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The role of myeloperoxidase as a biomarker in atherosclerotic cardiovascular disease

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Abstract

Myeloperoxidase (MPO), a heme-containing protein integral to the innate immune system, plays a pivotal role in both bactericidal activity and inflammation regulation. In this comprehensive review, we aim to delve into the extensive literature on MPO's involvement in cardiovascular disease. Through exhaustive searches of reputable databases such as PubMed, Scopus, and Web of Science, we conducted a thorough analysis to elucidate MPO's potential as a predictor for cardiovascular disease risk. Evidence suggests that MPO serves as a valuable biomarker, particularly in identifying vulnerable lesions predisposed to rupture,leading to myocardial infarction, especially in the context of acute coronary syndrome. Its early release in response to symptom onset positions MPO as a promising tool for triaging chest pain patients in emergency departments, offering advantages over conventional markers like cardiac troponins. Moreover, studies have demonstrated a correlation between elevated MPO levels and higher mortality rates in patients with acute coronary syndrome, both in short- and long-term follow-up. However, the widespread adoption of MPO as a routine clinical biomarker faces significant challenges. Standardizing measurement techniques and determining the optimal timing of assessments are crucial for ensuring reliability and comparability across studies. Furthermore, while MPO shows promise as a complement to existing risk stratification protocols, questions remain regarding its superiority over troponins and its utility in stable coronary artery disease. Addressing these issues necessitates large-scale prospective cohort studies to clarify MPO's comparative effectiveness alongside conventional biomarkers. Despite these challenges, MPO emerges as a potentially valuable addition to risk assessment strategies, particularly for patients with persistently negative troponin levels, helping guide therapeutic interventions and enhancing clinical decision-making in cardiovascular medicine.

Keywords: Myeloperoxidase; Coronary artery disease; Unstable angina; Primary prevention; Cardiovascular risk

INTRODUCTION

Atherosclerosis is a pathologic condition in which inflammation has a main role from the very early beginning to the very late stage, and subsequent atheroma rupture as well. In this process initiated by lipid accumulation and inflammatory processes, innate and adaptive immune responses are involved. Several novel biomarkers have been utilized to predict the risk of cardiovascular (CV) events, like fibrinogen, high-sensitivity C-reactive protein (hs-CRP), myeloperoxidase (MPO), and matrix metalloproteinase (MMP). Among these predicting factors, MPO has emerged as a potential factor in the promotion and/or propagation of atherosclerosis, attracting researchers' consideration[1].

MPO, a unique heme-containing protein and proinflammatory enzyme, is expressed mainly by neutrophils during myeloid cell differentiation in bone marrow. MPO, as the main protein of neutrophil, accounts for 5% of its dry weight. The crucial role of MPO as a component of the innate immune response to foreign invasion was first described nearly 4 decades ago[2]. Through years after that, many investigations were performed to identify the role and activity of this unique protein. Daugherty *et al*.[3] initially found the enriched presence of enzymatically active MPO within human atheroma. An active form of MPO is involved in producing reactive oxygen species (ROS) and the oxidation of biological material. While MPO is essential to host defense, it causes tissue damage alongside its anti-pathogen activities[4]. MPO and its reactive oxidants have been implicated as contributors to tissue damage during inflammatory disorders[5].

Several studies have implicated the role of MPO in the process of atherosclerosis in humans. For example, individuals who develop total or partial MPO deficiency are considered to be less likely to be at risk of cardiovascular disease (CVD)[6]. On the other hand, increasing

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systemic levels of MPO have also been reported to predict the presence of angiographic coronary artery disease (CAD)[7]. Furthermore, two oxidized forms of amino acids, 3-chlorotyrosine, and 3-nitrotyrosine, that are characteristics of MPO activity, occur at high levels in atherosclerotic lesions[8].

Hence, there is an abundance of literature reporting the relevance of MPO concentration to different facets of atherosclerosis and identifying the role of this protein in estimating CV risk.

MECHANISM OF MPO PRODUCTION AND ACTION

For the first time, in 1941, the purification of an intensely green iron-containing protein with peroxidase activity was defined by Agner[9] in the purulent fluid of patients with tuberculous emphysema. As this protein had a green color, it was initially called verdoperoxidase. However, it was later determined that the expression of this protein is restricted to myeloid lineage, so its name was changed to MPO[10]. It took around 30 years to identify the function of the MPO-H_2O_2-halide complex as a powerful antimicrobial system, particularly in neutrophils[2].

The gene coding for human MPO is located on the long arm of chromosome 17 segment q23.1[11]. During myelopoiesis in the bone marrow, MPO is actively synthesized in the promonocytes and promyelocytes. The primary source of MPO is polymorphonuclear neutrophils (PMNs), which account for nearly 2% to 5% of PMNs' weight. However, MPO is also found in monocytes in a far smaller amount than neutrophils[12].

As long as PMNs, as the first immune cells migrating to the site of infection, are in their inactive state and H_2O_2 is absent, MPO is also in its inactive and quiescent state. When PMNs meet pathogens, they engulf them and phagosomes are formed. Intracellular granules of PMNs containing bactericidal substances then fuse with the phagosomes and phagolysosomal compartments are formed. Simultaneously, the different elements of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex are recruited both to the internal phagolysosomal membrane surface and to the plasma membrane resulting in the production of superoxide radicals (O_2 ·). O_2 · also generates hydrogen peroxide (H_2O_2) which is the essential substrate for the generation of MPO-derived oxidants through either spontaneous or superoxidase (SOD)-catalyzed dismutation of O_2 ·[13].

There are at least three different types of granules located within PMNs: the primary or the azurophilic granules, secondary or specific granules, and tertiary or gelatinase granules all released via an increase in intracellular calcium concentration[14]. While, for the discharge of primary and secondary granules, a higher quantity of calcium is needed, the tertiary granules are released with a lower concentration of intracellular calcium. The secondary and tertiary granules, which do not contain peroxidase, are those that firstly discharge[4].

MPO by itself has little bactericidal properties, but enzymatic reaction with H_2O_2 and halide (Cl^-, Br^-, I^-) or pseudohalide (SCN^-) ions produces hypohalous acids including hypochlorous acid (HOCl), hypobromous acid (HOBr), hypoiodous acid (HOI), and hypothiocyanous acid (HOSCN) which elicit most of the anti-bactericidal activity of neutrophils[2]. A complete MPO system consists of MPO, H_2O_2, and an oxidizable element. In fact, MPO catalyzes the reaction between H_2O_2 and physiological halides (such as Cl^-) to form reactive oxidants such as HOCl[2] (Figure 1).

HOCl is the most abundant product of MPO reaction due to the tremendously higher amount of Cl^- in plasma than other halides. HOCl is a highly reactive compound capable of oxidizing any oxidizable factor in any substrate such as heme groups and unsaturated fatty acids. Oxidation by HOCl results in changing the protein activity and consequently altering microbial and cellular actions. HOCl reacts with several compounds including nitrogen-containing compounds such as deoxyribonucleic acid (DNA), ribonucleic acid (RNA), glycosaminoglycans, and phospholipids[15]. In addition, HOCl and other MPO-derived oxidants alter immune responses and inflammatory reactions and the function of various immune cells[1].

Under physiologic states, another important substrate for MPO is nitric oxide (NO) and its major oxidized product nitrite (NO_2^-)[16]. NO_2^- is formed by the oxidation of NO_2^-, which is highly unstable but is very powerful in promoting protein nitration and lipid peroxidation[17].

Disproportionate MPO-derived oxidants have been associated with tissue damage, particularly in diseases involving acute or chronic inflammation[4]. The concentration of circulating MPO depends on several factors such as age, gender, smoking, and oral contraceptive use in women[4]. Neutrophil MPO activity is more prominent in women than men and increases with advanced age in both men and women. MPO within the circulation binds to albumin, plasma lipoproteins, erythrocytes, or within the circulating neutrophil microparticles. Although a number of polymorphisms have been recognized, mainly single nucleotide polymorphisms within or near the promoter region, it appears that heredity plays a modest role in determining the circulating concentration of MPO in healthy populations[4].

