

**RELEASE OF CARDIAC BIOMARKERS AND INFLAMMATORY RESPONSE
DURING CARDIOPULMONARY BYPASS: COMPARISON OF DIFFERENT
BIOCOMPATIBLE MATERIALS USED IN CARDIOPULMONARY BYPASS**

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Saskatoon

By

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ABSTRACTS

Coronary Artery Bypass Grafting (CABG) is an effective and invasive cardiac surgery to salvage blocked coronary artery. Cardiopulmonary bypass (CPB) is usually applied to support circulation during temporary cardiac arrest. Studies have demonstrated that cardiac injury, inflammation, and oxidative stress could be induced during CABG with CPB. We conducted two studies to investigate the release of cardiac biochemical markers and inflammatory response as well as to compare the effect of different coating biomaterial of CPB on the induction of inflammation and oxidative stress during CPB. We investigated the release patterns and the serum levels of cardiac markers as well as inflammatory markers in patients undergoing elective CABG at different time points after initiation of CPB. In this study, we demonstrated that cardiac markers such as creatine kinase isoenzyme MB (CK-MB), and cardiac troponin I (cTnI) and inflammatory markers such as tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), and high sensitivity C-reactive protein (hsCRP) were highly elevated after CPB. Moreover, we confirmed that cTnI is still a better biochemical marker for cardiac injury than others following CABG with CPB. Other nonspecific but highly sensitive markers such as lactate dehydrogenase (LDH), lactate, TNF- α , IL-6, and hsCRP could be potential surrogate markers for evaluation of cardiac injury following CPB.

Based on these findings, we conducted a further investigation to demonstrate our hypothesis that different biocompatible materials used in CPB may affect the inflammation and oxidative stress differently. Biocompatible materials are thinly coated on CPB tubes to provide similar environment like endothelial cells during cardiac surgery. There are several biocompatible materials available in the market. Each of them has unique characteristics. Inflammatory response is one of the body's fundamental defense mechanisms against foreign invaders. However, inappropriate or excessive response can lead to harmful, potentially life-threatening consequences due to severe inflammatory tissue destruction. CPB-induced inflammatory response can be one of the factors, which can affect surgical outcomes. Depending on the presence of different biocompatible materials in CPB circuits, the degree of immunoreactions can be varied.

In this study, we analyzed hsCRP, an acute phase protein, and tau protein, a marker of neurocognitive deficiency. Furthermore we analyzed inflammatory cytokines including TNF- α , IL-6, IL-10, and interferon-gamma (IFN- γ) to evaluate the levels of inflammation. Serum levels of oxidized nitric oxide as a marker of oxidative stress were also assessed. We demonstrated that different biocompatible material has different impacts on inflammation and oxidative stress. In the aspect of anti-inflammation, heparin-coated biocompatible material is better than others whereas surface-modifying additives biocompatible material is worse than others. Overall, different coating biomaterial of CPB results in various inflammatory response. In terms of oxidative stress, we did not observe significant difference between different biomaterial-coated CPB.

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LIST OF ABBREVIATIONS

8-iso-PGF_{2α}	8-iso-prostaglandin F_{2α}
AAT	Alpha 1-antitrypsin
ACS	Acute coronary syndrome
ASA	Acetyl salicylic acid
CABG	Coronary artery bypass grafting
CK-MB	Creatine kinase isoenzyme MB (muscle-brain)
CNS	Central nervous system
CPB	Cardiopulmonary bypass
CSF	Cerebrospinal fluid
cTnI	Cardiac troponin I
cTnT	Cardiac troponinT
DEHP	Di-2-ethyl-hexyl-phthalate
ELISA	Enzyme-linked immunosorbent assay
eNOS	Endothelial nitric oxide synthase
ET-1	Endothelin-1
FVII	Coagulation factor VII
HMWK	High molecular weight kininogen
hsCRP	High sensitivity C reactive protein
ICAM-1	Intercellular adhesion molecule-1

IFN-γ	Interferon-γ
IL-10	Interleukin-10
IL-6	Interleukin-6
iNOS	Inducible nitric oxide synthase
IV	Intravenous
LDH	Lactate dehydrogenase
LPS	Lipopolysaccharide
MAC	Membrane attack complex
MCP-1	Monocyte chemoattractant protein-1
MEIA	Microparticle enzyme immunoassay
MI	Myocardial infraction
MPO	Myeloperoxidase
NADPH oxidase	Nicotinamide adenine dinucleotide oxidase
NCD	Neurocognitive dysfunction (deficiency)
nNOS	Neuronal nitric oxide synthase
NSE	Neuron-specific enolase
OP	Operation (cardiac surgery)
OPCAB	Off-pump coronary bypass grafting
PAF	Platelet activating factor
PEO	Polyethylene oxide
PGI₂	Prostacyclin₂

PMEA	Polymethoxyethyl acrylate
PSGL-1	P-selectin glycoprotein ligand-1
PVC	Polyvinyl chloride
ROS	Reactive oxidative stress
SMA	Surface-modifying additive
SOD	Superoxide dismutase
TCC	Terminal complement complex
TF	Tissue factor
TFPI	Tissue factor pathway inhibitor
TNF-α	Tumor necrosis factor-α
ULN	Upper limit of normal
VCAM-1	Vascular cell adhesion molecule-1

1. REVIEW OF LITERATURE

Coronary Artery Bypass Graft (CABG) surgery is an invasive but effective measure to salvage human beings with coronary artery disease. In most of the cases, the patient heart theoretically has to be stopped during cardiac surgery to provide better access and handling for surgeons. Patient's blood has to be bypassed through heart-lung machine that is substituted for a patient's heart. Therefore, Cardiopulmonary bypass (CPB) is usually applied to support circulation during temporary cardiac arrest. CPB, heart-lung machine, becomes the most required equipment for the heart surgery.

During the coronary artery bypass grafting surgery (CABG), arteries or veins from elsewhere in the patient's body are grafted to the coronary arteries to bypass and improve the blood supply to the coronary circulation. In this procedure, CPB provides better access to the patient heart for the surgeon because it acts as a patient heart and lung during surgical operation. However, even though CABG and CPB are inevitably needed to salvage blocked coronary artery, cardiac injury occurs through CABG with CPB. There are many studies, which show that cardiac injury, inflammation, and oxidative stress could be induced during CABG with CPB.

CPB (Figure 1), as it was called heart-lung machine, is one of the most important technical innovations in healthcare history. Even though it was invented and has been developing for over 50 years with the strong support from scientific and engineering research, we are still facing the unavoidable systemic responses to CPB such as inflammatory reaction and complement activation. Furthermore, after one of popular natural anticoagulants, heparin, was discovered and commercially produced, these two innovations provided better treatment of cardiovascular disease for patients (Edmunds *et al.* 2004). After commercial production of heparin was introduced, study of the blood-heparin interaction received much attention from researchers to reduce the unwanted outcomes after surgery. Therefore, a couple of alternatives, so called biocompatible materials, for the heparin were developed and optimized for clinical use. Nowadays there are several biocompatible materials available and used in CPB circuits, and their effects have not been fully assessed and compared.

Basically blood cells are destined to meet unfamiliar environment while they are circulating through CPB. No matter how similar biocompatible materials we develop, normally blood cells only touch endothelial cells, a monolayer cell line of the entire cardiovascular system, and have not met and experienced those artificial materials before. When blood cells meet any other foreign surface such as CPB, both thrombotic and an inflammatory response are triggered (Edmunds *et al.* 2004). The thrombotic (clotting) response is attenuated by the use of heparin, but heparin does not completely prevent thrombosis, complement activation, or inflammation which is occurred mainly by neutrophils and monocytes (Paparella *et al.* 2004). This response produces a wide range of cytokines, cell-signaling proteins, and vasoactive substances that circulate and disrupt interstitial fluid balance and homeostasis. Both thrombotic and inflammatory responses produce thousands of micro-embolic particles consisting of fibrin fragments, plates, and others. Microparticles obstruct arterioles in some cases, and finally can damage organs and tissues (Rubens *et al.* 2004, Paparella *et al.* 2004, Landis 2007).

Most patients are recovered from the surgery after using of CPB, but a few show subtle neurocognitive deficiency or worsened kidney function (Paparella *et al.* 2004). Compared to the control group that does not use any biocompatible material in CPB circuit, other groups coating biocompatible materials in CPB circuit show there a fewer patients suffering from neurocognitive deficiency (Ramlawi *et al.* 2006a). Our research group has been conducting the research to evaluate the neurocognitive defect of different biocompatible materials. Our data has proved that there are some differences between different biocompatible materials in terms of inflammatory response and neurocognitive deficiency in patients undergoing CABG with CPB.

Many factors affect the intensity of the thrombotic and inflammatory responses. The intensity varies depending on the biomaterials used. The problem is that every biocompatible material, which is resistant to thrombotic response triggers clotting and even inflammation (Landis 2007). Basically, we can suppress the thrombotic and inflammatory responses for short periods by using biocompatible materials and manipulating the biochemical and hematological characteristics of blood. However, because of the delicate balance between coagulation and fibrinolysis pathways in human

body, we have to run risks of bleeding or infectious complications. Complete suppression of thrombin generation by new and better anticoagulants and the administration of specific inhibitors of complement, neutrophil and monocyte activation could prevent or reduce complications caused by contact between blood and the biocompatible material surface (Paparella *et al.* 2004, Landis 2007).

Much effort has been made to improve the biocompatible materials used in CPB circuits and several kinds of commercial biomaterials are available in market. Preliminary evaluation of each product was conducted by each manufacturer. However, a comprehensive evaluation and comparison for the currently available 5 different biocompatible materials has not been done. A simple diagram of typical cardiopulmonary bypass circuit is provided in Figure 1. Depending on the surgical circumstances, much device is attached to CPB.

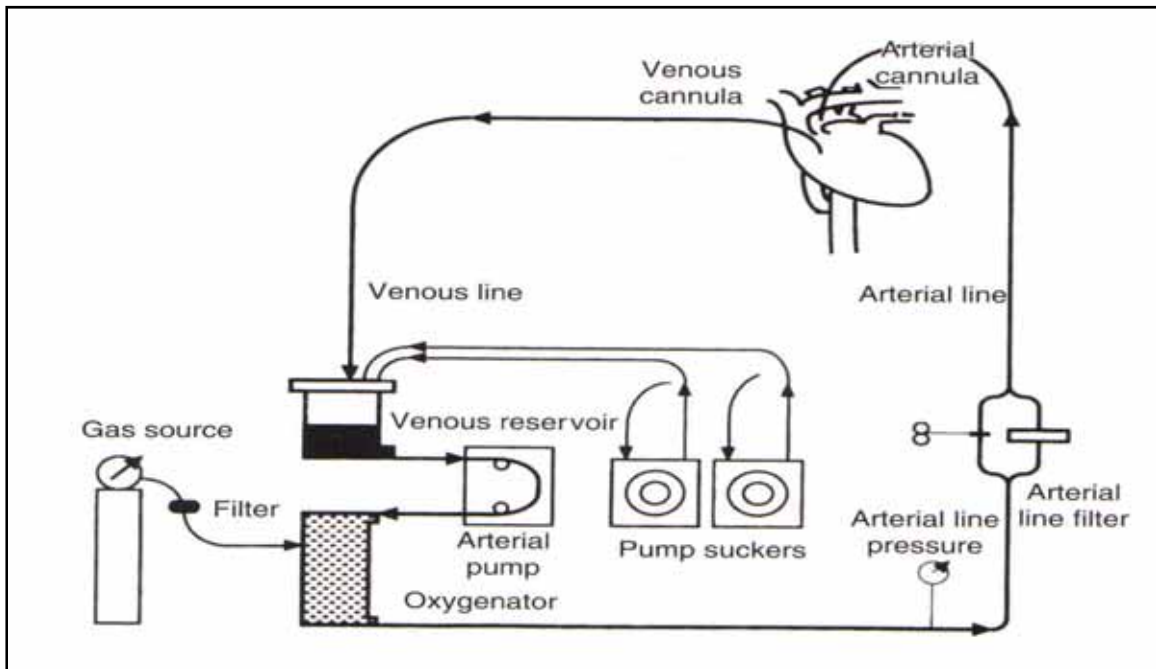


Figure 1. A typical cardiopulmonary bypass circuit (DiNardo *et al.* Anesthesia 2008).

1. 1. Heart attack and cardiac surgery

A heart attack that is also known as a myocardial infarction (MI) is the death of heart muscle, which results from ischemia (lack of blood flow), the sudden blockage of a coronary artery by a blood clot (Kumar *et al.* 2005). Coronary arteries are blood vessels that supply the heart muscle with blood and oxygen. The predominant underlying cause of coronary heart disease is atherosclerosis, which can result in myocardial infarction. Clinical interventions, which are used to re-supply blood to ischemic myocardium (heart muscle), include thrombolysis (remove the thrombus, blood clot), coronary angioplasty, and coronary bypass surgery (Verma *et al.* 2002). Among those clinical interventions, coronary bypass surgery has been widely used. Coronary artery bypass grafting surgery, so called CABG, is a surgical procedure performed to resume blood supply and reduce the risk of death from coronary artery disease. Arteries or veins from elsewhere in the patient's body are grafted to the coronary arteries to bypass atherosclerotic narrowings and improve the blood supply to the coronary circulation supplying the myocardium (Kumar *et al.* 2005). However, reperfusion of the ischemic heart can induce myocardial injury. This injury becomes worsened during open-heart surgery when the myocardium is exposed more stressful conditions (Verma *et al.* 2004). Myocardial reperfusion injury activates neutrophils, triggering an inflammatory response which results in generation of reactive oxygen species (ROS), cytokine release, and complement activation that make cardiac injury more exacerbated (Franke *et al.* 2005).

1.2. Cardiac injury and cardiac markers during CPB

Cardiac markers are the intracellular macromolecules that leak out of fatally injured myocardial cells through damaged cell membranes which can be detected in the blood. These molecules include myoglobin, cardiac troponins I and T (cTnI, cTnT), creatine kinase (CK), lactate dehydrogenase, and many others (Kumar *et al.* 2005). Some of the markers have become sensitive indicators of myocardial damage. From a biochemical perspective, the diagnosis of myocardial injury is established when blood levels of sensitive and specific biomarkers, such as cardiac troponin and the MB fraction

of creatine kinase (CK-MB), are increased in the clinical setting of acute ischemia (Kumar *et al.* 2005). The preferred biomarkers for myocardial damage are cardiac-specific proteins, particularly cTnI and cTnT. Troponins are the proteins that regulate calcium-mediated contraction of cardiac and skeletal muscle. These markers have nearly complete tissue specificity and high sensitivity. cTnI and cTnT are not normally detectable in the circulation, but after acute MI, levels of both cardiac troponins rise at 4 to 6 hours and peak at 48 hours. Troponin levels remain elevated for 7 to 10 days after the acute event (Kumar *et al.* 2005, McCance *et al.* 2006).

Creatine kinase (CK) is an enzyme that is highly concentrated in brain, myocardium, and skeletal muscle and is composed of two dimers, designated “M” and “B”. Total CK activity is sensitive but not specific, as CK is elevated in other conditions such as skeletal muscle injury. The isoenzyme CK-MM is derived predominantly from skeletal muscle and heart; CK-BB from brain, lung and many other tissues; and CK-MB principally from myocardium, although variable amounts of the MB form are also present in skeletal muscle. Formerly the “gold standard”, cardiac creatine kinase (CK-MB) remains the best alternative to troponin measurement. CK-MB activity begins to rise within 4 to 6 hours of onset of MI, peaks at about 24 hours, and returns to normal within approximately 72 hours (Kumar *et al.* 2005, McCance *et al.* 2006). Although the diagnostic sensitivities of cardiac troponin and CK-MB measurements are similar in the early stages of MI, cardiac troponins possess higher specificity than CK-MB. Elevated troponin levels remain for approximately 10 days after acute MI. The peak of either troponin or CK-MB is accelerated in patients who have had reperfusion, owing to washing out of the enzyme from the necrotic tissue (Kumar *et al.* 2005). In addition to CK-MB and cTn, C-reactive protein (CRP) may serve as a marker to predict the risk of myocardial infarct in patients with atherosclerosis. Using highly sensitive methods, serum CRP, levels of more than 3 mg/L are associated with the highest risk of cardiovascular disease, while levels of 1 to 3 mg/L are associated with moderate risk (Ridker *et al.* 2003, Levy *et al.* 2003, Denesh *et al.* 2004, Franke *et al.* 2005, Deblrier *et al.* 2006).

