FORUM REVIEW ARTICLE

MYELOPEROXIDASE AS A MULTI-FACETED TARGET FOR CARDIOVASCULAR PROTECTION

Chrishan J.A. Ramachandra[1,2#], Myu Mai Ja KP[1], Jasper Chua[1,3], Shuo Cong[1,4], Winston Shim[5], Derek J. Hausenloy[1,2,6-9]

[1]National Heart Research Institute Singapore, National Heart Centre Singapore, Singapore

[2]Cardiovascular & Metabolic Disorders Program, Duke-National University of Singapore Medical School, Singapore

[3]Faculty of Science, National University of Singapore, Singapore

[4]Department of Cardiac Surgery, Zhongshan Hospital, Fudan University, Shanghai, China

[5]Health and Social Sciences Cluster, Singapore Institute of Technology, Singapore

[6]Yong Loo Lin School of Medicine, National University Singapore, Singapore.

[7]The Hatter Cardiovascular Institute, University College London, London, UK.

[8]Cardiovascular Research Center, College of Medical and Health Sciences, Asia University, Taiwan.

[9]Tecnologico de Monterrey, Centro de Biotecnologia-FEMSA, Nuevo Leon, Mexico

Keywords: Myeloperoxidase; cardiovascular disease; inflammation; cardioprotection; oxidative stress; biomarker

Running title: Myeloperoxidase and cardiovascular protection

[#]Corresponding author:

Dr. Chrishan J.A. Ramachandra

National Heart Centre Singapore,

5 Hospital Drive, Singapore 169609

Email: chrishan.ramachandra@nhcs.com.sg

Phone: +65 6704 2202; Fax: +65 6844 9173

Word count: 6325

Reference numbers: 132

Color illustration: 5 (online 5 and hardcopy 0)

2

ABSTRACT

Significance: Myeloperoxidase (MPO) is a heme peroxidase that is primarily expressed by neutrophils. It has the capacity to generate several reactive species, essential for its inherent antimicrobial activity and innate host defense. Dysregulated MPO release however, can lead to tissue damage, as seen in several diseases. Increased MPO levels in circulation is therefore, widely associated with conditions of increased oxidative stress and inflammation.

Recent Advances: Several studies have shown a strong correlation between MPO and cardiovascular disease (CVD), whereby elevated levels of circulating MPO are linked to poor prognosis with increased risk of CVD-related mortality. Accordingly, circulating MPO is considered a 'high-risk' biomarker for patients with acute coronary syndrome, atherosclerosis, heart failure, hypertension and stroke, thereby implicating MPO as a multifaceted target for cardiovascular protection. Consistently, recent studies that target MPO in animal models of CVD have demonstrated favorable outcomes with regard to disease progression.

Critical Issues: Although most of these studies have established a critical link between circulating MPO and worsening cardiac outcomes, the mechanisms by which MPO exerts its detrimental effects in CVD remain unclear.

Future Directions: Elucidating the mechanisms by which elevated MPO leads to poor prognosis and conversely, investigating the beneficial effects of therapeutic MPO inhibition on alleviating disease phenotype, will facilitate future MPO-targeted clinical trials for improving CVD-related outcomes.

3

INTRODUCTION

Cardiovascular disease (CVD) remains the leading cause of death and disability globally, accounting for approximately 30% mortality, with risk factors, such as smoking, hypertension, diabetes, abdominal obesity and dyslipidemia contributing to its clinical outcome. It is a common term used to identify a number of linked pathologies such as coronary heart disease (CHD), cerebrovascular disease, peripheral arterial disease and rheumatic and congenital heart diseases and venous thromboembolism(116). It is also a major economic burden accounting for $555 billion in the United States in 2016, a cost which is projected to reach $1.1 trillion by 2035. Elucidating the underpinning mechanisms of various CVDs may provide novel insights into disease progression, as well as pinpoint potential targets for therapeutic intervention.

Myeloperoxidase (MPO) is a member of the superfamily of heme peroxidases that is mainly stored in azurophilic granules of leukocytes. It is secreted upon leukocyte activation and plays an important role in innate immunity(85). MPO is primarily found in neutrophils and to a lesser extent in monocytes and macrophages(108). In addition to these inflammatory cell types, MPO expression has also been observed in neurons(41) and endothelial cells(60). During inflammation, MPO is released from leukocytes and catalyzes the formation of several reactive species, including hypochlorous acid (HOCl) and hypothiocyanous acid which can post-translationally modify target proteins(1,66). While this function is essential for its antimicrobial activity and innate immunity, dysregulated MPO release can also lead to tissue damage, as observed in various diseases(4). Increased MPO levels in circulation are therefore, widely associated with conditions of increased oxidative stress and inflammation.

Several studies have reported a strong link between MPO and a wide variety of CVD, whereby increased circulating MPO levels are associated with poor prognosis with increased risk of CVD-related mortality (Figure 1)(80). Accordingly, MPO has been considered to be a biomarker for risk stratification in various CVDs, including acute coronary syndrome(9,13), atherosclerosis, heart failure(120), hypertension, and stroke (Table 1). In this review we discuss the recent associations of MPO with various CVD, as

4

well as provide an overview of the mechanisms by which MPO exerts its detrimental effects, and highlight MPO as a potential multifaceted target for cardiovascular protection.

CATALYSIS FUNCTION OF MPO

Human MPO is encoded by a single gene located on chromosome 17, whose main translation product is a single protein which undergoes N-linked glycosylation to produce a 92-kDa glycoprotein(79). This pro-MPO then undergoes proteolytic maturation to give rise to the native MPO, a homo-dimeric protein which is formed from two monomers that are linked via a cysteine bridge. Each monomer itself consists of a heavy chain and a light chain, as well as a functionally identical heme group (Figure 2)(27). Substrates that are oxidized by MPO bind to a hydrophobic pocket which lies at the entrance to the distal heme cavity.

MPO has limited antimicrobial activity on its own, however upon interaction with hydrogen peroxide, native MPO is converted to Compound I, which can then enter the 'halogenation cycle' or the 'peroxidation cycle'. When entering the halogenation cycle, Compound I can undergo two electron reductions with halides/pseudohalides such as chloride (Cl^-), bromide (Br^-), iodide (I^-) and thiocyanate (SCN^-) to generate corresponding hypohalous acids(32). When entering the peroxidation cycle, Compound I (via the formation of a Compound II intermediate), can undergo two successive one-electron reductions to generate several radicals(20) (Figure 3). Irrespective of the cycle, all one- and two-electron oxidation reactions catalyzed by MPO must be thermodynamically favorable, in accordance with the redox properties of the reactants(5). Studies assessing the redox potential of MPO have revealed that the standard reduction potentials of the redox couples (Compound I/native MPO, Compound I/Compound II and Compound II/native MPO) are critical determinants as to which substrate is oxidized (6,31). At a pH of 7, the Compound I/Compound II redox couple was found to have the highest standard reduction potential, which would suggest a preference towards the peroxidation cycle. Consistent with these findings, MPO exhibited higher affinity towards peroxidase substrate guaiacol (and not chloride ions) at a pH of 7.4. However, at an acidic pH, the chlorination activity was predominant over the peroxidase activity (125). Within the halogenation cycle itself,

5

there is a preferential substrate oxidation hierarchy, which can be displaced in response to changes in substrate concentration and pH(32,110). It would therefore seem that apart from redox and thermodynamic properties of the reactants, other factors such as pH and substrate concentration could favor for a particular cycle.

Despite the ability to catalyze several chemical reactions, the primary action of MPO is the generation of hypochlorous acid (HOCl). HOCl which is generated via Compound I interaction with Cl^-, is a potent antimicrobial oxidant, capable of modifying DNA, lipids and lipoproteins(68). HOCl can react rapidly with sulfur and nitrogen atoms (nucleophile groups) which are present in thiols and thioethers (cysteine and methionine residues respectively) as well as amines and amides(90-92). Modification of thiol groups, can have opposing effects on cellular components. For instance, oxidation of cysteine residues can lead to inactivation of cellular enzymes(94) and conversely, induce activation of matrix metalloproteinases(30). Similarly, modification of thioethers (oxidation of methionine residues) can lead to enzyme inactivation(45), inhibition of proteinases(124) as well as alterations to signaling cascades(71). Modification of amines and amides by HOCl that result in the generation of chloramines and chloramides respectively, could be formed on virtually all organic biological components, which could have serious cellular implications.

Importantly, oxidation of tyrosine residues by HOCl leads to the formation of 3-chlorotyrosine, which is considered a marker for MPO activity, as it is a secondary product that is far more stable than primary MPO generated species. Similarly, by entering the peroxidation cycle, MPO can give rise to nitrogen dioxide radicals ($^.NO_2$) that are capable of nitrating proteins and involved in lipid peroxidation. Much like 3-chlorotyrosine, nitrotyrosine which is the result of tyrosine oxidation via $^.NO_2$ is considered a marker of MPO-mediated oxidative stress. 3-chlorotyrosine and 3-nitrotyrosine has been shown to alter the function of apolipoprotein A-I by depleting ABCA1-dependent cholesterol efflux activity(111,129). Furthermore, x-ray crystallography studies have revealed that modifications on tyrosine residues could alter protein structure and function by inducing conformational changes and causing steric hindrance(95). Hence, while species generated by MPO are critical for the innate host defense system, their off-target release could lead

6

to compromised cellular/tissue function. For a comprehensive review on the reactive species generated by MPO, we would like to direct the reader to the following excellent reviews(20,68).

MPO – A BIOMARKER FOR RISK STRATIFICATION

Acute coronary syndrome

Acute coronary syndrome (ACS), which refers to a spectrum of clinical presentations(3,28) ranging from ST-segment elevation myocardial infarction (STEMI), non–ST-segment elevation myocardial infarction (NSTEMI) or unstable angina, is usually due to rupture of an unstable atherosclerotic plaque and partial or complete thrombosis of the infarct-related artery(19,62). In ACS, oxidative stress and inflammation play an important role in the destabilization of coronary plaques(40) and hence, identifying therapeutic targets that contribute to this pathophysiology may have direct clinical implications. Elevated plasma MPO levels have been investigated as a potential biomarker for discriminating chest pain due to ACS from other causes(63), and as a prognostic factor(9).

In an attempt to identify ACS in patients presenting with angina, researchers have investigated the diagnostic efficiency of plasma MPO levels alone or in combination with cardiac troponin I (cTnI) within 6h of hospital admission. Both MPO and cTnI were found to be significantly lower in non-ACS patients. Interestingly, higher MPO concentrations were observed in troponin-negative ACS patients who became troponin-positive after 6h, which suggested that combining both MPO and cTnI was more sensitive than assessment of cTnI alone for detecting ACS(107). Elevated MPO levels were also able to distinguish between chest pain due to acute myocardial infarction (AMI) from that due to unstable or stable angina(40). In this study, elevated MPO levels were also associated with subsequent complications such as heart failure, arrhythmias and renal failure. In another study, MPO levels assessed on admission and 6h later were able to discriminate between ACS due to angina from other causes(16).

Additionally, though elevated plasma MPO levels have been associated with increased incidence of several CVD, studies investigating the association of intracellular neutrophil or

7

monocyte MPO (mMPO) with CVD are less common. When researchers used flow cytometry, to measure mMPO in 1,465 subjects over a median follow-up of 9.6 years, no significant association was observed between mMPO and incident CHD, heart failure or all-cause mortality(88). Similarly, a study assessing 123 patients with chronic ischemic heart disease (IHD) actually reported a reduction in neutrophil MPO activity(11). Since inflammation is known to occur in these CVDs, it can be speculated that de-granulation and subsequent release of MPO may have already taken place, resulting in the observed intracellular MPO levels.

