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RESEARCH ARTICLE

The Association between Peripheral Blood Cells and the Frailty Syndrome in Patients with Cardiovascular Diseases

Constantin Bodolea[1,2], Elisabeta I. Hiriscau[2,3,*], Elena-Cristina Buzdugan[4,5], Alin I. Grosu[4,5], Laurențiu Stoicescu[4,5], Ștefan Vesa[6] and Omar Cauli[7]

[1]ICU Department, "Iuliu Haţieganu", University of Medicine and Pharmacy, Cluj-Napoca, Romania; [2]ICU Department, "Iuliu Haţieganu" University Clinical Municipal Hospital, Cluj-Napoca, Romania; [3]Nursing Department, "Iuliu Haţieganu", University of Medicine and Pharmacy, Cluj-Napoca, Romania; [4]Internal Medicine Department, "Iuliu Haţieganu"University of Medicine and Pharmacy, Cluj-Napoca, Romania; [5]Cardiology Unit, University Clinical Municipal Hospital, Cluj-Napoca, Romania; [6]Department of Clinical Pharmacology,"Iuliu Haţieganu"University of Medicine and Pharmacy, Cluj-Napoca, Romania; [7]Department of Nursing, University of Valencia, Valencia, Spain

Abstract: ***Background*:** Frailty syndrome is characterized by multisystem dysregulation frequently found in older individuals or even in younger patients with chronic disabling diseases such as cardiovascular diseases.

***Objective*:** To determine whether peripheral blood cell count, and its subpopulations, red blood cell and platelets, morphology and different ratios (neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio and red blood distribution width-to-platelet ratio) are associated with cardiac frail patients, and through this to improve the prediction of frailty status in patients with cardiovascular diseases.

***Methods*:** An observational, retrospective, cohort study enrolling 179 patients with cardiovascular disease divided into two groups: non-frail group (100 pts) and frail group (79 pts), a cohort detached from the Frail.RO study. The frailty was evaluated based on the Fried criteria; haematological markers, sociodemographic data, and variables related to cardiovascular diseases and comorbidities were also recorded.

Results: Lower lymphocytes, platelet count, and neutrophil-to-lymphocyte ratio were significantly associated with a more severe frailty syndrome. Regarding red blood cells, haemoglobin concentration and red cell distribution width significantly correlated with the severity of the frailty syndrome. Receiver operating characteristic curve analysis for these markers associated with the frailty syndrome revealed an acceptable sensitivity of 66 % and specificity of 65% to identify frail individuals. Malnutrition and hypercholesterolemia are relevant predictors for identifying frailty in hospitalized cardiovascular patients.

***Conclusion*:** The evaluation of peripheral blood cell composition routinely measured in clinical practice can represent a valuable, but limited indicator, to diagnose frailty syndrome and eventually, the effects of interventions in frail patients with cardiovascular diseases.

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1. INTRODUCTION

Frailty is a common geriatric syndrome affecting many elderly people, characterized by the decline of the physiological homeostatic reserve, resulting in a vulnerability to adverse outcomes, and consequently, to an inadequate response to different stressors [1, 2]. Regardless of the different frailty assessment tools, most of the studies have shown a strong association between the frailty syndrome and prolonged hospital stays, functional decline at discharge, increased healthcare costs and a significant mortality rate on medium- and long-term in the elderly and very ill inpatients [3-6].

From a pathophysiological point of view, frailty syndrome is characterized by multiple dysfunctions at musculoskeletal, neuroendocrine, haematological, immune and cardiovascular levels, due to a state of chronic, low-grade and non-infectious inflammation, expressed through the in-

*Address correspondence to this author at the Department of Nursing, Faculty of Medicine, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania; Tel/Fax: +40-743-720-545;
E-mails: ioanahiriscau@gmail.com, ihiriscau@umfcluj.ro

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creased inflammatory biomarkers such as C-reactive protein (CRP), interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α). Evidence supporting the systemic inflammation in the pathogenesis of frailty has been recently published in a study pointing out the temporal relationship between elevated inflammatory markers in midlife and an increased risk for developing frailty syndrome in old age [7]. Moreover, arguments have arisen, demonstrating a progressive relationship between the frailty syndrome, inflammation, and the malfunctioning of the immune system [7-10], this relationship yielding consequences upon the composition of the peripheral white blood cell (WBC) count.

Studies undertaken in community–dwelling older women have demonstrated an independent association of neutrophil or monocyte counts and frailty syndrome, pro-inflammatory, other than IL-6 mediated, as the underlying mechanism [11-13]. In prostate cancer patients undergoing androgen deprivation therapy, it has been observed that peripheral inflammatory markers are modified in those with a frail condition [14].

Changes in leukocyte count have been found to be associated with the physical phenotype of frailty in older individuals with good mobility levels [15]. No significant correlation between lymphocyte, eosinophil and basophil counts, and frailty occurrence has been identified in community-dwelling disabled older women [16]. Previously, the same authors demonstrated an association between the increased neutrophils level and the decrease in lymphocytes count with a five–year mortality [17], and a positive correlation between the increase in the total WBC and the incidence of frailty [18].

Neutrophil-to-lymphocyte ratio (NLR) was reported as an early marker of cardiovascular risk and a systemic endothelial dysfunction in asymptomatic individuals [19]. In patients with cardiovascular diseases (CVD) NRL has been found to be an independent predictor of the outcome in stable coronary artery disease (CAD), as well as a predictor of short- and long-term mortality in patients with acute coronary syndromes. High NRL values were associated with higher mortality in patients admitted with advanced heart failure (HF) [20].

Eosinopenia has been identified as a sensitive biomarker of sepsis, being strongly associated with an outcome and mortality rate in critically ill patients with a stroke [21], vascular surgery [22], traumatic intracranial hemorrhage [23], bacteremia [24] and *Clostridium difficile* infection [25].

Red cell distribution width (RDW) has been validated in many previous studies as an independent predictor of morbidity and mortality in HF [26], acute respiratory distress syndrome [27], critically ill surgical and non-surgical adults [28-30] and pediatric patients [31]. RDW is frequently associated with markers of chronic low-grade inflammation, oxidative stress, malnutrition, increased CRP, IL-6 and N-terminal pro–brain natriuretic peptide, which are common features described in frail patients. Only one previous study demonstrated an association between the RDW modifications and the occurrence of frailty in elderly patients [32]. Protein malnutrition, a frequent frailty-related condition, causing the alteration of the hematopoiesis process, leads to anemia, leukopenia, and bone marrow hypoplasia [33]. Due to the decrease of erythrocytes production as a result of protein malnutrition, it is likely that modified RDW levels are found in patients with chronic inflammation.

