Neutrophil-lymphocyte ratio as a prognostic marker of outcome in infrapopliteal percutaneous interventions for critical limb ischemia

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Background: Endovascular intervention has become a frequently used treatment of critical limb ischemia (CLI) in recent times. The recent Bypass vs Angioplasty in Severe Ischaemia of the Leg (BASIL) trial consensus recommended endovascular treatment as a first-line treatment in patients who have a life expectancy that was limited to <2 years. Despite these recommendations, there still remains limited data available to clinicians when seeking to risk stratify patients who present with CLI. The neutrophil-lymphocyte ratio (NLR) has been suggested to be a marker for predicting mortality and patency. This study aimed to investigate the use of the NLR as a prognostic marker for primary patency and mortality after an infrapopliteal endovascular intervention in patients with CLI.

Methods: All patients who underwent tibial angioplasty for CLI were retrospectively analyzed. Demographics, degrees of stenosis, vessel patency rates, mortality, and comorbidities were recorded. NLRs were calculated from preoperative blood samples. Primary end points were all-cause mortality, primary patency, and amputation-free survival (AFS) within the follow-up period of 12 months. Multivariate Cox proportional hazard models were used to identify independent predictors. Overall survival, AFS, and the probability of a vessel remaining patent were evaluated by standard Kaplan-Meier survival curves and groups compared by the log-rank test.

Results: Eighty-three patients were monitored for 12 months. Ninety limbs were identified, with 104 procedural events and 127 vessels undergoing successful angioplasty. The technical success rate was 86%, and patency at 1 year was 19%. Survival at 1 year was 76% and AFS was 61%. Patients with a NLR <5.25 had an increased risk of death (hazard ratio, 1.97; 95% confidence interval, 1.08-3.62; P = .03) compared with those with a NLR of <5.25. Furthermore, those with lymphocytes counts of <1.5 \times 10^9/L had higher mortality (hazard ratio, 1.88; 95% confidence interval, 1.02-3.70; P = .045) than those with lymphocyte counts >1.5 \times 10^9/L. The NLR and absolute lymphocyte counts are potentially valuable prognostic indicators for risk stratification of patient’s presenting with CLI undergoing infrapopliteal angioplasty. (J Vasc Surg 2014;60:661-8.)

Critical limb ischemia (CLI) is associated with significant morbidity and mortality. If left untreated, the natural history of CLI is a 25% to 45% chance of major amputation1 and a 25% chance of death at 1 year.2,3 The Bypass vs Angioplasty in Severe Ischaemia of the Leg (BASIL) trial,4 although not specifically looking at tibial interventions, suggested that endovascular intervention should be considered the primary treatment if life expectancy were limited to <2 years. Endovascular intervention is becoming a common approach for CLI associated with infrapopliteal disease, with the primary goal of limb salvage rather than long-term patency.5 Endovascular intervention is considered to be cheaper and safer than bypass, with lower associated morbidity, mortality, and hospital stays while achieving limb salvage rates comparable to bypass without precluding later distal bypass surgery.6-11

Restenosis after treatment is a major limitation of tibial endovascular therapy. Restenosis rates are high, with reported rates between 30% and 80%12 at 1 year, but clinical success rates are often higher, with tissue healing persisting despite reocclusion,3 depending on the length of the segment involved and the degree of stenosis.

Predicting outcomes after an endovascular intervention for CLI has proven difficult. An association of diabetes mellitus, gangrene, and the presence and degree of renal dysfunction with poor outcomes has been suggested.7,13 The neutrophil-lymphocyte ratio (NLR) is another such potential prognostic marker identified as a useful tool in recognizing patients with potential for adverse outcomes when treated for CLI. Spark et al14 showed the NLR independently predicted death in chronic CLI. The NLR is thought to be a marker of systemic inflammatory burden, which, if elevated, may lead to more aggressive neointimal hyperplasia and restenosis.14 However, the NLR has not been validated as a predictive marker for outcomes in tibial angioplasty for CLI.
The aim of this study was to determine whether NLRs are a valid prognostic indicator for restenosis, limb salvage, or death in patients undergoing an endovascular intervention of the infrapopliteal vessel that present as CLI.