Researchers have compared the diagnostic accuracy of AMI using high-sensitivity cardiac troponin T (hs-cTnT), MPO and pregnancy-associated plasma protein A (PAPP-A) in patients at the time of presentation at the emergency department, and found that patients with AMI had higher levels of all 3 markers, with hs-cTnT demonstrating a better diagnostic performance than MPO and PAPP-A for patients with AMI(53). The time-course of MPO elevation in STEMI treated by primary percutaneous intervention (PPCI) has been shown to display a biphasic pattern, with the highest levels seen at 4h and 24h after PPCI, with marked decreases at 8h and 12h, before reaching the lowest level at 168h. The 24h MPO level correlated with troponin I, as well as with heart failure and hence, in this study, MPO was considered an independent predictor of in-hospital mortality rate(115). In another study of STEMI patients, elevated MPO and C-reactive protein (CRP) levels were shown to be associated with major cardiovascular events, and MPO appeared to be a better predictor than NT-proBNP levels(49,52). However not all studies have been positive with some studies showing no correlation of MPO levels with clinical outcomes following ACS(98,128).

Atherosclerosis

Atherosclerosis is a chronic arterial disease and a leading cause of vascular disease-related death globally, resulting in major clinical manifestations such as ischemic heart disease (IHD), ischemic stroke and peripheral arterial disease(46) of which, the former remains the leading cause of premature adult mortality(74). Since oxidative stress plays a critical role in the initiation and progression of atherosclerosis, researchers have assessed the role of

8

MPO as a marker of oxidative stress, and whether its circulating levels correlates with the risk of CHD.

In the MONICA/KORA Augsburg study, serum MPO levels were measured in healthy men and women. During the mean follow-up of 10.8 years, in which 333 subjects went on to develop CHD (1727 did not), elevated serum MPO was found to be independently associated with increased risk of incident CHD(50). Given that most patients with recognized atherosclerotic burden are not indicated for revascularization interventions, it is necessary to identify patients at risk for major adverse cardiac events. When assessing plasma MPO concentrations of 1895 patients with known atherosclerotic burden undergoing elective coronary angiography, researchers observed an increased MPO level to be significantly associated with the incidence of major adverse events over a 3-year follow-up. This suggests that MPO could serve as a prognostic marker for adverse events in stable patients with coronary artery disease (CAD)(121). Conversely, in the Dallas Heart Study, where plasma MPO was measured in 3294 subjects, an increased level of MPO was highly associated with aortic, but not coronary atherosclerosis, suggesting MPO to be a potential risk factor in peripheral arterial disease(17). This is consistent with an earlier study involving 156 peripheral artery disease patients, where MPO (but not C-reactive protein) was found to predict an increased risk of major cardiac events(14). Similarly, elevated circulating MPO has been reported in patients with atherosclerosis obliterans (ASO), when compared to those who did not have ASO(127).

Since MPO is reported to induce dysfunctional high-density lipoprotein (HDL)(25), a cohort study was performed in a large multi-ethnic population of 2924 adults free of CVD, to assess whether serum MPO levels indexed to HDL particle concentration (MPO/HDLp) could be correlated with increased CVD risk(54). At a median follow-up of 9.4 years, MPO/HDLp was found to be associated with a 74% increase in incident atherosclerotic CVD (first non-fatal myocardial infarction, non-fatal stroke, coronary revascularization, or CVD death) and a 9% increase in total incident CVD, suggesting increased MPO/HDLp at baseline to be associated with increased risk of incident CVD events, in a population initially free of CVD. Additional studies have also been conducted to assess as to whether

9

MPO could also be associated with other risk factors, such as elevated glucose and lipid concentrations. However, when basal levels of MPO were measured in 53 IHD patients over a period of 24 weeks, although MPO was found to be a marker of inflammation in patients with IHD, it could not be associated with other risk factors of atherosclerosis, and had no correlation with glucose or lipid values(106). In a similar study, which investigated possible associations of serum MPO levels with atherosclerotic plaques in 40 patients without risk factors for atherosclerosis (mainly diabetes, hypertension, obesity and hyperlipidemia), although researchers found increased MPO levels in patients with atherosclerosis, it could not be used as a predictor of coronary artery disease in patients without the risk factors(44).

Heart failure

Heart failure (HF), which arises from cardiac dysfunction, affects multiple organ systems and presents symptoms such as edema, dyspnea and fatigue. The prevalence of HF is rapidly increasing in ageing populations, and hence understanding its etiology and identifying prognostic markers is essential for effective management strategies. During advanced stages of HF, inflammatory and immune responses have been reported(22) and as a result, various inflammatory biomarkers may be used to predict the prognosis of patients with acute and chronic HF (CHF). When 140 patients with chronic systolic HF were examined for correlations between plasma MPO levels and echocardiographic indexes as well as long-term clinical outcomes, elevated plasma MPO levels, apart from being a predictor of adverse clinical outcomes, was also found to be associated with a higher probability of more advanced HF(120). Similarly, when assessing the combined effect of high-sensitivity C-reactive protein (hsCRP) and MPO in 136 CHF patients over 34 months, researchers observed a 6-fold increase in risk when both markers were elevated(119). Further stratification of patients according to hsCRP, MPO and NT-proBNP cut-off values provided an increment of risk prediction in adverse events. The study concluded that the combinatorial analysis of hsCRP and MPO measurements provided a complementary prognostic value in these patients. Furthermore, in an attempt to investigate the relationship between MPO and other myocardial damage biomarkers, such as heart-type fatty acid-binding protein (H-FABP) and troponin T (TnT), MPO was significantly associated

10

with serum H-FABP, but not TnT in 42 CHF patients(34). In a separate study involving 86 patients with different stages of CHF, in addition to differences in MPO levels being observed between CHF patients and healthy subjects, a positive correlation was established between CHF severity and MPO levels, as MPO was significantly elevated in patients with higher mortality(8). Consistently, when assessing MPO activity in HF, heightened MPO chlorinating activity was found in 81 CHF patients compared to healthy subjects along with a positive correlation with ceruloplasmin, inflammatory, neurohormonal and nitrosative parameters, suggesting a role of MPO chlorinating activity in HF progression(15). Alternatively, when investigating the probability of MPO levels to predict the risk of developing HF in 3733 healthy elderly subjects over an approximate 7-year follow-up, increased systemic MPO levels was independently associated with the development of HF, especially in subjects without traditional cardiovascular risk factors(118).

Though HF has conventionally been associated with reduced systolic function, HF with preserved systolic function/ejection fraction (PRESYF/HFpEF) which currently accounts for approximately 50% of all HF cases, is a leading cause of morbidity and mortality(86). Diastolic dysfunction is a key pathophysiological feature of HFpEF that arises from incomplete myocardial relaxation and subsequent impaired rate of ventricular filling, which in turn could result in dyspnea, fatigue and exercise intolerance(59). As identification of biomarkers for early diagnosis of diastolic dysfunction could lead to improved patient management strategies, researchers assessed the levels of serum MPO in 91 patients diagnosed with diastolic dysfunction. When MPO values were correlated with echocardiography parameters, serum MPO levels were found to be independently or in correlation with the echocardiography parameters associated with diastolic dysfunction(18).

Contrary to these findings, a study using canine models, failed to observe a positive correlation with MPO and HF severity(89). Instead, when 69 canines with different stages of HF, due to chronic mitral valvular insufficiency (CVMI) were compared against 13 healthy controls, a negative correlation between MPO levels and HF severity was observed, as serum MPO levels decreased as the HF increased in severity.

Hypertension

Hypertension is a main contributor to CVD, in both developed and developing countries. Being associated with more than 40% of CVD-related deaths and renal diseases, it is one of the leading causes of mortality globally. Since it is largely asymptomatic, many hypertensive patients are undiagnosed and hence, untreated, resulting in premature deaths, due to various complications(77). With the aim of understanding the relationship between the MPO reaction system and oxidative modifications of serum lipoproteins, researchers attempted to compare the peroxidation and chlorination activity of MPO, against various oxidative and anti-oxidative stress-related indices in atherosclerotic patients with hypertension(64). Interestingly, the peroxidation activity, rather than the chlorination activity correlated with progressive low-density lipoprotein modification in the patient cohort.

Given that obesity is a risk factor for preeclampsia, and that neutrophil infiltration into blood vessels occurs in both obese and preeclamptic women, a study attempted to investigate the effects of MPO under these conditions(112). Interestingly, MPO distribution in blood vessels was found to be different in obese compared to overweight (but not obese) and normal (non-overweight) pregnant women, as immunohistochemical staining showed the most abundant amounts of MPO in the subcutaneous fat of blood vessels of obese pregnant women, followed by overweight and then normal pregnant women. Furthermore, leukocyte infiltration was absent in normal pregnant women, while in obese and preeclamptic pregnant women, leukocytes were found in the lumen, flattened and adhered to the endothelium and infiltrated into the vessel wall. In general, due to neutrophil infiltration, both obese and preeclamptic women had increased MPO in the systemic vasculature. MPO expression was also increased in the maternal blood vessels of preeclamptic women than normal pregnant or normal nonpregnant women. In a similar study, when plasma MPO concentrations and activity was measured in 219 healthy pregnant women, 130 gestational hypertension and 143 preeclampsia patients, it was found that preeclampsia and gestational hypertension patients not receiving anti-hypertensive treatment had higher MPO levels and increased MPO activity respectively(101). Furthermore, higher levels of MPO was in the supernatant obtained

12

from human umbilical vein endothelial cell cultures incubated with plasma from preeclampsia group compared to healthy pregnant women. The study also demonstrated that elevated MPO levels may contribute to endothelial dysfunction via nitric oxide impairment, as inhibition of MPO activity *in vitro* improved nitric oxide bioavailability. Interestingly, antihypertensive drugs seemed to decrease MPO levels in preeclampsia and gestational hypertension patients, but with no impact on MPO activity(101). In a study aimed at understanding the effects of MPO in hypertensive pregnancy, researchers observed increased cardiac MPO in the left but not right ventricle of hypertensive pregnant rats(131). Increased circulating MPO activities were associated with concomitantly lower number of viable fetuses, litter size, fetal and placenta weights as well as decreases in nitric oxide in hypertensive rats. Circulating sFlt-1 and VEGF was also increased in the hypertensive pregnant group, which cumulatively suggested preeclampsia to be associated with an inflammatory response.

Stroke is one of the main causes of mortality and morbidity worldwide, with hypertension being an important risk factor. Since stroke survivors have increased risk of recurrent stroke, dementia and other vascular diseases(132), it is important to identify markers that could predict such events. Consistently, when assessing 13 plasma biomarkers in 2176 participants of which 562 had outcome strokes, MPO was independently associated with the risk of recurrent stroke(33). Alternatively, MPO can also be used as an imaging biomarker to risk-stratify vulnerable stroke patients(12).

Diabetes mellitus

Obesity has become a major public health concern globally, affecting approximately 1.5 billion people. Given that obesity is a risk factor for various diseases, including type 2 diabetes, the increase in obesity has projected the number of people affected by type 2 diabetes to increase from 366 million in 2011 to 552 million in 2030(65). An initial study demonstrated MPO to bind to red blood cells (RBC) that resulted in alterations to their biophysical properties. A correlation between plasma MPO concentration and RBC rigidity index in type-2 diabetes mellitus (DM) patients with CHD was also observed, suggesting MPO to alter components of the microcirculation under pathological stress conditions(38).

13

In a study assessing neutrophil activity in type-2 DM patients with and without CHD, researchers found plasma MPO levels to be elevated in those with CHD, suggesting plasma MPO levels to be an additional biomarker of oxidative stress and cardiovascular risk in DM patients(37).