It has been shown that MPO has many effects other than its catalytic activity, including effects on various cell signaling, cell-cell interactions, and modulating inflammatory responses[4]. For instance, Lefkowitz *et al.*[18–19] provided evidence that MPO can modulate immune responses by activating macrophages. They found that in vitro exposure of macrophages to MPO led to the

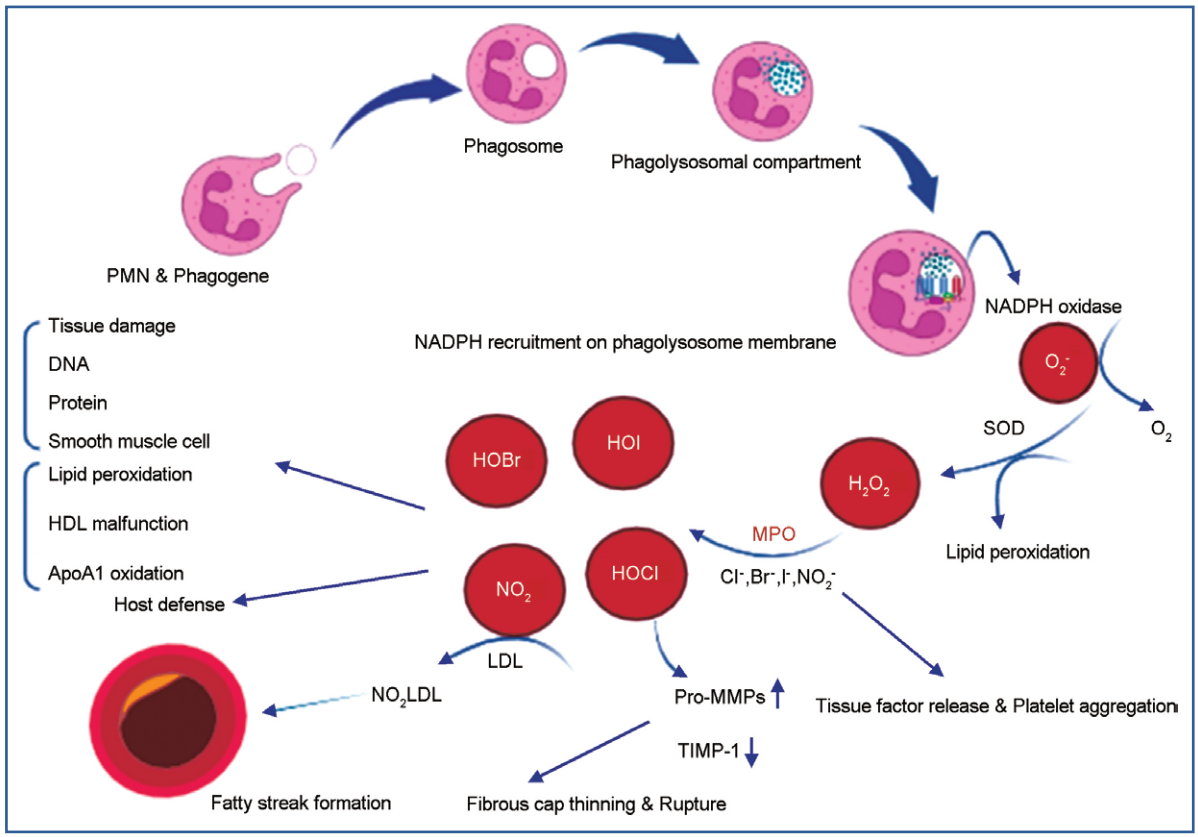

Figure 1. MPO biological function and its role in atherosclerosis.
ApoA1: apoprotein A1; DNA: deoxyribonucleic acid; HDL: high-density lipoprotein; LDL: low-density lipoprotein; MPO: myeloperoxidase; NADPH: nicotinamide adenine dinucleotide phosphate; PMN: polymorphonuclear neutrophil; Pro-MMP: pro-matrix metalloproteinase; SOD: superoxide dismutase; TIMP-1: tissue inhibitor of metalloproteinase-1.

release of tumor necrosis factor (TNF)-α and low levels of interferon (IFN)-ϒ in conjunction with boosted macrophage-dependent cytotoxicity. Despite the vital role of MPO in host defense against different bacteria and fungi, it can result in tissue damage by creating reactive halogenating and nitrating agents in inflammatory processes[20]. MPO, as a dual-edged sword, contributes to many chronic inflammatory diseases such as rheumatoid arthritis, chronic kidney disease, metabolic syndrome, and CVD[21].

MPO is also considered as a potential contributor in the promotion and/or proliferation of atherosclerosis. Understanding the role of MPO in pathogenesis of atherosclerosis might influence the preventive and therapeutic measures in CVD.

THE ROLE OF MPO IN THE PATHOGENESIS OF ATHEROSCLEROSIS

MPO has been implicated in multiple processes throughout the evolution of atherosclerosis, including the initiation and progression of plaque formation, lipid peroxidation, generation of atherogenic lipoproteins and dysfunctional high-density lipoprotein (HDL), and catalytic consumption of NO that results in limiting NO bioavailability and endothelial dysfunction[22].

Altering NO bioavailability

MPO contributes to the process of atherosclerosis by altering the bioavailability of NO which is an essential factor for a healthy endothelium, regulating vascular tone, reducing platelet aggregation, inhibiting endothelial inflammatory responses, and controlling intimal smooth muscle cell proliferation. MPO restricts the bioavailability of NO by inhibiting the activation of NO synthase (NOS), generating oxidants, and reducing the bioavailability of NADPH which is an essential cofactor for NOS[23]. In this regard, Vita *et al.*[24] demonstrated a robust and independent relationship between serum MPO levels and endothelial dysfunction measured by NO-dependent flow-mediated dilation of the brachial artery. There was greater than a six-time increase in the occurrence of endothelial dysfunction in patients in the highest quartile of MPO compared with those in the lowest quartile of MPO[24].

Moreover, it was reported that in patients with symptomatic CAD, acetylcholine-stimulated forearm blood flow was inversely associated with plasma concentrations of MPO[25]. Endothelial dysfunction due to the restriction of NO bioavailability is not only connected with serum MPO level but also is linked with subendothelial-located MPO which oxidizes NO and leads to the alteration of NO function and endothelial dysfunction[4].

Interruption of the fibrous cap

MPO has also been suggested to have a role in converting a mature atherosclerotic plaque to a vulnerable state. The herald lesion of plaque rupture is a thin cap fibro-atheroma with a necrotic core infiltrated by macrophages and few or absent smooth muscle cells within the cap[26]. Burke *et al.*[27] reported a significant presence of monocytes and PMNs in the fibrous cap by MPO staining. They identified a greater density of MPO-positive monocytes and neutrophils in occlusive thrombi. Within arteries, the density of MPO-positive cells was much greater in the occlusive than non-occlusive disrupted fibrous cap. The length of the thrombus was positively associated with intra-clot MPO-positive cells also[27]. The mechanism by which MPO could induce fibrous cap weakening, thinning, and rupture might be in part explained by the study conducted by Sugiyama *et al.*[28] They demonstrated that MPO-positive macrophages produce HOCl, which activates pro-matrix metalloproteinase (pro-MMPs) and inactivates tissue inhibitors of metalloproteinase-1 (TIMP-1), leading to extracellular matrix degradation. A noticeable infiltration of neutrophils and colocalization of MPO- and HOCl-modified proteins with macrophages in culprit lesions associated with thrombi have been detected in pathological studies[29] (Figure 1).

MPO may assist a thrombogenic environment by releasing tissue factor from endothelial cells and by priming platelet aggregation. Additionally, MPO may promote endothelial cell apoptosis along with the activation of protease cascades, thus interrupting of the fibrous cap and ischemic events[30].

Lipoprotein modification

The strong relationship between hypercholesterolemia and atherosclerosis has been well-established[31]. Moreover, a major role in the modification of low-density lipoprotein (LDL) and its uptake (especially oxidized LDL) that results in the accumulation of lipids in foam cells has been identified in the pathogenesis of atherosclerosis[32].

MPO, as a stimulator of dysfunctional and altered lipoproteins, could adversely affect the progression of atherosclerosis. Several studies have demonstrated the effect of MPO on the generation of atherogenic LDL particles and dysfunctional HDL through MPO-catalyzed pathways[32]. MPO-generated products such as H_2O_2, HOCl, and NO_2 stimulate lipid peroxidation, the transformation of LDL to a high-uptake form, and diminish the ability of apoprotein A1 (apoA1) to promote cholesterol efflux[33]. Zhang *et al.*[34] demonstrated that PMNs isolated from individuals with MPO deficiency do not promote lipid peroxidation when activated ex vivo in plasma but they initiated peroxidation of lipid when exogenous catalytic levels of MPO were added.

Different forms of modified LDL, including oxidized LDL, glycoxidized LDL, and carbamylated LDL, have been recognized. The latter was first described in uremic patients under hemodialysis, but it has been confirmed that carbamylation of LDL also occurs in healthy subjects[35]. Carbamylation of any target protein changes its structure and function. LDL is a target of carbamylation, especially at the site of atherosclerotic lesions, and its product, carbamylated LDL, is the most common isoform of LDL[36]. The carbamylation of LDL cholesterol due to MPO-catalyzed pathway has been described as a process to produce proatherogenic LDL particles through which the high-uptake forms of LDL are generated by converting the LDL into a ligand for the scavenger receptor SRA-1, leading to augmentation and progression of atherosclerotic plaque[34]. NO_2 LDL has been demonstrated to participate in fatty streak formation and atherosclerotic lesion development through selective spotting by the scavenger receptor CD36[37].

Functional impairment of HDL is another mechanism by which MPO imposes its effect on the formation of atherosclerotic plaques. HDL is the major atheroprotective particle in plasma built on a backbone of predominantly apoA1. It exerts its protective effect through a reverse cholesterol transport (RCT) pathway which is the net transport of cholesterol from peripheral tissues to the liver for final removal into the intestinal lumen as biliary cholesterol for excretion in feces[38]. It is established that HDL becomes dysfunctional in the case of having fewer anti-inflammatory and endothelial protective properties compared with normal HDL function[39]. Undurti *et al.*[40] also established that the loss of non-cholesterol efflux-related activities and the gain of proinflammatory functions are also the results of MPO-catalyzed oxidation of HDL. HDL is also susceptible to MPO-catalyzed carbamylation and has been found to play a role in atherosclerosis. Carbamylated HDL can also promote aortic endothelial cell apoptosis which is a critical hallmark of atherosclerotic lesions[34].

Plasma apoA1 as a discerning target for MPO is modified from a small monomer to dimer and multimer cross-linked forms that make the lipoprotein susceptible to oxidative modification and loss of cholesterol efflux function in human plasma and atherosclerotic plaque[35]. Despite the consensus on the role of MPO in apoA1 oxidative modification and dysfunction, the precise mechanism of HDL function impairment still remains to be established[24].

