Platelet-to-lymphocyte ratio, neutrophil-to-lymphocyte ratio and monocyte-to-HDL cholesterol ratio as markers of peripheral artery disease in elderly patients

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Abstract. Solid evidence underlines the pivotal role played by inflammation regarding atherosclerosis. Peripheral artery disease (PAD) is one of atherosclerotic cardiovascular diseases (CVDs), it is highly frequently diagnosed in older individuals. In the present study we carried out an investigation on the association between platelet-to-lymphocytes ratio (PLR), neutrophil-to-lymphocyte ratio (NLR), monocyte-to-HDL cholesterol ratio (MHR) with PAD as favourable markers. We identified 300 subjects aged over 70 years, without any concomitant CVDs. The PLR, NLR and MHR were assessed from peripheral venous blood routinely drawn in the ward during hospitalization. Patients were divided in groups according to ankle brachial index (ABI) value (>0.9; 0.9-0.99; 1-1.4; >1.4). Higher PLR (P=0.007), NLR (P=0.0001) and MHR (P=0.0001) were associated with <0.9 ABI. Patients with a >1.4 ABI showed NLR values higher compared to >0.9l ABI (P<0.01). Univariate linear regression analysis demonstrated the direct correlation between increase in PLR (P=0.0023) and MHR (P<0.0001) with the decrease in ABI value. In multivariate linear regression analysis including main cardiovascular risk factors we found that PLR, NLR and MHR were independently associated with lower ABI (P=0.0011). Results show and suggest that the elevated PLR, NLR and MHR are related to PAD evaluated with ABI measurement. PLR and MHR seem to be more reliable markers than NLR in PAD. NLR seems to be more related to incompressibility of arterial wall. It is hypothesized that these three indexes may play a role as simple and repetitive markers of PAD.

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Key words: peripheral artery disease, platelet-to-lymphocyte ratio, neutrophil-to-lymphocyte ratio, monocyte-to-HDL cholesterol ratio, inflammation, elderly, ankle-brachial index, atherosclerosis

Introduction

Cardiovascular diseases (CVDs) represent the major causes of mortality worldwide ranging to 17.3 million of deaths per year, although in more progressed countries a consistent and progressive decline is witnessed (1). However, both coronary artery disease and cerebrovascular disease remain the two main causes of death with 1.8 and 1.0 million per year respectively (2). Atherosclerotic clinical symptoms are preceded by the so-called subclinical atherosclerosis, an early stage without clinical evidence. It is crucial to evaluate the subclinical condition to achieve a better clinical outcome of patients. Inflammation plays a pivotal role for starting and progressing of atherosclerosis disease. High sensitive C-reactive protein (hsCRP) stimulates and increases phagocytosis and chemotaxis. Elevated levels of hsCRP represent a risk marker for potential atherosclerotic plaque instability in patients with coronary artery disease (3,4). It is known that fibrinogen (F) is associated with incremental risk of coronary artery disease. Subjects with high plasma level of F have a 3-fold increase of global cardiovascular disease (5). Furthermore, homocysteine, leptin, adiponectin, tumor necrosis factor alfa (TNF-α), interleukin-1 (IL-1) and interleukin-6 (IL-6) act in determining both higher prevalence and severity of CVDs (6,7). Platelet-to-lymphocyte ratio (PLR), neutrophil-to-lymphocyte ratio (NLR) and monocyt-to-HDL cholesterol ratio (MHR) have been widely studied as markers of inflammation, and they may be considered as simply and

helpful biomarkers available with routinely blood tests (8-10). PLR is the ratio between absolute count of platelets (tiny blood cells related to thrombotic events) and lymphocytes (a decrease in lymphocyte count is associated to generalized stress). NLR is calculated by dividing the number of neutrophils (notoriously increased in inflammatory events) by number of lymphocytes and is used as a marker of subclinical inflammation. The utility of MHR is related to an evaluation of inflammatory state (monocytes absolute count) and to HDL-cholesterol, a well-known cardiovascular protecting factor. Both NLR and PLR were considered to be linked to the severity and progression of peripheral arterial disease (PAD) in general population (11,12). There are limited data examining the role of these two markers in elderly patients and also the predictive value of MHR in PAD. In the present study we determined the association between PLR, NLR, MHR with PAD. PAD was diagnosed by measuring the ankle brachial index (ABI).