Heart transplant and graft rejection

Rates of graft rejection are high among recipients of heart transplants. As a result, following heart transplantation, endomyocardial biopsy (EMB) is the standard method to diagnose acute graft rejection, and hence, there is an inherent need to develop a less or non-invasive alternative method of diagnosis. With the aim of achieving this, researchers measured serum MPO and carbonyl proteins (CP) in 28 patients who had undergone heart transplantation and had experienced various grades of rejection(58). Serum MPO and CP levels in post-transplant patients with Grade 2R rejection were significantly elevated at the time of rejection, compared to those measured a month earlier. This highlighted the possibility that serum MPO and CP levels could be used to predict Grade 2R rejection, thereby, suggesting the use of these markers to monitor rejection events non-invasively. MPO imaging represents another potential alternative to invasive techniques for diagnosing graft rejection, as mice which underwent heart transplant showed that a specific monocyte subpopulation could accumulate in the allografts and express high levels of MPO(58).

The longevity of heart transplants is heavily dependent on chronic rejection in the form of cardiac allograft vasculopathy (CAV)(70). CAV has high prevalence among heart transplant patients due to both immunological and non-immunological factors(123). When assessing 47 heart transplant recipients, researchers observed patients with CAV to have a higher oxidative stress index (OSI), MPO levels and catalase activity, when compared to those who did not have CAV. Upon further stratification of CAV patients to mild and severe, the latter group was associated with higher OSI, and a moderate correlation was observed between the CAV grade and OSI, MPO, and catalase activity(51).

14

MPO – A TARGET FOR CARDIOVASCULAR PROTECTION

Atherosclerosis

In one of the first studies aimed at identifying proteins which are directly targeted by MPO, researchers found apolipoprotein A-I (apoA-I); the primary protein constituent of HDL to be a selective target for MPO-catalyzed nitration and chlorination(129). Furthermore, these MPO-catalyzed modifications that lead to the oxidation of HDL and apoA-I, resulted in the inhibition of ABCA1-dependent cholesterol efflux from macrophages. Interestingly, MPO was found to exert its effects by directly binding to apoA-I, which resulted in the latter's oxidation. In a more recent study, apoA-I nitration and chlorination levels were found to be significantly increased in the serum of diabetic patients, with a concurrent decrease in apoA-I concentration and cholesterol efflux activity(67). Further, *in vitro* studies suggested MPO-catalyzed modifications to impair anti-apoptotic properties of HDL. HDL found in atherosclerotic plaques are considered to be cardioprotective(24), an effect that is compromised upon MPO-mediated nitration and chlorination(130). When the effects of nitrated and chlorinated HDL on vascular smooth muscle cell (VSMC) migration, proliferation, apoptosis and plaque stability was investigated, researchers found these modifications to inhibit VSMC migration and proliferation, though apoptosis was not affected(130). This was attributed to a reduction in ERK1/2 phosphorylation, which was observed when VSMCs were incubated with oxidized HDL. Consistent with this, the migration defects could be rescued with a MAPK inhibitor; PD98059. Furthermore, in aortic VSMCs that were deficient for scavenger receptor BI (SR-BI), the migration differences observed when VSMCs were treated with either native or oxidized HDL was diminished, suggesting SR-BI to play a role in VSMC migration. Oxidized HDL induced neointima formation and reduced SMCs in atherosclerotic plaque, resulting in an elevated vulnerable index of the plaque, which suggested oxidized HDL to contribute to plaque instability(130). In another study, researchers investigated the role of MPO to regulate atherosclerotic lesion formation and composition in LDLR$^{-/-}$ mice; a model for atherosclerosis(103). Upon treatment with a selective small molecule MPO inhibitor; PF-06282999, though there was no effect on the lesion area, a reduced necrotic core was observed in the aortic root. Furthermore, MPO inhibition did not seem to alter leukocyte homing and macrophage content in the plaques. This seemed to suggest that although

15

MPO inhibition had no effect on plaque area nor the homing of inflammatory cells, it could alter the level of inflammation in the lesions, and hence, MPO inhibition could stabilize the plaque and prevent its rupture. In a more recent study, researchers attempted to target MPO in ruptured atherosclerotic plaques(97). In a tandem stenosis $Apoe^{-/-}$ model of atherosclerotic plaque instability, MPO activity was found to be greater than 2-fold when plaques were rendered unstable. Genetic deletion of MPO as well as pharmacological treatment with an MPO inhibitor, AZM198 resulted in increased fibrous cap thickness, decreased fibrin and hemosiderin in unstable plaques, suggesting that inhibition of plaque MPO activity could be a potential management strategy for patients with high-risk coronary artery disease.

Ischemia/reperfusion injury

Apart from directly inhibiting MPO, researchers have also investigated alternative approaches by which to target MPO activity. Since endothelin (ET) receptor antagonists have been reported to limit myocardial ischemia/reperfusion (I/R) injury(43,105), a study was aimed at assessing the cardioprotective effect of such antagonists, and their ability to inhibit MPO activity during I/R. When pigs were subjected to acute myocardial ischemia followed by reperfusion, infiltration of MPO^+ cells into the ischemic region was observed. However, when a selective ET(A) receptor antagonist LU135252 was administered into the left anterior descending coronary artery during the final 10 min of ischemia and the first 5 min of reperfusion, a marked reduction in final infarct size was achieved in comparison to the vehicle group. Interestingly, MPO activity in the ischemic myocardium was significantly lower in the LU135252 treated group, which suggested a positive correlation between infarct size and MPO activity(36). Hence, by targeting the ET(A) receptor, a cardioprotective effect could be achieved by reducing injury mediated by MPO activity. In a separate study, the cAMP phosphodiesterase inhibitor; BMY21190 was also found to reduce the infarct zone in a canine model of I/R injury(72), and much like the previous study, MPO activity was significantly lower in the area at risk in the BMY21190 treated group. As phloroglucinol has been reported to have anti-inflammatory and anti-oxidant properties(55), its effects were investigated in a rat model of I/R injury(61). Post-I/R, in addition to having a large infarct size, control rats comprised of increased MPO levels and

16

activity in the plasma and myocardium, which could be alleviated by pre-treatment with phloroglucinol.

Diabetes mellitus

In the case of diabetes mellitus (DM), given that vascular dysfunction and inflammation are hallmarks of the disease, and as overexpression of adenosine A3 receptor (A3AR) has been shown to mediate such conditions, researchers attempted to study the role of MPO in adenosine-dependent vasomotor function in a murine model of DM(82). When wild-type and MPO-deficient mice were treated with Streptozotocin, an increase in MPO plasma levels was observed in the former group, which lead to an enhanced aortic superoxide production. Furthermore, vasoconstriction of aortic segments was increased in diabetic wild-type mice as compared to diabetic MPO-deficient mice, which was attributed to MPO-mediated increases in vascular A3AR expression that lead to enhanced vasoconstriction and vascular dysfunction(82).

Atrial fibrillation

In an attempt to assess whether inflammation is the cause of atrial fibrillation, researchers treated mice with angiotensin II to induce leukocyte activation (104). Upon doing so, wild-type mice exhibited higher levels of plasma MPO, abundant levels of 3-chlorotyrosine in the atrial tissue as well as increased matrix metalloproteinase (MMP) activity and atrial fibrosis. Contrary to this, MPO$^{-/-}$ mice showed a reduction in all endpoints and were also protected against atrial fibrillation, an effect that was lost upon intravenous supplementation of recombinant MPO.

Myocardial infarction

Following MI, the adverse remodeling of the left ventricle and its subsequent dilation is a major cause of congestive heart failure. In an attempt to determine a link between left ventricle remodeling and inflammation, researchers investigated the effects of MPO in a chronic coronary artery ligation model(7). Interestingly, MPO$^{-/-}$ mice demonstrated decreased leukocyte infiltration and improved left ventricle function, that was attributed to decreased tissue plasmin activity. Furthermore, MPO and plasminogen activator

inhibitor 1 (PAI-1) seemed to have contrasting roles, as MPO^{-/-} mice demonstrated delayed myocardial rupture, whereas PAI-1^{-/-} demonstrated accelerated rupture. Similarly, MPO inhibitor molecules have also been reported to alleviate cardiac remodeling and improve function in a mouse MI model(2). When using PF-1355; an oral inhibitor of MPO, 7 days of treatment saw a decrease in the number of inflammatory cells and attenuated left ventricular dilation, while 21 days of treatment saw further improvement in cardiac function and remodeling. Furthermore, early administration of the inhibitor as opposed to later treatment (1h vs 25h post-MI) seemed to result in better outcomes. In an attempt to assess the effects of MPO on post-ischemic arrhythmogenic ventricular remodeling, researchers found the ventricles of MPO^{-/-} mice to exhibit improved electrical conduction in the peri-infarct zone post-MI, compared to wild-type mice(73). This was attributed to the maintenance of Connexin 43, which was broken down in the wild-type mice by an MPO-mediated activation of MMP-7. Furthermore, MPO was capable of transdifferentiating fibroblasts to myofibroblasts, the latter of which was reduced in MPO^{-/-} mice, which coincided with decreased post-ischemic fibrosis. In addition to these small animal models of MI, MPO^{+} infiltrating cells can also be detected in cardiac tissue obtained from Yorkshire pigs that underwent myocardial injury due coronary artery ligation (Figure 4)(76). Future studies on large animal models of CVD could help ascertain the reason for this infiltration, identify mechanisms by which these cells impede cardiac function as well as assess potential therapeutic outcomes through MPO inhibition strategies.

Pulmonary arterial hypertension

More recently, a study aimed to assess whether MPO is causally liked to pulmonary arterial hypertension (PAH) as two independent clinical cohorts where found to have elevated plasma MPO(57). When attempting to decipher the mechanism by which MPO causes PAH, researchers observed that upon hypoxia, the right ventricular pressure was less increased in MPO^{-/-} mice, when compared to wild-type mice. Furthermore, hypoxia induced activation of the Rho-kinase pathway that is reported to be involved in vasoconstriction and structural vascular remodeling(83). Consistently, while this pathway was blunted in MPO^{-/-} mice, infusion of MPO activated this pathway and increased right ventricular pressure, which could be prevented by the Rho-kinase inhibitor; Y-27632.

18

Furthermore, the MPO inhibitor AZM198 attenuated PAH, in a Sugen5416/hypoxia rat model, demonstrating tight interplay between MPO, the Rho-kinase pathway and vascular function.

ANTIOXIDANTS AND CARDIOVASCULAR PROTECTION

Under normal cellular conditions, a balance exists between the generation of reactive species and protection by antioxidants. An imbalance in this process, either due to an increased generation of reactive species or impairment of the antioxidative protective pathways, results in oxidative stress(78). Oxidative stress, though initially confined to reactive oxygen species (ROS), now includes reactive nitrogen species (RNS) as well. ROS are by-products of oxidative metabolism, while RNS are produced by nitric oxide synthase which is initiated from L-arginine(102). ROS and RNS include (but are not limited to) hydrogen peroxide, superoxide, nitric oxide and peroxynitrite, all of which could be incorporated into the MPO reaction cycle to generate 3-chlorotyrosine and 3-nitrotyrosine (Figure 5)(117). Since these reactive species have also been shown to be responsible for the pathophysiology of various CVDs(48,75), the targeting of oxidative stress via the use of antioxidants could be considered an alternative cardioprotective strategy. However, despite positive findings in pre-clinical and small clinical trials, reports on large clinical trials assessing the protective properties of antioxidants, alone or in combination, have largely been disappointing, with certain antioxidants such as beta-carotene found to increase the risk of death, in subjects who had a previous MI(75,113). Diverse speculations have been made as to why antioxidant therapies have not been successful, including several generalizations, such as; (i) not all reactive species are universally harmful, (ii) excessive amounts of antioxidants could render the cells in a highly reduced harmful state and that (iii) antioxidants may be metabolized prior to reaching its target(39). Having said this, the endoplasmic reticulum-resident selenoproteins, are rapidly emerging as cardioprotective agents, as several studies have linked their dysfunction with susceptibility to oxidative stress and CVD(100). More recently, selenoprotein T was found to exert cardioprotective effects in a rat model of I/R injury(99). Following the use of a peptide encompassing the redox motif of selenoprotein T, researchers observed significant contractile recovery and reduction in infarct size. This protective effect was accompanied

19

by modulation of several favorable signaling pathways, the suppression of pro-apoptotic factors with concurrent stimulation of anti-apoptotic proteins, as well as the reduction of several oxidative and nitrosative stress markers(99). This clearly suggests that the targeting of oxidative stress for cardioprotection does not encompass one pathway, but an intricate network of multiple pathways, and hence, it would be interesting to see if selenoproteins, would fare better than their contemporary antioxidants in improving CVD-related outcomes.