METHODS

Ethics approval for this study and the study design was obtained from the Institutional Review Board. Written consent was obtained from all patients contributing to the study.

Study design. The case notes of consecutive patients treated by the Department of Vascular Surgery in a tertiary teaching hospital in South Australia were retrospectively reviewed. All patients who underwent infrapopliteal angioplasty for the treatment of CLI between August 2001 and January 2010 were identified from the Department of Vascular Surgery prospectively collected patient information system. Patients who had infrapopliteal stents were excluded from the study.

Patient and laboratory data. Preprocedural and post-procedural angioplasty images were reviewed, and stenoses were graded using the TransAtlantic Inter-Society Consensus (TASC) for the Management of Peripheral Arterial Disease (TASC II) classification. The severity of disease was stratified according to the Rutherford classification. Blood samples were taken routinely at preadmission clinics for elective interventional cases and at the time of admission for emergency cases. Demographic characteristics, laboratory studies, including leukocyte counts, absolute neutrophil count, and absolute lymphocyte counts, were collected from the hospital patient information system. The NLRs were calculated from the laboratory data (absolute neutrophil count/absolute lymphocyte count). Patients were then stratified by their NLR as <5.25 or ≥5.25 according to previous published reports on the prognostic value of the NLR.

Interventions and medical therapy. Infrapopliteal interventions were performed using an antegrade or retrograde approach at the discretion of the treating physician. Heparin was routinely administered during all procedures. After the procedure, routine practice was for patients to be loaded with 300 mg of clopidogrel and to be discharged on a 4-week course of combination antiplatelet therapy consisting of aspirin (100 mg) and clopidogrel (75 mg). Adjuvant medical therapies were prescribed with the end points targeted according to the recommendations from the TASC II guidelines. These included the use of β-blockers, antiglycemic therapies, antihypertensives, diuretics, and cholesterol-lowering medications, as indicated on a patient-to-patient basis. Nonsteroidal anti-inflammatory drugs were not routinely prescribed and were often contraindicated in light of renal dysfunction in our population; however, whether they were prescribed in between follow-up visits was not recorded. Furthermore, additional wound care clinics and follow-up was arranged for patients with persistent ulcers, as indicated.

Surveillance protocol. After the interventions, patients were admitted to the wards for observation and discharged when deemed medically fit by the treating surgeon. Patients were routinely followed up at 3-month intervals in outpatient clinics, at which stage arterial duplex scans were performed to assess vessel patency. The degree of stenosis was assessed by measuring the peak systolic velocity (PSV) and calculating the peak velocity ratio (intrastenotic PSV divided by the proximally recorded PSV). A peak velocity ratio of >2.4 was reported as a stenosis of >50% according to Ranke et al. These patients then underwent angiography at the discretion of the operating surgeon if findings correlated clinically.

Complications. Major complications were defined as access site complication requiring surgery, bleeding complications with a drop in hemoglobin levels of ≥2 g/dL, macroembolization requiring further revascularization or immediate amputation postprocedure. Minor complications were defined as perioperatively complications that did not require further recanalization, including superficial hematoma, minor embolization, and local dissection.

Study end points. Primary outcomes were technical success, all-cause mortality, 12-month primary patency, and amputation-free survival (AFS). Technical success was defined as a successful recanalization with <50% residual narrowing and restoration of antegrade perfusion as measured on ultrasound imaging. Primary patency was defined as uninterrupted patency with no procedure at the treated site or on a segment adjacent to it.

Statistical analysis. A two-sided log-rank test with an overall sample size of 83 patients achieved 80% power at a 0.05 significance level to detect a minimum hazard ratio (HR) of 1.8 when the NLR was ≥5.25 and a HR of 1.00 for a NLR of <5.25. Statistical analyses were performed using STATA 12.0 software (StataCorp LP, College Station, Tex) and R 2.15.1 software (The R Foundation for Statistical Computing, http://www.r-project.org/foundation/). Descriptive statistics for interval scale measurements are expressed as mean and standard deviation, and percentages were calculated for categoric data.

