Growth Differential Factor 15 (GDF%15), Possible Biomarker in Heart Failure

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The present study aims at determining on the one hand the growth differentiation factor 15 significance as possible risk biomarker for this condition and, on the other hand, the degree of correlation between its serum concentration and the class of inotropism deficit.The value of the current research stems from the very selected theme, the activity of GDF-15, member of the superfamily of cytokines TGF-β recognized as having implication in atherosclerosis, but almost unexplored as role in the myocardiumremodeling processes, more precisely in fibrosis.

Keywords:cytokines receptor superfamily, myocardial oxidative stress,myocardium remodeling, myocardial ventricular contraction deficit, risk biomarker

Worldwideresearch regarding biomarkers in heartfailure is oriented towards the study of processes, respectively the parameters, which give information about the relationship with: inflammation, remodeling cardio-vascular remodeling, cellular signaling mediated by ion channels, such as those of calcium and/ orcytoskeletal receptors [1-3].

TGF%â member of the cytokines superfamily, induced myocardially by ischemia oroverexertion, TGF beta is activated in response to stress. In pathological conditions, it integrates information from different pathophysiological pathways. It is produced in cardiomyocytes, smooth muscle cells and endothelial cells [2].

Given the increased incidence of cardiovascular diseases, research in this field concerns are directed towards :

- their prevention and their possible complications after occurrence, complications which are often debilitating and socially costly, if not lead to patient's death;
- the identification of blood or laboratory parameters which allow for the possibility of assessing, in particular of the risk;
- to identify new ways and means of real effective therapy that can be defined as the new *therapeutic targets*.

Achieving these objectives requires the availability of certain means of risk identification/ evolving prognosis, such as markers, specific revealing parameters in order to label a biological or laboratory parameter as indicator of risk in a particular condition.

The concept of biomarker (biological marker) was introduced as a medical term in 1989, defined as follows: m*easurable and quantifiable biological parameter which serves as an indicator in assessing the risk of disease, exposure to the environment and its effects, diagnosis of disease, metabolic processes, substances abuse etc.* [1].

In 2001, the Bethesda working group for biomarkers standardized the concept, redefining it as t*he biomarker is a size/feature determined and assessed objectively as an indicator of normal biological processes or the pathological ones or of pharmacological responses to a therapeutic intervention* [1].

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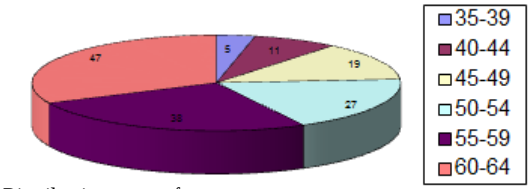
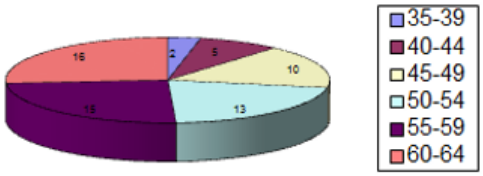
Experimental part

Material and method

The case studyincludes a total of 208 patients of both genders, hospitalized and diagnosed with heart failure having as a single etiology chronic ischemic heart disease, whose age ranged between 35 and 64 (fig. 1).



Distribution no. of men



Distribution no. of women

Fig. 1. Constituency of the study batch by gender and age of patients

Selective recruitment into the study group of the patients having as cause of the myocardial contractile deficit only theischemic cardiomyopathy was imposed in order to put the whole patients batch in the same etiopathogenic conditions, and in order to avoid having different initial sources are producing radical oxidants. Thepo-orchronicirrigation withfunctional contractile compensatedmyocardium, thepredominant source of producingROS (oxygenfree radicals) / RNS (nitrogen free radicals)iscoronaryatherosclerosis; byonsetof heartfailure additionallyoccur at leasttwoothersources of ROS/RNS:

- rise in the myocardium deficit irrigation by reducing the cardiac output value and diastole duration; the latter is the expression of tachycardia installed as reactive compensation mechanism.
- reducing the organflows, which enables to be admitted that, in cardiac dysfunction with reduced ejection fraction, the ROS/ RNStissue synthesis is generalized.

