Serum Concentration of hsCRP - Possible Marker for Therapy Evaluation in Left Ventricular Dysfunction with Preserved Ejection Fraction

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The study on a subset of 208 patients with diastolic dysfunction with the ejection fraction preserved discusses the possibility of circulating CRP levels to be accepted as a diagnostic marker. In order to carry out a comparative analysis of the significance of hsCRP serum values, the study also included a subgroup labeled as control, consisting of the same number of patients, but diagnosed only with painful chronic ischemic cardiopathy - a stable form of angina pectoris. The conclusion of the study is that the circulating level of hs-CRP can be accepted as a diagnostic marker for both ischemic cardiopathy and diastolic dysfunction with preserved ejection fraction. The result, corroborated with data from other studies that recognize the serum concentration of hs-CRP as a predictor marker in cardiovascular disease, supports its usefulness as a marker for cardiac insufficiency with a history of ischemic cardiopathy, without being a marker for assessing the degree of myocardial contractility deficiency.

Keywords: biomarker, hs-CRP (high-sensitive CRP), cardiac insufficiency with preserved ejection fraction (CI with preserved EF), chronic ischemic heart disease

Atherosclerosis, as the main cause of cardiovascular disease that occurs as a complication (of it), has inflammation as a turning pathogenic mechanism because the process participates in all stages of the degenerative disease development, from initiating the lesion to the progression and destabilization of the plaque [1-4]. Worldwide, based on this pathogenic role in the development of cardiovascular disease, one of the research goals of the past decades has been to evaluate certain factors labeled as inflammatory in the prediction of cardiovascular risk (table 1) [4-8].

Among the markers mentioned in the table below, in the form of coronary atherosclerosis, CRP is considered to be the coronary inflammatory syndrome marker with the highest predictive value of risk of myocardial infarction and/or sudden death [5,6].

At the level of the myocardium subjected to hypoxia/ischemia, the contractility deficit is unanimously attributed to the remodeling process, whose mechanisms are still insufficiently recognized but involve the role of inflammation and changes in the interstitial matrix [4,7].

Experimental part

Material and method

After the introduction of biomarkers in 1989 as a means of diagnosing and evaluating prognosis in cardiac insufficiency, although a huge number of serum compounds (cytokines, chemokines or even hormones) were studied to be used as markers, only the circulating natriuretic peptides were admitted as specifically expressing the existing myocardial contractile deficit.

Hs-CRP, although accepted by the European Cardiology Guide as an overall survival indicator biomarker in cardiovascular disease, is not (also) a risk indicator for cardiac insufficiency (table 1). This fact motivated the investigation of the practical utility of hs-CRP serum determination in cardiac insufficiency, having chronic ischemic cardiopathy as its etiology and the preservation of the ejection fraction as a feature.

In addition, to support such an objective for the study there also are:

- the ease and low cost of the hs-CRP determination technique;
- the certainty of intervention in inflammatory processes, including those of remodeling the myocardium that is under oxidative stress;
- the interest of day-to-day medical practice to benefit from parameters to ease its assessment of the intensity of the inflammatory process depending on the variation in plasma concentrations of various acute phase reactants, like the reactive C protein [9-15].

From the point of view of the usefulness of determining serum hs-CRP for medical practice, the study can also be justified by the need to identify biomarkers that allow, on the one hand, early evaluation of cardiac insufficiency, and

Marker utility	Name of identified marker
predictive markers for cardiovascular disease	- CRP, IL-18
markers of atheromatous plaque instability	- high plasma levels: IL-6, IL-7, IL-12, IL-1, IL-18; - low plasma levels: IL-10:
independent markers of ischemic coronary disease	- IL-6, IL-8

Table 1
USE OF INFLAMMATORY MARKERS IN THE ASSESSMENT OF THE STRATIFICATION RISK AND THE PROGNOSIS OF CARDIOVASCULAR DISEASE

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Functional class	Total target subgroup patients		The average value of hsCRP
	no. abs	%	mg/L
II	100	48,08	7.3
III	108	51,92	7.9
Total	208	100	7.6

