UNIVERSITATEA DE MEDICINĂ ȘI FARMACIE
GRIGORE T. POPA IAȘI

THE PRACTICAL RELEVANCE OF CARDIAC BIOMARKERS IN VARIOUS TYPES OF HEART FAILURE

- PHD THESIS SUMMARY -

Scientific coordinator:
Prof. Univ. Dr. Laurenţiu ŞORODOC

Candidate:
Asist. Univ. Dr. Alexandra ROTARIU

2019

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The doctoral thesis includes:

- A general overview structured in 5 chapters and 41 41 pages
- Original research structured in 8 chapters and 78 pages
- 21 tables, 41 figures
- 271 bibliographic references
- The list of articles published during the PhD research period, indexed in international databases and ISI

Note: The bibliography and figures included in this abstract are a relevant selection from the thesis. Their original numbering and placement in the contents of the main text are preserved.

Keywords, selected according to MeSH: heart failure, biomarker, NT-proBNP, galectin 3, cystatin C.

INTRODUCTION. CURRENT STATE OF KNOWLEDGE

According to European Society of Cardiology, the definition of heart failure involves a complex clinical syndrome comprising typical signs and symptoms associated with objective proofs of structural or functional cardiac abnormalities at rest. By default, asymptomatic stages and atypical forms of heart failure raise diagnosis and therapeutic behavior problems.

The rapid and correct differentiation of heart failure from other pathologies with similar clinical presentation remains a challenge. After the urgent evaluation of the patient's symptomatology, the clinical examination, the standard electrocardiogram (ECG) and the chest radiography, the clinician may face a series of uncertainties related to the diagnosis, having as a direct consequence the erroneous diagnosis or the delay of the initiation of a specific treatment. Since it is estimated that the diagnosis of heart failure is omitted in about 15-20% of cases, a better stratification of the cardiovascular risk is absolutely necessary in patients presenting with atypical chest pain, dyspnea or non-specific cardiac symptoms.

Within the diagnostic algorithm of a newly appeared cardiac dysfunction, the biomarkers have benefited in the last decades a major interest from the researchers, conferring additional value to the standard methods of evaluation of the heart failure.

Cardiac biomarkers already implemented in current medical practice (BNP and NT-proBNP

natriuretic peptides, expression of increased intracavitary pressure) have diagnostic and prognostic value in heart failure, but there is no recognized cut-off value for either of the two natriuretic peptides. Moreover, their serum levels are influenced by non-cardiac pathologies, such as renal failure, thyroid impairment, anemia, acute cerebrovascular disease, obesity.

In order to carry out this work, we have focused on two cardiac biomarkers of recent interest (cystatin C and galectin-3), a decision motivated by numerous studies that certify their qualities, the last years being relevant in the outline of the pathophysiological mechanisms leading to heart failure. Studies that have so far compared galectin-3 and cystatin C with conventional markers (such as NT-proBNP) support the diagnostic and prognostic benefits of multi-marker assessment, but there are multiple unknowns in this direction.

MOTIVATION AND PURPOSES OF DOCTORAL RESEARCH. This clinical study aims to identify the extent to which the comparison of two new cardiac biomarkers (cystatin C and galectin 3) with conventional biomarkers (NT-proBNP), in a multi-marker approach, facilitates the diagnosis of acute heart failure, as well as whether this strategy can be implemented in routine medical practice.

The primary outcome of the study was to establish the utility of the multi-marker asessment in specifying the diagnosis in patients with suspected heart failure.

The secondary outcomes of the study were:

a) Identification of patients with high risk of morbidity-mortality through cardiovascular events based on biomarkers analysis, allowing intensification of therapeutic efforts;
b) Comparative analysis of the diagnostic performance related to the three biomarkers in the two major pathophysiological entities: cardiac failure with preserved ejection fraction *versus* heart failure with reduced ejection fraction;
c) Facilitating differential diagnosis with non-cardiac pathologies, in an uncertain clinical context;
d) Methods of implementation of new biomarkers in current medical practice;
e) Avoiding the irrational use of complex imaging investigations in well-defined clinical situations;
f) Identification of selection criteria for patients in the early stages of myocardial injury, beneficiaries of long-term prevention therapy of cardio-vascular adverse events.