Furthermore, specific cardiac enzymes, creatine kinase isoenzymes MB (CK-MB), and cardiac troponins I (cTnI) and T (cTnT) are generally elevated in the serum of

patients who have undergone cardiac surgery. Their postoperative values are frequently above the levels normally used to define MI (Taggart 2000). The release of cTn in the setting of open heart surgery not only reflects MI but may also result from myocardial cell injury attributable to incomplete cardioprotection, reperfusion injury, unavoidable surgical trauma, and direct current defibrillation. The association between increased postoperative cTn concentrations and increased postoperative mortality and morbidity has been well established in recent trials (Greenon *et al.* 2001, Fellahi *et al.* 2003). These data indicate that cTn concentration can serve as a valuable marker for the diagnosis of postoperative MI or myocardial injury in CABG patients (Jacquet *et al.* 1998). Although cTn has been recommended as the gold standard for the diagnosis of acute MI, it is still considered to be a necrotic marker for myocardial injury and is not a preferred marker for the diagnosis of reperfusion injury or re-infarction. Yet there are limited data available to assess the use of cTn as a marker of myocardial ischemia after open heart surgery (Etievent *et al.* 1995, Vermes *et al.* 2000). The significance of cardiac enzyme levels in the postoperative phase is considered uncertain as several factors (poor myocardial protection, early graft failure, intracoronary embolization, incomplete revascularization, and surgical trauma) may contribute to their release (Paparella *et al.* 2005). Injury or trauma during CABG triggers an acute phase reaction (Teoh *et al.* 1995). Inflammatory sensitive plasma protein such as alpha 1-antitrypsin (AAT), ceruloplasmin, and high sensitivity C-reactive protein (hsCRP) have been demonstrated to be elevated in acute coronary syndrome (ACS) (Engstrom *et al.* 2002, Engstrom *et al.* 2004, Lind *et al.* 2004).

1.3. Inflammatory response with CPB

Inflammation is one of healing process in our body. Unlike adaptive immunity, inflammatory response does not need much specificity. Instead it needs quick response to remove the invaders or healing the injury. Therefore, inflammation process could be in the innate immunity category (McCance *et al.* 2006). CPB is a technique that temporarily takes over the function of the heart and lungs during surgery, maintaining the circulation of blood and the oxygen content of the body (Gravlee *et al.* 2000). The CPB pump itself

is often referred to as a heart-lung machine. During the cardiac surgery with CPB, the inflammatory response is very important to our body for healing, not harming. However, this inflammatory response may trigger a significant systemic inflammatory response when using CPB during open-heart surgery (Gravlee *et al.* 2000, Rubens *et al.* 2004) (Figure 2). The CPB-induced inflammatory response could further generate myocardial injury and induce myocardial dysfunction because cardiac surgery without CPB appears to be associated with reduced myocardial injury (Suleiman *et al.* 2007).

It has been proposed that surgery without CPB would significantly reduce the stress response associated with open-heart surgery (Akila *et al.* 2007). Now it is widely accepted that beating heart surgery that is performed without CPB attenuates cytokine and stress responses (Yamaguchi *et al.* 2005, Hoel *et al.* 2007). However, since the inflammatory response is only reduced and not eliminated, it is likely to continue to influence cardiac function and clinical outcome (Quaniers *et al.* 2006). The main source is surgical trauma, which will continue to trigger a stress response which is mediated by the release of various cytokines and hormones. Therefore, not employing CPB does not necessarily mean the absence of inflammatory response (Quaniers *et al.* 2006).

A more effective way of counteracting the inflammatory response and oxidative stress may be the omission of CPB. This idea provides the reintroduction of off-pump coronary artery bypass grafting (OPCAB) which does not use CPB during surgery, but it was rapidly off staged by on-pump CABG soon after the invention of the CPB owing to the attraction of operating on a still heart in a bloodless field (Raja *et al.* 2007). Moreover, even though OPCAG reduces inflammation and oxidative stress, but cannot prevent those responses (Akila *et al.* 2007). The main cause of inflammatory and oxidative stress in OPCAB is surgical trauma (Prondzinsky *et al.* 2005, Berg *et al.* 2006). Figure 2 shows an overview of the inflammatory response when using CPB during cardiac surgery.

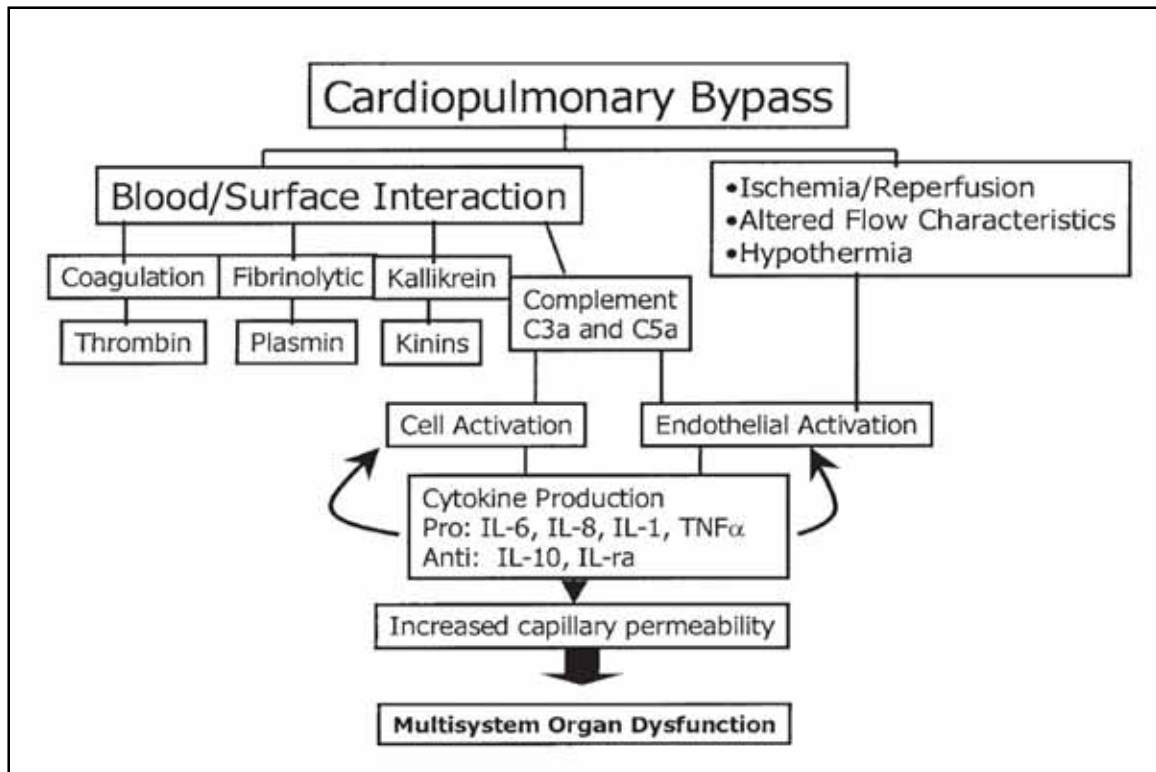


Figure 2. An overview of the inflammatory response to CPB (Kozik *et al.* 2006).

1.3.1. Cellular components of blood involved in inflammation during CPB

1.3.1.1. Erythrocytes

Erythrocytes (Red blood cells) are mainly damaged during CPB by shear stress (Gravlee *et al.* 2000). Erythrocytes deformability is reduced by CPB because of mechanical damage to erythrocytes. This has the effect of inducing changes to ionic pumps at the cell surface, which leads to abnormal accumulation of intracellular cations (Gravlee *et al.* 2000). Another damaging factor to erythrocytes is membrane attack complex (MAC), which is produced by activation of complement (Gravlee *et al.* 2000). Therefore, the life span of erythrocytes is reduced. After erythrocytes are dead, the hemoglobin is released into blood stream. This free hemoglobin may damage tissue function by increasing plasma pressure and viscosity. Also cytotoxic free radicals are released from auto-oxidation of hemoglobin because hemoglobin could be a sink of nitric

oxide. So the anemia could be seen frequently after cardiac surgery (Gravlee *et al.* 2000, Pacher *et al.* 2007)

1.3.1.2. Neutrophils

The cellular immune system is crucial to the inflammatory response during and following cardiac surgery (Laffey *et al.* 2002, Rinder *et al.* 2006, Warren *et al.* 2007). Leukocyte counts decrease in response to hemodilution during CPB and increase slightly after operation (Gravlee *et al.* 2000). Only a few neutrophils attach to synthetic surface. Nevertheless, neutrophils are strongly activated during CPB mainly by C5a which is produced by the complement activation. Other agonists include IL-1 β , TNF- α , IL-8, C5b-9, heparin, histamine, hypochlorous acid, and products of arachidonate metabolism such as thromboxane A2 (Menasche *et al.* 2003, Warren *et al.* 2007). Neutrophil activation releases potent proteolytic and cytotoxic substances such as lysozyme, myeloperoxidase, elastase, collagenase, integrins, histaminases, heparanase, complement activator, and membrane-associated NADPH (nicotinamide adenine dinucleotide) phosphate oxidase from specific granules (Birregaard *et al.* 1997, Warren *et al.* 2007). Activated neutrophils also produce cytotoxic reactive oxygen and nitrogen intermediates such as superoxide anion, hydrogen peroxide, hydroxyl radicals, hypochlorous acid and peroxynitrite (Babior 2000, Warren *et al.* 2007). Finally neutrophils produce arachidonate metabolites, prostaglandins, leukotrienes, and plate-activating factor. All of these vasoactive and cytotoxic substances are produced and released into the extracellular environment (Asimakopoulos *et al.* 2002, Menasche *et al.* 2003, Chen *et al.* 2004, Gorbet *et al.* 2004, Warren *et al.* 2007).

1.3.1.3. Platelets

The platelet numbers are counted during cardiac surgery because the platelet count could accounts for possibility of severe bleeding during and after surgery (Gravlee *et al.* 2000). The number of platelets decreased right after CPB but increased slightly later

on (Gravlee *et al.* 2000, Hundelshausen *et al.* 2007). The platelets are used for coagulation process to protect our body from bleeding during cardiac surgery. The platelets are activated during CPB by a variety of agonists such as thrombin, platelet activating factor (PAF), serotonin, and thromboxane A2 (Blockmans *et al.* 1995). Internally generated thromboxane A2 is known as a strong activator of platelets. Also platelets have several proteaseactivated receptors to most of these agonists, which has an important role in adhesion and thrombus formation (Menasche *et al.* 2003, Landis *et al.* 2007). Platelets contribute to the inflammatory response by synthesis and release of serotonin from dense granules, IL-1 β and IL-8 from alpha granules (Blockmans *et al.* 1995, Hundelshausen *et al.* 2007). These platelet-secreted cytokines may be involved in the inflammatory response to CPB because the platelets are strongly activated in both wound and perfusion circuit (Menasche *et al.* 2003). Circulating neutrophils and monocytes constitutively express P-selectin glycoprotein ligand-1 (PSGL-1), which interacts with aggregated platelets via P-selectin expressed on activated platelets (Yang *et al.* 1999, Hundelshausen *et al.* 2007). Platelets aggregate using platelet GPIIb/IIIa receptors attached to symmetrical fibrinogen molecules to form bridges between platelets. During CPB, platelets aggregate with each other and to monocytes and neutrophils to trigger and accelerate the inflammatory response (Landis *et al.* 2001, Gorbet *et al.* 2004, Colman *et al.* 2006, Edmunds *et al.* 2006, Hundelshausen *et al.* 2007).

1.3.1.4. Endothelial cells

Basically, the endothelial activation is a process of healing mechanisms in our body. Through this mechanism, our body tries to remove the stressful factors and get back to normal physiological condition (Schmid *et al.* 2006). The vascular endothelium is involved in a variety of physiological and pathological processes and also it is involved in many of the biological events, which affect the perioperative course of the cardiac surgery (Chen *et al.* 2004, Schmid *et al.* 2006). Therefore, few biocompatible materials use the mimicry of vascular endothelium to reduce the inflammatory response by providing real microenvironments of vascular endothelium to blood cells. The endothelium controls vascular tone and permeability, regulates coagulation and

thrombosis, and direct accessing of leukocytes into inflammatory area through the expression of surface proteins and secretion of soluble mediators (Laffey *et al.* 2002, Schmid *et al.* 2006). The inflammatory response to CPB is characterized by activation of endothelial cells and endothelium dysfunction. Endothelial cells are activated during CPB by a variety of agonists such as thrombin, C5a and cytokines IL-1 β and TNF- α (Menasche *et al.* 2003, Schmid *et al.* 2006). Also shear stress produced by the pumps in CPB can trigger endothelium activation (Gravlee *et al.* 2000). IL-1 β and TNF- α induce the early expression of P-selectin and the later synthesis and expression of E-selectin, which are involved in the initial stages of neutrophil and monocyte adhesion to endothelium (Menasche *et al.* 2003, Schmid *et al.* 2006). These two cytokines also induce expression of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), which firmly bind neutrophils and monocytes to the endothelial cells and initiate leukocyte trafficking to the extravascular space (Eikomo *et al.* 2004, Rankin 2004, Schmid *et al.* 2006). Moreover, IL-1 β and TNF- α induce endothelial cell production of the chemotactic proteins such as IL-8 and monocyte chemoattractant protein-1 (MCP-1), and induce production of prostacyclin (PGI₂) by the cyclooxygenase pathway and nitric oxide by nitric oxide synthase (NOS). These two vasodilators reduce shear stress and increase vascular permeability and enhance leukocyte adhesion and transmigration (Vane *et al.* 1990, Menasche *et al.* 2003, Hooper 2004, Schmid *et al.* 2006). In addition to NO and PGI₂, endothelial cells produce the vasoconstrictor endothelin-1 (ET-1) and inactivate other vasoactive mediators such as histamine and bradykinin (Vane *et al.* 1990). Furthermore, IL-1 β and TNF- α stimulate endothelial cell production of pro-inflammatory cytokines such as IL-1, IL-6, IL-8, MCP-1, and PAF (Hooper 2004, Edmunds *et al.* 2006, Schmid *et al.* 2006).

1.3.2. Humoral components of blood involved in inflammation during CPB

1.3.2.1. Complement

Complement was discovered many years ago as a heat-labile component of normal plasma initiating the opsonization of bacteria by antibodies and allows antibodies to kill some bacteria (Janeway *et al.* 2001). This activity was said to ‘complement’ the antibacterial activity of antibody. However, complement can also be activated early in infection in the absence of antibodies. Therefore, nowadays, it is accepted that complement first evolved as part of the innate immune system, where it still plays an important role (Janeway *et al.* 2001). The complement system consists of more than 30 plasma proteins that interact to produce powerful vasoactive anaphylatoxins (C3a, C4a, and C5a), opsonin (C3b), and the terminal complement complex (TCC), C5b-9, which acts as a membrane attack complex (MAC) (Walport 2001). C5a and C5b-9 play major roles in promoting neutrophil-endothelial cell interactions through upregulating specific adhesion molecules. C5b-9 may also activate platelets and promote platelet-monocyte aggregates (Walport 2001). These complement proteins contribute to neutrophil loss from blood circulation by adhesion to surface-bound platelets and more importantly endothelial cells. The interaction between complement proteins and neutrophils contributes to postoperative organ damage after CPB (Walport 2001, Colman *et al.* 2006, Edmunds *et al.* 2006, Edmunds *et al.* 2008).

Complement is activated by three pathways, but only the classical and alternative pathways are involved in CPB, although a role for the manose-lectin pathway has not been excluded (Levy *et al.* 2003). Direct contact between heparinized blood and the synthetic surfaces of the CPB activates the contact plasma proteins and the classical complement pathway (Goor *et al.* 2004, Gorbet *et al.* 2004, McCance *et al.* 2006, Nilsson *et al.* 2007). Then the generation of C3b activates the alternative pathway. During CPB complement is largely activated by the alternative pathways (Goor *et al.* 2004, Gorbet *et al.* 2004, Nilsson *et al.* 2007). There are three phases for complement activation during CPB and cardiac surgery: during blood contact with non-endothelial cell surfaces; after protamine administration and formation of the protamine-heparin complex; and after

reperfusion of the ischemic heart. CPB and myocardial reperfusion activates complement by both classical and alternative pathways and the protamine-heparin complex activates complement by the classical pathway. Other agonists activating the classical pathway during CPB include endotoxin, apoptotic cells, and C-reactive protein (Menasche *et al.* 2003, Colman *et al.* 2006, Edmunds *et al.* 2006, Edmunds *et al.* 2008).

1.3.2.2. Coagulation

The coagulation system is a group of plasma proteins finally forming a fibrinous mesh at an injured or inflamed site (McCance *et al.* 2006). Generally, the coagulation system is known as the function of stopping bleeding at injured site. However the coagulation system also prevents the spread of infection to adjacent tissues, keeps foreign bodies at the site of inflammatory cell activity, provides a framework for future healing. The main substance in this fibrinous mesh is an insoluble protein such as fibrin that is the end product of the coagulation pathways (Gorbet *et al.* 2004, Nilsson *et al.* 2007). Furthermore, the coagulation pathways can be activated by many substances which are released during tissue destruction and infection, including collagen, proteinases, kallikrein, plasmin and bacterial products, endotoxins (Gorbet *et al.* 2004, Colman *et al.* 2006, Edmunds *et al.* 2006, Nilsson *et al.* 2007, Edmunds *et al.* 2008).

