The Lymphocyte Count and Neutrophil/lymphocyte Ratio are Independent Predictors for Adverse Cardiac Events in Ischemic Heart Failure but not with Non-ischemic Heart Failure

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The predictive value of some biomarkers in heart failure is not yet established. A high value of white blood cell (WBC) count and neutrophil/lymphocyte ratio (NLR) was found to be a marker of higher long term cardiovascular mortality in patients with ischemic heart disease. Recent data suggests that some specific types of leucocytes have different predictive values for the cardiovascular risk. This value is improved by using NLR. The purpose of the study was to evaluate the predictive values of WBC count, NLR, WBC differential formula (WBC-DF) for cardiovascular events in patients with non-ischemic heart failure (NIHF) as compared with ischemic heart failure (IHF). Our study included 256 patients with HF (147 with IHF and 109 with NIHF), NYHA class II-IV. They signed informed consent and underwent clinical and laboratory assessment: lipid profile, NT-proBNP, CRP, WBC, NLR and WBC-DF. The patients were followed up for one year. In patients with IHF, significantly higher levels of NT-proBNP ($p<0.001$), CRP ($p<0.005$), WBC ($p<0.005$), lymphocytes ($p<0.05$) and NRL values (1.27 (1.2-1.36) vs 0.99 (0.4 – 1.15), $p<0.02$) were found as compared with NIHF. A significant difference between NLR values was found only in patients in NYHA class II vs III. A positive correlation between NRL and NT-proBNP level was found ($p<0.05$). The lymphocyte count, NLR and NYHA class ($p<0.001$) represent independent predictors for the rehospitalization and cardiac events in HF patients. Our study has shown that for patients in the same NYHA class, the lymphocyte count and NLR are independent predictors for adverse cardiac events only in IHF.

Keywords: lymphocyte count, neutrophil/lymphocyte ratio, biomarkers, predictive factors, ischemic heart failure, non-ischemic heart failure

Heart failure is an endemic clinical condition and a major cause of invalidity, rehospitalization and fatality, causing a severe burden on health care [1].

In spite of new therapies, the mortality and need for rehospitalization in HF patients still remain high (25% in the first 30 days, increasing to 35-40% in the first 90 days after discharge), therefore this interval is aptly called *the vulnerable phase* [2, 3].

Each rehospitalization for HF decompensation leads to further deterioration of heart performance and renal function [3], and proportional increase of the risk for mortality and rehospitalization, which is highest in the first 30 days and decreases in the next 3 to 6 months [4,5].

Therefore is essential to identify predictive markers (clinical, hemodynamic and biological) for patients with high risk of poor outcome. Papers published in the last decade have proposed and identified biomarkers [6-10], but the widespread use of some of them is restricted due to cost and availability [11,12].

Several studies have evaluated the predictive ability of WBC count [13] and WBC-DF [14-17]. Increased WBC count , a marker for inflammatory state, was proven to be a predictor for long-term cardiovascular mortality in patients with stable ischemic heart disease or acute myocardial infarction [13].

Recent studies suggest some specific leukocyte populations are predictors for cardiovascular risk [14-18] - lymphopenia (found in all patients with HF) was associated with poor outcome [17,18].

NLR was proven to have good predictive value for mortality in cardiac [19-24] and non-cardiac conditions [26,27]. The predictive value of NLR (a low-cost and widely available marker) was not sufficiently studied in patients with HF.

The goal of our study was to evaluate the predictive value of NLR and WBC populations for cardiovascular events and rehospitalization in patients with ischemic and non-ischemic HF.

Experimental part

Our study included 256 HF patients in NYHA class II-IV. Inclusion criteria were: age > 18 years and hospitalization for worsening HF. Exclusion criteria were: patients with acute inflammatory or infectious diseases; treatments which influence WBC count; patients with neoplasia; patients with interventional or surgical procedures in the last 3 months; patients with acute myocardial, peripheral or cerebral ischemia; vasculitis or connective tissue disease. All patients underwent echocardiography (Siemens Acuson X300 with a 2-5 MHz probe) to evaluate morphologic abnormalities (heart chambers and valves), systolic and diastolic function. Laboratory workup was performed at hospital admission: NT-proBNP, renal and liver function tests, complete blood count with WBC-DF, inflammatory markers (erythrocyte sedimentation rate, CRP, and fibrinogen), lipid profile and glucose. NLR was computed as the ratio of neutrophils to lymphocytes.

