SERUM AND PLASMA MYELOPEROXIDASE IN ACUTE CORONARY SYNDROME AND CHRONIC STABLE ANGINA

by

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Table of Contents

CHAPTER THREE:RESULTS

CHAPTER FIVE: LIMITATION AND RECOMMENDATIONS

List of Tables

List of Figures

List of Abbreviations and Acronyms

ACS	Acute Coronary Syndrome
CRP	C-reactive protein
CSA	Chronic Stable Angina
CV	Coefficient of Variation
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked Immunoassay
IC	Intracoronary Artery
MPO	Myeloperoxidase
OD	Optical Density
PA	Peripheral Artery/Femoral Artery
PBS	Phosphate Buffer Solution
PV	Peripheral Vein/Antecubital Vein
S.D.	Standard Deviation
S.D.S-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
TEMED	Tetramethylethylenediamine
TMB	Tetra-methyl Benzidine
vs.	Versus

List of Symbols

%	Percentage
bpm	Beat Per Minutes
kDa	Kilo Dalton
mmHg	Milimeter Mercury
n	Nano
°C	Degree Celsius
V	Voltage
α	Alpha
β	Beta
μ	Micro

ABSTRAK

Mieloperoksida (MPO) memainkan peranan penting dalam patogenesis destabilisasi plak koronari. Kajian kes-kawalan ini bertujuan untuk mengetahui perbezaan tahap MPO plasma-EDTA di antara pesakit sindrom koronari akut (ACS) dan angina stabil kronik (CSA). Penyelidikan ini juga bertujuan untuk mengetahui perbezaan tahap MPO dalam sampel serum dan plasma-EDTA antara ACS dan CSA. Kes melibatkan 9 pesakit ACS (umur 52.8±11.9 tahun; min±S.D.) yang menjalani angioplasti primer, sementara 9 pesakit CSA (umur 58.3±11.4 tahun; min±S.D.) sebagai kawalan. MPO dalam plasma-EDTA dan serum yang diambil dari arteri femoralis, vena antekubital dan arteri intrakoronari ditentukan dengan menggunakan ELISA sandwich. Protein C-reaktif (CRP) berfungsi sebagai rujukan. Kepekatan serum MPO adalah lebih tinggi signifikan berbandingkan plasma-EDTA bagi ACS dan CSA. Bagi ACS, kepekatan MPO (mean±S.D.) dalam serum adalah lebih tinggi signifikan berbanding plasma-EDTA bagi sampel darah dari vena antekubital (2864.64±1777.40 ng/ml *vs.* 1321.31±319.02 ng/ml, p=0.021) dan darah intrakoronari (2949.31±2170.21 ng/ml *vs.* 1230.08±383.85 ng/ml, p=0.033). Namun, tiada perbezaan signifikan (p=0.171) di antara serum dan plasma-EDTA bagi darah yang diambil dari arteri femoralis. Bagi CSA, terdapat perbezaan MPO yang signifikan antara serum dan plasma-EDTA bagi sampel darah dari arteri femoralis (2521.29±1266.97 ng/ml *vs.* 549.65±526.09 ng/ml, p=0.001), vena antekubital (2171.25±983.27 ng/ml *vs.* 725.27±671.56 ng/ml, p=0.004) dan arteri intrakoronari (1979.59±912.41 ng/ml *vs.* 621.00±528.93 ng/ml, p=0.001). Kepekatan MPO plasma-EDTA adalah lebih tinggi signifikan untuk ACS jika dibandingkan dengan CSA bagi sampel darah dari arteri

femoralis (1259±405.49 ng/ml *vs.* 549.65±526.09 ng/ml, p=0.005), vena antekubital (1321.31±319.02 ng/ml *vs.* 725.27±671.56 ng/ml, p=0.031) dan arteri intrakoronari (1321.31±319.02 ng/ml *vs.* 725.27±671.56 ng/ml, p=0.031). Walaubagaimanapun, tiada hubungan yang signifikan di antara MPO plasma-EDTA dan CRP serum untuk ACS dan CSA. Penemuan ini menunjukkan bahawa kepekatan MPO dalam plasma-EDTA adalah berbeza dengan yang ditentukan dalam sampel serum untuk pesakit yang sama. Plasma-EDTA merupakan specimen yang lebih sesuai untuk pengukuran MPO kerana MPO plasma-EDTA lebih terkawal dengan pelepasan in vivo dari leukosit berbanding dengan serum. Penyelidikan ini juga menunjukkan bahawa MPO plasma-EDTA secara signifikan lebih tinggi dalam pesakit ACS berbanding dengan CSA. Penemuan ini menunjukkan bahawa MPO berpotensi sebagai penanda pertumbuhan plak atheromatous dan ketahanan. Kajian kohort yang melibatkan saiz sampel yang besar diperlukan untuk menandakan kepentingan MPO dalam patogenesis dan destabilisasi plak secara klinikal.

ABSTRACT

Myeloperoxidase (MPO) plays essential roles in the pathogenesis of coronary plaque destabilization. This case-control study aimed to investigate the differences in EDTA-plasma MPO level in acute coronary syndrome (ACS) and chronic stable angina (CSA). This study also aimed to investigate the differences in the MPO levels in serum and EDTA-plasma samples among ACS and CSA patients. Cases involved 9 ACS patients (age 52.8±11.9 years; mean±S.D.) who underwent primary angioplasty, while 9 CSA patients (age 58.3±11.4 years; mean±S.D.) were recruited as controls. The MPO was measured in EDTA-plasma and matched serum samples collected from femoral artery, antecubital vein and at the culprit coronary artery using in-house developed and validated sandwich ELISA. C-reactive protein (CRP) served as reference protein. Serum MPO level was significantly higher compared to EDTA-plasma in ACS and CSA. For ACS, the MPO level (mean±SD) in serum was significantly higher in venous blood (2864.64±1777.40 ng/ml *vs.* 1321.31±319.02 ng/ml, p=0.021) and intracoronary blood (2949.31±2170.21 ng/ml *vs.* 1230.08±383.85 ng/ml, p=0.033) compared to EDTA-plasma. However, there was no significant difference (p=0.171) between serum and EDTA-plasma MPO level for blood taken from femoral artery. For CSA, there were significant differences between serum and EDTA-plasma MPO level in femoral arterial blood (2521.29±1266.97 ng/ml *vs.* 549.65±526.09 ng/ml, p=0.001), antecubital venous blood (2171.25±983.27 ng/ml *vs.* 725.27±671.56 ng/ml, p=0.004) and intracoronary arterial blood (1979.59±912.41 ng/ml *vs.* 621.00±528.93 ng/ml, p=0.001). The EDTA-plasma MPO concentration was significantly higher in ACS compared to CSA for blood sampled from femoral artery (1259.19±405.49