CONCLUSIONS

Being a prognostic biomarker in a wide variety CVDs, MPO has the potential to be a multifaceted target for cardiovascular protection. It is important to note however, that while several studies support MPO to be a predictor of worsened cardiovascular outcomes, this has not been a universal occurrence and hence, care should be taken when determining its use as a biomarker for assessing disease severity and heightened risk. This is further confounded by reports which demonstrate that the sample type, the manner in which it was acquired, as well as the storage conditions can affect MPO measurements(126). While these variables can probably be overcome with the development of cost-effective, rapid assays that are resistant to exogenous MPO inhibitors(35), the findings reported from various animal models of CVD, in which inhibition of MPO or its depletion resulted in an alleviated disease phenotype, is highly encouraging. While we wait in anticipation of the results from the clinical trial (NCT03611153) aimed at investigating the effect of MPO inhibition on resting and exercise hemodynamics in patients with HFpEF, further studies aimed at elucidating the mechanisms by which elevated MPO leads to poor prognosis and conversely, studying the effects of MPO inhibition on alleviating disease phenotype in other CVDs will facilitate future MPO-targeted clinical trials for improving CVD-related outcomes.

ACKNOWLEDGEMENTS

20

AUTHOR DISCLOSURE STATEMENT

The authors disclose no competing financial interests.

21

ABBREVIATIONS

A3AR   Adenosine A3 receptor

ABCA1 ATP Binding Cassette Subfamily A Member 1

ACS   Acute coronary syndrome

AMI   Acute myocardial infarction

apoA-I   Apolipoprotein A-I

Apoe   Apolipoprotein E

ASO   Atherosclerosis obliterans

CAD   Coronary artery disease

CAV   Cardiac allograft vasculopathy

CHD   Coronary heart disease

CHF   Chronic heart failure

CP   Carbonyl proteins

CRP   C-reactive protein

cTnI   Cardiac troponin I

CVD   Cardiovascular disease

DM   Diabetes mellitus

ET   Endothelin

HDL   High-density lipoprotein

HF   Heart failure

H-FABP   Heart-type fatty acid-binding protein

HFpEF   Heart failure with preserved ejection fraction

HOCl   Hypochlorous acid

hsCRP   High-sensitivity C-reactive protein

hs-cTnT   High-sensitivity cardiac troponin T

I/R   Ischemia/reperfusion

IHD   Ischemic heart disease

LDLR   Low-density lipoprotein receptor

MI   Myocardial infarction

MMP   Matrix metalloproteinase

MPO   Myeloperoxidase

22

NSTEMI	Non-ST-segment elevation myocardial infarction
NT-proBNP	N-terminal prohormone of brain natriuretic peptide
OSI	Oxidative stress index
PAH	Pulmonary arterial hypertension
PAI-1	Plasminogen activator inhibitor 1
PAPP-A	Pregnancy-associated plasma protein A
PPCI	Primary percutaneous intervention
RBC	Red blood cell
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
sFlt-1	Soluble fms-like tyrosine kinase-1
SR-BI	Scavenger receptor class B, type I
STEMI	ST-segment elevation myocardial infarction
TnT	Troponin T
VEGF	Vascular endothelial growth factor
VSMC	Vascular smooth muscle cells

REFERENCES

1. Abdo AI, Rayner BS, van Reyk DM, Hawkins CL. Low-density lipoprotein modified by myeloperoxidase oxidants induces endothelial dysfunction. *Redox Biol* 13: 623-632, 2017.

2. Ali M, Pulli B, Courties G, Tricot B, Sebas M, Iwamoto Y, Hilgendorf I, Schob S, Dong A, Zheng W, Skoura A, Kalgukar A, Cortes C, Ruggeri R, Swirski FK, Nahrendorf M, Buckbinder L, Chen JW. Myeloperoxidase Inhibition Improves Ventricular Function and Remodeling After Experimental Myocardial Infarction. *JACC Basic Transl Sci* 1: 633-643, 2016.

3. Allen LA, O'Donnell CJ, Camargo CA, Jr., Giugliano RP, Lloyd-Jones DM. Comparison of long-term mortality across the spectrum of acute coronary syndromes. *Am Heart J* 151: 1065-71, 2006.

4. Aratani Y. Myeloperoxidase: Its role for host defense, inflammation, and neutrophil function. *Arch Biochem Biophys* 640: 47-52, 2018.

5. Arnhold J, Furtmuller PG, Obinger C. Redox properties of myeloperoxidase. *Redox Rep* 8: 179-86, 2003.

6. Arnhold J, Furtmuller PG, Regelsberger G, Obinger C. Redox properties of the couple compound I/native enzyme of myeloperoxidase and eosinophil peroxidase. *Eur J Biochem* 268: 5142-8, 2001.

7. Askari AT, Brennan ML, Zhou X, Drinko J, Morehead A, Thomas JD, Topol EJ, Hazen SL, Penn MS. Myeloperoxidase and plasminogen activator inhibitor 1 play a central role in ventricular remodeling after myocardial infarction. *J Exp Med* 197: 615-24, 2003.

8. Avaliani T, Talakvadze T, Tabagari S. Prognostic Value of Plasma Myeloperoxidase Level's and Echocardiographic Determinants in Chronic Heart Failure Patients. *Georgian Med News*: 55-60, 2019.

9. Baldus S, Heeschen C, Meinertz T, Zeiher AM, Eiserich JP, Munzel T, Simoons ML, Hamm CW, Investigators C. Myeloperoxidase serum levels predict risk in patients with acute coronary syndromes. *Circulation* 108: 1440-5, 2003.

24

10. Barbato E, Bartunek J, Mangiacapra F, Sciarretta S, Stanzione R, Delrue L, Cotugno M, Marchitti S, Iaccarino G, Sirico G, Di Castro S, Evangelista A, Lambrechts D, Sinnaeve P, De Bruyne B, Van De Werf F, Janssens S, Fox KA, Wijns W, Volpe M, Rubattu S. Influence of rs5065 atrial natriuretic peptide gene variant on coronary artery disease. *J Am Coll Cardiol* 59: 1763-70, 2012.

11. Bazarnyi VV, Tikhonina EN, Kondrashov KV. [The detection of myeloperoxidase in case of surgical treatment of ischemic heart disease]. *Klin Lab Diagn*: 8-10, 2012.

12. Breckwoldt MO, Chen JW, Stangenberg L, Aikawa E, Rodriguez E, Qiu S, Moskowitz MA, Weissleder R. Tracking the inflammatory response in stroke in vivo by sensing the enzyme myeloperoxidase. *Proc Natl Acad Sci U S A* 105: 18584-9, 2008.

13. Brennan ML, Penn MS, Van Lente F, Nambi V, Shishehbor MH, Aviles RJ, Goormastic M, Pepoy ML, McErlean ES, Topol EJ, Nissen SE, Hazen SL. Prognostic value of myeloperoxidase in patients with chest pain. *N Engl J Med* 349: 1595-604, 2003.

14. Brevetti G, Schiano V, Laurenzano E, Giugliano G, Petretta M, Scopacasa F, Chiariello M. Myeloperoxidase, but not C-reactive protein, predicts cardiovascular risk in peripheral arterial disease. *Eur Heart J* 29: 224-30, 2008.

15. Cabassi A, Binno SM, Tedeschi S, Graiani G, Galizia C, Bianconcini M, Coghi P, Fellini F, Ruffini L, Govoni P, Piepoli M, Perlini S, Regolisti G, Fiaccadori E. Myeloperoxidase-Related Chlorination Activity Is Positively Associated with Circulating Ceruloplasmin in Chronic Heart Failure Patients: Relationship with Neurohormonal, Inflammatory, and Nutritional Parameters. *Biomed Res Int* 2015: 691693, 2015.

16. Calmarza P, Lapresta C, Martinez M, Lahoz R, Povar J. Utility of myeloperoxidase in the differential diagnosis of acute coronary syndrome. *Arch Cardiol Mex* 88: 391-396, 2018.

17. Chen LQ, Rohatgi A, Ayers CR, Das SR, Khera A, Berry JD, McGuire DK, de Lemos JA. Race-specific associations of myeloperoxidase with atherosclerosis in a population-based sample: the Dallas Heart Study. *Atherosclerosis* 219: 833-8, 2011.

18. Coculescu BI, Dinca GV, Balaet C, Manole G, Balaet M, Stocheci CM. Myeloperoxidase, a possible biomarker for the early diagnosis of cardiac diastolic dysfunction with preserved ejection fraction. *J Enzyme Inhib Med Chem* 33: 1292-1298, 2018.

25

19.	Crea F, Libby P. Acute Coronary Syndromes: The Way Forward From Mechanisms to Precision Treatment. *Circulation* 136: 1155-1166, 2017.

20.	Davies MJ. Myeloperoxidase-derived oxidation: mechanisms of biological damage and its prevention. *J Clin Biochem Nutr* 48: 8-19, 2011.

21.	Derosa G, Mugellini A, Pesce RM, D'Angelo A, Maffioli P. A study about the relevance of adding acetylsalicylic acid in primary prevention in subjects with type 2 diabetes mellitus: effects on some new emerging biomarkers of cardiovascular risk. *Cardiovasc Diabetol* 14: 95, 2015.

22.	Dick SA, Epelman S. Chronic Heart Failure and Inflammation: What Do We Really Know? *Circ Res* 119: 159-76, 2016.

23.	Duivenvoorden R, Mani V, Woodward M, Kallend D, Suchankova G, Fuster V, Rudd JHF, Tawakol A, Farkouh ME, Fayad ZA. Relationship of serum inflammatory biomarkers with plaque inflammation assessed by FDG PET/CT: the dal-PLAQUE study. *JACC Cardiovasc Imaging* 6: 1087-1094, 2013.

24.	Feig JE, Feig JL, Dangas GD. The role of HDL in plaque stabilization and regression: basic mechanisms and clinical implications. *Coron Artery Dis* 27: 592-603, 2016.

25.	Feng H, Li XA. Dysfunctional high-density lipoprotein. *Curr Opin Endocrinol Diabetes Obes* 16: 156-62, 2009.

26.	Ferrante G, Nakano M, Prati F, Niccoli G, Mallus MT, Ramazzotti V, Montone RA, Kolodgie FD, Virmani R, Crea F. High levels of systemic myeloperoxidase are associated with coronary plaque erosion in patients with acute coronary syndromes: a clinicopathological study. *Circulation* 122: 2505-13, 2010.

27.	Fiedler TJ, Davey CA, Fenna RE. X-ray crystal structure and characterization of halide-binding sites of human myeloperoxidase at 1.8 A resolution. *J Biol Chem* 275: 11964-71, 2000.