Contribution to adverse myocardial remodeling and heart failure after fibrous cap rupture

MPO likely contributes to the pathological events that occur after rupture of the fibrous cap and luminal occlusion. MPO is capable of activating protease and promoting degradation of the extracellular matrix, and thus

has an important role in the progression of myocardial necrosis to adverse remodeling and heart failure. MPO also induces endothelial dysfunction and promotes the expression of p-selectin on the endothelial surface, resulting in platelet adhesion[41].

Askari *et al*. demonstrated the contribution of MPO to adverse ventricular remodeling after myocardial infarction in a mouse model[42]. Using a chronic coronary artery ligation model in MPO-knockout mice, a significant decline in leukocyte infiltration and left ventricular dilatation associated with delayed myocardial rupture and preservation of systolic function has been observed.

THE PREDICTIVE VALUE OF MPO MEASUREMENT IN THE SETTING OF PRIMARY PREVENTION

Using a scoring system in primary prevention/point of care has been very useful in the risk stratification of patients with CVD. However, even after implementing a comprehensive risk score assessment, there has remained a notable number of patients with residual CVD risk. In fact, the optimal goal is identifying this group of patients and utilizing efficacious medications to reduce the future risk of events. In this matter, biomarkers including novel lipid markers, inflammatory biomarkers such as hs-CRP, and methods to detect subclinical organ damage are some examples of using new methods to reclassify patients in the setting of primary prevention[43]. MPO as a novel inflammatory biomarker has been studied to recognize its capability to strategize patients at risk in the primary prevention venue. Although only a few studies have been conducted to evaluate the predictive role of MPO in developing CAD in apparently healthy subjects, the results of these studies are significant. A landmark nested case-control study among participants of the European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk Study was conducted by Meuwese *et al*.[44] In this study, the baseline MPO concentration was measured in apparently healthy subjects who did not have any history of heart attack or stroke at the baseline clinical visit. The case subjects were 1,138 patients in whom fatal or non-fatal CAD developed during follow-up. Two thousand two hundred and thirty-seven subjects were considered as controls who remained free of CAD during follow-up. The results showed that individuals in the top quartile of MPO had an odds ratio (OR) of 1.49 (95% confidence interval [95% CI]: 1.20–1.84) compared with those in the bottom quartile (P = 0.001). The significant correlation between MPO concentration and the incidence of heart attack remained substantial after adjustment for other CVD risk factors (OR: 1.36, 95% CI: 1.07–1.73 for top vs. bottom quartile; P < 0.001). This association was stronger in patients with fatal CAD than in patients with non-fatal CAD.

Another study by Wong *et al*.[45] was conducted to evaluate MPO as a predictor marker and coronary artery calcium (CAC) scoring. They enrolled 1,302 asymptomatic healthy adults who were followed for an average of 3.8 years. Subjects with MPO levels at or above the median level were more likely female and had much higher systolic and diastolic blood pressure, lower HDL cholesterol, higher LDL cholesterol, and higher body mass index. There was a higher incidence of CVD events in the subjects with MPO level at or above the median level even after adjustment for traditional risk factors (hazard ratio [HR]: 1.9, 95% CI: 1.0–3.6, P = 0.04). In addition, it was observed that there was a positive correlation between the MPO concentration and CAC score. Using CAC score and MPO as a combination of CVD risk predictors, the highest incidence of CVD events was reported in those patients with high CAC score (≥100) and MPO levels at or above the median. (adjusted HR: 19.5, P < 0.0001).

In conclusion, MPO appears to be a valuable marker for predicting CVD events in the setting of primary prevention/point of care. More studies are needed to determine its use in the risk stratification of subclinical atherosclerotic patients.

THE PREDICTIVE VALUE OF MPO MEASUREMENT IN CHRONIC STABLE CADs

Patients with stable coronary artery diseases (SCAD) are heterogeneous groups with regard to pathology such as the progression rate of the disease, the severity of coronary lesions, and the incidence of acute coronary syndrome (ACS)[46]. Despite the appreciable improvements in the treatment strategies of CAD by surgical and percutaneous revascularization methods, many patients do not fulfill the criteria to use these treatment methods. These patients might be evaluated for intensive CV risk factor modification and ideal medical treatment[46]. Boden *et al*.[47] reported comparable benefits in terms of CV outcomes among patients with significant SCAD randomized to treatment with either aggressive preventive medical intervention or percutaneous coronary intervention plus aggressive preventive medical intervention. Therefore, it appears that identifying patients at increased risk for major CV events helps clinicians prescribe medications or other therapeutic strategies to reduce morbidity and mortality.

More recently, there has been an emerging number of studies focusing on the role of MPO measurement in the risk prediction of CAD. MPO is considered to detect high-risk patients with SCAD and categorize them for planning treatment strategies. The first epidemiological study measuring the association between MPO and CVD was published by Zhang *et al*.[7]. In this case-control study, the authors investigated the relation of MPO, expressed as mass per milligram of neutrophil

protein or as MPO mass per milliliter of blood, with CAD risk in 158 patients with established CAD and 175 controls. They found that CAD patients had significantly higher concentrations of MPO compared to the controls. MPO was associated with an 11.9-fold increased risk of CAD for leukocyte MPO and a 20.4-fold increased risk of CAD for blood MPO after multivariate logistic regression analysis adjusted for traditional CVD risk factors. Kimak *et al.*[48] examined the association between inflammatory markers and MPO. They concluded, based on the results of the study, that increased levels of inflammatory markers might result in a surge of MPO concentration, which decreases apoA1 and HDL cholesterol levels. On the other hand, moderate dyslipidemia and dyslipoproteinemia worsen inflammation, which consequently leads to increased MPO concentration. High levels of MPO and MPO/HDL and MPO/apoA1 ratios could differentiate the patients with SCAD who are at risk of stroke or ACS. MPO is also considered to have a predictive role in the severity of coronary atherosclerotic lesions in patients with SCAD[49].

In a large cohort study consisting of 1,895 patients with angiographically significant CAD who were medically treated, MPO plasma level was identified to correlate with the rate of major adverse cardiovascular events (MACEs)[50]. It was reported that at an optimal cut-off point of 322 pmol/L, MPO was predictive of higher 3-year MACEs (HR: 1.78, 95% CI: 1.33–2.37, $P < 0.001$). This association remained significant even after adjustment for other risk factors.

However, not all studies have proven this independent correlation. One cohort study by Stefanescu *et al.*[51], consisting of 382 SCAD patients in whom MPO blood level was measured, showed increased all-cause mortality among patients with high tertile of MPO (>75.0 µg/L) during a median follow-up of 3.5 years. Nonetheless, MPO was not an independent marker for all-cause mortality after adjusting for other CV risk factors, ejection fraction, hs-CRP, and baseline creatinine level by the Cox proportional hazards model. Yet, they concluded that MPO could be considered as an index of increased CVD risk because its increased plasma level is accompanied by a more adverse CV risk profile.

Another small study by Hasanpour *et al.*[52], consisting of 40 patients without CV risk factors who underwent coronary angiography, showed that MPO level was not meaningfully associated with coronary atherosclerosis (OR of 0.67 for CAD [95% CI: 0.17–2.50]). Muller *et al.*[53] used fractional flow reserve (FFR) as an index for the severity of atherosclerotic lesions in 197 patients with SCAD with single vessel disease to evaluate the correlation between MPO concentration and severity of lesions. MPO concentration was similar in patients with SCAD whose FFR was >0.8 vs. patients with FFR ≤0.8[53].

Considering these studies, it seems that the independent correlation of MPO concentration with different aspects of SCAD, including the severity of coronary lesions, is not a consistent finding.

THE PREDICTIVE VALUE OF MPO MEASUREMENT IN ACS

ACS is caused by plaque rupture that results in a critical and sudden diminished blood flow at the distal part of the plaque. This event could manifest in different types of clinical symptoms with different degrees of severity from complete obstruction of the vessel lumen leading to acute myocardial infarction (AMI) to an incomplete myocardial infarction or non-ST elevation myocardial infarction (NSTEMI) and unstable angina. Cardiac enzymes such as troponin T (TnT) and troponin I (TnI) will increase in the setting of myocardial infarction both NSTEMI and AMI. However, diagnosing of ACS in the emergency department (ED) is not always straightforward. Cardiac troponins have low sensitivity, which makes it difficult to diagnose ACS at a very early stage. Also, these enzymes have a prolonged release pattern that adds to the difficulty of diagnosing ACS at the early stage[54]. Importantly, in a subset of patients with ACS, cardiac troponin does not increase, but these patients develop AMI leading to death or revascularization. Therefore, it seems that there is a necessity to consider another biomarker to distinguish ACS in the ED, both for triage of patients suspected of ACS and to recognize high-risk ACS patients to plan appropriate treatment strategies during hospitalization and aftercare. Among different types of biomarkers, MPO appears to be a good candidate in this regard, as it is involved in the process of activation of metalloproteinase, plaque instability, and rupture[55].

Sugiyama *et al.*[28] demonstrated the increased numbers of MPO-expressing macrophages in eroded or ruptured plaques while macrophages within the fatty streak contained little or no MPO. Other authors have reported an increased concentration of MPO in plasma has been reported in ACS patients[56]. A small study by Ferrante *et al.*[57] showed a significantly higher concentration of MPO in patients with ACS who had eroded lesions than in patients with ruptured plaques. Also, in a post-mortem study of coronary lesion specimens, superimposed luminal thrombi on eroded lesions contained much more MPO-containing cells than intraluminal thrombi superimposed on the ruptured plaques[57].