Materials and methods

Study population. We consecutively screened 300 subjects aged over 70 years, without manifest concomitant atherosclerotic disease, hospitalized from July 1, 2015 to December 31, 2018, in the Geriatric Department, ARNAS Garibaldi, Catania. Patients were informed on research, they were asked to give their verbal informed consent. Verbal informed consent was obtained from all patients. The study was conducted acording to the ethical guidelines of the 1975 Declaration of Helsinki. To avoid confounding data, we excluded patients with malignancies, inflammatory disease, autoimmune disease, hematologic disease, hepatic insufficiency, chronic kidney disease, acute coronary syndrome, severe consequences of type 2 diabetes, and patients who had received immunosuppressive drugs or corticosteroids in the previous three months. Gender, age, weight, body mass index (BMI), and cardiovascular risk factors (diabetes, arterial hypertension, smoke, dyslipidaemia) were recorded. The diagnosis of diabetes was made according to the criteria of American Diabetes Association (13). Hypertension and dyslipidaemia were diagnosed according to the seventh report of the Joint National Committee on prevention, detection, evaluation, and treatment of high blood pressure (JNC 7) and guideline of the National Cholesterol Education Program (ATP III), respectively (14,15). BMI was calculated using the formula of weight/height^2 (kg/m^2). Blood pressure was measured manually on the non-dominant arm in a seated position after a 10-min rest.

Clinical and biochemical assessments. Blood samples were collected when patients were admitted to the hospital division. Complete blood count, serum glucose, blood urea nitrogen, creatinine, total cholesterol, low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides, high sensitive hsCRP, erythrocytes sedimentation rate (ESR), and F were assessed. Cell count was performed by flow cytometry; in particular PLR was calculated by platelets counts (x10^3/l)/total lymphocyte counts (x10^3/l). NLR was calculated by neutrophil counts (x10^3/l)/total lymphocyte count (x10^3/l). MHR was calculated by monocyte counts (x10^3/l)/HDL-C (mg/dl).

ABI measurement. ABI measurements were performed using a B-mode tomographic ultrasound system (Esaote Mylab 5) with a linear 7-12 MHz transducer and measuring systolic blood pressure in non-dominant arm brachial, posterior and anterior tibial arteries. The ABI was calculated as the ratio of the ankle (lower value between posterior and anterior tibial artery) and brachial (brachial artery) systolic blood pressure. ABI is considered as normal in a range between 1 and 1.4. The ABI value ≤0.9 is used to diagnose the low-extremity artery disease (LEAD), as a marker of lower extremity artery stenosis (>50% stenosis). ABI values ranging from 0.90 to 0.99 are considered 'borderline', while values higher than 1.4 indicate non-compressible arteries (16). Patients were divided into four groups, according to the ABI values: ≤0.9 (g1); 0.9-0.99 (g2); 1-1.4 (g3) and >1.4 (g4).

Statistical analysis. Continuous variables were presented as mean ± standard deviation (SD) and categorical data were summarized as frequencies (percentage). For more than two level variables, analysis by ANOVA test, followed by post hoc (Tukey) were performed. Differences among groups were analysed by non-parametric Wilcoxon test.

For continuous variables, correlation was evaluated with simply linear univariate, Pearson's correlation method (ϱ) and multivariate regression (included age, history of hypertension, smoking, diabetes mellitus, dyslipidaemia, high sensitive hsCRP, ESR, and F. P-value <0.05 was considered statistically significant. Statistical analysis was performed by using 'R' software.

Results

A total of 300 patients, 132 men (44%) and 168 women (56%) were analysed in the study. Mean age was 81.2 (±6.9) years. Among these subjects, 167 (56%) were affected by arterial hypertension, 92 (31%) by type 2 diabetes, 141 (47%) by dyslipidaemia and 85 (28.3%) were smokers. The mean and SD of PLR, NLR and MHR were, respectively, 184.92 (±70.84), 3.91 (±2.15) and 26.39 (±19.38). The demographic, clinical and laboratory characteristics and PLR, NLR, MHR means with SD of four groups (g1-g4) are summarized in Table I.

Comparison between groups shows higher PLR in patients with ABI ≤0.9 (g1) than in patients with a >0.9 ABI (P=0007). Tukey's post hoc analysis revealed a statistically significant difference between g1 and g2 (P=0.0098), g1 and g3 (P=0.0013), but a non-significant difference between g1 and g4 (P=0.8594). PLR values in g4 patients were higher than in subjects of g2 (P=0.5323) and g3 (P=0.6183) with non-statistically relevant differences (Fig. 1).