Like complement cascade, the coagulation system is a cascade reaction and can be activated through different pathways: extrinsic pathway and intrinsic pathway. Intrinsic pathway is commonly considered that high molecular weight kininogen (HMWK), prekallikrein and Factor XII require contact with negatively charged surfaces (Landis *et al.* 2007). However, since the occurrence of negatively charged surfaces in vivo is limited, the importance of the intrinsic pathway to normal blood coagulation remains speculative. Collagen present in the subendothelium after vessel injury could be the surface required for this reaction (Gorbet *et al.* 2004). On the other hand, the extrinsic pathway is initiated by tissue factor (TF) (Somer *et al.* 2002b), which is expressed on damaged cell surfaces at the site of vascular injury. Plasma Factor VII (FVII) binds to this TF on the cell membranes and activates another inactive precursor factor. The

extrinsic pathway and intrinsic pathway are not independent of each other, and also this coagulation pathway interacts with complement pathway through generated thrombin (Gorbet *et al.* 2004, Colman *et al.* 2006, Edmunds *et al.* 2006, Nilsson *et al.* 2007).

1.3.3. Release of cytokines during CPB

Cytokines are soluble proteins and polypeptides that act as paracrine messengers of the immune system and are produced by a large variety of cell types. These cells include activated monocytes, tissue macrophages, lymphocytes, and endothelial cells (Laffey *et al.* 2002). Individual cytokines may exert either pro-inflammatory or anti-inflammatory effects. Cytokines are essential for immunologic and physiologic homeostasis, and they are produced in response to a variety of physiological and pathological stimuli (Miller *et al.* 1997). IL-1 β and TNF- α are early response cytokines that are promptly produced at the site of infection or injury by resident macrophages (Asimakopoulos *et al.* 2001). These cytokines then stimulate surrounding stromal and parenchymal cells to produce more IL-1 β and TNF- α and chemokines, particularly IL-8 and MCP-1, which are powerful chemoattractants for neutrophils and macrophages, respectively (Janeway *et al.* 2001). Together with IL-6, the cytokine that regulates production of acute phase proteins such as C-reactive protein and α 2-macroglobulin by the liver. The five cytokines (IL-1 β , TNF- α , IL-6, IL-8, and MCP-1) are the major pro-inflammatory mediators which are involved in the acute inflammatory response to CPB (Holmes *et al.* 2002, Levy *et al.* 2003, Maharaj *et al.* 2004, Deblier *et al.* 2006, Landis *et al.* 2007). The major anti-inflammatory cytokine involved during CPB is IL-10. IL-10 inhibits synthesis of pro-inflammatory cytokines and induces production of IL-1 receptor antagonist IL-1ra, which downgrades the response to IL-1 (Holmes *et al.* 2002, Levy *et al.* 2003, Maharaj *et al.* 2004, Deblier *et al.* 2006, Landis *et al.* 2007). Pro-inflammatory cytokines increase during and after cardiac surgery using CPB with peak concentrations usually 12 to 24 hours after CPB ends (Menasche *et al.* 2003). Generally, the levels of cytokines in serum during CPB differ greatly in timing, within and between studies. Many factors can affect the production of cytokine during CPB, for instance, the differences in the duration of CPB, perfusion temperatures, perfusion equipment, and

aortic cross-clamp times, differences in methods of myocardial protection, priming solution, anesthesia, and intravascular drugs (Menasche *et al.* 2003). The ischemic/reperfused heart is a major source of inflammatory cytokines and reactive oxidants (Schulze *et al.* 2000). In addition to inflammatory cytokines, IFN- α is thought to possess neuroregulatory functions including behavior, temperature regulation, and control of feeding patterns (Wang *et al.* 2000). There is no article dealing with the serum levels of interferon (IFN) during CPB. It is more popular in the field of neuroscience. Administration of IFN- α to humans results in an array of central nervous system (CNS) side effects. Recipients of IFN- α therapy typically report reduced alertness, and after several weeks of treatment, a syndrome of subjective memory loss has been identified (Valentine *et al.* 1999). In 2002 Wilson *et al.* proposed that the mechanisms of IFN- α -induced neurotoxicity may include cytokine, neurotransmitter, or neuroendocrine effects or a combination of thereof. Also they proposed that some of these CNS effects may occur via induction of IL-1, IFN- γ , and TNF- α , all of which are known to produce potent CNS effects

1.3.4. Oxidative stress during CPB

Oxidative stress means the loss of the balance between oxidants and antioxidants. It occurs in cells when the cell's natural antioxidant defense is overwhelmed by the generation of reactive oxygen species (ROS). Oxygen derived free radicals such as superoxide anion ($O_2^{\cdot-}$) and hydroxyl radical ($\cdot OH$) and related non-radical such as hydrogen peroxide (H_2O_2) compounds are referred to as ROS. In our human body, ROS are generated mainly by phagocytic cells such as monocytes, macropahages, neutrophils and eosinophils through NADPH oxidase (Berg *et al.* 2006, Deblier *et al.* 2006). These ROS are very important to remove the foreign invaders in our body. During the cardiac surgery with CPB, the artificial surface in CPB stimulates blood cells such as neutrophils and monocytes to produce ROS and other inflammatory agents. Under normal physiological condition, these ROS can be neutralized by antioxidants such as SOD and catalase (Berg *et al.* 2006, Deblier *et al.* 2006). Moreover, whether the CPB is used or not

during cardiac surgery, surgical trauma itself causes oxidative stress by endothelial dysfunction and blood cell activation (Prondzinsky *et al.* 2005, Berg *et al.* 2006).

In this study, we analyzed nitric oxide as a marker of oxidative stress during CPB in the patients undergoing cardiac surgery. Nitric oxide is one of gaseous transmitters together with carbon monoxide and hydrogen sulfide. In the cardiovascular system, nitric oxide is known as one of strong muscle cell relaxants through decreasing the intracellular calcium concentration. Even though nitric oxide is very important and beneficial gas to human body, the higher level of nitric oxide damages our body through inflammatory response (Hayashi *et al.* 2001, Ricciardolo *et al.* 2004, Deblieer *et al.* 2006, Pacher *et al.* 2007). Usually, nitric oxide is very easy to penetrate the cell membrane and highly reactive free radical gas. In the absence of other nitric oxide scavenger, the major breakdown product of nitric oxide in aqueous solutions is NO_x including nitrite (NO₂) and nitrate (NO₃) (Ricciardolo *et al.* 2004, Pacher *et al.* 2007). However, in the inflammatory condition where there is higher level of free radicals such as superoxide anion (O₂[•]) that is produced by phagocytic cells NADPH oxidase, nitric oxide formed peroxynitrite (ONOO[•]) that is one of radicals (Ricciardolo *et al.* 2004, Pacher *et al.* 2007). This peroxynitrite may decompose to form nitrite (NO₂) and hydroxyl radical (•OH) from peroxynitrous acid (ONOOH) or produce nitrate (NO₃) (Ricciardolo *et al.* 2004, Willcox *et al.* 2004, Pacher *et al.* 2007). Therefore, high level of NO_x is an indication of increased oxidative stress.

1.3.5. Other important factors associated with inflammation during CPB

CPB depends on two basic requirements: adequate blood volume to maintain appropriate blood flow and adequate gas flow to maintain appropriate gas exchange (Gravlee *et al.* 2000). Blood flow affects the growth of thrombi and the deposition of fibrin. Since patient's heart does not strongly beat during cardiac surgery, the pumps are needed to suction blood from patient's heart and supply blood to the body. Blood flow determines the rates of transport of cells and proteins to the artificial surface, but it also changes the level of receptor expression on platelets and leukocytes (Gorbet *et al.* 2004).

As platelets are an important part of the thrombus, the effect of shear stress by blood flow on platelets has been studied extensively. Higher shear stress results in higher platelet deposition and lower fibrin deposition, while at lower shear the inverse (Turitto *et al.* 1980, Hanson *et al.* 1998). Moreover, this shear stress activates blood cells such as neutrophils and monocytes as well as endothelial cells leading to inflammatory response (DiNardo *et al.* 2008).

Another important factor is air leading to microemboli. Gaseous and particle microemboli are a major cause for neuropsychological dysfunction after CPB procedure in pediatric and adult cardiac patients and a correlation between the number of intravascular microemboli detected during CPB and the incidence of postoperative brain injury, patient mortality and morbidity have been demonstrated (Borger *et al.* 2001, Stump 2005, Whitaker *et al.* 2006). Even though the air occurs through surgical procedure and anesthetic procedure, most of the air leading to emboli originates from the pump in CPB (Gravlee *et al.* 2000). In the very recent study with porcine model conducted by Bierbach and colleagues, it was demonstrated that emboli formation rather than inflammatory mediators are responsible for increased cerebral water content after conventional myocardial revascularization. In this study they demonstrated most of the emboli were generated after initiation of CPB, and determined and compared the cerebral water content (cerebral edema) and inflammatory mediators such as TNF- α , IL-6, and IL-8 between on-pump CABG and off-pump CABG (Bierbach *et al.* 2008)

1. 4. Development of biocompatible materials used in CPB

One of the most attractive areas in biomaterial research is the study of blood-biomaterial interactions (Figure 3). However, depending on the presence or absence of biocompatible materials in CPB circuits, there are very unique and clear distinctions between groups in terms of the level of inflammation. Therefore, in spite of much research and investigation for decades, we still do not fully understand the pathophysiological aspects of these interactions. In other words, the limitations, which are related to blood reactions at the biomaterial interface such as cell activation,

inflammation and thrombosis still exist and remain uncontrollable. We have seen and faced unwanted effects from using either biomaterials or biomaterials and cardiopulmonary bypass at the same time even though we may perfectly mimic the microenvironmental conditions in blood stream into the biomaterial coatings.

CPB, acts as heart-lung machine during cardiac surgery, provides fresh oxygen and removes the carbon dioxide from the patient's blood during operation time (Gravlee *et al.* 2000). In the earliest CPB device, both in vitro and in vivo circuits were prepared with stainless steel and glass. Polymers were first introduced for creating disposable circuits so that cleaning and re-sterilizing was not necessary. The biocompatibility was not much considered at that time (Rubens *et al.* 2002). To connect between patient's heart and CPB machine, we need a long and flexible tube that is made of polyvinyl chloride (PVC). To make this tube flexible, it needs adding plasticizer, di-2-ethyl-hexyl-phthalate (DEHP), but the problem is that this plasticizer causes strong inflammatory response. Gourlay demonstrated the expression level of CD11b on neutrophils decreased when they removed DEHP from the surface of the PVC. Also, this finding is consistent with previous studies. (Tsuji *et al.* 1984, Lakshmi *et al.* 1998, Gourlay 2001).

Now there are several kinds of biocompatible coating circuits available in market. Each biocompatible material has its unique design and effects during CPB (Gourlay *et al.* 2001, Rubens 2002, Gunaydin *et al.* 2004, Belway *et al.* 2006, Jordan *et al.* 2007). The most popular one with much research is the heparin coated. Basically, heparin is a co-factor which is involved in coagulation pathway. Heparin binds to anti-thrombin III and protects thrombin from being activated. Like intact endothelial cell surface, heparin-coated circuit has much negative charge on biomaterial surface, which is believed to inhibit thrombosis (Gourlay *et al.* 2001, Rubens 2002, Belway *et al.* 2006, Jordan *et al.* 2007). Even though there are three primary ways in which heparin may be bound to polymer surface, nowadays the heparin-immobilized technique becomes popular. In this technique, heparin is immobilized permanently upon the biomaterial surface (Rubens 2002). Since Larm *et al.*, first described the preparation of covalently bound surface heparin in 1983, heparin-coated circuit has been developed so far. Polyethylene oxide (PEO) was utilized as a spacer group as its hydrophilicity and its dynamic motion would

further inhibit platelet interactions with this surface (Rubens 2002, Belway *et al.* 2006, Jordan *et al.* 2007). In this technology group, Trillium™ (Medtronic, Minneapolis, MN) and Bioline™ (Jostra, Germany) are the examples. Another approach is a surface-modifying additive (SMA). Based on this technology, biomaterial has both polar and non-polar micro-domains on its surface (Gunaydin *et al.* 2004). Basically this coating material inhibits protein contact activation, and X coating™ (Terumo, Tokyo, Japan) is one of commercial product in this group. The concept of this biocompatible material involves creating localized alternating hydrophobic and hydrophilic microdomains on the blood-contacting surface. Surfaces are coated with poly (2-methoxyethylacrylate), which has hydrophobic polyethylene backbone, and its residue has mild hydrophilicity with no chemical functional groups such as –OH or –NH₂. Therefore, the outer side of the PMEA molecule becomes inactive chemically, and the artificial surface could not interact with blood cells easily. Also a competitive interaction whereby one microdomain inhibits the effect of the other is created (Rubens 2002, Belway *et al.* 2006, Jordan *et al.* 2007). Another novel approach that has been introduced recently for CPB is the designing biomaterials that mimic the non-thrombogenic nature of cardiovascular endothelium. The chosen substrate biomaterial is coated with a derivative of phosphorylcholine, which is the major lipid head group component presenting on the outer surface of biological cell membrane (Rubens 2002). In this group, we evaluated Memsys™ (Sorin Biomedica) in our study. Several studies have been conducted to evaluate each product feature in hemostasis but comprehensive comparison of these biomaterials is lacking (Tsai *et al.* 1994, Spijker *et al.* 1997, Gu *et al.* 1998, Rubens *et al.* 1999a, Rubens *et al.* 1999b, Wimmer *et al.* 1999, Somer *et al.* 2000, Hsu *et al.* 2001, Heyer *et al.* 2002, Somer *et al.* 2002a, Feyrer *et al.* 2003, Palatianos *et al.* 2003, Ikuta *et al.* 2004, Ueyama *et al.* 2004, Vroege *et al.* 2004, Vroege *et al.* 2005, Kutay *et al.* 2006, Kutay *et al.* 2006, Pappalardo *et al.* 2006).

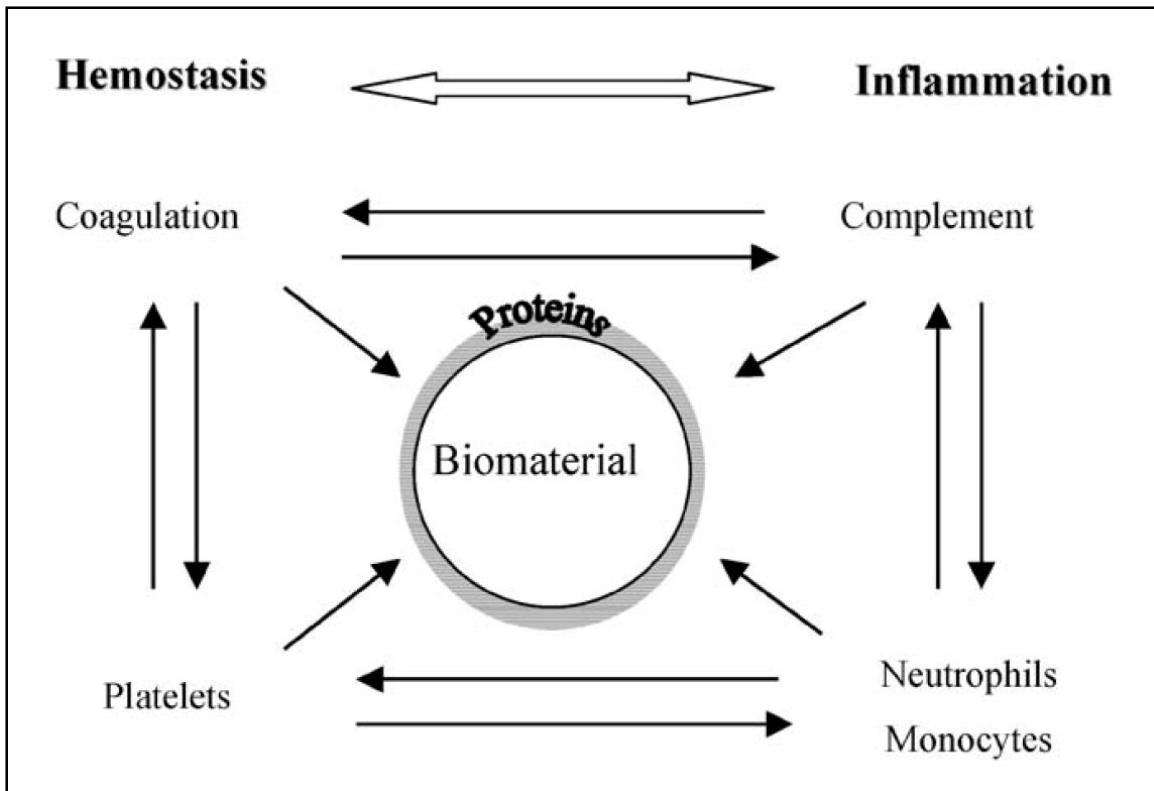


Figure 3. Overview of blood-material interactions (Gorbet *et al.* 2004).

2. OBJECTIVES

2.1. Release of cardiac markers during CABG with CPB

Determination of cardiac markers can assess cardiac injury induced by CPB during CABG. However, the markers and their release patterns are not well defined. There is little information on the release patterns and serum levels of cardiac biochemical, inflammatory, and oxidative stress markers during and after CABG with CPB. This study was aimed to assess the release and timing of cardiac biochemical and inflammatory markers in patients undergoing elective CABG with CPB.