ng/ml *vs.* 549.65±526.09 ng/ml, p=0.005), antecubital vein (1321.31±319.02 ng/ml *vs.* 725.27±671.56 ng/ml, p=0.031) and intracoronary artery (1321.31±319.02 ng/ml *vs.* 725.27±671.56 ng/ml, p=0.013). There was however no significant association between MPO and CRP concentration in ACS and CSA. These findings suggest that MPO concentration in EDTA-plasma were dissonant with those measured in matched serum samples. The EDTA-plasma is the preferred specimen for MPO measurement as its value is not confounded by poorly controllable ex vivo release of MPO from leucocytes as in serum. This study also shows that EDTA-plasma MPO is significantly higher in patients with ACS compared with CSA patients. These findings suggest a potential role of MPO as a marker of atheromatous plaque growth and vulnerability. Large cohort studies are required to establish the clinical importance and pathogenic significance of MPO in plaque destabilization.

CHAPTER ONE

LITERATURE REVIEW

1.1 Introduction

In humans, atherosclerotic plaques are characterized by a fibrous cap covering a raised lesion inside the internal elastic lamina composed of fibrous tissue, with or without a core filled with macrophage and smooth muscle cell derived foam cells and extracellular lipid deposits (Monaco *et al.*, 2005). Plaque rupture has been described as the disruption of the fibrocellular cap of the atherosclerotic lesion allowing direct contact between blood and the lipid-rich core (Monaco *et al.*, 2005).

Inflammation plays a critical role in acute myocardial infarction. Systemic inflammation is associated with a high risk of future primary and secondary events, including stable and unstable coronary heart disease syndromes and risk of coronary events in stable and unstable angina (Haverkate *et al.*, 1997).

Oxidative stress and inflammation play important roles in the pathogenesis of destabilization of coronary artery disease leading to acute coronary syndrome (ACS). Infiltrating macrophages and neutrophils participate in the transformation of stable coronary artery plaques to unstable lesions (Naruko *et al.*, 2002, Sugiyama *et al.*, 2001). Myeloperoxidase (MPO), a proinflammatory enzyme is abundant in ruptured plaque and can be measured in peripheral blood (Loria *et al.*, 2008).

Acute coronary syndrome is characterized by evidence of leukocyte activation. Early studies found that markers of inflammation such as CRP correlate with unstable angina pectoris in ACS (de Beer *et al.*, 1982, Berk *et al.*, 1990, Buffon *et al.*, 1999).

Markers of myocyte necrosis, such as creatine kinase-myocardial band and cardiac troponin, are invaluable diagnostic tools for ACS patients and are routinely used for risk stratification. However, even cardiac troponin, a highly specific marker of cardiac myocyte necrosis, has a relatively low diagnostic sensitivity for ACS (Hamm *et al.*, 1997, Hamm *et al.*, 1999, Antman *et al.*, 1996, Ohman *et al.*, 1996). Hence, many patients with troponin negative ACS, who have vulnerable coronary plaques, remain at high risk for future ischemic events.

Myeloperoxidase has been implicated in initiation and propagation of atherosclerosis in a number of epidemiological studies. Coronary artery disease (CAD) patients had significantly higher concentrations of MPO compared to the normal human (Zhang *et al.*, 2001). MPO was increased in patients with CAD and correlated to the extent and severity of atherosclerosis of the coronary vessels. In a multivariate model, MPO was the strongest independent predictor of cardiovascular disease outcome (Schindhelm *et al.*, 2009).

Myeloperoxidase was present and colocalized with macrophages in human atherosclerotic lesions. It was catalytically active and capable of modifying cells and lipoproteins within the atheroma (Brennan and Hazen, 2007). In advanced plaques, neutrophils have been detected at sites of fissuring and rupture, and both intracellular and degranulated MPO are present (Takahashi *et al.*, 2005). This suggests that MPO

derived from both monocytes and neutrophils may contribute to cardiovascular disease but at different critical stages of the plaque.

High MPO levels are reported to be a risk factor for early cardiac events in patients with ACS (Samimi-Fard *et al.*, 2009). MPO appear to be related to the short term risk of patients with ACS. These individuals with evidence for inflamed and unstable atherosclerotic plaque formation could benefit most from an aggressive medical treatment, and the benefits of an early invasive strategy might also be greatest among those with elevated levels of inflammatory biomarkers (Fichtlscherer *et al.*, 2004).

Plasma MPO levels are not elevated in patients with stable CAD compared to non-CAD patients (Kubala *et al.*, 2008). CAD remains a heterogenous disease with a wide range of clinical presentations and outcomes. Various systemic markers of inflammation have been investigated and linked to identify patients at risk of CAD and predict future cardiovascular events (Fichtlscherer *et al.*, 2004).

Thus, this study is carried out to compare the serum MPO levels in patients presented with ACS and patients with stable CAD. This study is also aimed to investigate the correlation between MPO levels with several important clinical features like serum cholesterol and degree of blockage. Besides that, this study also aimed to identify and compare MPO levels in three different blood sources, which are intracoronary blood, peripheral artery and peripheral venous blood.