Platelets (PLT) regulate the homeostatic function and inflammatory response, which are altered in elderly frail adults and may increase the risk of CVD by promoting prothrombotic and pro-inflammatory status in this population [34]. The mechanism of PLT activation is due to oxidative stress, highly expressed in frail elderly patients [35].

Considering that PLT and lymphocytes are strongly involved in the inflammation process, the platelet-to-lymphocyte ratio (PLR) was used as an indicator of inflammation in oncological, cardiac [36, 37] and diabetic patients [38]. PLR was also found to be associated with an increased risk of sarcopenia in older adults.

RDW–to-platelet ratio (RPR) is a marker that has been found to be suggestive of a risk predictor of liver fibrosis [39]. This parameter has never been used in the frailty assessment in patients with CVD.

1.1. Frailty in Cardiac Patients

Identifying frailty syndrome in CVD patients has relevant implications for clinical practice. The presence of frailty may limit the benefits of surgery in some cardiac diseases because of the risk of falls, functional and cognitive impairment, or other specific frailty-related changes that can increase the risk of adverse outcomes [40, 41]. Therefore, frailty assessment may be particularly useful in risk prediction and decision-making in clinical settings.

In cardiac pathology, frailty is generally associated with negative outcomes in HF and an increased mortality [42]. Approximately 25% of older patients with HF exhibit evidence of frailty, which in turn aggravates the prognosis of the disease [43, 44]. Prestigious cardiology and geriatric societies support the idea that future guidelines should consider the multimodal assessment of frailty in the management of HF patients [45].

Although the diagnosis of frailty is generally made on criteria depending on its conceptualization, the use of frailty assessment for clinical decision-making regarding the care planning and management is rarely applied in clinical settings [46]. While frailty assessment in cardiac patients has provided a value-added utility in clinical decision-making, it remains insufficiently performed in cardiology settings or is not implemented at all. This might be explained, firstly, by the lack of evidence and guidance on how to incorporate frailty status information within a cardiology setting, and on the other hand, by the selection of the most appropriate frailty instrument that can be used specifically for cardiac patients [47]. Given that the frailty syndrome is considered a chronic low-grade inflammation, an evaluation of the blood composition, as well as the alterations in WBC counts and their subtypes, might be valuable indicators in identifying frailty early in CVD patients. The major advantage is that the blood tests are easily and routinely performed in any clinical setting.

The proportion of peripheral differential leukocytes count (neutrophils, lymphocytes, eosinophils), RDW, PLT count, some specific ratios such as the NLR, PLR and RPR, have been lately considered accessible and reliable markers of the immunological response in different pathological conditions (neoplasms, chronic liver disease, diabetes, CVD). Although the association between frailty syndrome and the changes in peripheral WBC, RDW or PLT has already been demonstrated in some studies, cardiac patients have been less investigated in this regard. A higher incidence of the frail condition has been reported among the patients with HF, but a relation between frailty and the changes in the blood cell count has not been searched systematically in cardiac patients. Considering frailty a clinical condition of reduced resilience in elderly, it is expected that the presence of frailty and its severity are related to the modified level of inflammation of this population. We chose to test this hypothesis in hospitalized patients with CVD who were assessed prior to the frailty detection. In conjunction with this condition, we analyzed the WBC count, the different leukocyte subtypes, haemoglobin (Hb) level, PLT count, RDW and related nutritional status.

We conducted a retrospective study aiming to investigate the relationship between the peripheral differential leukocytes count (neutrophils, lymphocytes, eosinophils, basophiles and monocytes), RDW, NLR, PLR, and RPR as a hallmark of immune response, and severity of frailty in patients suffering from CVD. The main objectives were the following:

1. Evaluation of the relationship between total WBC count, the leukocytes subtypes, RDW, NLR, PLR, RPR, and frailty syndrome in CVD patients.
2. Identification of the diagnostic accuracy of the haematological biomarkers associated with the frailty syndrome to identify frail patients.

2. METHOD

2.1. Study Design

An observational, retrospective, cohort study was carried out to identify changes in the complete blood count (CBC) including Hb, total WBC count, leukocytes subtypes, PLT count, and RDW in frail patients with CVD. This study is part of the Frail.ro mother-study, an institutional project developed between 2016-2019 within the University Clinical Municipal Hospital, Cluj-Napoca, Romania. The main objective of the Frail.ro study aimed to identify the incidence of the frailty syndrome in the hospitalized population in different medical units (geriatric, cardiology, surgery and urology) of the above mentioned institution.

The study was conducted in accordance with the ethical principles stated by the Declaration of Helsinki in conducting medical research. We obtained written consent from each patient enrolled in the Frail.ro study after they had been informed about the purpose of the study and all the procedures involved. Patients were also informed about the confidentiality and its limitations regarding the provided data. The study protocol was approved by the Local Ethics Committee of the University Clinical Municipal Hospital, Cluj-Napoca, Romania (reference Protocol nr. 5/2017, the approval of the study 20.02.2017).

According to the research protocol, the assessment for identifying frailty in hospitalized cardiac patients was carried out between July-December 2017.

The inclusion criteria for the enrolment in the study were as follows:

- Patients admitted to the cardiology unit during July-December 2017;
- Patients previously diagnosed with heart symptoms or CVD ((Ischemic heart disease, Percutaneous transluminal coronary angioplasty (PTCA), Hypertension, Valvulopaties, Arrhythmias, Conduction disorders, Pacemaker, Congestive heart failure));
- Patients who agreed to participate and be assessed for frailty, and signed the informed consent.

The patients' cardiovascular conditions were established according to clinical and history antecedents and definitions proposed by guidelines as following [45]:

a) Coronary heart disease if antecedent of angina, myocardial infarction (MI), coronary revascularization, or positive stress test was present;

b) Arterial hypertension if systolic blood pressure was >140 mmHg and/or diastolic blood pressure was >90 mmHg;

c) Valvular heart disease if conclusive data was obtained following history, symptomatic status, physical examination, and echocardiography evaluation;

d) HF if symptoms such as breathlessness were accompanied by signs such as pulmonary crackles and peripheral edema, in the presence of structural and/or functional cardiac abnormality, and a decreasing of cardiac output, and/or increasing in intracardiac pressures at rest or during stress;

e) Atrial fibrillation or any other arrhythmias and/or cardiac conduction disorders if the diagnosis was present in a medical report or any electrocardiographic registration.

