prospective randomized trials reviewed the outcomes following CABG with CPB or OP. Scientifically, statistically valid studies observe longer period after multivessel procedure, examine multiple aspects and are prospective and randomized or meta-analyses of several trial. The first randomized trial was published in1995 by Vural [75] which was followed by appearance of other trials. In review paper of Chassot 14 trials with high level of evidence were designated [73]. The biggest trials involve the "beating heart against cardioplegic arrest studies" (BHACAS 1 and 2) [80], trials of Puskas (SMART trial) [80], van Dijk [45, 76], Diegeler [80], Jaegere (OCTOPUS trial) [80], Straka (Prague-4 Trial) [77], Lancey [78], Nathoe [79], moreover studies of Cleveland, Mach and Patel enrolling 118140, 12540 and 10941 patients [74].

The present section is attempted to evaluate whether the theoretical advantages from eliminating CPB from coronary revascularization are translated into a decreased incidence of certain complications. Because, small sample size and short –term follow –up are the limitation our examinations about patient's outcome, related literature will be reviewed in this part of the thesis. There are several clinical trials and meta-analyses, which provides additional statistical power to appraise statistically significant differences for some outcomes.

These comparisons help us to assess the clinically manifest side-effects of CPB the incidence of prolonged, impaired recovery, and improve our understanding about what kind of complications occur as a result of inflammatory reactions after CPB.

3.2 Patients and methods

For statistical comparison of patient's outcomes receiving operation with CPB or OP technique pre, peri, and postoperative outcomes of 50 patients (25 operated wit CPB and 25 with OP) were analyzed retrospectively, at random.

3.2.1 Anesthesia and operative technique

Both CPB and OP patients received 10 mg midazolam 1 hour before operation. Anaesthesia was induced with midazolam 0.1 mg kg^{-1}, fentanyl 2μg kg^{-1} and propofol 2 mg kg^{-1}. After adequate neuromuscular block with pipecuronium 0.1 mg kg^{-1} endotracheal tube was inserted.

In patients undergoing CPB anesthesia was maintained with continuous infusion of propofol. Coagulation was suspended by administration of heparin to achieve an activated clotting time longer than 400 seconds. Heparin was neutralised with protamin sulphate after CPB. Hollow fibre oxygenator and roller pumps were used to accomplish moderate hypothermic CPB, with pulsatile flow of 2.2-2.4 l/m^2/minute. Myocardial preservation was performed with cold, antegrade, crystalloid cardiolplegia and topical cooling.

In patients receiving beating-heart surgery, anesthesia was maintained with sevoflurane 0.5-1 % and nitrous oxide 60 % in oxygen. Using Octopus (Medtronic, Inc., MI, USA) cardiac stabilizer coronary arteries were occluded for less than 20 minutes per distal anastomosis.

Patients were further cared according to same, standardized protocol.

3.2.2 Preoperative data

To analyze whether two groups of patients are statistically comparable, following preoperative data were acquired: Age, gender, severity of coronary artery disease (number of diseased vessel), classification of angina (according to Canadian Cardiovascular Society Classification System if stable angina was present), prior surgery or percutan coronary intervention (PCI), existence and type of diabetes (type I or II of diabetes mellitus), preoperative risk score (Euro score), preoperative ejection fraction (EF) (%).

3.2.3 Perioperative and early-postoperative outcomes

With regard data of operation number of grafts, arterial grafts, time of operation (from incision to closure) were compared.

Postoperative blood loss, transfusion requirements, the time of ventilatory support, length of intensive care unit stay and hospitalization, were documented furthermore ejection fraction on the sixth postoperative day was calculated in both groups.

Table 1. Preoperative data of patients

	CPB group	OP group
Age (year)	62,64±1,96	63,36±1,83
Gender (female/male)	22/3	19/6
Euro score	2,78±0,35	2,6±0,49
CCS (average of score)	2,33±0,24	2,61±0,21
Preoperative EF (%)	55,82±2.14	54,52±2,31
Diagnosis:		
1-vessel disease	1	1
2-vessel disease	4	8
3-vessel disease	20	16

CCS: Canadian Cardiovascular Society Classification System; EF: ejection fraction;

3.2.4 Determination of myocardial injury

Plasma levels of troponin I (TI), creatine- phosphokinase myocardial specific isoenzyme (CKMB), glutamine oxalacetate transferase (GOT) were measured as a part of the routinely used clinical investigations. The TI level in plasma was demonstrated in unit per liter (U/l). Blood samples were taken for enzyme determinations just before operation, during the early reperfusion and on the first and second postoperative days.

3.2.5 Statistical analysis

The data were presented as mean ± standard error of the mean. The data between the two groups were compared with unpaired Student's t test. In a given group comparisons between the control data were made using paired Student's t test. Differences were considered significant at p values less than 0,05

3.3 Results

3.3.1 Preoperative data

Preoperative characteristic of patients are shown on Table 1. There were no differences in age, gender, Euro score, ratio of stable angina, preoperative left ventricular function (EF). Only a little divergence could be observed in number of occluded vessels. 2 patients in CPB group and one patient in OP group suffered form type I of diabetes, furthermore seven patients in CPB and 9 in OP group develop type II of diabetes. Tree patient had vascular surgical operation in both groups previously. Four patients in CPB and seven in OP group undergone prior PCI.

3.3.2 Early postoperative outcomes

The average number of distal graft, and the number of arterial graft (left internal mammary artery in all cases) applied were similar and are presented on Table 2.

There was no hospital mortality, myocardial infarction or major neurological complication in the two study groups. Re-hospitalisation during the one year long follow-up period was necessary in one patient in both groups (for 5 days in OP group and 2 days in CPB group).

The main postoperative characteristics of patients are shown on Table 2. Intubation-time was significantly higher in CPB group related to OP group. In addition the postoperative blood loss of CPB group through the chest drain exceeded the level of OP group markedly however this difference was not proved statistically significant. 18 patients in CPB group and 11 in OP group required blood transfusion, and the quantities of transfusion was significantly higher in CPB group (Table 2). Despite the length of ICU stay of CPB group was longer no significant difference could be established. Patients operated with conventional method applying CPB spent significantly longer period in the hospital. Postoperative EF of patients operated with CPB decreased significantly related to preoperative values and it was also significantly lover compared to OP group.

3.3.3 Alteration of myocardial necrosis enzyme levels

These sensitive indicators of myocardial damage differed significantly between the two groups. TI levels of CPB group were many fold higher compared with OP group in all time points. Most intense elevation in CPB group can be observed on the morning of 1st postoperative day (Fig. 4). Increased appearance of myocardial injury during and following CPB was determined by CK-MB plasma concentrations (Fig 5). Intra and postoperative levels

of CK-MB differed significantly from preoperative values in both groups except the 2nd postoperative day of OP group. OP was advantageous regarding CK and GOT levels (Fig 4b and 4a.). Statistically significantly difference were found in operative and postoperative values of all enzyme levels between two groups

Table 2. Intra and postoperative characteristics of patient

	CPB group	OP group
Number of grafts (distal anastomoses)	3,04±0,19	3,24±0,16
Number of arterial grafts	1	1±0,05
Length of operation (minutes)	199,56±10,05	202,68±9,03
Blood loss (ml)	781,66±86,23	636,57±29,57
Transfusion (units of RBC)	2,41±0,36	0,86±0,28
Time to extubation (hours)	11,4±1,11	9,87±0,97
Length of ICU stay (hours)	54,4±5,95	47,48±1,71
Length of hosp. (hours)	272,01±11,45	238,89±9,38
Postoperative EF (%)	45,69±1,93	50,83±1,98

RBC: red blood cell, ICU: intensive care unit, hosp-hospitalisation, EF: ejection fraction

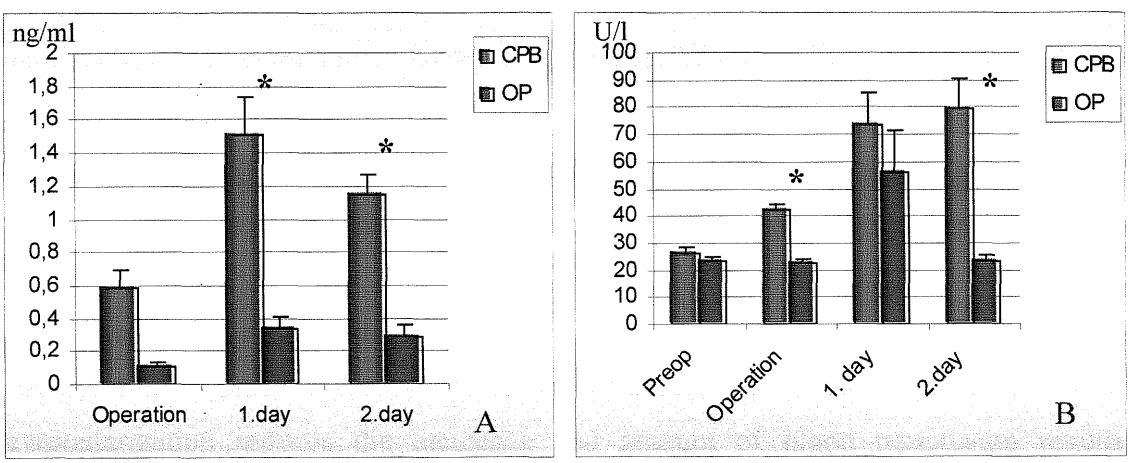


*Figure 4. : Plasma level of troponin I (A) and GOT (B) (mean ± SEM). CPB: operation with cardiopulmonary bypass; OP: Off: pump operation; * p<0,05 between the two groups, # p<0,05 compared to the control value.*

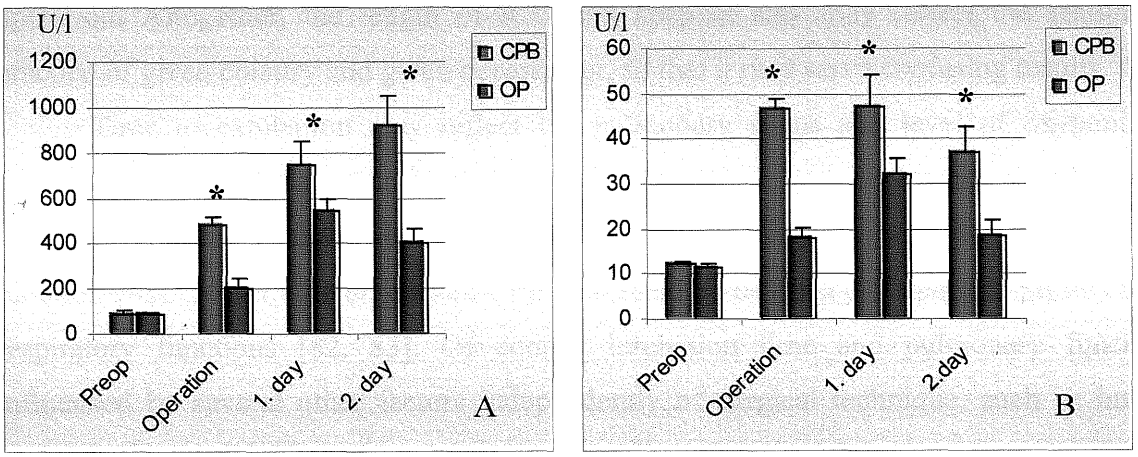


*Figure 5. : Plasma level of creatinin kinase (CK) (A) and CK-MB (B) (mean ± SEM). CPB: operation with cardiopulmonary bypass; OP: Off- pump operation; * $p<0,05$ between the two groups, # $p<0,05$ compared to the control value*

3.4 Discussion

Our present results provide evidence that the application of CPB alone induces significant increase in myocardial injury. These finding are associated with evident intensification of postoperative blood loss, increased need for transfusions, lengthened ventilation time and hospitalization. Besides the more intensive signs of myocardial damage, deteriorated myocardial function can be observed following CPB. Our trial described here, entered to OPCAB trialist collaboration controlled by Geert van derHeijden. This collaboration involves several European cardiac surgical centers and is aimed to perform a meta-analysis with large number of patients. Previous results of van der Heijden has been published yet [178].

Accumulating evidences confirm certain benefit of avoiding of CPB by applying OP surgery. Almost uniformly, majority of papers concluded that eliminating CPB in coronary revascularization reduces the incidence and amount of blood transfusion requirements. Studies further support that application of CPB is associated with larger amount of intra and postoperative blood loss [73, 80]. Only Bouchard and co-workers found lack of evidence of decreased bleeding complication in course of OP [179]. It is also supported in the literature that use of CPB prolongs ICU and/or hospital length of stay [73, 80]. ICU length of stay and total time to discharge from ward may represent the condition and recovery of patient after

operation. Altogether, the length of ICU and hospital stay may reflect the features and practice of given country and given department, so that it may serve confusing results.

Time to extubation may reflect the pulmonary status and level of oxygenization. Although numerous papers postulated extended intubation and mechanical ventilation time after conventional CABG with CPB [80, 83], studies aimed to compare the pulmonary function after CABG with or without CPB suggested no obvious difference in preservation of respiratory functions [82, 83]. Of course, intubation time and pulmonary function is influenced by several other factors independently of surgical technique, such as intra and postoperative medication.

Overwhelming majority of publications confirmed the more intensive increase in myocardial necrosis enzymes following CPB related to OP surgery [78, 80, 84]. Langenbach and coworkers also experienced the postoperative shrinkage in EF [80]. These observation suggest functional and/or structural damage of myocardium during the early period following CPB. Other interesting findings of Selvanayagam and co-authors documented increased TI levels and decreased cardiac index in patients having CABG with CPB compared to OP patients however they did not find any long-lasting or irreversible myocardial damage as assessed by contrast-enhanced cardiovascular magnetic resonance imaging (MRI) [85].Vural, Angelini, Zamvar experienced higher requirement of inotropic support or intraaortic balloon pump following open heart surgery representing deteriorated systolic function [74, 80]. Further studies suggest that myocardial preservation of OP procedure is associated with reduction of inflammatory processes and oxidative injury in myocardium [81]. According to our observation we found an important elevation of necrosis enzyme levels following CPB. On the other hand decrease of postoperative EF was observed after CPB compared to OP group which might be clinically irrelevant.

Until now, The OP approach has not been proven to reduce incidence of arrhythmias. While several papers report decreased incidence of atrial fibrillation others showed no difference with open-heart CABG [81].

CPB is known to cause generation of processes leading to impair renal function. OP operation is probably associated with reduced incidence of renal failure [86]. As described by Ascione, glomerular filtration was much better and tubular readsorption much less impaired after off-pump procedures [87]. Increase of creatinin and urea levels was proven to be diminished by applying OP [87].

One of the major advantages of OP is the reduced incidence of neurologic complications and severe stroke, especially in high-risk patients [80]. It is worth mentioning that no study has ever determined higher incidence of neurological impairment after OP.

Patients randomised to OPCAB and CABG-CPB had similar symptoms, generic and disease-specific qualitity of life scores by use of generic questionnaires even 1 month, 2 or 3 years after intervention [86, 89].

Cost-effectiveness of OP can be hardly studies because of health care systems of given countries. Despite, some paper concluded that may be cheaper because of the reduced postoperative disturbances and not due to intraoperative instrumental savings [81]. Weimar wrote in his paper that reduction of just strokes by using OP could save a two-digit amount of million Euros [80].

On the other hand, Kim and associates published that OP may decrease patency rate of saphenous vein grafts 1 year after surgery, which can be the major counter-argument for OP technique [180]. Later examinations, however, attest a good quality of anastomoses and patency rates comparing well to conventional method [90]. Puskás reported a patency rate of 98,8 % at the time of hospital discharge [81]. Long term results of patency rates should be clarified in meta-analyses.