The outcomes of interest were mortality and primary patency of vessels attempted. Time to event (survival time) was calculated in days from the date of the procedure (baseline) to the survival date, which was date of death, loss to follow-up, or end of follow-up at 12 months. Kaplan-Meier survival curves were used to estimate survival rates, likelihood of limb salvage, and the probability of vessel patency. Log-rank tests for trend of survival function across categories of covariates were performed.

Multivariate Cox proportional hazards regression analysis was applied to calculate the HR for categories of covariates. The models were adjusted by age, gender, Rutherford classification at presentation, TASC score, prescribing rates, year of intervention, and patient comorbidities, which included ischemic heart disease, chronic renal insufficiency, diabetes mellitus type 2, dyslipidemia, and hypertension. Proportional hazard assumptions for the data set were also tested and confirmed to satisfy assumptions.

RESULTS

We identified 83 patients with ischemia affecting 90 limbs. Patient demographics were typical of patients with
severe peripheral arterial disease (Table I). Most patients had ischemic ulceration or minor gangrene. Of 104 procedures, 71 (68%) angioplasty procedures were performed for isolated infrapopliteal disease, and the remainder (32%) involved concurrent angioplasty of the superficial femoral artery, the popliteal artery, or both. Eight patients underwent multiple angioplasties, including four with bilateral disease and 14 with angioplasty on the ipsilateral side.

The tibial vessels that were targeted ranged from a TASC score of B to D, with 16 unable to be assessed due to unavailability of imaging. The most common vessel to undergo angioplasty was the anterior tibial artery, followed by the tibioperoneal trunk and the peroneal artery, with the posterior tibial least involved. The overall initial technical success rate for the study was 85%, with 127 of 149 successfully treated lesions.

Survival at 1 year postangioplasty was 76% (Fig 1). AFS was 68% (Fig 2). Patency rates were 55% at 3 months, 44% at 6 months, 28% at 9 months, and 19% at 1 year (Fig 3). Of the 13 failed angioplasty procedures, three limbs (23%) underwent bypass, and two (15%) required amputation. In contrast, for the 77 limbs with successful procedures, 14 (18%) had repeat angioplasty, four (5%) had bypass, and five (6%) had amputations.

There were no major procedural complications. Of the 23 minor complications, nonocclusive dissections occurred in nine target vessels (7%), and one patient required superficial femoral artery stenting. Distal embolism occurred in six patients (5%), who were treated with suction embolectomy and glyceryl trinitrate. A postprocedural hematoma developed in five patients (4%), and all were treated conservatively. An arteriovenous fistula formed at the treated segment in two patients (2%), without any clinical consequence. One anterior tibial artery (1%) was perforated. None of the minor complications were associated with any significant clinical consequences and did not require any further revascularization.

Patients with a NLR of $\leq 5.25$ had a 12-month mortality risk that was nearly double that of those with a NLR of $>5.25$ (1-year mortality of 39% vs 17%; respectively; HR, 1.97; 95% confidence interval [CI], 1.08-3.62; $P = .03$; Fig 4).

Table I. Demographic and clinical characteristics of 83 patients with 104 admissions