Exclusion criteria were dementia condition and delirium, chronic inflamatory diseases and individuals that refused the comprehensive assessment for frailty.

2.2. Measurement of Frailty

The measurement of frailty was performed based on the Fried criteria [1]. This model operationalizes physical frailty through the measurement of five characteristics: loss of weight, exhaustion, low physical activity, weak grip strength and slow walking speed.

1. Unintentional weight loss: the criterion was considered positive if the weight loss was 4.5 kg or more in the last year.
2. Self-reported exhaustion: the criterion was considered positive if the patient answered yes to either of the following questions extracted from the Center for Epidemiologic Studies Depression Scale [48]: “I felt that everything I did was an effort” or “I could not get going”.

Patients responding positively were asked "How often in the last week did you feel this way?" the possible responses varying from 0 to 3. The score 0 = rarely or none of the time (<1 day) or 1 = some or a little of the time (1–2 days) was considered negative for this criterion; the score 2 = a moderate amount of the time (3–4 days) or 3 = most of the time was considered positive for the exhaustion criterion within the frailty assessment. We defined the severity of exhaustion as the sum of these item scores.

3. Low physical activity: physical activity (PA) is defined as any bodily movement produced by skeletal muscles that result in energy expenditure (kilocalories), which varies continuously from low to high [49]. PA was quantified using the International Physical Activity Questionnaire (IPAQ) short form [50] translated in the Romanian language for assessing PA level undertaken across the following dimensions: leisure time, domestic and gardening (yard) activities, work, and transportation. Specific types of activity assessed within these four dimensions were walking (at work, at home, to travel from place to place), moderate-intensity activities (carrying light loads, bicycling at a regular pace, or doubles tennis) and vigorous-intensity activities (heavy lifting, digging, aerobics, or fast bicycling). We assessed the PA level reported to 24 hours (hours per day or minutes per day spending on a specific activity) and to a week (days per week). For the computation of the MET-min/week we used the IPAQ automatic report provided by the IPAQ platform (created by Andrea Di Blasio, Francesco Di Donato and Christian Marzocco at "Endocrinology Unit" of the "Department of Medicine and Aging Sciences" of "G. d'Annunzio" University of Chieti-Pescara (Italy)). In assessing the PA level, each patient was asked to provide corresponding information prior to the admission in the hospital.

The scoring categorizing the level of PA was the following:

- Low physical activity: individuals who do not meet criteria for moderate or vigorous activity.
- Moderate physical activity: 3 or more days of vigorous activity of at least 20 minutes per day or 5 or more days or any combination of walking, moderate- or vigorous-intensity activities achieving a minimum of at least 600 MET-min/week.
- High physical activity: vigorous-intensity activity on at least 3 days and accumulating at least 1500 MET-minutes/week or 7 days or any combination of walking, moderate- or vigorous-intensity activities achieving a minimum of at least 3000 MET-min/week.

4. Slowed motor performance (slowness) was measured by performing the 4-6m speed test, adjusted for sex and height [51].
5. Weakness was measured as the mean grip strength of the dominant hand three times with a Jamar hydraulic dynamometer [52].

2.3. Variables

At baseline, we collected socio-demographic data, age and gender, origin environment, level of education, marital status, and the number of medications consumed daily (a number equal or more than three medications daily being considered a positive criterion). Body mass index (BMI = weight/height^2) was reported according to the classification proposed by Mini Nutritional Assessment (which was used for the nutritional status evaluation within the Frail.ro mother study): 0 = BMI less than 19; 1 = BMI 19 to less than 21; 2 = BMI 21 to less than 23; 3 = BMI 23 or greater. We measured frailty in each study participant based on the Fried criteria [1]. Other variables considered within the frailty assessment included those related to cardiac disease, comorbidities, measured by their presence or absence (categorical variables). We also used Charlson Comorbidy Index (CCI), which had a prognostic value for cardiovascular complications after acute myocardial infarction, being considered an independent predictor of long-term survival. Nutritional status was appraised with Mini Nutritional Assessment (MNA) which distinguishes between patients with an adequate nutritional status (MNA = 24-30), at risk of malnutrition (MNA = 17-23.5) and malnourished (MNA < 17). MNA shows a high sensitivity (96%) and specificity (98%), and a predictive value of 97% [53].

2.4. Measurement of the Blood Markers

Fasting blood samples are a routine procedure and were performed in each patient admitted to the cardiology department. Blood samples were collected by venipuncture in dry collection tubes and EDTA tubes after the patient was considered eligible and his/her consent had been obtained for participating in the study. The WBC and the fractions (neutrophils, lymphocytes, eosinophils, basophils, and monocytes), Hb, RDW, PLT, were obtained after the blood analysis had been performed in the hospital laboratory (SYSMEX XT 4000i). RDW (normal range 9-15%) was expressed as continuous and categorical variable in quartile (Q1= 0-13.3%, Q2 = 13.3%-13.9%, Q3 = 13.9%- 14.9%, Q4 = > 14.9%). We calculated NLR, PLR and RPR based on the results provided by the hospital laboratory. NLR, PLR and RPR were also analyzed as continuous and categorical variables (Table **1**).

2.5. Statistical Analysis

Continuous variables were expressed as the mean and standard deviation, and categorical variables as the absolute value with their percentage. For our study we considered two groups, the non-frail group, which included patients fulfilling 2 frailty criteria or less, and the frail group with 3 or more criteria. Independent-samples t-tests were performed to compare the groups. We used the chi-squared test for comparing categorical variables. The linear correlations between continuous variables have been evaluated by using the Spearman rank test; the non-parametric Mann-Whitney U test was used for the differences between the frail vs the non-frail group for continuous data for independent samples. Predictive models were developed as follows: variables showing high correlation were checked independently in a linear regression

Table 1. Distribution in quartiles of ratios variables (NRL, PLR, RPR).