Naruko *et al.*[58] considered angiographically determined morphology of lesions in ACS patients and its association with MPO concentration. They found that median interquartile range (IQR) plasma MPO levels in patients with unstable angina pectoris with a complex lesion were significantly higher than in patients with simple lesions. In the atherectomy specimens, they observed a higher number of MPO-positive cells in complex

lesions compared with simple lesions. Furthermore, they reported a significant correlation between plasma MPO concentration and the number of MPO-positive cells in atherectomy specimens.

Although cardiac troponin rises within 3 to 6 hours after the onset of symptoms, MPO starts rising within just 2 hours after the onset of chest pain[57]. In this regard, the MPO concentration was measured in 83 patients with presenting non-traumatic chest pain in the ED over a 6-hours interval. A significant difference in MPO concentration between non-ACS patients and ACS patients, especially at 6 hours, was reported. Also, there was a significant difference in MPO level in patients with ACS and SCAD[57].

Another study showed that baseline MPO is higher in patients presenting with chest pain compared with control subjects, and among patients who experienced AMI within 16 hours after presentation compared with patients who did not[59]. The increasing incidence of AMI was proportionate to the concentration of MPO. Patients who had negative cardiac troponin T (cTnT) at the time of presentation but gradually had a measurable cTnT increase were more likely to be in the third or fourth quartile of MPO level than in the first or second quartile. In addition, baseline MPO concentration was higher in subjects who experienced MACEs and needed revascularization in the succeeding 30-day and 6-month periods than those who did not experience those complications. After using multivariate logistic regression for various risk factors, elevated levels of MPO remained a predictor of increased risk of myocardial infarction, the need for revascularization, and major adverse coronary outcomes within 30 days and 6 months after presentation ($P < 0.001$). It was demonstrated that MPO was not only an independent predictor of adverse CV events in patients with positive cTnT, but it remained as a risk marker in patients who were persistently negative for TnT. So, it appears that MPO is likely a predictor for vulnerable plaque pathology independent of cardiac troponin. Interestingly, hs-CRP could not predict adverse outcomes in patients with consistently negative cTnT. It can be concluded that baseline MPO could be used as a useful marker for triage of patients in ED due to a more rapid and elevated baseline concentration of MPO rather than cardiac troponin that needs time to increase.

Nonetheless, it might be assumed that if sensitive cardiac troponin were used to evaluate patients who present with chest pain in ED, the predictive value of MPO concentration might be reduced. However, Searle *et al.*[59] showed that the MPO has a significant independent value inpredicting MACEs in patients with acute chest pain even if serial sensitive cardiac troponin I (cTnI) is negative. In fact, it might be beneficial to keep the MPO-positive patients with negative serial cTnI in hospital for further care and evaluation instead of discharging them to avoid major CV events.

If MPO is considered a biomarker in the risk stratification of patients with chest pain in ED, it must be superior to the cardiac troponins. Cardiac troponins are not sensitive enough in the early stage of myocardial damage, and this is a main limitation of this biomarker. However, while some studies have reported the value of MPO concentration for the rapid triage of patients with suspected ACS in ED, some studies have not[60–61].

Peacock *et al.*[61] found a significant association between MPO concentration among patients who developed positive troponin from a negative baseline troponin compared with patients who remained troponin negative. However, because of large IQR overlap results, it is an insufficient and unacceptable marker for application in clinical settings. They concluded that MPO could not be a useful marker in the triage of patients with symptoms of chest pain in ED. The superiority of this study is the heterogeneous pattern of subjects included in the study, which is in contrast to some studies in which highly selected patients were enrolled. The latter could induce spectrum bias. For instance, in the study by Brennan *et al.*[62] non-cardiac chest pain (NCCP) was reported in just 21.5% which is much less than those reported in other studies. In addition, the 23.5% MI rate (>300% than Phenotypes of Chronic Obstructive Pulmonary Disease in Central and Eastern Europe Study) presents a significant bias to an adverse outcomes population. Moreover, in the study of Brennan *et al.*,[62] insensitive cardiac troponin was used could overestimate the predictive effect of MPO. If they had used a high-sensitive assay of cardiac troponin, they could have recognized myocardial necrosis, and then MPO may not have been a more important predictor[62].

What if we use cardiac troponin and MPO concentration for risk stratification of patients with chest pain? In this matter, Sawicki *et al.*[60] reported an improvement in sensitivity of the combined use of MPO and cTnI compared with the assessment of cTnI alone in all patients with ACS. However, patients were enrolled in this study after they were triaged in the ED, resulting in bias due to the non-heterogeneous inclusion of participants.

Nicholls *et al.*[63] demonstrated that serial measurement of MPO is superior to single point measurement of MPO. The MACEs rate was higher in patients with increased MPO concentration in serial measurement than those with unchanged MPO from baseline. An interesting point was that patients who had a low level of MPO at each time point after presentation had fewer MACEs regardless of the high baseline MPO level. Combined serial measurement of both cTnI and MPO improved accuracy compared with measuring each marker alone as it captured all patients who experienced MACEs during 6-months follow-up. While an increased concentration of MPO in >90% patients who had MACEs during 6 months follow-up was observed, a baseline increased cTnI was manifested in only 55.6% of patients who experienced MACEs during 6 months follow-up. The

least missed MACEs occurred with a negative baseline MPO and at least one other time point negative MPO.

Baldus *et al.*[64] recruited 1,090 patients with ACS in a prospective cohort study. This study established that serum MPO is a powerful and independent prognostic determinant of clinical outcome in patients with ACS, even independent of systemic inflammatory biomarkers such as CRP. The seminal result of the study was the capability of MPO in re-stratifying of patients with ACS who had low TnT levels.

A recent meta-analysis included 13 studies with 9,090 subjects and a median follow-up of 11.4 months has been recently performed by Kolodziej *et al.*[65] In this study, the prognostic value of MPO in ACS patients has been evaluated. The results of this meta-analysis showed that a high level of MPO meaningfully predicted mortality (OR: 2.03; 95% CI: 1.4–2.94, $P < 0.001$). Nonetheless, it did not have significant predictive value for major adverse events and recurrent MI (OR: 1.28, 95% CI: 0.92–1.77 and OR: 1.23, 95% CI: 0.96–1.58, respectively). The insignificant results in terms of MACEs and recurrent MI might be in part due to individual studies that were heterogeneous and consisted of ACS and non-ACS patients. This meta-analysis suggests MPO could be included in the risk stratification models that guide therapy of high-risk ACS patients. Finally, the results of the most important studies in terms of the predictive value of MPO for CVD risk are summarized in Table 1.

SENSITIVITY AND SPECIFICITY OF MPO IN COMPARISON WITH CARDIAC TROPONINS

Khan *et al.*[66] compared the sensitivity and specificity of MPO and high-sensitive cardiac troponin T (hs-cTnT) in patients suspected of AMI. They showed higher sensitivity and specificity of hs-cTnT (87% and 98%) compared to MPO (82% and 84%) within the first 4 hours of onset of chest pain at the ED. The optimal cut-off value for hs-cTnT and MPO were 14 ng/L and 564 pmol/L, respectively[72].

In terms of differential diagnosis of patients presenting with chest pain between ACS and NCCP, Peacock *et al.*[61] reported 90% specificity with a cut-off point of 210 ng/mL, 0.18 sensitivity, 0.69 negative predictive value, and 0.47 positive predictive value for MPO.

Assessing the early diagnostic efficacy of plasma MPO alone or in combination with cTnI for detecting ACS in patients presenting with chest pain, Sawicki *et al.*[60] reported the calculated sensitivity and specificity of 55% and 100%, at the 97.5th percentile for MPO, with positive and negative predictive values of 100% and 47%. While for cTnI at the 99th percentile cutoff, the sensitivity and specificity were 66% and 100%, respectively, with positive and negative predictive values of 100% and 54%. They stated that diagnostic accuracy was increased for the combination of cTnI and MPO.

ISSUES FACING MEASUREMENT OF MPO ACTIVITY

MPO expression or the measurement of its activity provides valuable information in terms of MPO quantity. However, even at the same level of MPO, there is an inter-individual difference in MPO enzymatic activity[72]. Multiple factors influence MPO (either increased or decreased activity), including age, gender, multiple polymorphisms, and endogenous MPO-inhibitory factors[73]. Endogenous MPO-inhibitory proteins, including ceruloplasmin and antioxidants such as ascorbic acid, could inhibit circulating MPO[74].

Despite the crucial role of MPO measurement in detecting its effect on inflammation, there is no consensus on the best methods of MPO measurement. Methodology and pre-analytical sample handling might influence MPO results[75]. The most common technique used to measure the concentration of MPO is enzyme-linked immunosorbent assay (ELISA)[76]. This costly and time-consuming technique uses ROS generated by immune-conjugate horseradish peroxidase (HRP) instead of directly measuring MPO-derived ROS[77]. There are various commercially used ELISA available, but these assays require several hours to complete and so are not suitable for routine clinical chemistry[78].

A recent MPO assay, Architect MPO (Abbott Laboratories Diagnostics, Abbott Park, IL, USA) is stated to be an accurate and expedient automated method for measuring MPO in plasma[76]. In terms of MPO results, a good correlation of this automated method with the ELISA method has been demonstrated[76,77]. The superiority of the Architect MPO assay over the ELISA assay is that it takes 15 minutes compared with ELISA, which takes 5 hours. Hence, this technique could be a suitable alternative to the reference method of ELISA because of its ease of use, reliable analytical performance, and speed[78].

