Myeloperoxidase and Coronary Arterial Disease: From Research to Clinical Practice

Raquel Melchior Roman[1,4], Andrea Elisabet Wendland[2,4], Carisi Anne Polanczyk[3,4]

Department of Cardiology – Hospital São Vicente de Paulo, Passo Fundo-RS[1], Laboratory of Clinical Pathology - Hospital de Clínicas de Porto Alegre, Porto Alegre-RS[2], Department of Cardiology – Hospital de Clínicas de Porto Alegre, Porto Alegre-RS[3], Post-Graduate Program in Cardiology of the Universidade Federal do Rio Grande do Sul, Porto Alegre, RS[4] - Brazil

Abstract

Myeloperoxidase (MPO) is an enzyme derived of leukocytes that catalyze formation of numerous reactive oxidant species. Besides members of the innate host defense, evidences have been proving the contribution of these oxidants to tissue injury during inflammation. MPO participates in proatherogenic biological activities related to the evolution of cardiovascular disease, including initiation, propagation and acute complications of atherosclerotic process. Thereby, MPO and its inflammatory cascade represents an attractive target for prognostical investigation and therapeutics in atherosclerotic cardiovascular disease. In this review, we present the state of the art in the understanding of biological actions to clinical evidences of the relationship between MPO and coronary arterial disease. Several studies point to the independent effect of MPO levels in the evolution of disease and incidence of events in patients with acute coronary syndrome. However, the additional predictive value of MPO levels in the cardiovascular risk assessment, to incorporate it to the clinical practice as marker of plaque vulnerability, is still not consistent. Additional studies are necessary to confirm its role in the different forms of presentation of ischemic disease, besides the standardization of the assay, fundamental point for transition of this marker from research atmosphere to use in clinical routine: : from laboratory to clinical practice.

Introduction

Inflammation has been related to several stages of development of atherosclerotic plaque, from lipid deposit to rupture of the plaque and their thrombotic complications. Several epidemiologic trials have been evaluating the inflammatory markers (C-reactive protein, cytokines, adhesion molecules, total leucocyte count) and its clinical applicability as risk predictors for cardiovascular disease (CVD)[1-3].

Reagents of acute phase as the C-reactive protein (CRP), sensitive inflammation markers, but little specific, they are increased in early and late stages of atherosclerotic lesions. Its detection as risk predictor serum marker for coronary arterial disease in epidemic studies has based its use as part of the preventive evaluation of cardiovascular risk[4]. However, many patients with risk of hard cardiovascular events are not yet earlier identified and the sudden manifestations frequently demand search of emergency services. Thus, there is need to identify additional markers mainly for risk evaluation of cardiovascular events related to plaque vulnerability[5-7].

The rupture or erosion of plaque with intramural thrombus formation represent the most important morphologic modification in the transformation of stable coronary lesions into clinically unstable. The anatomopathological substrate of these complications is heterogeneous with respect to the architecture and composition of the plaque, but the presence of the localized inflammation has been appearing as common denominator. While lymphocytes and monocytes have been considered important contributors specially in the physiopathology of cardiovascular disease mainly for generation of pro-inflammatory cytokines; the polymorfonuclear neutrophil can modulate and signal inflammatory pathway by the secretion of enzymes that interact in target-organ. The function of neutrophils at site of tissue lesion is quite complex, but can be summarized by endocytosis of foreign material and secretion of intracellular enzymes as elastase, endopeptidase and myeloperoxidase (MPO)[8].

The synthesis of MPO occurs during the myeloid differentiation in the bone marrow and it is complete within the granulocytes, previously to its entrance in the circulation. This enzyme is found predominantly in neutrophils, monocytes and some subtypes of tissue macrophages. Represents more than 5% of total protein content of the cell in neutrophils and 1% in monocytes[9].

MPO is a cationic protein, with molecular weight of 144 kD, that consists of two identical dimmers linked by a disulfide bridge, each dimmer composed of a subunit of light and a heavy chain, with functionally identical heme groups. It is the main component of the azurophilic granules of the neutrophils, promptly liberated after activation by different antagonists contributing to the innate immune response of the organism[9-11].