28.	Fox KA. Coronary disease. Acute coronary syndromes: presentation--clinical spectrum and management. *Heart* 84: 93-100, 2000.

29.	Freedman JE, Larson MG, Tanriverdi K, O'Donnell CJ, Morin K, Hakanson AS, Vasan RS, Johnson AD, Iafrati MD, Benjamin EJ. Relation of platelet and leukocyte inflammatory transcripts to body mass index in the Framingham heart study. *Circulation* 122: 119-29, 2010.

26

30. Fu X, Kassim SY, Parks WC, Heinecke JW. Hypochlorous acid oxygenates the cysteine switch domain of pro-matrilysin (MMP-7). A mechanism for matrix metalloproteinase activation and atherosclerotic plaque rupture by myeloperoxidase. *J Biol Chem* 276: 41279-87, 2001.

31. Furtmuller PG, Arnhold J, Jantschko W, Pichler H, Obinger C. Redox properties of the couples compound I/compound II and compound II/native enzyme of human myeloperoxidase. *Biochem Biophys Res Commun* 301: 551-7, 2003.

32. Furtmuller PG, Burner U, Obinger C. Reaction of myeloperoxidase compound I with chloride, bromide, iodide, and thiocyanate. *Biochemistry* 37: 17923-30, 1998.

33. Ganz P, Amarenco P, Goldstein LB, Sillesen H, Bao W, Preston GM, Welch KMA, Committee SS. Association of Osteopontin, Neopterin, and Myeloperoxidase With Stroke Risk in Patients With Prior Stroke or Transient Ischemic Attacks: Results of an Analysis of 13 Biomarkers From the Stroke Prevention by Aggressive Reduction in Cholesterol Levels Trial. *Stroke* 48: 3223-3231, 2017.

34. Gedikli O, Kiris A, Hosoglu Y, Karahan C, Kaplan S. Serum myeloperoxidase level is associated with heart-type fatty acid-binding protein but not troponin T in patients with chronic heart failure. *Med Princ Pract* 24: 42-6, 2015.

35. Goiffon RJ, Martinez SC, Piwnica-Worms D. A rapid bioluminescence assay for measuring myeloperoxidase activity in human plasma. *Nat Commun* 6: 6271, 2015.

36. Gonon AT, Gourine AV, Middelveld RJ, Alving K, Pernow J. Limitation of infarct size and attenuation of myeloperoxidase activity by an endothelin A receptor antagonist following ischaemia and reperfusion. *Basic Res Cardiol* 96: 454-62, 2001.

37. Gorudko IV, Kostevich VA, Sokolov AV, Shamova EV, Buko IV, Konstantinova EE, Vasiliev VB, Cherenkevich SN, Panasenko OM. Functional activity of neutrophils in diabetes mellitus and coronary heart disease: role of myeloperoxidase in the development of oxidative stress. *Bull Exp Biol Med* 154: 23-6, 2012.

38. Gorudko IV, Sokolov AV, Shamova EV, Grigorieva DV, Mironova EV, Kudryavtsev IV, Gusev SA, Gusev AA, Chekanov AV, Vasilyev VB, Cherenkevich SN, Panasenko OM, Timoshenko AV. Binding of human myeloperoxidase to red blood cells: Molecular targets and biophysical consequences at the plasma membrane level. *Arch Biochem Biophys* 591: 87-97, 2016.

39. Goszcz K, Deakin SJ, Duthie GG, Stewart D, Leslie SJ, Megson IL. Antioxidants in Cardiovascular Therapy: Panacea or False Hope? *Front Cardiovasc Med* 2: 29, 2015.

40. Govindarajan S, Raghavan VM, Rao AC. Plasma Myeloperoxidase and Total Sialic Acid as Prognostic Indicators in Acute Coronary Syndrome. *J Clin Diagn Res* 10: BC09-13, 2016.

41. Green PS, Mendez AJ, Jacob JS, Crowley JR, Growdon W, Hyman BT, Heinecke JW. Neuronal expression of myeloperoxidase is increased in Alzheimer's disease. *J Neurochem* 90: 724-33, 2004.

42. Grishkovskaya I, Paumann-Page M, Tscheliessnig R, Stampler J, Hofbauer S, Soudi M, Sevcnikar B, Oostenbrink C, Furtmuller PG, Djinovic-Carugo K, Nauseef WM, Obinger C. Structure of human promyeloperoxidase (proMPO) and the role of the propeptide in processing and maturation. *J Biol Chem* 292: 8244-8261, 2017.

43. Han H, Neubauer S, Braeker B, Ertl G. Endothelin-1 contributes to ischemia/reperfusion injury in isolated rat heart-attenuation of ischemic injury by the endothelin-1 antagonists BQ123 and BQ610. *J Mol Cell Cardiol* 27: 761-6, 1995.

44. Hasanpour Z, Javanmard SH, Gharaaty M, Sadeghi M. Association between serum myeloperoxidase levels and coronary artery disease in patients without diabetes, hypertension, obesity, and hyperlipidemia. *Adv Biomed Res* 5: 103, 2016.

45. Hawkins CL, Davies MJ. Inactivation of protease inhibitors and lysozyme by hypochlorous acid: role of side-chain oxidation and protein unfolding in loss of biological function. *Chem Res Toxicol* 18: 1600-10, 2005.

46. Herrington W, Lacey B, Sherliker P, Armitage J, Lewington S. Epidemiology of Atherosclerosis and the Potential to Reduce the Global Burden of Atherothrombotic Disease. *Circ Res* 118: 535-46, 2016.

47. Heslop CL, Frohlich JJ, Hill JS. Myeloperoxidase and C-reactive protein have combined utility for long-term prediction of cardiovascular mortality after coronary angiography. *J Am Coll Cardiol* 55: 1102-9, 2010.

48. Jain AK, Mehra NK, Swarnakar NK. Role of Antioxidants for the Treatment of Cardiovascular Diseases: Challenges and Opportunities. *Curr Pharm Des* 21: 4441-55, 2015.

28

49. Kacprzak M, Zielinska M. Prognostic value of myeloperoxidase concentration in patients with ST-segment elevation myocardial infarction treated with primary percutaneous coronary intervention. *Int J Cardiol* 223: 452-457, 2016.

50. Karakas M, Koenig W, Zierer A, Herder C, Rottbauer W, Baumert J, Meisinger C, Thorand B. Myeloperoxidase is associated with incident coronary heart disease independently of traditional risk factors: results from the MONICA/KORA Augsburg study. *J Intern Med* 271: 43-50, 2012.

51. Kargin R, Emiroglu MY, Evlice M, Celik M, Toprak AE, Avci A, Ayturk M, Kulahcioglu S, Bulut M, Caliskan M. Role of the oxidative stress index, myeloperoxidase, catalase activity for cardiac allograft vasculopathy in heart transplant recipients. *Clin Transplant* 32: e13273, 2018.

52. Kaya MG, Yalcin R, Okyay K, Poyraz F, Bayraktar N, Pasaoglu H, Boyaci B, Cengel A. Potential role of plasma myeloperoxidase level in predicting long-term outcome of acute myocardial infarction. *Tex Heart Inst J* 39: 500-6, 2012.

53. Khan DA, Sharif MS, Khan FA. Diagnostic performance of high-sensitivity troponin T, myeloperoxidase, and pregnancy-associated plasma protein A assays for triage of patients with acute myocardial infarction. *Korean J Lab Med* 31: 172-8, 2011.

54. Khine HW, Teiber JF, Haley RW, Khera A, Ayers CR, Rohatgi A. Association of the serum myeloperoxidase/high-density lipoprotein particle ratio and incident cardiovascular events in a multi-ethnic population: Observations from the Dallas Heart Study. *Atherosclerosis* 263: 156-162, 2017.

55. Kim MM, Kim SK. Effect of phloroglucinol on oxidative stress and inflammation. *Food Chem Toxicol* 48: 2925-33, 2010.

56. King CC, Piper ME, Gepner AD, Fiore MC, Baker TB, Stein JH. Longitudinal Impact of Smoking and Smoking Cessation on Inflammatory Markers of Cardiovascular Disease Risk. *Arterioscler Thromb Vasc Biol* 37: 374-379, 2017.

29

57. Klinke A, Berghausen E, Friedrichs K, Molz S, Lau D, Remane L, Berlin M, Kaltwasser C, Adam M, Mehrkens D, Mollenhauer M, Manchanda K, Ravekes T, Heresi GA, Aytekin M, Dweik RA, Hennigs JK, Kubala L, Michaelsson E, Rosenkranz S, Rudolph TK, Hazen SL, Klose H, Schermuly RT, Rudolph V, Baldus S. Myeloperoxidase aggravates pulmonary arterial hypertension by activation of vascular Rho-kinase. *JCI Insight* 3, 2018.

58. Koestenbauer S, Stiegler P, Stadlbauer V, Mayrhauser U, Leber B, Schweiger M, Wasler A, Prenner G, Sereinigg M, Zelzer S, Stojakovic T, Scarpatetti M, Griesbacher A, Greilberger J, Tscheliessnigg K. Myeloperoxidase and carbonyl proteins: promising markers for non-invasive monitoring of graft rejection after heart transplantation. *J Heart Lung Transplant* 29: 1352-7, 2010.

59. Kotecha D, Lam CS, Van Veldhuisen DJ, Van Gelder IC, Voors AA, Rienstra M. Heart Failure With Preserved Ejection Fraction and Atrial Fibrillation: Vicious Twins. *J Am Coll Cardiol* 68: 2217-2228, 2016.

60. La Rocca G, Di Stefano A, Eleuteri E, Anzalone R, Magno F, Corrao S, Loria T, Martorana A, Di Gangi C, Colombo M, Sansone F, Patane F, Farina F, Rinaldi M, Cappello F, Giannuzzi P, Zummo G. Oxidative stress induces myeloperoxidase expression in endocardial endothelial cells from patients with chronic heart failure. *Basic Res Cardiol* 104: 307-20, 2009.

61. Li TT, Zhang YS, He L, Li NS, Peng J, Li YJ. Protective effect of phloroglucinol against myocardial ischaemia-reperfusion injury is related to inhibition of myeloperoxidase activity and inflammatory cell infiltration. *Clin Exp Pharmacol Physiol* 38: 27-33, 2011.

62. Libby P, Pasterkamp G, Crea F, Jang IK. Reassessing the Mechanisms of Acute Coronary Syndromes. *Circ Res* 124: 150-160, 2019.

63. Liu C, Xie G, Huang W, Yang Y, Li P, Tu Z. Elevated serum myeloperoxidase activities are significantly associated with the prevalence of ACS and High LDL-C levels in CHD patients. *J Atheroscler Thromb* 19: 435-43, 2012.

30

64. Liu Q, Liu Y, Shi J, Gao M, Liu Y, Cong Y, Li Y, Wang Y, Yu M, Lu Y, Wang D, Chen S, Zheng Y, Cheng Y. Entire Peroxidation Reaction System of Myeloperoxidase Correlates with Progressive Low-Density Lipoprotein Modifications via Reactive Aldehydes in Atherosclerotic Patients with Hypertension. *Cell Physiol Biochem* 50: 1245-1254, 2018.

65. Loh M, Zhou L, Ng HK, Chambers JC. Epigenetic disturbances in obesity and diabetes: Epidemiological and functional insights. *Molecular Metabolism* 27: S33-S41, 2019.

66. Love DT, Barrett TJ, White MY, Cordwell SJ, Davies MJ, Hawkins CL. Cellular targets of the myeloperoxidase-derived oxidant hypothiocyanous acid (HOSCN) and its role in the inhibition of glycolysis in macrophages. *Free Radic Biol Med* 94: 88-98, 2016.