It is evidenced that mortality rates for OP and CPB technique are similar, nonetheless case and risk matched trials reported that lower mortality for OP [74, 81].

Superiority of OP techniques lies on the management of high-risk patients. There are persuasive evidences that OP offers prognostic advantage in patients with recent myocardial infarction, poor left ventricular function, chronic renal failure, obstructive pulmonary disease, obesity, and in elderly patients and patients receiving redo-CABG [74, 80, 81]. One of the greatest utility for OPCAB is probably the severely calcified or diseased aorta in which manipulation or clamping of the aorta can be associated with neurological consequences. To diminish of damaging effects and exhausting inflammatory processes has great importance in these patients.

In low or intermediate-risk patients, nevertheless no obvious difference could be clarified in long term outcomes. In these patients OP offers a good clinical outcomes.

In sum, CPB may disturb the normal recovery of patients after heavy intervention for short or mid-term period causing prolonged hospital stay, increased loss of blood, and worsened heart function. Longer-term follow up studies however revealed no difference

between two types of operation. High risk patient have impaired physiological protection against the damaging effects of CPB and clinical signs of pulmonary, renal neurological dysfunction, expressed myocardial damage, abnormal bleeding, tendency to infections can be manifested. Furthermore the number of trials and enrolled subjects are growing following up longer period to expose the real advantages and disadvantages of off-pump operation. According to the present results we can suppose there some disturbances occur early after applying of CPB. Understanding of the pathophysiological, biochemical events of CPB is essential therefore, which processes may contribute to impaired recovery or clinical complications.

4. CYTOKINE NETWORK AND MYOCARDIAL CYTOKINE-PRODUCTION IN PATIENTS RECEIVING CONVENTIONAL OR OFF-PUMP OPERATION

(Several parts of this section has been published in paper number 4 as mentioned in section 11, List of publications, Manuscript related to thesis)

4.1 Introduction

Regarding inflammatory manifestation to CPB, central importance belongs to release of different cytokines regarding mediators which orchestrate the inflammatory processes, cellular activation, and leukocyte migration. Pro-inflammatory cytokines in extremely elevated concentrations can modulate the function of organs [23, 24, 91]. Dominant anti-inflammatory effects however can blunt adequate immune response impairing defensive mechanisms and healing processes. The balance between pro- and anti-inflammatory cytokines is essential to appraise the genuine effect of different cytokines and the characteristics of cytokine network. Temporal change of the balance between pro and anti-inflammatory cytokines has been less investigated.

One of the hallmarks of CPB-mediated inflammatory response is the alteration of IL-12 playing a crucial role in cell-mediated immune response [93]. Few studies have been carried out comparing the effects of coronary operations with or without CPB on IL-12 levels leaving certain key questions unanswered.

Studies have shown that CABG performed without CPB, known as off-pump (OP) surgery helps avoiding unwanted effects such as over activation of inflammatory response [92].

Increased evidence suggests that the myocardium is capable of synthesizing biologically active cytokines [94]. The effect of OP surgery on myocardial cytokine production has not been yet investigated in details. Therefore, this study investigates the association between CPB or OP surgery and cytokine production by the heart.

The aims of the present section were: 1) to measure pro/anti- inflammatory cytokine ratios in patients who underwent operation with or without CPB up to the end of first postoperative week, 2) to determine the alteration in IL-12 levels during and after both operations, and 3) to ascertain the myocardial outflow of cytokines in course of operation with or without CPB.

4.2 Patients and methods

4.2.1 Patients

A prospective randomized study was performed, on all patients undergoing CABG with or without CPB operated in two cardiac centers: the Department of Cardiac Surgery of Zala County Hospital and the Heart Institute of the University of Pecs. The study protocol was approved by the Ethics Committees of both hospitals. All patients were provided with oral and written informed consent.

The total of 30 subjects participated in the study (63, 2±2,5 years, 7 females, 23 males); they received elective CABG. 20 of the patients were operated with conventional method using CPB (CPB group), while 10 patients underwent operation with OP technique (OP group). There was no significant difference in the preoperative data of the patients with respect their age, gender, the number of performed grafts (3,21±0,22 in CPB group, 3±0,42 in OP group), the number of arterial graft (LIMA) (0,88±0,09 in CPB group, 0,92±0,0,08 in OP group) and in the preoperative risk score (Euro score: 2,6±0,54 in CPB group, 2,54±0,31 in OP group). All participants received standard preoperative treatment and the technique was randomly chosen for each patient. Exclusion criteria were: immunological disease, recent myocardial infarction (<3 month), acute operation, reoperation, previous stroke, infection, coagulopathy, tumor, acute or chronic renal failure and respiratory impairment.

4.2.2 Anaesthesia and surgical technique

Each group of patients received the same protocol as described in the section 3 (page 26).

4.2.3 Blood sampling

In CPB group, blood samples from peripheral vein were taken just after the induction of anaesthesia and 5 minutes after the cessation of aorta cross clamping, and on the 1st, 2nd, 3rd, and 7th postoperative days. In OP group, blood samples were collected after the induction of anaesthesia, 5 minutes after completion of the last graft, and on the 1st, 2nd, 3rd, and 7th postoperative days.

Further blood samples were taken from coronary sinus (CS) using a catheter in both groups: In CPB group, 5 minutes after the declamping of aorta and in OP group, 5 minutes after the completion of the last graft.

All the samples were collected in sterile vacuum tubes containing sodium heparin.

4.2.4 Measurement of cytokines

Blood samples from peripheral vein were first incubated on 37 °C for 4 hours, then stimulated with phorbol 12-myristate13-acetate (PMA, 111 ng ml-1). After this period of stimulation, tubes were centrifuged at 3000 g for 10 minutes; then, the supernatant was separated into vials and frozen immediately to -75°C and stored at that temperature until the day of measurement (within 2 months). The plasma concentrations of stimulated cytokines were determined using the Becton Dickinson cytometric bead array (CBA Human Inflammatory Kit, BD Biosciences, Pharmingen, USA) following the instructions of manufacturers. This newly developed method enables reliable measurement of six human cytokine levels: tumor necrosis factor α, interleukin –1β, 6 ,8, 10, 12p70 (TNF, IL-1, IL-6, IL-8, IL-10, IL-12) simultaneously from small sample volumes. PMA stimulation is highly influenced by white blood cell count. Our outcomes were corrected to cell count thus expressing the final values in pg ml^{-1} per 10^6 cells.

Besides the monitoring of the absolute concentration of given cytokines the pro/anti-inflammatory cytokine balance was worked out in all samples by dividing the plasma concentration of different pro-inflammatory cytokines with the concentration of interleukin-10.

During CS blood sampling (in the fifth minute of reperfusion), other peripheral blood samples were collected to compare the plasma concentration of cytokines between CS and peripheral vein samples. PMA stimulation releases cytokines produced by white blood cells. To obtain the cytokines secreted only by the myocardium, PMA stimulation was neglected both in CS and peripheral vein samples. These samples were measured as described above.

The cytokine levels of CS samples were compared to their corresponding peripheral vein levels thus expressing the values in percentage. The concentration of unstimulated peripheral vein samples was considered to be 100% in each sample. The cytokine levels of CS samples were compared to their corresponding peripheral vein levels, expressing the values in per cent.

4.2.5 Statistical analysis

The data are presented on tables and figures as mean ± standard error of mean (SEM). The data between the two groups were compared with unpaired Student's t test. In a given group comparisons between control data were made using paired Student's t test. Differences were considered significant at p values less than 0,05.

4.3 Results

4.3.1 Concentration of cytokines from samples of peripheral vein

Absolute concentrations of measured cytokines corrected to white blood cell count are presented in Table 3.

Table 3. Plasma concentration of tumour necrosis factor α, interleukin -1, IL-6, IL-8, IL-12 and IL-10 in patients undergoing coronary artery bypass grafting with or without cardiopulmonary bypass (pg/ml).

		Control	Rep5	POD1	POD2	POD3	POD7
TNF	CPB	**26,04±5,37** *	**8,84±2,01** *#	54,03±11,45	26,88±0,90	27,99±2,08	18,74±4
	OP	20,19±2,2	17,99±5,15	16,18±5,39	21,01±2,93	10,76±3,60	16,11±3,97
IL-1	CPB	12,95±2,11	**2,34±0,45** **	**22,60±2,21** **##	7,55±1,77	10,68±0,82	14,21±1,38
	OP	10,39±1,57	5,42±2,54	6,37±2,25	11,67±5,59	13,62±5,58	16,76±10,93
IL-6	CPB	9,86±1,23	7,34±1,83	**39,89±12,42** **	10,83±2,04	23,64±8,45	18,95±1,5 *
	OP	9,40±2,97	15,54±3,45	**27,50±7,07** *	20,12±3,59	14,18±1,73	9,16±3,3
IL-8	CPB	1627,6±128,3	**1919,6±263,9** #	**1355±125**	**1090,3±99,7** **	1621±204,3	1835,9±247,3
	OP	1520±190,3	1297,53±191,59	1344,32±143,1	1199,2±75,5	1374,7±92	1866,4±263,1
IL-12	CPB	0,13±0,03	**0,24±0,04** *	**0,26±0,03** **	0,15±0,04	0,24±0,07	0,11±0,05
	OP	0,14±0,11	0,11±0,05	0,09±0,06	0,11±0,08	0,17±0,08	0,17±0,08
IL-10	CPB	4,04±0,78	**23,3±5,28** **##	6,37±1,35	**1,53±0,22** **	**1,59±0,41** *	1,17±0,24 *
	OP	4,05±0,65	4,77±1,65	**8,98±1,50** *	**8,08±1,11** #	2,65±0,11	1,55±0,34

Data are presented as mean ± standard error of the mean. * = $p<0,05$ compared to preoperative values (control); ** = $p<0,03$ related to preoperative values (control); # = $p<0,05$ compared to other group at same time point; ## = intergroup difference was $p<0,03$ (# and ## were placed to the higher values). Control: before surgery; CPB: Cardiopulmonary bypass; IL: interleukin; OP: off-pump; POD1,2,3,7: on the 1st, 2nd 3rd, 7th postoperative day; Rep5: time point 5 minute after the beginning of reperfusion; TNF: tumor necrosis factor α.

4.3.2 Assessment of cytokine balance

The balance between inflammatory and anti-inflammatory forces was determined by calculating pro-inflammatory cytokine/IL-10 ratio. All ratios were similar in the CPB and OP group. TNF/IL-10, IL-6/IL-10 and IL-8/IL-10 ratios are shown in Figures 6, 7 and 8. In CPB group, an early drop was observed during surgery and afterwards the ratio increased extremely throughout the observation period. During surgery in the fifth minute of reperfusion the decrease in TNF/IL-10, IL-1/IL-10, IL-6/IL-10, IL-8/IL-10 ratios was significant when compared with the corresponding preoperative ratios. In OP group, the ratio of given pro-inflammatory cytokine and IL-10 tended to decrease reaching it's minimum value on the 1st or 2nd postoperative day thereafter it normalised gradually. Statistical analysis revealed consequential differences between groups A and B firstly in TNF/IL-10 ratios on the 1st, 2nd, 3rd and 7th post-operative days (Fig. 6), secondly in IL-6/IL-10 ratio on the 2nd and 3rd post-operative day (Fig. 7) and in IL-8/IL-10 ratios in the 5th minute of reperfusion and on the 1st and 2nd post-operative days (Fig. 8).

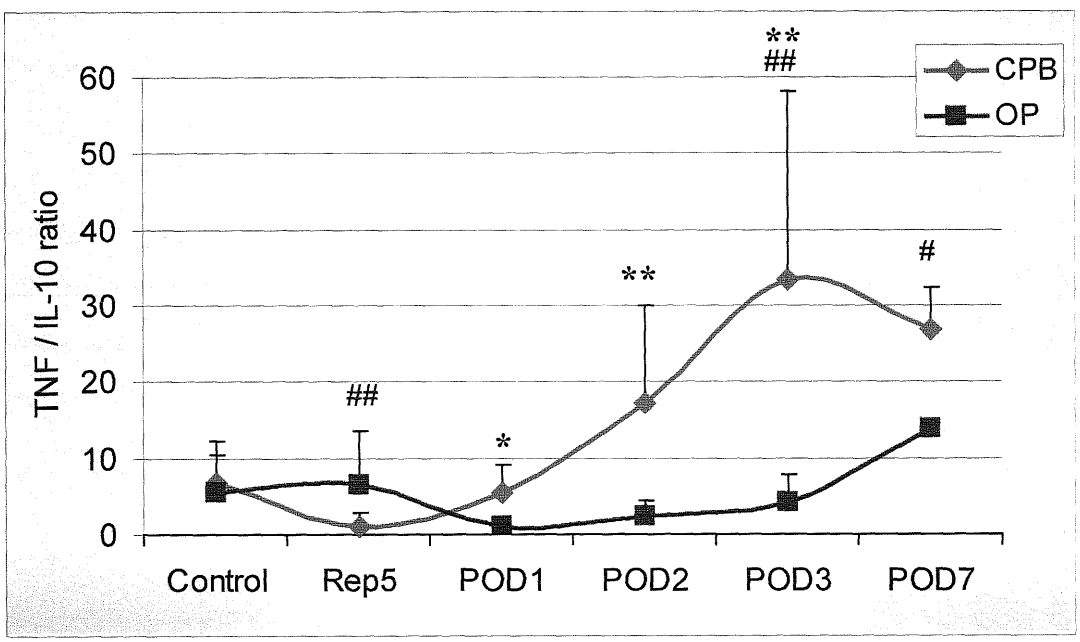


*Figure 6. Tumour necrosis factor α and interleukin-10 ratio over the time in patients undergoing coronary artery bypass grafting with cardiopulmonary bypass (CPB group) or off-pump technique (OP group). Data are presented as mean ± standard error of the mean. * = p<0,05 compared to preoperative values (control); ## = p<0,03 related to preoperative values (control); * = p<0,05 compared to other group at same time point; ** = intergroup difference was p<0,03.*

Control: before surgery; CPB: Cardiopulmonary bypass; IL: interleukin; OP: off-pump; POD1,2,3,7: on the 1st, 2nd 3rd, 7th postoperative day; Rep5: time point 5 minute after the beginning of reperfusion; TNF: tumor necrosis factor α.