Pre-analytical sample handling also affects the MPO results. MPO concentrations were consistently higher in samples collected in serum and heparin plasma tubes compared to samples collected in ethylenediaminetetraacetic acid (EDTA) or citrate tubes due to MPO leakage from leukocytes[79]. In order to avoid inaccuracy in the results of MPO, it is ideal to centrifuge the whole blood sample immediately after sampling. In these situations, it is advised that the sample would be protected in ice immediately after sampling[80]. It has been observed that by keeping the sample at room temperature, MPO concentrations will gradually increase due to the leakage from leukocytes into the sample[77]. Another point regarding the pre-analytical sample handling is the comparison of the measurement of MPO in serum and heparinized plasma. Measuring MPO in serum would alter the MPO concentrations because it is essential to keep the sample for at least 30 minutes at room temperature for clotting before centrifuge, which could result in a higher concentration of MPO[79]. Importantly, MPO concentrations have been reported to be much more stable in EDTA than in citrate or serum-containing collection tubes[80].

Table 1.

The most prominent studies investigating the role of MPO in predicting CVD risk

Setting of study	Authors	Type of study	Number of participants	MPO measurement/technique of measurement	Outcome measure (s) or objective (s)	Duration of follow-up	Correlation of interested outcome with MPO level after adjustment for CVD risk factors (P value)
Apparently healthy individuals	Meuwese *et al.*[44]	Nested case-control	1,138 cases vs. 2,237 controls	Serum MPO/ELISA	CAD events	8 years	<0.001
	Wong *et al.*[45]	Cohort	1,302	Serum MPO/immunoassay	CVD events	3.8 years	0.04
Stable coronary artery disease	Zhang *et al.*[7]	Case-control	158 cases with established CAD vs. 175 controls	Mass per milligram of neutrophil protein or as MPO mass per milliliter of blood	CAD risk	-	<0.001
	Stefanescu *et al.*[51]	Cohort	382	Serum MPO/ELISA	MACEs	3 years	>0.05
	Hasanpour *et al.*[52]	Case-control	18 cases with CAD vs. 22 controls without CAD	Serum MPO/ELISA	MACEs	3.5 years	0.002
	Khan *et al.*[96]	Cohort	1,465	Intracellular monocyte MPO	Incident cardiovascular disease	9.6 years	>0.05
	Calmarza *et al.*[54]	Cross-sectional	197	MPO serum concentration/single-step sandwich enzyme immunoassay	Severity of coronary lesion (FFR)	-	>0.05
	Uydu *et al.*[67]	Case-control	111 cases vs. 68 controls	Serum MPO/ELISA	Severity of coronary lesion (visual angiographically)	-	0.77
	Wiersma *et al.*[68]	Cross-sectional	267	Serum MPO/ELISA	Scintigraphic myocardial perfusion abnormalities	-	>0.05
	Kubala *et al.*[69]	Cross-sectional	557	Serum MPO/ELISA	CVD (angiographically)	-	>0.05
	Roman *et al.*[70]	Cohort	178	Serum MPO/ELISA	MACEs	13 ± 4 months	>0.05

(Continued)

Table 1.
(Continued)

Setting of study	Authors	Type of study	Number of participants	MPO measurement/technique of measurement	Outcome measure (s) or objective (s)	Duration of follow-up	Correlation of interested outcome with MPO level after adjustment for CVD risk factors (P value)
Unstable angina	Searle *et al.*[59]	Cross-control	236 unstable angina 146 stable angina vs. 85 controls	MPO serum concentration/not specified	Complexity of lesions	-	0.015
	Nicholls *et al.*[63]	Case-control	604 cases vs. 115 controls	MPO serum concentration/ELISA	Acute myocardial infarction	During hospitalization, 30 days, 6 months	0.001
	Roman *et al.*[70]	Cohort	130	Serum MPO/ELISA	MACEs	Hospitalization period	<0.001
	Sawicki *et al.*[60]	Case-control	253 ACS and 47 other heart diseases or unspecified Chest pain as cases vs. 124 controls	Serum MPO/automated chemiluminescent microparticle immunoassay with ARCHITECT ci8200 system	Diagnostic efficacy of MPO for diagnosis of ACS	Hospitalization period	0.0001
	Peacock *et al.*[61]	Cohort	604	Serum MPO/ELISA	MACEs	30 days, 6 months	<0.001 (MACEs) in 30 days and 6 months
	Baldus *et al.*[64]	Cohort	1,090	Serial serum MPO/ELISA	MACEs	6 months	0.003
	Kolodziej *et al.*[65]	Meta-analysis	9,090	Serum MPO measurement/not identified	MACEs, recurrent MI, all-cause mortality	11.4 months	<0.001 for all-cause mortality, >0.1 for MACEs and recurrent MI
	Calmarza *et al.*[54]	Cohort	83	Serum MPO/single-step sandwich enzyme immunoassay	Discrimination between ACS and non-ACS patients	Not identified	<0.05
	Khan *et al.*[66]	Cohort	180	Serum MPO/ARCHITECT ci4100 immunoassay	Acute myocardial infarction	Hospitalization period	0.028
	Sawicki *et al.*[60]	Cohort	425	Serum MPO measurement/ELISA	MACEs	6 weeks	<0.001
	Govindarajan *et al.*[71]	Cohort	93	Serum MPO activity/spectrophotometry	MACEs	Hospitalization period	<0.001

ACS: acute coronary syndrome; CAD: coronary artery disease; CVD: cardiovascular disease; ELISA: enzyme-linked immunosorbent assay; FFR: fractional flow reserve; MACEs: major adverse cardiovascular events; MI: myocardial infarction; MPO: myeloperoxidase.

MPO AS A TARGET FOR TREATMENT

MPO, in its role as a marker of inflammation, is essential to defend the human body against pathogens. However, its persistent activation and elevation could result in pathologic conditions in vivo. Therefore, it is prudent to focus on targeting MPO as a promising strategy for the prevention or treatment of inflammatory conditions including CVDs. According to several studies, MPO plays an important role in altering vascular and pulmonary NO bioavailability through oxidizing NO[81]. NO plays a vital role in CV homeostasis by regulating arterial tone and arterial pressure, inhibiting platelet aggregation, vascular smooth muscle cell proliferation, and leukocyte-endothelial interaction[82]. Decreased NO bioavailability which is presented as decreased endothelial-dependent vasodilation could predict CV events in patients with CAD[83].

Several studies demonstrated the inverse correlation between levels of circulating and vessel-bound MPO with endothelium-dependent vasodilatation in CAD patients[25]. In this regard, the effect of some pharmacologic agents with established activity as MPO inhibitors of MPO NO oxidase activity under physiological conditions has been investigated. The objective of these studies was to find pharmacological interventions to prohibit NO consumption catalyzed by MPO. This pathway focuses on the mechanism of NO consumption by MPO that is critically dependent on the availability of endogenous radical scavengers and MPO substrates[84].

Maiocchi *et al.*[85] studied the effect of structurally diverse pharmacological agents with activities such as MPO substrates/inhibitors or antioxidants on MPO NO oxidase activity in human plasma. Among the investigated pharmacological agents, 2-thioxantine strongly inhibited MPO turnover and NO consumption. Acetaminophen firstly increased the consumption of NO by increasing the activity of MPO NO oxidase, but after MPO addition, the overall extent of NO consumption diminished due to H_2O_2 depletion. Interestingly, ascorbate and acetaminophen showed synergistic action in depleting NO consumption as ascorbate is capable of rapidly scavenging acetaminophen radicals to produce ascorbyl radicals which consume NO with relatively low efficiency. In fact, the overall mechanism of ascorbate and acetaminophen is by diverting MPO to an inefficient pathway of NO consumption that results in the reduction of NO depletion[82]. The same process was observed with resveratrol as it first increased the NO consumption and MPO turnover, but after the addition of MPO, it diminished the rate of NO consumption. Acetaminophen, resveratrol, and ascorbate also had synergistic actions [84]. Trolox which is an analog of vitamin E inhibited MPO NO oxidase activity by scavenging the NO-consuming tyrosyl and urate radicals in ascorbate-depleted fluids[85].

Nitroxides, like tempol, are a class of stable synthetic radicals that have been demonstrated to augment vascular NO bioavailability in oxidative stress in animal models. Nitroxides can act as superoxide dismutase mimetics and, as a consequence, diminish NO consumption[86].

Furthermore, some physiologic substances act as MPO inhibitors mainly by operating as free radical scavengers. The most well-known compound in this regard is melatonin which is capable of inhibiting H_2O_2-induced lipid peroxidation and lipoprotein modification[87]. The role of melatonin as a neurohormonal substance has been investigated in multiple studies. The positive effect of melatonin on lipid metabolism, hypertension, and pulmonary hypertension has been demonstrated[88]. There is also a strong inverse relationship between endogenous melatonin levels and CVD[89]. In addition, melatonin has been shown to have protective effects against ischemia-reperfusion injury in various organs, including the heart as it can reduce the infarct size and the incidence of reperfusion arrhythmias in ST elevation myocardial infarction likely due to its ability in scavenging free radicals and express antioxidant enzymes. Melatonin supplementation is accepted as an effective treatment for cardiac diseases in experimental animal studies[90,91].