1.2 Acute Coronary Syndrome

1.2.1 Classification of Acute Coronary Syndrome

Acute coronary syndrome is a pathophysiologic continuum that results from rupture of an atherosclerotic plaque, with subsequent platelet aggregation and thrombus formation (Epstein *et al.*, 1992). It can lead to clinical presentations ranging from entirely asymptomatic to unstable angina to acute myocardial infarction to sudden cardiac death attributable to arrhythmias. Plaque rupture or erosion with mural thrombus formation is considered to represent the most important morphological changes that underlie the transformation of stable coronary lesions into clinically unstable lesions, causing unstable angina pectoris or acute myocardial infarction (Davies and Thomas, 1985).

The term "acute coronary syndrome" is a broad term meant to encompass the symptoms that manifest as a result of acute myocardial ischemia (Antman *et al.*, 2004). In patients with myocardial ischemia as a result of decreased blood supply, the initial 12-lead electrocardiography typically shows predominant ST-segment elevation as part of ST-elevation ACS. These patients are classified as having either aborted myocardial infarction or ST-elevation myocardial infarction (STEMI), a term that refers to the electrocardiographic manifestations of coronary artery occlusion (Filippone and Farina, 2003), based on the absence or presence of biomarkers of myocardial necrosis . ST-segment elevation ACS has the homogeneous etiology of transmural ischemia typically caused by fibrin-rich thrombus occluding the infarct-related artery (Waxman, 1999).

Electrocardiography with no predominant ST-segment elevation or non-ST elevation ACS patients are classified as having either unstable angina (UA) or non-ST elevation myocardial infarction (NSTEMI), based on the presence of biomarkers of myocardial

necrosis (Bassand *et al.*, 2007). Non-ST-segment elevation ACS has heterogeneous etiologies of predominantly subendocardial ischemia frequently caused by a platelet-rich thrombus (Abela *et al.*, 1999, Johnstone *et al.*, 2007).

According to National Cardiovascular Database, in 2006, they found that 42% of patients were admitted with STEMI, 39% with NSTEMI, and 25% with UA (Wan Azman and Sim, 2006). The three clinical conditions have similar pathophysiologies, although each has different clinical features, therapies, and prognosis. The presence of atherothrombotic coronary artery occlusion due to plaque rupture and superimposed thrombosis with or without distal embolization is the common pathophysiology of ACS (Gotlieb, 2005).

1.2.2 Unstable Angina

Unstable angina (UA) has been described as a clinical syndrome between stable angina and acute myocardial infarction. The patients present with varying histories and reflect complex pathophysiological mechanisms. In UA, the plaque is unstable causing platelet aggregation and activation of the coagulation systems, resulting in the formation of a platelet-rich thrombus. A thrombus rich in fibrin and erythrocytes may evolve and extend up- or down-stream in the artery (Roe *et al.*, 2001). Extensive local thrombosis rich in platelets will result in episodic flow-limiting coronary stenosis and myocardial ischemia associated symptomatically with unstable angina or with necrosis that characterizes non-Q wave myocardial infarction. If the plaque ruptures and thrombosis is extensive and rich in fibrin, the coronary artery may fully occlude and result in Q-wave myocardial infarction.

1.2.3 Non-ST Elevation Myocardial Infarction

Non-ST elevation myocardial infarction have clots that are platelet rich (Mizuno *et al.*, 1992). Therefore therapies in non-ST patients are aimed at inhibiting aggregation. In NSTEMI, a thrombus is incompletely or intermittently occludes a coronary artery, causing non-transmural or short lasting transmural myocardial ischemia (Agewall, 2008). Patients with NSTEMI have a much worse prognosis since 10-15% experience death or non fatal myocardial infarction within 1 year after admission.

1.2.4 ST-Elevation Myocardial Infarction

ST elevation patients generally have thrombus that is rich in fibrin (Mizuno *et al.*, 1992). The thrombus is completely and permanently occludes a coronary artery, causing transmural ischemia, and the thrombus will benefit from an acute percutaneous coronary intervention or thrombolysis (Agewall, 2008). Early fibrinolytic therapy using intravenous streptokinase or tissue plasminogen activator is efficacious in ST elevation patients.

1.2.5 Chronic Stable Angina

Chronic stable angina (CSA) usually involves stenosis caused by plaques resulting from years of atherosclerotic deposition in coronary arteries. These plaques are usually subocclusive and are typically stable. Clinical symptoms of CSA occur when the oxygen demand of heart tissue cannot be adequately supplied by the coronary artery blood flow and is characterized by transient episodes of chest pain precipitated by exercise or increased activity. The pain is usually resolved with rest or nitroglycerin (Azzazy and Christenson, 2002).

1.3 Pathophysiology of Acute Coronary Syndrome

The basis for the approach to the diagnosis and treatment of ACS is the culprit lesion, the atherosclerotic plaque embedded in the wall of the coronary artery. Patients with stable angina have a plaque with a smooth surface, composed mostly of collagen, and these plaques tend to be stable. This type of plaque may permit sufficient coronary blood flow at rest or with minimal exertion (Filippone and Farina, 2003).

Compared to stable plaques, complex plaques in patients who present with UA/NSTEMI or STEMI, have an irregular surface, with core-rich in cholesterol and esters as well as inflammatory cells. An increase in pressure within the plaque from inflammation or bleeding from vaso-vasorum and the shearing forces on the wall of plaque can cause the plaque to rupture. Hence, causes releasing of thrombogenic material that can cause platelet aggregation and thrombus formation (Filippone and Farina, 2003). If the thrombus totally occludes the lumen then the patient presents with STEMI. If the lumen is only partially occluded, then the patient presents with UA/NSTEMI.

Patients with ACS are characterized by increased platelet activation and aggregation within the coronary circulation (Libby *et al.*, 2002). Thrombus formation at a ruptured or eroded plaque and distal embolization of platelet aggregates eventually lead to myocyte necrosis (Davies *et al.*, 1986).

Pathophysiologically, acute coronary disease is reflected by increased plaque vulnerability, elevated platelet activation, and by recruitment and activation of leukocytes (Danesh *et al.*, 1998, Buffon *et al.*, 2002). Polymorphonuclear neutrophils

(PMN) were found to undergo site-specific activation and degranulation in patients with unstable CAD and were localized to culprit lesions (Buffon *et al.*, 2002, Leckie *et al.*, 2004, Biasucci *et al.*, 1996).