2.2. Comparison of biocompatible materials used in CPB during CABG

Immune responses and inflammation can be induced by CPB machine which is coated with different biocompatible materials. However, there has been no study conducted to compare the effects of different biocompatibilities on initiation of inflammation at different time points. The aim of this study is to compare the impact of different biocompatible material coating circuit used in CPB on inflammatory response and oxidative stress in patients undergoing cardiac surgery

3. MATERIALS AND METHODS

3. 1. SUBJECTS

3.1.1 Patients population

3.1.1.1. Release of cardiac markers during CABG with CPB

This is a prospective cohort study that was approved by Research Ethics Boards of the University of Saskatchewan and Saskatoon Health Region. Patients scheduled to undergo elective CABG surgery with CPB at Royal University Hospital were recruited between February and June 2006. Written informed consents were obtained from all study participants. Exclusion criteria were: onset of acute MI within 2 weeks; non-elective cardiac surgery; CABG associated with any other cardiac surgical procedures such as valve repair or replacement, congestive heart failure (ejection fraction < 30%), and off-pump/beating heart CABG. Patients with a chronic inflammatory disease on steroid therapy or those were hemodynamically unstable were also excluded from this study. Hemodynamic instability was defined as the need to use intra-aortic balloon pump to keep the systolic blood pressure to at least 90 mmHg in the presence of symptoms of

low cardiac output. Patients with an intra-aortic balloon pump were not included in this study. Forty patients undergoing elective CABG were included in this study. Under the current experimental conditions and determinations, the sample size of 40 subjects was sufficient to detect a significant difference of 0.9 at a two-sided significance level of 0.05 and at a power of 0.8.

3.1.1.2. Comparison of biocompatible materials used in CPB during CABG

One hundred and seventy six subjects who were scheduled to undergo elective CABG with CPB in the Department of Cardiac Surgery at the Royal University Hospital were recruited for the study from August 2007 to April 2008. All subjects were classified into six different groups according to the types of biocompatible materials used in CPB: Trillium, Phosphocholine, Bioline, Hyaluronan, PMEA (Polymethoxyethyl acrylate), and the control group. The number of subjects assigned into each biocompatible group is as follows: 33 for Trillium coating, 31 for Bioline coating, 32 for Phosphocholine coating, 32 for PMEA coating, 10 for Hyaluroan coating, and 38 for the control without CPB coating. For Tau protein measurement, all 176 subjects were included. For inflammation assessment, 78 of the 176 subjects were selected for this study. Patients were randomly selected and grouped. The exact number of each group used for this thesis, 16 for Trillium coating, 16 for Bioline coating, 16 for Phosphocholine coating, 16 for PMEA coating and 14 for the control group. We did collect 234 serum samples from above mentioned 78 patients. We excluded Hyaluroan coating group in this study because of severe problem such as severe decrease of platelet counts when using this coating. This study was approved by Biomedical Research Ethics Board (Bio-REB) of the University of Saskatchewan. Written informed consents were obtained from all subjects undergoing cardiac surgery. All subjects selected are greater than 50 years of age with the usual morbidities associated with cardiac disease (increased cholesterol, hypertension, smoking, diabetes, previous myocardial infarctions). Subjects who are being admitted as emergent or who suffer from a chronic inflammatory disease (rheumatoid arthritis, systemic lupus arthrematosus, Chrohn's disease, Wegener's granulomatosis, sarcoidosis, osteoarthritis, chronic obstructive pulmonary disease, ulcerative colitis, psoriasis and multiple sclerosis) were excluded from this study. Also, subjects who have suffered a stroke or who have a

narrowing of the carotid arteries which might decrease blood flow to the brain were excluded.

3.1.2. Surgical technique

The operations were performed by two cardiac surgeons. Under anesthesia, CPB was instituted with an ascending aortic cannula and a two-stage right-atrial cannula. Internal mammary artery and saphenous vein grafts were used in all patients. Distal and proximal graft anastomoses were performed using a single cross-clamp technique. Myocardial protection was achieved using antegrade and/or retrograde cold blood cardioplegia. Mild systemic hypothermia with a core temperature of 33°C was maintained during CPB.

3.1.3. Samples collection

3.1.3.1. Release of cardiac markers during CABG with CPB

Blood samples were collected from each patient before the induction (T0), 1 hour after starting of CPB (T1), 6 hours after starting of CPB (T2), 12 hours after starting of CPB (T3), and 24 hours after starting of CPB (T4). Blood samples were collected into tubes containing no anticoagulant and serum was prepared. For lactate measurement, blood samples were collected into sodium fluoride/potassium oxalate tube and immediately cooled to 4°C. After preparation, specimens were frozen and stored at -80°C if not immediately analyzed.

3.1.3.2. Comparison of biocompatible materials used in CPB during CABG

Blood is drawn from each patient at three different time points: before CPB (T1), 6 hours after surgery (T2), and 72 hours after surgery (T3). The first two samples of blood were drawn from an existing intravenous (IV) site and the third blood sample was

drawn from either an indwelling IV site or a vein. All blood samples were collected into tubes containing no anticoagulant and then serum was prepared after centrifugation. All serum samples were transferred into small cryogenic vials, and frozen, and then stored at -80 °C if not analyzed immediately.

3.2. METHODS

3.2.1 Biochemical analyses

3.2.1.1. Release of cardiac markers during CABG with CPB

Cardiac biochemical markers including total CK, CK-MB, lactate, and LDH were run on Beckman Synchron LX20 (Beckman, Palo Alto, CA). The upper limit of normal (ULN) for CK was 200 U/L, CK-MB 15 U/L, lactate 2.4 mmol/L, and LDH 200 U/L, respectively. Serum cTnI was measured using microparticle enzyme immunoassay (MEIA) technology on Abbott AxSYM (Abbott Laboratories, Abbott Park, IL) (ULN < 0.4 µg/L). Serum CK-MB mass was determined by MEIA on Abbott AxSYM (Abbott Laboratories, Abbott Park, IL) and the ULN was set at 6 µg/L. Serum hsCRP levels were determined using a near infrared particle immunoassay on Beckman Synchron LX20 (Beckman, Palo Alto, CA). The ULN for hsCRP was 7.0 mg/L. To evaluate the inflammatory response, serum concentrations of the proinflammatory cytokines TNF- α and IL-6 were measured by enzyme-linked immunosorbent assay (ELISA) using commercial ELISA kits (R&D Systems, Minneapolis, MN). The ULN for Both TNF- α and IL-6 was 5.0 ng/L. The AAT and ceruloplasmin were quantitated by nephelometry on Array (Beckman, Palo Alto, CA). The ULN for AAT and ceruloplasmin were 2.00 g/L and 630 mg/L, respectively. During CPB, moderate hemodilution with hematocrit level maintained between 20% and 25%. The results were not corrected for hemodilution, since we intended to document in vivo levels as organ function and the occurrence of post-operative complications appear to depend first and foremost on actual effective

concentrations. The UNL (upper limit of normal) unit of different analytes was used to compare the release patterns upon time on the same graph. Also the data of TNF- α and IL-6 were expressed as nanogram per liter (ng/L), not default unit such as picogram per milliliter (pg/ml), to show with hsCRP on the same graph.

3.2.1.2. Comparison of biocompatible materials used in CPB during CABG

Serum hsCRP levels were determined using a near infrared particle immunoassay on Beckman Synchron LX20 (Beckman, Palo Alto, CA). Tau protein was assayed by ELISA (BioSource International, Camarillo, CA). Serum samples were assayed for TNF- α , IL-6, IL-10, IFN- γ (R & D systems, Minneapolis, MN) and Nitric oxide (Cayman Chemical Company, Ann Arbor, MI) in duplicate using a commercially available ELISA kits. All tests were performed by following the each ELISA kit's manual and got the results by using 4-parameter-algorithm method when getting an internal standard curve fit. The data of oxidized nitric oxide was obtained with linear regression curve fit, not 4-parameter-algorithm method. The analytical detection limits of those items are 12 pg/ml for tau, 1.6 pg/ml for TNF- α , 0.7 pg/ml for IL-6, 3.9 pg/ml for IL-10, 8.0 pg/ml for IFN- γ , and 2.5 umol/l for nitric oxide.

3.2.2. Statistical analysis

In the study of evaluating the release of cardiac markers during CABG with CPB, results are expressed as mean \pm SEM or as folds over each individual analyte ULN (Upper limit of normal). Data were analyzed using analysis of variance (ANOVA) and the difference between the means of the two time points was compared using Student's *t* test. Another study, comparison of biocompatible materials used in CPB during CABG, results are expressed as mean \pm SD (standard deviation). Then data were analyzed using analysis of variance (ANOVA) and the difference between the means of the three time points was compared using Student's *t* test. We compared *p* values between biocompatible material groups at the same time points in addition to the different time points of each group. In both studies, statistical significance was considered when *p* value was less than 0.05.

4. RESULTS AND DISCUSSION

4.1. Patient characteristics

4.1.1. Release of cardiac markers during CABG with CPB

A total of 40 patients underwent elective CABG with the use of CPB were included in this study. The patients ranged in age from 30-75 (60 ± 8) years with body mass index 26.8 ± 3.4 kg/m². Of these, 33 were male and 7 were female. Ten (25%) patients had diabetes mellitus, 32 (80%) with hypertension history, and 34 (85%) on statins therapy. Fasting plasma glucose levels were 6.5 ± 0.7 mol/L. None of the patients in this study group had renal failure or chronic obstructive pulmonary disease. The number of diseased vessels ranged from 1-5 with 1-4 grafts completed at the time of surgery. Complete revascularization was achieved in all patients. Duration of CPB and aortic cross-clamping was 90 ± 20 and 80 ± 20 minutes, respectively. Eight patients required defibrillation after removal of the aortic cross-clamp. One patient required re-exploration for bleeding after operation. There was no death or major complication in these patients. The length of stay in intensive care unit and hospital was 18 hours and 4 days, respectively. All baseline levels of cardiac markers such as cTnI, CK, CK-MB were within the normal reference intervals. None of them had suffered an acute MI prior to CABG with CPB.

4.1.2. Comparison of biocompatible materials used in CPB during CABG

There were 176 patients enrolled in this prospective study. Six groups were formed. Serum hsCRP and tau protein were analyzed from 176 patients' samples while only 78 patients in five groups excluding hyaluronan group were included for analyses of TNF- α , IL-6, IL-10, IFN- γ , and oxidized nitric oxide. There were no statistically significant differences between the groups with respect to age, gender, body mass index, and operative data such as total bypass time and amounts of heparin and protamine dose. Furthermore, the risk factors such as previous myocardial infarction, hypertension,

hypercholesterolemia, diabetes mellitus, smoking as well as medication history were analyzed by chi-square test. Trillum and Bioline groups showed significant differences in hypercholesterolemia and Phosphocholine group showed a significant difference in diabetes mellitus compared to the control group ($p < 0.05$ Table 1).

Table 1. Patients' demographic and operative data (mean \pm standard deviation)

Variable	Control (14)	Trillum (16)	Bioline (16)	Phosphocholine (16)	PMEA (16)
Age (years)	63.6 \pm 8.8	63.4 \pm 9.4	67.9 \pm 9.7	63.4 \pm 11.0	64.7 \pm 10.5
Male/female (number)	8/6	12/4	11/5	12/4	11/5
Body mass index (BMI)	30.3 \pm 4.4	30.9 \pm 5.0	30.5 \pm 6.2	30.4 \pm 3.9	31.1 \pm 3.7
Previous MI	7/14 (50.0%)	8/16 (50.0%)	9/16 (56.3%)	9/16 (56.3%)	8/16 (50.0%)
Hypertension	10/14 (71.4%)	14/16 (87.5%)	14/16 (87.5%)	11/16 (68.8%)	11/16 (68.8%)
Hypercholesterolemia	6/14 (42.9%)	13/16 (81.3%)*	14/16 (87.5%)*	9/16 (56.3%)	11/16 (68.8%)
Diabetes mellitus	3/14 (21.4%)	6/16 (37.5%)	6/16 (37.5%)	12/16 (75.0%)*	1/16 (6.3%)
Smoking	3/14 (21.4%)	5/16 (31.3%)	4/16 (25.0%)	3/16 (18.8%)	2/16 (12.5%)
Acetyl salicylic acid	9/14 (64.3%)	9/16 (56.3%)	13/16 (81.3%)	13/16 (81.3%)	9/16 (56.3%)
Clopidogrel	2/14 (14.3%)	1/16 (6.3%)	0/16 (0.0%)	3/16 (18.8%)	1/16 (6.3%)
Total bypass time (min)	110.7 \pm 25.6	117.1 \pm 39.5	117.9 \pm 31.1	104.2 \pm 29.3	109.6 \pm 36.7
Aortic cross clamp time (min)	86.9 \pm 18.0	94.4 \pm 33.1	94.3 \pm 24.9	80.6 \pm 27.0	85.7 \pm 25.2
Ejection fraction (%)	56.9 \pm 11.2	54.3 \pm 13.1	58.9 \pm 8.4	59.9 \pm 8.2	57.1 \pm 12.4
Total heparin dose (IU)	42786 \pm 9705	46000 \pm 12044	44625 \pm 10308	45125 \pm 12559	43375 \pm 8115
Total protamine dose (mg)	364 \pm 58	370 \pm 103	370 \pm 99	360 \pm 96	363 \pm 80

4.2. Serum levels of cardiac biochemical, inflammatory, and oxidative stress markers during CABG with CPB

Serum levels of cardiac biochemical markers as well as inflammatory markers during CABG with CPB were provided in Table 2. All data is expressed as mean \pm SEM with different time points: T0 (Before induction), T1 (1 hour after CPB), T2 (6 hours after CPB), T3 (12 hours after CPB), and T4 (24 hours after CPB). Single asterisk (*) stands for p value is less than 0.05 and double asterisks (**) stands for p value is less than 0.01 versus their corresponding baseline values.

Table 2. Serum levels of cardiac biochemical, inflammatory, and oxidative stress markers after CABG

Biomarkers	T0	T1	T2	T3	T4
CK (U/L)	95.40 (13.20)	110.00 (13.05)	411.60 (61.27)**	523.00 (31.03)**	695.20 (81.41)**
CK-MB (U/L)	6.60 (0.79)	21.00 (3.10)*	29.70 (3.79)**	25.90 (2.98)**	17.67 (1.70)*
CK-MB mass (μ g/L)	1.25 (0.26)	4.63 (0.64)*	21.38 (3.70)**	15.10 (2.01)**	13.00 (1.81)**
cTnI (mg/L)	0.02 (0.00)	0.73 (0.34)*	4.78 (1.63)**	6.12 (1.68)**	3.38 (0.92)**
Lactate (mmol/L)	1.12 (0.10)	1.15 (0.07)	3.12 (0.21)**	2.97 (0.18)**	2.58 (0.25)*
LDH (U/L)	86.67 (7.74)	98.33 (14.94)	166.67 (8.32)*	230.50 (18.26)**	165.50 (11.36)*
TNF- α (ng/L)	4.28 (0.59)	8.24 (1.13)*	10.35 (1.35)**	8.83 (1.29)*	6.70 (0.85)*
IL-6 (ng/L)	0.45 (0.14)	14.62 (1.54)**	32.87 (2.49)**	14.68 (2.12)**	2.39 (0.71)*
hsCRP (mg/L)	3.70 (0.75)	3.22 (0.85)	3.72 (0.98)	13.98 (1.95)**	106.92 (13.81)**
AAT (g/L)	1.83 (0.10)	1.08 (0.09)	1.30 (0.18)	1.75 (0.13)	2.09 (0.22)*
Ceruloplasmin (mg/L)	389.60 (21.73)	227.10 (16.51)	273.10 (36.92)	339.20 (33.80)	347.20 (37.13)

4.2.1. Serum levels of CK, CK-MB, CK-MB mass, and cTnI after CPB

Serum levels of CK, CK-MB, CK-MB mass, and cTnI increased significantly after CPB (Figure 4 and Table 2). Serum CK levels started to increase significantly 6 hours after initiation of CPB ($p < 0.01$) and continued to increase until 24 hours after CPB ($p < 0.01$). Serum CK-MB levels increased 1 hour after CPB ($p < 0.05$) and reached the peak 6 hours after CPB ($p < 0.01$). Serum CK-MB remained elevated until 24 hours after CPB ($p < 0.05$). Serum CK-MB mass was released in a pattern similar to CK-MB. Serum CK-MB mass significantly increased 1 hour after CPB ($p < 0.05$) and reached the maximum 6 hours after CPB ($p < 0.01$). Serum cTnI levels were significantly increased 1 hour after CPB ($p < 0.05$) and reached the maximum 12 hours after CPB. Serum cTnI levels remained elevated until 24 hours after CPB ($p < 0.01$). Serum cTnI had the highest magnitude of increase compared to other cardiac markers.

