Neutrophil-to-lymphocyte, platelet-to-lymphocyte and lymphocyte-to-monocyte ratios are associated with amputation rates in patients with peripheral arterial disease and diabetes mellitus who underwent revascularization: A Romanian regional center study

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Abstract. Inflammation plays an important role in peripheral artery disease (PAD), contributing to the onset and progression of atherosclerosis, as well as to the rupture of atherosclerotic plaques. Studies have revealed that due to their inflammatory nature, leucocytes play an important role in the development of atherosclerosis. A retrospective study was conducted involving 203 patients with PAD admitted to Targu Mures Emergency County Hospital for revascularization surgery between January 2017 and June 2019 (of which 47 were treated by endovascular intervention, and 156 underwent classical surgical intervention). Among all patients included in the study, 47 patients required amputation following the revascularization intervention. The results indicated that though the mean patient age in the non-amputation group was higher than that in the amputation group, that the difference was not significant. With regard to sex distribution, 72% of the patients from the amputation group were male, while from the non-amputation group, 74% were male. The neutrophil-to-lymphocyte ratio (NLR) cut-off value for the prediction of amputation in PAD was 3.485 (sensitivity, 60.42%; specificity 72.44%), whereas the platelet-to-lymphocyte ratio (PLR) value was 152, (sensitivity, 54.17%; specificity, 71.79%), and was 2.55 for the lymphocyte-to-monocyte ration (LMR; sensitivity, 56.25%; specificity, 66.88%). The study concluded that in patients with PAD, the NLR and PLR were increased, while the LMR was decreased, which was also associated with a higher rate of amputation after revascularization, despite the lack of correlation between these factors, Fontaine classification and the number of damaged vessels. Therefore, pre-operative alterations in NLR, PLR and LMR may predict the need for amputation in patients with PAD, or those who underwent a revascularization intervention.

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Abbreviations: PAD, peripheral artery disease; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio; HDL, high-density lipoprotein; LDL, low-density lipoprotein

Key words: PAD, amputation, NLR, thrombocyte-to-lymphocyte ratio, LMR

Introduction

It is well known that peripheral artery disease (PAD) is defined as the partial or complete obstruction of one or multiple peripheral arteries. Previous studies have indicated that >200 million individuals worldwide are affected by PAD, a disease which integrates a large specter of symptoms (1). Studies have shown that due to their important role in inflammation, leucocytes play an important role in the development of atherosclerosis. Furthermore, as the neutrophil-to-lymphocyte ratio (NLR) mirrors the severity of inflammation, studies have also concluded that the NLR is associated with a high risk of cardiovascular pathology, as well as a higher mortality rate among patients with PAD (2,3). Furthermore, NLR and PLR could predict poor outcome in kidney disease (4), acute limb ischemia (5) and PAD (6).

As they have also been correlated with PAD, the platelet-to-lymphocyte ratio (PLR) and lymphocyte-to-monocyte ratio (LMR) are also of clinical relevance (7,8). Both the NLR and PLR have predictive value regarding the need for amputation in patients with diabetes, where higher values were exhibited in patients requiring amputation than those requiring debridement only (6-9). As far as the LMR is concerned, there is still no sufficient data regarding its correlation with PAD, despite the study of a representative population from the USA, where monocytes were the only leucocytes to be independently associated with PAD (6-9).

Nonetheless, considering that NLR, PLR and LMR evaluation are low-cost, consistently reproducible and easily used on a large scale in clinical practice, they may be considered as important steps in assessing the possibility of developing vascular events on the strength of post-operative complications. As a result, the aim of the present study was to evaluate the association between NLR, PLR, LMR and the amputation rate among patients with PAD who underwent a revascularization procedure.