Another potential MPO inhibitor, PF1355, which is an orally bioavailable inhibitor of MPO enzymatic activity, was examined by Ali *et al.*[92] to detect its benefit on ischemia-reperfusion injury in the mouse model. It was reported that a short duration of oral drug treatment for 7 days diminished inflammation and cardiac dilation during early infarct healing. Prolonged 21-day treatment improved ejection fraction (about 44%) and decreased end-diastolic volume (about 53%) and left ventricular mass (about 33%) compared with untreated control animals. In addition, an enhanced therapeutic effect was also attained by starting treatment as early as 1 hour after the initial ischemic insult. However, PF-06282999, another MPO inhibitor that is a structural analog to PF1355, did not affect the lesion area in LDL receptor-deficient ($Ldlr^{-/-}$) mice, while a reduced necrotic core area was observed in aortic root sections after MPO inhibitor treatment[93].

A study has revealed the beneficial effect of sodium thiocyanate (NaSCN) administration to apoenzyme (APOE) knockout ($ApoE^{-/-}$) mice on the reduction of atherosclerotic plaque size in the aortic root. Thiocyanate is an MPO inhibitor that competitively inhibits HOCl formation through oxidation of SCN^- to HOSCN[94]. The most recently published preclinical study in the mouse model showed that the treatment with AZM198, an MPO inhibitor, stabilized the previously unstable plaques. However, the inflammatory cell content of plaques was not changed by MPO inhibition[75].

Unfortunately, there have been few clinical trials involving humans in this area. Lam *et al.*[95] conducted the Safety and Tolerability Study of AZD4831 in Patients With Heart Failure (SATELLITE) Trial, where

patients with heart failure preserved ejection fraction (HFpEF) with left ventricular ejection fraction (LVEF) ≥40% were randomized 2:1 to receive an MPO inhibitor, mitiperstat 5 mg once daily treatment, which led to a substantial reduction in plasma MPO activity, showing promising target engagement. While no improvements were observed in coronary flow reserve or 6-minute walk distance, there was a trend toward improvement in the overall summary score of the Kansas City Cardiomyopathy Questionnaire (KCCQ). However, the study was underpowered for these efficacy outcomes, and the comparison was not statistically significant.

There are a number of important considerations with regard to targeting MPO specifically. Firstly, it is important to note that atherosclerosis is a multifactorial disease process in which numerous causes exist from the very early stage of the disease to the plaque vulnerability and subsequent rupture, myocardial infarction, and heart failure. Considering the possible treatment strategy for any aspect of this complex process is a significant challenge that needs to consider all of the elements contributing to the process[94]. Secondly, as MPO is an essential part of the innate immune system response needed to fight against pathogens MPO inhibition might have unintended consequences throughout immune system cascades[94]. Thirdly, MPO levels of the atherosclerotic lesions in ApoE knock-out mice or Ldlr$^{-/-}$ mice are far lower than the MPO content in human atherosclerotic lesions. In addition, in comparison to human leukocytes, murine leukocytes contain 10- to 20-fold less MPO content, yet a decrease in atherosclerotic lesions in the murine model has been observed[30]. Hence a similar level of MPO inhibition utilized in the murine model might not be satisfactory in humans[90].

CONCLUSION AND FUTURE PERSPECTIVES

MPO, a crucial immune protein, has emerged as a potential biomarker for CVD. Higher MPO levels correlate with the severity of atherosclerotic lesions, increased risk of CV events, and CAC scores in humans. However, its effectiveness in chest pain triage requires further investigation. Despite limited evidence in chest pain triage, MPO's earlier release compared to troponin justifies further investigation. Moving forward, we need standardization by establishing consistent methods for measuring MPO concentration and activity, conducting large-scale studies including diverse populations, and exploring the therapeutic potential of MPO inhibitors with careful consideration of their impact on the immune system.

MPO holds promise for improved CVD risk stratification. Further research on standardization and early diagnosis can pave the way for its clinical application. Despite current challenges, advancements in MPO measurement and potential therapeutic applications are on the horizon.

AUTHOR CONTRIBUTIONS

HA contributed by conceiving the idea, conducting the literature search, data extraction, and writing the manuscript. KJN contributed through the supervision of the literature search and article selection, providing review, feedback and editing on the first draft, and proofreading/editing the final manuscript. All authors read and approved the final manuscript.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest with regard to the content of this manuscript.

DATA SHARING STATEMENT

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

REFERENCES

[1] Schindhelm RK, van der Zwan LP, Teerlink T, *et al*. Myeloperoxidase: a useful biomarker for cardiovascular disease risk stratification? Clin Chem 2009;55(8):1462–1470. doi:10.1373/clinchem.2009.126029.
[2] Klebanoff SJ. Myeloperoxidase-halide-hydrogen peroxide antibacterial system. J Bacteriol 1968;95(6):2131–2138. doi:10.1128/jb.95.6.2131-2138.1968.
[3] Daugherty A, Dunn JL, Rateri DL, *et al*. Myeloperoxidase, a catalyst for lipoprotein oxidation, is expressed in human atherosclerotic lesions. J Clin Invest 1994;94(1):437–444. doi:10.1172/JCI117342.
[4] van der Veen BS, de Winther MP, Heeringa P. Myeloperoxidase: molecular mechanisms of action and their relevance to human health and disease. Antioxid Redox Signal 2009;11(11):2899–2937. doi:10.1089/ars.2009.2538.
[5] Zhang R, Shen Z, Nauseef WM, *et al*. Defects in leukocyte-mediated initiation of lipid peroxidation in plasma as studied in myeloperoxidase-deficient subjects: systematic identification of multiple endogenous diffusible substrates for myeloperoxidase in plasma. Blood 2002;99(5):1802–1810.
[6] Kutter D, Devaquet P, Vanderstocken G, *et al*. Consequences of total and subtotal myeloperoxidase deficiency: risk or benefit? Acta Haematol 2000;104(1):10–15. doi:10.1159/000041062.
[7] Zhang R, Brennan ML, Fu X, *et al*. Association between myeloperoxidase levels and risk of coronary artery disease. JAMA 2001;286(17):2136–2142. doi:10.1001/jama.286.17.2136.
[8] Bergt C, Pennathur S, Fu X, *et al*. The myeloperoxidase product hypochlorous acid oxidizes HDL in the human artery wall and impairs ABCA1-dependent cholesterol transport. Proc Natl Acad Sci U S A 2004;101(35):13032–13037. doi:10.1073/pnas.0405292101.
[9] Agner K. Verdoperoxidase: a ferment isolated from leucocytes. Acta Physiol Scand 1941;2(suppl 8):1–62.
[10] Andreadis AA, Hazen SL, Comhair SA, *et al*. Oxidative and nitrosative events in asthma. Free Radic Biol Med 2003;35(3):213–225. doi:10.1016/s0891-5849(03)00278-8.
[11] Vanhamme L, Zouaoui Boudjeltia K, Van Antwerpen P, *et al*. The other myeloperoxidase: emerging functions. Arch Biochem Biophys 2018;649:1–14. doi:10.1016/j.abb.2018.03.037.
[12] Dale DC, Boxer L, Liles WC. The phagocytes: neutrophils and monocytes. Blood 2008;112(4):935–945. doi:10.1182/blood-2007-12-077917.
[13] Borregaard N, Cowland JB. Granules of the human neutrophilic polymorphonuclear leukocyte. Blood 1997;89(10):3503–3521.
[14] Jantschko W, Furtmüller PG, Zederbauer M, *et al*. Exploitation of the unusual properties of human myeloperoxidase in inhibitor design. Biochem Pharmacol 2005;69(8):1149–1157. doi:10.1016/j.bcp.2005.02.006.
[15] Witko-Sarsat V, Descamps-Latscha B. Neutrophil-derived oxidants and proteinases as immunomodulatory mediators in