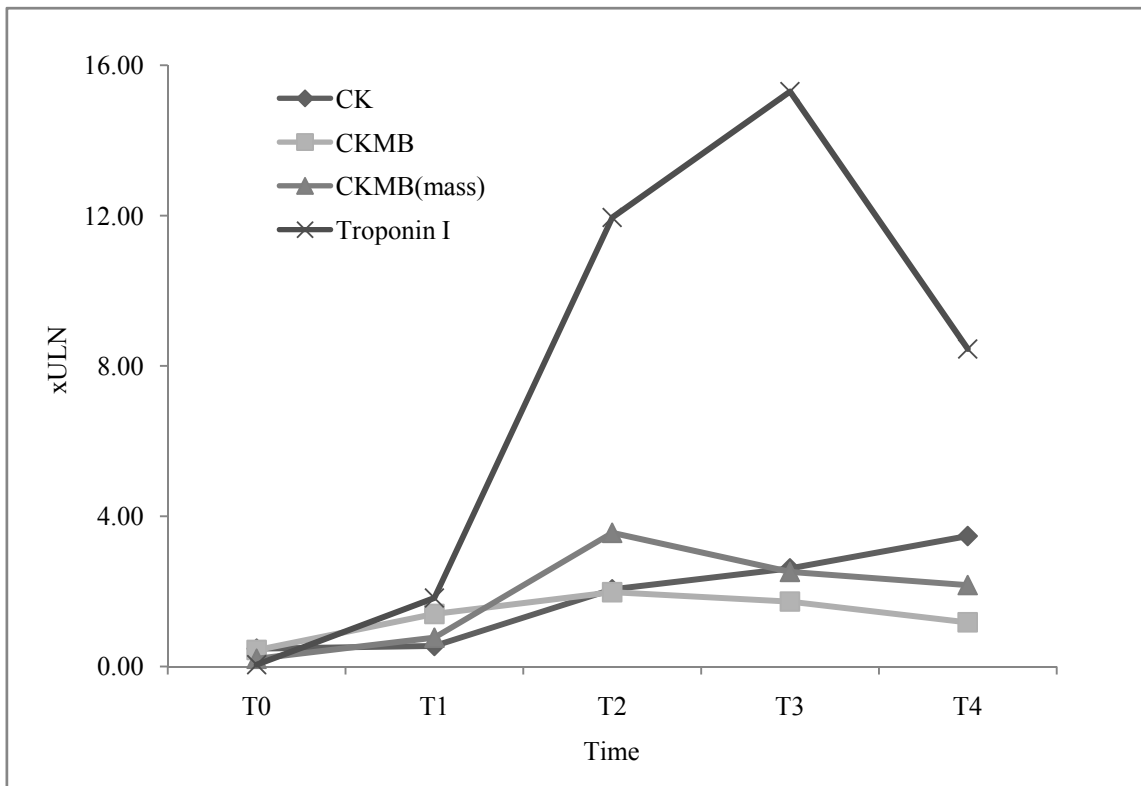


Figure 4. Serum levels of CK, CK-MB, CK-MB mass, and cTnI after CPB.

4.2.2. Serum levels of lactate and LDH after CPB

Serum levels of lactate and LDH increased significantly after CPB (Figure 5 and Table 2). Serum lactate levels increased significantly 6 hours after CPB ($p < 0.01$) and the elevated levels of lactate remained for over 24 hours after CPB ($p < 0.05$). Serum LDH levels significantly increased 6 hours after CPB ($p < 0.01$) and reached the maximum 12 hours after CPB. Thereafter, the serum levels of LDH gradually declined but remained significantly elevated until 24 hours after CPB.

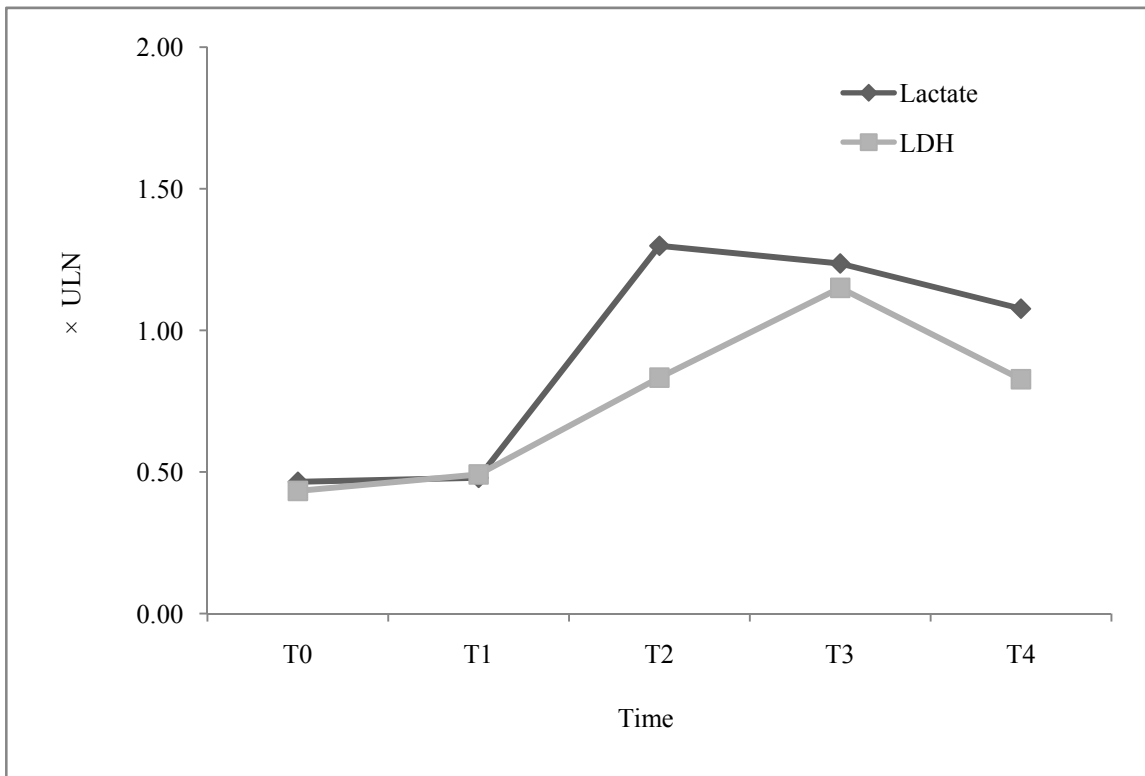


Figure 5. Serum levels of lactate and LDH after CPB.

4.2.3. Serum levels of TNF- α , IL-6, and hsCRP after CPB

Serum levels of TNF- α and IL-6 significantly increased 1 hour and reached the peak 6 hours after initiation of CPB ($p < 0.01$). The release of these markers then gradually decreased but remained elevated until 24 hours after initiation of CPB (Figure 6 and Table 2). Serum levels of hsCRP started to increase at 12 hours after initiation of CPB ($p < 0.01$) and continued to increase 24 hours after CPB (Figure 6 and Table 2).

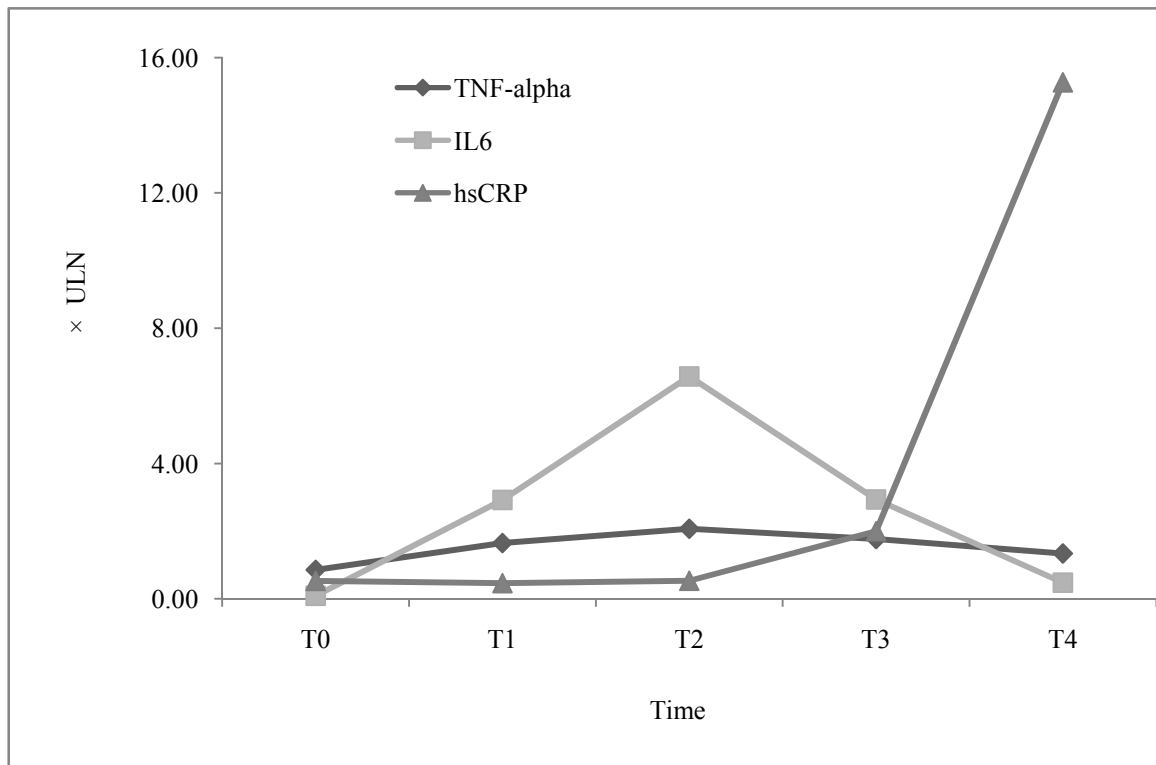


Figure 6. Serum levels of TNF- α , IL-6, and hsCRP after CPB.

4.2.4. Serum levels of AAT and ceruloplasmin after CPB

Serum AAT levels decreased 1 hour after CPB and remained at lower levels until 6 hours after CPB (Figure 7 and Table 2). At 12 hours after CPB, the levels of AAT increased comparably to the baseline level and reached significantly higher level than the baseline level 24 hours after CPB ($p < 0.05$). Serum ceruloplasmin levels decreased after CPB and reached the baseline level 24 hours after CPB (Figure 7 and Table 2).

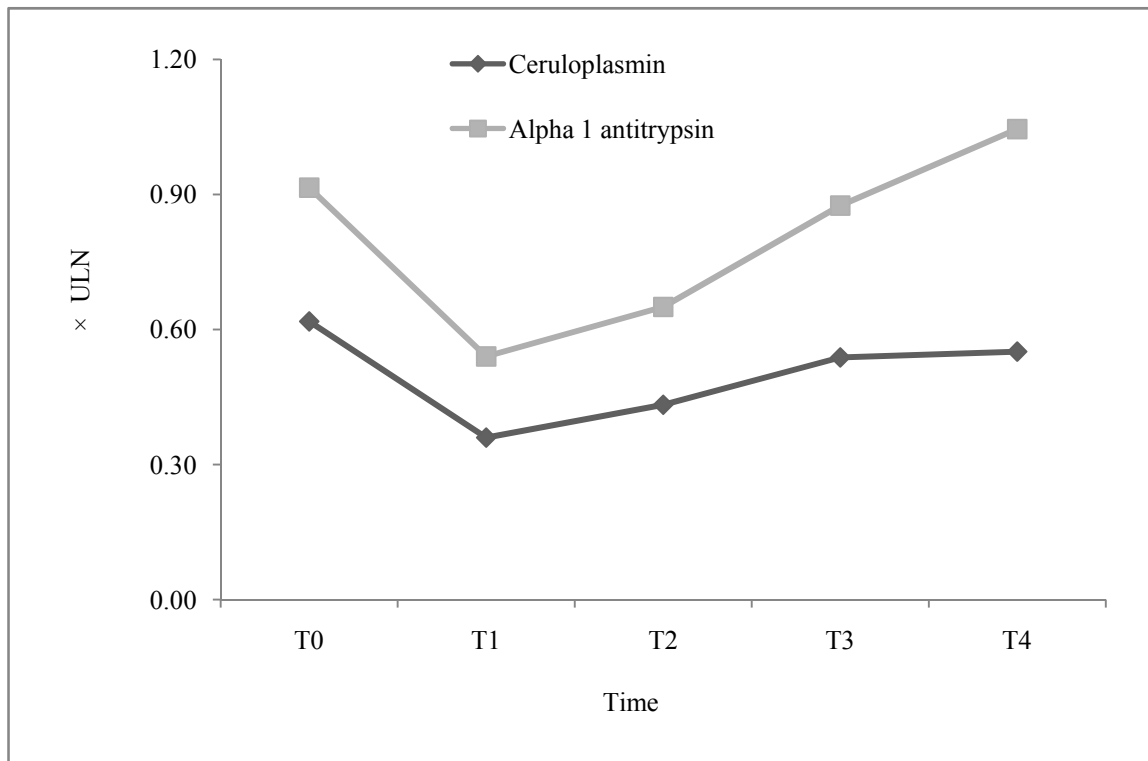


Figure 7. Serum levels of AAT and ceruloplasmin after CPB.

4.3. Comparison of biocompatible materials used in CPB during CABG

4.3.1. Serum Tau protein presence during CABG with CPB coated with different biomaterials

Total tau results were expressed as a dichotomous variable such as presence or absence of the protein in serum. So the tau protein result is defined as positive if the tau protein level is over the cut-off (25 pg/mL). As shown in Table 3, the percentage of the presence of tau protein increased after during CPB and then generally decreased 72 hours after CPB. Furthermore, after 6 hours after surgery (T2), tau protein was less detected in the heparin-coated groups such as Trillium and Bioline compared to other biocompatible groups employing new and recent technology such as Phosphocholine, and PMEA groups. In contrast, the control group without using biocompatible material in CPB circuit showed lowest presence of tau protein in both T2 and T3 stages except T1. Statistical analysis of tau protein presence for each group and at the different time points could not be applied because tau protein analysis was not quantitative but qualitative.

Table 3. Serum Tau protein presence during CABG with CPB coated with different biomaterials

Group	Time points	No. of total patients	No. of positive	Percentage*
Control	T1	38	5	13%
	T2	38	8	21%
	T3	38	6	16%
Trillium	T1	33	4	12%
	T2	33	8	24%
	T3	33	5	15%
Bioline	T1	31	3	10%
	T2	31	9	29%
	T3	31	6	19%
Phosphocholine	T1	32	8	25%
	T2	32	12	38%
	T3	32	7	22%
PMEA	T1	32	9	28%
	T2	32	15	47%
	T3	32	11	34%

Abbreviations: T1 (Before CPB), T2 (6 hrs post OP), and T3 (72 hrs post OP). * The percentage means the ratio of positive reaction, presence of tau protein, out of total patients in each group at different time points.

4.3.2. Serum TNF- α levels during CABG with CPB coated with different biomaterials

Serum levels of TNF- α gradually increased after initiating CPB and continued to increase 72 hours after cardiac surgery in all groups. Depending on the biocompatible material and the time after initiation of CPB, the increment of serum TNF- α levels was different (Figure 8). As shown in Table 4, the levels of TNF- α in Trillium group were significantly increased at different time points ($p < 0.05$). Serum levels of TNF- α in Bioline and Phosphocholine groups significantly increased only 72 hrs after CPB ($p < 0.05$). However, there was no statistical significance in comparison of p values of TNF- α between groups at different time points.

Table 4. Comparison of TNF- α levels in patients underwent CABG with CPB coated with different biocompatible materials at different time points

TNF- α	T1 (Before CPB)	T2 (6 hrs post OP)	T3 (72 hrs post OP)
Control	1.82 \pm 4.18	2.40 \pm 4.57	2.85 \pm 3.55
Trillium	1.30 \pm 2.97	4.04 \pm 7.28*	4.48 \pm 5.51 [†]
Bioline	2.05 \pm 2.49	2.11 \pm 3.10	7.10 \pm 7.85 ^{†§}
Phosphocholine	1.45 \pm 2.93	1.65 \pm 2.42	3.37 \pm 1.99 ^{†§}
PMEA	2.34 \pm 2.36	3.37 \pm 6.98	6.12 \pm 8.96

expressed as mean \pm SD; t-Test (tails:two-tailed distribution, type:paired).

* significant difference between T2 and T1 ($p < 0.05$).

[†] significant difference between T3 and T1 ($p < 0.05$).

[§] significant difference between T3 and T2 ($p < 0.05$).