67. Lu N, Xie S, Li J, Tian R, Peng YY. Myeloperoxidase-mediated oxidation targets serum apolipoprotein A-I in diabetic patients and represents a potential mechanism leading to impaired anti-apoptotic activity of high density lipoprotein. *Clin Chim Acta* 441: 163-70, 2015.

68. Malle E, Furtmuller PG, Sattler W, Obinger C. Myeloperoxidase: a target for new drug development? *Br J Pharmacol* 152: 838-54, 2007.

69. McLaughlin K, Baczyk D, Potts A, Hladunewich M, Parker JD, Kingdom JC. Low Molecular Weight Heparin Improves Endothelial Function in Pregnant Women at High Risk of Preeclampsia. *Hypertension* 69: 180-188, 2017.

70. Merola J, Jane-Wit DD, Pober JS. Recent advances in allograft vasculopathy. *Curr Opin Organ Transplant* 22: 1-7, 2017.

71. Midwinter RG, Cheah FC, Moskovitz J, Vissers MC, Winterbourn CC. IkappaB is a sensitive target for oxidation by cell-permeable chloramines: inhibition of NF-kappaB activity by glycine chloramine through methionine oxidation. *Biochem J* 396: 71-8, 2006.

72. Minami M, Driscoll EM, Simpson PJ, Hoff PT, Lucchesi BR. Effects of BMY21190, an inhibitor of cAMP phosphodiesterase, on infarct size and myeloperoxidase activity in the ischemic myocardium of a canine occlusion-reperfusion model. *Pharmacology* 50: 24-33, 1995.

73. Mollenhauer M, Friedrichs K, Lange M, Gesenberg J, Remane L, Kerkenpass C, Krause J, Schneider J, Ravekes T, Maass M, Halbach M, Peinkofer G, Saric T, Mehrkens D, Adam M, Deuschl FG, Lau D, Geertz B, Manchanda K, Eschenhagen T, Kubala L, Rudolph TK, Wu Y, Tang WHW, Hazen SL, Baldus S, Klinke A, Rudolph V. Myeloperoxidase Mediates Postischemic Arrhythmogenic Ventricular Remodeling. *Circ Res* 121: 56-70, 2017.

74. Mortality GBD, Causes of Death C. Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet* 385: 117-71, 2015.

75. Munzel T, Gori T, Bruno RM, Taddei S. Is oxidative stress a therapeutic target in cardiovascular disease? *Eur Heart J* 31: 2741-8, 2010.

76. Myu Mai Ja KP, Lim KP, Chen A, Ting S, Li SQ, Tee N, Ramachandra C, Mehta A, Wong P, Oh S, Shim W. Construction of a vascularized hydrogel for cardiac tissue formation in a porcine model. *J Tissue Eng Regen Med* 12: e2029-e2038, 2018.

77. Nahimana MR, Nyandwi A, Muhimpundu MA, Olu O, Condo JU, Rusanganwa A, Koama JB, Ngoc CT, Gasherebuka JB, Ota MO, Okeibunor JC. A population-based national estimate of the prevalence and risk factors associated with hypertension in Rwanda: implications for prevention and control. *BMC Public Health* 18: 2, 2017.

78. Nathan C, Cunningham-Bussel A. Beyond oxidative stress: an immunologist's guide to reactive oxygen species. *Nat Rev Immunol* 13: 349-61, 2013.

79. Nauseef WM, Olsson I, Arnljots K. Biosynthesis and processing of myeloperoxidase--a marker for myeloid cell differentiation. *Eur J Haematol* 40: 97-110, 1988.

80. Ndrepepa G. Myeloperoxidase - A bridge linking inflammation and oxidative stress with cardiovascular disease. *Clin Chim Acta* 493: 36-51, 2019.

81. Nilsson L, Hallen J, Atar D, Jonasson L, Swahn E. Early measurements of plasma matrix metalloproteinase-2 predict infarct size and ventricular dysfunction in ST-elevation myocardial infarction. *Heart* 98: 31-6, 2012.

82. Nishat S, Klinke A, Baldus S, Khan LA, Basir SF. Increased A3AR-dependent vasoconstriction in diabetic mice is promoted by myeloperoxidase. *J Cardiovasc Pharmacol* 64: 465-72, 2014.

32

83. Nunes KP, Rigsby CS, Webb RC. RhoA/Rho-kinase and vascular diseases: what is the link? *Cell Mol Life Sci* 67: 3823-36, 2010.

84. O'Malley RG, Bonaca MP, Scirica BM, Murphy SA, Jarolim P, Sabatine MS, Braunwald E, Morrow DA. Prognostic performance of multiple biomarkers in patients with non-ST-segment elevation acute coronary syndrome: analysis from the MERLIN-TIMI 36 trial (Metabolic Efficiency With Ranolazine for Less Ischemia in Non-ST-Elevation Acute Coronary Syndromes-Thrombolysis In Myocardial Infarction 36). *J Am Coll Cardiol* 63: 1644-53, 2014.

85. Odobasic D, Kitching AR, Holdsworth SR. Neutrophil-Mediated Regulation of Innate and Adaptive Immunity: The Role of Myeloperoxidase. *J Immunol Res* 2016: 2349817, 2016.

86. Oktay AA, Rich JD, Shah SJ. The emerging epidemic of heart failure with preserved ejection fraction. *Curr Heart Fail Rep* 10: 401-10, 2013.

87. Olza J, Aguilera CM, Gil-Campos M, Leis R, Bueno G, Martinez-Jimenez MD, Valle M, Canete R, Tojo R, Moreno LA, Gil A. Myeloperoxidase is an early biomarker of inflammation and cardiovascular risk in prepubertal obese children. *Diabetes Care* 35: 2373-6, 2012.

88. Oyenuga AO, Couper D, Matsushita K, Boerwinkle E, Folsom AR. Association of monocyte myeloperoxidase with incident cardiovascular disease: The Atherosclerosis Risk in Communities Study. *PLoS One* 13: e0205310, 2018.

89. Park JI, Suh SI, Hyun C. Evaluation of serum myeloperoxidase concentration in dogs with heart failure due to chronic mitral valvular insufficiency. *Can J Vet Res* 81: 37-40, 2017.

90. Pattison DI, Davies MJ. Absolute rate constants for the reaction of hypochlorous acid with protein side chains and peptide bonds. *Chem Res Toxicol* 14: 1453-64, 2001.

91. Pattison DI, Davies MJ. Reactions of myeloperoxidase-derived oxidants with biological substrates: gaining chemical insight into human inflammatory diseases. *Curr Med Chem* 13: 3271-90, 2006.

92. Pattison DI, Hawkins CL, Davies MJ. Hypochlorous acid-mediated oxidation of lipid components and antioxidants present in low-density lipoproteins: absolute rate constants, product analysis, and computational modeling. *Chem Res Toxicol* 16: 439-49, 2003.

93. Phuah CL, Dave T, Malik R, Raffeld MR, Ayres AM, Goldstein JN, Viswanathan A, Greenberg SM, Jagiella JM, Hansen BM, Norrving B, Jimenez-Conde J, Roquer J, Pichler A, Enzinger C, Montaner J, Fernandez-Cadenas I, Lindgren A, Slowik A, Schmidt R, Biffi A, Rost N, Langefeld CD, Markus HS, Mitchell BD, Worrall BB, Kittner SJ, Woo D, Dichgans M, Rosand J, Anderson CD, Metastroke, Consortium NI-S, International Stroke Genetics C. Genetic variants influencing elevated myeloperoxidase levels increase risk of stroke. *Brain* 140: 2663-2672, 2017.

94. Pullar JM, Winterbourn CC, Vissers MC. Loss of GSH and thiol enzymes in endothelial cells exposed to sublethal concentrations of hypochlorous acid. *Am J Physiol* 277: H1505-12, 1999.

95. Quint P, Reutzel R, Mikulski R, McKenna R, Silverman DN. Crystal structure of nitrated human manganese superoxide dismutase: mechanism of inactivation. *Free Radic Biol Med* 40: 453-8, 2006.

96. Rana JS, Arsenault BJ, Despres JP, Cote M, Talmud PJ, Ninio E, Wouter Jukema J, Wareham NJ, Kastelein JJ, Khaw KT, Boekholdt SM. Inflammatory biomarkers, physical activity, waist circumference, and risk of future coronary heart disease in healthy men and women. *Eur Heart J* 32: 336-44, 2011.

97. Rashid I, Maghzal GJ, Chen YC, Cheng D, Talib J, Newington D, Ren M, Vajandar SK, Searle A, Maluenda A, Lindstedt EL, Jabbour A, Kettle AJ, Bongers A, Power C, Michaelsson E, Peter K, Stocker R. Myeloperoxidase is a potential molecular imaging and therapeutic target for the identification and stabilization of high-risk atherosclerotic plaque. *Eur Heart J* 39: 3301-3310, 2018.

98. Rathnayake N, Gustafsson A, Norhammar A, Kjellstrom B, Klinge B, Ryden L, Tervahartiala T, Sorsa T, Group PS. Salivary Matrix Metalloproteinase-8 and -9 and Myeloperoxidase in Relation to Coronary Heart and Periodontal Diseases: A Subgroup Report from the PAROKRANK Study (Periodontitis and Its Relation to Coronary Artery Disease). *PLoS One* 10: e0126370, 2015.

34

99. Rocca C, Boukhzar L, Granieri MC, Alsharif I, Mazza R, Lefranc B, Tota B, Leprince J, Cerra MC, Anouar Y, Angelone T. A selenoprotein T-derived peptide protects the heart against ischaemia/reperfusion injury through inhibition of apoptosis and oxidative stress. *Acta Physiol (Oxf)* 223: e13067, 2018.

100. Rocca C, Pasqua T, Boukhzar L, Anouar Y, Angelone T. Progress in the emerging role of selenoproteins in cardiovascular disease: focus on endoplasmic reticulum-resident selenoproteins. *Cell Mol Life Sci* 76: 3969-3985, 2019.

101. Rocha-Penha L, Caldeira-Dias M, Tanus-Santos JE, de Carvalho Cavalli R, Sandrim VC. Myeloperoxidase in Hypertensive Disorders of Pregnancy and Its Relation With Nitric Oxide. *Hypertension* 69: 1173-1180, 2017.

102. Rosen GM, Tsai P, Pou S. Mechanism of free-radical generation by nitric oxide synthase. *Chem Rev* 102: 1191-200, 2002.

103. Roth Flach RJ, Su C, Bollinger E, Cortes C, Robertson AW, Opsahl AC, Coskran TM, Maresca KP, Keliher EJ, Yates PD, Kim AM, Kalgutkar AS, Buckbinder L. Myeloperoxidase inhibition in mice alters atherosclerotic lesion composition. *PLoS One* 14: e0214150, 2019.

104. Rudolph V, Andrie RP, Rudolph TK, Friedrichs K, Klinke A, Hirsch-Hoffmann B, Schwoerer AP, Lau D, Fu X, Klingel K, Sydow K, Didie M, Seniuk A, von Leitner EC, Szoecs K, Schrickel JW, Treede H, Wenzel U, Lewalter T, Nickenig G, Zimmermann WH, Meinertz T, Boger RH, Reichenspurner H, Freeman BA, Eschenhagen T, Ehmke H, Hazen SL, Willems S, Baldus S. Myeloperoxidase acts as a profibrotic mediator of atrial fibrillation. *Nat Med* 16: 470-4, 2010.

105. Ryu SM, Kim HJ, Cho KR, Jo WM. Myocardial protective effect of tezosentan, an endothelin receptor antagonist, for ischemia-reperfusion injury in experimental heart failure models. *J Korean Med Sci* 24: 782-8, 2009.

