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Myeloperoxidase (MPO) – possible diagnosis biomarker and risk stratification in myocardial inotropism deficit induced by chronic ischemic heart disease

Gabi Valeriu DINCA[1], Adriana DIACONU[2,3], Bogdan-Ioan COCULESCU[4,5], Gheorghe MANOLE[6,7], Horatiu VULTUR[2], Elena Claudia COCULESCU[8]

[1]Faculty of Medicine, "Titu Maiorescu" University, Bucharest, Romania
[2]Faculty of Medicine, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania
[3]Fundeni Clinical Institute, Bucharest, Romania
[4]Faculty of Midwifery and Nursing, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania
[5]Cantacuzino National Medico-Military Institute for Research and Development, Bucharest, Romania
[6]Romanian Academy of Medical Sciences, Bucharest, Romania
[7]Faculty of General Nursing, Bioterra University, Bucharest, Romania
[8]Faculty of Dental Medicine, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania

Abstract

The myeloperoxidase levels of 208 patients with ischemic heart disease hospitalized for congestive heart failure were tested at admission, and discharge after 14 days. The calculations for the serum average concentration/lot/date, were made, subsequent, using the values of the tested parameters regarding MPO (admission and discharge) the mean concentration/lot was made, regardless of time of determination. The calculated average values analyses of the three MPO serum parameters were constantly elevated and permanently matched the myocardial contractility deficiency class. The integrated data of the study, allows us to propose the MPO's circulating concentration as a diagnosis and risk assessment biomarker in the etiology of myocardial ischemia for chronic cardiac failure.

Keywords: myeloperoxidase (MPO), chronic ischemic cardiomyopathy, inotropism deficiency, oxidative stress

INTRODUCTION

In the diseases incidence hierarchy classification, cardiomyopathies represent a major cause regarding morbidity and mortality.

Atherosclerosis as a main etiology of the category, has his onset with puberty, progressing through age and through endothelial dysfunction generated at coronary level, becoming responsible for at least 90% of myocardial pathologies, regularly with high risk. Such a marked evolution recognizes as pathogenic mechanisms at the least the following:

- local oxidative stress;
- locally induced inflammation;
- immune involvement.

The coordinated and intricate action of the above mentioned at coronary level, leads subsequently to myocardial remodeling, through myocardial syncytial ma-

Corresponding authors:
Bogdan-Ioan Coculescu
E-mail: bogdancoculescu@yahoo.fr

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trix's fibrosis exacerbation, but also through induced apoptosis. Functionally, the myocardial normo-structure destruction manifests itself through different clinical forms, among which we can find the signs and symptoms that define an inotropism deficiency.

Myocardial remodeling is defined as a cardio-vascular myopathy progression predictive process, consisting in myocardial geometry and structure modifications, further developing fibrous tissue, highlighting the diminished myocardial contractility [1].

Systolic flow reduction in heart failure, leads to all organs flow decrease, including the myocardium. This implies an O_2 reduction in cardiomyocytes, potentially accelerated by tachycardia as a compensation mechanism during heart failure, but which, decreases the coronary flow through diastole reduction [2].

The myocardial metabolism conduction during hypoxia, leads to oxidative stress increase, which successively induces:

- cell infiltration of the phagocyte mononuclear system;
- triggering their metamorphosis;
- matrix proteins proteolysis, secondary inflammation defining processes [3,4].

In the cellular cytotoxicity, therefore in the myocardial fiber, the role of MPO is unanimously accepted, the hydrogen peroxide turning into hypochlorous acid under the enzyme role; a powerful cytotoxic oxidant [5].

$$H_2O_2 + Cl^- + H + HOCl + H_2O$$

Existing macrophages at myocardial syncytial level, as in atherosclerotic coronary lesions, have co-localized, both MPO and isozymes of the metalloproteinase matrix (MMPs) family, such as:

- matrilysin (MMP-7);
- collagenase-1 (MMP-1);
- collagenase-2 (MMP-8);
- gelatinase-B (MMP-9) etc.

Physiologically, the 20 family members of MMPs along with MPO, are in inactive state, but all activated by hypochlorous acid, through cysteine's thiol radical covalent modification of this enzymes structure (cysteine switching domain) [6,7].

From the above mentioned isozymes, during physiological and pathological processes, a substantial role for tissue allostasis is attributed to collagen destructive enzyme MMP-1 type 1, as a major protean structural tissue component barrier during diapedesis and cell migration, including in myocardial pathologies [7,8].