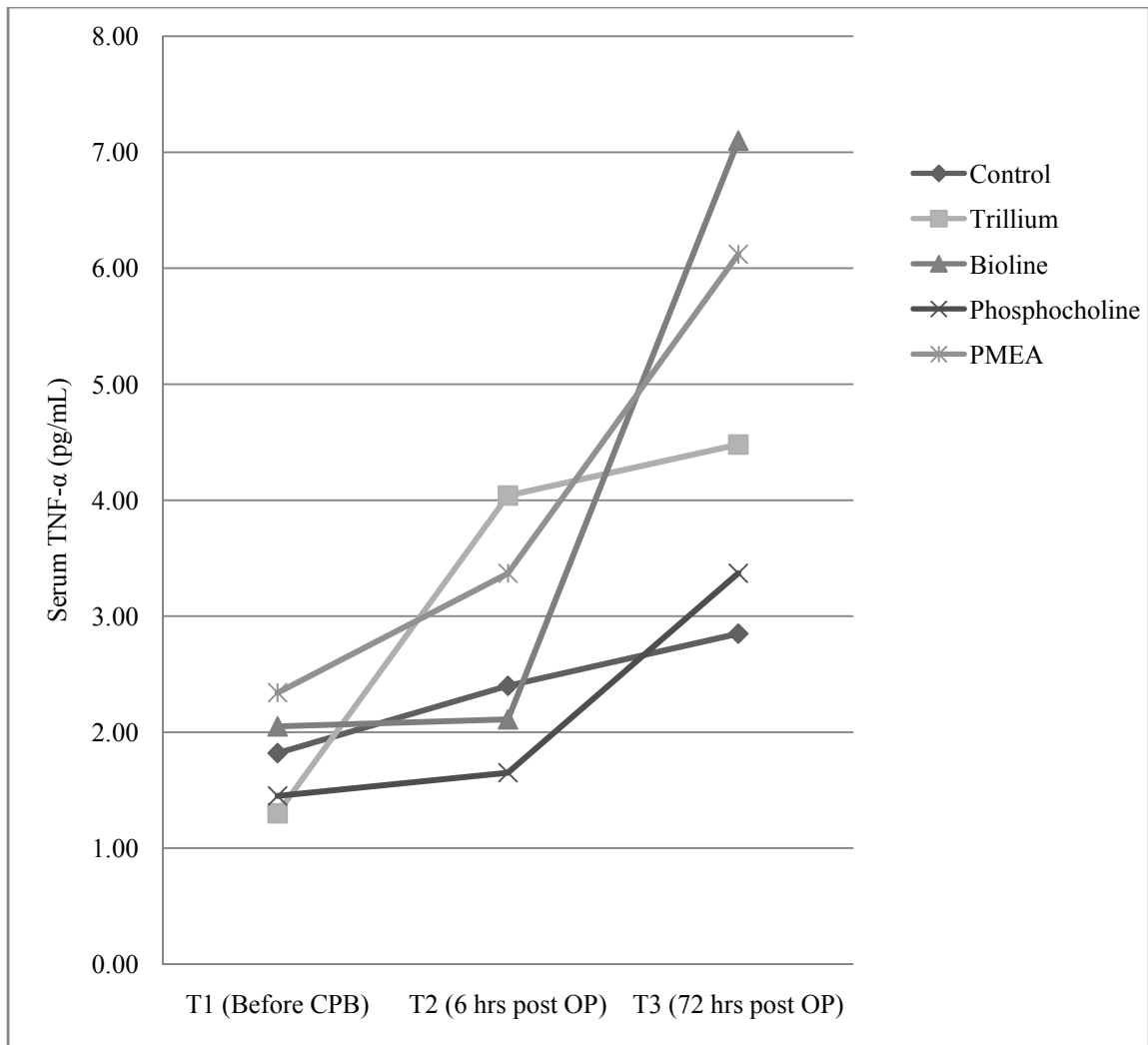


Figure 8. Serum TNF- α levels during CABG with CPB coated with different biomaterials.

4.3.3. Serum IL-6 levels during CABG with CPB coated with different biomaterials

Serum levels of IL-6 increased after initiation of CPB and gradually decreased but remained elevated after termination of CPB by 72 hours after cardiac surgery. The serum levels of IL-6 before the initiation of CPB did not differ significantly among four different biocompatible groups as well as the control group (Figure 9). All groups showed statistical increases in IL-6 levels at T2 (6 hrs post OP) and T3 (72 hrs post OP) in comparison of T1 (Before CPB) ($p < 0.01$ with exception of $p < 0.05$ in Trillium group at T3 vs. T2) (Table 5). However, there was no statistical significance in comparison of p values of IL-6 between groups at different time points.

Table 5. Comparison of IL-6 levels in patients underwent CABG with CPB coated with different biocompatible materials at different time points

IL-6	T1 (Before CPB)	T2 (6 hrs post OP)	T3 (72hrs post OP)
Control	1.82 ± 2.46	187.48 ± 133.91 ^{**}	55.61 ± 32.57 ^{††§§}
Trillium	3.59 ± 7.00	226.97 ± 165.83 ^{**}	102.87 ± 131.68 ^{††§}
Bioline	4.40 ± 6.80	164.94 ± 84.81 ^{**}	58.34 ± 28.93 ^{††§§}
Phosphocholine	3.59 ± 4.75	168.39 ± 100.49 ^{**}	60.77 ± 69.03 ^{††§§}
PMEA	16.37 ± 55.27	206.26 ± 150.66 ^{**}	61.14 ± 68.77 ^{§§}

expressed as mean ± SD; t-Test (tails:two-tailed distribution, type:paired).

** significant difference between T2 and T1 ($p < 0.01$).

†† significant difference between T3 and T1 ($p < 0.01$).

§§ significant difference between T3 and T2 ($p < 0.01$).

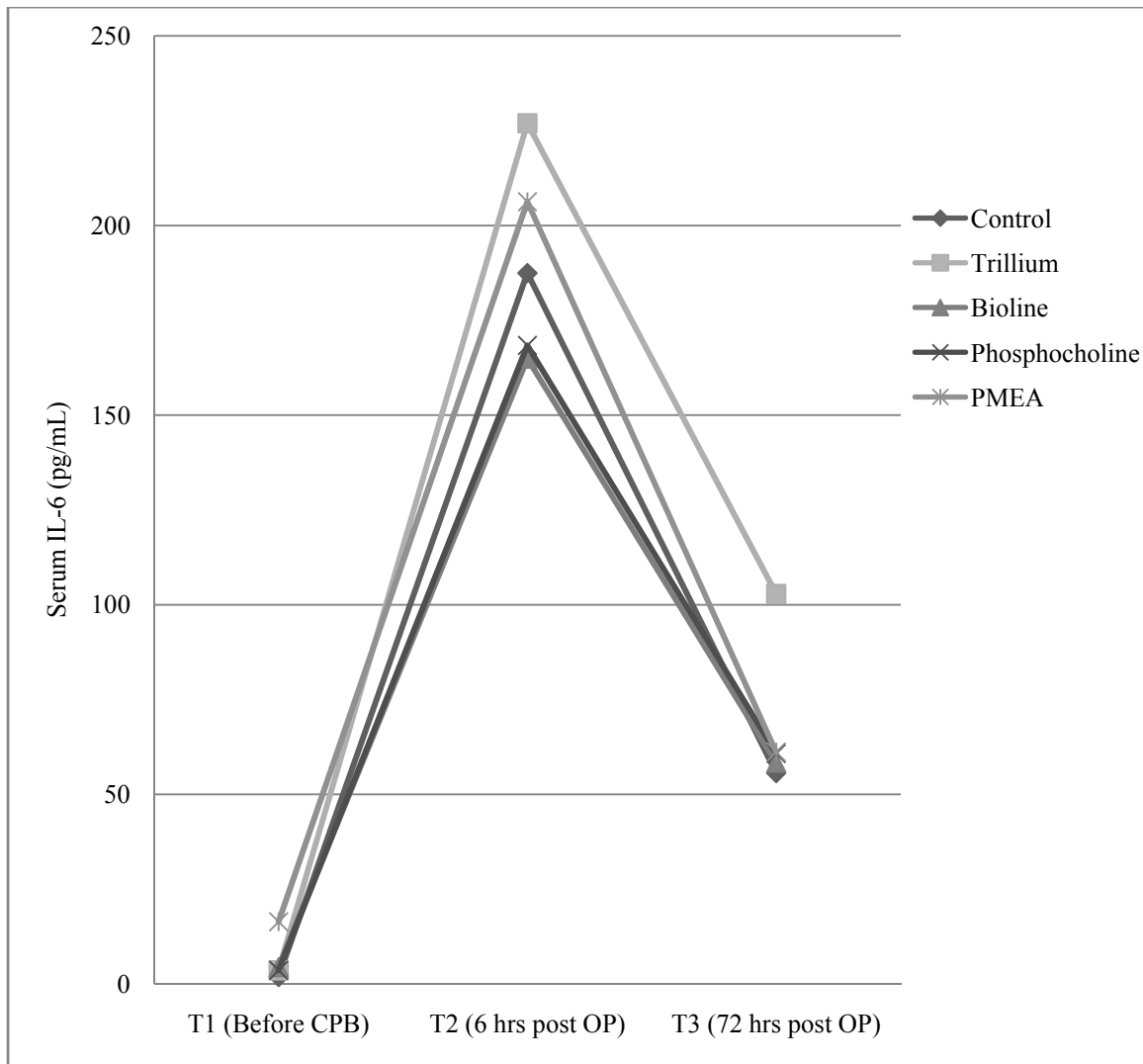


Figure 9. Serum IL-6 levels during CABG with CPB coated with different biomaterials.

4.3.4. Serum IL-10 levels during CABG with CPB coated with different biomaterials

Serum levels of IL-10 increased after initiation of CPB and gradually decreased but remained elevated after termination of CPB by 72 hours after cardiac surgery. The serum levels of IL-10 before the initiation of CPB did not differ significantly among four different biocompatible groups as well as the control group (Figure 10). All groups showed statistical increases in IL-10 levels between T2 (6 hrs post OP) and T1 (Before CPB) (PMEA: $p < 0.05$, others: $p < 0.01$). Serum levels of IL-10 at T3 (72 hrs post OP) decreased compared with those at T2 (6 hrs post OP) ($p < 0.01$) but continued to be highly elevated compared to that of T1 ($p < 0.01$) with exception of PMEA group (Table 6). Both Trillium and Bioline groups showed significant increases of IL-10 levels when compared to the control group at time point T3 (72 hrs post OP) ($p < 0.05$).

Table 6. Comparison of IL-10 levels in patients underwent CABG with CPB coated with different biocompatible materials at different time points

IL-10	T1 (Before CPB)	T2 (6 hrs post OP)	T3 (72 hrs post OP)
Control	11.48 ± 4.14	87.24 ± 67.10**	25.49 ± 8.22 ^{††§§}
Trillium	13.00 ± 4.01	97.98 ± 75.10**	37.30 ± 18.66 ^{††§§}
Bioline	9.15 ± 6.35	61.37 ± 32.72**	25.69 ± 7.77 ^{††§§}
Phosphocholine	10.91 ± 21.56	101.83 ± 75.70**	26.00 ± 13.84 ^{††§§}
PMEA	10.70 ± 6.11	110.12 ± 147.58*	48.41 ± 84.88 ^{§§}

expressed as mean ± SD; t-Test (tails:two-tailed distribution, type:paired).

* significant difference between T2 and T1 ($p < 0.05$).

** significant difference between T2 and T1 ($p < 0.01$).

†† significant difference between T3 and T1 ($p < 0.01$).

§§ significant difference between T3 and T2 ($p < 0.01$).

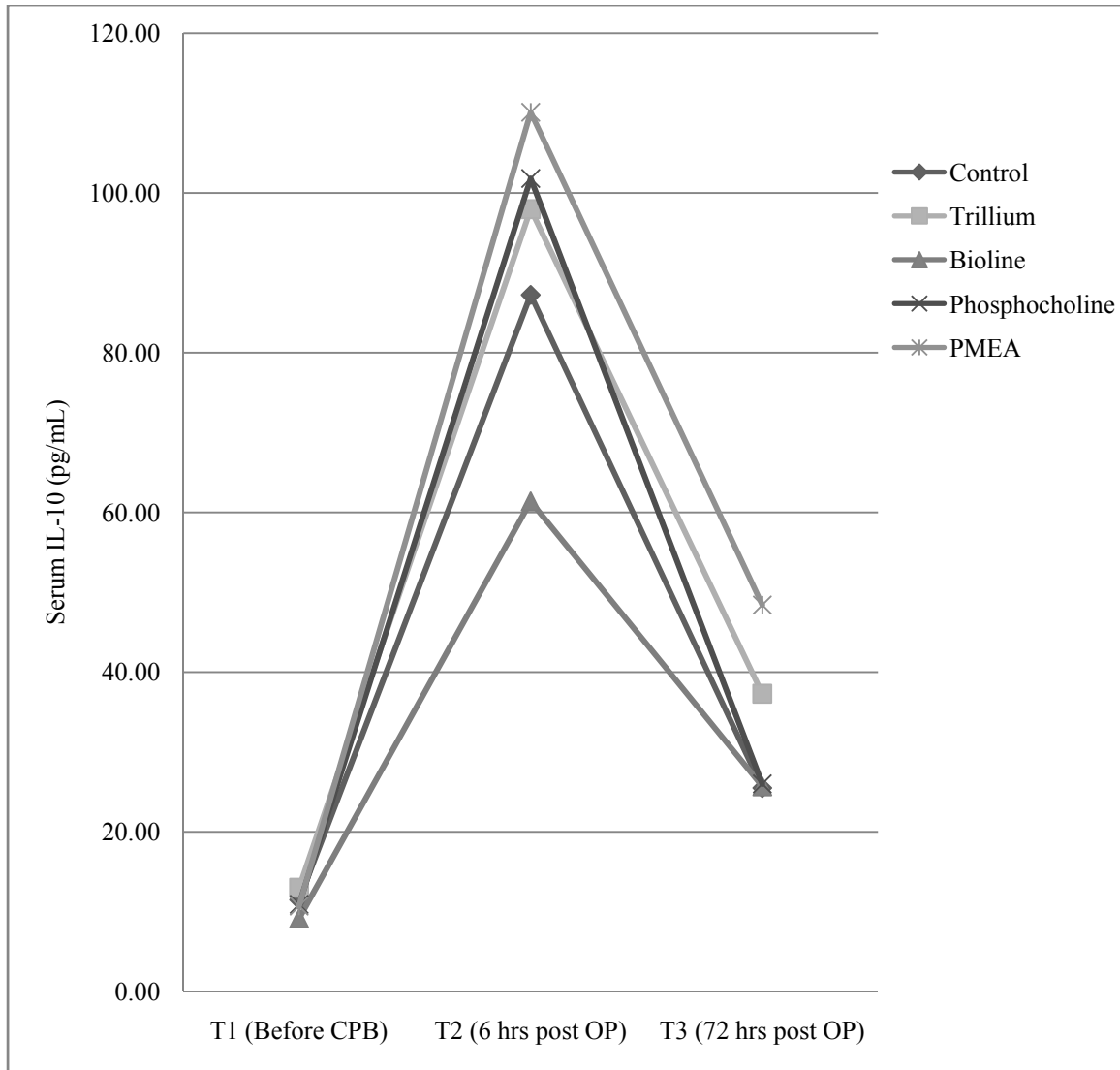


Figure 10. Serum IL-10 levels during CABG with CPB coated with different biomaterials.

4.3.5. Serum IFN- γ levels during CABG with CPB coated with different biomaterials

No statistical differences were found in the level of serum IFN- γ in patients undergoing cardiac surgery with CPB. Moreover, there was no statistical difference between biocompatible groups as well as within same group at different time points. There was no patient in Bioline group who showed serum IFN- γ , and only one or two patients out of 14 or 16 patients showed low levels of serum IFN- γ in other groups.

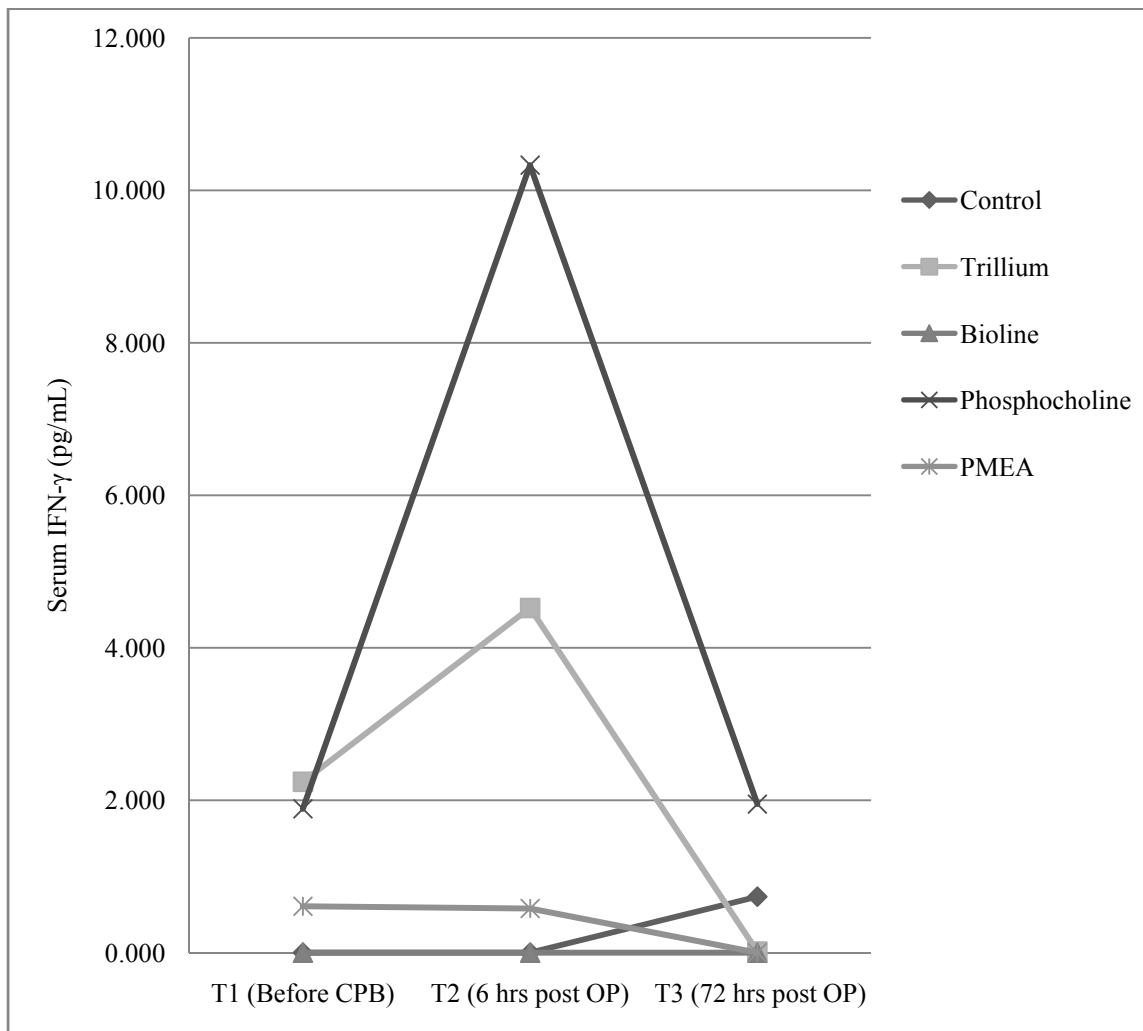


Figure 11. Serum IFN- γ levels during CABG with CPB coated with different biomaterials.