MATERIAL AND METHOD

The clinical study is of observational prospective type conducted between November 1, 2016 - March 1, 2018, including patients over 18 years of age who presented at the Emergency County Clinical Hospital „Sf. Spiridon“, Iași, within the Emergency Department or 2nd Internal Medicine Clinic, for dyspnea, palpitations, fatigue, chest pain, edema, clinical manifestations with acute onset (less than 48 hours) or exacerbated on a pre-existing cardiovascular pathological background.

The acute nature of chronic failure was considered in the following situations:

a) Admission of patient was considered necessary (within the 2nd Internal Medicine Clinic) for a clinical picture suggestive of heart failure;
b) Changes in the cardiovascular therapeutic scheme were necessary (in the sense of increasing the doses of the chronic medication or of the association of new classes of cardiovascular drugs);
c) There was a favorable response to standard treatment of heart failure (eg, the use of diuretic medication allowed the control of symptoms, improvement of clinical and radiological signs of pulmonary / systemic congestion, reduction of oxygen requirement, and gradually weight loss).

The study protocol was approved by the Research Ethics Committee of the University of Medicine and Pharmacy "Grigore T. Popa" - Iași (13.03.2016), respectively from the Emergency Clinical County Hospital "Sf. Spiridon". The "Good Clinical Practice" regulations regarding the design and conduct of the study, as well as those regarding the reporting of the obtained data were respected.

The general inclusion criteria consisted of: 1) adulthood (> 18 years old); 2) emergency presentation (in the Emergency Department of "Sf. Spiridon" Hospital / 2nd Internal Medicine Clinic); 3) presence of symptoms / signs suggestive or that have imposed differential diagnosis with acute/chronic acute heart failure: dyspnea,

palpitations, fatigue, chest pain, edema, etc.; 4) indication of admission in 2nd Internal Medicine Clinic.

The exclusion criteria consisted of:
1) Impossibility to perform transthoracic echocardiography within up to 48 hours from the time of admission (technical reasons, admission of patients on guard during legal free days, temporary lack of doctors with competence in echocardiography);
2) Active documented neoplastic pathology (due to interferences with the interpretation of galectin 3 values);
3) Active hepatitis (transaminases > 5 times the upper limit normal, ULN);
4) Pathologies with multiorgan fibrotic disease (pulmonary fibrosis, colagenosis, ș.a) to avoid false positive results of increasing serum galectin level 3;
5) Laboratory interference of cystatin C and galectin 3 according to the manufacturer's instructions for use.

For each participant was prepared a standardized file in which **demographic** and **epidemiological** parameters, **clinical** parameters were introduced (of which we mention the degree of urgency evaluated by the national protocol of triage of patients using the color algorithm, the degree of dyspnoea and the clinical status upon discharge: ameliorated, aggravated or deceased), as well as **paraclinical** parameters (standard biochemical profile, according to guideline recommendations in the evaluation of the patient with heart failure, glomerular filtration rate, GFR (ml/min/1,73m^2) calculated by the formula MDRD, Modification of Diet in Renal Disease, serum determinations of cystatin C, galectin 3, transthoracic echocardiography). Moreover, **indicators**

of economic-financial performance/ medical services/ quality (number of days of hospitalization, total costs per patient, mortality rate of cardiac cause compared to the mortality of non-cardiac causes) were evaluated.

PRINCIPLES OF SAMPLING AND ANALYSIS OF CARDIAC BIOMARKERS (CYSTATIN C, GALECTIN 3, NT-proBNP)

A) NT-proBNP. Quantitative determination of NT-proBNP was performed on the analyzer PATHFAST (Mitsubishi, Tokyo, Japonia) using reagents PATHFAST. The principle of the method is based on immunoenzymatic analysis by chemiluminescence (CLEIA) using the methodology MAGTRATION. The normal ranges of NT-proBNP were between 15 – 30000 pg/mL.

The cut-off values of NT-proBNP used in the study were determined differently depending on the age category, for reasons of superior diagnostic accuracy scientifically documented in the specialized literature (Chow, 2017). Thus, the following reference values were used: a) < 50 years old: > 450 pg/mL; b) 50-75 years old: > 900 pg/mL; c) > 75 years old: > 1800 pg/mL.