Materials and methods

Study population. A total of 203 patients with PAD and diabetes mellitus, admitted to Targu Mures Emergency County Clinical Hospital for a surgical intervention of revascularization between January 2017 and June 2019, were included in this retrospective study, of which 47 were treated in an endovascular manner and 156 underwent classical surgical intervention. The inclusion criteria were as follows: i) Patients diagnosed with PAD, who underwent a surgical revascularization procedure; ii) available smoking history, where patients were 'smoker' were considered active smokers as well as passive smokers; iii) in terms of hypertension, patients with systolic arterial pressure values ≥140 mmHg, diastolic ≥90 mm Hg or using anti-hypertensive medication; and iv) regarding diabetes, patients with a resting glucose level of ≥126 mg/dl, a plasmatic glucose level of ≥200 mg/dl after 2 h from the oral glucose tolerance test, symptomatic hyperglycemia with random plasmatic glucose levels of ≥200 mg/dl, or those using anti-diabetic medication. Exclusion criteria included: i) Patients with other inflammatory conditions other vascular pathologies; ii) patients <18 years of age; and iii) patients with incomplete leucocytes count data.

Data collection. The clinical characteristics of the patients, more specifically, age, sex, body mass index (BMI), smoking history, hypertension, chronic renal disease, heart failure, history of myocardial infarction, history of stroke and previous medication, were collected from medical files. Pre-operative results consisting of the number of neutrophils, lymphocytes, thrombocytes and monocytes were collected and evaluated. Data related to the type of revascularization intervention and the need for postoperative amputation were also noted. After the determination of NLR, PLR and LMR, their association with the amputation rate was evaluated.

Study design. A retrospective study was conducted, which analyzed data from 203 patients with PAD who underwent a revascularization procedure; two groups were assigned based on the 203 consecutive patients diagnosed with PAD, who were admitted for a revascularization procedure: i) Patients requiring an amputation procedure following revascularization intervention (n=47); and ii) patients who did not require amputation (n=156).

Statistical analysis: Descriptive and analytical statistics were performed using SPSS v17.0 (SPSS, Inc.), GraphPad Prism 8 (GraphPad Software, Inc.) and XLSTAT-Lite 2015 software for Windows 10 Pro. Grubbs test was performed to eliminate aberrant values. Categorical variables are presented as absolute values or percentages, and non-Gaussian distribution data are presented as median values [confidence intervals (CI)]. Kolmogorov-Smirnov test was performed to determine distribution normality. In order to compare the clinical traits of the patients, and the use of medication prior to hospital admission, Fisher's exact test was performed for categorical variables, and the Mann Whitney U test for ordinal values. The Mann Whitney test was also used to compare the leucocyte ratios between the two patient groups. Spearman's receiver operating characteristic (ROC) curve analysis was used to determine the cut-off values. Spearman's correlation coefficient was used to determine correlations between the NLR, PLR, LMR and Fontaine class, the number of affected vessels, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) values. P<0.05 was considered to indicate a statistically significant difference.

Results

Although the average patient age of the non-amputation group (66.5 years; 95% CI, 62-70) was higher than that of the amputation group (64 years; 95% CI, 60-70), the difference was not statistically significant (P=0.32; Fig. 1A), so age did not influence the technique of amputation/non-amputation selected.

Regarding the sex distribution, there was no significant difference between the amputation group, which consisted of 72% male patients, and the non-amputation group, where male patients represented 74% of the group (P=0.85; Fig. 1B), suggesting that the sex of the patient did not influence the choice of surgery.

According to the BMI classification, which was applied to the entire study group, both amputation (27.4; 95% CI, 24.9-29.2) and non-amputation (26.8; 95% CI, 25.2-29.1) group patients were within the overweight category, with median