Table 2
THE DISTRIBUTION OF hs-CRP AVERAGE SERUM CONCENTRATIONS VALUES TO THE TARGET SUBGROUP'S COMPONENT CASUISTRY, DEPENDING ON THE CARDIAC INSUFFICIENCY CLASS (NYHA CLASSIFICATION)

on the other, the optimization of treatment in order to delay the appearance of the irreducible form, which has multiple consequences, such as significant deterioration of the quality of life, high medical and social costs etc. [3,16-18].

a. Casuistry of the study:

The batch was composed of 416 patients, of whom:

- 208 (control subgroup) with the main affliction being chronic painful ischemic cardiopathy clinically manifested as a stable angina pectoris. Anamnestically, during the general clinical and echocardiographic examination, the patients did not have symptoms/signs of decompensation of myocardial contractility;
- the remaining 208 patients (target subgroup) were diagnosed as suffering from congestive cardiac insufficiency, grades II-III (NYHA classification) with an etiology of chronic cardiac ischemia (table 2).

Only patients who, echocardiographically, presented a preserved volume of systolic blood flow were allowed in this subgroup.

The distribution in the two subgroups, according to the gender and age of the patients, was shown in figure 1a and 1b, respectively (fig. 1).

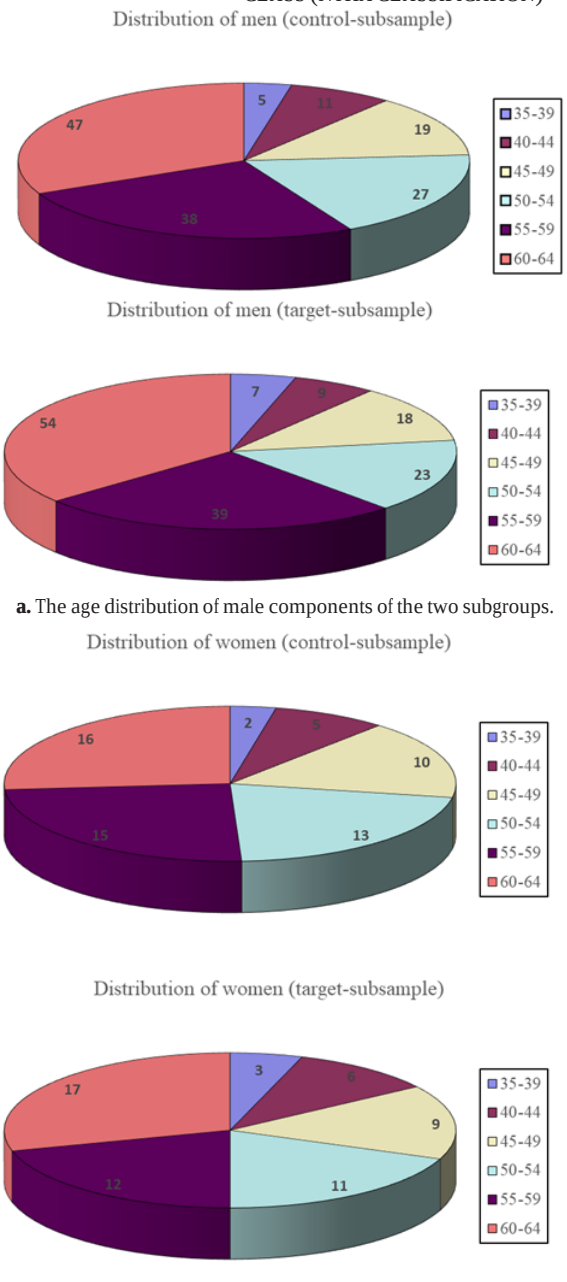
In order to minimize the influence of the age factor as much as possible, we observed the results of the studies that indicate that it corresponds to the 45-65 years range in the selection of the patients; at the level of the study group, the average age was 54, 55. Even though hospitalization provided the assurance that patients were *subjected* to a relatively identical controlled lifestyle (effort, diet), and identical treatment, the investigation performed was an outpatient investigation. This is because the serum level of hs-CRP is stable over long periods of time, has no circadian variation and is not influenced by food intake.