B) Galectin 3. Quantitative determination of galectin 3 in human serum/plasma (obtained from whole blood collected in plastic tubes on EDTA medium) uses the technology CMIA (Immunological test based on chemiluminescence), optimized using flexible testing protocols integrated under the name of CHEMIFLEX. ARCHITECT *i* System was used for these determinations (Abbott Laboratories, Chicago, IL, Statele Unite ale

Americii). The detection limit of galectin 3 was 1,1 ng/mL.

Galectin 3 values were expressed in ng/mL with division into 2 major categories of clinical performance: a) ≤ 17,8 ng/mL, values considered suggestive for *low* risk of heart failure; b) > 17,8 ng/mL, values considered suggestive for *high* risk of heart failure. According to the literature data, for the purposes of statistical processing, patients with high levels of galectin 3 were further divided into two subgroups (moderate risk 17,8-25,9 ng/mL and high risk for galectin 3 values > 25,9 ng/mL) (McCullough, 2011).

C) Cystatin C. The method used for the analysis of cystatin C consisted of quantitative immunoturbidimetric determination in human serum or plasma. (PETIA – particle enhanced turbidimetric immunoassay). ARCHITECT c System was used for these determinations. The detection limit of the device was 0,05 mg/L.

The reference values were different depending on the patient's gender. Thus, for women the normal range was considered between 0,40-0,99 mg/L, and in men, depending on the age category, normal values were found in the range 0,31-0,79 mg/L (< 49 years old), respectively 0,41-0,99 mg/L (> 50 years old).

PRINCIPLES OF ECOCARDIOGRAPHICAL DATA PROCESSING AND ANALYSIS

Echocardiography was performed in all patients included in the study (regardless of the cardiac or non-

cardiac etiology of the clinical picture), and the echocardiographic parameters of interest were:

- ✓ Confirmation or rejection of the presence of a cardiac structural or functional abnormality (within the limits of ultrasound examination: transthoracic examination / resting hemodynamic conditions)Funcţia sistolică a ventriculului stâng estimată prin fracţia de ejecţie a ventriculului stâng, FEVS.
- ✓ The diastolic function of the left ventricle was estimated by the report E/A < 1 (diastolic dysfunction with abnormal relaxation).

According to the systolic function of the left ventricle, the patients were divided into 4 subgroups: 1) reduced EF (<40%); 2) „mid range" EF (40-50%); 3) preserved EF (50-60%); 4) normal EF (> 60%) (Ponikowski et al., 2016).

In this context, in order to achieve the objectives of the study, we divided the group of patients into two subgroups: cardiac and non-cardiac. The group of cardiac patients included patients with newly diagnosed heart failure or having an exacerbation of a chronic heart failure. The group of non-cardiac patients included patients whose clinical manifestations were explained by diagnoses of a cause other than cardiac.

For establishing the diagnosis of heart failure, the medical records of the patients were consulted by two internal medicine physicians (A.S. and O.S.). The clinical, biological data were analyzed (in the absence of availability of results for cystatin C and galectin 3), imaging data (electrocardiogram, chest X ray,

echocardiography), as well as the response to the therapy administered during the hospitalization period.

There were no limitations regarding the etiology of heart failure, including patients with various stages of heart dysfunction.

STATISTICAL DATA ANALYSIS

IBM SPSS Statistics for Windows, versiunea 19.0 (IBM Corp, Armonk, NY, SUA) and MedCalc version 18.2.1 (MedCalc Software bvba, Ostend, Belgia) were used for data analysis.

The diagnostic performance of the three biomarkers was evaluated by analyzing the ROC curve (Receiver operating characteristic) and by comparing the areas under the curve (AUC) on both the total study group and the risk subgroups.

Statistical processing uses two-tailed tests, and the statistical significance threshold was considered at a p value < 0,05.

RESULTS

The study included 208 patients aged between 41 and 94 years. Of these, 123 patients were female (59.1%) and 85 were male (40.9%).

Diagnostic criteria for cardiac etiology of acute clinical manifestations were met in 61.1% of patients (n=127 pacients), while the group of non-cardiac patients included 38.9% of the patients (n=81 pacients) (Fig.VIII.5).

The severity of dyspnoea was assessed using the NYHA functional classification, with an equal

distribution of NYHA class III-IV patients compared to NYHA class I-II patients (49,6% versus 50,4%).