4.3.6. Serum Nitric oxide levels during CABG with CPB coated with different biomaterials

Serum levels of oxidized nitric oxide (nitrite + nitrate) before the initiation of CPB did differ among four different biocompatible groups as well as the control group. With exception of PMEA group, all other groups showed the decrease of the serum levels of oxidized nitric oxide to different degree in a timely dependent matter (Figure 12). Interestingly, as shown in Table 7, Phosphocholine group showed a significant decrease between T2 (6 hrs post OP) and T1 (Before CPB) ($p < 0.05$) while PMEA group showed a statistical increase in the same time points ($p < 0.05$). Both Bioline and Phosphocholine groups showed statistical decreases in the levels of serum nitrite and nitrate compared with the control group at T2 (6 hrs post OP) ($p < 0.05$).

Table 7. Comparison of nitric oxide levels in patients underwent CABG with CPB coated with different biocompatible materials at different time points

NOx	T1 (Before CPB)	T2 (6 hrs post OP)	T3 (72 hrs post OP)
Control	39.00 ± 12.95	38.69 ± 16.11	33.36 ± 12.99
Trillium	33.05 ± 14.64	33.22 ± 17.75	25.34 ± 12.02 [†]
Bioline	27.16 ± 4.65	25.05 ± 3.00	18.97 ± 9.05
Phosphocholine	33.05 ± 9.53	26.05 ± 6.32 [*]	25.41 ± 12.63
PMEA	30.33 ± 15.55	36.95 ± 23.28 [*]	24.11 ± 8.16 [§]

expressed as mean ± SD; t-Test (tails:two-tailed distribution, type:paired).

* significant difference between T2 and T1 ($p < 0.05$).

† significant difference between T3 and T1 ($p < 0.05$).

§ significant difference between T3 and T2 ($p < 0.05$).

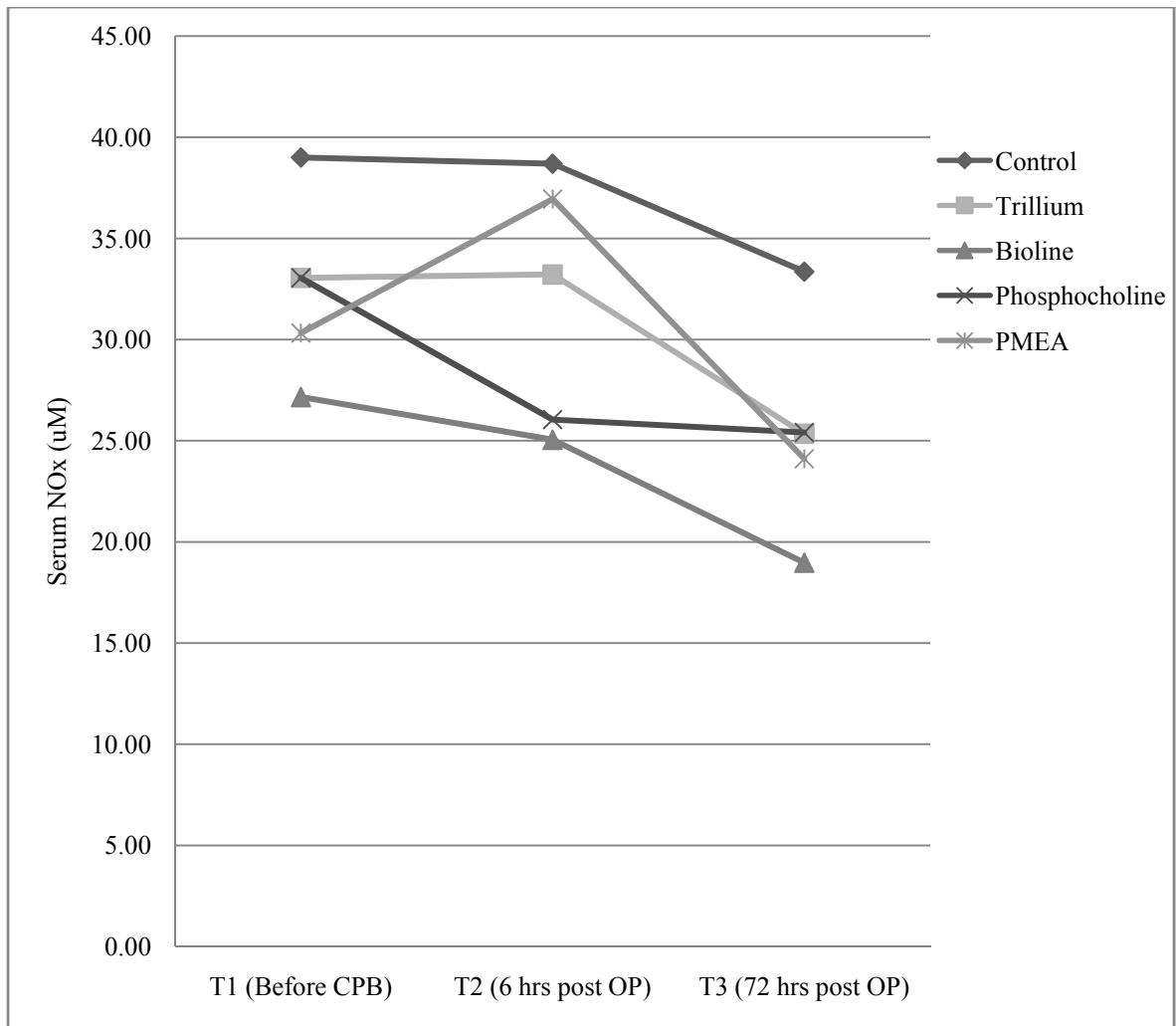


Figure 12. Serum Nitric oxide levels during CABG with CPB coated with different biomaterials.

4.3.7. Serum hsCRP levels during CABG with CPB coated with different biomaterials

Serum hsCRP levels were significantly increased in all groups between T2 (6 hrs post OP) and T1 (Before CPB) ($p < 0.01$) except PMEA group (Figure 13). Furthermore, serum hsCRP levels increased to a higher degree at T3 (72 hrs post OP) compared to T1 and T2 (6 hrs post OP) ($p < 0.01$) in all groups. When comparing the hsCRP levels between groups at different time points, only Trillium group had a statistical significance at 6 hrs after surgery (T2) compared with the control group ($p < 0.05$).

Table 8. Comparison of hsCRP levels in patients underwent CABG with CPB coated with different biocompatible materials at different time points

hsCRP	T1 (Before CPB)	T2 (6 hrs post OP)	T3 (72 hrs post OP)
Control	4.2 ± 6.4	9.5 ± 5.2**	192.8 ± 84.1 ^{††§§}
Trillium	5.2 ± 6.2	17.9 ± 12.4**	211.9 ± 50.4 ^{††§§}
Bioline	5.4 ± 14.5	17.4 ± 20.6**	206.0 ± 57.5 ^{††§§}
Phosphocholine	5.8 ± 12.2	10.3 ± 9.5**	172.7 ± 63.1 ^{††§§}
PMEA	2.6 ± 4.7	16.8 ± 27.8	181.3 ± 89.3 ^{††§§}

expressed as mean ± SD; t-Test (tails:two-tailed distribution, type:paired).

** significant difference between T2 and T1 ($p < 0.01$).

†† significant difference between T3 and T1 ($p < 0.01$).

§§ significant difference between T3 and T2 ($p < 0.01$).

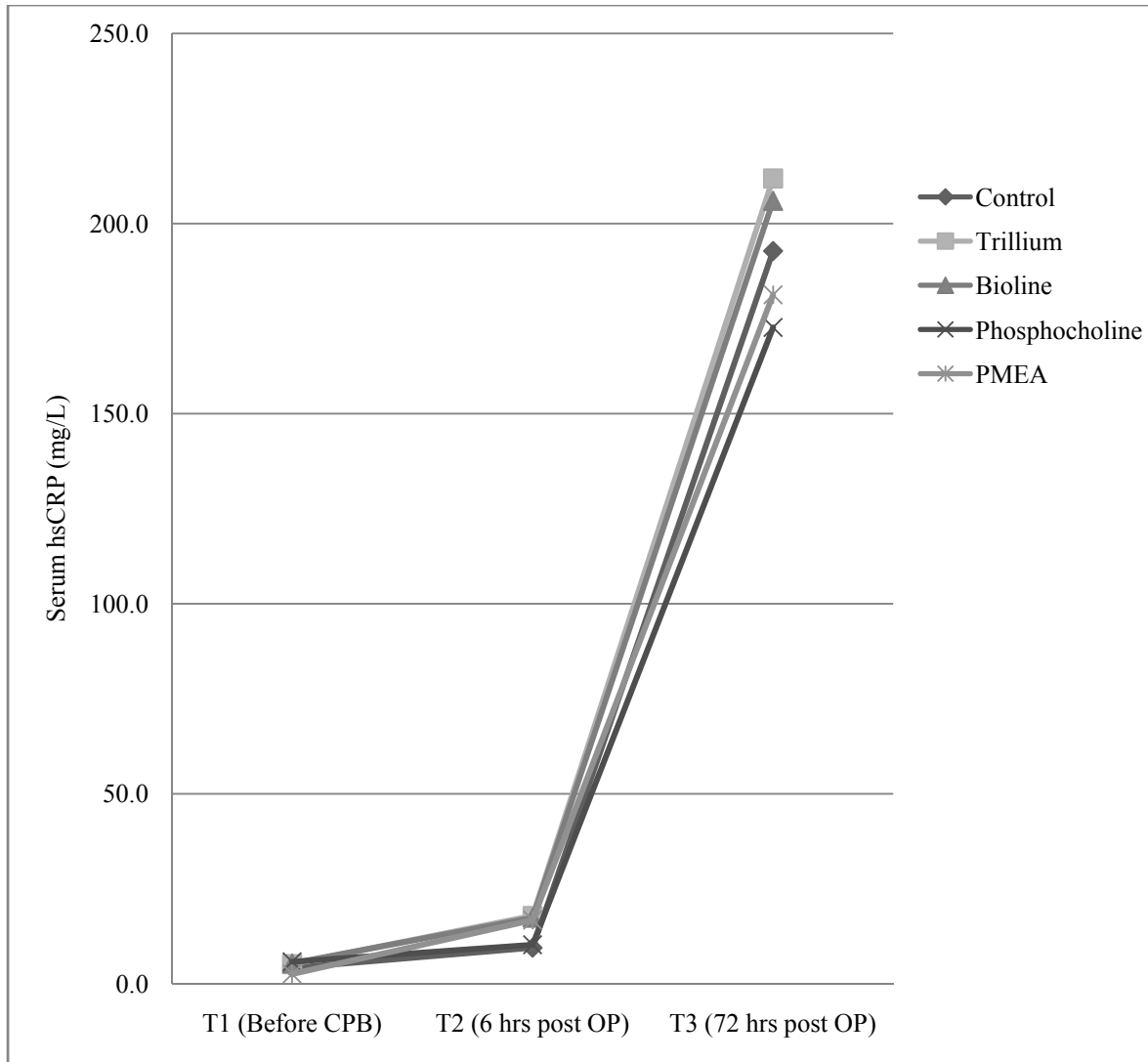


Figure 13. Serum hsCRP levels during CABG with CPB coated with different biomaterials.

4.4. DISCUSSION

4.4.1. Release of cardiac markers during CABG with CPB

Measurements of serum levels of CK and CK-MB have long been used for diagnosis of MI. However, elevated levels of CK and CK-MB are also seen in skeletal muscular disorders and in other conditions such as renal failure. This lack of absolute cardiac specificity in complex clinical situations has led to the development of more specific assays. In our study, we observed that serum lactate and LDH levels significantly increased 6 hours after initiation of CPB and remained elevated till 24 hours after initiation of CPB, indicating that lactate and LDH are potentially useful markers for cardiac injury during CABG. Nevertheless, like CK and CK-MB, the release of lactate and LDH could also occur in many other parts of the body, including the kidneys, red blood cells, brain, stomach, and skeletal muscle. Because of poor specificity, measurement of CK or LDH has largely been replaced by cTn (Morrow *et al.* 2007).

Substantial evidence demonstrates that cTn predicts myocardial damage and mortality after CABG and suggests that a single postoperative cTn measurement can be used to estimate myocardial damage after CABG (Bimmel *et al.* 2003, Fellahi *et al.* 2003, Kathiresan *et al.* 2004, Onorati *et al.* 2005, Salamonsen *et al.* 2005). Other studies have shown that while cTnI is a valuable marker for immediate myocardial damage after coronary bypass operations, it does not predict midterm outcome (Kovacevic *et al.* 2004, Noora *et al.* 2005, Paparella *et al.* 2005). One study has observed no correlation between the necrotic cardiac markers (cTn and CK-MB) and myocardial function (Karu *et al.* 2005). Data also suggest that in those patients who have not had an acute MI but have suffered cardiac trauma such as that associated with open heart surgery, cTn can predict myocardial damage (Carrier *et al.* 2000, Horvath *et al.* 2000). A recent study has shown that an earlier and greater cTnI release potentially predicts the cardiac reperfusion injury associated with CABG (Costa *et al.* 2001). The release of cTn can be attributed to iatrogenic stressors (Taggart 2000), myocardial stunning (Abu *et al.* 2002), and elevated preload, independently of cardiac ischemia (Khan *et al.* 2004). We observed a significant release of cTnI as early as 1 hour after the initiation of CPB and this release reached the

maximum in 12 hours. Serum cTnI has the highest magnitude of increase compared to other cardiac markers. Considering the high sensitivity, high specificity, and strong predictive value of cTnI for myocardial injury, cTnI could be a strong predictor for assessment of myocardial injury after CABG with CPB. Protective measures such as aprotinin, a serine protease inhibitor, can thus be administered accordingly, as demonstrated in a recent study (Karaca *et al.* 2006).

Due to the combination of local trauma, mechanical stimulation from extracorporeal circulation, and pulmonary and myocardial reperfusion, cardiac surgery results in substantial inflammatory responses, leading possibly to postoperative complications. Proinflammatory cytokines, such as TNF- α , IL-6, and IL-8 play a key role in the inflammatory cascade after CPB (Celik *et al.* 2004, Colagrande *et al.* 2006, Franke *et al.* 2005). Sharma *et al.* demonstrated the release of proinflammatory mediators during myocardial ischemia/reperfusion in CABG surgery (Sharma *et al.* 2003). As reported by us and others, serum levels of cytokines and CRP are highly elevated during CABG (Colagrande *et al.* 2006, Paparella *et al.* 2005) and are correlated to the degree of myocardial damage and clinical outcomes (Levy *et al.* 2003, Quaniers *et al.* 2006). In this study, we observed a significant release of serum TNF- α and IL-6 just 1 hour after initiation of CPB, reaching maximum levels at 6 hours after initiation of CPB. The release of serum hsCRP was delayed compared with that of TNF- α and IL-6. It reached statistical significance 12 hours after initiation of CPB. The magnitude of hsCRP release at 24 hours after CPB is comparable to that of cTnI though the peak release is delayed. Our findings indicate that the release of these inflammatory markers could potentially be clinically useful as a monitor for myocardial injury during CABG with CPB. Inflammation sensitive plasma proteins such as AAT and ceruloplasmin have been shown to be increased in MI (Engstrom *et al.* 2002, Engstrom *et al.* 2004, Lind *et al.* 2004) but have never been studied in CABG with CPB. We show that serum levels of AAT were decreased in the beginning of CPB and then increase slowly and reach statistical significance 24 hours after initiation of CPB. This finding indicates that inflammation sensitive plasma proteins may also be useful markers for evaluation of cardiac injury during CABG. However, further studies are needed to establish the correlation between

serum levels of these markers and the extent of myocardial injury. The role of these markers needs to be evaluated in the mid- or long-term prognosis.