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NLR = neutrophils number ($x10^9$ /L)/ lymphocytes number ($x10^9$ /L)

Biochemical analysis was performed on a Beckman-Coulter AU 680 device. Clinical and demographic data was collected from the patients admission charts. Ischemic heart disease was present if a diagnosis of stable angina, unstable angina, previous myocardial infarction or coronary heart disease with uni-, bi- or multivascular disease diagnosed at coronarography was found. Patients were followed for one year for cardiovascular events and rehospitalizations. All patients signed an informed consent. Study methodology was approved by the Ethical Committee.

Statistical analysis

Statistical analysis was performed with SPSS 16. Group comparison was done using the chi-square test for categorial variables, Student test for continuous variables with normal distribution and Mann-Whitney U test for continuous variables with abnormal distribution and ordinal variables. Probability of outcome with no rehospitalization was evaluated by Kaplan-Meier method, and differences between Kaplan-Meier curves – by log rank test. A value of $p < 0.05$ was deemed significant; confidence intervals were calculated for $p=0.05$ as threshold.

Results and discussions

Average age for the 256 patients (47.3% males and 52.7% females) was 67 ± 15 years. Based on HF etiology, patients were assigned to two groups: ischemic HF (IHF) and non-ischemic HF (NIHF). Patients demographic, clinical and biological data are presented in table 1. There were no significant differences between the two groups regarding gender, age and BMI.

Patients with NIHF had significantly higher levels of total cholesterol and LDL-cholesterol ($p < 0.005$), while patients with IHF had significantly higher levels of WBC ($p < 0.001$), Lymphocytes ($p < 0.05$), PMN ($p < 0.005$), NRL ($p < 0.02$), NT-proBNP ($p < 0.001$) and CRP ($p < 0.005$). We found NLR had a significant positive correlation with NT-proBNP ($r = 0.43$, $p < 0.05$) and NYHA class ($r = 0.253$, $p = 0.004$). We also found NLR had significant differences between classes NYHA II and III only (table 2).

Univariate analysis showed that lymphocyte count, NLR and NYHA class ($p<0.001$) are independent predictors for event-free outcome in patients with HF (table 3). After adjusting for NYHA class we found that lymphocyte count and NLR remained predictors for rehospitalization only in patients with IHF.

In our study the lymphocyte count, NLR and NYHA class are predictors for event-free outcome in patients with HF. Further, after adjusting for NYHA class, we found that the lymphocyte count and NLR remained predictors only in patients with IHF. To the best of our knowledge, this is the first study to analyze the predictive capacity of NLR and WBC-DF in HF patients according to the ischemic or non-ischemic etiology of HF.

There are many studies that prove WBC and their subpopulations (PMN, lymphocytes) are useful biomarkers for evaluating inflammation involved in atherosclerosis and ischemic heart disease [13, 14] (stable and unstable angina, previous myocardial infarction, coronary bypass surgery). Increased WBC count has proven to be an independent predictor for future cardiovascular events in patients with ischemic HF.

Chronic activation of inflammatory responses in HF plays a major pathophysiological part in disease progression by releasing cytokines and activation of apoptosis [16, 28-30].

Long-term sympathetic activation [31,32] and increased levels of cytokines (especially TNF-α) are involved in decreasing the lymphocyte count and variability of WBC populations [32].

Lymphopenia found in every patient with acute and chronic HF, irrespective of etiology [28,29,33], correlates with disease severity [23] and was proven to be a negative prognostic marker, associated with increased mortality, both in-hospital [18] and post-discharge (short-term [29,

	Patients with NIHF	Patients with IHF	p
Age (years)	68.11 ± 15.85	67.12 ±13.87	NS
Gender - women (number)	71 (73.9%)	1. (68.9%)	NS
Gender - men (number)	25 (26.1%)	29 (31.1%)	NS
BMI (kg/m^2)	20.63 ± 14.75	22.03 ± 11.82	NS
Duration of hospitalization (days)	6.66 ±2.77	7.54 ±3.84	NS
Hemoglobin (g/dl)	13.10 ± 2.57	12.94 ± 3.37	NS
Hematocrit (%)	33.48 ± 14.51	31.32 ± 14.49	NS
WBC ($x10^9$ /l)	6.71 ± 2.08x 10^9	7.68 ± 3.07 $x10^9$	< 0.001
PMN (%)	52.6 ± 31.3	57.8 ± 29.4	< 0.005
Lymphocytes (%)	13.8±10	16.9 ± 10.2	< 0.05
NLR (value and range)	0.99 (0.4-1.15)	1.27 (1.2-1.36)	< 0.02
CRP (mg/dl)	0.54 ± 0.11	0.72 ± 0.26	< 0.001
NTproBNP (ng/ml)	2978.33 ± 1106.20	5126.85 ± 209.16	< 0.001
Total cholesterol (mg/dl)	161.48 ± 65.01	140.27 ± 70.12	< 0.001
LDL cholesterol (mg/dl)	106.9 ± 56.24	87.9 ± 43.59	< 0.001