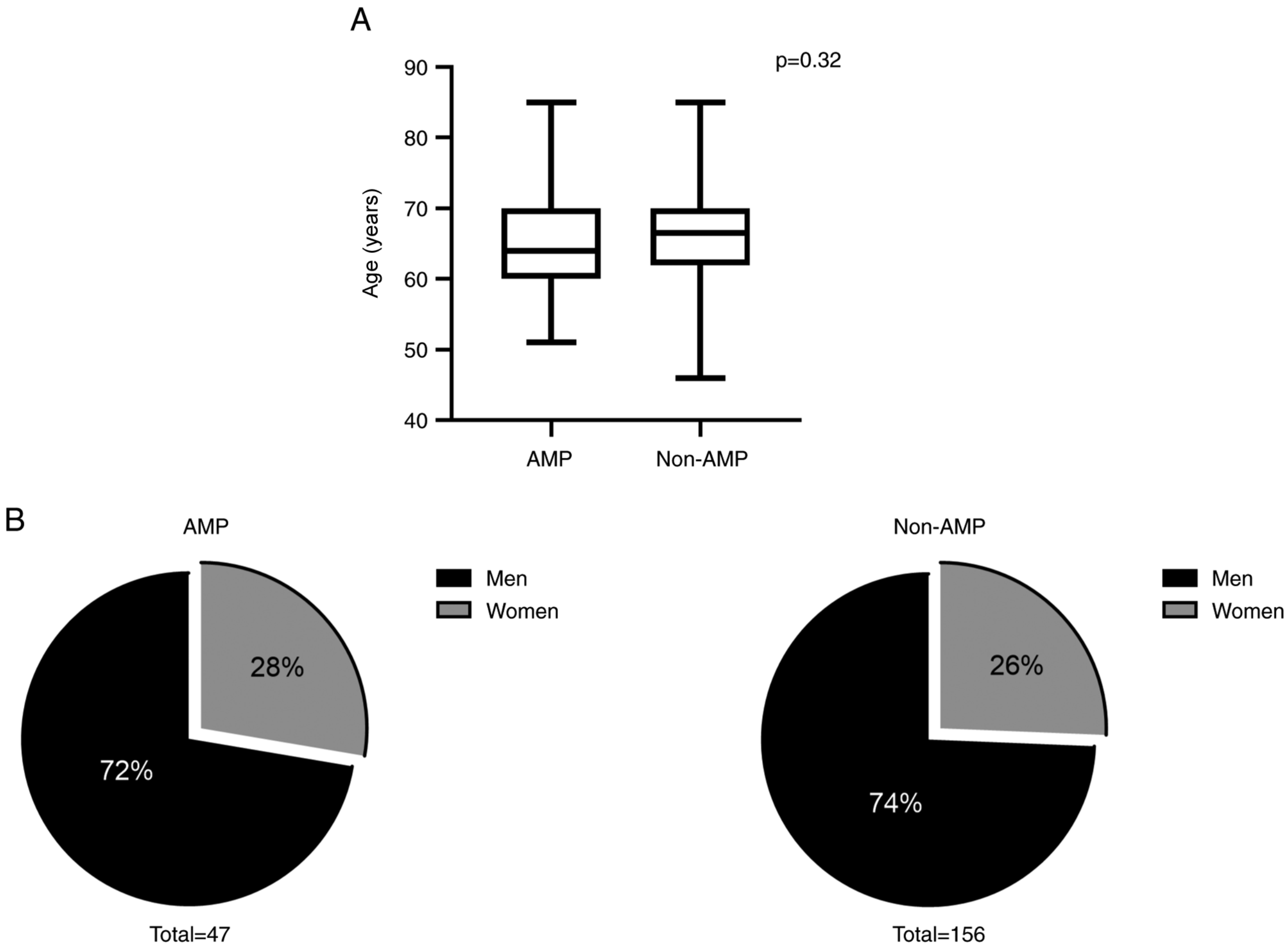

Figure 1. Distribution of patients by (A) age and (B) sex in the AMP and non-AMP groups. AMP, amputation.

values of the BMI ranging between 25 and 30 kg/m^2. Although there was a difference between the two median values, this could not be considered significant (P=0.93; Fig. 2), meaning that the BMI categorization did not affect the choice of amputation vs. non-amputation procedure.

Therefore, it can be concluded that demographic features such as age, sex, and body mass index did not influence the choice of amputation/non-amputation method.

The study also revealed a similar percentage of patients (53 vs. 58%, respectively; P=0.61) who were either active smokers or passive smokers in both groups. When taking into consideration the hypertensive condition of the patients, 87% in the amputation group were smokers, compared with 94% in the non-amputation group, though the difference was not significant (P=0.21; Fig. 3). In conclusion, smoking status and arterial hypertension did not influence the choice of procedure.

Diabetes mellitus type 2, a risk factor for PAD, was more frequent in the amputation group than in the non-amputation group, though once again, the difference was not significant (83% vs. 72%, respectively; P=0.18; Fig. 4). In terms of other encountered comorbidities, there were no significant differences between the two groups regarding the distribution of a history of myocardial infarction or stroke, heart failure or chronic kidney disease (all P>0.05; Table I). Therefore, prior diabetes and the aforementioned comorbidities had no effect on the choice of procedure performed.