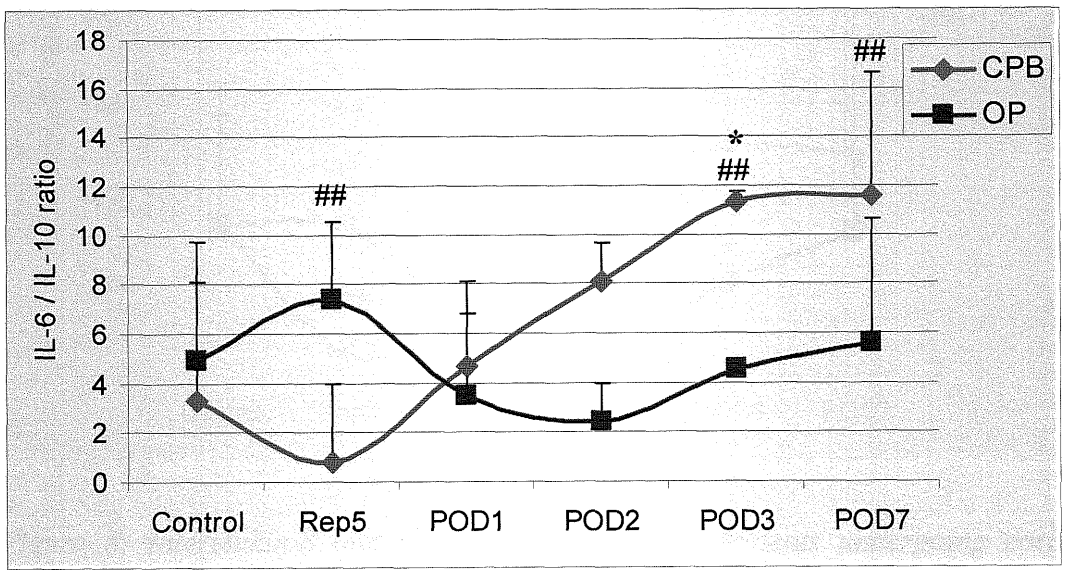


Figure 7: Interleukin-6 and interleukin-10 ratio in patients undergoing coronary artery bypass grafting with cardiopulmonary bypass (CPB group) or off-pump technique (OP group). Data are presented as mean ± standard error of the mean. # = p<0,05 compared to preoperative values (control); ## = p<0,03 related to preoperative values (control); = p<0,05 compared to other group at same time point. Control: before surgery; CPB: Cardiopulmonary bypass; IL: interleukin; OP: off-pump; POD1,2,3,7: on the 1st, 2nd 3rd, 7th postoperative day; Rep5: time point 5 minute after the beginning of reperfusion; TNF: tumor necrosis factor α.*

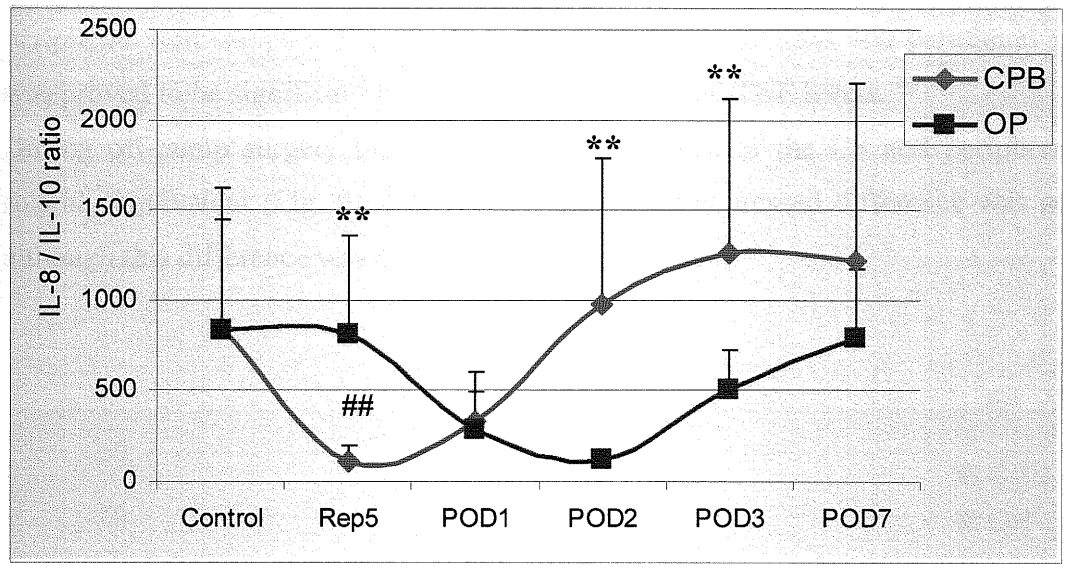


*Figure 8: Interleukin-8 and interleukin-10 ratio in patients undergoing coronary artery bypass grafting with cardiopulmonary bypass (CPB group) or off-pump technique (OP group). Data are presented as mean ± standard error of the mean. ## = p<0,03 compared to preoperative values (control); ** = p<0, 03 related to other group at same time. Control: before surgery; CPB: Cardiopulmonary bypass; IL: interleukin; OP: off-pump; POD1,2,3,7: on the 1st, 2nd 3rd, 7th postoperative day; Rep5: time point 5 minute after the beginning of reperfusion;*

4.3.3. Concentration of cytokines from samples of coronary sinus

In CPB group, all of observed cytokines from CS exceeded the concentrations of peripheral vein samples (Fig. 9). The difference between sinus and peripheral vein samples was proved to be significant for the IL-1, IL-6, IL-8 and TNF levels.

During off-pump surgery the cytokine concentrations of the CS and peripheral vein were roughly equivalent (Fig. 9). Interestingly, the most expressed difference was seen in IL-10 although this difference was not statistically significant.

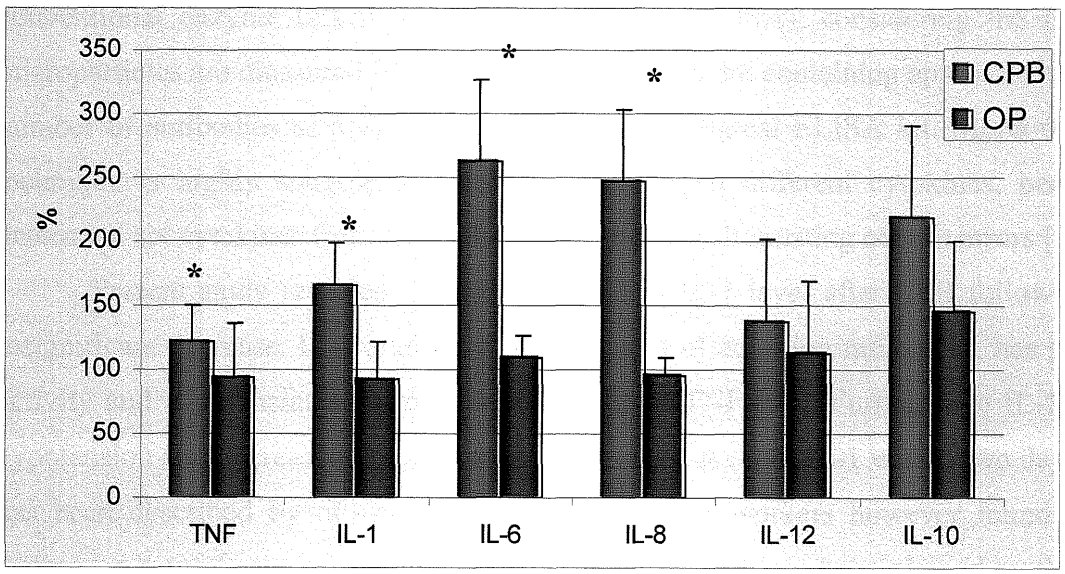


*Figure 9. Cytokine levels of coronary sinus blood during coronary artery bypass grafting with cardiopulmonary bypass (CPB group) and with off-pump technique (OP group). Dark bars show CPB group and light bars represent OP group. Concentrations are presented as per cent of peripheral venous blood level of given cytokine at the same time. Data are shown as mean ± standard error of the mean. * = $p<0,05$ compared to the concentration of peripheral venous blood. IL: interleukin; TNF: tumour necrosis factor α.*

4.4 Discussion

The present study showed that CPB caused a prolonged pro-inflammatory predominant response. Pro/anti-inflammatory ratios could be balanced by minimal invasive off-pump technique. Our investigations also demonstrated that application of CPB and not off-pump technique was associated with cytokine production of myocardium.

A completely novel method was demonstrated in this paper to measure six cytokines simultaneously from a single sample with cytometry using CBA technique [95]. This method appears to be a more reliable and easy technique to measure cytokines compared to conventional enzyme linked immunosobent assay (ELISA), considering the fact that 300 microparticles are measured for each cytokine, all of them containing approximately the same number of antibodies as present in one well of an original ELISA kit. Moreover, the CBA technique is highly suitable to assess ratios between different cytokines, because it can determine six cytokines from the same sample thereby eliminating certain errors [95].

Present study revealed marked elevation in IL-1 level after CPB following an acute insignificant decrease. IL-1 augments the synthesis of adhesion molecules, has procoagulant activity and it potenciates the deleterious effect of TNF [96]. Furthermore IL-1 can induce hypotension and decreased vascular tone [97]. Raised level of IL-1 within two days after CPB has been described by others [65]. Guliemos and co-workers however found no obvious difference between the off-pump and CPB patients, which can be explained by the short time of CPB, because they examined single-graft bypass procedures. Interestingly, we did not find the elevation in IL-1 level after off-pump surgery as illustrated by Guliemos.

IL-6 raised markedly until the 1st postoperative day, thereafter it normalised gradually in both groups. CPB provoked higher elevation but there was no significant difference between the groups. Besides, IL-6 is known as a good marker of surgical invasivity it controls the immunological processes via numerous ways [24]. IL-6 regulates the synthesis of acute phase proteins, causes T cell activation and promotes the growth of haemopoietic cells and fibroblasts [14]. In the literature no or non-obvious differences were found in IL-6 level of patients operated with or without CPB [65] and our results agree with these findings. Even though studies established significant difference in IL-6 level comparing conventional operation and MIDCAB surgery furthermore higher IL-6 concentration was documented in patients received thoracotomy versus sternotomy [102]. The above findings suggest that the IL-6 release is rather influenced by the surgical invasivity than by reperfusion or myocardial

injury or use of CPB alone [25] explaining our results that failed to determine any main difference between the two observed groups.

Level of IL-8 demonstrated significant elevation during and after CPB however off-pump surgery is associated with consequential increase. IL-8 is the first known and reasonably potent chemoatractant protein controlling the trafficking of leukocytes trough endothelium [98]. IL-8 has been shown to intensify neutrophil cells for oxidative product, mainly superoxide production and also it is proved that the level of IL-8 correlate highly with the myocardial injury after myocardial infarction [98]. With respect to central role of IL-8 in myocardial damage in course of cardiac operation its kinetics is well-investigated even after conventional and after off-pump coronary bypass. It is documented by several authors that CPB induces extreme elevation in peripheral level of IL-8 early postoperatively. Since Ascione described no relevant change due to off-pump operation, others evidenced major raise in early stage after off-pump surgery [15, 18]. Although majority of publications established an early postoperative increase of IL-8 to CPB we however have found long-lasting elevation. This contrast can be explained by different methods. We used 4 hours long stimulation with PMA of whole blood which released the synthesised cytokines without new protein synthesis. We believe comparable stimulation may occur when leukocytes are passing trough the activated endothelium of injured organs, in course of CPB in lung and heart. This method may better model the leukocyte response to such a stimulus. It is proved that the stimulated level of cytokines is more important and on the site of damaged organ it is rather true to fact than the unstimulated level of cytokine released by peripheral leukocytes of resting condition. In this manner, our results suggest that the IL-8-secreting capacity is markedly higher within the 1st three postoperative days in CPB patients compared with preoperative values. This conclusion is inconsistent with the result of other studies measuring the absolute level of IL-8 (without stimulation). Since these investigations confirmed higher IL-8 level in the early hours after surgery in CPB patients with similar levels between CPB and OP at later times [103, 104]. In contrary, stimulated levels of IL-8 were significantly higher both in CPB and in OP patients within the 1st postoperative day, however it's levels remained elevated only after CPB in later period while in OP patients it tends to recover over the postoperative days.

Investigations about the cell-mediated immunity and activation of lymphocytes are essential to assess the characteristic of inflammatory response to CPB. IL-12 showed intensive elevation after CPB whereas off-pump surgery provokes unsignificant change in IL-12 plasma level. IL-12 is known to be the activator of the type-1 T helper-cell (TH1)-

mediated immune response. TH1-mediated response is essential for activating natural killer cells and cytotoxic T cells moreover for enhancing cytotoxicity. Some articles suggest that IL-12 level drops early after weaning from CPB [57, 99, 103]. In SIRS patients after CPB nonetheless elevated IL-12 level is determined compared with uncomplied patients after CPB. Myocardial infarction also causes IL-12 elevation [99, 103].

According to our finding the TH1/TH2 response changes in two phases after CPB: firstly the TH2-mediated response is stronger due to elevated IL-10 level subsequently, after the 1st postoperative day the TH1-mediated response tends to be upregulated. Likewise, in course of off-pump surgery is associated with more moderate shift in TH1/TH2 response determined by cytokine pattern as estimated by relation between IL-12 and IL-10.

Besides the recognition pro-inflammatory cytokine release the investigation of anti-inflammatory cytokines kinetics gained increased attention. Our outcomes about the change of IL-10 levels can be described as follows: CPB is associated with a pronounced elevation at early period and with IL-10 levels below the preoperative values in longer terms. OP technique nevertheless causes later elevation in IL-10 level. IL-10 exerts protective role by suppressing the production of pro-inflammatory cytokines and by inhibiting neutrophil-endothelial cell adhesion. IL-10 also serves as an essential function in healing of vascular anastomoses and jeopardised tissue by inhibiting of vascular smooth muscle proliferation and influencing matrix metalloproteinase activity [100, 101]. Above results correlate to other studies about the short-lived but considerable raise of IL-10 during and after CPB furthermore about the lack of IL-10 release in the very early postoperative period after off pump surgery [102]. Most of them applied short observation period to measure IL-10 level lasting mainly not longer than the 1st postoperative day. Diegeler documented similar changes concerning the CPB patients over the postoperative days [104]. However non significant difference was found following off pump technique all the same on the 2nd postoperative day the plasma level of IL-10 was obviously higher than before surgery. By way of explanation on ambiguous results we can refer to different methods.

The absolute concentration of cytokines is considered to be less important than their relative balance, which may better reflect the net effect of cytokine response [105]. The novelty of our study lies on the acknowledgement, that the increased and continuously elevating force of pro-inflammatory response is balanced only during the early postoperative period by IL-10 in patients undergoing CABG with CPB. Our results about the anti-inflammatory predominant response to CPB up to the 1st postoperative day agree with the outcomes of other studies [105]. Moreover, Hövers-Gürich and associates reported similar

alterations regarding the later events observing infant patients, specifically elevation in pro-inflammatory cytokines without the counterbalance of IL-10 [105]. In addition less alteration in the balance of pro and anti-inflammatory responses can be observed after off-pump operation with dominating anti-inflammatory forces.

These results may have therapeutic consequences. Steroid administration is known to reduce the generation of pro-inflammatory cytokines with the exaggeration of IL-10 and anti-inflammatory cytokine response thereby reducing the ratio of pro and anti-inflammatory cytokines [106]. In most studies examining the efficacy of steroid treatment in patients receiving CPB, steroid was administered before or during operation [107]. Although majority of these investigations confirmed beneficial effect of preoperative steroid treatment, our findings suggest the necessity of eventual longer-term administration of corticosteroids. With respect to the aspect of cytokine balance, steroid administration or anti-inflammatory treatment should be required only from the 2nd postoperative day up to the end of the first week. Cytokine response after off-pump operation however does not necessitate any anti-inflammatory treatment because it is balanced with anti-inflammatory batteries. The above are theoretical hypotheses and further clinical studies about the longer-term administration of steroids should be performed to evaluate the warranty of this presumption. The adverse effect of steroid treatment is also known. Perioperative treatment with dexamethasone resulted in more pronounced postoperative pulmonary dysfunction, initiated postoperative hyperglycemia [12,181]

The other interesting finding of the present study is the inspection of the increased myocardial outflow of different cytokines during CPB. No obvious differences could be observed in the OP group between cytokines of peripheral-venous and CS blood. To our knowledge, this is the first study comparing the myocardial production of cytokines during CPB and OP technique. Despite cardio protection with cardioplegia and cooling, the heart is exposed to relatively long-term and global ischemia as a result of cross clamping of the aorta. Before aorta cross clamping the myocardium is perfused with blood, whose leukocytes are primed by extracorporeal circulation. In course of off-pump operation however, myocardial and cardiac endothelial cells respond to brief and partial ischemic periods. It is well-known that myocardial cells can produce cytokines after ischemia-reperfusion because of the activation of nuclear factor- kappa B (NF-KB) [108]. Oxygen species, free radicals generate the signal leading to NF-KB activation [108]. Meldrum and colleagues initially described the NF-KB activation during CABG operation with CPB [109]. In the review article of Kukreja [108] it was established that only a large amount of free radicals and marked inflammation

can contribute to cytokine expression of myocardium. Brief periods of coronary ligatures during ischemic preconditioning may result in late protection via protein production with protective effects and without expression of cytokines. Further investigations are needed to evaluate the cellular mechanisms in the myocardium responding to operations with CPB or OP surgery.