Markers	Quartile 1	Quartile 2	Quartile 3	Quartile 4
NLR	0-2.1	2.1-3.1	3.1-4.4	> 4.4
PLR	0-100.4	100.4- 136.9	136.9- 194.3	> 194.3
RPR	0-0.0485	0.0485- 0.0594	0.0594- 0.0747	> 0.0747

and then were entered in the binary logistic regression to build the model. Regression models were subsequently adjusted for age and gender. Receiver operating characteristic (ROC) curve analysis was used to identify the diagnostic accuracy of blood tests for frailty. Statistical significance was set at a *P*-value of less than 0.05. Data analyses were carried out using the IBM SPPS Statistics 22.

3. RESULTS

3.1. Baseline Characteristics

Of the 179 patients enrolled in the study, 78 (43.6%) were men and 101 (56.4%) were women, aged between 23 and 90 years old. The sample was divided into two groups, the non-frail group, which included 100 (55.9%) patients, and the frail group including 79 (44.1%) patients identified with a frail condition; all patients had been previously diagnosed with heart symptoms or cardiac disease. 109 patients of the total sample had a normal nutritional status, 59 were identified at risk of malnutrition, and 11 were malnourished. The sample baseline characteristics are illustrated in Table **2**.

3.2. Comparisons between Groups

No differences between the groups were observed related to sex and the origin environment. The level of education and marital status were found statistically significant ($p<0.001$). Regarding the CVD between the non-frail and frail group significant differences were identified related to ischemic cardiac disease ($p<0.002$), cardiac valvulopathies ($p<0.001$), arrythmias ($p<0.01$), and congestive heart failure ($p<0.001$); related to comorbidities were hypercholesterolemia ($p<0.005$), pulmonary disease ($p<0.008$), cerebrovascular diseases ($p<0.05$), and diabetes ($p<0.05$). The results are presented in Table **3**.

3.3. Correlations between Frailty, Blood Parameters, Socio-Demographic Data, Polypharmacy, CCI, and Nutritional Status

A negative correlation was found between the frailty score and lymphocytes (Rho = -0.27, $p< 0.001$; Spearman test; Fig. **1**). Between frailty and NLR a positive correlation was found (Rho = 0.26, $p<0.001$; Spearman test). No other correlations were observed between frailty, total WBC count and leukocytes subtypes: leukocytes (Rho = -0.04, p = .59), neutrophils (Rho = 0.08, p = .27), basophiles (Rho = -0.01, p = .89), monocytes (Rho = 0.003, p = .98) and eosinophils (Rho = -0.033, p = .67).

Related to haematological parameters, a negative correlation was found between frailty and Hb (Rho = - 0.22, $p<0.003$), and positive correlations between RDW-CV (Rho = 0.29, $p<0.001$; Spearman test), RDW-SD (Rho = 0.25, $p<0.001$; Spearman test), and RPR (Rho = 0.23, $p<0.002$; Spearman test). A positive correlation has been identified between PLT count (Rho = - 0.17, $p<0.02$; Spearman test) and frailty.

Positive correlations were observed between frailty and age (Rho = 0.35, $p<0.001$; Spearman test), polypharmacy (Rho = 0.25, $p<0.01$; Spearman test) and CCI (Rho = 0.26, $p<0.001$; Spearman test). Nutritional status was found to be positively correlated with frailty (Rho = 0.34, $p<0.001$, Spearman test), and no significant correlation has been observed between frailty and BMI (Rho = - 0.14, p= .053, Spearman test) (data are provided in supplementary materials).

Comparing the non-frail vs frail group significant differences (Mann-Whitney *U* test) have been observed regarding the age, education level and marital status ($p<0.001$). Related to immunological blood parameters, significant differences (Mann-Whitney *U* test) between groups have been identified related to lymphocyte count (Lc) ($p<0.001$), NLR ($p<0.001$), Hb ($p<0.003$), RDW-CV ($p<0.001$), RDW-SD ($p<0.001$), PLT count ($p=0.02$), and RPR ($p<0.001$). No statistical significance was found for PLR. Polypharmacy, CCI and nutritional status were also found to be statistically significant ($p<0.001$; Mann-Whitney *U* test). The data is presented in Table **4**.

Related to cardiac diseases, our data showed significant differences between groups for ischemic cardiac disease ($p=0.002$), arrhythmias ($p=0.007$), cardiac valvulopaties and congestive heart failure ($p<0.001$; Mann-Whitney *U* test); as comorbidities, statistically significant were diabetes and cerebrovascular diseases ($p<0.05$; Mann-Whitney *U* test), respectively, hypercholesterolemia and pulmonary disease ($p<0.005$; Mann-Whitney *U* test) (data shown in supplementary materials).

Between NLR and frailty an association in Q1 ($p<0.003$) and Q4 ($p<0.003$), respectively was found, between RPR and frailty in Q1 ($p<0.009$) and Q4 ($p<0.006$). Patients with the frail condition had a statistically significant higher RDW level in Q1 ($p<0.001$) and Q4 ($p<0.001$) than the non-frail patients.

Table 2. Baseline characteristics of the sample including in the analysis. Quantitative variables are expressed by mean (M) and standard deviation (SD), and qualitative variables as a percentage.

Baseline Characteristics	
Age (years)	M = 65.07 SD = 12.9
Gender	Males = 78 (43.6%) Females = 101 (56.4%)
Origin environment	Rural = 92 (51.4%) Urban = 87 (48.6%)
Marital/Civil status	Married = 115 (64.2%) Single = 64 (35.8%)
Level of education	Elementary education = 75 (41.9%) Secondary education = 76 (42.5%) Bachelor (and over) = 28 (15.6%)
BMI (kg/m^2)	0 (BMI<19) = 0.6% 1 (BMI 19-21) = 3.9% 2 (BMI 21-23) = 10.1% 3 (BMI >23) = 85.5%
Nutritional status	0 (normal nutritional status) = 60.9% 1 (at risk of malnutrition) = 33% 2 (malnourished) = 6.1%
Polypharmacy expressed as a number of daily intake medications (considered positive $\geq$3 medications daily)	Polypharmacy = 132 (73.7%)
Number of frail (fulfilled ≥3 Fried criteria) and non-frail (fulfilled 0, 1 or 2 Fried criteria) individuals	Frail = 79 (44.1%) Non-frail = 100 (55.9%)
Cardiac Diseases	
(Documented) Ischemic heart disease	111 (62%)
Percutaneous transluminal coronary angioplasty (PTCA)	5 (2.8%)
Arterial hypertension	143 (79.9%)
Cardiac valvulophaties	111 (62%)
Arrhythmias	93 (52%)
Conduction disorders	13 (7.3%)
Pacemaker	10 (5.6%)
Congestive heart failure	71 (39.6%)
Comorbidities	
Cerebrovascular/Neurological diseases	31 (18.3%)
Diabetes	52 (29.1%)
Hypercholesterolemia	66 (36.9%)
Hypertriglyceridemia	37 (20.7%)
Digestive disorders	73 (40.8%)
Pulmonary disease	68 (37%)
Kidney disease	85 (47.5%)
Neoplasia	32 (17.9%)

Table (2) contd….