Growing evidences demonstrate the action of MPO as central participant of the link between inflammation and cardiovascular disease. The myeloperoxidase, through the reaction with hydrogen peroxide, form free radicals and diffusible oxidative substances with antimicrobial activity, but

Key words

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Mailing address: Raquel Melchior Roman •
Rua Capitão Eleutério, 111/202 - Centro - 99010-060 - Passo Fundo, RS - Brazil
E-mail: rmelchior@cardiol.br
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it also promotes oxidative damage of host tissue by exercising pleiothropic effects in the vascular system with potential impact in the atherosclerosis development, endothelial dysfunction, plaque unstabilization and response in ventricular remodeling after ischemic injury[12-14] (Figure 1).

Biochemical mechanisms of the relationship between myeloperoxidase and cardiovascular disease

The participation of myeloperoxidase in the composition of lipid content of the vascular atheroma, in the proteases activation and in vasoconstriction mechanisms and thrombosis makes the involvement of this heme protein in development of atherosclerotic disease and their thrombotic complications very consistent (Table 1).

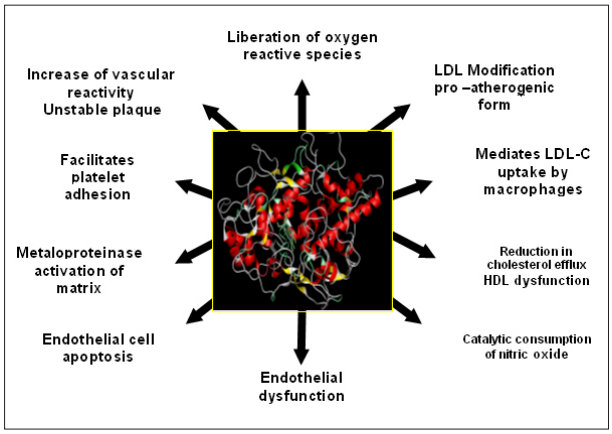

Figure 1 - Potential mechanisms of myeloperoxidase in atherogenesis. Adapted from Hasson et al[9] and Nicholls and Hazen[12].

Myeloperoxidase as catalytic of lipid oxidation: effects on LDL and HDL

The oxidative modification of LDL leads to the increase of its uptake and degradation by macrophages resulting in cholesterol deposit and formation of foam cells, the cellular mark of the fatty streaks. Recent studies point to probable mechanisms by which myeloperoxidase is capable to promote oxidation of lipoproteins *in vivo*[15-21]. Macrophages use NADPH oxidase to produce superoxide (O_2-) that can dismutate and form hydrogen peroxide (H_2O_2). MPO catalyzes reactions with H_2O_2 to generate more potent cytotoxic oxidizers like HOCl (hypochlorous acid) and tyrosyl radical, the only human enzyme capable to generate HOCl. Through assays of high sensibility and specificity, several stable final products generated by these species have been detected in atherosclerotic plaque[15-17].

MPO has the ability to modify oxidatively the amino acid tyrosine of the apolipoprotein B-100 (apo B-100) using H_2O_2 and ion Cl^- to generate 3-chlorotyrosine. It also can nitrate the tyrosine oxidating nitrite (NO_2^-), final product of the metabolism of the nitric oxide (NO), generating 3-nitrotyrosine[18-19]. Besides markers of MPO activity, the 3-chlorotyrosine and 3-nitrotyrosine have clinical importance for promoting oxidative damage contributing to atherosclerosis.

Podrez et al[18,20] characterized the system MPO - H_2O_2-NO_2 as preferential pathway, used by monocytes, to convert LDL in atherogenic forms with greater affinity with the receiver CD36, main macrophage receiver for oxidized LDL directly involved in the formation of foam cells *in vivo*.

More recently, it was demonstrated that HDL is also susceptible to oxidative modifications mediated by myeloperoxidase by nitration or halogenation of tyrosine residues in the apolipoprotein A-I (Apo A-I). These harm the ability of the protein to promote the ABCA-1 dependent reverse transport of cholesterol contributing to the formation of atherosclerotic lesions[21-23].