One of the principal enzymes released upon PMN activation is MPO, a highly abundant redox-active hemoprotein (Nicholls and Hazen, 2005, Podrez *et al.*, 2000a). The importance of PMN degranulation of MPO in the coronary circulation is illustrated by the fact that systemic MPO plasma and serum are markedly elevated and have emerged as powerful predictors of adverse outcome in patients with acute CAD (Buffon *et al.*, 2002, Brennan *et al.*, 2003, Baldus *et al.*, 2006, Cavusoglu *et al.*, 2007, Tang *et al.*, 2007).

Coronary artery plaques develop from atherogenesis, which begins early in life when lipid-rich deposits containing macrophages and T-lymphocytes exist in the aorta shortly after birth and increase with age. By early adulthood, fatty streaks are present in a large proportion of individuals (Azzazy and Christenson, 2002). With increasing age, expanding lesions become more numerous and may affect normal laminar blood flow. The lesions contain smooth muscle cells form a fibrous plaque. In more advanced lesions, the fibrous plaque may become vascularized, and the size of the lipid-rich core increases depending on anatomic location and a number of genetic and life-style risk factors including age, gender, family history, blood pressure, smoking habit, diabetes mellitus and blood lipid concentrations (NCEP, 2001).

Rupture of unstable coronary plaques and the resulting thrombus formation are the causal pathologies underlying myocardial infarction. Plaques may become unstable and

rupture at any age causing ACS, a continuum of ischemic disease ranging from unstable angina, associated with reversible myocardial cell injury, to frank myocardial infarction with large areas of necrosis (Azzazy and Christenson, 2002).

Progressive occlusion of the coronary artery causes most heart attacks to occur after plaque rupture and ensuing thrombus formation in vulnerable lesions of moderate stenosis (Libby *et al.*, 1997). Plaque rupture is a process of chronic inflammation. Inflammation pathways involved in attracting leukocytes to the arterial wall. Adhesion of circulating leukocytes to the endothelium is as a response to the elaboration of chemoattractant cytokines by endothelial cells and other cells in the vessel wall. Increased expression of adhesion molecules in endothelial cells of plaque microvessels or in endothelial cells overlying the lipid core may contribute to further leukocyte recruitment to sites of atherosclerosis (van der Wal *et al.*, 1992, O'Brien *et al.*, 1993, Poston *et al.*, 1992). Cytokines also contribute to the response by activating endothelial cells and modulating macrophage and vascular smooth-muscle cell function (Libby, 1995, Epstein and Ross, 1999).

Once in the vessel wall, monocytes develop into macrophages as they take up oxidized low density lipoprotein (LDL) and differentiate into foam cells. Macrophages and lipid-laden foam cells are heavily implicated as prime culprits in the molecular events that promote and complicate atherosclerosis. Monocytes or macrophages are also a source of cytokines that inhibits vascular smooth-muscle cell production of collagen and other extracellular matrix components of the fibrous cap, thus weakening the structure that separates the highly coagulable necrotic lipid core from the circulating coagulation system (Plutzky, 2001).

Destabilization of the fibrous cap decreased collagen production by vascular smooth muscle cells and increased collagen and matrix degradation, primarily by monocytes or macrophages. Destabilization of the fibrous plaque and plaque rupture may due to matrix-metalloproteinase secretion by monocytes or macrophages (Knox *et al.*, 1997, Aikawa *et al.*, 1998, Libby, 2000a). When circulating blood contacts the lipid core, a thrombus is formed (Libby, 2000a). Complete occlusion of the artery by thrombus results in ST-segment elevation myocardial infarction, sometimes accompanied by sudden death. A partial thrombus may lead to presentations of unstable angina and non-ST segment elevation myocardial infarction. The summary for the pathophysiology of ACS is as shown in figure 1.1.