106. Salonen I, Huttunen K, Hirvonen MR, Dufva J, Groundstroem K, Dufva H, Pekkanen J, Salonen RO. Serum myeloperoxidase is independent of the risk factors of atherosclerosis. *Coron Artery Dis* 23: 251-8, 2012.

107. Sawicki M, Sypniewska G, Kozinski M, Gruszka M, Krintus M, Obonska K, Pilaczynska-Cemel M, Kubica J. Diagnostic efficacy of myeloperoxidase for the detection of acute coronary syndromes. *Eur J Clin Invest* 41: 667-71, 2011.

35

108. Schultz J, Kaminker K. Myeloperoxidase of the leucocyte of normal human blood. I. Content and localization. *Arch Biochem Biophys* 96: 465-7, 1962.

109. Scirica BM, Sabatine MS, Jarolim P, Murphy SA, de Lemos JL, Braunwald E, Morrow DA. Assessment of multiple cardiac biomarkers in non-ST-segment elevation acute coronary syndromes: observations from the MERLIN-TIMI 36 trial. *Eur Heart J* 32: 697-705, 2011.

110. Senthilmohan R, Kettle AJ. Bromination and chlorination reactions of myeloperoxidase at physiological concentrations of bromide and chloride. *Arch Biochem Biophys* 445: 235-44, 2006.

111. Shao B, Bergt C, Fu X, Green P, Voss JC, Oda MN, Oram JF, Heinecke JW. Tyrosine 192 in apolipoprotein A-I is the major site of nitration and chlorination by myeloperoxidase, but only chlorination markedly impairs ABCA1-dependent cholesterol transport. *J Biol Chem* 280: 5983-93, 2005.

112. Shukla J, Walsh SW. Neutrophil release of myeloperoxidase in systemic vasculature of obese women may put them at risk for preeclampsia. *Reprod Sci* 22: 300-7, 2015.

113. Siti HN, Kamisah Y, Kamsiah J. The role of oxidative stress, antioxidants and vascular inflammation in cardiovascular disease (a review). *Vascul Pharmacol* 71: 40-56, 2015.

114. Sorrentino SA, Besler C, Rohrer L, Meyer M, Heinrich K, Bahlmann FH, Mueller M, Horvath T, Doerries C, Heinemann M, Flemmer S, Markowski A, Manes C, Bahr MJ, Haller H, von Eckardstein A, Drexler H, Landmesser U. Endothelial-vasoprotective effects of high-density lipoprotein are impaired in patients with type 2 diabetes mellitus but are improved after extended-release niacin therapy. *Circulation* 121: 110-22, 2010.

115. Stankovic S, Asanin M, Trifunovic D, Majkic-Singh N, Ignjatovic S, Mrdovic I, Matic D, Savic L, Marinkovic J, Ostojic M, Vasiljevic Z. Time-dependent changes of myeloperoxidase in relation to in-hospital mortality in patients with the first anterior ST-segment elevation myocardial infarction treated by primary percutaneous coronary intervention. *Clin Biochem* 45: 547-51, 2012.

36

116.   Stewart J, Manmathan G, Wilkinson P. Primary prevention of cardiovascular disease: A review of contemporary guidance and literature. *JRSM Cardiovasc Dis* 6: 2048004016687211, 2017.

117.   Syslova K, Bohmova A, Mikoska M, Kuzma M, Pelclova D, Kacer P. Multimarker screening of oxidative stress in aging. *Oxid Med Cell Longev* 2014: 562860, 2014.

118.   Tang WH, Katz R, Brennan ML, Aviles RJ, Tracy RP, Psaty BM, Hazen SL. Usefulness of myeloperoxidase levels in healthy elderly subjects to predict risk of developing heart failure. *Am J Cardiol* 103: 1269-74, 2009.

119.   Tang WH, Shrestha K, Troughton RW, Borowski AG, Klein AL. Integrating plasma high-sensitivity C-reactive protein and myeloperoxidase for risk prediction in chronic systolic heart failure. *Congest Heart Fail* 17: 105-9, 2011.

120.   Tang WH, Tong W, Troughton RW, Martin MG, Shrestha K, Borowski A, Jasper S, Hazen SL, Klein AL. Prognostic value and echocardiographic determinants of plasma myeloperoxidase levels in chronic heart failure. *J Am Coll Cardiol* 49: 2364-70, 2007.

121.   Tang WH, Wu Y, Nicholls SJ, Hazen SL. Plasma myeloperoxidase predicts incident cardiovascular risks in stable patients undergoing medical management for coronary artery disease. *Clin Chem* 57: 33-9, 2011.

122.   Tsimikas S, Mallat Z, Talmud PJ, Kastelein JJ, Wareham NJ, Sandhu MS, Miller ER, Benessiano J, Tedgui A, Witztum JL, Khaw KT, Boekholdt SM. Oxidation-specific biomarkers, lipoprotein(a), and risk of fatal and nonfatal coronary events. *J Am Coll Cardiol* 56: 946-55, 2010.

123.   Van Keer JM, Van Aelst LNL, Rega F, Droogne W, Voros G, Meyns B, Vanhaecke J, Emonds MP, Janssens S, Naesens M, Van Cleemput J. Long-term outcome of cardiac allograft vasculopathy: Importance of the International Society for Heart and Lung Transplantation angiographic grading scale. *J Heart Lung Transplant*, 2019.

124.   Vissers MC, Winterbourn CC. Myeloperoxidase-dependent oxidative inactivation of neutrophil neutral proteinases and microbicidal enzymes. *Biochem J* 245: 277-80, 1987.

125.   Vlasova, II, Arnhold J, Osipov AN, Panasenko OM. pH-dependent regulation of myeloperoxidase activity. *Biochemistry (Mosc)* 71: 667-77, 2006.

126. Wendland AE, Camargo JL, Polanczyk CA. Effect of preanalytical variables on myeloperoxidase levels. *Clin Chim Acta* 411: 1650-5, 2010.

127. Yonezawa K, Morimoto N, Matsui K, Tenjin T, Yoneda M, Emoto T, Sawada T, Nomura T, Okamoto H, Takarada A, Ikeda H, Ito M. Significance of the neutrophil myeloperoxidase index in patients with atherosclerotic diseases. *Kobe J Med Sci* 58: E128-37, 2013.

128. Zelzer S, Enko D, Pilz S, Tomaschitz A, Marz W, Meinitzer A. Myeloperoxidase, asymmetric dimethyl-arginine and the renin-angiotensin-aldosterone-system in cardiovascular risk patients: Cross-sectional findings from the Ludwigshafen Risk and Cardiovascular Health (LURIC) study. *Clin Biochem* 50: 739-745, 2017.

129. Zheng L, Nukuna B, Brennan ML, Sun M, Goormastic M, Settle M, Schmitt D, Fu X, Thomson L, Fox PL, Ischiropoulos H, Smith JD, Kinter M, Hazen SL. Apolipoprotein A-I is a selective target for myeloperoxidase-catalyzed oxidation and functional impairment in subjects with cardiovascular disease. *J Clin Invest* 114: 529-41, 2004.

130. Zhou B, Zu L, Chen Y, Zheng X, Wang Y, Pan B, Dong M, Zhou E, Zhao M, Zhang Y, Zheng L, Gao W. Myeloperoxidase-oxidized high density lipoprotein impairs atherosclerotic plaque stability by inhibiting smooth muscle cell migration. *Lipids Health Dis* 16: 3, 2017.

131. Zhu ML, Zhao JP, Cui N, Goncalves-Rizzi VH, Possomato-Vieira JS, Nascimento RA, Dias-Junior CA. Cardiac myeloperoxidase activity is elevated in hypertensive pregnant rats. *J Huazhong Univ Sci Technolog Med Sci* 37: 904-909, 2017.

132. Zonneveld TP, Richard E, Vergouwen MD, Nederkoorn PJ, de Haan R, Roos YB, Kruyt ND. Blood pressure-lowering treatment for preventing recurrent stroke, major vascular events, and dementia in patients with a history of stroke or transient ischaemic attack. *Cochrane Database Syst Rev* 7: CD007858, 2018.

38

	Type of disease	Type of study	Number of subjects	Major outcomes	References
Table 1: Studies assessing the prognostic value of MPO in cardiovascular disease					
Heslop, C.L. *et al.* (2010)	ACS	Prospective cohort	885	MPO independently predicted cardiovascular mortality risk in coronary angiography patients. Combination of MPO with C-reactive protein was a stronger predictor of cardiovascular mortality risk	(47)
Rana, J.S. *et al.* (2011)	ACS	Prospective case-control study	2861	Circulating MPO levels (men only) are associated with an	(96)

39

				increased risk for CHD	
Scirica, B.M. *et al.* (2011)	ACS	Randomized controlled trial	4352	Plasma MPO levels are independently associated with some adverse cardiovascular outcomes in non-ST-segment elevation ACS patients, but do not provide substantial incremental prognostic information when evaluated together with cTnI and NT-proBNP	(109)
Nilsson, L. *et al.* (2012)	ACS	Randomized controlled trial	58	Plasma MPO levels did	(81)

40

				not correlate with measures of infarct size, left ventricular dysfunction or remodeling	
O'Malley, R.G. *et al.* (2014)	ACS	Randomized controlled trial	4432	Plasma MPO levels are not independently associated with risks of cardiovascular death and heart failure in patients with non-ST-segment elevation ACS	(84)
Mollenhauer, M. *et al.* (2017)	ACS	Retrospective cohort	2622	Plasma MPO levels in post-ischemic	(73)

				patients are independently associated with a history of ventricular arrhythmias, sudden cardiac death or implantation of an implantable cardioverter defibrillator	
Ferrante, G. et al. (2010)	Atherosclerosis/ACS	Prospective cohort	25	Systemic MPO levels are significantly elevated in patients with ACS presenting with eroded culprit plaque compared to patients presenting	(26)

42

				with ruptured culprit plaque	
Tsimikas, S. *et al.* (2010)	Atherosclerosis/ACS	Prospective case-control study	2160	MPO mass is higher in patients with CAD, but they only have a weak potentiation of risk when combined with other oxidative biomarkers	(122)
Barbato, E. *et al.* (2012)	Atherosclerosis/ACS	Randomized controlled trial	1397	Plasma MPO levels are significantly higher in patients who harbor a specific variant in the atrial natriuretic peptide gene that is associated	(10)

43

				with major adverse cardiovascular events	
Duivenvoorden, R. *et al.* (2013)	Atherosclerosis/ACS	Randomized controlled trial	130	Circulating MPO levels are associated with carotid plaque inflammation and is independent of traditional cardiovascular disease risk factors in atherosclerotic plaque	(23)
Sorrentino, S. A. *et al.* (2010)	Diabetes mellitus	Case-control study/randomized controlled trial	43	HDL-associated MPO activity and protein content which alter endothelial NO	(114)

|				production are significantly increased in diabetic patients and are reduced after extended-release niacin therapy	
Derosa, G. *et al.* (2015)	Diabetes mellitus/hypertension	Randomized controlled trial	213	MPO identified as a new emerging marker of cardiovascular risk and is reduced in patients with type 2 diabetes mellitus and hypertension after treatment with acetylsalicylic acid	(21)

45

McLaughlin, K. *et al.* (2017)	Hypertension	Randomized controlled trial	45	Low molecular weight heparin induced significant increases in plasma MPO levels in high-risk preeclampsia pregnant women resulting in improved endothelial function	(69)
Ganz, P. *et al.* (2017)	Stroke	Case–cohort study	2176	Plasma MPO levels are independently associated with the risk of recurrent stroke and improve risk classification when added to a clinical risk	(33)