In the process of co-opting patients for the study, we took into account that the circulating level of hs-CRP may be reduced by the administration, during therapy, of various drugs such as statins, fibrates, niacin and thiazolidin-ediones. For this reason, patients who were receiving such medication were not co-opted in the group.

b. Method: The quantitative determination of the reactive C protein highly sensitive in serum was performed via the latex-immuno-turbidimetry method using the Enzyme Linked Immunosorbent Assay (ELISA). This is because the literature recommends this method as being the most appropriate to estimate risk/progression in vascular diseases as it has an analytical sensitivity around the value of 0.1 mg/L [19].

As normal serum hs-CRP values, levels of 0.5-0.6 mg/dL were accepted, and the deviation limits established on three gradual levels are similar to the reference data from the medical literature [6,20,21]. Thus, we allowed as decision intervals the areas recommended by CDC/AHA serving as a risk marker in cardiovascular disease:

hs-CRP = 0.6-1 mg/L, with the average field value of: 0.8 mg/L), low risk;



a. The age distribution of male components of the two subgroups.

b. The age distribution of female components of the two subgroups

Fig. 1. Composition of the studied subgroups according to the gender and age of the patients

hs-CRP = 1.1-3 mg/L, moderate risk;
hs-CRP > 3.1 mg/L, high risk [6].

Results and discussions

a. The calculated average circulating value of hs-CRP for the casuistry of the entire group and of the two subgroups is shown in the table 3.

Unit name/unit of measurement and average normal biomarker reference value	Average calculated value for component cases of the		
	Whole group (n= 416)	Control subgroup (n=208)	Target subgroup (n=208)
< 0.6 mg/L	7.55mg/L	7.5 mg/L	7.6 mg/L

Table 3
THE VARIATION LIMITS OF hs-CRP SERUM VALUE IN THE CASES SELECTED FOR STUDY

b. Referring the calculated average value of hs-CRP for the two subgroups to the values that define the areas as risk markers in cardiovascular diseases recommended by the CDC/AHA, we identified:

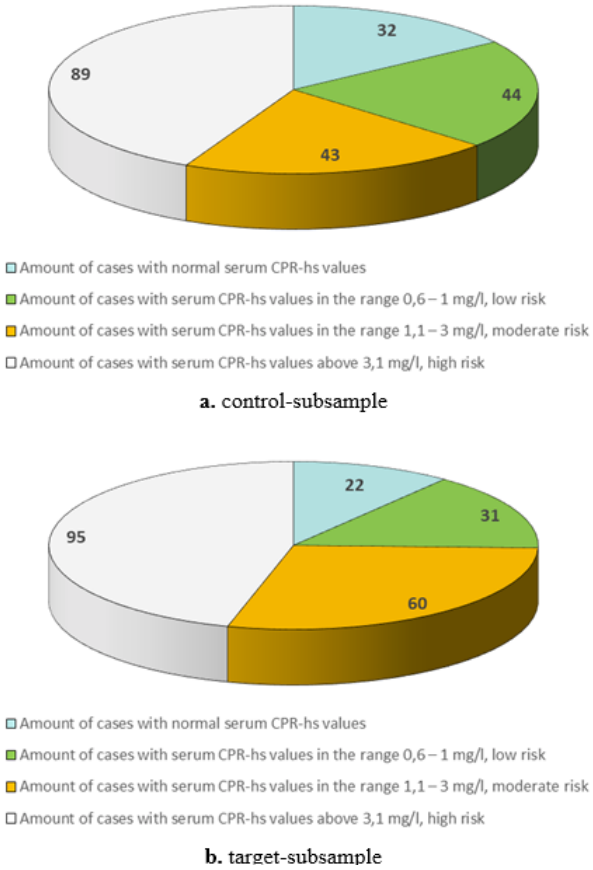


Fig. 2. The distribution of casuistry in the two subgroups in relation to the circulating level of hs-CRP structured on the gradual domains that define it as a risk marker in cardiovascular diseases recommended by the CDC/AHA.

c. By calculating the average serum circulating value of hs-CRP for the whole casuistry with diastolic ventricular dysfunction, depending on the contractility deficiency class, the incidence recorded in the following table was identified.