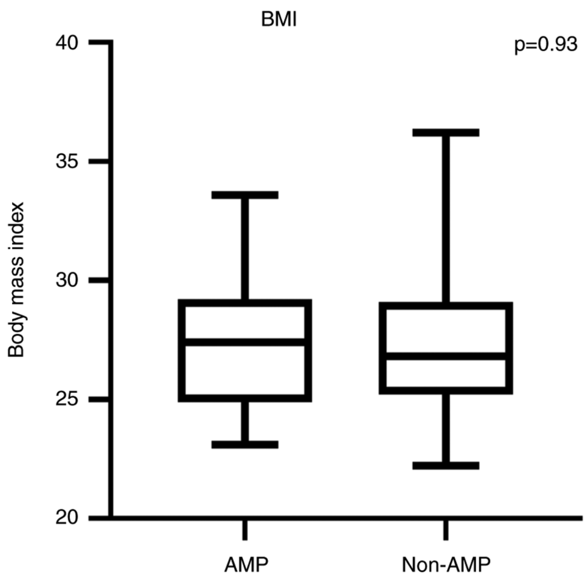

Figure 2. Body mass index of patients in the AMP and non-AMP groups. AMP, amputation.

Regarding the type of revascularization procedure, 91% of patients in the amputation group underwent open surgery (femuro-popliteal bypass and endarterectomy), while 70%

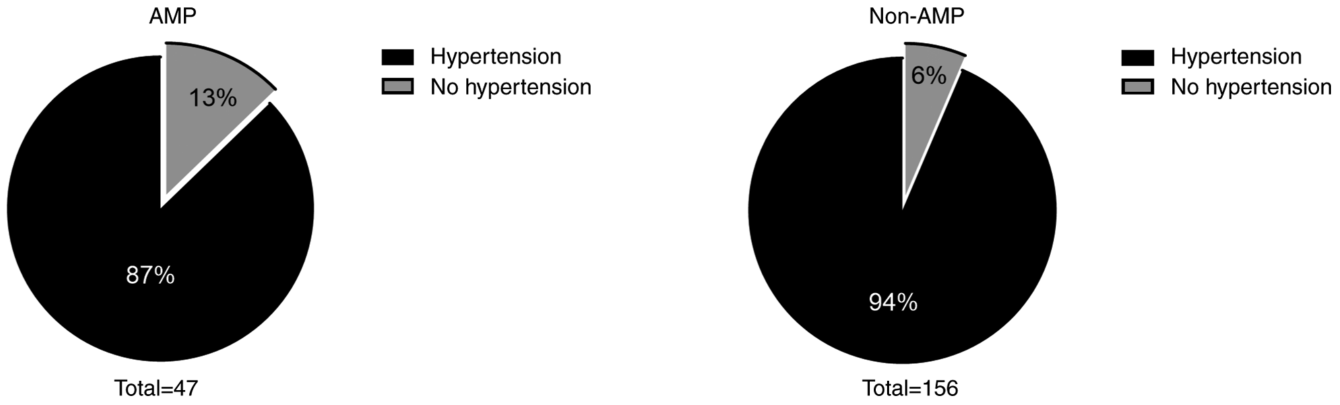

Figure 3. Distribution of hypertensive patients in the AMP and non-AMP groups. AMP, amputation.