MMP activity modulation achievement is made through regulating transcription and TIMPs binding. MMP/TIMP 1:1 binding, inhibits MMP enzymes action, controlling the proteolytic activity from the extracellular matrix level [9,10]. Through activation, MMP develops proteolytic action that on one hand becomes responsible for tissue continuity disruption, and on the other hand, as a result of tissue destruction, it generates new products, as:

- collagens;
- laminin;
- fibronectin;
- vitronectin;
- tenectin;
- elastin;
- proteoglycans [6].

MATERIALS AND METHODS

The myeloperoxidase serum level is unanimously accepted as a predictive biological marker during coronary atherosclerosis risk assessment, and for two of its major complications: myocardial infarction and death. Starting from the MPO's developed cytotoxic action and lack of more published studies, the medical literature is still reluctant to include the myeloperoxidase serum concentration, also, as a marker of myocardial inotropism deficiency; we appreciate that research on such an objective is up to date.

The undertaken study, following the above mentioned objective, included 208 patients with heart failure, developed over chronic ischemic cardiomyopathy of atherosclerotic cause.

a. Inclusion criteria for the study group. The contractile myocardial deficit assessment for this group was based on the described symptoms, clinical exam, EKG and especially on the cardiac ultrasound results due to the accurate appreciation of the ventricular systolic function, which through the presented values largely for the left ventricle offers valuable and precise information over the outcome of ischemic cardiac disease for patients. In conditions of myocardial mal-irrigation, highlighting not only the value of the systolic flow, but also the existence of local transient hypokinesis or segmental akinesia of the left ventricular walls, the cardiac ultrasound provides indirect data of the local dynamics of the regional myocardial ischemia (exacerbation or resolution).

Cardiac contractile functional decompensation classes were those of NYHA classification, the patients included in the study being placed in the IInd-IVth classes (Table 1).

TABLE 1. *Chronic heart failure classification (NYHA criteria)*

Inotropic deficit class	The defining elements of the class
I	Asymptomatic patient at rest, as well as at exercise
II	Asymptomatic patient at rest, with dyspnea at high and moderate exertion; or prolonged
III	Asymptomatic patient at rest, with dyspnea at slight exertion, regardless of their duration
IV	Sick with symptoms and at rest

The cardiac failure can take two clinical forms depending on the value of the systolic flow, as preserved ejection fraction and reduced ejection fraction; in the study only the patients with reduced ejection fraction were included [11-15].

b. Exclusion criteria. Patients which did not consent with the study or agreed with the reevaluation with the necessary investigation tests in 14 days.

Patients receiving nitro derivative antianginal medication, knowing that by metabolism it generates radicals from the reactive species categories of nitrogen. Patients with a history of myocardial infarction were excluded from the group.

Patients diagnosed with ICC which received, prior to the diagnosis of ICC, statins, immunoglobulins or phosphodiesterase inhibitors. Voluntary exclusion is justified by the following medication actions:

1. for hypolipemic agents:
- development of pleiotropic effects, by improving the endothelial function, as a result of cNOS gene transcription induction [11];
- decrease of ROS production in the arterial wall;
- PCR serum values drop regarding hypercholesterolemia associated disease;
- reduction, most likely, at the macrophage level of TNFα, IL-1 and IL-6 production [12-14].

2. Exclusion from the study group of the patients who had received phosphodiesterase inhibitors during the last 3 months was imposed because of the cytokines production inhibition, especially of IL-6, IL-1β and TNFα.

3. ICC cases that received within the last 3 months immunoglobulin i.v.; the drug interferes with the inflammatory balance, regardless the disease, thereby for ICC also [13].

The present study appoints as a prospective one following ethical principles derived from the Declaration of Helsinki (Committee on Human Investigation), stipulated in the international and national regulations in force. Likewise the norms on "human research" issued by the ethics commissions of each hospital that provided us with patients willing to participate in the study.

Prior to inclusion in the study, the patients signed the informed consent regarding the use of the clinical data, the acceptance of the set of investigations necessary for the study and the processing of the results of the paraclinical and/or serum determinations for the purpose of the research, in full knowledge of the case.

Patients associating chronic inflammatory pathologies as lupus erythematosus, rheumatoid arthritis, ankylopoietic spondylitis, infectious endocarditis, psoriasis etc., could determine false PCR results and fibrinogen high level.