Several papers recorded significantly elevated IL-6 and TNF concentrations of CS compared with arterial blood [94, 110, 111]. Karube and co-workers conversely documented no significant difference between sinus and arterial cytokine concentrations [110] just as Wan and colleagues did not find deviance in IL-8 and IL-10 [94]. Since cytokine levels are elevated in the CS, the local concentration of these cytokines may increase more noticeably during and after CPB causing augmented reactions and injurious effects in the reperfused heart.

One limitation of this study is the relatively small number of patients investigated. Although the two groups of patients were comparable regarding the preoperative data, this size is not sufficient to permit a general conclusion and assess clinical outcomes of the patients comparing their clinical parameters. A larger number of patients could enable the examination of cytokine balance and myocardial cytokine production of high risk patients and those patients having prolonged recovery, postoperative myocardial dysfunction, respiratory failure, etc. A further important limitation is that the anaesthetic technique was not completely the same in all the groups. Because of the different techniques used it was difficult to standardise the anaesthesia and intraoperative treatment. Studies investigating the effect of anaesthesia on cytokine production state early change of selected cytokines after anaesthesia with different agents. Most of these studies applied less then 24 hours observation periods and one of the examined cytokines changed just after wound closure as a direct effect of chosen anaesthetic management [112-114]. The effect of steroid treatment was not obtained. The anti-inflammatory batteries were followed incomprehensively. Besides the investigation of IL-10, IL-1 receptor antigen and soluble TNF receptor I and II provide a reliable method to ascertain the changes in anti-inflammatory forces during longer periods after off-pump or conventional CABG.

Present study tries to give a comprehensive view about alteration of cytokine network during and after CABG operation with and without CPB using a novel method, the CBA technique. Our investigations establish the efficacy of this technique also in clinical practice for obtaining pro and anti-inflammatory response because of its reliability and simplicity.

1) Hence in course of CPB the elevation of pro-inflammatory cytokine is highly counterbalanced by systematic release of IL-10 during and in the early period following the operation. Consequently, after the 1st postoperative day, significantly elevated pro/anti-inflammatory cytokine ratios were measured. In contrast, off-pump surgery is associated with a rather balanced relation of pro and anti-inflammatory response.

2) The concentration of IL-12 was also higher following CPB.

3) Our study revealed the myocardial outflow of certain pro-inflammatory cytokines during CPB, as manifested by higher sinus level of IL-1, IL-6, IL-8 and TNF, while it was undetected in course of off-pump surgery. These results may reflect the different cellular effects of the two procedures and try to improve our understanding about the impact of CPB on patients

5. EXPRESSION OF CD97 AND ADHESION MOLECULES ON CIRCULATING LEUKOCYTES IN PATIENTS UNDERGOING CORONARY ARTERY BYPASS SURGERY WITH OR WITHOUT CARDIOPULMONARY BYPASS

(Several part of this section has been published in paper number 3 as mentioned in section 11, List of publications, Manuscript related to thesis)

5.1 Introduction

Evidence suggests that the activation of WBC may be the major and central contributor of the above process [91]. Responding to CPB PMN release oxidants and proteases result in tissue damage [19]. MC also play a pivotal role in the regulation of inflammatory processes, however, the exact change in the phenotype of monocytes during CPB is not well determined [20, 21]. The crucial role of lymphocytes (peripheral blood lymphocytes, PBL) in the pathophisiology of reperfusion is now well established, even if the modification of lymphocytes' phenotype under CPB appears to be ambiguous [22].

A molecule that may be involved in inflammatory processes of CPB is CD97, which can help to assess the response of different populations of WBC to CPB, in particular, the activation of PBL. CD97 is a member of epidermal growth factor seven-span transmembrane protein (EGF-TM7) family [116, 117]. Expression of CD97 is restricted to myeloid cells and it is presented only at a low level by resting lymphocytes [117]. Expression of CD97 is induced distinctly and rapidly by various stimuli on activated lymphocytes followed by both redistribution of preformed protein and increased mRNA synthesis. CD97 is suggested to play a very important role within immune system, inflammation and WBC sequestration. The change in CD97 expression of different leukocyte populations in course of acute inflammation such as surgery has not been investigated, nevertheless, we believe this marker is going to be highly relevant following the activation of leukocytes. Moreover, this molecule is a sensitive marker of lymphocyte activation which process is indefinite under CPB.

On the other hand, PMNs extend tissue damage via expression of adhesion molecules (CD11, CD18) [93].

The purpose of this study is to determine the involvement of CD97 in the inflammatory processes caused by CPB and to assess the expression of adhesion molecules

during and after cardiovascular surgery. Our results can be compared with those observed in patients operated without CPB (OP).

5.2 Patients and methods

5.2.1 Patients

Thirty patients undergoing first time, elective CABG were enrolled in the study. Subjects were randomly selected into two groups. CPB group consisted of patients who received conventional CABG using CPB (20 subjects) and OP group underwent OP surgery (10 patients). There were no significant differences in the preoperative data of patients as demonstrated in section 4 (page 35). Patients with immunological disease, recent myocardial infarction (<3 months), previous stroke, or those receiving acute operation or reoperation, those who have developed infection, coagulopathy, tumour, acute or chronic renal failure and respiratory impairment were excluded from the study.

The protocol of the study was approved by the Ethics Committee of the University of Pecs and the Zala County Hospital. All patients were provided with oral and written informed consent and were informed clearly about the details of study and blood sampling. Investigations were performed in accordance with the Declaration of Helsinki.

5.2.2 Anesthesia, operative technique

Each group of patients received the same protocol as described in the section 3 (page 26).

5.2.3 Blood sampling

In CPB group, blood samples from the peripheral vein were taken just after the induction of anesthesia, during ischemia and 5, 30 minutes after the cessation of aorta cross clamping, and on the 1st, 2nd, 3rd, and 7th postoperative days. In OP group, blood samples were collected after the induction of anesthesia, during ischemia and 5, 30 minutes after completion of the last graft, and on the 1st, 2nd, 3rd, and 7th postoperative days.
All the samples were collected in sterile vacuum tubes containing sodium citrate.

5.2.4 White blood cell count measurement

The measurement of WBC count was part of the clinical investigation routine. The WBC count is determined in 10^9 cells per litre. Number of different WBC subsets were presented preoperatively, during the surgery (30 minutes after reperfusion) and on the 1st, 2nd, 3rd and 7th postoperative days.

5.2.5 Assessment of CD 97 expression

Coagulation of the blood was attenuated by sodium citrate. The samples were incubated with monoclonal antibodies against human CD 97 molecules (BD Biosciences, Pharmingen, USA) for 15 minutes. The antibodies were stained with fluorescein-isotiocianate (FITC). The red blood cells were lysed by hypoosmotic ammonium chloride solution (BD Biosciences, Pharmingen, USA) for 12 minutes. For the more regular distinguishing between the leukocyte subpopulations monocyte-specific staining of CD14-phycoerithrin (PE) was applied. Each sample was centrifuged at 300g for 7 minutes. Thereafter the pellet was diluted by 2 ml of phosphate buffer solution (PBS) and centrifuged once more (at 300g for 7 minutes). The pellet was fixed in 0,5% formaldehyde solution, and stored in dark, on 4 ° C. The samples were measured by BD FacsCalibur (BD Biosciences, USA) flowcytometer within 5 days. To determine the aspecific marking mouse anti IgG_1 κ staining was used according to the above protocol. The results were analyzed by Cellquest software (BD Biosciences, USA), and are expressed in arbitrary unit (AU). The percentage of CD97 positive lymphocytes was also estimated in all samples counting lymphocytes on which the expression of CD97 exceeded a certain level.

5.2.6 Measurement of adhesion molecules

Anticoagulated whole blood samples were stained with FITC- labeled monoclonal antibodies against CD11a and CD18 (BD Biosciences, Pharmingen, USA) according to the above protocol.

5.2.7 Statistical analysis

The data is presented in the tables and figures as mean ± standard error of mean (SEM).

The data between the two groups were compared with unpaired Student's t test. In a given group comparisons between control data were made using paired Student's t test. Differences were considered significant at p values less than 0,05.

5.3 **Results**

5.3.1 Alteration in leukocyte subsets

There were no hospital mortalities pulmonary insufficiencies or neurological complications in either CPB group or in OP group.

The total WBC count as well as PMN count increased rapidly after reperfusion in both groups and the highest value could be registered in CPB group on the 2nd postoperative day. Later the WBC and PMN count decreased gradually. The PMN counts in CPB group were significantly higher in all time points compared to the preoperative control, sample, meanwhile in OP group it exceeded the control value on the 1st, 2nd and 3rd postoperative days (Table 4).

Mean value of the MC count (Table 4) rose markedly on the 1st postoperative day concerning both groups. It reached the maximum in both groups on the 3rd postoperative day with significant difference in all samples in CPB group and on the 2nd, 3rd and 7th days in OP group (Table 4).

The number of PBL in CPB group decreased immediately during surgery and remained remarkably low while reaching its nadir on the 1st postoperative day when it was accounted as 58% of the control value. Thereafter, it tended to normalize progressively reaching the control value at the end of the first postoperative week (Table 4). Comparing the control samples statistically significant decreases were found during surgery and on the 1st, 2nd and 3rd postoperative days. Similarly, an intensive drop in PBL count was documented in OP group, however, the decrease was less marked than in CPB group. The lowest level was observed on the 1st postoperative day, which was proven to be significant, compared to preoperative value. After this period the number of PBL gradually returned to the normal value.

Table 4.: Changes in leukocytes count

	CPB group (CPB)	OP group (OP)	P value
PMN count after anesthesia	4,81±0,26	4,79±0,23	0,484
PMN count 30 minutes after reperfusion	7,38±0,86	6,65±0,63	0,33
PMN count on the POD1	10,63±0,32	10,30±0,44	0,31
PMN count on the POD2	9,79±0,54	10,847±0,41	0,274
PMN count on the POD3	8,69±0,58	7,82±0,42	0,134
PMN count on the POD7	6,06±0,5	5,27±0,34	0,142
MC count after anesthesia	0,53±0,02	0,55±0,04	0,386
MC count 30 minutes after reperfusion	0,34±0,04	0,47±0,04	0,127
MC 1 count on the POD1	1±0,09	0,7±0,1	0,192
MC count on the POD2	0,97±0,05	1,02±0,07	0,432
MC count on the POD3	1,05±0,05	1,04±0,09	0,458
MC count on the POD7	0,93±0,06	0,8±0,06	0,104
PBL count after anesthesia	2,23±0,13	2,15±0,12	0,329
PBL count 30 minutes after reperfusion	1,4±0,15	1,87±0,14	0,082
PBL count on the POD1	1,37±0,04	1,54±0,08	0,227
PBL count on the POD2	1,41±0,11	1,86±0,12	0,147
PBL count on the POD3	1,98±0,16	1,99±0,16	0,490
PBL count on the POD7	2,07±0,31	1,97±0,19	0,41

Data are presented as mean ± SEM. CPB group: operation with cardiopulmonary bypass. OP group: Off- pump operation. P value vas calculated CPB group versus OP group. PMN: polymorphonuclear leukocytes; MC: monocytes; PBL: lymphocytes; POD: postoperative day

5.3.2 Appearance of CD97 on granulocytes and monocytes

Biphasic modification in surface expression of CD97 was noted on myeloid cells (PMN and MC) in CPB group (Figure 10).

First, an intensive drop was present in CD97 activity of PMN in course of CPB (Figure 10). The values of the two intra-operative samples during reperfusion were significantly lower compared to the control sample. On the first day, the CD97 level of PMN was close to the control value and subsequently it started to rise considerably, reaching its

maximum 3 days after operation with significant difference on the 2nd and 3rd days in relation to control. In OP group the PMN CD97 consistency showed marked decrease in the ischaemic period differing significantly from control. It finally remained around the control value. Statistically, the values of CPB group were considerably lower during the early reperfusion period than the values of OP group. In addition it exceeded the values of OP group significantly on the 2nd and 3rd postoperative days.

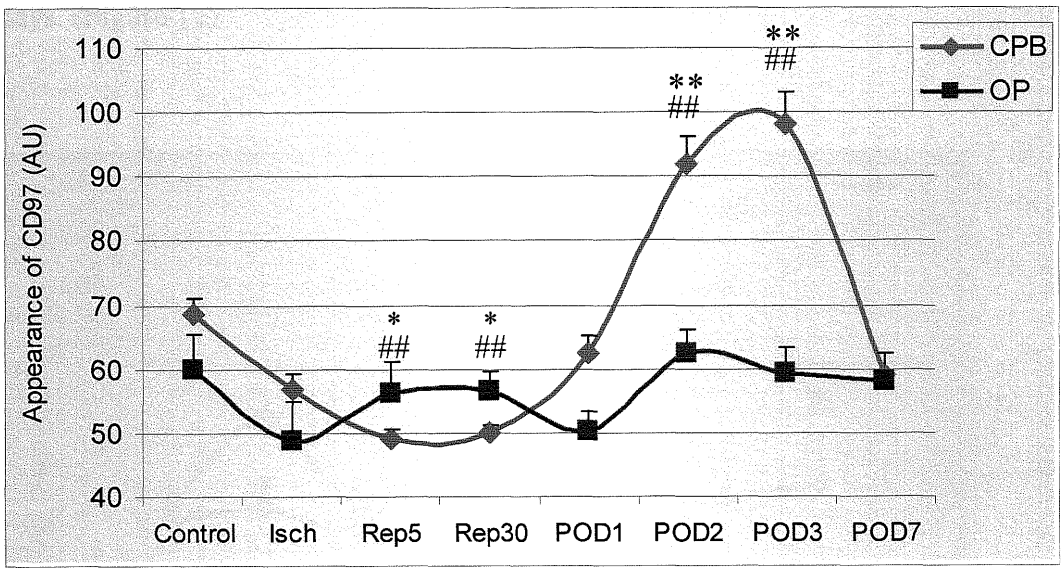


*Figure 10.: Expression of CD97 on granulocytes (mean ± SEM). CPB: operation with cardiopulmonary bypass. OP : Off- pump operation. * p<0,05 between the two groups, ** p<0,01 between the two groups, # p<0,05 compared to the control value, ## p<0,01 compared to the control value. Cont: preoperative; Isch: ischaemic; Rep5, Rep30: 5 and 30 minutes after reperfusion; POD: postoperative days.*

The acute, intraoperative decrease in CD97 expression was less expressed on MC than observed on PMN in CPB group. Exactly as it appeared in PMN, in the case of MC there was a significant difference during the reperfusion and on the 2nd and 3rd days compared to the control value. Differences between the two groups were proved significant on the 2nd and 3rd postoperative days.

5.3.3 Percentage of CD97 positive lymphocytes

In CPB group, the rate of active CD97 positive PBL (Figure 11) tended to rise gradually and markedly from the beginning of reperfusion to the 3rd postoperative days when 30,12±5,86% was determined to CD97 positive (preoperative value: 8,3±1.56%).