Table 1 – Biochemical mechanisms of MPO and relation to cardiovascular disease

Mechanisms	Biochemical pathways	Consequence
Catalytic lipid oxidation	Halogenated composition (HOCl) Radical thyrosyl Nitrogen reative species	LDL oxidated with greater affinity CD36 → Formation foam cells HDL: Target oxidation APO A1 → Reduction reverse transport ABCA-1
Endothelial dysfunction	Physiologic oxidative NO consumption Reduction NO-sinthetase activity and cofactors(NADPH)	Impaired vasodilation Platelet aggregation Recruitment and leukocyte activation
Promotion Oxidative Stress	Generation Nitrated Oxidants: (ON2) and nitrotirosyne	Vascular injury and atherogenesis promotion
Modulation Proteases Cascade	Inactivation protease inhibitors (α-1-antitripsyne, TIMs, PAI-1) Ativation latent forms I (proeslastases e MMPs)	Degradation and rupture of fibrous layer of atherosclerotic plaque Ventricular remodeling
Increase thrombogenecity	HOCL: apoptose cels. endoteliais Aumento expressão e ativação fator tecidual Aumento expressão endotelial de moléculas de adesão (selectinas)	Activation and platelet aggregation – intraluminal thromb formation

Myeloperoxidase and nitric oxide metabolism: contribution to endothelial dysfunction

The vascular endothelial dysfunction, affecting the vasomotor response to the nitric oxide (NO), is a well established phenomenon in cardiovascular disease and some peroxidase-mediated mechanisms have been studied recently. Abu-Soud and Hazen[24], demonstrated that all members of the heme peroxidase superfamily, of which the myeloperoxidase is the prototype, are capable of catalytically consume NO under physiologic conditions, limiting its bioavailability. Besides the catalytic consumption, oxidants generated are capable to inhibit the activity of the NO synthetase and to reduce their cofactors like NADPH. Histopathological studies demonstrate the accumulation of MPO in the vascular endothelium and its combination with nitrotyrosine in the subendothelial space of atherosclerotic lesions interfering locally in the effect of NO in the vessel wall[25].

Serum levels of MPO measured in 298 individuals demonstrated it to be independent predictor of endothelial dysfunction, appraised by brachial artery flow-mediated dilation determined by ultrasound. After adjustment for presence of traditional cardiovascular risk factors, medications and presence of artery coronary disease, individuals with MPO levels in the highest quartile had probability 6,4 times greater of presenting endothelial dysfunction in relation to the lowest[26].

Myeloperoxidase and plaque vulnerability

Most of the studies that evaluate myeloperoxidase action in cardiovascular disease focus the early stage of the process. However, recent studies emphasize the modulating action of MPO over the proteases cascade participating in the acute complications of atherosclerosis, stimulus to thrombogenecity and plaque vulnerability[27].

An extensive infiltration of monocytes and neutrophils is typically described in fissured and thrombosed plaques in autopsy of patients with acute coronary syndromes (ACS)[28,29]. In situations of leukocyte activation, MPO is secreted from cytoplasmatic granules to phagolysosome and extracelular space with extensive impregnation at sites of plaque rupture. Buffon et al[30] documented the reduction of intracellular content of leucocitary MPO in patients with unstable angina submitted to coronary angiography in samples collected in coronary sinus and of femoral artery. The activation gradient was independent of location of the stenosis responsible for ischemia, introducing the concept of widespread leucocytary activation in coronary bed, systemically inflamed in ACS.

The link of MPO with activation of the proteases cascade occurs through the oxidative inactivation of proteases inhibitors [α1-antitrypsin, tissue inhibitors of metalloproteases (TIMs) and plasminogen activator inhibitor-1 (PAI-1)] and activation of latent proelastases and metalloproteinases (MMPs). MMPs affect the remodeling and stability of atherosclerotic plaque. Fu et al[31] demonstrate the generation of oxidative species of HOCl by myeloperoxidase activating the pro-matrilysin (MMP-7) capable of promoting degradation of extracelular matrix of the fibrous layer potentially serving as a mechanism for atherosclerotic plaque rupture.

In vitro study demonstrated that incubation of endothelial cells with low doses of MPO or macrophages expressing MPO result in increase of expression and activity of the tissue factor, favoring thrombogenicity. HOCl, derived of MPO, can induce death of endothelial cells and desquamation by either apoptotic or oncotic cell-death pathways, promotion activation and platelets aggregation[32].

The ability of MPO to reduce the bioavailability of NO changes the endothelial surface, usually antithrombotic, thrombogenic by expression of several prothrombotic and antifibrinolytic factors.