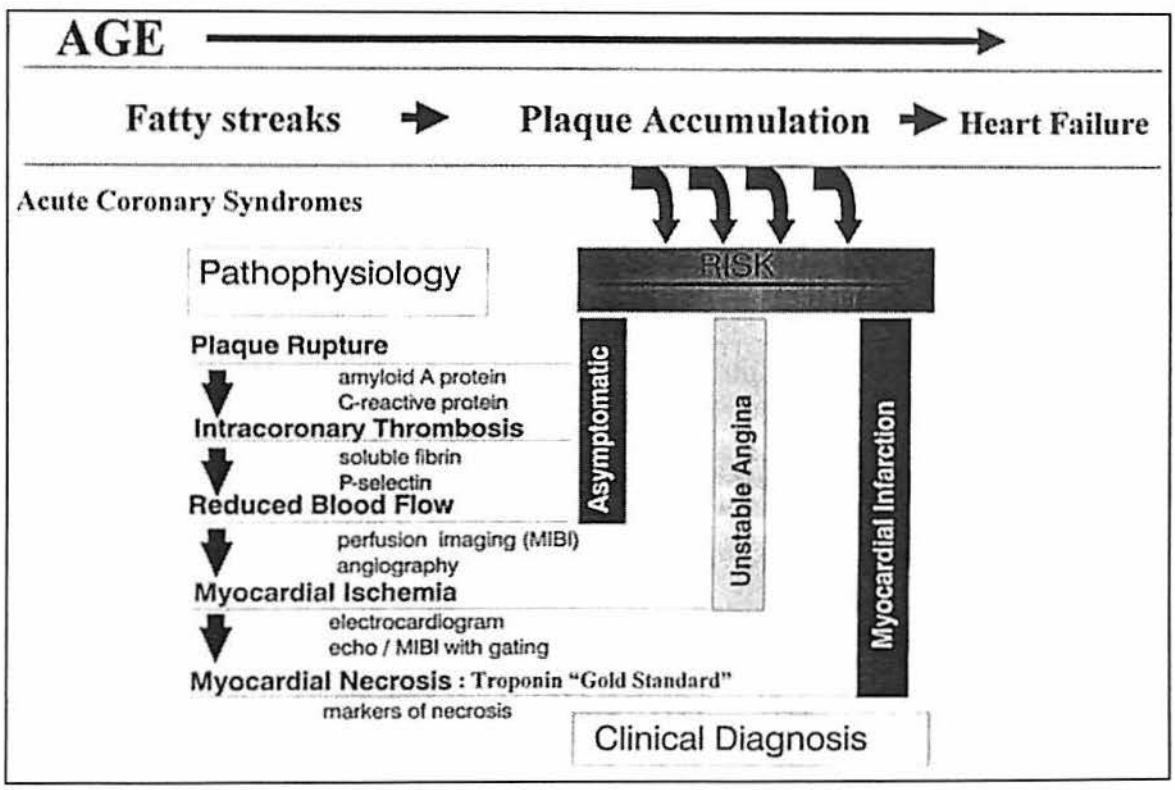

Figure 1.1 The pathophysiology of ACS. Accumulation of fatty streaks leads to the formation of plaques that when rupture lead to ACS. Necrosis caused by myocardial infarction may cause heart failure (adapted from Azzazy and Christenson, 2002)

1.4 Prevalence of Acute Coronary Syndrome

Acute coronary syndrome is the leading cause of mortality in developed countries. At least 2 million individuals are hospitalized each year with ACS and 1 in 5 deaths in the United States as a result of ischemic heart disease (Rosamond *et al.*, 2007).

In 2006, there were a total of 31186 admissions to the 73 coronary care units in Malaysia, of which 12534 admissions were due to ACS. The incidence of ACS admission was 47.1 per 100,000 populations in 2006 in Malaysia reported by the National Cardiovascular Database (Wan Azman and Sim, 2006).

1.5 Cardiac Markers of Acute Coronary Syndromes

The characteristic of the ideal marker are summarized in Table 1.1. Plaque instability and platelet activation are common physiologic features in the ACS. Therefore markers reflecting plaque stability and activated platelets would have great clinical potential. Inflammation markers such as CRP have demonstrated use for assessment of long-term risk, but may also have a role in assessing the suspected ACS patient (de Winter *et al.*, 2000).

Table 1.1 Characteristics of the ideal cardiac marker (adapted from Azzazy and Christenson, 2002)

Characteristic	Description
Size	Smaller markers are released faster from injured tissues
Cellular localization	Soluble cytoplasmic marker is preferred to structural marker
Absolute cardiac tissue specificity	Marker should not exist in other tissues under physiological or pathological conditions
High tissue sensitivity	Abundance in cardiac tissue and absence from plasma
Specificity for irreversible damage	Must be able to differentiate between reversible (ischemia) and irreversible (necrosis) damage
Release	Its release from the myocardium should be complete following the injury and in direct proportion to the size of injury (infarct sizing)
Stability	Marker should reach peak levels shortly after the injury and persists in circulation for few hours to allow a suitable diagnostic window
Clearance	Marker should be cleared rapidly to allow diagnosis of recurrent injury
Applications	Should permit monitoring of reperfusion, reocclusion, and both early and late diagnosis of cardiac injury
Detectability	Rapid whole blood assay that is quantitative and cost-effective must be available for the marker

Inflammation plays an important role in all stages of atherosclerosis from initiation to progression and, eventually, plaque rupture resulting in ACS (Libby *et al.*, 2002). It is generally believed that inflammation markers, detected in elevated levels in the systemic blood, are released from ruptured plaques in coronary arteries, but this assumption has never been proven. Another assumption is that inflammation markers are released from noncoronary sources inducing a proinflammatory milieu thereby affecting coronary plaques, which are transformed onto an unstable form (Kirbiš *et al.*, 2010).

Kirbis *et al.* observed that excess circulating inflammation markers, being characteristic of unstable coronary artery disease, are released from non coronary sources. Thus, it may be speculated that systemic inflammation precedes local inflammation at the plaques, thereby transforming coronary disease from stable to an unstable form (Kirbis *et al.*, 2010). Kirbis *et al.* found out that, in patients with ACS, there was no significant difference between systemic and intracoronary blood levels of any inflammatory marker. In patients with stable angina there was also no difference between intracoronary and systemic values of any measured inflammation marker (Kirbiš *et al.*, 2010).

1.6 Myeloperoxidase as Biomarker in Acute Coronary Syndrome

1.6.1 Myeloperoxidase

Myeloperoxidase (MPO) is primarily hosted and stored in human polymorphonuclear neutrophils (PMN) and by representing 5 % of total protein, is one of the most abundant proteins in PMN. MPO has also been localized to monocytes and tissue macrophages (Lau and Baldus, 2006).

The mature enzyme is a 140 kDa heme-containing homodimer, with each monomer consisting of heavy (55-64 kDa, 466 amino acids) and a light (10-15 kDa, 108 amino acids) subunit (Akin and Kinkade, 1986, Koeffler *et al.*, 1985). MPO belongs to mammalian family of peroxidases. The reaction optimum of MPO is at pH 5.5, but the enzyme remains active over a wide range of pH (Deby-Dupont *et al.*, 1999).

Myeloperoxidase synthesis occurs during myeloid differentiation in bone marrow and is completed within granulocytes prior to their entry into the circulation. The enzyme is stored in primary granules of neutrophils and monocytes and is not released until leukocyte activation and degranulation (Funayama *et al.*, 2010).

Myeloperoxidase synthesis occurs in neutrophil and monocyte granules, prior to phagocyte trafficking from the bone marrow. MPO granules are lost as monocytes differentiate into macrophages, but the enzyme is present in certain subsets of macrophages. Following stimulation and cellular activation, MPO is secreted into the phagolysosome and extracellular space, where it interacts with hydrogen peroxide and endogeneous substrates, results in the generation of diffusible reactive species such as hypochlorous acid, tyrosyl radical, and nitrogen dioxide. These species can interact with wide variety of targets including proteins, lipids, and DNA, and they alter cell structure, integrity, viability, and signaling (Brennan and Hazen, 2007).