Of the 208 patients, 4.3% of patients died (8 patients with cardiovascular causes, 1 patient with respiratory causes), 2,9% (n = 6 pacients) presented aggravated general condition upon discharge from the 2nd Internal Medicine Clinic – (requiring transfer to coronary intensive care units, with the diagnosis of acute coronary syndrome). The remaining 193 patients (92.8%) were discharged with improved general condition.

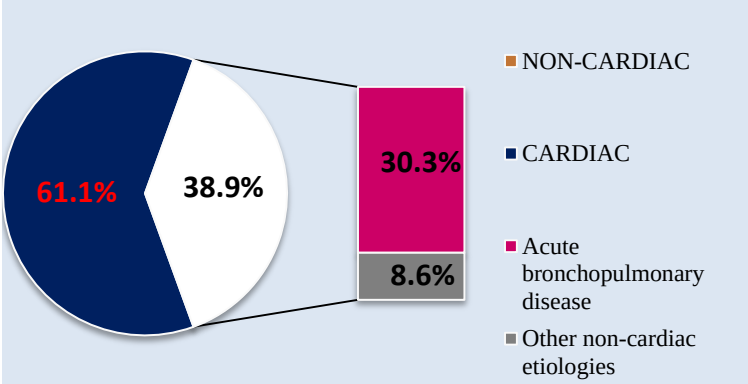

Fig.VIII.5. Distribution of patients according to cardiac or non-cardiac etiology of dyspnea

For NT-proBNP values between 12-30000 pg/mL were recorded. The serum level of galectin 3 was in the range 7.5-86.6ng/mL, while for cystatin C the range of values was between 0.74-9.67 mg/L.

The percentage distribution of patients according to the normal or increased values of the biomarkers can be found in Fig.VIII.9.

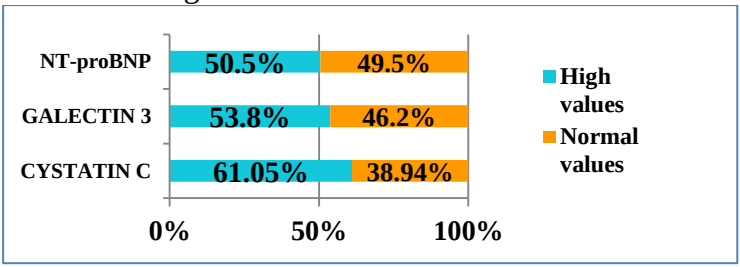

Fig. VIII.9. Distribution of patients according to biomarker values

DESCRIPTIVE STATISTICAL ANALYSIS ON SUB-GROUPS REPORTED TO CARDIAC OR NON-CARDIAC ETIOLOGY

The basic characteristics of the group of confirmed patients with acute heart failure showed some differences with significant statistical power regarding demographic, biochemical or echocardiographic data when compared to patients without acute heart failure. Patients with acute heart failure were older and required longer hospitalization compared to the group of patients without acute heart failure. Also, the heart patients had lower values of the ejection fraction of the left ventricle, together with a higher heart rate. The glomerular filtration rate was lower in cardiac patients (p = 0,009) (Table VIII.1.).

Table VIII.1. Comparative analysis between the groups with cardiac and non-cardiac dyspnea

	Total pacients	Acute cardiac dyspnea	Acute non-	*P*

			cardiac dyspnea	
Age (years)	72,96 ± 11,11	75,96 ± 10,18	69,14 ± 11,49	**0,000***
Length of admission, (days)	7,27 ± 3,45	7,61 ± 3,42	6,74±3,46	**0,032***
Heart rate (bpm)	91,99 ± 25,78	98,06 ± 27,68	82,46 ± 19,06	**0,000***
GFR (ml/min/ 1.73m²)	72,69 ± 25,68	67,50 ± 26,93	80,82 ± 21,33	**0,009***
LVEF (%)	50,38 ± 9,76	46,60 ± 9,43	56,32 ± 6,95	**0,000***
Serum urea (mg/dL)	50,47 ± 26,94	56,70 ± 30,71	40,71 ± 15,28	**0,000***
Serum uric acid (mg/dL)	5,89 ± 2,66	6,26 ± 2,92	5,30 ± 2,07	**0,000***

GFR, glomerular filtration rate,
LVEF, lef ventricular ejection fraction; * *p<0,05*

After controlling for the differences between cardiac and non-cardiac patients determined by age (more advanced in cardiac patients) and glomerular filtration rate (lower in cardiac patients), ANCOVA method was performed to evaluate the extent to which patients in the acute heart failure group maintain elevated levels of NT-proBNP and / or galectin 3, at a significant level compared to non-cardiac patients. The result of the analysis showed statistically significant differences between the groups regarding the increased value of the two biomarkers after removing the influences that the age

or the deterioration of the renal function, expressed by the glomerular filtration rate, could have. (NT-proBNP: F = 16,81(1,204), p = 0,000; galectina 3: F=22,45(1,204), p = 0,000).