The pathogenic mechanism of interconditionality between coronary atherosclerotic lesions and inotropic deficiency is shown in the figure 3 [5,22].

During the evolution of chronic ischemia cardiopathy, the main pathogenic processes that belong to the histological type-where fibrosis and myocardial hypertrophy (myocardial remodeling) are present - are also associated with the disruption of those generating energy, responsible for the proper functioning of the ionic sarcolemale pumps (fig. 4).

Affecting the ventricular contractile property is evolutionary-gradual, allowing the presence of three evolutionary stages to be identified:

1. disruption of ion-trans-membrane migration;
2. acquiring the interest of the proto-diastolic relaxation process of the ventricular myocardium, with a double consequence:

- increasing the time of iso-volume relaxation, an active energy consuming process.
- reducing myocardial compliance.

3. increase of ventricular telediastolic pressure, secondary to the previous process, a pathogenic mechanism responsible for the occurrence of myocardial contractile deficit.

As motivational arguments for the selection of hs-CRP for the study we bring in, aside from those mentioned in the material and method subchapter, the double role developed by the highly sensitive isoform:

- to intervene in the immune mechanisms via the C_1q fraction of the complement;
- to contribute to the repair of the endothelium affected by oxidative stress [4, 5, 22-24].

Pathogenically, these hs-CRP are beneficial in the early development stages of arteriosclerosis, also at coronary

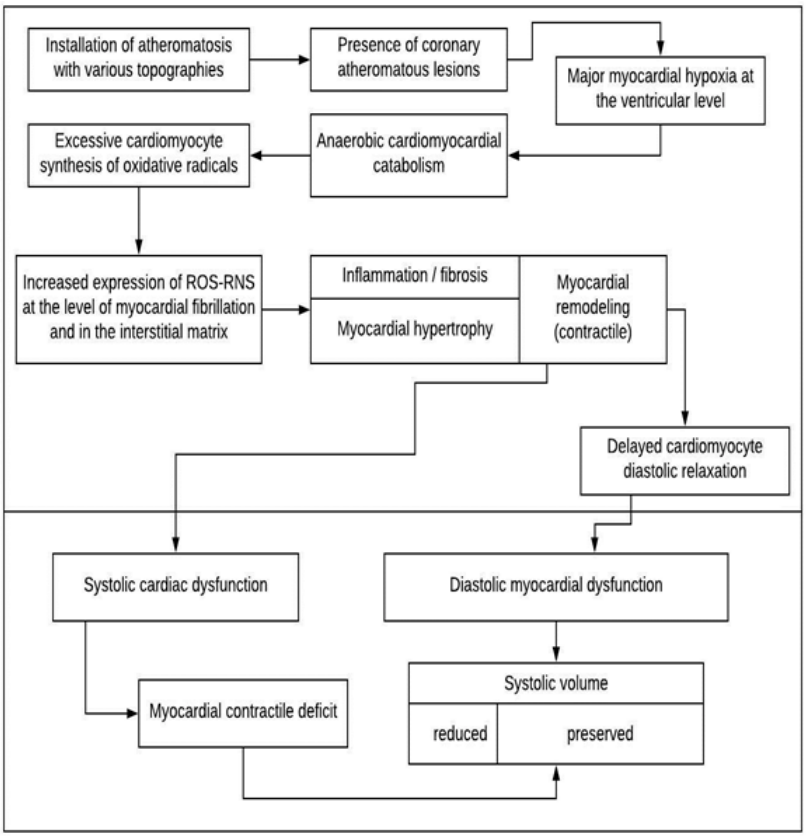


Fig. 3. Sequence of pathogenic mechanisms in cardiac insufficiency due to myocardial ischemia [16]