During cellular activation and degranulation, MPO is released into phagocytic vacuoles as well as into the extracellular space (Nauseef, 1998). MPO is synthesized as a precursor during myeloid differentiation in bone marrow, and its processing is completed prior to PMN entering the circulation (Lau and Baldus, 2006). Monocytes

gradually lose their MPO during maturation into macrophages (Nakagawara *et al.*, 1981). In macrophages, which have lost the ability to synthesize MPO, the latter is taken up by endocytosis of whole neutrophils or of MPO alone (Nauseef, 1998).

Myeloperoxidase is a highly abundant redox-active hemoprotein (Nicholls and Hazen, 2005, Podrez *et al.*, 2000a). MPO and its products display a diversity of pro-inflammatory and pro-atherogenic properties including catalytic consumption of endothelium-derived nitric oxide, LDL oxidation, modulation of metalloproteinase activities, and activation of PMN in a cytokine-like manner independent of the catalytic activity (Podrez *et al.*, 2000a, Zhang *et al.*, 2001, Sugiyama *et al.*, 2001).

Myeloperoxidase is an enzyme linked to both inflammation and oxidative stress. It is abundantly expressed in the azurophilic granules of most leukocyte subspecies, including neutrophils and monocytes (Klebanoff, 2005). MPO is released by leukocytes in a state of inflammation and catalyzes the formation of several reactive species, including hypochlorous acid (Klebanoff, 2005).

1.6.2 Role of Myleperoxidase

(a) Myeloperoxidase and Low Density Lipoprotein

Minimally modified low density lipoprotein (LDL) particles in the intima may trigger the influx of monocytes that mature into resident macrophages, some of which express MPO. Neutrophils in the blood stream are attracted and bound to sites of damaged endothelium. MPO released by these adherent leukocytes is initially bound to the vascular endothelium and subsequently transcytosed to the subendothelial matrix

(Schindhelm *et al.*, 2009). Therefore, both local releases by resident macrophages and trancytosis of intraluminally produced MPO are sources of MPO in the vascular wall.

The oxidative modification of LDL is an early event in atherosclerosis, and oxidized LDL contributes to atherogenesis by promoting cholesterol deposition and transformation of macrophages into foam cells (Stocker and Keaney, 2004), which effects at the initiation and propagation stage and complication stage (Abu-Soud and Hazen, 2000). Degranulation of MPO displays potent pro-atherogenic properties. MPO can oxidize LDL cholesterol, thereby propagating uptake by macrophages and perpetuating foam cell formation (Podrez *et al.*, 2000b).

The association of MPO with LDL may enhance oxidation of this lipoprotein (Carr *et al.*, 2000). Both the lipid and protein components of LDL are subject to oxidation by MPO (Brennan and Hazen, 2007). Entrapment of the lipoprotein and initiation of lipid and protein oxidation are thought to occur at the very earliest stages of plaque formation. Under physiologic conditions, activated human monocytes also use MPO-generated reactive nitrogen species to render LDL atherogenic, which converts it to a high-uptake form for macrophages while simultaneously promoting both apolipoprotein B-100 protein nitration and initiation of LDL lipid peroxidation. The oxidized phospholipids are enriched within atherosclerotic lesions. Protein modification of LDL also includes nitrotyrosine and dityrosine, both are elevated in the LDL-isolated form atheroma when compared with levels in healthy controls (Hazen and Heinecke, 1997).

(b) Myeloperoxidase and High Density Lipoprotein

High Density Lipoprotein (HDL) is responsible for cholesterol efflux from cells. HDL has been identified as a target for site specific modification by MPO-derived oxidants in the artery wall (Zheng *et al.*, 2004, Bergt *et al.*, 2004). MPO is involved in rendering HDL dysfunctional (Panzenboeck *et al.*, 1997, Nicholls *et al.*, 2005). HDL isolated from atherosclerotic lesions contains bound MPO and numerous MPO-derived peptides, including site specific oxidative modifications by reactive chlorinating and nitrating species. This is correlated with the degree of functional impairment in HDL cholesterol efflux function and the frequency of coronary artery disease and cardiovascular disease (Brennan and Hazen, 2007).

The binding of MPO to high-density lipoprotein is thought to facilitate the selective targeting of apoliprotein A-I for oxidative modification and impairment in reverse cholesterol efflux function of the particle (Zheng *et al.*, 2004, Bergt *et al.*, 2004). Tyrosine nitration of the apolipoprotein moiety in HDL cholesterol resulted in the loss of its anti-inflammatory properties (Lau and Baldus, 2006). Systemic MPO levels were associated with atherosclerotic burden and disease progression. Elevated MPO levels, especially in the presence of low levels of HDL cholesterol, were associated with 2.6 time higher risk of plaque progression (Brennan and Hazen, 2007).

(c) Myeloperoxidase and Nitric Oxide

Nitric oxide (NO) serves as a substrate for peroxidases. It is able to use nitric oxide as a physiologic substrate. Hence, permits MPO to catalytically consume NO, resulting in endothelial dysfunction (Abu-Soud and Hazen, 2000). MPO catalytically consumes endothelial-derived nitric oxide, thereby reducing nitric oxide bioavailability and

impairing its vasodilatory and anti-inflammatory function (Abu-Soud and Hazen, 2000). As MPO can oxidize nitric oxide to nitrate, an increase in MPO may compete with the vasodilating activity of nitric oxide, thus providing a reduction in blood flow within the myocardium (Funayama *et al.*, 2010).

Scavenging of NO by MPO-derived reactive substances may further reduce the bioavailability of NO. Hypochlorus acid can react with nitrogen atoms of the nitric oxide synthase (NOS) substrate arginine to produce chlorinated arginine species that are inhibitors of all isoforms of NOS. Hypochlorus acid is a potent inducer of uncoupling of endothelial NOS, thereby turning NOS into a superoxide-producing enzyme (Xu *et al.*, 2006).