The diagnostic performance of the two biomarkers was tested on the total group of patients, but also on subgroups considered with high cardiovascular risk and with difficulties in establishing the positive diagnosis of heart failure.

The predictive diagnostic value of cardiac dyspnea is similar between NT-proBNP (area under curbe, AUC = 0.781, p = 0.000) and galectin 3 (AUC = 0.803, p = 0.000) in the total group of patients. (Table VIII.5).

Table VIII.5. Diagnostic performance of NT-proBNP and galectin 3 for cardiac dyspnea

	Biomarker	AUC (95% CI)	p	**Sensi-tivity (%)**	**Speci-ficity (%)**	**Opti-mal cut-off**	p
Total number of patients	NT-proBNP (pg/mL)	0.781 (0.718-0.835)	0.55	66.13	82.14	1538	0.000**
	Galectin-3 (ng/mL)	0.803 (0.742-0.855)		72.6	84.52	18.8	0.000**
Age > 60 years	NT-proBNP (pg/mL)	0.745 (0.675-0.807)	0.13	66.7	77.9	1538	0.000**
	Galectin-3 (ng/mL)	0.811 (0.746-0.866)		75.7	83.8	18.8	0.000**
Obesity (BMI > 30kg/m²)	NT-proBNP (pg/mL)	0.781 (0.659-0.875)	0.44	58.97	87.5	1081	0.000**
	Galectin-3 (ng/mL)	0.841 (0.727-0.921)		74.36	91.67	19.5	0.000**

GFR < 60ml/ min/ 1.73m²	NT-proBNP (pg/mL)	0.671 (0.545-0.781)	0.28	75.0	60.0	1538	0.023*
	Galectin-3 (ng/mL)	0.769 (0.650-0.863)		80.77	73.33	18.6	0.006*
LVEF < 40%	NT-proBNP (pg/mL)	0.792 (0.597-0.921)	0.23	83.33	75	2065	0.071
	Galectin-3 (ng/mL)	0.651 (0.449-0.820)		75	75	19.5	0.481
Supra-ventri-cular rythm disorders (atrial fibrilla-tion / flutter)	NT-proBNP (pg/mL)	0.697 (0.605-0.779)	0.07	51.06	86.36	3228	0.0007 *
	Galectin-3 (ng/mL)	0.825 (0.743-0.889)		71.28	90.91	17.9	0.000 **
Diabetes mellitus type 2	NT-proBNP (pg/mL)	0.838 (0.719-0.921)	0.64	67.57	90.91	1648	0.000 **
	Galectin-3 (ng/mL)	0.803 (0.679-0.895)		75.68	81.82	18.5	0.000 **

* p<0.05; ** p<0.0001

There were no statistically significant differences between the areas under the curve (AUCs) for galectin 3 and NT-proBNP in the high risk subgroups analyzed.

Additionally, the summation of the two components in a single latent variable was associated with an increase in AUC proving superior diagnostic accuracy (AUC = 0.859, p = 0.000) compared to the independent analysis of the two biomarkers. (Fig.VIII.23).

The analysis of the areas under the curve did not identify significant differences regarding the diagnostic

accuracy between NT-proBNP and cystatin C on the total group of patients.