inflammation. Mediators Inflamm 1994;3(4):257–273. doi:10.1155/S0962935194000360.
[16] Abu-Soud HM, Hazen SL. Nitric oxide modulates the catalytic activity of myeloperoxidase. J Biol Chem 2003;275(8):5425–5430. doi:10.1074/jbc.275.8.5425.
[17] Augusto O, Bonini MG, Amanso AM, *et al*. Nitrogen dioxide and carbonate radical anion: two emerging radicals in biology. Free Radic Biol Med 2002;32(9):841–859. doi:10.1016/s0891-5849(02)00786-4.
[18] Lefkowitz DL, Gelderman MP, Fuhrmann SR, *et al*. Neutrophilic myeloperoxidase-macrophage interactions perpetuate chronic inflammation associated with experimental arthritis. Clin Immunol 1991;91(2):145–155. doi:10.1006/clim.1999.4696.
[19] Lefkowitz DL, Mills KC, Moguilevsky N, *et al*. Regulation of macrophage function by human recombinant myeloperoxidase. Immunol Lett 1993;36(1):43–49. doi:10.1016/0165-2478(93)90067-c.
[20] Domigan M, Charlton TS, Duncan MW, *et al*. Chlorination of tyrosyl residues in peptides by myeloperoxidase and human neutrophils. J Biol Chem 1995;270(28):16542–16548. doi:10.1074/jbc.270.28.16542.
[21] Arnhold J. The dual role of myeloperoxidase in immune response. Int J Mol Sci 2020;21(21):8057. doi:10.3390/ijms21218057.
[22] Nicholls SJ, Hazen SL. Myeloperoxidase, modified lipoproteins, and atherogenesis. J Lipid Res 2009;50(Suppl):S346–S351. doi:10.1194/jlr.R800086-JLR200.
[23] Auchère F, Capeillère-Blandin C. NADPH as a co-substrate for studies of the chlorinating activity of myeloperoxidase. Biochem J 1999;343(Pt 3):603–613.
[24] Vita JA, Brennan ML, Gokce N, *et al*. Serum myeloperoxidase levels independently predict endothelial dysfunction in humans. Circulation 2004;110(9):1134–1139. doi:10.1161/01.CIR.0000140262.20831.8F.
[25] Baldus S, Heitzer T, Eiserich JP, *et al*. Myeloperoxidase enhances nitric oxide catabolism during myocardial ischemia and reperfusion. Free Radic Biol Med 2004;37(6):902–911. doi:10.1016/j.freeradbiomed.2004.06.003.
[26] Burke AP, Farb A, Malcom GT, *et al*. Coronary risk factors and plaque morphology in men with coronary disease who died suddenly. N Engl J Med 1997;336(18):1276–1282. doi:10.1056/NEJM199705013361802.
[27] Burke AP, Kolodgie FD, Farb A, *et al*. Role of circulating myeloperoxidase positive monocytes and neutrophils in occlusive coronary thrombi. J Am Coll Cardiol 2002;39(Supplement_1):256. doi:10.1016/s0735-1097(02)81147-0.
[28] Sugiyama S, Okada Y, Sukhova GK, *et al*. Macrophage myeloperoxidase regulation by granulocyte macrophage colony-stimulating factor in human atherosclerosis and implications in acute coronary syndromes. Am J Pathol 2001;158(3):879–891. doi:10.1016/S0002-9440(10)64036-9.
[29] Naruko T, Ueda M, Haze K, *et al*. Neutrophil infiltration of culprit lesions in acute coronary syndromes. Circulation 2002;106(23):2894–2900. doi:10.1161/01.cir.0000042674.89762.20.
[30] Nicholls SJ, Hazen SL. Myeloproxidase and cardiovascular disease. Arterioscler Thromb Vasc Biol 2005;25(6):1102–1111. doi:10.1161/01.ATV.0000163262.83456.6d.
[31] Butler WJ, Ostrander LDJ, Carman WJ, *et al*. Mortality from coronary heart disease in the Tecumseh Study: long–term effect of diabetes mellitus, glucose tolerance and other risk factors. Am J Epidemiol 1985;121(4):541–547. doi:10.1093/oxfordjournals.aje.a114031.
[32] Avogaro P, Cazzolato G, Bittolo-Bon G. Some questions concerning a small, more electronegative LDL circulating in human plasma. Atherosclerosis 1991;91(1-2):163–171. doi:10.1016/0021-9150(91)90198-c.
[33] Zheng L, Nukuna B, Brennan ML, *et al*. Apolipoprotein A-I is a selective target for myeloperoxidase-catalyzed oxidation and functional impairment in subjects with cardiovascular disease. J Clin Invest 2004;114(4):529–541. doi:10.1172/JCI21109.
[34] Zhang R, Brennan ML, Shen Z, *et al*. Myeloperoxidase functions as a major enzymatic catalyst for initiation of lipid peroxidation at sites of inflammation. J Biol Chem 2002;277(48):46116–46122. doi:10.1074/jbc.M209124200.
[35] Apostolov EO, Shah SV, Ok E, *et al*. Quantification of carbamylated LDL in human sera by a new sandwich ELISA. Clin Chem 2005;51(4):719–728. doi:10.1373/clinchem.2004.044032.
[36] Kalim S, Karumanchi SA, Thadhani RI, *et al*. Protein carbamylation in kidney disease: pathogenesis and clinical implications. Am J Kidney Dis 2014;64(5):793–803. doi:10.1053/j.ajkd.2014.04.034.
[37] Podrez EA, Febbraio M, Sheibani N, *et al*. Macrophage scavenger receptor CD36 is the major receptor for LDL modified by monocyte-generated reactive nitrogen species. J Clin Invest 2000;105(8):1095–1108. doi:10.1172/JCI8574.
[38] Fielding CJ, Fielding PE. Molecular physiology of reverse cholesterol transport. J Lipid Res 1995;36(2):211–228.
[39] Sorrentino SA, Besler C, Rohrer L, *et al*. 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 2010;121(1):110–122. doi:10.1161/CIRCULATIONAHA.108.836346.
[40] Undurti A, Huang Y, Lupica JA, *et al*. Modification of high density lipoprotein by myeloperoxidase generates a pro-inflammatory particle. J Biol Chem 2009;284(45):30825–30835. doi:10.1074/jbc.M109.047605.
[41] Peng DQ, Wu Z, Brubaker G, *et al*. Tyrosine modification is not required for myeloperoxidase-induced loss of apolipoprotein A-I functional activities. J Biol Chem 2005;280(40):33775–33784. doi:10.1074/jbc.M504092200.
[42] Askari AT, Brennan ML, Zhou X, *et al*. Myeloperoxidase and plasminogen activator inhibitor 1 play a central role in ventricular remodeling after myocardial infarction. J Exp Med 2003;197(5):615–624. doi:10.1084/jem.20021426.
[43] Zannad F, De Backer G, Graham I, *et al*. the ESC Working Group on CardioVascular Pharmacology and Drug Therapy. Risk stratification in cardiovascular disease primary prevention – scoring systems, novel markers, and imaging techniques. Fundam Clin Pharmacol 2012;26(2):163–174. doi:10.1111/j.1472-8206.2011.01023.x.
[44] Meuwese MC, Stroes ES, Hazen SL, *et al*. Serum myeloperoxidase levels are associated with the future risk of coronary artery disease in apparently healthy individuals: the EPIC-Norfolk Prospective Population Study. J Am Coll Cardiol 2007;50(2):159–165. doi:10.1016/j.jacc.2007.03.033.
[45] Wong ND, Gransar H, Narula J, *et al*. Myeloproxidase, subclinical atherosclerosis, and cardiovascular disease events. JACC Cardiovasc Imaging 2009;2(9):1093–1099. doi:10.1016/j.jcmg.2009.05.012.
[46] Tonkin AM, Blankenberg S, Kirby A, *et al*. LIPID study investigators. Biomarkers in stable coronary heart disease, their modulation and cardiovascular risk: the LIPID biomarker study. Int J Cardiol 2015;201:499–507. doi:10.1016/j.ijcard.2015.07.080.
[47] Boden WE, O'Rourke RA, Teo KK, *et al*. COURAGE Trial Research Group. Optimal medical therapy with or without PCI for stable coronary disease. N Engl J Med 2007;356(15):1503–1516. doi:10.1056/NEJMoa070829.
[48] Kimak E, Zięba B, Duma D, *et al*. Myeloperoxidase level and inflammatory markers and lipid and lipoprotein parameters in stable coronary artery disease. Lipids Health Dis 2018;17(1):71. doi:10.1186/s12944-018-0718-4.
[49] Baseri M, Heidari R, Mahaki B, *et al*. Myeloperoxidase levels predict angiographic severity of coronary artery disease in patients with chronic stable angina. Adv Biomed Res 2004;3:139. doi:10.4103/2277-9175.135155.
[50] Tang WH, Wu Y, Nicholls SJ, *et al*. Plasma myeloperoxidase predicts incident cardiovascular risks in stable patients undergoing medical management for coronary artery disease. Clin Chem 2011;57(1):33–39. doi:10.1373/clinchem.2010.152827.
[51] Stefanescu A, Braun S, Ndrepepa G, *et al*. Prognostic value of plasma myeloperoxidase concentration in patients with stable coronary artery disease. Am Heart J 2008;155(2):356–360. doi:10.1016/j.ahj.2007.10.017.
[52] Hasanpour Z, Javanmard SH, Gharaaty M, *et al*. Association between serum myeloperoxidase levels and coronary artery disease in patients without diabetes, hypertension, obesity, and hyperlipidemia. Adv Biomed Res 2016;5:103. doi:10.4103/2277-9175.183663.
[53] Muller O, Ntalianis A, Wijns W, *et al*. Association of biomarkers of lipid modification with functional and morphological indices of coronary stenosis severity in stable coronary artery disease. J Cardiovasc Transl Res 2013;6(4):536–544. doi:10.1007/s12265-013-9468-x.

[54] Calmarza P, Lapresta C, Martinez M, *et al*. Utility of myeloperoxidase in the differential diagnosis of acute coronary syndrome. Arch Cardiol Mex 2018;88(5):391–396. doi:10.1016/j.acmx.2017.11.003.

[55] Teng N, Maghzal GJ, Talib J, *et al*. The role of myeloperoxidase in coronary artery disease and its potential implication in plaque rupture. Redox Rep 2017;22(2):51–73. doi:10.1080/13510002.2016.1256119.

[56] Brennan ML, Hazen SL. Emerging role of myeloperoxidase and oxidant stress markers in cardiovascular risk assessment. Curr Opin Lipidol 2003;14(4):353–359. doi:10.1097/00041433-200308000-00003.

[57] Ferrante G, Nakano M, Prati F, *et al*. High level of systemic myeloperoxidase is associated with coronary plaque erosion in patients with acute coronary syndromes. Circulation 2010;122(24):2505–2513. doi:10.1161/CIRCULATIONAHA.110.955302.

[58] Naruko T, Furukawa A, Yunoki K, *et al*. Increased expression and plasma levels of myeloperoxidase are closely related to the presence of angiographically-detected complex lesion morphology in unstable angina. Heart 2010;96(21):1716–1722. doi:10.1136/hrt.2009.187609.