In OP group, the percentage of active CD97 positive PBL showed a peak during operation at 30 minutes of reperfusion and it was also slightly elevated on the 1st postoperative day (10,44±1,97% versus control value of 7.9±0,65%) (Figure 11). The rate of CD97 positive PBL in CPB group exceeded significantly those of OP group on the 2nd and 3rd days. (Figure 12)

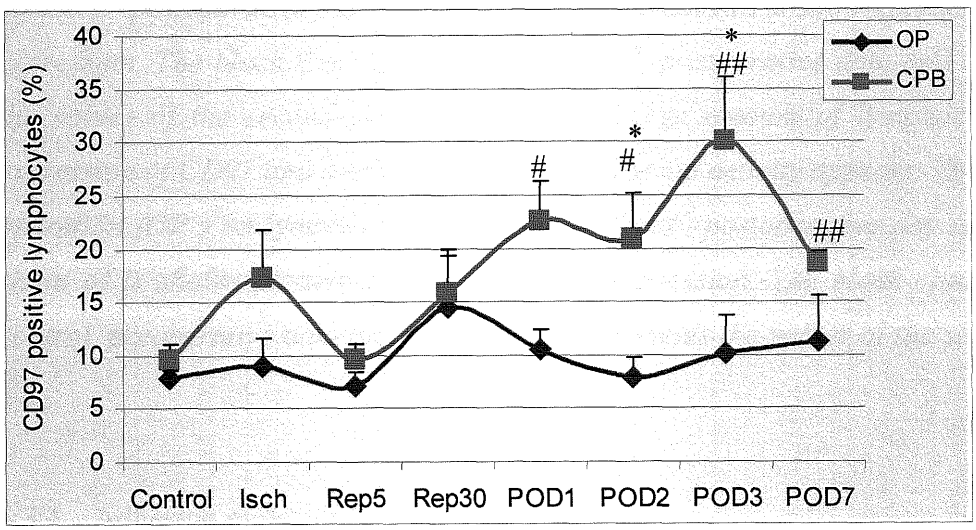


*Figure 11.: Change in CD97 expression of lymphocytes. CPB: operation with cardiopulmonary bypass. OP : Off-pump operation. (A) Percentage of CD97 – positive lymphocytes. * p<0,05 between the two groups, # p<0,05 compared to the control value, ## p<0,01 compared to the control value. Cont: preoperative; Isch: ischaemia; Rep5, Rep30: 5 and 30 minutes after reperfusion; PD: postoperative day.*

A. **B.**

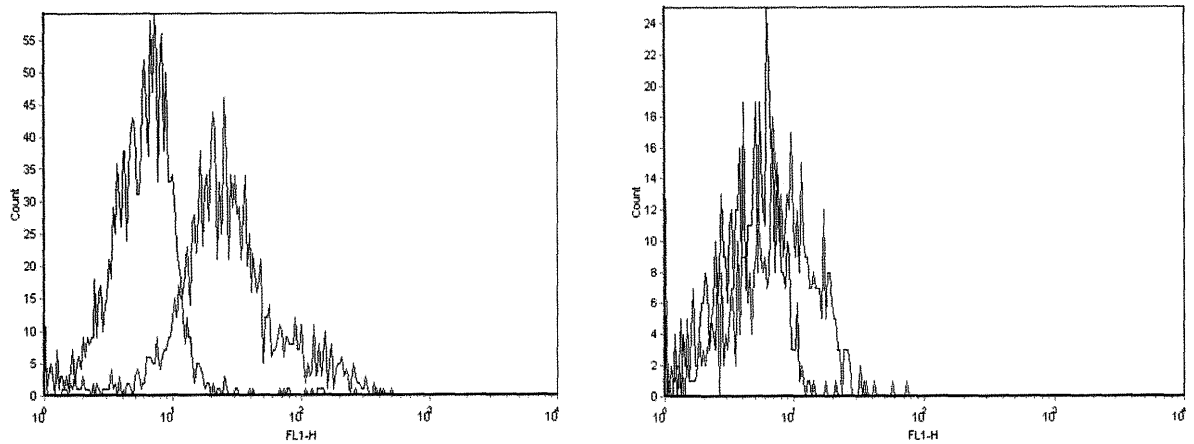


Figure 12.: The effect of conventional operation using CPB (CPB) on lymphocyte activation on the 3rd postoperative day (A). The count of lymphocytes is shown plotted against the CD97 expression. The black line represents the control, preoperative data and the blue line shows the values of the second postoperative day. Large amount of lymphocytes increase their expression of CD97 and a new population of immense activity appears. The effect of off-pump operation (OP) on lymphocyte activation on the 3rd postoperative day (B). The lymphocyte count of is shown plotted against the CD97 expression. The black line demonstrates the control, preoperative data and the blue line represents the values of the second postoperative day.

5.3.4 Adhesion molecules

The surface appearance of CD11a and CD18 adhesion molecules expressed by granulocytes was characteristic in both groups. The expression of CD11a and CD18 changed almost the same way in each population of leukocytes. The integrin levels on the surface of PMN (Figure 13a, b) tended to decrease in the early phase of reperfusion and afterwards they increased. The expression of both integrins was markedly higher on granulocytes of CPB group than of OP group, especially on the 2nd postoperative day. Expression of CD11a and CD18 on monocytes were similar (expression of CD18 is shown in Figure 13c). Lymphocytes increased their expression of integrins gradually with maximum on the 2nd postoperative day in CPB group and on the 3rd day in OP group (Figure 13d). On the 2nd postoperative day, the data of CPB group were statistically and significantly higher than the values of OP group.

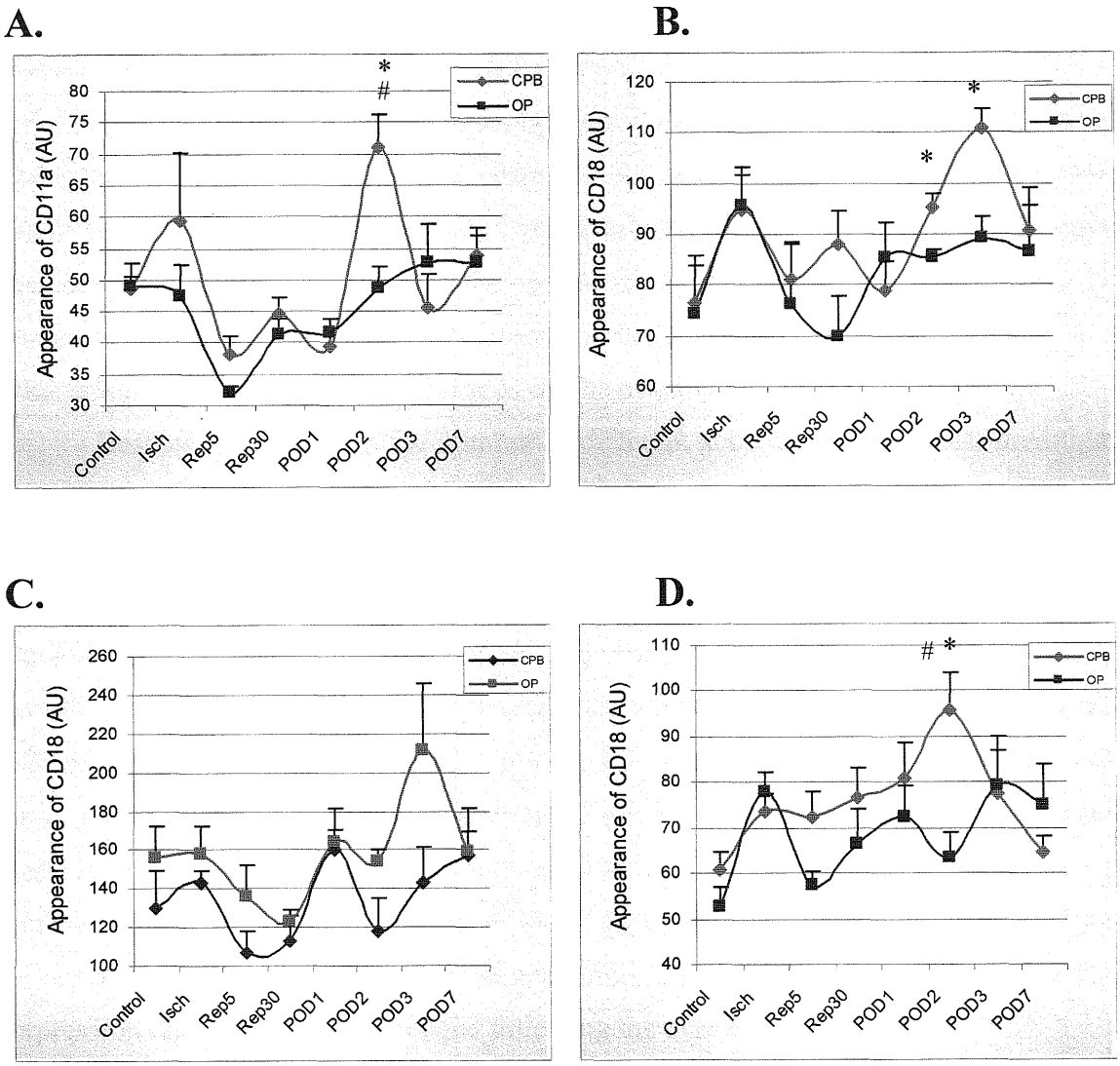


*Figure 13.: Changes in expression of adhesion molecules (mean ± SEM). CPB: operation with cardiopulmonary bypass. OP : Off- pump operation. * $p<0,05$ between the two groups, ** $p<0,01$ between the two groups, # $p<0,05$ compared to the control value, ## $p<0,01$ compared to the control value. (A) CD11a expression on granulocytes. (B) CD18 expression on granulocytes (C) CD18 expression on monocytes. (D) CD18 expression on lymphocytes. Cont: preoperative; Isch: ischaemia; Rep5, Rep30: 5 and 30 minutes after reperfusion; POD: postoperative day.*

5.4 Discussion

This study shows a considerable increase in the total WBC count principally on the 2nd and 3rd postoperative days of CPB. It occurs mainly as a result of elevation of PMN cells. MC count increased noticeably after the 1st postoperative day. A considerable lymphocytopenia was observed on the first days after the operation. Operation of similar surgical invasivity but without CPB (OP) causes moderate elevation in PMN and MC count and a reasonable drop in PBL count. These findings correspond to results of other papers [12, 20, 93, 118].

Besides the change in the number of different subsets of leukocytes, investigation of the modification in activation of leukocyte population is essential to assess the characteristic of inflammatory response to CPB. Publications dealing with the potential activation or deactivation of lymphocytes during or after CPB seem contradictory.

Several authors determined the surface expression of interleukin-2 receptor (CD25) on lymphocytes and also interleukin-2 plasma level with the result that it decreased or remained unchanged during the operation and in quite early in the postoperative period [119, 120]. Chu et al. however, found significant elevation of soluble CD25 observing the first 2-week long periods [120]. Further marker of lymphocyte activation, the function of which is not fully understood, is the CD69, also known as the "very early activation antigen" designating that it is a sensitive indicator of early cellular activation [121]. Studies demonstrated that the CD69 expression raise or remain unchanged following the use of CPB [122].

Other mediators to assess the state of lymphocyte activity are the adhesion molecules. Therefore lymphocyte-derived expression of members of the integrin family (CD11, CD18) was determined [18]. Toft and associates described that the integrin expression rose noticeably during surgery. According to the results of this study, the surface appearance of CD11a and CD18 increased markedly after operation especially on the 2nd day, which considerably exceeded the level of off-pump operation [182].

A novel marker reliably indicating the early activation of lymphocytes may be the CD97. The aim of this study was to assess the lymphocyte activation during and after CPB using this novel surface molecule. The rate of CD97 positive lymphocytes increased after surgery with CPB until the 3rd postoperative day and remained elevated throughout the first postoperative week. The group that underwent OP surgery showed less pronounced change.

Sancho and associates studied the surface appearance of peripheral T cells because of the interaction with stimulated endothelial cells in their inventive in vitro study. In this work CD97 was routinely applied to assess the early stage of T cell activation [122].

According to the observations of the present study, the expression of CD97, PMN and MC show early decrease followed by an evident elevation. The change in the phenotype of monocytes as a result of CPB is debated in the literature. In most cases early decrease in the activity state of the MC population is observed by numerous publications using different markers (HLA-DR, CD11c, CD18, CD64) [23, 123]. Most of them conclude recovery of these markers until the end of first day after surgery. Hiersmayr and co-workers, however, found further decrease in HLA-DR and adhesion molecule expression after the first postoperative day. However, most studies observing longer postoperative period suggest monocyte activation after CPB and that the activated monocytes are present in circulation for 2-3 days correlates with our findings about the monocyte-derived expression of CD97[124].

The activation of PMN population obviously occurs in the course of CPB [91] and it can also be followed by CD97 expression of PMN. Interestingly our results demonstrated decreased CD97 expression in the course of CPB during surgery and within 24 hours after operation. Presumably, the depletion of these cells can be explained by haemodilution or selective sequestration of the still active cells or even by the adsorption of activated leukocytes to the extracorporeal circuit [125, 126]. During the first postoperative days circulating PMNs becomes extremely abundant in CD97 when the production and surface presentation of the molecule can take place.

The importance of binding of CD97 to CD55 (decay accelerating factor, DAF) has recently gained attention. Activated leukocytes richly presenting CD97 on cellular surface can attach to the activated endothelial cells, which presumably express increased amount of CD55 thus facilitating leukocytes transendothelial migration [12, 14, 93, 127]. Moreover, it has been identified that CD97 bind to chondroitin sulfate, which is abundantly situated on cellular surface and extracellular matrix, thereby augments extracellular anchorage of white blood cells.

In addition, CD97 plays an important role in intracellular signalling of WBC via the G protein dependent pathway [116, 117]. The CD97 receptor has a G-protein coupled receptor related moiety, which may be activated through binding ligands on N-terminal EGF-like domain of molecule. It is conceivable that this process can lead to further activation thus amplifying inflammatory response and to tissue damage during and after CPB [93].

Advantageous results of studies dealing with blocking of CD97 with antibody suggest the important role of the molecule within the immune system. The effect of treatment with monoclonal antibodies against CD97 was studied in inflammatory diseases [128, 129].

Neutrophil adhesion and function could be prevented by blocking of CD97 both in dextran sulphate sodium induced colitis and in pneumonia.

A second target of this study was the monitoring of the modification of adhesion molecules responding to conventional or off-pump operation. The observed integrin molecules (CD11, CD18) form the LFA-1, which is known to contribute to the development of firm adhesion between endothelial cells and WBC by joining to intercellular adhesion molecule-1 (ICAM). Matata and associates established elevated concentration of ICAM-1 following CPB by obtaining its soluble level in plasma [18]. According to the findings of this study, supposedly the increased LFA-1 expression is a causative factor that PMN can migrate through vascular wall at a greater degree. Moreover, the investigations of the present study showed that monocytes and lymphocytes take place in the process of sequestration after CPB. It is recognized that extreme adhesion of WBC can lead to leukocyte aggregation, leukostasis producing disturbance in capillary flow.

Several authors described significant decrease in LFA-1 expression taking blood samples on the early postoperative period, within the first day [124, 125]. Results of Tarnok and associates showed decreased expression of LFA-1 during operation, furthermore no increase was observed on the 7^{th} day coinciding this study on these time points. The most attractive findings of our present report is that the CD11a and CD18 expression showed significant increase in all subsets of leukocytes on the 2^{nd} or 3^{rd} postoperative days in the course of CPB.