4.4.2. Comparison of biocompatible materials used in CPB during CABG

Following our initial study on the release of inflammatory markers, we extended further research to compare the impact of different biocompatible materials used in CPB on initiation of inflammation. Contrary to expectation, the initial control group without biocompatible coating circuit showed less pro-inflammatory cytokine release compared to some biocompatible material groups. Eventually, we found that there had been a mistake made when setting up the control group CPB circuit. In the control group, the oxygenator that is a part of the CPB was already coated with Trillium biocompatible material, which compromised our data for the control group. We found our mistake when we already finished collecting control samples, so we did collect new control samples again. There was no significant difference both in tau protein presence and in hsCRP levels between previously collected control samples and newly collected control samples.

In 2005 Baufreton *et al.* proposed that the increase of complement activation and inflammation due to the contact between blood and the artificial surfaces of heart-lung machine (CPB) has been linked to neurocognitive dysfunction (NCD) after cardiac surgery. Another research conducted by Ramlawi *et al.* 2006a examined the association between biochemical markers of brain injury and the inflammatory response in relation to neurocognitive deficiency after CPB. They evaluated three potential brain injury markers such as S100 β , neuron-specific enolase (NSE), and tau protein, and insisted NSE and tau protein are better markers than S100 β to evaluate brain injury. But total tau results were used as a dichotomous variable reflecting presence or absence of the protein in serum. That was because the exact pharmacokinetics of tau protein are not yet known, so drawing correlations and conclusions on the basis of the quantitative levels would not have been justified (Ramlawi *et al.* 2006a). In another article, conducted by the same research group, they demonstrated that CRP and inflammatory cytokines are associated with NCD (Ramlawi *et al.* 2006b). CRP, a marker of inflammation, is considered a potential marker for risk assessment of coronary heart disease (Ridker *et al.* 2003, Danesh *et al.* 2004). We show in our study that hsCRP levels were significantly elevated

after initiation of CPB in all group regardless of the biomaterials used in CPB circuits. This indicates that inflammation is inevitable and may also be caused by factors other than CPB itself. Therapeutic measures should be applied to prevent cardiac dysfunction caused by CPB-induced inflammation.

Generally cells release the cytokine for only a few hours after appropriate stimulation. So, individual cytokine cannot provide exact inflammation status in our body during CPBs. Also each patient shows different non-specific innate immune responses at different levels against the same stimulation such as CPB. Thus, comparing the difference of inflammatory response in patients undergoing cardiac surgery with CPB with only using some cytokine levels is almost impossible. However, if we consider tau protein results, we could draw a conclusion even though it is still hard to insist, because except Hyaluronan group tau results were gained from at least over 31 patients in each group and tau results could account for neurocognitive deficiency that is one of the side effects of cardiac surgery and when using CPB this unwanted effect is more common. Also neurocognitive deficiency is caused by complicated mechanisms of whole inflammatory response through coagulation cascade, complement activation, and neutrophil and monocyte activation and endothelial activation during cardiac surgery with or without CPB (Baufreton *et al.* 2005, Ramlawi *et al.* 2006a, Ramlawi *et al.* 2006b).

In our study, we have demonstrated positive tau protein in patients with CPB. However, tau protein was less detected in the heparin-coated groups such as Trillium and Bioline compared to other biocompatible groups employing new and recent technology such as Phosphocholine, PMEA, and Hyaluronan groups. A larger study needs to be done to assess mid and long term prediction of tau on neurocognitive deficiency.

In addition, our study has shown that the serum levels of TNF- α and IL-6 as pro-inflammatory cytokines were increased after initiation of CPB but remained elevated by 72 hours after termination of CPB. The serum levels of IL-10, which is classified as an anti-inflammatory cytokine, showed similar release pattern like pro-inflammatory cytokines. It seems that this is a natural defense mechanism in our body when exposure to harmful situation such as CPB and cardiac surgery. Serum levels of TNF- α , IL-6, and 10 did not differ significantly among four different biocompatible groups as well as the

control group before the initiation of CPB. In addition to pro-inflammatory and anti-inflammatory cytokine analysis, we examined the serum levels of IFN- γ to evaluate whether IFN- γ is involved in neurotoxicity by inflammation as Wilson *et al.* 2002 proposed. However, IFN- γ did not show the statistical significance in any biocompatible groups as well as the control group.

There was no technical concern when analyzing cytokines with serum samples, but when gathering and analyzing data, especially TNF- α , we had to double check the overall process of analysis. Cells nearly always produce cytokine for only a few hours after appropriate stimulation. The problem of cytokine detection at the protein level is their short half-life. The biological half-life of cytokines is very short, generally not exceeding a few minutes in plasma except IL-12 whose half-life is several hours. Cytokines do not only occur in free and native form but also bind to numerous carrier proteins and can undergo rapid proteolytic cleavage. Also, a wide variety of cells secrete soluble cytokine receptors after activation. These generally have neutralizing properties (Asadullah *et al.* 2002). On the other hand, they may prolong activity by inhibiting cytokine elimination. Cytokines can also bind to other plasma proteins. Such proteins cover certain epitopes on the cytokine surface that may or may not still be recognized, depending on the epitope specificity and affinity of the cytokine-complex antibodies used in the ELISA (Asadullah *et al.* 2002). Therefore, the interpretation of cytokine concentrations in body fluids such as plasma poses a problem when the cytokine level is low. However, nowadays thanks to the science development, the new generation of ELISAs is actually very sensitive and has high specificity, so can detect cytokines by picomolar level. Thus most cytokines are detectable even in the plasma of healthy volunteers. It has been well established that individual cytokines can have multiple and overlapping functions and in many cases, depending on cell types and concentration, these functions may appear to be contradictory. The information which is carried by individual cytokines can be modified by other cytokines and proteins present within the network at any given moment in space and time (Hooper 2004).

To examine the antioxidant effects of biocompatible materials, we analyzed the serum levels of oxidative stress during cardiac surgery with CPB which was coated with

or without biocompatible materials. The nitric oxide was decreased even in the control group which did not use coated circuit in CPB as time passed by, but there was no statistical significance. Nitric oxide level was decreased significantly at T2 in Phosphocholine group. However PMEA group showed the increase of nitric oxide production after initiation of CPB. Whether the anti or pro-oxidant effects can be attributed to the biomaterials coated on the CPB circuits needs to be confirmed by further study. In addition, cardiac surgery itself may cause more production of oxidative stress rather than use of CPB. Basically nitric oxide cannot be rigidly classified as a pro-inflammatory or anti-inflammatory molecule (Cirino *et al.* 2006). This is because NO has been shown that its production was either increased or decreased during inflammation. Nitric oxide has three different iso-enzymes for production, and these enzymes have different characteristics. During the cardiac surgery, generally either eNOS or iNOS, or both eNOS and iNOS could be involved. The eNOS is constitutively expressed in endothelial cells and synthesizes NO in response to an increase in calcium or some cases to calcium independent stimuli such as shear stress. However, iNOS is the inducible that is generally not constitutive and is typically synthesized in response to inflammatory mediators (Cirino *et al.* 2006, Pacher *et al.* 2007). There are numerous studies on the biological function of nitric oxide. However, there were few articles dealing with nitric oxide biology in patient undergoing cardiac surgery with CPB. Moreover, most of those articles showed data with animal model such as rats, not real human samples. In spite of rat animal study, Hayashi *et al.* 2001, Hayashi *et al.* 2004 demonstrated that cardiac surgery with CPB increased nitric oxide production while the nitric oxide levels in cardiac surgery without CPB did not change from before the initiation of CPB to at the termination of CPB, and to 3 hours after termination of CPB. In contrast to their findings, our study showed there was no statistically significant effect in nitric oxide production during CPB except PMEA coating group. When considering the development of the components of CPB machine such as oxygenator as well as membrane, pumps, and coating circuit, the data difference between previous research and our research could be acceptable. Also the sensitivity and specificity of ELISA kits used for analysis has been improved even though most of the ELISA kits for assessment of oxidized nitric oxide (nitrite and nitrate) adopted Griess method.

More importantly, there are more studies which support our data. Berg *et al* in 2006 demonstrated the levels of oxidative stress during cardiac surgery with CPB to examine the importance of surgical trauma compared to CPB use. There were 20 patients undergoing cardiac surgery with CPB and collected blood samples 14 times from each patient at 14 different time points such as from the beginning of the surgery to 2 days after surgery. Then they examined the serum levels of 8-iso-prostaglandin $F_{2\alpha}$ (8-iso-PGF $_{2\alpha}$) which is accepted as a highly reliable biomarker of oxidative stress (Morrow *et al.* 1990, Basu 1998, Basu *et al.* 2005). According to their data, oxidative stress sharply increased right after initiation of cardiac surgery, remained increased levels by CPB on, gradually decreased during CPB and reached to the baseline when the terminating CPB (Berg *et al.* 2006).

Furthermore, we examined the serum levels of hsCRP, one of acute phase proteins. The hsCRP was slightly increased after initiation of CPB and significantly increased after termination of CPB by 72 hours post operation although there was no statistical significance between biocompatible groups in serum levels of hsCRP. This observation fits in with the previous works (Ridker *et al.* 2003, Levy *et al.* 2003, Denesh *et al.* 2004, Franke *et al.* 2005, Deblier *et al.* 2006). Also, this hsCRP data showed almost same pattern with previous our study, release of cardiac markers during CABG with CPB. Studies have demonstrated that the IL-6 is involved in the production of C-reactive protein in liver during inflammatory process. In other words, when the serum levels of IL-6 were decreased after termination of CPB, the serum levels of hsCRP sharply were increased by 72 hours after cardiac surgery.

Generally, during the cardiac surgery with CPB, our body has to meet and cope with non-endothelial environments such as synthetic peptide in CPB. Operative trauma is combined with systemic activation of the various blood components and ischemic injury to organs, especially the lung, brain, and kidneys. Overlaying all of these concerns is the possibility of thrombotic occlusion to the grafted vessels (Landis 2007). A central challenge for the surgeon is to control bleeding, which is exacerbated by the hyperfibrinolytic state and loss of platelet function because of thrombin activation in the bypass circuit. Antifibrinolytic agents such as aprotinin have been successfully used to

prevent bleeding during cardiac surgery. The issue of platelet preservation during CPB is especially important in antiplatelet agents such as aspirin, which surgeons want to maintain patients on for as long as possible before surgery (Landis 2007). In this study, we asked and got information about previous medication history of acetyl salicylic acid (ASA) and clopidogrel (Plavix), recommended for patients with coronary heart disease as anti-coagulants from patients (Table 1), and found out the patient history whether he/she has been taking medication ASA before cardiac surgery could be a benefit to the patient when he/she got cardiac surgery with CPB. That is because the PMEA group taking less ASA medication compared to other groups showed higher level of inflammation and oxidative stress. The more interesting evidence supporting this could be the heparin, which was administered to patients during cardiac surgery. All patients including the control group received heparin as an anti-coagulant during surgery and then this heparin was neutralized by protamine. Therefore, thanks to the heparin, even the control group which did not use coated biocompatible material in CPB showed the middle range of inflammation. The study conducted by Karlsson *et al.* supports our interpretation. With healthy human males, they demonstrated that intravenous injection of heparin leads to a prompt increase in plasma extracellular superoxide dismutase (SOD) activity. Also they confirmed that heparin induced no release of extracellular superoxide dismutase from blood cells, nor does it activate extracellular superoxide dismutase in plasma. Therefore it indicated that the source of the released enzyme is the endothelial cell surfaces (Karlsson *et al.* 1987).

Moreover, as Prondzinsky and colleagues proposed in 2005, surgical trauma of conventional surgical procedures is a more potent activator of the inflammatory response after cardiac surgery rather than CPB. But it is true that CPB accounts for inflammatory response, because the inflammatory response decreases when we do not use CPB during cardiac surgery. The main concern of surgical trauma affecting the outcomes of cardiac surgery with or without CPB is that coagulation cascade could be initiated from surgical trauma where the tissue factor (TF) is expressed on damaged cells at the site of vascular injury. Furthermore, because of endothelial injury, endothelial cells cannot normally produce tissue factor pathway inhibitor (TFPI) and thrombomodulin which are very

important and strong inhibitors of coagulation pathway (Gorbet *et al.* 2004). Thus extrinsic coagulation cannot be controlled.

The limitation of our present study is that our data shows higher standard deviations in serum cytokine levels, even though we could explain the reason for this based on the fact that the immune reactions are so widely different from individuals to individuals. For instance, the serum levels of TNF- α in both Bioline and PME A groups at T3 (72 hrs post OP) showed higher standard deviations: Bioline is 7.10 ± 7.85 and PME A is 6.12 ± 8.96 . Considering the overall low levels of serum TNF- α in the beginning of inflammatory response, these data with high standard deviation is not good. Especially, 2 out of 16 patients in Bioline group contributed to the data with higher standard deviations at 72 hours post operation. Moreover, the serum levels of IL-6 in Trillium group at T3 (72 hrs post OP) showed extremely high standard deviations like 102.87 ± 131.68 and the levels of IL-6 in all of the groups showed high standard deviations at the termination of CPB (Table 5). As shown in Table 6, the serum levels of IL-10 also showed similar trend in PME A group at both T2 (6 hours post OP/ 110.12 ± 147.58) and T3 (72 hrs post OP/ 48.41 ± 84.88). Taken together, either on the termination of CPB or 72 hours after operation time points, the serum levels of cytokines in some biocompatible groups showed high levels of standard deviations. It means that each patient has different levels of defense mechanism against stressful situations such as cardiac surgery. Especially, when we see the cytokine data at time point T3 (72 hrs post OP), we can estimate that each patient has different levels of healing processes after cardiac surgery, so their serum levels of cytokines which account for inflammation could be different with each other. Specimen preparation and stability may also contribute to the variation of the results. Sample size can be another factor to make the standard deviation of the measurement wide. However, our data is still valuable as this is the first study to compare the effects of different biocompatible materials used in CPB with different time points on inflammation and oxidation stress.

In the future, since there are many factors affecting inflammatory response and oxidative stress during CPB, we need to add more analytical parameters to evaluate the difference of effects on inflammation and oxidative stress of biocompatible materials.

Moreover, we need to increase patient population to get reasonable data which has lower standard deviation. We already analyzed cytokines such as TNF- α , IL-6, IL-10 and IFN- γ and one of oxidative stress marker, NO. Since the membrane attack complex (MAC) mainly causes cell damage, first of all, we need to analyze the activity of TCC (terminal complement complex). Also, the activity of neutrophils could be another useful marker when evaluating inflammation. There are several kinds of analytical items to examine the neutrophil activity such as myeloperoxidase, elastase, or CD 11b. Especially, myeloperoxidase (MPO) could be useful when we evaluate oxidative stress because myeloperoxidase is involved in producing hypochlorous acid (HOCl) and hydroxyl radical (\cdot OH) from hydrogen peroxide (H_2O_2), oxygen (O_2) and chloride ion (Cl⁻). Another marker of neutrophil activity is the level of activation of cell surface marker, CD11b. The expression level of CD11b on neutrophils is strongly related to the level of inflammation. Finally the other prospective marker to evaluate the effects of biocompatible materials could be a soluble VCAM-1 or ICAM-1. The activation of VCAM-1 and ICAM-1 during CPB and even cardiac surgery without using CPB is connected to the activation of neutrophils and activation of phagocytic cells and other inflammatory agents such as cytokines and chemokines. Through analyzing soluble endothelial cell adhesion molecules, we can evaluate the level of endothelial activation leading to endothelial dysfunction which is related to the inflammation procedure. Also the levels of endothelial dysfunction could be fit well with surgical trauma during cardiac surgery as well as CPB. If we successfully analyze those all markers in serum and re-analyze data which we already gained, then we could compare the effects of biocompatible materials on patients undergoing cardiac surgery with CPB.

5. SUMMARY AND CONCLUSIONS

Systemic production and release of an array of cardiac biochemical and inflammatory markers was found in patients undergoing CABG, which could indicate the pathophysiological changes of myocardial function after CPB. The release patterns and the serum levels of cardiac markers as well as inflammatory markers in patients undergoing elective CABG with CPB were established. Our data indicate that cTnI is still a better biochemical marker for cardiac injury than others following CABG with CPB. Also we found that other nonspecific but highly sensitive markers such as LDH, lactate, TNF- α , IL-6, and hsCRP could be potential surrogate markers for evaluation of cardiac injury following CPB. Monitoring these markers may provide useful information to clinicians for patient management and prognosis after CABG with CPB.

In the comparison of different biocompatible materials in the aspects of anti-inflammation, our data suggests that heparin-coated biocompatible materials are better than others which employed recent technologies such as surface-modifying additives and mimicry of cell membrane. Moreover, in terms of oxidative stress, biocompatible materials do not show anti-oxidant effects during CPB, but it seems that administered heparin could act as an ant-oxidant during cardiac surgery with CPB coated with or without biocompatible material. To date there is no clear evidence whether biocompatible materials reliably reduce thrombin formation and the associated consumption of platelets and fibrinogen or attenuate complement activation and initiation of systemic inflammation. In our study, we were not able to demonstrate some of the beneficial features such as anti-inflammatory function of the biocompatible materials claimed by the manufacturers or reported by other studies. Hence, a larger study is needed to evaluate the protective effects and efficacy of those biocompatible materials used in CPB.

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