Mieloperoxidase and ventricular remodeling

Leukocyte migration to peri-necrotic zones and reperfusion of an occluded artery exposes the ischemic territory to further inflammatory and oxidative stresses. It is possible that the increase in MPO activity that accompanies models of ischemia reperfusion injury contributes to the resulting tissue damage and infarct extension.

MPO can contribute to myocardial dysfunction and adverse ventricular remodeling after infarction through several potentials mechanisms. MPO generates several oxidants reagents and cytotoxic species, including aldehydes, that can modify covalently critical residues in key ionic channels or transporters contributing to damage of the contractile function after ischemic episodes. The ischemic phenomena and myocardial reperfusion stimulate the recruitment of polymorphonuclear cells (PMNs) and the associated microvascular dysfunction has also been attributed to the decrease of the bioavailability of vascular NO. The predominant mechanism is through the fast reaction with superoxide derived from NADPH oxidase of recruited and activated PMNs neutrophils in inflamed tissues [33].

Besides, MPO can affect post-infarction ventricular remodeling by activation of the protease. The inactivation of the PAI-1 by oxidation catalyzed by MPO, results in increase of the plasmin activity, that accelerates the degradation of the matrix, a requirement for the thinning of the ventricular wall and dilation of the chamber. Askari et al[34] studying MPO knockout mice (MPO -/-) after myocardial infarction demonstrated reduction of leukocyte infiltration and left ventricular dilation associated with preservation of the systolic function.

Vasilyev et al[35] demonstrated that there is significant increase in the generation of aldehydes in post-infarction tissues of wild rat in relation to MPO - / -, clearly indicating the role of MPO in the formation of these species. The study demonstrates that aldehydes derived from oxidation of common amine acids, catalyzed by MPO, represent a fast and relevant mechanism for the generation of cytotoxic species in inflammatory sites.

Myeloperoxidase: polymorphisms and mutations

Although previously described as a rare genetic disorder, the hereditary deficiency of MPO is relatively common in the United States and Europe, with prevalence of 1:2000 to 1: 5000 individuals, and less frequent in Japan, 1:55000[9].

A variety of mutations that result in MPO deficiency have

been related. These affect the biosyntese of MPO, impeding that the enzyme be processed until the mature phase or present low peroxidase activity[9]. Cross- study with 92 individuals with myeloperoxidase deficiency demonstrated significant reduction of prevalence of cardiovascular disease[36].

Some polymorphisms are described for the gene of MPO, including a located functional polymorphism in the promoter area of the gene that affects its transcription[37].

The polymorphism - 463 G/A, that consists of the substitution of G for A in the position 463pb, leads to reduction of MPO expression in the genotype AA, intermediate levels in GA and larger amount of intracellular MPO in the genotype GG. Nikpoor et al[38] demonstrated in case-control study the association between polymorphism of the MPO gene and CAD. Comparing 229 patients with coronary disease and 217 controls, they found protecting effect of the allele A, and individuals with two copies of the allele A presented lower probability of CAD when compared with individuals AG or GG.

In a cohort study was evaluated the risk of cardiovascular events associated to the genotype GG. 139 patients with coronary disease were studied, 89 (64%) GG, 45 (32%) GA and 5 (4%) AA, followed for 45±19 months. Patients with genotype GG presented higher rate of events (death, infarction, hospitalization for unstable angina) than the group GA/AA, 19 vs. 4%, p = 0.02[39].

Myeloperoxidase in atherosclerotic plaque and in "culprit lesions"

Naruko et al[29] analyzed the neutrophils in 126 segments of coronary arteries obtained in autopsies or during atherectomy procedures. The immunohistochemical analysis with different antibodies was used for neutrophil identification: anti-CD66b, elastase, myeloperoxidase mono and polyclonal and CD 11b. All ruptured or eroded plaques of patients with MI expressed neutrophils infiltration compared with rare detection in coronary lesions of patients with non-cardiac death. Similar observation were made in atherectomy specimens in 35 patients with stable angina (SA) and 32 with unstable angina (UA). In patients with UA the neutrophyle expression was documented only in 44% of the "culprit lesions" and with SA in 6%. Those observations suggest that the neutrophils infiltration is actively associated with acute coronary events.