46

				algorithm	
Phuah, C. L. *et al.* (2017)	Stroke	Retrospective cohort/Prospective cohort	3033/174	Common genetic variants that result in increased MPO levels, increase risk of primary intracerebral hemorrhage and lacunar stroke	(93)
Freedman, J. E. *et al.* (2010)	Obesity/cardiovascular disease	Prospective cohort	1846	MPO and other specific inflammatory platelet-derived transcripts including ICAM1, IFNG, IL1R1, IL6, COX2, TNF, TLR2 and TLR4, were significantly	(29)

|					
				associated with higher body mass index	
Olza, J. *et al.* (2012)	Obesity/cardiovascular disease	Prospective case-control study	446	MPO is an early biomarker of inflammation associated with CVD risk in obese children at the prepubertal age	(87)
King, C. C. *et al.* (2017)	Smoking/cardiovascular disease	Longitudinal cohort	1652	Smoking heaviness is associated with increased MPO	(56)
Abbreviations: ACS- Acute coronary syndromes; CHD- Coronary heart disease; CAD- Coronary artery disease; HDL- High-density lipoprotein; NO- nitric oxide; CVD- Cardiovascular disease					

FIGURE LEGENDS

Figure 1
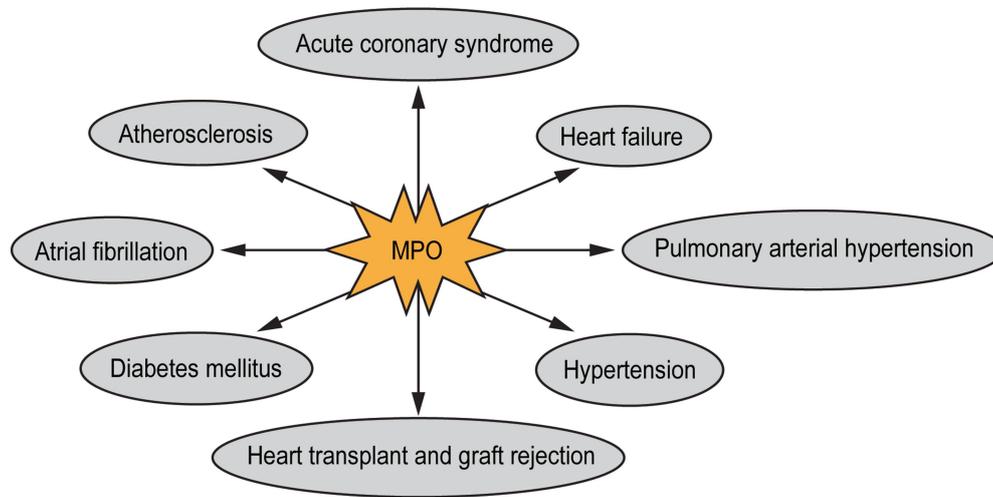
Figure 1: Schematic representation of cardiovascular diseases in which elevated MPO levels have been detected in circulation.

49
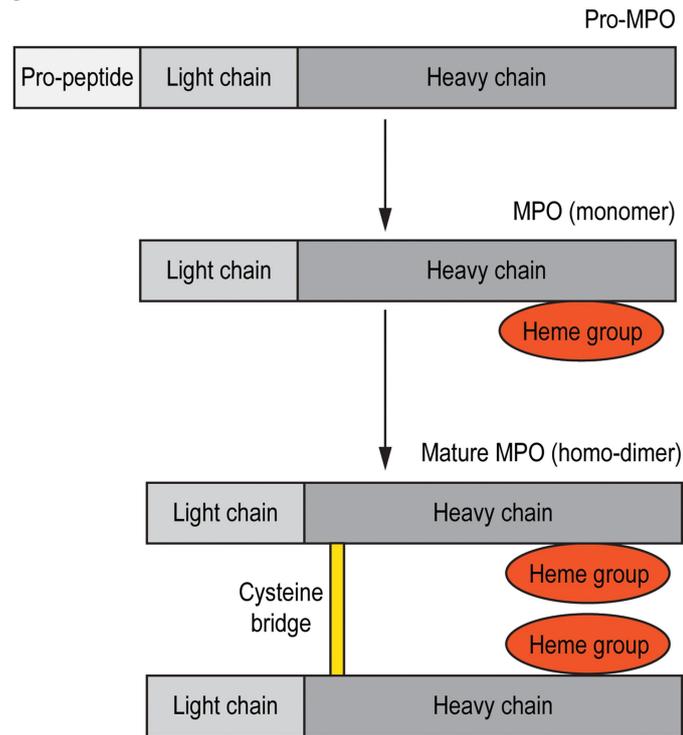
Figure 2

Figure 2: Schematic illustration of MPO processing. The Pro-MPO undergoes proteolytic cleavage to give rise to the MPO monomer comprising heavy and light chains as well as a heme group. The mature MPO homodimer comprises two monomers that are linked by a cysteine bridge. Adapted and modified from following study (42).
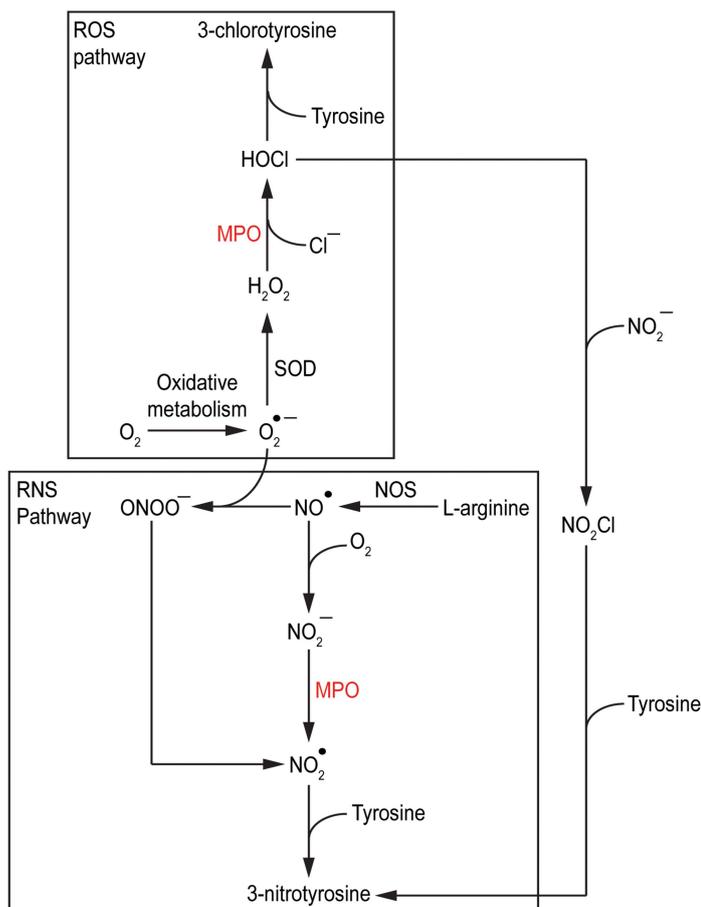
Figure 3
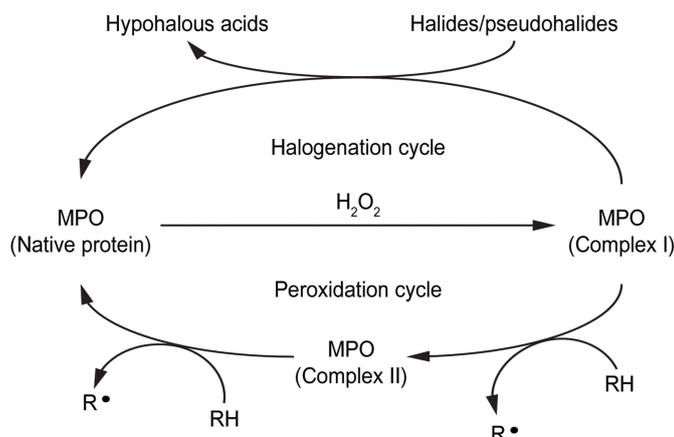
Figure 3: Schematic illustration of MPO catalyzed reactions. Upon interaction with hydrogen peroxide, native MPO is converted to Complex I, which can be converted back to the native state by entering either the halogenation cycle (one-step reaction), or the peroxidation cycle (two-step reactions) with the latter giving rise to an intermediary Complex II. The halogenation cycle produces hypohalous acids upon Complex I interaction with halides and pseudohalides, while the peroxidation cycle gives rise to reactive species/radicals (R$^{\cdot}$) upon oxidation of organic peroxidation substrates (RH) such as tyrosine.

51
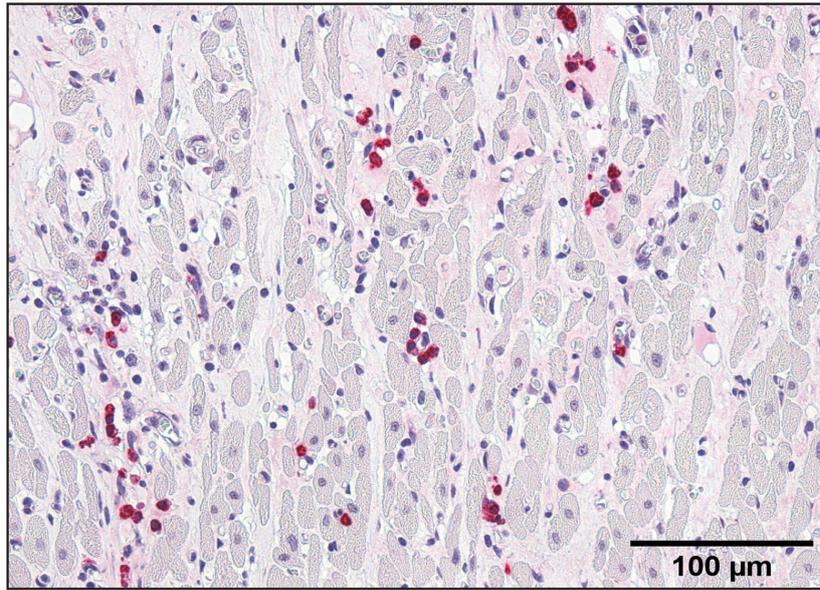
Figure 4

Figure 4: Immunohistochemistry pictograph showing infiltrating MPO$^+$ cells (red) in porcine myocardium 2 weeks post-myocardial injury. Image captured from cardiac sections used in following study (76).

Figure 5

Figure 5: Schematic illustration as to how reactive species generated from the ROS/RNS pathways act as substrates for MPO catalyzed reactions, giving rise to 3-chlorotyrosine and 3-nitrotyrosine. In the ROS pathway, superoxide ($.O_2^-$) is produced through oxidative metabolism and converted into hydrogen peroxide (H_2O_2) by superoxide dismutase (SOD). MPO in the presence of hydrogen peroxide and chloride ions (Cl^-) is capable of generating hypochlorous acid (HOCl), which can modify tyrosine residues into 3-chlorotyrosine. In the RNS pathway, nitric oxide synthase (NOS) catalyzes the production of nitric oxide (.NO) from L-arginine. Nitric oxide is converted into nitrite ions (NO_2^-) in the presence of oxygen, followed by conversion into nitrogen dioxide ($.NO_2$), a reaction catalyzed by MPO. Nitrogen dioxide can modify tyrosine residues into 3-nitrotyrosine. Alternatively, nitric oxide, in the presence of superoxide gives rise to peroxynitrite ($ONOO^-$), which is broken down into nitrogen dioxide. Hypochlorous acid itself can react with nitrite ions to form nitryl chloride (NO_2Cl), which can modify tyrosine residues into 3-nitrotyrosine.