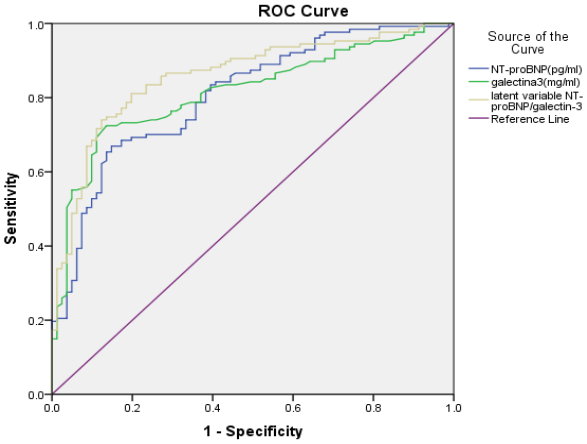

Fig. VIII.23. ROC curve analysis of NT-proBNP, galectin 3 and latent variables in acute cardiac dyspnea

DISCUSIONS

Our prospective observational study analyzed the diagnostic performance of NT-proBNP, galectin 3 and cystatin C in establishing the cardiac etiology of acute cardiopulmonary manifestations (or acute on a chronic pathological background) in a group of 208 patients. Also, the diagnostic accuracy of the three biomarkers was also studied on subgroups of patients considered to have high cardiovascular risk, in relation to the potential interferences in the interpretation of natriuretic peptide values.

Most studies that analyzed the combination of NT-proBNP, galectin 3 and cystatin C biomarkers included patients diagnosed with heart failure and evaluated their role in establishing the prognosis and

stratifying the risk of cardiovascular adverse events. To our knowledge, this is the first study to analyze the diagnostic implications of this combination of biomarkers in patients who present in the Emergency Department with suspected acute heart failure.

From the point of view of gender distribution, our patient group included a higher percentage of female patients, without this result reaching statistical significance. There was no association between patient gender and cardiac etiology of acute dyspnea.

Acute heart failure in the renal patient

One of the strengths of our study is reflected in maintaining the statistically significant differences of NT-proBNP and galectin 3 levels even after the influence of age and renal function was removed by the ANCOVA covariance test. Certainly, the impact of age and degradation of renal function on these biomarkers cannot be completely excluded, but we can say that the type of acute dyspnoea significantly influences the serum level of these markers. For these reasons, both galectin 3 and NT-proBNP can be considered as effective indicators of assessing the risk of acute cardiac dyspnea and, consequently, of acute heart failure.

Heart failure with preserved versus reduced ejection fraction

The distribution of patients in our study according to the left ventricular ejection fraction showed that most patients had preserved ventricular systolic function. (38,5%). The results obtained do not identify statistically significant differences between the sex of the patient and the left ventricular systolic function, expressed by the ejection fraction of the left ventricle.

The use of the three biomarkers, independently or in combination, allowed the identification of differences with strong statistical significance between the groups of patients related to the ejection fraction of the left ventricle.

Severity criteria in the patient with acute heart failure

Acute heart failure is the main reason for hospitalization in elderly patients (Farmakis et al., 2015). Intra-hospital mortality rate in patients with acute heart failure varies between 4 and 11%, and of patients who survive about 30% will die within one year. (Allen et al., 2015; Farmakis et al., 2018). The mortality rate in the patients in our study was 4.3% (n = 9), of which 3.85% died from cardiovascular causes.

The plasma level analysis of the three biomarkers showed significantly increased values in patients with acute cardiac dyspnea, suggesting their potential to identify patients with high cardiovascular risk.

Our results did not identify significant differences between the left ventricular ejection fraction and the patient's condition at discharge (improved, aggravated, deceased) (p = 0,187). Also, there was no association between the patient's gender and the condition at discharge.

Diagnostic performance of biomarkers in acute heart failure

The existence of a strong reciprocal correlation between NT-proBNP, galectin 3 and cystatin C provides the scientific support for the usefulness of a multimarker approach (which includes all three biomarkers) to establish the diagnosis of acute heart failure.

With these arguments, our research continued with the analysis of the ROC curve in order to evaluate the diagnostic performance of the three biomarkers.

In the total group of patients, the diagnostic performances of NT-proBNP and galectin 3 or NT-proBNP and cystatin C or galectin 3 and cystatin C did not show statistically significant differences, indicating that all three biomarkers are reliable tools for concerns regarding the diagnosis of acute heart failure.

For NT-proBNP and galectin 3 the results were comparable in the analysis of the total number of patients, as well as in the analysis of the subgroups with high cardiovascular risk. The associated comorbidities created the premise of higher optimal cut-off values, without affecting the diagnostic accuracy of NT-proBNP or galectin 3.

After independent analysis of NT-proBNP and galectin 3 confirmed the diagnostic accuracy of acute cardiac dyspnea, a composite variable of the two biomarkers was found to have superior predictive diagnostic performance. Moreover, we can state that plasma determination of galectin 3 improves the diagnosis of acute heart failure when used in combination with NT-proBNP.