Blood Counts	
Leukocytes (10^9/L)	8.1 ± 4
Neutrophils (10^9/L)	5.4 ± 2.2
Lymphocytes (10^9/L)	1.8 ± 0.7
Monocytes (10^9/L)	0.7 ± 0.3
Eosinophils (10^9/L)	0.2 ± 0.2
Basophils (10^9/L)	0.02 ± 0.01
Platelets (10^9/L)	241.5 ± 73
RDW-CV (%)	14.5 ± 1.8
RDW-SD (fl)	47.2 ± 7.2
Neutrophils/Lymphocytes ratio	3.5 ± 2.2
Platelets/Lymphocytes ratio	158 ± 83
RDW-CV/platelets ratio	0.07 ± 0.065

RDW, red blood cell distribution width; PLT, platelets; NLR, neutrophils-to-lymphocytes ratio; PLR, platelets-to-lymphocytes ratio, RPR, red blood cell distribution width-to-platelets ratio, BMI, Body Mass Index.

Table 3. Comparison of non-frail and frail groups regarding cardiac diseases and comorbidities.

	Non-frail (n=100)	**Frail (n=79)**	**p value**
Cardiac diseases			
(Documented) Ischemic cardiac disease	52	59	**0.002**
Percutaneous Transluminal Coronary Angioplasty (PTCA)	2	3	0.65
Arterial hypertension	76	67	0.19
Cardiac valvulopathies	51	60	**0.001**
Arrhythmias	43	50	**0.01**
Conduction disorders	8	5	0.76
Pacemaker	5	5	0.75
Congestive heart failure	36	51	**0.001**
Comorbidities			
Cerebrovascular/Neurological diseases	10	21	**0.048**
Diabetes	23	29	**0.049**
Hypercholesterolemia	46	20	**0.005**
Hypertriglyceridemia	21	16	1.000
Digestive disorders	43	30	0.542
Pulmonary disease	29	39	**0.008**
Kidney disease	45	40	0.547
Neoplasia	15	17	0.326

Statistically significant **P values** (P<0.05) are indicated in bold.

Table 4. Comparisons of non-frail and frail group (Mann-Whitney *U* test). Quantitative variables are expressed by mean (M) and standard deviation (SD), and qualitative variables as a percentage.

-	Frail Condition	*N*	Mean Value	SD	*P*
Age (years)	Non-frail	100	61.23	11.673	**0.001**
	Frail	79	69.92	13.002	
Education (years)	Non-frail	100	11.08	3.32	**0.001**
	Frail	79	8.59	3.76	
Marital status (married)	Non-frail	76/100 (76%)	-	-	**0.001**
	Frail	39/79 (49, 4%)			
Leukocytes	Non-frail	100	7.953	2.329	0.58
	Frail	79	8.258	5.492	
Neutrophils	Non-frail	100	5.254	2.228	0.26
	Frail	79	5.530	2.178	
Lymphocytes	Non-frail	100	1.945	0.759	**<0.001**
	Frail	79	1.568	0.700	
Monocytes	Non-frail	100	0.665	0.225	0.97
	Frail	79	0.688	0.310	
Eosinophils	Non-frail	100	0.154	0.119	0.66
	Frail	79	0.176	0.205	
Basophils	Non-frail	100	0.028	0.015	0.88
	Frail	79	0.029	0.018	
Haemoglobin	Non-frail	100	13.938	1.803	**0.003**
	Frail	79	12.915	2.346	
RDW-CV	Non-frail	100	14.013	1.745	**<0.001**
	Frail	79	14.198	1.835	
RDW-SD	Non-frail	100	45.658	6.245	**0.001**
	Frail	79	49.249	7.805	
PLT	Non-frail	100	254.00	70.608	**0.022**
	Frail	79	225.65	73.496	
NLR	Non-frail	100	3.2	2.17	**0.001**
	Frail	79	4.1	2.19	
PLR	Non-frail	100	153.203	84.680	0.21
	Frail	79	164.134	79.776	
RPR	Non-frail	100	0.059	0.021	**0.001**
	Frail	79	0.086	0.092	
Polypharmacy (≥3 medications daily)	Non-frail	64/100 (64%)	-	-	**0.001**
	Frail	68/79 (86.1%)			
Charlson Comorbidity Index	Non-frail	100	4.93	2.306	**0.001**
	Frail	79	6.33	2.62	
Nutritional status	Non-frail	100	0.27	0.489	**0.001**
	Frail	79	0.68	0.671	
BMI	Non-frail	100	2.87	0.418	0.053
	Frail	79	2.72	0.619	

Statistically significant **P values** ($P<0.05$) are indicated in bold.

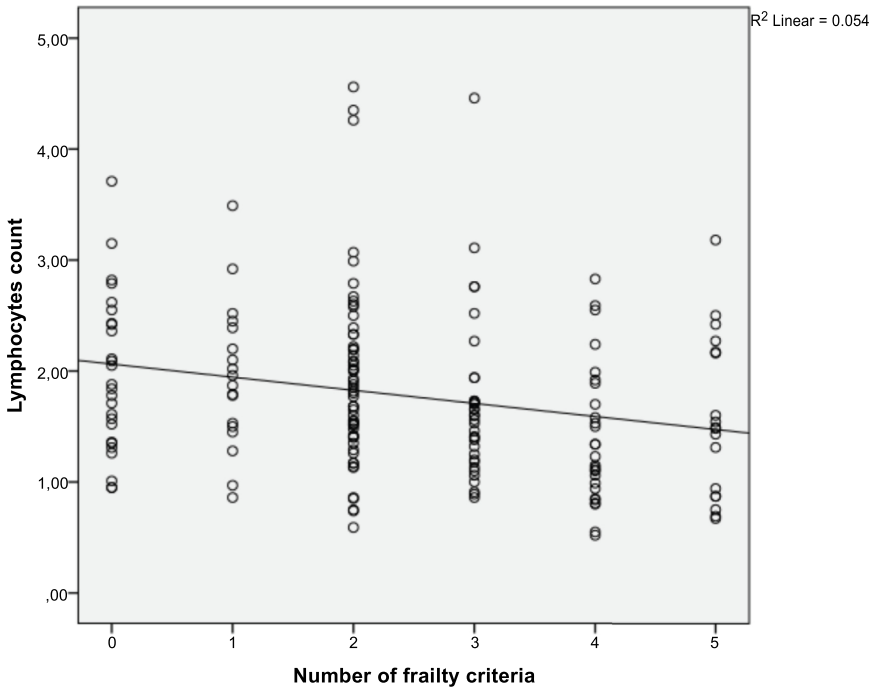

Fig. (1). Correlation between lymphocytes count and frailty criteria. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