A greater NLR was found in patients with ABI ≤0.9 (g1) than in patients with a 0.9-1.4 ABI (P=0.0001). Tukey's post hoc analysis showed a statistically significant difference between g1 and g3 (P=0.0099) and a non-statistically difference between g1 and g2 (P=0.2460).

Subjects of g4 presented NLR values higher than other groups. Statistically significant differences were shown between g4 and g2 (P=0.0056), g4 and g3 (P=0.0007); statistically non-significant variations were found between g4 and g1 (Fig. 2).

Table I. Baseline clinical and laboratory findings reported as mean and standard deviation (SD).

Baseline characteristics	Mean ± SD
Age (years)	81.2±6.9
Sex	F 168 (56%); M 132 (44%)
Weight (MHz)	71.9±12.2
Height (cm)	161.2±8.1
Body mass index - BMI (kg/m^2)	28±5
Abdominal circumference (cm)	97±13
Cardiovascular (CV) risk factors	
Arterial hypertension	167 (56%)
Diabetes mellitus	92 (31%)
Smoking	85 (28.3%)
Dyslipidaemia	141 (47%)
Laboratory findings	
Platelets	227±93.066x10^3/ml
Lymphocytes	1.625±0.698x10^3/ml
Neutrophil	6.012±1.851x10^3/ml
Monocytes	0.8±0.3x10^3/ml
HDL-cholesterol	26.5±11.2 mg/dl
Creatinine	1.3±0.9 mg/dl
Erythrocyte sedimentation rate (ESR)	32±19 mm/h
C-reactive protein (CRP)	2.72±3.42 mg/dl
Fibrinogen (F)	449.5±108.5 mg/dl
Platelet-to-lymphocyte ratio (PLR)	184.92±70.84
Neutrophil-to-lymphocyte ratio (NLR)	3.91±2.15
Monocytes-to-HDL-C ratio (MHR)	26.39±19.38
PLR	
G1	225.61±157.71
G2	151.04±85.08
G3	160.53±127.21
G4	206.54±141.77
NLR	
G1	4.58±3.55
G2	3.44±2.70
G3	3.17±1.98
G4	6.96±9.21
MHR	
G1	31.58±15.61
G2	24.01±9.89
G3	22.77±12.46
G4	27.92±14.21

Baseline clinical and laboratory findings reported as mean and SD of PLR, NLR and MHR in G1, G2, G3 and G4.