Functional class	Distribution within the batch depending on the gender						Entire batch	
	males			females				
	no. abs.	% out of		nr. abs.	% out of		no. abs.	%
		entire batch (n = 208)	only out of males		entire batch (n = 208)	only out of females		
II	63	30.3	35.33	17	8.17	29.3	80	38.46
III	71	34.13	47.33	29	13.9	50	100	48.08
IV	16	7.7	10.6	12	5.77	20.69	28	13.4
Total	150	72.21	100	58	27.84	100	208	100

Table 1
DISTRIBUTION OF THE STUDY BATCH ON GENDER AND FUNCTIONAL CLASS OF THE HEART PUMP, ASSESSED ACCORDING TO NYHA CLASSIFICATION CRITERIA

Selecting the patients' age groups is justified by the fact that the circulating levels of GDF-15 varies withage; agingresulting in a reduction in serumactivitylevels [6].

In order to interpret the significance of serum values determined for GDF-15 in the studied cases, we have referred to the normal corresponding average age of our group, which was 54.2 years/batch, and, depending on the gender of the patients 55 years for males and 53.38 for females.

In our study, we have included only patients who had an average weight, or at most overweight, which is known as the body mass index influence on serum GDF-15 activity [4].

Furthermore, knowing the records in medical literature that the serum activity of GDF- 15 can be modified by smoking or by taking statins, all patients included in the study batch were non-smokers at leastfor thelasttwoyears or, in thelastquarter, hadnotbeenunderlipidlowering-medication [5].

Toassesstheseverity of contractile deficit, weused NYHA classificationcriteria(New York Heart Association); distribution in relationtotheseverity of ventricularmyocardial contractile deficit, depending on genderisshown in table 1.

Determining the circulating GDF-15 values was carried out from the blood serum collected in the morning, on an empty stomach, twice over a period of 14 days, which is the length of hospitalization period. For each set of measurements, when admitting in hospital, respectively on the 14th day, subsequently was calculated the average concentration, thereby producing two parameters, conventionally referred to as:

- average serum concentration of GDF-15 at hospital admission;
- average serum concentration of GDF-15 at hospital discharge.

Tightening the first determination to be performedwithin the first two days of hospitalization is justified by the chance that could have developed by the treatment instituted for the disease that is by changing the values determined for the enzyme. By imposing this requirement we believe that we excluded the possible action developed by morphological-functional component therapy, yet reversible in the poor contractile myocardium.

Subsequently, the values of two mentioned parameters calculated have led to a third one: the mean serum concentration of GDF-15 per batch, regardless of the collecting time.

Dynamic tracking of the three parameters values in serum, within fortnight, allowed on the one handto have a perspective on the directions on how the serum activity of GDF-15 correlate with the clinical course of heart failure under therapy, on the other hand, by determinations when admitting in hospital identify a possible correlation between the circulating activity and isozyme class of functional contractile deficit. Interpretation of the survey data was conducted both by comparing each value of the three mentioneddetermined/calculated parameters, and by reference to the circulatingnormal value. We admitted as level indicating absent risk concentrations in excess of 1200 ± 101 pg/mL, and in order to assess the risk value below the lower limit of normal, gradually:

- values 1200 ± 101 pg / mL: minimal risk;
- the circulating levels with values> 1200 ± 101 pg/ mL: increased risk;
- and serum concentrations <1200 ± 101 pg / mL: high risk.

Determination of GDF-15 – ELISA method

Method of dosing uses using ELISA immunoassay technique (enzyme linked immunosorbent assay). A kit *Quantikine Human GDF-15 Immunoassay* R & D Systems was used. The microplates contain immobilized monoclonal antibodies specific for GDF-15. On adding the addition of serum to each spot plate GDF-15 present in the serum shall react with the monoclonal antibodies and will be retained. By washing, all substances which do not reacted are removed and polyclonal antibody specific for GDF-15 marked enzymatically with horseradish peroxidase shall be added. Polyclonal antibody, which does not react, marked with peroxidase is removed. By the adding substrate (tetramethylbenzidine) and incubated for 30 min colour is developed. The reaction is stopped and the color intensity is measured by reading the extinction at 450 nm. The color intensity is proportional to the concentration of GDF-15.

Results and discussions

Comparison of the three mean serum PON_1 concentrations: on the entire batch, those at admission or discharge are summarized in the figure below (fig. 2).

In relation to the ejection fraction value, the numerical distribution of the mean concentration and of the standard deviation, in the target group,isshown in the following table (table 2).

Determination of serum activity of GDF- 15 in batch measured by the average dose values determined at admission or discharge, allowed us the next *stratification* of distribution, depending on the areas selected.

Interpretation of the study results regarding the the variation of the serum GDF- 15marker

Comparing this value of the average serum variation of the GDF-15 biomarker in the target group with the normal circulating GDF- 15 (1200 ± 101pg/mL), the former presented a growth rate of 26 % of itsvalue (1551 ± 433.41pg/mL) (fig. 4).