All the predictors (variables showing correlation with frailty) were entered one by one in the linear regression for identifying confounders; the interest variables were selected and then univariate and stepwise multivariate logistic were performed to determine independent factors for frailty. Covariates included CVD (ischemic heart disease, arrhythmias, valvulopaties, and congestive heart failure), comorbidities (diabetes, cerebrovascular diseases, hypercholesterolemia, and pulmonary disease), socio-demographic data, polypharmacy, CCI, and nutritional status. The variables showing statistical significance were entered into binary logistic regression. Within this model 80, 4 % of the variance from the outcome variable (frailty status) was explained by the predictors. The B coefficients for nutrition and hypercholesterolemia were positive and statistically significant with corresponding Odd ratios over 1. Although the NRL, polypharmacy, and CCI contributed to the variance, they are not statistically relevant. The data is presented in Table **5**.

Comparison of ROC curves for the discrimination performance of lymphocytes, Hb, and PLT count (observed data tend to decrease for frailty) is shown in Fig. (**2**), respectively, the ROC curves for the accuracy test of RDW-CV, RDW-SD, NLR and RWD/PLT (observed data tend to increase for frailty) are shown in Fig. (**3**). Area under the curve values were the following: Lymphocytes (0.661), Hb (0.629), PLT (0.600), RDW-CV (0.687), RDW-SD (0.644), RPR (0.647), and NLR (0.651). The RDW cut-off value of 13.95% corresponds to a sensitivity of 65, 8% and a specificity of 65% for a frail condition.

4. DISCUSSION

In this article we present the results from a retrospective, cohort study, conducted on hospitalized patients with CVD regarding the relationship between the frailty and immunological response expressed by changes in WBC count, peripheral differential leukocytes count, Hb, PLT and RDW. We investigated whether the above mentioned markers or a combination among them can be reliable criteria for identifying frailty in cardiac patients.

The CBC has been proved to be an important biological marker for the prediction of incident all-cause mortality in a moderate- to high-risk cardiovascular population. In a cohort study including patients with CAD, Anderson *et al*. [54] analyzed the CBC parameters, developing a risk score model that demonstrated an excellent predictive ability (AUC 0.712) of concurrently considered total WBC count, WBC subtypes (monocyte, neutrophil and lymphocyte counts), PLT count, hematocrit, mean corpuscular volume, and RDW. In their analysis WBC count, PLT and RDW have been found to be predictive markers within the risk score for all-cause mortality at 30 days and one year, not only for the study population, but also for those without CAD. Consistently with this data, we report the relevant changes in CBC only in the PLT count ($p=0.022$) and RDW ($p<0.001$) related to frailty, the WBC count alone ($p=0.58$) showing no significance in our analysis. The relevant change within leukocytes subtypes in relation with the frail condition was LYc, this indicator being negatively correlated with the severity of frailty ($p<0.001$). Regarding the modified level of lymphocytes our data is consistent with those reported by Fernandez-Garrido *et al*. [13] in a study conducted in institutionalized frail elderly women. In their investigation on the relation between the frailty syndrome and immune system alterations, especially the WBC subtypes, the severity of frailty syndrome was inversely associated with a reduced lymphocytes level, but with no other WBC fractions or red blood cell markers. Interestingly here, in comparison with the

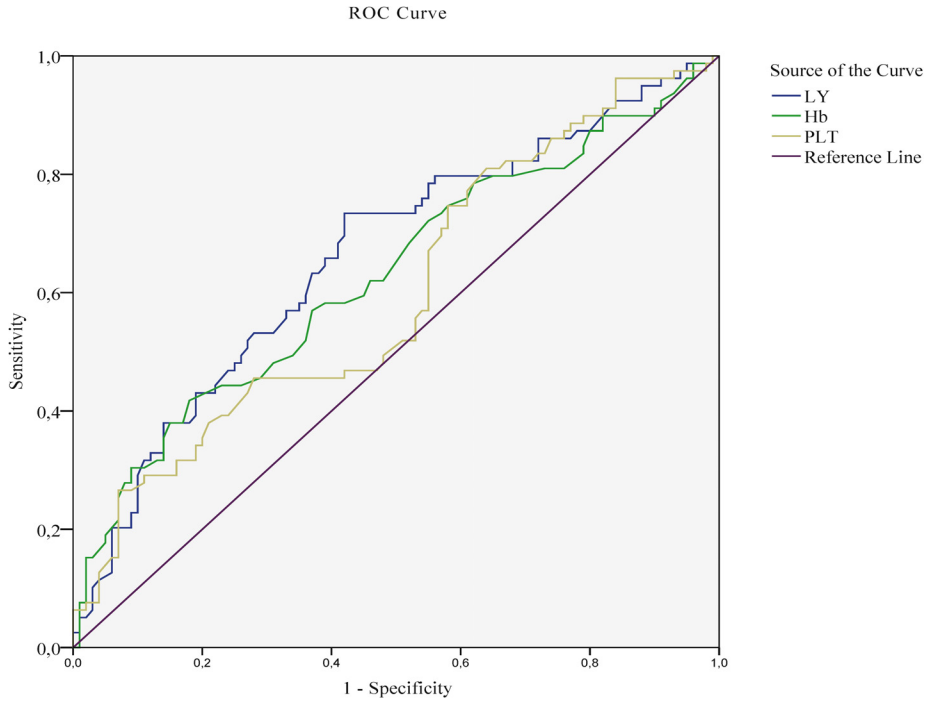

Fig. (2). Comparisons of ROC curves for lymphocytes, hemoglobin and platelets. Hb, Hemoglobin; LY, Lymphocytes, PLT, platelets. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