(d) Myeloperoxidase and Hypochlorous Acid

Myeloperoxidase functions to catalyze the conversion of chloride and hydrogen peroxide to hypochlorite (Apple *et al.*, 2005). The MPO-catalyzed oxidation of chloride by H_2O_2 results in formation of hypochlorous acid, a powerful chlorinating oxidants which is believed to be critically important for the microbicidal and viricidal properties of neutrophils (Deby-Dupont *et al.*, 1999). Hypochlorous acid (HOCl) is able to oxidize the apolipoprotein moiety of very low density lipoproteins, leading to enhanced cholesterol ester uptake by macrophages (Lau and Baldus, 2006).

MPO and hypochlorite-modified proteins are colocalizes in human atherosclerotic lesions (Malle *et al.*, 2000). Higher concentration of proteins modified by hypochlorous acid are found in eroded and ruptured plaques compared to stable plaques (Schindhelm *et al.*, 2009).

Hydrogen peroxide is the co-substrate for all MPO-catalyzed reactions. MPO amplifies the oxidative potential of hydrogen peroxide by producing a variety of reactive oxidants, including chlorinating and nitrating species (Schindhelm *et al.*, 2009, Zhang *et al.*, 2002, Podrez *et al.*, 2000a). MPO is catalytically active within human atheroma because chlorotyrosine, a specific product of protein modification by MPO-generated halogenating oxidants, is enriched within human atherosclerotic lesions compared to normal arterial intima (Hazen and Heinecke, 1997). MPO-derived reactive species as shown in figure 1.2, contribute to plaque destabilization and rupture by activating various protease cascades that affect the stability and thrombogenicity of plaques (Fu *et al.*, 2001).

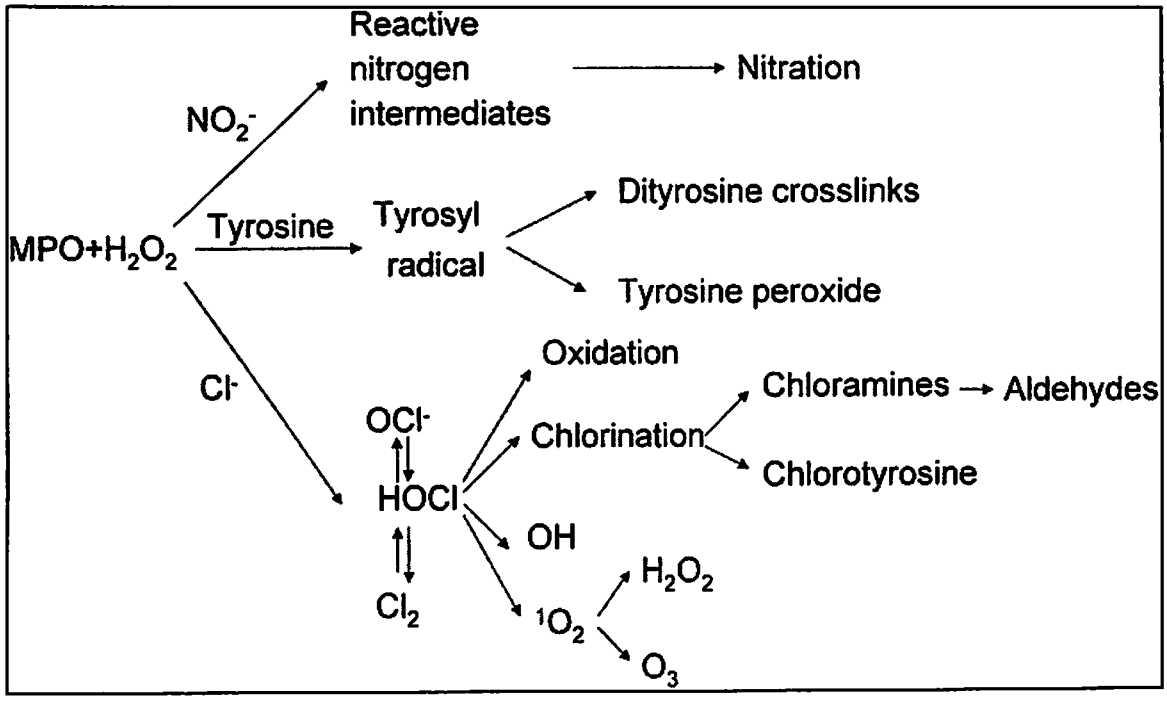

Figure 1.2 Products of the MPO-mediated system (adapted from Klebanoff *et al.*, 2005).

(e) Myeloperoxidase and Matrix Metalloproteases

Patients with acute myocardial infarction has a higher unstable plaques (Rossi *et al.*, 2006). MPO may play role in plaque destabilization by activating metalloproteinases, thereby weakening the fibrous cap (Rudolph *et al.*, 2007). MPO-generated oxidants capable of converting latent matrix metalloproteases (MMP) into active forms and inhibiting the activity of matrix metalloprotease tissue inhibitors (Brennan and Hazen, 2007). MPO has been shown to activate MMP and promote destabilization and rupture of the atherosclerotic plaque surface (Fu *et al.*, 2001, Libby, 1995, Libby, 2000b).

1.6.3 Myeloperoxidase and Plaque instability

MPO is present in activate neutrophils and monocytes. It has been identified in human plaques (Daugherty *et al.*, 1994). It is a marker of plaque destabilization and is released from macrophages into the extracellular fluid and general circulating during inflammatory conditions (Apple *et al.*, 2005).

Myeloperoxidase enriched within culprit lesions of subjects who experience sudden cardiac death (Sugiyama *et al.*, 2001). It has been linked to activation of protease cascades and both proapototic and prothrombotic pathways that are believed to be involved in plaque fissuring (Fu *et al.*, 2001, Rudolph *et al.*, 2007), development of superficial erosions (Sugiyama *et al.*, 2001), intracoronary thrombus generation during sudden cardiac death (Nicholls and Hazen, 2005), promote catalytic consumption of nitric oxide, leading to development of endothelial dysfunction (Baldus *et al.*, 2004, Abu-Soud and Hazen, 2000).

Localization of MPO within human artheroslerotic plaque and biochemical studies that MPO-generated reactive oxidant species promote tissue injury during cardiovascular disease. Oxidative stress and inflammation play important roles in the pathogenesis of the destabilization of coronary artery disease leading to ACS. Infiltrating macrophages and neutrophil participate in the transformation of stable coronary artery plaque to unstable lesions with a thin fibrous cap, so MPO is a promising marker of inflammation in patients with ACS, with a good correlation with short, medium, and long-term prognosis (Morrow *et al.*, 2008).