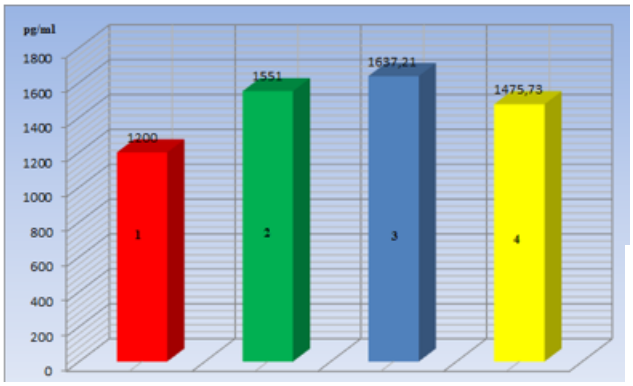


Fig. 2. Comparison of the three mean serum PON1 concentrations depending on the determination time

Legend:
1 ■ = Normal serumlevels of GDF-15 (pg/mL);
2■ = The meanserumconcentration GDF-15 entire lot (pg/mL);
3■ = The meanserumconcentration GDF-15 admission (pg/mL);
4■ = The meanserumconcentration GDF-15 discharge (pg/mL).

Table 2

REPRESENTATION OF THE COMPONENT CASES OF THE TARGET-BATCH DEPENDING ON THE VALUE OF THE AVERAGE SERUM CONCENTRATION GDF- 15

Name/ measurement, biomarker normal reference interval		Incidence determined for the batch cases							
		în întreg lotul (n = 208)		la bolnavi cu ICC şi					
				FE > 50% (sub-batch I : 91cases)			FE< 50% (sub-batch II: 117cases)		
		no. abs.	%	no. abs.	% out of		no. abs.	% out of	
					*	**		*	**
GDF-15	>1200±101pg/ml	178	85.6	75	83.5	36	103	89	50
	<1200±101pg/ml	13	6.2	7	7.7	3.4	6	5.2	2.9
	normal: 1200±101pg/ml	17	8.17	9	9.9	4.4	8	6.9	3.9

Legend: * = % out of the entire target-sub-batch; ** = % out of the entire target-batch;
Table no.2–Representation of the component cases ofthe target-batch depending on the value of the average serum concentration GDF- 15

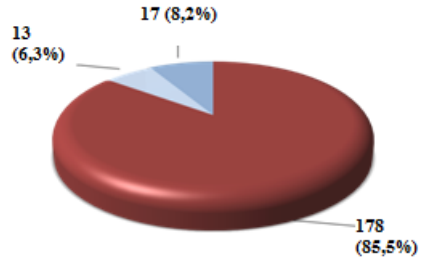


Fig. 3. Numerical distribution of the average seric concentration values and standard deviation of GDF -15 biomarker in the target group, regardless of the patients' gender

Legend:
- circle area=the overall number of patients in the target batch;
- sector■=number of patients in the target group who had normal values of serum concentration (Normal: 1200 ± 101pg/ml);
- sector■=the number of patients in the target group who had higher than normal mean serum values (>1200 ± 101pg/ml);
- sector■ =the number of patients in the target group who had lower than normal mean serum values (<1200 ± 101pg/ml).

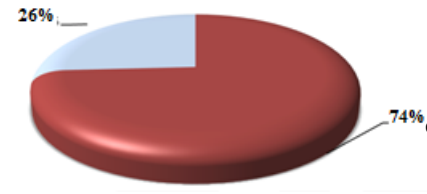
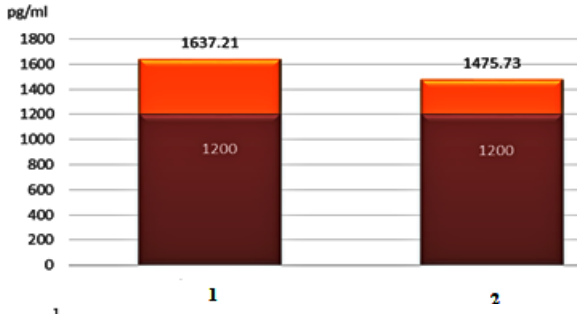


Fig.4. Comparative representation of the increase value of the average serum concentration of GDF -15 in patients with chronic heart failure being investigated in relation to normal circulating value

Legend:
■ =normal serum concentration value;
■ =increase share value above normal serum mean GDF-15



Legend:
1 = value of the average serum concentration of GDF-15 calculated as a result of dosages conducted at admission
2 = value of the average serum concentration of GDF-15 after dosages calculated performed at discharge ;
■ = value of the normal circulant of GDF-15;
■= the rate of increase above the normal of the average of the serum concentration of GDF-15

Fig.5. Highlighting share value increase mean serum concentration of GDF -15 based on the results of measurements performed at admission or discharge for patients with heart failure , regardless of gender or systolic volume

Increase of GDF- 15 is above the circulating normal value of 36% for the average of serum concentration calculated on the basis of dosages performed on admission and almost 23% for the one determined at discharge (fig. 5).