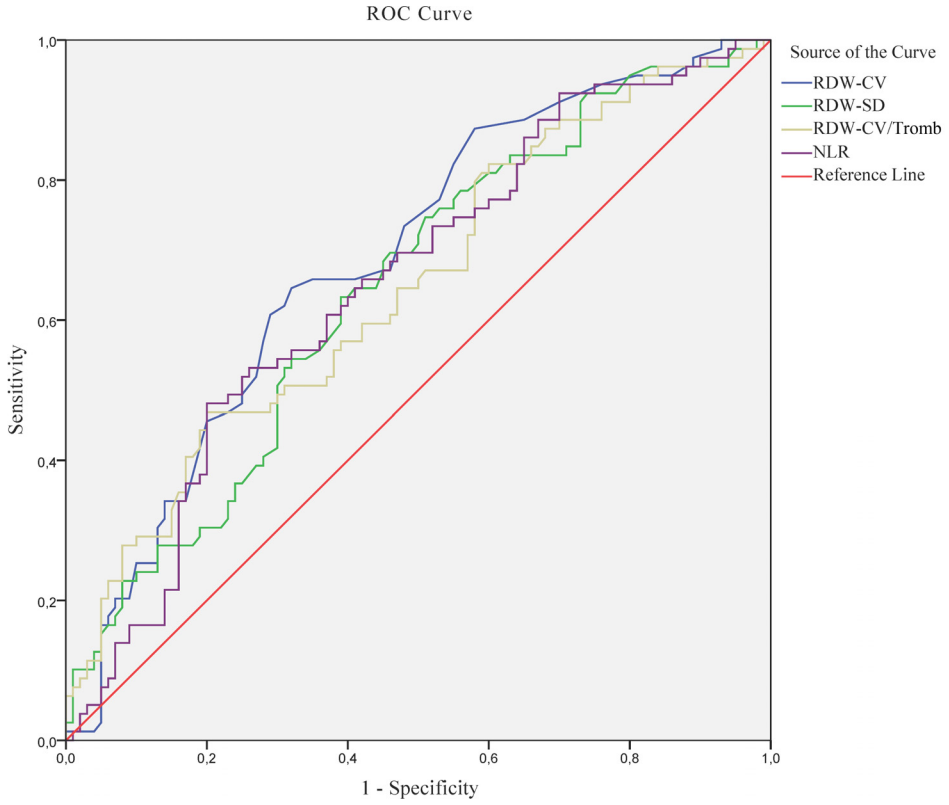

Fig. (3). Comparisons of ROC curves for RDW-CV, RDW-SD, RPR and NLR. RDW, red cell distribution width; RPR, red cell distribution-to-platelets ratio, NLR, neutrophils-to-lymphocytes ratio. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Table 5. Results of predictors including in the model.

Variables	B	Sig. (P value)	Logistic Regression Analysis EXP(B)	95% CI for EXP(B)
Nutritional status	1,260	**0.001**	3,526	1,926-6,454
Educational level	-0,145	**0.005**	0,865	0,783-0,956
Haemoglobin	-0,194	**0.03**	0,823	0,690-0,983
PLT	-0,008	**0.001**	0,992	0,987-0,997
NLR	0,147	0.98	1,158	0,973-1,379
Congestive heart failure	-0,804	**0.03**	0,447	0,220-0,911
Cardiac valvulopathies	-1,053	**0.005**	0,349	0,168–0,723
Pulmonary disease	-1,028	**0.005**	0,358	0,175–0,732
Cerebrovascular diseases	-2,269	**0.003**	0,103	0,023-0,460
Hypercholesterolemia	0,746	**0.04**	2,109	1,045–4,256
Diabetes	-0,945	**0.02**	0,389	0,170–0,886
Polypharmacy	-0,867	0.70	0,420	0,164-1,074
CCI	0,064	0.40	1,066	0,917-1,240

Statistically significant **P values** (P<0.05) are indicated in bold.

results reported above, the critical level of lymphocytes in relation with the number of frailty criteria in our study, a slight change in LYc corresponding to 0, 1 or 2 criteria and an important decreased level at the occurrence of 3 criteria was observed. This might have a clinical relevance in approaching CVD patients with frailty syndrome; a decreased LYc could signalize a potential depreciation of frailty which can be limited, at least temporarily, through implementing the appropriate interventions. In patients with MI, a high neutrophil count and a low LYc were identified as stronger independent predictors of cardiovascular death, an elevated NLR being considered the most powerful single WBC count predictor in this regard [20, 55]. In our study, the WBC count and neutrophil count were not found to be statistically relevant with frailty characteristics (p=0.58, respectively, p=0.26). However, the high number of neutrophils has been strongly associated with certain CVDs even within normal limits, especially in HF [56, 57]. A possible explanation of the results obtained in our study is related to the fact that some authors, in a recent study, reported changes in the leukocytes and neutrophils count positively correlated with the evolution of CVD, but without referring to the frailty status [56]. The analysis from our study refers only to the one set of laboratory blood tests performed in hospitalized cardiac patients (during admission), and then the search for potential changes in the WBC subtypes related to the frail condition at that moment. Some prospective studies pointed out the relation between the increase in both the lymphocytes and neutrophils count, in dynamics and the progress of cardiovascular disease [57]. Regarding the NLR, we found this marker significantly higher in CVD patients with a frail condition. NRL was also found to be an important predictor for frailty, the CVD patients with frail condition being 1.15 times (15.8% probability) more likely to have a high NLR level than those without frailty.

Although PLR is considered an important indicator of systemic inflammation [36], especially associated with a greater risk of sarcopenia in geriatric individuals, our findings evidenced no relevant association between PLR and frailty or the risk of being frail.

Unlike Fernandez-Garrido *et al*. in their study performed in institutionalized older individuals (a study sample not specific for individuals with CVD as in the present study), we found a significant Hb alteration between the non-frail and frail group, the Hb level being identified as a strong and independent risk factor for mortality in patients with HF and acute MI [55]. Anemia was defined as Hb concentration less than 13 g/dL in men and less than 12 g/dL in women, respectively. Our findings show a negative correlation between Hb and frailty (p<0.003), 27.8% of frail patients exhibited anemia compared with 9% of the non-frail patients.