Sugiyama et al[28] demonstrated in atherosclerotic plaques of patients with sudden death, larger expression of MPO in the rupture sites, in superficial erosions and in the lipid core while fatty streaks exhibited smaller expression. In addition, association of MPO expression in macrophages and HOCl was demonstrate by immunohistochemical techniques in culprit lesions.

Myeloperoxidase in stable coronary disease

Zhang et al[40] conducted a case-control study to determine the association between MPO levels and the prevalence of chronic artery disease in 158 patients submitted to cardiac catheterism with established CAD and 175 patients without angiographically significant CAD. In multivariate models, adjusted for traditional risk factors, risk score of Framingham and leukocyte count, MPO levels were significantly associated with the presence of DAC with RC 12 for the highest quartil of leucocitary MPO and 20 for the serum MPO.

However, the prognostic value of MPO for occurrence of events in stable patients is not still clear. In a cohort of 178 patients with stable angina, in following in specialized clinic of ischemic cardiopathy in Brazil, the prognostic value of myeloperoxidase levels was evaluated. During the average surveillance of 12±5 months, 26 (14%) of the patients presented a major cardiovascular event (death, infarction, revascularization, acute coronary or peripheric vascular syndrome), however there was no significant difference in MPO levels among the group with or without events[41].

Myeloperoxidase in patients with chest pain

Brennan et al[42] evaluated the value of serum MPO levels as risk predictor for cardiovascular events in 604 consecutive patients assisted in the emergency unit for chest pain of suspected cardiac origin. The measurement of MPO on admission, average time of 4 hours after the onset of the pain, was independent risk predictor for major coronary events in 30 days (infarction, need of revascularization or death); RC 2ºqtl 1,7 (1,1-2,8), 3ºqtl 3,2 (2,0-5,4) and 4ºqtl 4,7 (2,8-7,7) and in 6 months. In patients without necrosis evidence, defined by negative dosage of troponin in 16 hours of monitorization, the risk of revascularization and other major adverse cardiac events in 30 days and 6 months was also greater with the increase of the quartil of MPO. Using the cutoff point of 198 pM derived of the ROC curve, the myeloperoxidase addition to troponin, as screening test, improved the ability to identify patient with risk of events in 30 days of 58% for 84,5% (p <0,001). That was the first study of the literature that demonstrated the usefulness of MPO dosage in patient selection in the emergency for risk stratification for cardiovascular events. Even in patients where infarction was excluded, based on the troponin measure, the increase of MPO levels in the presentation was predictor of events. Another advantage is that while the circulating levels of troponin increase in 3 to 6 hours after myocardial injury, the MPO levels were significantly high in the presentation (even in 2 hours from the onset of the symptoms) in patients with initially negative troponin. Those findings suggest that MPO is a marker of ACS, preceding the necrosis, as such, a predictor of vulnerable plaque.

Myeloperoxidase in acute coronary syndrome

Investigators of the CAPTURE study, that included 1090 patients with ACS and recurrent angina underwent percutaneous coronary intervention, evaluated the prognostic value of MPO levels. MPO levels correlate with traditional risk factors, but they did not correlate with troponin T, CD 40 soluble ligand, C reactive protein (CRP) or electrocardiographic alterations. However, patients with elevated MPO levels, 31,3% of the sample, experienced an increased risk for death or infarction in 72 hours up to 6 months, RR 2.25 (1,32-3,8). Particularly, MPO levels of >350μg/l identified a subgroup of patients with increased risk for events even with normal

troponin (15.9% vs 2%, p=0.001)[43].

In patients with ACS, levels of MPO predict increase risk for subsequent cardiovascular events and they extend the prognostic information of other traditional biomarkers. With troponin, MPO identified 95% of all of the adverse events of the CAPTURE study. Risk stratification in patients with ACS remains a major objective for the selection of medical and interventional treatment regimens. Troponin are the most established prognostic marker to predict events and to identify patient with greater benefit from aggressive strategies. As it reflects myocardial necrosis, efforts have been established to identify patient in risk during earlier stages of the disease. Myeloperoxidase signals and identifies the state of acute inflammation of the coronary circulation by the increase of neutrophil activation that precedes the myocardial injury.