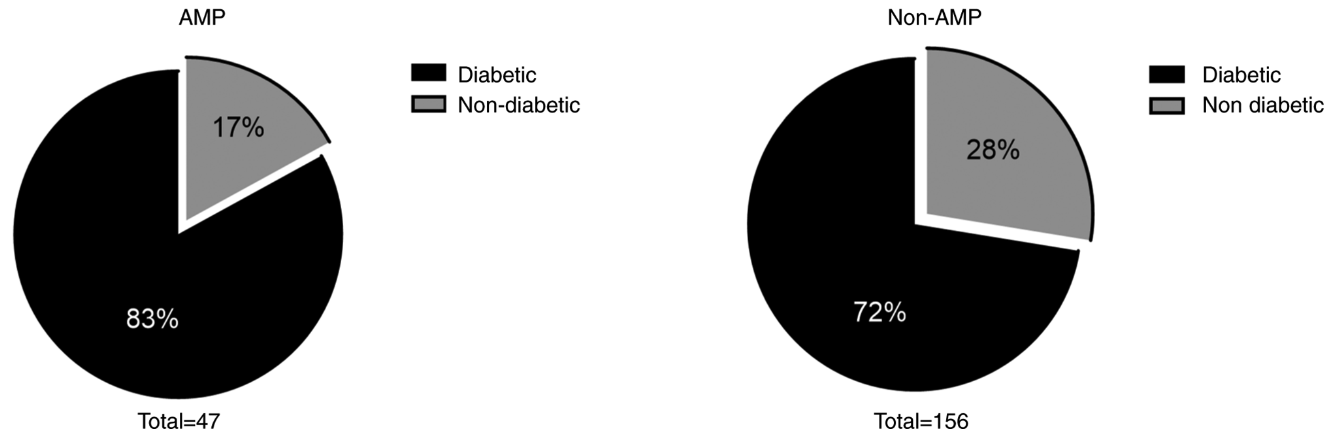

Figure 4. Distribution of patients with diabetes in the AMP and non-AMP groups. AMP, amputation.

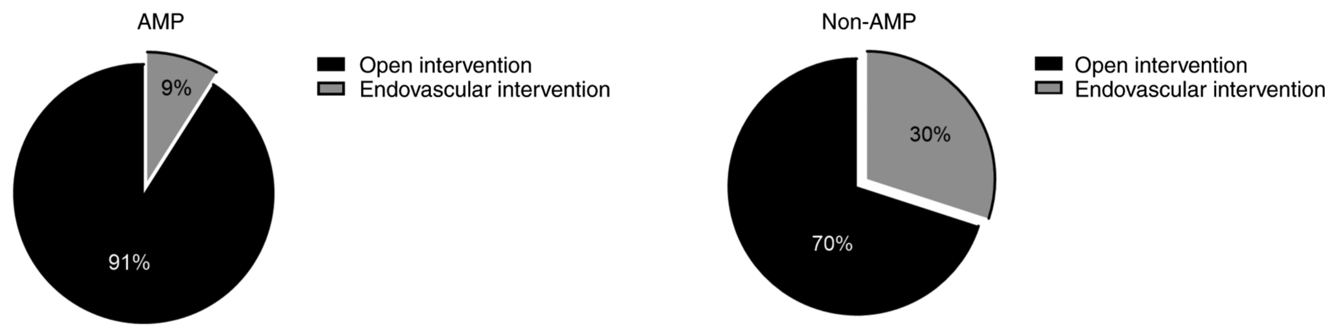

Figure 5. Type of surgery received by patients in the AMP and non-AMP groups. AMP, amputation.

of patients in the non-amputation group underwent the same intervention type (Fig. 5). Moreover, the percentage of patients using medication on the administration to the hospital was similar in both groups (all P>0.05), with >80% of the patients receiving Cilostazol and >90% using statins and antiplatelet treatment. On the other hand, Pentoxifylline was administered to a lower number of patients (Table II).

Higher NLR values were observed in patients who underwent post-operative amputation (4.11; 95% CI, 2.57-6.47) in comparison to patients who underwent a successful revascularization procedure (2.68; 95% CI, 1.91-3.85) (P<0.0001; Fig. 6A). Moreover, an increase in PLR in patients who required amputation compared with those who did not required amputation after surgical revascularization of the inferior extremity was observed (170.2; 95% CI, 104.00-226.5 vs. 110.9; 95% CI, 87.31-162.30) (P<0.01: Fig. 6B). By contrast, LMR was reduced in patients who underwent amputation compared with patients who did not require amputation (2.12; 95% CI, 1.67-4.40 vs. 3.22; 95% CI 2.22-5.08) (P<0.01; Fig. 6C). Therefore, while the NLR and PLR values were higher in amputated patients, LMR was lower in these patients.