Our data show that a cut-off value of NT-proBNP of 2065 pg / mL is needed as a predictor for LVEF <40%, with a sensitivity of 84% and a specificity of 100%. Differences in the inclusion of patients according to the LVEF value may provide an explanation for the cut-off differences recorded.

In the group of patients with reduced ejection fraction (<40%), the best diagnostic performance was obtained for NT-proBNP.

Differential diagnosis of acute heart failure

The main differential diagnosis of acute heart failure is represented by the acute respiratory pathology, aspect confirmed also by the results of our study. In the general population, it is expected that there is a common cardiopulmonary pathological association, by combining in about 30% of cases the heart failure with the chronic obstructive airway diseases. The occurrence of an episode of infectious exacerbation of chronic respiratory pathology can have a negative effect on cardiac function, the reverse situation being also valid. Diagnostic confusions in the emergency care unit in a patient with dyspnoea may occur in about 50% of patients. The echocardiographic confirmation of a certain degree of cardiac dysfunction may be the expression of a pre-existing cardiac pathology, without further degradation during the acute respiratory episode.

In our study, the proportion of patients with chronic lung disease was reduced (10.1%), while acute bronchopulmonary disease was the main differential diagnosis of acute dyspnea (one in three patients). Most cases of acute respiratory disease have had an infectious context, either through acute community infections of the lower respiratory tract or through infectious exacerbations of chronic obstructive diseases.

FINAL CONCLUSIONS

1. The unitary approach from the therapeutic point of view of the patients with heart failure continues to

present limitations, most probably, of the impossibility of including a pathophysiological complex under a single therapeutic "umbrella". Thus, in order to be able to achieve progress in addressing patients with heart failure, it is of real interest to identify the early stages and to characterize, from a physiopathological perspective, the mechanisms involved.

2. A number of peculiarities of the cardiac patient are found in our study: a) the acute episodes of chronic heart failure were, more frequently, reason for presentation in urgency compared to the purely acute forms of heart failure; b) cardiac failure with preserved ejection fraction has characterized most heart patients; c) the group of cardiac patients presented more advanced age and more significant deterioration of renal function compared to the group of non-cardiac patients.

3. Although the impact of age and renal function on serum level of biomarkers cannot be excluded, our study has shown that the cardiac etiology of acute clinical manifestations significantly influences their serum level.

4. The combined analysis of the 3 biomarkers (NT-proBNP, galectin 3 and cystatin C) allows rapid triage of patients who present, under emergency conditions, with suspected acute heart failure and identifies patients with high cardiovascular risk who require intensive treatment.

5. The multimarker approach can improve the short-term prognosis of patients with heart failure, by identifying those situations with severe paucisymptomatic cardiac pathology in which isolated determination of NT-proBNP is not eloquent, for various reasons.
6. The association of NT-proBNP and galectin 3 is superior to NT-proBNP in the identification of patients with acute forms of heart failure.
7. For patients with acute heart failure and renal impairment, the diagnostic performance of cystatin C and galectin 3 are similar.
8. The laboratory methods used for the serum determination of the 3 biomarkers, in particular galectin 3 and cystatin C for which there is less clinical experience, have proven to be easy to implement in medical practice and reliable.
9. The widespread use of new classes of drugs in the treatment of heart failure (sacubitril + valsartan) with proven effects on the biological profile of natriuretic peptides will result in a reduction in their diagnostic and prognostic performance, so it is necessary to use superimposed diagnostic tools, foldable on the profile of galectin 3 and cystatin C biomarkers.
10. The current costs of using biomarkers can be significantly reduced by better accepting their usefulness in medical practice. The increased justifiable interest for these analyzes and their dissemination in

different medical services (starting with the emergency services / clinics with a medical profile) is a first step in increasing the confidence of the practitioners regarding the potential of these biomarkers.

11. The major impact of these biomarkers, which will also be reflected on indicators of cost-efficiency, derives from a better management of the cases with heart failure, stratification of the cardiovascular risk (of a population at high risk), avoiding costly and consuming investigations, time and human resources.

12. The use of biomarkers is not recommended in patients with advanced stage heart failure, with an unfavorable prognosis already specified by previous evaluations, represented by persistently increased serum concentrations of biomarkers.

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