The share of growth occurred mainly based on the determinations of the existing values when admission; the average value of the concentrations determined at the moment of admission being of 1637.21 ± 453.76 pg/mL, what means a 36.4 percent increase over normal. Under the established therapy, the average serum concentration of circulating GDF-15 is reduced to a value of 1475.73 ± 51.64 pg/mL, yet remaining higher to the normal circulating levels, standing at a value of 22.1% (fig. 5).Variationof average serum biomarker GDF-15 in patients belonging to the target batch in relation to the time of collecting/ determination). The data presented allow us to admit that, under therapy, the biomarker reduces its serum concentration value, being acceptable as a marker of therapy evaluation, namely of evolution and prognosis.

Comparingthe same average serum GDF-15 biomarker concentrations existing in the batch cases with the one in the control group (1551 ± 433.41 pg/mL versus 1219 ± 70.7pg/mL) shows an increase of 27.7% of the normal bloodcytokine value levels. This means that heart failure with associate ischemic cardiomyopathy compared with uncomplicated chronic ischemic cardiomyopathy, the average serum cytokine increased by over a quarter. Otherwise formulated, chronic ischemic cardiomyopathy contributes to the rate of increase in average serum GDF-15 in patients with heart failure etiologically induced by deficit in irrigation of the myocardium with the value difference between: the average concentration of serum GDF-15 calculated for the entire group target - average serum concentration of GDF-15 for the entire control group = 1551 ± 433.41 pg/mL- 1219 ± 70.7pg/mL = 332 ± 362pg/mL, the percentage equivalent is 27.7% of normal circulating cytokine value.

To calculate the part that coronary atheroma contributes to the average serum concentration calculated for the entire group of patients with heart failure, it is necessary to take into account that, in its turn,the average serum concentration value of GDF-15 in patients withchronic ischemic cardiomyopathy(control group) consists also inthe normal blood cytokine value and a growth rate that is generated by the deficit of myocardium irrigation via oxidative stress. It results that the value share growth of 27.7% which is the rate of increase in serum GDF-15 in the process of changing from myocardial ischemia without contraction defict to myocardial ischaemia with heart failure, in which we should add the serum cytokine concentration value, which exceeded the normal circulating one. Calculation comes as follows:

- average serum GDF-15 concentration calculated for the entire targetgroup - normal serum concentration of GDF-15 = 1551 ± 433.41 pg/mL 1200 ± 101pg/mL = 551 ± 332.41pg/mL = 46% out of concentration normal serum GDF-15 concentration.

This value means that, by installing the etiology of heart failure, having as etiology ischemic cardiopathy,the share above the normal circulating of GDF-15 is almost half out of the normal circulating, varying with the severity of the contractility deficit.

Conclusions

Increases in serum biomarker activity of GDF-15 means/ is correlated with the process of myocardial fibrosis;

Reducing the amount of ejection fraction is the expression of a major myocardial contractile deficit, in which the adaptive compensation mechanisms of the heart were overcome and morphologically, as a result of hypoxia, via ROS/RNS the fibrosis process becomes predominantly functional.

To indicate whether heart failure take the form with conservation or reduction of systolic flow.

GDF-15 levels were significantly increased in patients with heart failure as compared to the to the control group, similarly in both groups.

The fact that, after therapy instituted for heart failure, serum concentration of GDF-15 did not become normal, remaining high, just like the fact that its value correlates with the degree of myocardial contractiledeficit (classes according to NYHA classification), allows us to agree with the researchers who, by the results of their studies, proposethe use of serum level as well as a useful prognostic marker of the disease.

Under the latter aspect, the utility GDF-15 serum as a prognostic biomarker in heart failure, the data in the medical literatureconsultedrecognizethat thevalue increaseswhenthereisa multimarkercomponet [7, 8].

The results of these biomarkert recommendthe usefulness of this biomarker determination in the diagnosis of heart failure and, in particular, in the form of preserved ejection fraction. The study has a limit, the low number of cases studied and very short duration of patients' trackingundertheinstitutedtreatment [8, 9].

However, the results achieved recommend the extending not only to deepen those observed, but also to identify certain sets of biomarkers useful in diagnosis, monitoring and prediction.

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