Off-pump surgery produces more moderate alteration in CD97 and also LFA expression. Al-Ruzzeh and his co-worker stated that circulating neutrophil-derived CD11b expression is lower in course of off-pump surgery until the 1^{st} day [132]. It can be assumed that off-pump surgery is associated with more intensive sequestration of leukocytes during surgery and at an early stage of reperfusion because cardioprotection is not present during ischaemia as during conventional operation. All the same this technique does not inflict leukocyte activation and late expression of adhesion molecules within the later period of reperfusion.

This study introduced the modification in CD97 expression of different leukocyte subsets during cardiovascular surgery. CD97 may be an important contributor of the regulation of inflammatory events during and after cardiac surgery. Circulating myeloid cells expressed decreased level of CD97 until the 1^{st} day after surgery, thereafter these proved to be in higher activity state. Although CABG operation performed with CPB is associated with significantly lower lymphocyte counts, these lymphocytes exhibit more activation markers.

Surface presentation of adhesion molecules showed analogous change in patients operated with CPB. Off-pump operation provokes more moderate activation both in myeloid cells and in lymphocytes. In one respect, our results show the beneficial effect of off-pump technique, which can correlate with the rare complications observed in patient compared to operation with CPB. The results about CD97 expression in course of CPB contribute to a more detailed understanding of the complex inflammatory response, blunted immunological functions during and after CPB. The functional role of CD97 during CBP can be elucidated in experimental model by treatment with monoclonal antibody against CD97, thus investigating the potential therapeutic effect of CD97 blocking.

6. OXIDATIVE STRESS DURING CORONARY ARTERY BYPASS GRAFTING WITH OR WITHOUT CARDIOPULMONARY BYPASS

6.1 Introduction

Previously, it was clarified that CPB induces profound and long-term activation of each subsets of leukocytes. Besides activated leukocytes plug on the microvasculature causing hypoperfusion, most important mechanism of WBC-mediated injury is the production of free radicals, especially by PMN. Evidences suggest that oxidative stress play a significant role in pathogenesis of CPB [39, 133]. Systemic occurrence of oxidative stress was demonstrated in course of CPB [39]. Ones, generation of ROS source from activated PMN during CPB. On the other hand increased release of great amount of ROS occurs at organ, cellular level as a result of myocardial IR, suspension of the circulation of lungs and hypoperfusion of organs [39]. The nature of these oxidative events leads to depletion of plasma antioxidants thus leading to development of systematic oxidative stress with cellular damage and appearance of metabolites like MDA [133]. There are only a few papers dealing with endogen antioxidants. These reported that antioxidant response is not sufficient to counteract the heavy oxidant attack during application of CPB [134, 135].

It has been suggested that the systemic oxidative stress caused by CPB result in a significant proportion of the adverse outcomes [39, 136]. It has moreover been demonstrated that on-pump procedure gives rise to a more pronounced systemic inflammation and oxidative stress than the off-pump procedure in the early postoperative period [11, 137]. The changes in activity of endogen and plasma antioxidants have also been observed in the acute phase of reperfusion [134, 138].

Less is known, however, about the time-course, duration of oxidative stress following CPB. Since we have observed prolonged inflammatory response and WBC activation after CPB, we aimed to investigate the effect of CPB on ROS generation and systemic antioxidant capacity. We therefore conducted a comprehensive analysis of the temporal profile and magnitude of oxidative stress during and after CPB comparing the results to OP technique.

6.2 Patients and methods

6.2.1 Patients

Thirty patients (63, 2±2,5 years, 7 females, 23 males) undergoing first time, elective CABG were enrolled in the study. Subjects were randomly selected into two groups. CPB group consisted of patients received conventional CABG using CPB (CPB group, 20 subjects) and OP group underwent OP surgery (OP group, 10 patients). There was no significant difference in the preoperative data of patients concerning the age, gender the number of performed grafts (3,58±0,18 in CPB group, 3,375±0,31 in OP group) and number of arterial graft (LIMA) (0,88±0,09 in CPB group, 0,92±0,0,08 in OP group) and preoperative risk score (EuroSCORE) score: 2,53±0,54 in CPB group, 2,59±0,31 in OP group). Patients with immunological disease, recent myocardial infarction (<3 mounts), previous stroke, receiving acute operation or reoperation, developing infection, coagulopathy, tumor, acute or chronic renal failure, respiratory impairment were excluded from study. Anesthesia and operative technique in both groups was performed as described in the section 3.

The protocol of study was approved by the Ethics Committee of the University of Pecs as well as the Zala County Hospital. All patients were provided with oral and written informed concern and were profusely inform about the details of study and blood sampling.

6.2.2 Anesthesia, operative technique

Each group of patients received the same protocol as described in the section 3 (page 26).

6.2.3 Blood sampling

Blood samples were collected in both groups at different time points during the surgery and first postoperative week. The protocol for blood sampling was designed that the time points of samples were equivalent in time according to the two groups of different techniques. In CPB group, samples from peripheral vein were taken just after the induction of anaesthesia furthermore 5 and 30 minutes after the cessation of aorta cross clamping, moreover on the morning of 1^{st}, 2^{nd}, 3^{rd}, and 7^{th} postoperative days. In OP group blood from peripheral vein were collected just after the induction of anaesthesia, 5 and 30 minutes after completion of the last graft, furthermore on the morning of 1^{st}, 2^{nd}, 3^{rd}, and 7^{th} postoperative days. All of the samples were collected in sterile vacuum tubes containing sodium citrate.

6.2.4 Measurement of reactive oxygen species producing capacity

Respiratory burst of leukocytes was assessed by measuring the amount of reactive oxygen species in whole blood via modified chemiluminescense (CL) method based upon the reaction of luminol with free radicals, as described by Dandona an associates [139]. Firstly, 20 µl of blood was diluted in 1400 ml Medium 199 (Sigma, USA) nutrient mixture at 37 °C. Thereafter 30 µl of 0.56mM 3-aminophtalhidrazide (Loba Feinchemie, Austria) was added to the cuvette and placed immediately to Chrono-Log Whole Blood Lumino-aggregometer (Model 560, Chrono-Log, USA) and was incubated at 37 °C with continuous mixing during measurement. The background chemiluminescence was recorded against time by the printer of luminometer. After a few minutes, 30 µl of 0.16 mM phorbol-12 myristate-13 acetate (PMA) (Sigma, USA) was injected promptly into the cuvette.

The intensity and rate of generation of free radicals were determined by peaked curve. Subsequently given papers with curve about free radical production were scanned into computer and the latency, rise of curve and the area under the curve were calculated applying Colim software (Fig. 14). The given values were divided by leukocyte count. The area under the curve represents the total free radical generating capacity, respiratory burst activity of standard number of leukocytes to standard stimulation of PMA. Thus this parameter was further compared between groups.

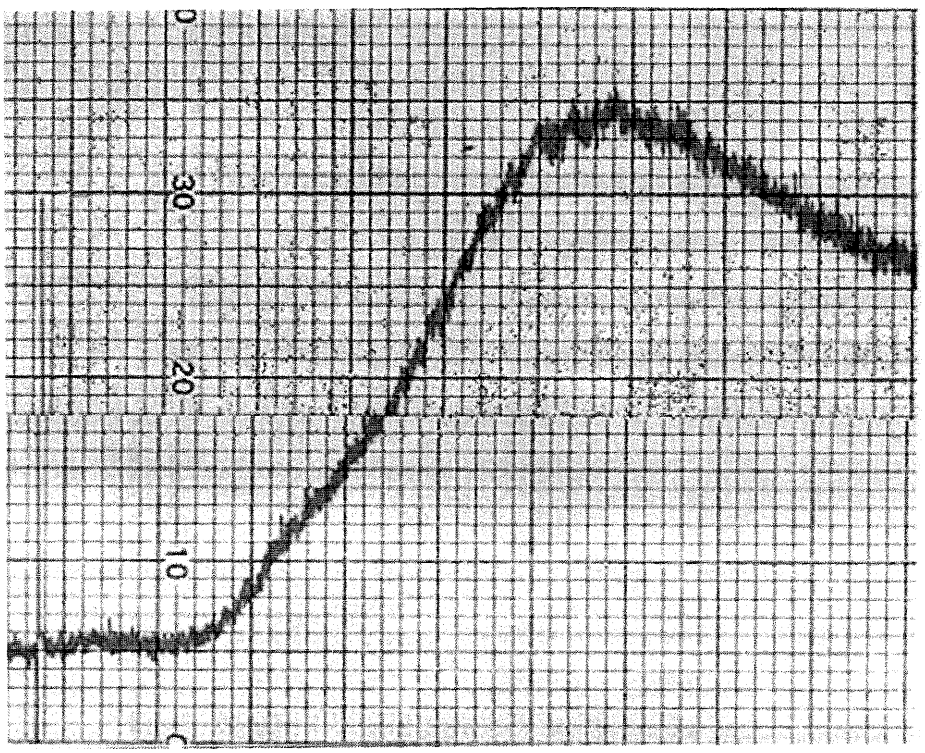


Figure 14.: Representative curve of oxidative burst.

6.2.5 Visualisation of ROS production in isolated PMN

Following blood sample collection, leukocytes were harvested from blood by placing large drops of blood on glass slides for 2 minutes at 37 °C. Preformed coagulum was removed from slides and slides were rinsed with saline to replace nonadherent cells. Cells were incubated in 20 mM cerium-chloride ($CeCl_3$) solution (Sigma, USA) and 50μM of propidium iodide (PI) (Sigma, USA) for nuclear staining. Preparations were then mounted in glycerol gelatin (Sigma, USA) supplemented with anti-fading agent of 1,4 diazabicyclo-octane (DABCO, USA). To control $CeCl_3$ staining negative control preparations, without Ce treatment and positive control preparations treated with 1μM PMA were carried out.

Validation of $CeCl_3$ staining was performed by confocal laser scanning microscopy as described by Telek et al [140]. The imaging and semiqantification of Ce-perhydroxide deposits was performed with Nicon Eclipse TE-300 inverted microscope attached to an MRC-1024ES confocal system (Bio-Rad, UK). Preparations were illuminated with at 488 and 457 nm with argon laser. Ce deposits were detected by reflectance. Detecting fluorescence above 550 nm, the appearance of PI stained nuclei could be detected. High-resolution images were

taken (2500xmagnification) and digital superposition to give a three-layer composite image using Confocal Assistant TM 4.0 software (Todd Clark Brelje, USA).

6.2.6 Assessment of lipid peroxidation

Plasma of blood samples were divided as described in section 4. Malondialdehyde (MDA) concentration was monitored from plasma-samples as indices of lipid peroxidation. MDA concentration was measured by spectrofotometry using original kit (CALBIOCHEM Lipid Peroxidation assay kit, EMD Biosciences, Inc. San Diego, USA) following the instructions of manufacturers. Standard curve was obtained to estimate the concentration of MDA in samples from adsorbance in μmol per liter

6.2.7 Determination of activation of antioxidant enzymes and reduced glutatione

Blood samples were centrifuged at 2225g for 10 minutes on 4 °C and the supernatant was aspirated and dropped. Thereafter the pellet was diluted with ice-cold saline and centrifuged once more (at 3000g for 7 minutes) followed by repeatedly aspiration of supernatant. The pellet was treated with hypo-osmotic solution of ammonium- chloride thus lysing red blood cells (RBC). The activity of superoxide dismutase (SOD) and catalase (CAT) were analyzed from haemolysates by colorimetric, original assays (CALBIOCHEM Catalase assay kit, CALBIOCHEM Superoxide dismutase assay kit, EMD Biosciences, Inc., USA). Moreover the level of reduced glutatione (GSH) level was quantified from haemolyzates by commercially available assay kit (CALBIOCHEM Glutatione assay kit, EMD Biosciences, Inc., USA). The final activities and concentrations were calculated after standardization. The activity of CAT and SOD were presented in unit per liter. GSH level was shown in nM per liter.

6.2.8 Statistical analysis

The data is presented in the tables and figures as mean ± standard error of mean (SEM).

The data between the two groups were compared with unpaired Student's t test. In a given group comparisons between control data were made using paired Student's t test. Differences were considered significant at p values less than 0,05.

6.3 Results

6.3.1 Production of reactive oxygen species

CPB induced extreme increase in ROS generating capacity of leukocytes. It elevated gradually during the intervention, 30 minutes after cessation of aorta cross-clamping. After the operation it rose further peaking on the 2nd POD (almost 20 fold increase related to control value). The values of all time points exceeded significantly the control level. OP operation was also associated with considerable elevation in ROS generation but it was definitely lower than in CPB group. It increased till the 3rd POD reaching its maximum, which was 9 fold higher than the preoperative value. The amounts of ROS were significantly higher on the 1st, 2nd, 3rd, 7th POD compared to the control. Comparisons between groups showed significantly higher levels in CPB group over the postoperative days (Figure 15).

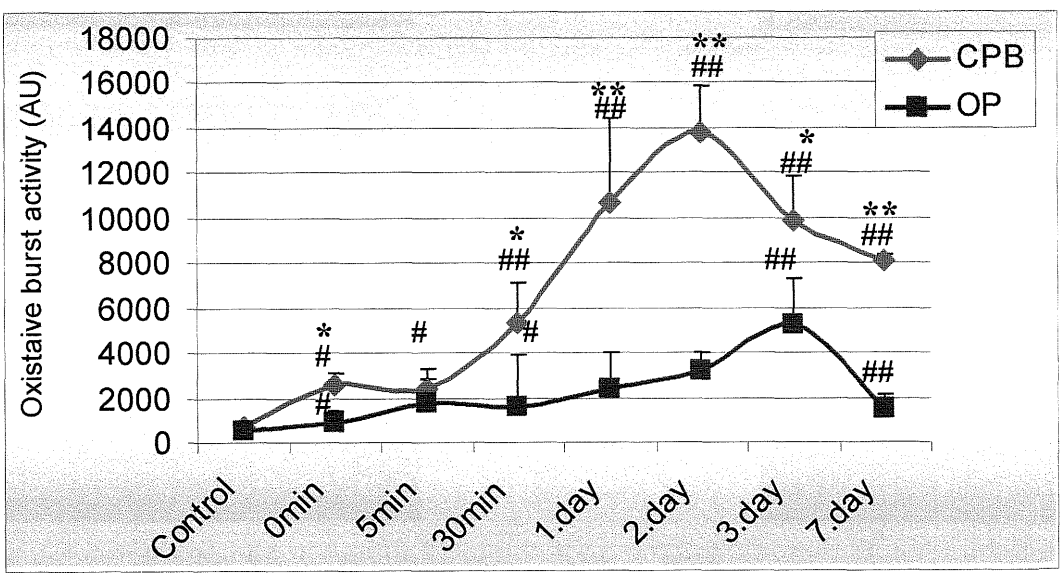


Figure 15.: Oxidative burst activity of PMN following CABG with or without CPB. Data are presented as mean ± SEM in arbitray unit (AU). CPB: operation with cardiopulmonary bypass; OP: Off- pump operation; $p<0,05$ between the two groups, .** $p<0,01$ between the two groups, # $p<0,05$ compared to the control value, ## $p<0,01$ compared to the control value.*

6.3.2 Visualisation of ROS production in isolated PMN

Small amount of Ce-deposits could be detected in control samples both of OP and CPB group (Fig. 16). Qualitative imaging, however showed dramatically increase in CPB groups during early reperfusion till the end of postoperative week. Semiqualitative analysis demonstrated peak values of reflectance signals of Ce-perhydroxide deposits on the first POD. Staining of Ce was markedly abrogated in OP group (Fig. 16).