Based on ROC curve analysis, the NLR cut-off value for predicting amputation requirement in patients with PAD was 3.485 (sensitivity, 60.42%; specificity, 72.44%), the PLR cut-off

Table I. Distribution of comorbidities in patients with peripheral artery disease.

Comorbidity	AMP, % (n=47)	Non-AMP, % (n=156)	P-value
Previous myocardial infarction	25.53	23.71	0.84
Heart failure	55.31	65.38	0.23
Previous stroke	19.14	17.30	0.82
Chronic kidney disease	8.51	16.02	0.24

AMP, amputation.

Table II. Treatment type of patients with peripheral artery disease on entry to hospital.

Treatment type	AMP, % (n=47)	Non-AMP, % (n=156)	P-value
Cilostazol	82.97	80.76	0.83
Pentoxifylline	10.63	17.30	0.36
Antiplatelet	100	98.07	>0.99
Anticoagulant	44.68	54.58	0.24
Statins	97.87	95.51	0.68

AMP, amputation.

Table III. Correlation between NLR, LMR and PLR, and Fontaine class, number of affected vessels, LDL and HDL.

	P-value			
Parameter	Fontaine class	Number of affected vessels	LDL (mg/dl)	HDL (mg/dl)
NLR	0.91	0.91	0.79	0.23
PLR	0.53	0.49	0.17	0.55
LMR	0.74	0.25	0.54	0.17

NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

value was 152 (sensitivity, 54.17%; specificity 71.79%), and the LMR cut-off value was 2.55 (sensitivity, 56.25%; specificity 66.88%) (Fig. 7). The study revealed that none of the three ratios were correlated with Fontaine class, the number of affected vessels, HDL or LDL levels (all P>0.05; Table III).

In terms of comorbidities, risk factors, type of surgery, Fontaine Classification, number of affected vessels, and history of medication, no statistically significant differences were observed between the two groups. However, a high NLR and PLR value, and a lower LMR value, were associated with amputation post-revascularization as shown in Figs. 6 and 7.

Discussion

Peripheral arterial disease is a public health problem, affecting many patients through its debilitating nature. Numerous studies have incriminated the systemic inflammatory response in the initiation and progression of PAD (7,8). Given the role of the inflammatory system in the development of this disease, the present study aimed to investigate the relationship between PAD and NLR, PLR and LMR, which are considered markers of inflammation (7-9).

Despite the need for amputation after revascularization, the primary clinical features of patients in the amputation and non-amputation groups was similar in the present study. The non-significant difference in risk factors (hypertension, diabetes and smoking) for PAD suggests that these factors did not dictate the rate of amputation in patients who have undergone revascularization.

More than half of the patients included in the present study were smokers. Amongst the risk factors, it is important to highlight the importance of smoking in the development of PAD, which is noted as one of the most important risk factors in all studies (10). Even though different studies approach different stages of smoking state (e.g., current, past or never) it has been highlighted that a history of smoking doubles the risk of PAD compared with non-smokers (10,11).

Diabetes mellitus is also considered to be associated with a high risk of developing PAD; Murabito *et al* (12) revealed an association between diabetes and age and sex, but not in multivariate models. Patients with type 2 diabetes mellitus were found to have a higher risk of PAD development. Via multivariate analysis, Rhee and Kim (13) revealed that diabetes mellitus was associated with PAD, whereas recently diagnosed diabetes mellitus had limited significance, and TTGO was not associated with PAD.

Arterial hypertension was also amongst the incriminated risk factors, with a study showing a significant and independent association between systolic arterial pressure and PAD, while diastolic pressure was not significantly associated with PAD (12).

In spite of the necessity for post-operative amputation, the primary clinical traits of the patients in the present study were similar in both the amputation and non-amputation groups. The amputation ratio of the patients who underwent the revascularization procedure was high, with 23.17% of the patients requiring postoperative amputation. However, the non-significant difference of these risk factors for PAD may suggest that they do not dictate the amputation ratio in patients who underwent the revascularization procedure. Furthermore, the association between medication at admission to hospital and PAD suggests that no previous therapy could reduce amputation risk. The results of the present study should be carefully interpreted, as in both studied groups, >80% of the patients were receiving Cilostazol treatment, >90% were following a statins and antiplatelet treatment plan, and only Pentoxifylline was administered to a small number of patients.