In our environment, the prognostic value of myeloperoxidase measurement was evaluated in a group of 130 patients with acute coronary syndrome (ACS). High levels of MPO at hospital admission confirmed risk increase of 3.8 times for incidence of cardiovascular events in-hospital (death, recurrent angina, heart failure and severe arrhythmia), independent of other appraised predictors as age, dyslipidemia, ischemic alterations in electrocardiogram, troponin and CRP. These findings reproduce the estimated risk by the CAPTURE study with similar magnitude in a unselected sample of patients with ACS[44].

Cavusoglu et al[45] appraised the long term prognostic value of MPO levels in 193 men with ACS and they demonstrated that the basal measurement of MPO was an independent predictor of infarction in 24 months.

Biasucci et al[46] conducted study to evaluate the prevalence and time course of neutrophil activation in the course of ACS and temporal relation between the episodes of recurring ischemia and the neutrophil activation. They evaluated the index of intracellular myeloperoxidase that quantifies the average activity of MPO in the whole neutrophil population. In normal individuals the index is close to zero. Positive values appear when the neutrophils are rich in MPO and negative values when there is depletion of the enzyme, that typically occurs after neutrophil activation. The intracellular index was significantly reduced in patients with unstable angina and myocardial infarction compared with patients with chronic stable angina and normal individuals. During coronary care unit admission, with electrocardiographic monitorization, there was no changes in the index of MPO before or after the ischemic episodes compared with basal values, nor correlation with time or length of the ischemia.

Gach et al[47] evaluated the activation of PMN in small sample of patients with unstable angina undergoing cardiac catheterization. Significant increase of MPO levels was documented in serial measures in the first 24 hours after stenting in relation to the group where just diagnostic catheterization was performed.

Khan et al[48] studied 384 patients post ST segment elevation myocardial infarction to determine the prognostic value of MPO levels and NT-BNP (N-terminal peptide natriuretic type pro-B). The average value of the measurements during 5 days of the onset of chest pain was used. The MPO levels were significantly higher in patients that presented the primary end point (death or readmission with infarction) compared with survivors without recurrent infarction. Using model of logistic regression, the combination of MPO and NT-BNP markers improved the accuracy to 76%, exceeding results of any peptide separately.

The long-term prognostic of MPO and markers of proteic oxidation was also evaluated by Mocatta et al[49] in 512 patients admitted with acute myocardial infarction. MPO and carbonil protein were higher in patients with infarction in 24-96 hour after admission than in controls. However, just the MPO levels but not the carbonil protein were independent predictors of mortality in 5 years's surveillance period. Patient with MPO levels above the average in combination with high levels of NT-proBNP and reduced ejection fraction presented significantly lesser survival. High MPO, therefore, added prognostical information for mortality in long term,when used with established markers as NT-proBNP and ventricular function.

Myeloperoxidase and heart failure

Tang et al[50] in cross-sectional study evaluated the MPO levels in 102 patients with diagnosis of heart failure (ejection fraction <50%) and 105 healthy controls. MPO levels were significantly higher in patients with chronic systolic heart failure (HF) (1,158 vs 204 pM, p< 0.0001). MPO levels increased with progression of functional class of New York Heart Association (NYHA) and they were correlated with BNP levels. This strong association of MPO levels with the prevalence of HF was independent of other factors as age and levels of BNP, OR 27(95%CI: 3.6-371).

In a prospective population study of screening HF in the community, 1,360 subjects were appraised through dosage of multiple markers. The measurement of MPO and CRP, additionally to BNP demonstrated the best specificity, 94.3%, for the diagnosis of ventricular systolic dysfunction[51].

Recently, investigators of EPIC (European Prospective Investigation into Cancer and Nutrition) published a case-control study including 3,375 healthy individuals of the population cohort of Norfolk - UK, demonstrating that high levels of MPO are associated with increase of future risk of cardiovascular events, independent of traditional risk factors[52].

Myeloperoxidase and therapeutics

The participation of MPO with its pro-atherogenic activities in several stages of the cardiovascular disease has been stimulating interest in development of specific anti-MPO therapeutics[12]. A great difficulty in the development of inhibitors is the concern that the medication may impairment the innate immune defense.

The statins have been shown to reduce levels of oxidants derived from MPO and NO, mainly nitrotyrosine, independent of the effects of lipid reduction, suggesting anti-inflammatory and antioxidant properties that should be included in the pleiothropic effects attributed to these drugs[53,54]. Zhou et al[55] evaluated the effect of atorvastatin in the levels of MPO and CRP in 78 patients with ACS. The patients were randomized

for conventional treatment and 10mg/day of atorvastatin or treatment without cholesterol-lowering drugs. Measurements demonstrated the additional reduction of MPO (16% vs. 8%, p=0.01) after a week of treatment.