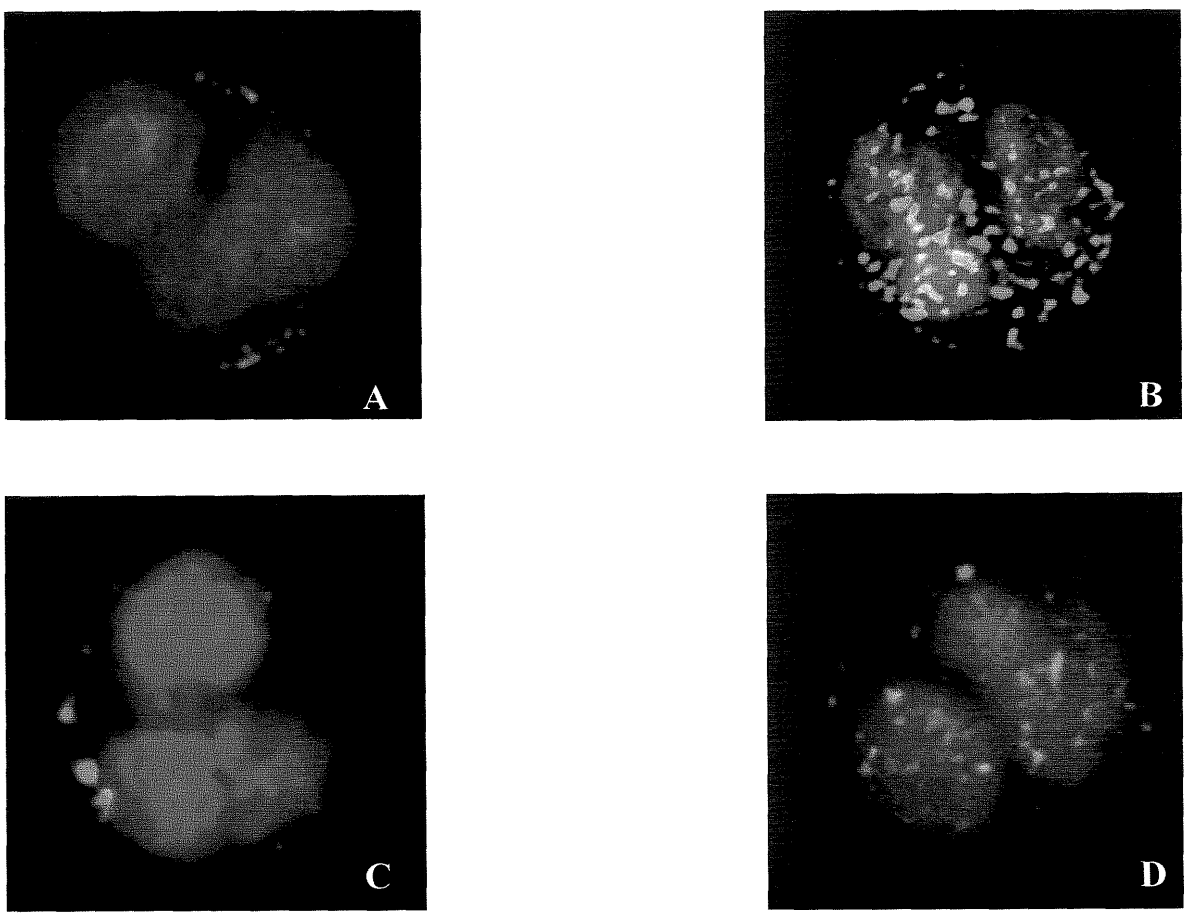


Figure 16.: Cerium histochemistry of PMN from blood of patients operated with conventional or off-pump surgery. Pictures show one PMN representative of patients before operation with CPB (A), on the 2nd postoperative day following conventional CABG (B) , before off-pump operation (C) and 2 days thereafter (D). Green pixels represent the reflectance of cerium-perhydroxide deposists. Red marking demonstrate the nuclear staining with propidium iodide.

6.3.3 Level of malondialdehyde

The changes in MDA plasma levels as marker of lipidperoxidation in both groups over the time are demonstrated on figure 17. MDA level of CPB group peaked 30 minutes after beginning of reperfusion and it remained highly elevated on the 1st, 2nd and 3rd POD with significant difference from the early reperfusion (5 and 30 minutes of reperfusion) to the 3rd POD. However, only a slight elevation can be documented in OP group peaking on the 2nd POD. Statistically significant deviation between groups can be reported on the 5th and 30th minutes of reperfusion furthermore on the 2nd POD.

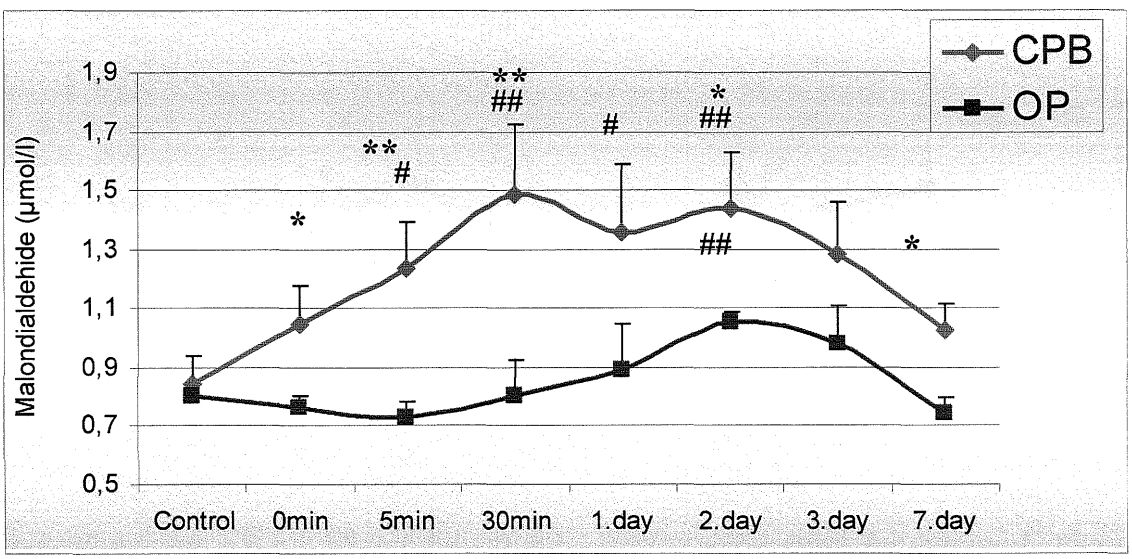


Figure 17. Level of malondialdehide in plasma (mean ± SEM). CPB: operation with cardiopulmonary bypass; OP: Off- pump operation; $p<0,05$ between the two groups, .** $p<0,01$ between the two groups, # $p<0,05$ compared to the control value, ## $p<0,01$ compared to the control value.*

6.3.4 Change in activity of antioxidant enzymes and level of reduced glutation

Contrasting alteration in SOD activity can be noted in two groups (Fig 18). In CPB group it decreased continuously reaching its nadir on the 2nd POD and it normalized to the 7th POD. Comparisons of data over the time with control (preoperative) sample showed significantly lower values 30 minutes and 2 days after reperfusion. With regard the OP group SOD activity rose gradually during the operation while on the 1st POD it was closed to control thereafter it increased again without any considerable difference to preoperative sample. The deviance between groups was proved significant on the 2nd and 3rd POD.

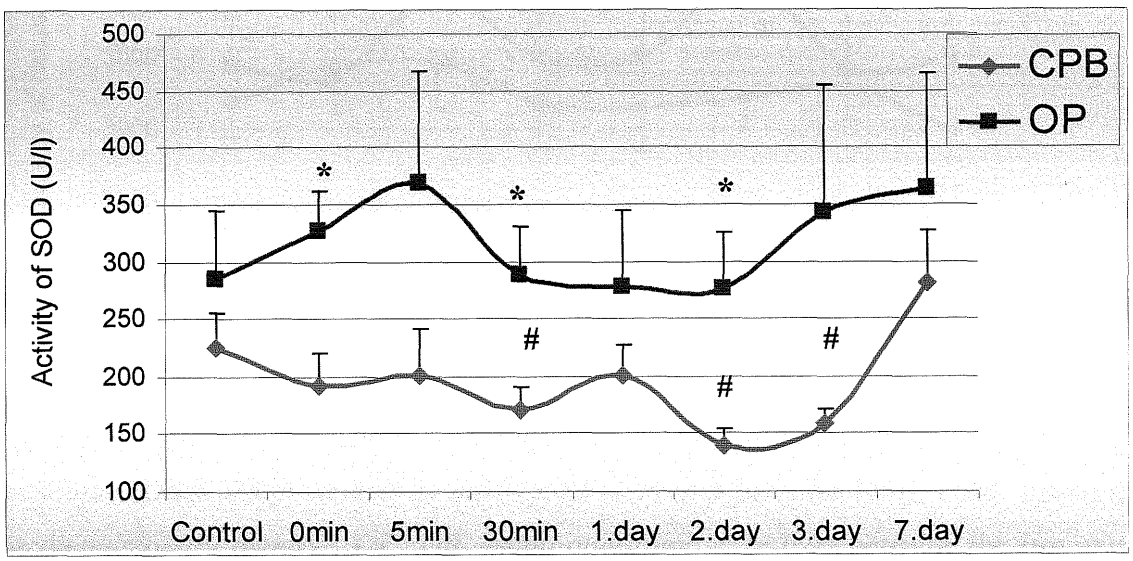


*Figure 18.: Determination of superoxide dismutase activity from haemolysates of patients receiving CABG carried on with or without CPB. Data are presented as mean ± SEM in unit per liter (U/l). CPB: operation with cardiopulmonary bypass; OP: Off- pump operation; * $p<0,05$ between the two groups, # $p<0,05$ compared to the control value.*

Less expressed change can be observed in CAT activity (Fig. 19.). Following an early insignificant elevation a moderate drop was documented in CPB group. CAT activity of off-pump patients peaked on the 2nd POD. Neither analysis of time points in a given group nor statistical calculations of differences between group revealed no trivial deviance

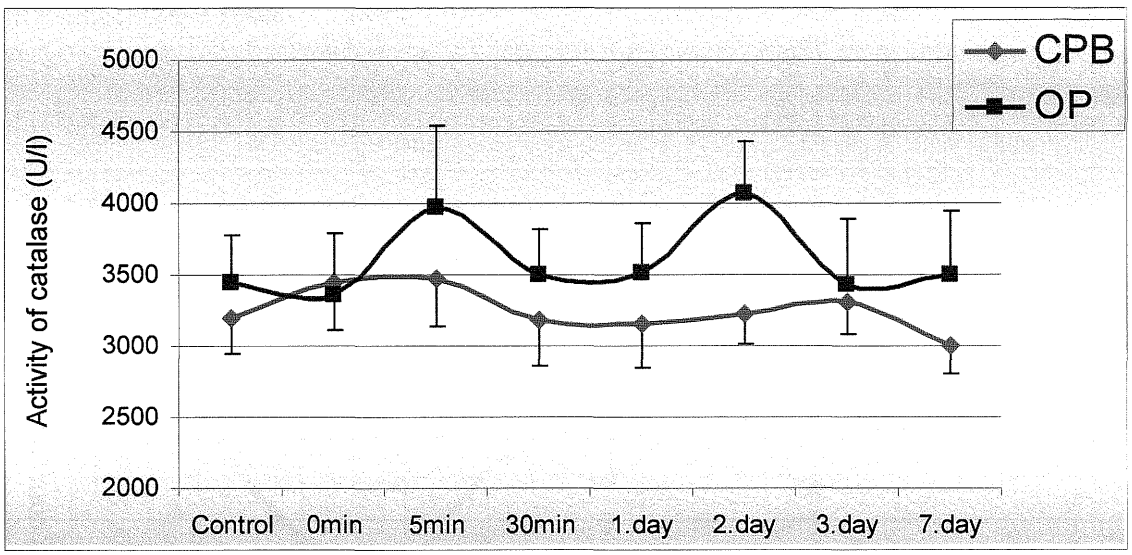


*Figure 19.: Activity of catalase folloving coronary revascularisation with CPB or off-pump technique. Data are presented as mean ± SEM in unit per liter (U/l). CPB: operation with cardiopulmonary bypass; OP: Off- pump operation; * $p<0,05$ between the two groups, # $p<0,05$ compared to the control value.*

GSH level decreased markedly soon after beginning of ischaemia in both groups (Fig. 20). Although in CPB group GSH levels remained decreased until the end of the first postoperative week the values of OP group tend to recover to the 1st POD. All of samples from beginning of reperfusion to the 2nd POD had significantly lower GSH levels related to preoperative values, considering CPB group. In OP group no statistically significant difference could be calculated related to control. On the 2nd, 3rd POD significant intergroup differences were found

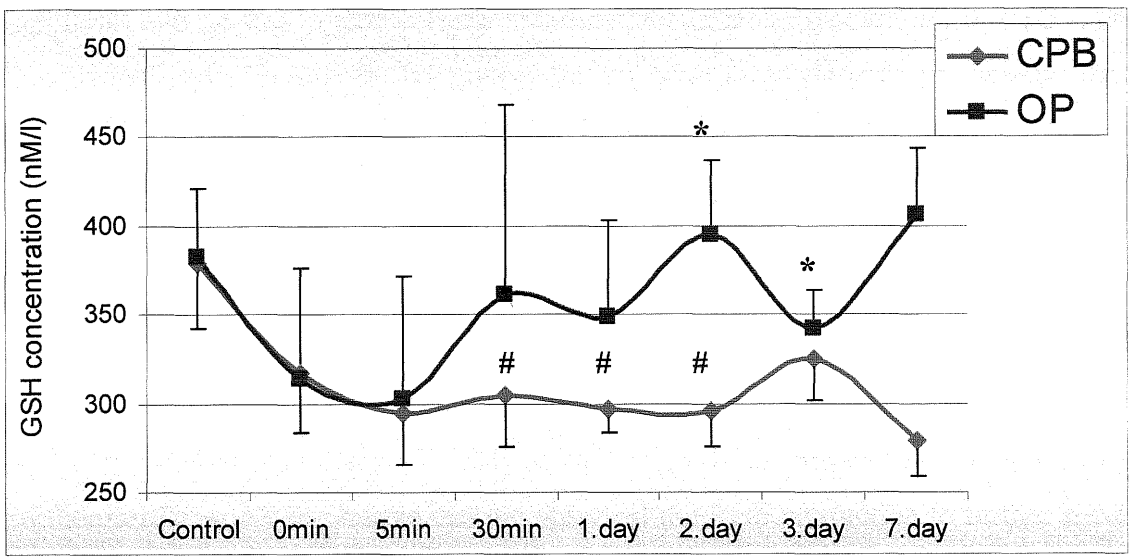


*Figure 20.: Reduced glutation concentration of haemolysates during and after CABG with or without CPB. Data are presented as mean ± SEM in nmol per liter (nM/l). CPB: operation with cardiopulmonary bypass; OP: Off- pump operation; * $p<0,05$ between the two groups, # $p<0,05$ compared to the control value.*

6.4 Discussion

Our results demonstrate that profound and long-lasting oxidative stress occurs in patients undergoing CABG with CPB. The antioxidant activity of these patients could not recover during the first days of postoperative period. OP surgery was able to reduce the increase of ROS release and to preserve antioxidant capacity.