Inflammation plays an important role in PAD, contributing to the onset and progression of atherosclerosis, as well as to the

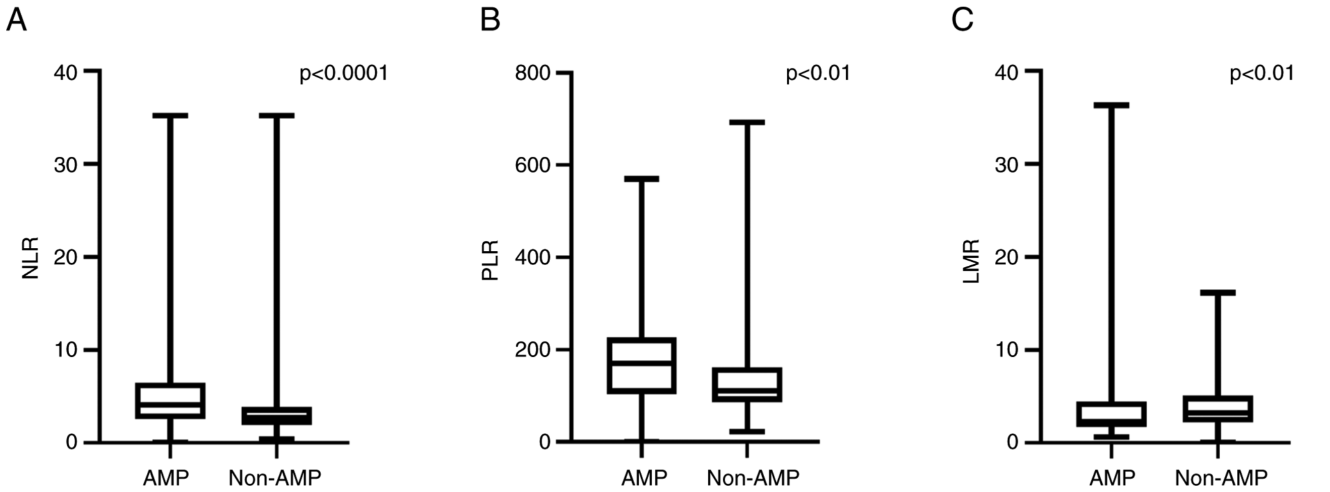

Figure 6. Association between (A) NLR, (B) PLR and (C) LMR and the AMP ratio. AMP, amputation; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio.

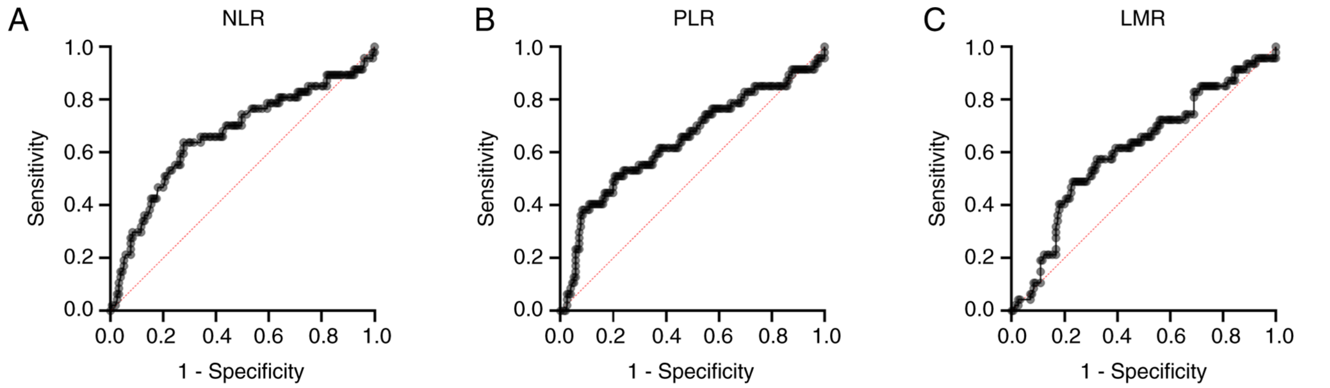

Figure 7. Receiver operating characteristic curve analysis for (A) NLR, (B) PLR and (C) LMR cut-off values to predict amputation requirement in patients with peripheral artery disease. NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio.