Patients exceeding more than 10% the ideal body weight taking into consideration the obesity – dyslipidemia correlation. The 1989 WHO selection report of the National Institute of Health (NIH) experts was respected applying the formula:

BMI = body weight expressed in Kg/height^2 = Kg/m^2

The patients were hospitalized for 2 weeks undergoing the determination of the selected serum levels as follows:
- serum concentration on the first day of admission;
- serum concentration on the last day of admission (14th);

Based on the obtained results, the average serum concentration was calculated;

For both testing were used two samples each as a measure of results accuracy.

Other possible criteria for participating until the end of the study are commented in the discussion section depending on the laboratory results and multiple investigations for establishing the positive, etiological and physiological diagnosis.

c. Method of determination. For quantifying the MPO, the immunoenzymatic fluorescence method (FEIA) was used. The method permits the anti-MPO antibodies detection, which are involved particularly in the vascular inflammatory pathogenesis, on the basis of their role as co-participants responsible for vascular dysfunction by atherosclerosis [4,16,17].

RESULTS AND DISCUSSIONS

The average serum concentration/lot and time of determination was calculated. Thereby, we have constituted two new parameters regarding the current level of MPO, whose values are shown in Table 2.

Subsequently, all this values allowed us to calculate the mean serum concentration with the standard deviation applied for whole the patients (Table 2).

TABLE 2. *The values of the average concentration of the MPO, depending on the moment of determinations*

The name/ unit of measurement		Normal values of biomarkers	The values determined in the cases of the component group (n = 208)		
			Average range of variation		
			for the whole group	upon admission	after discharge
MPO	IU/L	200-260	351,70 ± 24,75	383 ± 14,25	319 ± 23,85

Reading the results determined at admission and discharge from the hospital as per the abovementioned methods, we wanted to underline the following:
- The correlation degree of the serum level values with the pathology, highlighting the specificity;
- The correlation degree between the MPO's serum concentration variations and the clinical/

pathophysiological evolution stage of the cardiac failure, following the NYHA evaluation.

As a pathology whose incidence is increasing and whose prognosis leads relatively slowly, but surely to death, chronic heart failure has generated the channeling of researchers not only for the elucidation of the pathophysiological mechanisms by which the deficiency of inotropism occurs, but also for other objectives of study, between which those of identification the serum markers, able to identify as early as possible the progressive diminution of contractility of the myocardium or to evaluate the risk during the evolution of the disease, can be mentioned [18-25].

The comparison of the MPO's serum concentration mean value regardless the time of determination for the entire group, shows significantly higher levels in relation to normal circulating values (Table 2).

MPO's serum concentration mean value/lot was 13,5% higher than normal, the same as in the ratio calculation:

- mean serum concentration of MPO/patients on admission divided over the normal circulating MPO value = 14.7%.

As well as in:

- mean serum concentration of MPO/patients on discharge divided over the normal circulating MPO value = 12,3%

The values by which all the MPO's concentrations exceed the normal values approves the high statistical importance, which highlights the myocardial contractile failure due to this circulating enzyme level as a diagnosis marker

- in support of this idea we mention the existence of a high incidence of the mean enzyme serum concentration; 76,4% of patients.

Our findings mirror the results of the few existent international studies which admit that in heart failure the circulating level of MPO is increased and correlated with the ejection fraction reduction degree or the plasma concentration of natriuretic peptide B.

Following the mean serum concentration with the myocardial contractile deficit we observed that by the decreasing of the systolic volume, the circulating enzyme level still remains over the normal values. We are debating the following hypothesis:

- the result may be the expression of the therapeutic measures;
- the myocardial syntactic or interstitial process, especially the inflammatory one allows by decreasing its level, the development of the cardiomyocyte apoptosis-inducing process with the effect of myocardial contractile mass reduction.

CONCLUSIONS

The analysis of the constantly increased serum mean values of the MPO, regardless the determination time, but also as an average/study group shows a true correlation with the pathology but also with the pathophysiological evolutionary stages of the disease, manifesting itself as a specificity between the variation levels and the myocardial inotropism deficit class.

By this, we appreciate that broader population studies, could admit that the MPO's serum value concentration has a permanent practical utility as a diagnosis tool and prognostic determination.

One of the limits of the undertaken study in order to have a better interpretation of the analyzed values of the MPO would have been the comparative analysis of the same tests but only in patients with chronic ischemic heart disease, as a witness group.

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