The majority of clinical ischemic events result from the breakdown of the fibrous cap overlying the atherosclerotic plaque (Falk *et al.*, 1995). Pathological studies have established that culprit lesions are typically macrophage rich atheroma containing large amounts of matrix metalloproteinases and prothrombotic material (Aikawa and Libby, 2000). These plaques are more likely to undergo thinning and subsequent breakdown of the overlying fibrous cap. This exposes circulating blood to the plaque's thrombogenic core, resulting in thrombus formation, luminal compromise, and ischemia. The ability of systemic MPO levels to predict the likelihood of clinical events suggests that MPO plays a role in the transition of a mature atherosclerotic plaque to the vulnerable state (Nicholls and Hazen, 2005).

1.6.4 Myeloperoxidase in Acute Coronary Syndrome

Myeloperoxidase serum and plasma levels are markedly elevated in patients with ACS, forming a firm mechanistic link between PMN activation, MPO release, and compromised vascular reactivity (Baldus *et al.*, 2003, Brennan *et al.*, 2003, Baldus *et al.*, 2006, Cavusoglu *et al.*, 2007, Tang *et al.*, 2007).

A study by Baldus S. *et al.* established MPO serum levels as a powerful independent prognostic determinant of clinical outcome in patients with ACS. In patients with troponin T serum levels below 0.01mg/L, elevated MPO serum levels identify a subgroup of patients who have significantly increased cardiac risk (Baldus *et al.*, 2003, Brennan *et al.*, 2003). This indicates that elevated MPO serum levels are not temporally related to myocardial injury. MPO identify patients at risk for cardiovascular events who had low baseline troponin T serum levels. MPO release precedes myocardial injury and identifies patients with unstable atherosclerotic plaque formation even before complete microvascular obstruction. ECG evidence of myocardial ischemia did not correlate with MPO levels. Hence, MPO release is a prerequisite rather than a consequence of myocardial injury (Baldus *et al.*, 2003).

Plasma MPO concentrations provide independent prognostic value for the prediction of long-term incident major adverse cardiovascular events in stable, medically managed patient population with coronary artery disease (Tang *et al.*, 2010). Patients with progressive stenosis had significantly higher baseline MPO concentrations compared to patients with stable disease (Markus *et al.*, 2006). In patients undergoing elective coronary angiography, there is no significant differences in MPO concentrations for those with proven stable CAD compared to those without proven CAD (Kubala *et al.*, 2008).

In a study by Apple *et al.*, there is no additional diagnostic value of MPO compared to troponin I in patients with clinically diagnosed ACS (Apple *et al.*, 2009). Patients with increased MPO concentrations were at higher risk for non fatal myocardial infarction or re-hospitalization for ACS at 30 days (Apple *et al.*, 2007). An elevated concentration of

MPO measured early after presentation with ACS is associated with higher risk of death and recurrent ischemic events (Brennan *et al.*, 2003, Baldus *et al.*, 2003, Mocatta *et al.*, 2007).

Myeloperoxidase is elevated in culprit lesions that have fissured or ruptured in patients with sudden death from cardiac causes (Naruko *et al.*, 2002). The plasma levels of MPO at the culprit lesion of infarct-related coronary artery had a positive correlation with those in the peripheral venous. Local MPO is increased in culprit coronary artery in the patients with acute myocardial infarction. There is evidence for leukocyte activation and degranulation of mononuclear cells and elicits the release of MPO into the circulation (Baldus *et al.*, 2003, Brennan *et al.*, 2003, Naruko *et al.*, 2002, Buffon *et al.*, 2002).

Extensive monocyte and neutrophils filtration is seen in fissured, thrombosed plaques in the culprit coronary artery. MPO may be suggested to act as principal mediator. In the recent study by Funayama *et al.*, MPO was measured within 24 hours after the onset of infarction, and the concentration of plasma MPO in systemic circulation was increased. It was greater at the culprit lesions of infarct-related coronary artery than in the peripheral venous. This may implicate that leukocyte enzymes are secreted from infiltrated leukocytes around the ruptured plaque of culprit coronary artery (Funayama *et al.*, 2010).

Biasucci *et al.* (1996) observed that circulating neutrophils in patients with acute myocardial infarction and UA have a low MPO content, and therefore high MPO levels in the circulation, as compared with those with chronic stable angina and variant angina.

MPO is prevalently a marker of instability, oxidative stress and damage (Biasucci *et al.*, 1996).

In a study by Naruko *et al.*, they found that increased expression and plasma MPO levels are closely related to the presence of angiographically detected complex lesion morphology in patients with unstable angina (Naruko *et al.*, 2010). Elevated MPO plasma concentration is associated with an increased risk of death in patients with stable CAD. Elevated MPO concentration does not predict the increased risk of death independently of other known cardiovascular risk factor (Stefanescu *et al.*, 2008).

Immunohistochemical analyses localized PMN to culprit lesions of patients with ACS (Baldus *et al.*, 2004). PMN adhere to the vessel wall in acute coronary disease and undergo site-specific activation. Hence, PMN release MPO into the coronary circulation as evidenced by elegant studies measuring MPO content of PMN in aorta ascendances and coronary sinus (Buffon *et al.*, 2002, Deby-Dupont *et al.*, 1999). Given the release of MPO into the vessel lumen and its avid binding properties to endothelial cells it is likely that free MPO associates with the coronary endothelium and exerts its proinflammatory catalytic activity even in the absence of PMN (Lau and Baldus, 2006).

In patients with unstable angina and acute myocardial infarction, MPO released into the coronary circulation yielded elevated MPO plasma levels (Buffon *et al.*, 2002, Deby-Dupont *et al.*, 1999), with leukocyte MPO and blood MPO levels strongly correlating with the presence of coronary artery disease (Baldus *et al.*, 2003, Zhang *et al.*, 2001).