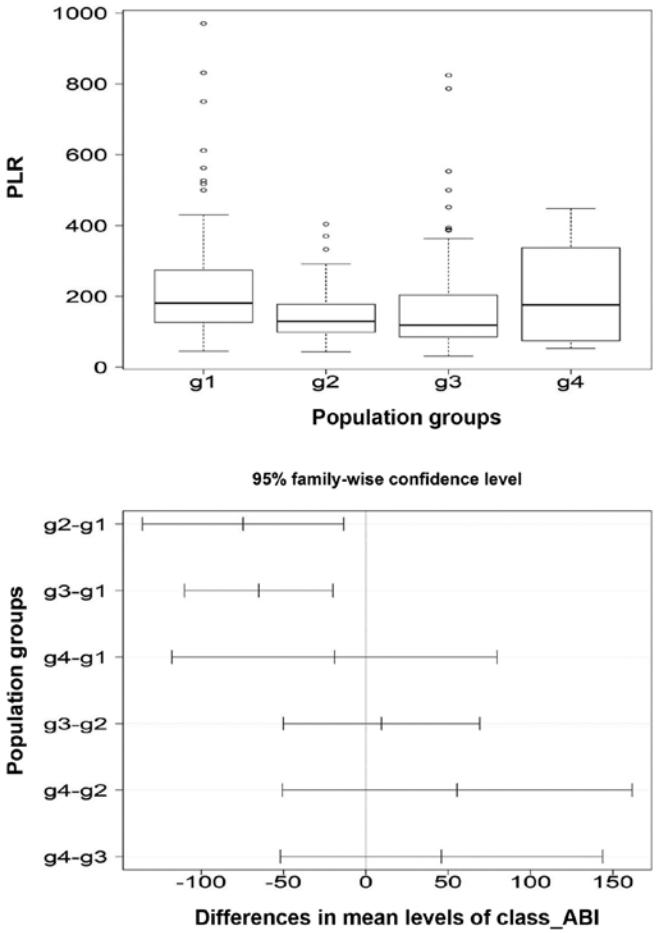

Figure 1. Boxplots. Comparison of PLR in subjects with ABI ≤0.9 (g1), ABI 0.9-0.99 (g2), ABI 1-1.4 (g3), ABI >1.4 (g4). Post hoc analysis for evaluation of differences in PLR mean levels among the subgroups of examined population. Population groups are indicated on x-axis, PLR values on y-axis. Higher PLR in patients with ABI≤0.9 (g1) than in patients with >0.9 ABI (P=0007). Tukey's post hoc analysis revealed a statistically significant difference between g1 and g2 (P=0.0098), g1 and g3 (P=0.0013), but a non-significant difference between g1 and g4 (P=0.8594). PLR values in g4 patients were higher than in subjects of g2 (P=0.5323) and g3 (P=0.6183) with non-statistically relevant differences. PLR, platelet-to-lymphocyte ratio; ABI, ankle-brachial index.

Comparison between groups highlights higher MHR values in patients with ABI ≤0.9 (g1) than in patients with a >0.9 ABI (P<0.0001). Tukey's post hoc analysis revealed a statistically significant difference between g1 and g2 (P<0.0001), between g1 and g3 (P<0.0001) and a non-statistically difference between g1 and g4. Subjects of g4 presented higher MHR than individuals of g2 and g3, but with no relevant statistical differences (Fig 3).

A negative correlation was found between PLR, NLR, MHR and ABI. The strongest correlation was found between the MHR and ABI (ϱ=-0.24) and PLR and ABI (ϱ=-0.18); the analysis demonstrated a weaker correlation between NLR and ABI (ϱ=-0.09). Univariate linear regression analysis demonstrated a strong correlation between an increase in PLR and a decrease in ABI (r=0.0276; F-statistic = 9.496; P=0.0023). Similar relationship was found for MHR (r=0.0551; F-statistic = 18.44; P<0.0001). For NLR a non-significant correlation was found (r=0.0055; F-statistic = 2.65; P=0.1046) (Fig. 4).

Non parametric analysis (Wilcoxon test) demonstrated a strong relation between pathological ABI with the increasing of PLR (P=2.2x10^{-16}), NLR (P=4.2x10^{-13}) and MHR (P=1.2x10^{-15} (Table IIA).