Evidences are continuously growing to prove that free radicals are produced after operation with CPB [38]. Since ROS are extremely reactive and have very short life spans they can only be quantified difficultly. In tissues, ROS can only be measured by electron spin resonance (ESR) spectroscopy as performed in patients receiving CABG carried out on CPB by Garlick and colleagues [141]. Other effective and accepted approach to measure free radicals is the chemiluminesce method when ROS, maily superoxide anions are reacted with luminol provoking scintillation [142]. ROS generating capacity of PMN were ascertained after provoked activation with PMA. This activation of PMN reflects the degree of their activation and in compliance with it the action of leukocytes when they pass through capillaries and appear in extracellular space of jeopardized myocardium or lung. The ability of activated PMN to endothelial transmigration have been clarified previously (section 5). Kuzuya and associates described the extracellular activation of PMN thus leading to enlarged free radical production in extracellular matrix. Moreover they revealed direct correlation between the ROS production of PMN and myocardial injury during reperfusion [143]. According to our results, patients received operation with CPB are more susceptible to increased oxidative burst activity of PMN. These data agree with the results of other studies using other methods [144, 145]. Investigations measuring ROS with ESR spectroscopy postulated similar consequences early after cessation of aorta-cross clamping [39, 146]. Furthermore it worth emphasize that we observed the ROS production to further elevate after the early minutes of reperfusion, over the first postoperative week peaking on the 2nd POD.

MDA is a marker of lipidperoxidation, which shows the extent of developed oxidative damage [147]. Our findings suggest that increased level of lipidperoxidation occur during the operation and first postoperative days in patients undergoing conventional CABG. Early increase of MDA was reported in other studies [148, 149]. By contrary, our findings address a prolonged elevation in plasma MDA level representing the existence of oxidative stress even at later postoperative period.

During normal condition and even in course of increase ROS production antioxidant enzymes act to minimize the likely harm of toxic ROS products. Our results about the endogen antioxidant battery are indicative of long-lasting and exhausting oxidative stress in course of CPB. Regarding off-pump surgery the GSH levels and antioxidant enzyme activities compensate the increased forces of free radical production.

SOD catalyzes the dismutation of superoxide anion to hydrogen peroxide and molecular oxygen. Developing hydrogen-peroxide is further degraded by CAT to water and molecular oxygen. Third main preventative antioxidant enzyme is the glutation peroxidase

promoting the reduction of hydrogen peroxide and lipid hydroroxides to water or lipid alcohol. In such a reaction GSH is utilized as reductant leading to appearance of oxidized glutation (GSSG). The function, activity of glutation peroxidase was obtained by measuring of GSH level in cell. Normally, cells maintain high level of GSH performed by depletion of NADPH as reducing agent thus eliminating the effect of continuously generating ROS [150]. In condition of hard oxidative stress, GSH and NADPH is depleted thereby leading to deteriorated functions or cellular death. During increased free radical production such as ischemia-reperfusion endogen antioxidant capacity of different tissues can be remarkably reduced as described by Das and co-authors [151]. Under this circumstance the endogen defense is impaired and cells can not cope with further raising level of ROS [152].

There is evidence in literature that CPB decrease the activity of SOD and CAT even in course of early and long reperfusion [153]. In abstract of Pechan and coworkers, the reduction of antioxidants was documented during the first postoperative day after CPB agreeing with our findings [154]. Regarding off-pump surgery marked reduction was observed in SOD activity. We however found enlarged activity of SOD and CAT in OP patients. This phenomenon increased endogen antioxidant battery can be explained by compensation of slightly increased free radical production. Alternatively, evidences are growing to suggest that brief and multiple periods of partial ischemia of heart result in cardioprotection partially via expanded activity of antioxidant enzymes, as reported by Kuzuya and co-authors [155]. In our OP patient the ischemic periods lasted 10 to 15 minutes on several occasion mainly 2 to 4 times. These ischemic effects may be enough to trigger preconditioning.

In conclusion, present study revealed extremely elevated and prolonged production of free radicals and exhausted, reduced activity of antioxidant defense following conventional CABG with CPB. Off pump operation can be characterized by moderately increased ROS production with appropriately enhanced antioxidant capacity. The most important novelty of this study is the establishment of long-lasting oxidative stress to CPB. It may be elucidated by prolonged WBC activation following CPB.

On the other hand, the appearance of profound oxidative stress during the first postoperative week in CPB patients may contribute to impaired recovery following intervention.

7. SYSTEMIC ACTIVATION OF POLY(ADP-RIBOSE) POLYMERASE IN PATIENTS RECEIVING CORONARY ARTERY BYPASS GRAFTING WITH OR WITHOUT CARDIOPULMONARY BYPASS

7.1 Introduction

Occurrence of oxidative stress during and after CPB strongly suggests the role of poly (ADP-ribose) polymerase (PARP) pathway. Free radicals are known to provoke DNA strand-breakage activating PARP. Under pathologic condition associated with marked free radical production, it can lead to cellular NAD+ and ATP depletion [42]. Depending on severity of DNA damage three different ways can be triggered through PARP activation. Mild oxidative injury resulted in DNA repair with preserved cellular functions, severe damage leads to apoptosis through p53 dependent and independent (apoptosis-inducing factor) pathways. Necrotic cell death is caused by more severe oxidative stress via overactivation of PARP with consecutive NAD depletion [42]. Moreover, PARP activation was reported to alter the function of transcription factors and upregulate the expression of several pro-inflammatory genes by direct protein-protein interaction or by poly ADP-ribosylation [156]. Recent studies suggest that pharmacological inhibition of PARP can beneficially influences the protein-kinase signaling in ischemic-reperfused hearts [157].

The central role of PARP activation in pathomechanism of myocardial ischemia-reperfusion has been widely established [42, 158]. It has been demonstrated by several authors that activation of PARP enzyme play crucial role in systemic inflammation, circulatory and endotoxin shock [43, 159]. Experimental studies suggest the importance of PARP and beneficial role of PARP inhibition during application of CPB and cardiac arrest [160, 161]. It was also clarified that local insult of myocardial ischemia-reperfusion is sufficient to induce the activation of PARP in circulating leukocytes during infarction [162]. Furthermore Hageman and coworkers [163] reported that systemic PARP activation is present in patient with chronic obstructive pulmonary disease, representing inflammatory disorder.

At present, data are lacking on the role of systemic PARP activation in patients receiving open-heart surgery and it is also unknown whether omission of CPB is eligible to reduce systemic PARP activation. To answer these questions, systemic activation of PARP was assessed in

peripheral lymphocytes of patients during and after operation with CPB and in patients undergoing OP surgery.

7.2 Patients and methods

7.2.1 Patients

Thirty patients were selected as described in section 6 (page 62)

7.2.2 Anesthesia, operative technique

Each group of patients received the same protocol as described in the section 3 (page 26).

7.2.3 Determination of PAR polymers

Venous blood sample for determination of PAR polymers was collected in EDTA-containing tubes, put immediately on ice until sample preparation. The mononuclear cells were separated using Ficoll-Paque (Amersham Bioscience, Sweden). Briefly, 6 mL of Ficoll-Paquette was placed into the bottom of a centrifuge tube, and 8 mL of 1:1 dilution of anticoagulated whole blood in PBS was pipetted onto the top with care taken to avoid mixing. The tubes were then centrifuged at 800 *g* for 15 min at 4°C. The band of mononuclear cells on the top of Ficoll-Paque below the level of plasma layer was aspirated.

Staining for flow cytometry was carried out as described by Ogata with modifications [164]. Briefly, mononuclear cells were pelleted by centrifugation (175 g, 5 minutes) and then fixed in 1% formaldehyde in PBS for 10 minutes at 37 °C. After 1 minute of chilling, cell suspensions were washed in PBS again by centrifugation. Cells were permeabilized applying 90 % methanol (Sigma) for 30 minutes at 4 °C. Thereafter cells were rinsed twice with 0.5 % bovine serum albumin (BSA) (Invitrogen, USA) followed by incubation in 0.5 % BSA for 10 minutes at room temperature. Subsequent to blocking of cells in BSA, polyclonal antibodies against PAR was used at dilutions 1:10 for 45 minutes. After centrifugation, supernatant was carefully aspirated and cells were resuspended in 100 µl 0.5 % BSA containing FITC conjugated secondary antibody (MolecularProbes, USA) at a dilution of 1:50, and were incubated for 30 minutes. Fluorescent staining of samples was quantified by flow cytometric measurement of 10 000 cells. To determine the non-specific marking of cells, secondary antibody was applied for 30 minutes

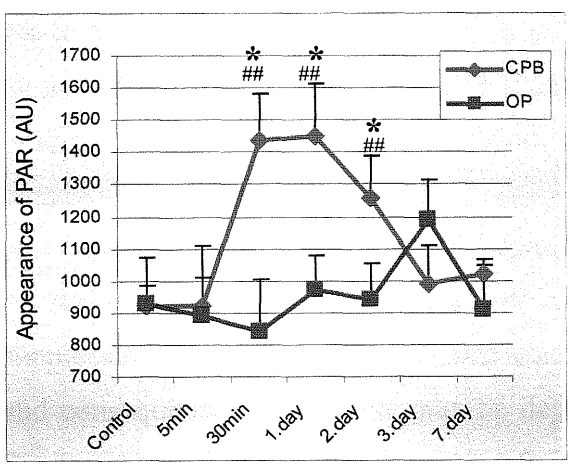
without primary antibody following permeabilization. Our results were analyzed by Cellquest software (BD Biosciences, USA) measuring the amount of PAR in the cells as mean fluorescence intensity (MFI).

7.2.4 Statistical analysis

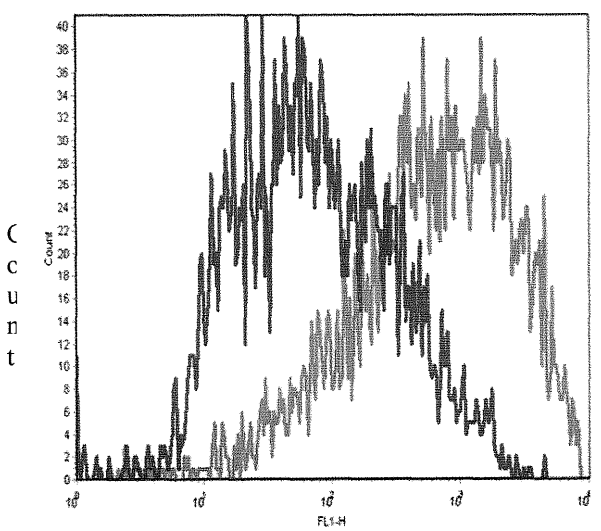
The data is presented in the tables and figures as mean ± standard error of mean (SEM). The data between the two groups were compared with unpaired Student's t test. In a given group comparisons between control data were made using paired Student's t test. Differences were considered significant at p values less than 0,05.

7.3 Results

The binding of PAR specific antibodies, which is indicative of PARP activation, was markedly increased in patient operated with open-heart surgery (Fig. 21). PAR staining was significantly stronger from the early reperfusion to the 2nd POD (Fig 21b). On the 3rd and 7th days PAR staining was around baseline level in patients operated with CPB. OP technique was able to abolish the increased activation of PARP. Considering OP patients, appearance of PAR did not differ from control level during the whole observation period. Mononuclear cells of CPB patients had significantly higher amount of PAR 30 minutes after aorta de-clamping and on the 1st and 2nd POD compared to leukocytes of OP patients..



A



B

(Fluorescense intensity)

Figure 21.: Appearance of PAR in lymphocytes of patients receiving CABG (A). CPB: operation with cardiopulmonary bypass. OP: Off- pump operation. $p<0,05$ between the two groups, # $p<0,05$ compared to the control value, ## $p<0,01$ compared to the control value.*

Activation of PARP enzyme on the 2nd postoperative day (B). The lymphocyte count of is shown plotted against the intensity of PAR. The red line demonstrates the population of lymphocytes of one patient representative of CPB group, blue line represents the values of the OP group.

7.4 Discussion

The present study showed that application of CPB resulted in significant and prolonged activation of PARP in circulating mononuclear cells of patients. OP technique abolished the systemic and long-term PARP activation. This study showed for the first time that CPB causes PARP activation in circulating leukocytes of human beings.

Activation of PARP enzyme has been identified as a crucial pathway in cellular response to oxidative injury. Previous studies have demonstrated the activation of PARP in myocardium following reperfusion [42, 165]. Recently, evidences suggest excessive PARP activation occurs in the tissues of heart and lung, during and following CPB [166-168]. Treatment with potent inhibitors of PARP had advantageous effect on postoperative cardiopulmonary functions. Furthermore Szabó and associates established the beneficial effect of PARP inhibition on mesenterial vascular function, improvement of coronary blood flow and renal function [169-171]. These findings suggest the importance of systemic PARP activation. Murthy reported that rat mononuclear cells showed increased staining against PAR following experimental myocardial infarction [162]. Several types of inflammatory diseases are associated with PARP activation in circulating leukocytes [163, 172].

Oxidative stress is primary responsible for activation of PARP as demonstrated in our previous study (section 6). PARP activation in cells receiving oxidative stress, however leads to suppression of cellular metabolic state via cellular NAD+ depletion. It is conceivable that NAD+ and consequently ADP levels may be depleted in the circulating lymphocytes following CPB. Suppression of metabolic function of circulating leukocytes can be advantageous due to inhibition of their contribution in inflammatory processes and increased elimination of inflammatory cells. On the other hand overactivation of PARP may also amplify inflammatory events via necrotic death of inflammatory cells [42, 173]. Even though, assessment of systemic PARP activation in circulating cells of patients may represent the PARP activation of systemic organs, which are exposed to same extent of oxidative damage. Importantly, there is an association between PARP activation and inflammatory processes. In its activated state, PARP triggers NFKB and AP-1 leading to increased expression of cytokines, iNOS, MIP-1α, and adhesion molecules [174]. Blockade of PARP influences the down-regulation of these inflammatory mediators contributing to delayed inflammatory processes [42, 162]. Without doubt, the effectiveness of PARP inhibition in diseases of inflammatory origin such as shock

sepsis and delayed phase of reperfusion has been proved by several authors [42, 156, 175]. Our results suggest that therapeutic usage of PARP inhibitors may provide effective approach in treatment of cardiac surgical patients. On the other hand, the assay presented by us can be useful to assess systemic PARP activation in individuals and also to follow the ability of agents to block catalytic activity of PARP, in setting of clinical study.

In conclusion, our study shows the over-activation of PARP in patients undergone open-heart surgery. Cardiac surgical intervention on beating heart without CPB does not induce significant activation of PARP enzyme.

8. NOVELTIES AND CLINICAL RELEVANCE

- Despite the early elevation of pro-inflammatory cytokines are counterbalanced by anti-inflammatory forces, prolonged and considerable pro-inflammatory processes are present during days following application of CPB. Moreover these findings can refer to timing of anti-inflammatory therapy after open-heart surgery.

- It was demonstrated that myocardial outflow of pro-inflammatory cytokines occur during CPB and it is less expressed during off-pump technique. Thus jeopardized myocardial tissue can contribute to inflammatory processes and amplify local inflammatory insults.

- It was proved for the first time that off-pump surgery can decrease IL-12 expression when compared to operation with CPB, thereby decreasing the contribution of cellular immune response.

- Novel to this work was the demonstration of CD97 activation on leukocytes after CPB representing activation state of white blood cells. Adhesion molecules are also presented markedly in course of CPB. Off-pump surgery decrease both CD97 and adhesion molecule expression related to surgery using CPB.

- We were able to demonstrate that activated leukocytes can exert condition of oxidative stress as a result of CPB. Oxidative injury remains significant over the postoperative days following CPB. Off-pump surgery is associated with more moderate oxidative processes.

- Exhaustible oxidative effects after CPB provoke decreased activity of antioxidant enzymes.

- Our biochemical measurements provided evidence for the first time that marked oxidative injury led to systemic PARP activation in patients receiving open-heart surgery. Off-pump surgery was able to reduce manifest activation of PARP enzyme.

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