Table 1
PATIENTS DEMOGRAPHIC, CLINICAL AND BIOLOGICAL DATA

Table 2
PATIENTS BIOLOGICAL DATA ACCORDING TO NYHA CLASS

	NYHA II	NYHA III	p	NYHA III	NYHA IV	p
Patients	115 (35.6 %)	150 (46.7%)		150 (46.7%)	58 (17.7%)	
NLR	3.97±3.10	10.82±7.01	<0.001	10.82±7.01	11.15±5.72	0.287
Lymphocytes (%)	15.24±11.71	14.65±9.17	<0.001	14.65±9.17	13.3±7.92	0.546
PMN (%)	50.11±31.1	58.56±28.22	<0,001	58.56±28.22	61.94±29.3	<0.05
PCR (mg/dl)	0.02±0.10	0.77±0.2	<0.001	0.77±0.2	1.17±0.44	0.134
NT proBNP (ng/dl)	33658±770.0	5070.2±2024.9	<0.01	5070.2±2024.9	6505.3±6442.9	<0.001
Duration of hospitalization	6.33±2.73	8.12±3.8	<0.001	8.12±3.8	8.41±3.5	0.145

Table 3
UNIVARIATE ANALYSIS OF PREDICTORS FOR REHOSPITALIZATION

	OR	p	95.0% CI	
			Lower	Upper
Lymphocytes (x10^9/L)	0.424	0.024	0.342	0.508
NLR (neutrophil / lymphocytes ratio)	0.687	0.021	0.458	0.917
NYHA class	0.703	0.029	0.543	0.868

33] and long-term [17, 22]). Furthermore, the study by Westenbrink et al. has proven that patients with ischemic HF have a profound and general bone marrow dysfunction [34] affecting simultaneously many cell lines that is correlated to HF severity (assessed by NT-proBNP and NYHA class). Therefore lymphopenia might be caused both by generalized hematopoetic dysfunction and chronic sympathetic activation in HF patients.

The ability of lymphopenia to predict rehospitalizations in the *vulnerable phasis* (6 months) was not studied yet. Our results prove lymphopenia predicts the need for rehospitalization for patients with ischemic and non-ischemic HF in the first 6 months after discharge.

NLR was recently proven to be a strong predictor for in-hospital [26] and long-term post-hospitalization [22] mortality in patients with acute [23] or chronic HF [24, 27], independent of other hemodynamic cardiovascular risk factors [19]. NLR provides information on two pathophysiologic pathways – neutrophils (linked to rapid immunologic response and increased levels of free radicals, responsible for tissue injury) and lymphocytes (linked to chronic adaptive immune response).

Durmus et al. [20] have found that in patients with acute decompensated HF the NLR is correlated with left ventricular systolic performance (assessed through ejection fraction). In our study we found a positive correlation between NLR and NT-proBNP level. We also found a significant difference in NLR between patients in classes NYHA II vs. III but no difference between patients in classes NYHA III and IV.

Our study has several limitations: it didn't have relatively similar numbers of patients in NYHA classes II-IV, it didn't follow-up the variation in WBC-DF and NLR during hospitalization and after compensation and it didn't analyze the predictive capacity of NLR at the time of hospital discharge.

Given that mortality and the need for rehospitalization in HF are still high in the *vulnerable phase,* it is extremely important to identify the high-risk patients. This is why many studies have analyzed the ability of clinical or biological markers to predict the post-hospitalization outcome of HF patients.

Conclusions

Performing WBC-DF and NLR at hospital admission is a simple, accessible and cost-efficient method to predict the event-free interval until rehospitalization in patients with ischemic HF.

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