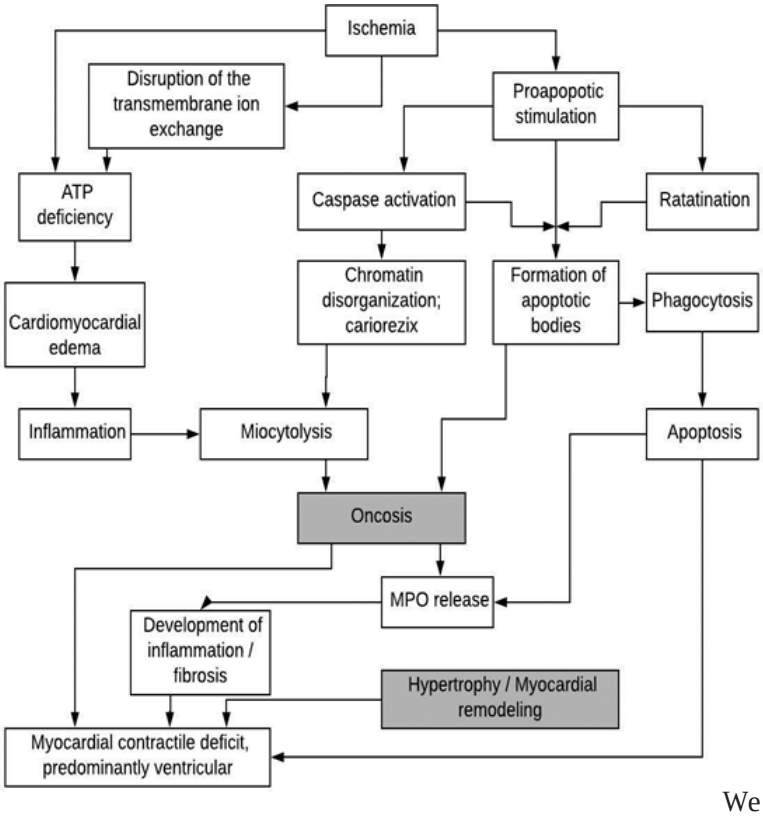


Fig. 4. The fundamental pathogenic processes responsible for the installation of myocardial contractile deficit [16].

level, as they influence coronary flow and thereby the properties of the myocardium, including inotropism.

Synthesized predominantly in the hepatocytes, but also in the atheromatous plaque, via induction by some cytokines such as IL-6 and TNF-α, hs-CRP, by reaching higher than normal serum values, transforms the marker, contributing mainly to the removal of LDL cholesterol in the atheromatous plaque, an effect which generates a slight benefit, but with major microembolar risk, also at the coronary branches level.

a. For the casuistry of the entire study group of 416 patients, the calculated average circulating value of hs-CRP was 7.55 mg/L, representing an increase of 12.6 times the normal serum baseline average, calculated on the basis of references accepted by the specialty literature as physiological. The augmentation rate brings as a statistical argument that the hs-CRP serum parameter is an indicator of the homeostasis imbalance existing at the myocardial level, whose common etiology is coronary atherosclerosis.

The aforementioned growth quota value is approximately identical when compared to the same reference average normal serum hs-CRP value of average values calculated for patients in the control subgroup or for those in the target subgroup (12.5 versus 12.6). The absence of a significant difference is explained by the fact that although the cases in the target subgroup have ventricular diastolic dysfunction they do not have a systolic volume deficiency, which allows maintaining the diastolic coronary flow to a level superposable to the one generated only by the presence of coronary atherosclerosis.

b. Comparing the maximum serum normal value of 0.6 mg/L with the average serum hs-CRP concentration in the selected patients:

- in the control subgroup there were statistically significant increases in 176 cases, i.e. in 84.6% of the cases with chronic ischemic cardiopathy.

- as for those of the target subgroup, there were increases in 186 cases, representing almost 89% of the subgroup (table/fig. 3).

We find that the incidence data of the calculated average serum concentration of hs-CRP increases for the two subgroups do not reveal any significant deviations. Just as with the previous finding, we explain this by the common etiological cause of the two categories of illness and the preservation of the ejection fraction.

c. In relation to the degree of cardiac inotropy deficiency (NYHA classification criteria), table/figure 4, data shows that: the calculated value of average serum concentration of hs-CRP was 7.3 mg/L in patients with Grade II myocardial diastolic dysfunction and the incidence was 48.08. For those in Grade III the incidence, although lower, 51.92%, was associated with a calculated value for the average concentration of hs-CRP of 7.9 mg/L. It is noted that by accentuating the myocardial contractility deficit the average serum concentration of hs-CRP increases with the value of the circulating normal, which can be evaluated as a risk indicator. Statistically however, the finding cannot be supported because the incidence of the increase quota is higher by only 3.8 percent compared to the casuistry of the control subgroup.