rupture of atherosclerotic plaques (14,15). Although inflammation is known to cause changes in the number of neutrophils, lymphocytes, monocytes and platelets, the significance of NLR, PLR and LMR is not well understood. Neutrophils release inflammatory mediators that influence endothelial dysfunction, and play an important role in increasing platelet count by stimulating megakaryocytes, creating a prothrombotic status (16). The role of platelets in the progression of atherosclerosis has been highlighted by demonstrating an interaction between platelets and endothelial cells and leukocytes, but also by highlighting the release of inflammatory substances by platelets, resulting in the adhesion and migration of monocytes (16,17).

Being involved in immune system regulation, the lymphocyte plays a crucial role in modulating the inflammatory response during atherosclerosis (14). Inflammation increases lymphocyte apoptosis, with low lymphocyte counts reflecting a repressed immune response, which has been shown to be associated with more severe clinical outcomes in various cardiovascular diseases (14-16). Monocytes also play an important role in the formation of atheroma plaque. Their recruitment and development into macrophages are the first events in atherosclerosis, which indicates the importance of inflammation in the onset of the pathology. Being involved in all stages of atherosclerotic progression, the number of monocytes is a useful marker for the evaluation of atherosclerosis (16).

Moreover, in the present study, the PLR and LMR were correlated with PAD. Both NLR and PLR had predictive values regarding the need for amputation in patients with diabetes, with higher values in the patients who required amputation than in those requiring debridement. As far as LMR is concerned, there is still no sufficient data regarding its correlation with PAD, although in a study conducted on a representative population from the USA, monocytes where the only leucocytes to be independently associated with PAD (9).

Previous studies regarding PAD with chronic evolution revealed that the NLR is an independent predictor for lesion complexity, highlighted by the TransAtlantic Inter Society Consensus-II (TASC-II) (6) classification, whereas Fontaine classification was not associated with this report (18). Moreover, a higher NLR was independently associated with a high probability of mortality in patients with chronic ischemia of the limbs or intermittent claudication, in a study conducted on 149 patients with chronic critical ischemia of the limbs (19).

It should also be highlighted that in the present study, NLR, PLR and LMR were not correlated with either Fontaine classification or with the number of affected vessels. Similar to the findings of the present study, a prospective study conducted on a smaller number of patients was not able to confirm the association between NLR and Fontaine classification (6). As a consequence, it should be taken into account that the NLR was correlated with TASC-II classification in a previous study (6). The aforementioned data suggest that the present study cannot entirely indicate the symptomatology or the number of lesions, but can predict the complexity of the existing arterial lesions in PAD.

Hirsch *et al* (20) indicated that 29% of the population are affected by PAD of the inferior limbs, and the fact that PAD prevalence raises with age, it is important to take into account both the need for improved prognostic indicators for patients with PAD, as well as the use of common inflammatory markers, such as those deriving from complete blood counts.

The primary limitation of the present retrospective study is the small amount of patients recruited, which is due to its nature as a single center study. Although NLR, PLR and LMR modifications can predict the amputation rate in PAD patients who underwent a revascularization procedure, the prognostic value of the study could not be evaluated. In order to confirm these results, future studies should be conducted with an increased number of patients. The retrospective projection of the study and the single blood sample taken for the assessment of NLR, PLR and LMR, are also considered study limitations.

In conclusion, the present study data confirm that in patients with PAD, higher NLR and PLR, as well as reduced LMR, are associated with a greater amputation rate following revascularization, despite the lack of correlation between these ratios, Fontaine classification and the number of affected vessels. The preoperative modifications reported for NLR, PLR and LMR may predict the requirement for amputation in patients with PAD who underwent a revascularization procedure.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

MC designed the study, participated in the collection of data, interpretation of data and writing of the manuscript. ER, RN and IB contributed to the collection of data. IH, BS, LP and EH participated in statistical analysis and interpretation of the results. AM, CM, NB and ITB participated in the study design and writing of the manuscript. All authors read and approved the final manuscript. All authors confirm the authenticity all of the raw data.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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