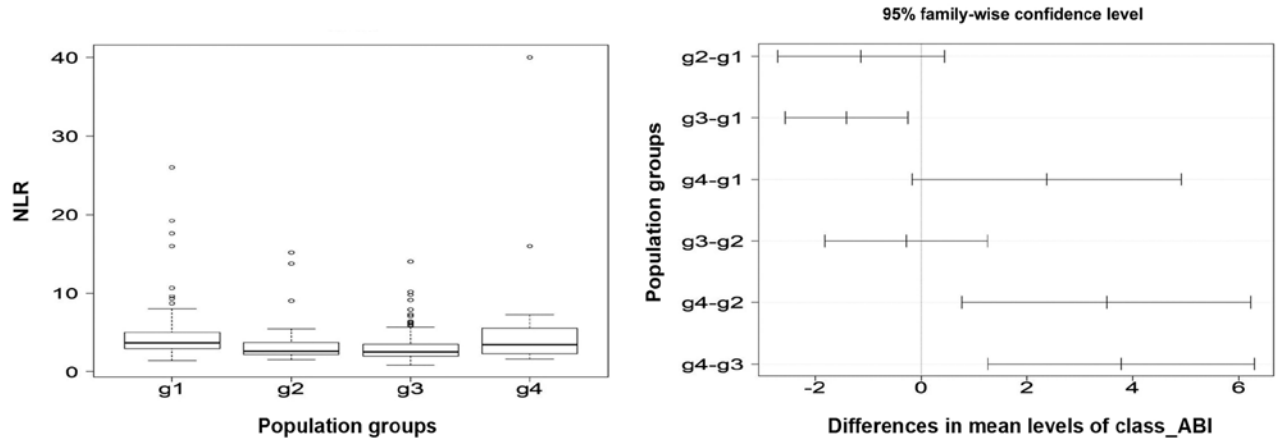

Figure 2. Boxplots. Comparison of NLR in subjects with ABI ≤0.9 (g1), ABI 0.9-0.99 (g2), ABI 1-1.4 (g3), ABI >1.4 (g4). Post hoc analysis for evaluation of differences in NLR mean levels among the subgroups of examined population. Population groups are indicated on x-axis, NLR values on y-axis. A greater NLR was found in patients with ABI ≤0.9 (g1) than in patients with a 0.9-1.4 ABI (P=0.0001). Tukey's post hoc analysis underlined a statistically significant difference between g1 and g3 (P=0.0099) and a non-statistically difference between g1 and g2 (P=0.2460). Subjects of g4 presented NLR values higher than the other groups. Statistically significant differences were shown between g4 and g2 (P=0.0056), g4 and g3 (P=0.0007); non-statistical variations were found between g4 and g1. NLR, neutrophil-to-lymphocyte ratio; ABI, ankle-brachial index.

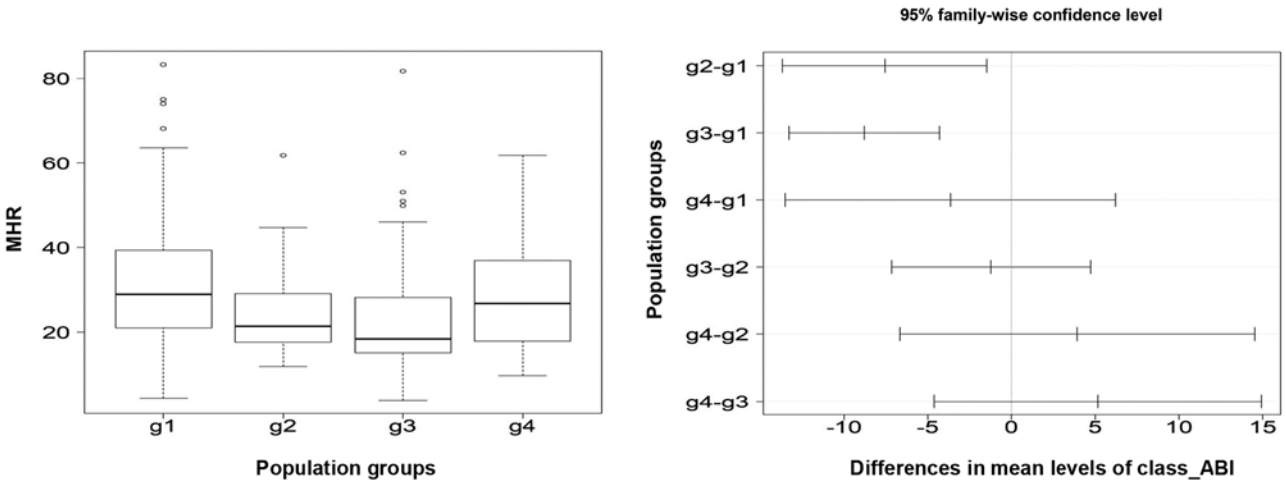

Figure 3. Boxplots. Comparison of MHR in subjects with ABI ≤0.9 (g1), ABI 0.9-0.99 (g2), ABI 1-1.4 (g3), ABI >1.4 (g4). Post hoc analysis for evaluation of differences in MHR mean levels among the subgroups of examined population. Population groups are indicated on x-axis, MHR values on y-axis. The figure shows higher MHR in patients with ABI ≤0.9 (g1) than in patients with a >0.9 ABI (P<0.0001). Tukey's post hoc analysis revealed a statistically significant difference between g1 and g2 (P<0.0001), between g1 and g3 (P<0.0001) and a non-statistical difference between g1 and g4. Subjects of g4 presented MHR higher than individuals of g2 and g3 with no relevant statistical difference. MHR, monocytes-to-HDL-C ratio; ABI, ankle-brachial index.