Baldus et al[56] evaluated the effect of heparin administration during cardiac catheterization in 109 patients, demonstrating increase in plasmatic MPO levels induced by heparin that was correlated with improvement of the endothelial function. MPO binding to the vessel wall is a prerequisite for MPO –dependent oxidation of endothelium-derived nitric oxide and impairment of endothelial function. This way, the mobilization of MPO from vascular compartments can represent a mechanism by which the heparin exercises anti-inflammatory effects and increases the bioavailability of vascular NO.

Recommendations of changes in lifestyle, including diet and exercises, have demonstrated benefit in the prevention and treatment of the coronary artery disease. Roberts et al[57] evaluated 31 obese men submitted to intensive modification of lifestyle, (diet rich in fibers, low fat diet and daily aerobic exercises) for three weeks, analyzing inflammatory and oxidative stress markers and endothelial function. After short intervention period, there was significant reduction of MPO levels (166 vs. 133 ng/mL, p <0.05) and of other biomarkers.

Technical considerations in myeloperoxidase measurements

Progress in the understanding of atherosclerosis physiopathology and of acute ischemic syndromes have been leading to an explosion in the development of assays of serum biomarkers, however, there is no consensus for the effectiveness of its use in clinical practice. When the use of a new marker is evaluated it is necessary to consider certain specifications, that should include validation of the analytical inaccuracy and detection limits, characterization of the calibrator, specificity of the assay and standardization, pre-analytical characteristics and appropriate studies of reference intervals[6,7,58].

Besides the blood dosage by ELISA method, the MPO content can be measured in the neutrophils as a degranulation index by flow cytometry in some hematologic analyzers[6,59].

The measurement of MPO still needs standardization. Studies evaluating its usefulness as risk or prognostic predictor, used assays with ELISA technique, still not available commercially (Oxis Health Products, Calbiochem and PrognostiX). There is no consensus as for the unit of measure to be used to express the results, determination of population reference values and probable pre-analytical factors that could interfere in its dosage, as the type of sample used (serum or plasma), the anticoagulant type and the stability of the analyte. Chang et al[60] found that the length of time keeping the blood in room temperature before centrifugation greatly affect the MPO content in the plasma. When the sample was placed on ice after collection, even with the centrifugation occuring in room temperature, there was no increase in the concentration of MPO. While in samples maintained in room temperature, apparently, MPO continued to release from the leukocytes into blood until centrifugation, what also explains higher values in the serum in relation to the plasma collected with heparin, because the sample stand at room temperature for longer period to coagulate.

Conclusions and future perspectives

By current knowledge, data suggest that MPO can act as much as marker of cardiovascular disease promoting independent information in the diagnosis and prognostic of patient, as also potential cause of the progression and instability of atherosclerotic lesions at time of acute ischemia.

There are future perspectives for evaluation of its usefulness to guide the actual cardiovascular therapeutic decision, besides the development of specific treatments aiming at the removal of MPO of the endothelial and subendothelial spaces, preventing the pro-inflammatory actions of MPO in blood vessel wall or by reduction of H_2O_2 sources, avoiding or reducing the MPO-dependent depletion of vascular ON levels.

Additional studies are necessary, mainly related to the standardization of the assay technique, collection and storage method and to the knowledge of eventual expected interferences. The uniformization of the units of measure and populational reference values should facilitate the clinical evaluation, and, in the future, the development of commercially available assays, standardized and automated, capable to increase agility and reduce the analytical imprecision.

Also, an inflammatory marker for evaluation of cardiovascular risk must to have, among other characteristics: independence of the established risk factors, ability to improve the risk prediction beyond the traditional risk factors, acceptable costs of the assay and generalization of results to several populational groups.

The additional predictive value of MPO levels in the stratification of cardiovascular risk to incorporate it to the clinical practice as marker of plaque vulnerability is still not clear. Additional studies are necessary to confirm its diagnostic and prognostic ability in the different forms of presentation of ischemic cardiopaty, besides the standardization of the assay that is still fundamental issue for the transition of this marker from the research context to use in the clinical routine.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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