Comparing the mentioned values, calculated based on measurements with the maximum normal serum value of 0.6 mg/L with the one representing the average serum concentration of hs-CRP, and taking into consideration the contractility deficit class, it was found that:

- for patients with Grade II chronic diastolic dysfunction (NYHA classification), the achieved level was 12.5 times higher than normal;

- for the patients suffering from diastolic dysfunction manifested by symptomatology specific to Grade III myocardial contractile deficit (NYHA classification), the dosed value was close to those with Grade II myocardial contractile deficit, being 12.7 times higher than the normal maximum.

Despite the fact that only 186 of the 208 cases with cardiac dysfunction with preserved systolic blood flow (86.5%) had hs-CRP serum values above the normal upper limit, the average value calculated for the whole subgroup was in the risk areas recommended by the CDC/AHA to serve as a risk marker in cardiovascular disease [6]. In the

same way, if the circulating value of hs-CRP for the lower limit of the increased risk range is > 3.1 mg/L, the average value calculated for the whole target subgroup was 2 times over it, standing at 7.6 mg/L (fig. 2).

The data presented entitle us to support the following two deductions:

- cardiac dysfunction is consistently accompanied by a significant increase in the circulating concentration of hs-CRP, which is an argument for being accepted as a disease-specific marker;

- the grade of myocardial contractile dysfunction does not correlate with the circulating level of hs-CRP, an argument in favour of the fact that although the marker may be admitted as having disease specificity, it is not a revealing one regarding the evolutionary stage of the disease.

d. Comparing the average hs-CRP serum value calculated for the whole group with the average serum concentrations of each subgroup does not highlight statistically significant changes:

- the average hs-CRP serum value for the whole group/ average hs-CRP serum value for the control subgroup = 7.55 mg/L versus 7.5 mg/L;

- the average hs-CRP serum value for the whole group/ average hs-CRP serum value for the target subgroup = 7.55 mg/L versus 7.6 mg/L;

e. An identical situation is also obtained if we compare the average hs-CRP serum values calculated for the sub-groups:

average hs-CRP serum value for the control subgroup/ average hs-CRP serum value for the target subgroup = 7.5 mg/L versus 7.6 mg/L.

We interpret this fact by expressing the real situation of common status for the two diseases that recognize a common cause of redox homeostasis disturbance, the atherosclerotic one (for this study, with coronary localization). For the cases co-opted in the target subgroup, the preservation of the ejection fraction did not result in a reduction in the coronary flow distributed to the myocardium during diastole. On the basis of this type of reasoning, we extended the study investigating the same parameters in a subgroup consisting of patients with chronic heart failure with the same etiology but low systolic flow. Until the publication of the results of this latest study on the variation in hs-CRP serum concentration, we can report that reduction of the ejection fraction is a further source of coronary flow reduction, which stacks with that induced by coronary atherosclerosis. With this type of patients with myocardial contractile deficiency, as a result of the reduction of the systolic flow and the installation of the tachycardia, a reduction of the diastolic filling of the coronaries which accentuates myocardial hypoxia induced by coronary arteriosclerosis results as a compensatory mechanism. The consequence is the increase in oxidative stress, because the rest of the organs are also associated with the flow, due to the fact that their flow has been reduced.

Conclusions

The value of the circulating concentration of hs-CRP did not show statistically significant variations between the two categories of patients in the study (with chronic painful ischemic cardiopathy or left ventricular diastolic dysfunction), but for both illnesses, circulating levels are augmented, which, statistically is very significant. This is an argument that supports that the hs-CRP serum level may be recorded as a diagnostic marker for each of the two categories of disease, although the study data is tilted to give it specificity for chronic ischemic heart disease. In cases of cardiac diastolic dysfunction with preserved ejection fraction, the increase of the hs-CRP serum levels expresses in particular the myocardial infarction caused by ischemic cardiopathy, as the coronary flow variation is not present.

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Manuscript received: 28.08.2018