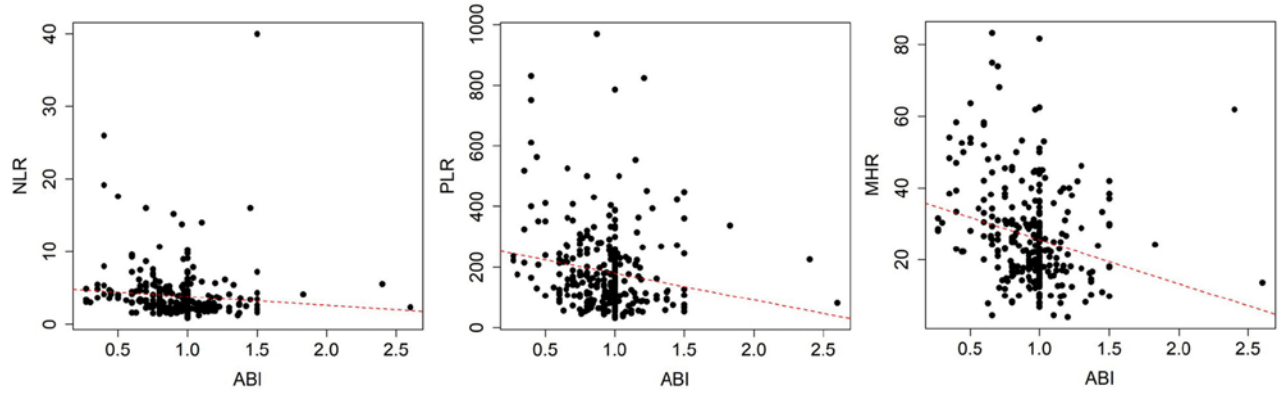

Figure 4. Univariate linear regression analysis to evaluate the correlation between PLR, NLR, MHR (values indicated on y-axis) and ABI (x-axis). Increased values of PLR and MHR correlated with the decrease of the ABI values (r=0.0276; F-statistic = 9.496; P=0.0023) and (r=0.0551; F-statistic = 18.44; P<0.0001). Increased NLR value was related to decreased ABI values. No significant correlation was found (r=0.0055; F-statistic = 2.65; P=0.1046). PLR, platelet-to-lymphocyte ratio; NLR, neutrophil-to-lymphocyte ratio; MHR, monocyte-to-HDL-C ratio; ABI, ankle-brachial index.

Table II. Results of the statistical analysis.

A, Non parametric analysis (Wilcoxon test) to evaluate correlation between pathological ABI and an increasing of PLR ($P=2.2x10^{-16}$), NLR ($P=4.2x10^{-13}$) and MHR ($P=1.2x10^{-15}$) values.

	≤0.9 ABI	
Markers	Wilcoxon	P-value
PLR	89.877	$2.2x10^{-16}$
NLR	84.547	$4.2x10^{-13}$
MHR	77.877	$1.2x10^{-15}$

B, Multivariate linear regression analysis for predicting a decreased ABI (adjusted R-squared = 0.06603, F-statistic = 2.922, P=0.0011).

Parameters	r	P-value
MHR	-2.993	0.00300
NLR	0.587	0.04557
PLR	-1.107	0.02691
Hypertension	-0.781	0.03560
Diabetes mellitus	-2.625	0.00912
Dyslipidemia	0.365	0.71551
Smoking	2.085	0.03793
ESR	-0.501	0.61691
CRP	0.265	0.79131
F	-1.090	0.27650
Age	0.288	0.77344

W, Wilcoxon test; ABI, ankle-brachial index; PLR, platelet-to-lymphocyte ratio; MHR, monocyte-to-HDL-C ratio; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; F, fibrinogen.

In multivariate linear regression analysis, it was found that hypertension, diabetes mellitus, smoke, PLR, NLR and MHR were independent factors for predicting a decreased ABI. The model shows an independent association with pathological values in ABI measurements (adjusted R-squared = 0.06603, F-statistic = 2.922, P=0.0011). These data are shown in Table IIB.

Discussion

The present study evaluated the three blood tests derived indexes (PLR, NLR and MHR) to predict the PAD diagnosed by using the ABI.

Platelets are able to release inflammatory mediators leading to adhesion and transmigration of monocytes and interact with endothelial barrier of the arterial wall promoting atherosclerotic lesions. Low lymphocyte count indicates generalized stress or various underlying illnesses. PLR, the ratio between platelet and lymphocyte absolute counts, is raised in non-dipper hypertensive patients, in patients with venous thromboembolism, in patients with chronic PAD or critical limb ischemia (CLI) and in patients suffering from chronic kidney disease (17,18). The >144 PLR ratio was found to be associated with raised mortality in patients treated with percutaneous coronary angioplasty after NSTEMI or STEMI myocardial infarction (19).