[59] Searle J, Shih J, Muller R, *et al*. The role of myeloperoxidase (MPO) for prognostic evaluation in sensitive cardiac troponin I negative chest pain patients in the emergency department. Eur Heart J Acute Cardiovasc Care 2013;2(3):203–210. doi:10.1177/2048872613484688.

[60] Sawicki M, Sypniewska G, Kozinski M, *et al*. Diagnostic efficacy of myeloperoxidase for the detection of acute coronary syndromes. Eur J Clin Invest 2011;41(6):667–671. doi:10.1111/j.1365-2362.2010.02457.x.

[61] Peacock WF, Nagurney J, Birkhahn R, *et al*. Myeloproxidase in diagnosis of acute coronary syndromes: the importance of spectrum. Am Heart J 2011;162(5):893–899. doi:10.1016/j.ahj.2011.08.017.

[62] Brennan ML, Penn MS, Van Lente F, *et al*. Prognostic value of myeloperoxidase in patients with chest pain. N Engl J Med 2003;349(17):1595–1604. doi:10.1056/NEJMoa035003.

[63] Nicholls SJ, Tang WH, Brennan D, *et al*. Risk prediction with serial myeloperoxidase monitoring in patients with acute chest pain. Clin Chem 2011;57(12):1762–1770. doi:10.1373/clinchem.2011.166827.

[64] Baldus S, Heeschen C, Meinertz T, *et al*. CAPTURE Investigators. Myeloperoxidase serum levels predict risk in patients with acute coronary syndromes. Circulation 2003;108(12):1440–1445. doi:10.1161/01.CIR.0000090690.67322.51.

[65] Kolodziej AR, Abo-Aly M, Elsawalhy E, *et al*. Prognostic role of elevated myeloperoxidase in patients with acute coronary syndrome: a systematic review and meta-analysis. Mediators Inflamm 2019;2019:2872607. doi:10.1155/2019/2872607.

[66] 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 2011;31(3):172–178. doi:10.3343/kjlm.2011.31.3.172.

[67] Uydu HA, Bostan M, Yilmaz A, *et al*. Comparison of inflammatory biomarkers for detection of coronary stenosis in patients with stable coronary artery disease. Eur Rev Med Pharmacol Sci 2013;17(1):112–118.

[68] Wiersma JJ, Verberne HJ, Meuwese MC, *et al*. Myeloperoxidase is not associated with scintigraphic myocardial perfusion abnormalities in type 2 diabetic patients with mild stable anginal complaints. Clin Chim Acta 2011;412(1-2):86–90. doi:10.1016/j.cca.2010.09.019.

[69] Kubala L, Lu G, Baldus S, *et al*. Plasma levels of myeloperoxidase are not elevated in patients with stable coronary artery disease. Clin Chim Acta 2008;394(1–2):59–62. doi:10.1016/j.cca.2008.04.001.

[70] Roman RM, Camargo PV, Borges FK, *et al*. Prognostic value of myeloperoxidase in coronary artery disease: comparison of unstable and stable angina patients. Coron Artery Dis 2010;21(3):129–136. doi:10.1097/MCA.0b013e328333f50d.

[71] Govindarajan S, Raghavan VM, Rao AC. Plasma myeloperoxidase and total sialic acid as prognostic indicators in acute coronary syndrome. J Clin Diagn Res 2016;10(8):BC09–BC13. doi:10.7860/JCDR/2016/20715.8347.

[72] Chevrier I, Tregouet DA, Massonnet-Castel S, *et al*. Myeloperoxidase genetic polymorphisms modulate human neutrophil enzyme activity: genetic determinants for atherosclerosis? Atherosclerosis 2006;188(1):150–154. doi:10.1016/j.atherosclerosis.2005.10.012.

[73] Piedrafita FJ, Molander RB, Vansant G, *et al*. An Alu element in the myeloperoxidase promoter contains a composite SP1-thyroid hormone-retinoic acid response element. J Biol Chem 1996;271(24):14412–14420. doi:10.1074/jbc.271.24.14412.

[74] Chapman AL, Mocatta TJ, Shiva S, *et al*. Ceruloplasmin is an endogenous inhibitor of myeloperoxidase. J Biol Chem 2013;288(9):6465–6477. doi:10.1074/jbc.M112.418970.

[75] Chen W, Tumanov S, Kong SMY, *et al*. Therapeutic inhibition of MPO stabilizes pre-existing high risk atherosclerotic plaque. Redox Biol 2022;58:102532. doi:10.1016/j.redox.2022.102532.

[76] Goiffon RJ, Martinez SC, Piwnica-Worms D. A rapid bioluminescence assay for measuring myeloperoxidase activity in human plasma. Nat Commun 2015;6:6271. doi:10.1038/ncomms7271.

[77] Zietzer A, Niepmann ST, Camara B, *et al*. Sodium thiocyanate treatment attenuates atherosclerotic plaque formation and improves endothelial regeneration in mice. PLoS One 2019;14(4):e0214476. doi:10.1371/journal.pone.0214476.

[78] Datwyler SA, Hsu SC, Matias MS, *et al*. Potential interference by antineutrophil cytoplasmic autoantibodies in myeloperoxidase immunoassays. Clin Chem 2008;54(12):2084–2086. doi:10.1373/clinchem.2008.110841.

[79] Shih J, Datwyler SA, Hsu SC, *et al*. Effect of collection tube type and pre-analytical handling on myeloperoxidase concentrations. Clin Chem 2008;54(6):1076–1079. doi:10.1373/clinchem.2007.101568.

[80] Chang PY, Wu TL, Hung CC, *et al*. Development of an ELISA for myeloperoxidase on microplate: normal reference values and effect of temperature on specimen preparation. Clin Chim Acta 2006;373(1-2):158–163. doi:10.1016/j.cca.2006.05.030.

[81] Abu-Soud HM, Hazen SL. Nitric oxide is a physiological substrate for mammalian peroxidases. J Biol Chem 2000;275(48):37524–37532. doi:10.1074/jbc.275.48.37524.

[82] Thomas SR, Witting PK, Drummond GR. Redox control of endothelial function and dysfunction: molecular mechanisms and therapeutic opportunities. Antioxid Redox Signal 2008;10(10):1713–1765. doi:10.1089/ars.2008.2027.

[83] Schächinger V, Britten MB, Zeiher AM. Prognostic impact of coronary vasodilatory dysfunction on adverse long-term outcome of coronary heart disease. Circulation 2000;101(16):1899–1906. doi:10.1161/01.cir.101.16.1899.

[84] Rees MD, Maiocchi SL, Kettle AJ, *et al*. Mechanism and regulation of peroxidase-catalyzed nitric oxide consumption in physiological fluids: critical protective actions of ascorbate and thiocyanate. Free Radic Biol Med 2014;72:91–103. doi:10.1016/j.freeradbiomed.2014.03.037.

[85] Maiocchi SL, Morris JC, Rees MD, *et al*. Regulation of nitric oxide oxidase activity of myeloperoxidase by pharmacological agents. Biochem Pharmacol 2017;135:90–115. doi:10.1016/j.bcp.2017.03.016.

[86] Wilcox CS, Pearlman A. Chemistry and antihypertensive effects of tempol and other nitroxides. Pharmacol Rev 2008;60(4):418–469. doi:10.1124/pr.108.000240.

[87] Galijasevic S. The development of myeloproxidase inhibitors. Bioorg Med Chem Lett 2019;29(1):1–7. doi:10.1016/j.bmcl.2018.11.031.

[88] Maarman G, Blackhurst D, Thienemann F, *et al*. Melatonin as a preventive and curative therapy against pulmonary hypertension. J Pineal Res 2015;59(3):343–353. doi:10.1111/jpi.12263.

[89] Dominguez-Rodriguez A, Abreu-Gonzalez P, Sanchez-Sanchez JJ, *et al*. Melatonin and circadian biology in human cardiovascular disease. J Pineal Res 2010;49(1):14–22. doi:10.1111/j.1600-079X.2010.00773.x.

[90] Sun H, Huang FF, Qu S. Melatonin: a potential intervention for hepatic steatosis. Lipids Health Dis 2015;14:75. doi:10.1186/s12944-015-0081-7.

[91] Tan DX, Manchester LC, Reiter RJ, *et al*. Ischemia/reperfusion-induced arrhythmias in the isolated rat heart: prevention by melatonin. J Pineal Res 1998;25(3):184–191. doi:10.1111/j.1600-079x.1998.tb00558.x.

[92] Ali M, Pulli B, Courtie G, *et al*. Myeloperoxidase inhibition improves ventricular function and remodeling after experimental

myocardial infarction. JACC Basic Transl Sci 2016;1(7):633–643. doi:10.1016/j.jacbts.2016.09.004.
[93] Roth Flach RJ, Su C, Bollinger E, *et al*. Myeloperoxidase inhibition in mice alters atherosclerotic lesion composition. PLoS One 2019;14(3):e0214150. doi:10.1371/journal.pone.0214150.
[94] van Dalen CJ, Whitehouse MW, Winterbourn CC, *et al*. Thiocyanate and chloride as competing substrates for myeloperoxidase. Biochem J 1997;327(Pt 2):487–492. doi:10.1042/bj3270487.
[95] Lam CSP, Lund LH, Shah SJ, *et al*. Myeloperoxidase inhibition in heart failure with preserved or mildly reduced ejection fraction: SATELLITE trial results. J Card Fail 2024;30(1):104–110. doi:10.1016/j.cardfail.2023.04.003.

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