In a meta-analysis regarding the RDW and mortality in older adults [57], a higher RDW was strongly associated with the risk of death and cardiovascular events in middle-aged and older adults [58–61]. This association was independent of the Hb concentration, although the association was significantly weaker in anemic than in nonanemic older adults. In this regard, our data reveal no association between the RDW and Hb in both groups, anemic individuals (31 out of 179 patients) (p=0.18) and nonanemic individuals (148 patients) (p=0.53). In our study, the RDW was moderately negatively correlated with Hb (Rho = - 0.39), without any evidence of any statistical interaction detected between both variables at p<0.05. This data is consistent with those re-

ported by Felker at al. (Rho = - 0.27 in the CHARM program[1], respectively, Rh0 = - 0.40 in the Duke Databank[2]), both, Hb and RDW, being significant predictors in all multivariable models after adjustment for all other predictors [60].

RDW is a good quality integrative marker of the malnutrition syndrome associated with inflammation. Correlation between the RDW and inflammation, malnutrition and blood diseases, have been recently emphasized by different studies [62]. We found a positive correlation between the RDW, nutritional status and frail condition, a higher RDW being found in cardiac patients with frailty in comparison with non-frail patients ($p<0.001$). Although a positive correlation between the RDW and nutritional status (Rho = 0.19, p=0.013) was found, no significant correlation was observed when the lymphocytes variable was controlled (Rho = 0.11, p=0.16). Nutritional status proved to be a relevant predictor for frailty; the CVD patients, malnourished or at risk of malnutrition are 3.52 times more likely to be frail than those with a normal nutritional status. Tonelli *et al*. reported an independent relation between elevated levels of RDW and the risk of HF, cardiovascular events and mortality in people with prior MI (without any evidence of HF at baseline) [63]. A high RDW is generally set at 14%, which corresponds to the 95th percentile of RDW for the general population. We found a significant association between the RDW level >14, 9% and frailty characteristics. Although the higher RDW is associated with advancing age, the mechanisms through which RDW increases with age and is associated with mortality remain insufficiently explained; one hypothesis is based on the role played by the oxidative stress and chronic inflammation given that both can reduce red blood cell (RBC) survival, leading to a more mixed population of RBC volumes in the circulation. As a result of inflammation status of frailty, the erythropoiesis is altered, and consecutively, the red blood cell circulation half-life, and red blood cell membrane deformability also, and these factors might increase anisocytosis [64, 65].

A decreased PLT count (along with mean platelet volume) was found to be associated with aging, particularly in the frail population. Even though the mechanism underlying the alteration of the PLT count with increasing age remains unclear, it is considered a reduced effect in hematopoietic stem cells in old age or a reduction of the thromboprotein from birth to adulthood [66]. RPR was referred to in a study conducted by Karakoz *et al*. as an inflammatory biomarker independently associated with the prediction of advanced hepatic fibrosis [39]. Considering frailty as a chronic low-grade inflammation, RPR might play a role in the prediction of frailty status in CVD patients, but further studies are required in this regard.

These haematological markers associated with the frailty syndrome could be useful in monitoring clinical intervention to prevent or delay the progression of the frailty syndrome in patients with CVD.

[1] Candesartan in Heart Failure: Assessment of Reduction in Mortality and Morbidity

[2] Duke databank for Cardiovascular Disease

5. STUDY LIMITATIONS

A few limitations warrant consideration. The study sample included only 179 patients with diverse cardiovascular pathology; due to the small number of participants, we could not group the patients depending on a specific cardiac illness for searching and reporting the blood parameters of interest to the respective disease. This permitted us to report only the biomarkers that characterize the population with CVD in general. On the other hand, the non-frail group included also patients with 1 or 2 frailty criteria, this aspect being able to influence the differences between the non-frail and frail groups related to some analyzed parameters that were at the limit to be relevant for a frail condition. Another shortcoming is related to the data collected. We analyzed only the first set of laboratory data, at the patient's admission in the hospital, but it may be that a serial measurements of the blood cell markers of interest would allow a better characterization of the association of RDW and RPR with a frail condition. Although the antiplatelet agents play an important role in the treatment of patients with acute coronary syndrome, and indirectly influencing the RDW level, we were not mainly focused to collect this data, given that all hospitalized cardiac patients received a minimal dose (75 mg) of Aspirin as part of the treatment protocol.

CONCLUSION

In summary, we made the observation that the evaluation of peripheral blood cells composition has proved to be a valuable, but limited indicator, which when combined with clinical aspects, might be useful in the risk prediction of CVD patients with frailty or at a high risk to be frail.

LIST OF ABBREVIATIONS

CAD	=	Coronary Artery Disease
CBC	=	Complete Blood Count
CCI	=	Charlson Comorbidity Index
CRP	=	C-Reactive Protein
CVD	=	Cardiovascular Disease
Hb	=	Haemoglobin
HF	=	Heart Failure
IL-6	=	Interleukin-6
LYc	=	Lymphocyte Count
MNA	=	Mini Nutritional Assessment
MI	=	Myocardial Infarction
NLR	=	Neutrophils-to-Lymphocytes Ratio
PA	=	Physical Activity
PLR	=	Platelet-to-Lymphocyte Ratio
PLT	=	Platelets
PTCA	=	Percutaneous Transluminal Coronary Angioplasty
RDW	=	Red Cell Distribution Width
RPR	=	Red Blood Distribution Width-to-Platelet Ratio
TNF-α	=	Tumor Necrosis Factor *alpha*
WBC	=	White Blood Cell

AUTHORS' CONTRIBUTION

C. Bodolea and E. I. Hiriscau prepared the original manuscript. E. Buzdugan, A.I. Grosu and L. Stoicescu evaluated data from hospitalized cardiac patients. E.I.H. entered the data in database, carried out the statistical analysis contributing to the drafting of the method, results and discussion sections. S. Vesa contributed also in providing the statistical analysis. O.C. contributed to the critical review and the finalization of the manuscript.

The authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the Local Ethics Committee of the University Clinical Municipal Hospital, Cluj-Napoca, Romania (Protocol Number: 5/2017).

HUMAN AND ANIMAL RIGHTS

No animals were used in the study. All human procedures were followed in accordance with the Helsinki Declaration of 1975 as revised in 2013 (http://ethics.iit.edu/ecodes/node/3931).

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

CONSENT FOR PUBLICATION

A written informed consent was obtained from all patients prior to the publication of the study.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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SUPPLEMENTARY MATERIAL

Supplementary material is available on the publisher's website along with the published article.

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