Neutrophils increase in acute inflammation, and NLR represents a helpful prognostic marker in patients with acute coronary syndrome (ACS). NLR >5.9 correlates with raised 90-day mortality in patients with ischemic stroke (20). Subjects suffering from increased PAD value of the NLR was found to be associated with a greater prevalence of CLI (21). Circulating monocytes as a source of various cytokines and molecules interact primarily with platelets and endothelial cells leading to aggravation of inflammatory, pro-thrombotic pathways. MHR is an index related to inflammatory processes, especially those linked to atherosclerotic lesions. The activation of Pattern Recognition Receptor (PRR) in the vascular wall is a pivotal event in atherogenesis. Activation of inflammation has been shown to induce production of monocyte chemoattractant protein 1 (MCP1), recruiting monocytes in vascular lumen (22). A significant amount of surface lymphocyte antigen 6 complex (Ly6Chi monocytes) is expressed on $CD14^{+}CD16^{-}$ monocytes, a subtype having pro-inflammatory activity, it was found elevated in patients with dyslipidaemia (23). MHR has been reported as a helpful marker to identify subjects with high risk for the major cardiovascular events (MACE) (24). Our results demonstrate a similar prevalence of principal CV risk factors (arterial hypertension, diabetes mellitus, dyslipidaemia, and smoking) compared to general population. Prevalence of PAD (ABI <0.90) was up to 34%, slightly higher compared to epidemiological data in non-geriatric patients. Prevalence of the PAD ranged between 20 and 28% (25). Recent studies showed a positive association between NLR and PLR with the severity of lower extremity PAD, poor prognosis, particularly with risk of one-year readmission. NLR also correlates with one-year mortality (26). According to ABI value, we obtained 4 subgroups (g1, g2, g3 and g4). Our results demonstrated that patients with ABI <0.9, a well-recognized PAD diagnostic index, showed higher PLR and MHR values. Increased NLR values were found to be associated to >1.4 ABI (lower limb arterial incompressibility). A statistically significant correlation was found between lower ABI values with the increase of PLR and MHR values; correlation was found as non-significant for NLR. In a model including well-known cardiovascular risk factors such as arterial hypertension, diabetes mellitus, dyslipidaemia, smoking, age and bio-humoral tests (ESR, CRP and F), we found that PLR, NLR and MHR represent independent variables for predicting PAD. This work highlighted that PLR and MHR represent two reliable markers of PAD. In particular, their increase is associated with decreased of the ABI values. NLR presented a weaker relationship with decreasing ABI (statistically non-significant), but an elevated value is strongly related to a condition of arterial incompressibility, likely due to medial calcification in subjects with long-lasting atherosclerotic disease.

Limitations and strength. It is noteworthy that the present study was planned as an observational study, so it may be considered a limitation. However, the study focused on value

of interesting blood cell parameters in patients affected by one of the atherosclerotic diseases. Additionally, the study shows the relationship between some of the above referred blood cell parameters with the ABI value as validated diagnostic tool for PAD. Limitation of this study could be related to the typology of research. Study enrolled 300 patients admitted to geriatric internal medicine unit, so we believe that the population was homogeneous and numerous. The present study was not planed as longitudinal study, it did not allow us to discuss the capability of the markers to predict both outcome and prognosis of PAD patients. Further evaluations will be targeted to achieving data concerning the bloodstream cells pathways related to the clinical outcome or prognosis in elderly patients.

In conclusion, the present study confirms that ultrasound plays a pivotal role in diagnosis and follow-up of PAD. The importance of inflammation is largely accepted as a key player leading to promotion and acceleration of atherosclerosis and consequences such as PAD. Our group has contributed to this concern showing increased plasma level of inflammatory, endothelial and metalloproteinase markers in PAD (27-29). The present study suggests that elevated PLR, NLR and MHR are related to PAD diagnosed by the ABI measurement. PLR and MHR were shown to be more reliable markers than NLR in PAD. NLR seems to be more related to the incompressibility condition of lower limb arteries. Based on our findings we can hypothesize that these complete blood counts may be considered, as favourable, simple, potential and repeatable markers for PAD.

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Availability of data and materials

The data used and/or analysed in this study are available from the corresponding author with reasonable request.

Authors' contributions

All authors (SS, AA, GB, CM, MDG, CGD, ESDV, MR, SL and SSS) made substantial contributions to the study design, data acquisition and interpretation of the data. Each author participated sufficiently in the work to take public responsibility for appropriate portions of the content and agree all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

Verbal informed consent was obtained from each patient and the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in *a priori* approval by the institution's human research committee.

Patient consent for publication

Identifying information, including names, initials, date of birth or hospital numbers, images or statements are not included in the manuscript.

Competing interests

The authors declare that they have no competing interests.

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