<table>
<thead>
<tr>
<th>Variablea</th>
<th>Overall</th>
<th>&lt;5.25</th>
<th>$\geq 5.25$</th>
<th>Pb</th>
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<td>Demographic characteristics</td>
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</tr>
<tr>
<td>Male</td>
<td>50 (64.9)</td>
<td>25 (55.6)</td>
<td>25 (78.1)</td>
<td>.04</td>
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<td>Age, years</td>
<td>81.0 (76.0-86.0)</td>
<td>81.0 (75.0-86.0)</td>
<td>82.0 (76.5-86.0)</td>
<td>.70</td>
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<td>Risk factors (n = 83 patients)</td>
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<tr>
<td>Hypertension</td>
<td>42 (54.6)</td>
<td>26 (57.8)</td>
<td>16 (50.0)</td>
<td>.50</td>
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<td>Ischemic heart disease</td>
<td>49 (63.6)</td>
<td>23 (51.1)</td>
<td>26 (81.3)</td>
<td>&lt;.01</td>
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<td>Diabetes mellitusd</td>
<td>46 (59.7)</td>
<td>27 (60.0)</td>
<td>19 (59.4)</td>
<td>.96</td>
</tr>
<tr>
<td>Chronic renal impairmente</td>
<td>12 (15.6)</td>
<td>9 (20.0)</td>
<td>3 (9.4)</td>
<td>.21</td>
</tr>
<tr>
<td>Dyslipidemiaf</td>
<td>29 (37.7)</td>
<td>17 (37.8)</td>
<td>12 (37.5)</td>
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<td>Rutherford classification</td>
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<td>IV</td>
<td>22 (24.8)</td>
<td>11 (19.0)</td>
<td>11 (28.2)</td>
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<tr>
<td>C</td>
<td>16 (19.8)</td>
<td>9 (17.0)</td>
<td>7 (26.9)</td>
<td>.46</td>
</tr>
<tr>
<td>D</td>
<td>61 (77.9)</td>
<td>42 (79.3)</td>
<td>19 (73.1)</td>
<td>.74</td>
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<tr>
<td>Disease and intervention</td>
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<tr>
<td>Anterior tibial</td>
<td>41 (42.3)</td>
<td>30 (51.7)</td>
<td>11 (28.2)</td>
<td>.02</td>
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<tr>
<td>Posterior tibial</td>
<td>21 (21.7)</td>
<td>11 (19.0)</td>
<td>10 (25.6)</td>
<td>.43</td>
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<tr>
<td>Tibioperoneal trunk</td>
<td>31 (32.0)</td>
<td>18 (31.0)</td>
<td>13 (33.3)</td>
<td>.81</td>
</tr>
<tr>
<td>Peroneal</td>
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<td>12 (20.7)</td>
<td>13 (33.3)</td>
<td>.16</td>
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<tr>
<td>No. of vessels attempted</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>62 (63.9)</td>
<td>37 (63.8)</td>
<td>25 (64.1)</td>
<td>.99</td>
</tr>
<tr>
<td>2</td>
<td>29 (29.9)</td>
<td>16 (27.6)</td>
<td>13 (33.3)</td>
<td>.70</td>
</tr>
<tr>
<td>3</td>
<td>6 (6.2)</td>
<td>5 (8.6)</td>
<td>1 (2.6)</td>
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NLR, Neutrophil-lymphocyte ratio; TASC, TransAtlantic Inter-Society Consensus.

aCategoric data are presented as number (%) and continuous data as median (interquartile range).

bP values are based on $\chi^2$ test for categories and Mann-Whitney $U$ test for age. P values are not available for missing cells: 83 patients for demographic characteristics and risk factors, 104 admissions for Rutherford classification, modified TASC classification, disease and intervention, and number of vessels attempted.

cDefined as serial blood pressure measurements >140/90 mm Hg.

dDefined as fasting blood glucose test result at or >7.0 mmol/L or a random blood test result at or >11.1 mmol/L.

eDefined as serum creatinine >1.5 mg/dL for >3 months.

fDefined as history of increased low-density lipoprotein or triglycerides, or low high-density lipoprotein or use of lipid-lowering agents, or both.
also had close to double the chance of death compared with those with lymphocyte counts $>1.5 \times 10^9/L$ (36% vs 14%, respectively; HR, 1.88; 95% CI, 1.02-3.70; $P = .045$; Fig 5). A high NLR was associated with a higher risk of the patient having ischemic heart disease ($P < .01$) and disease involving the anterior tibial artery ($P = .02$; Table I).

No statistical significance was demonstrated when stratified by absolute neutrophil count (HR, 1.19; 95% CI, 0.63-2.25; $P = .61$). Furthermore, primary patency at 1 year for the cohort showed no significant difference when stratified by NLR (HR, 1.03; 95% CI, 0.74-1.43; $P = .87$), lymphocyte (HR, 1.02; 95% CI, 0.73-1.43; $P = .91$), and neutrophil counts (HR, 0.86; 95% CI, 0.58-1.28; $P = .47$; Table II). The standard error for calculated Kaplan-Meier curves remained $<10\%$ at the 12-month follow-up for overall survival, AFS, and patency. However, during the 8 years, there was a significant difference in the severity of the Rutherford classification at presentation, with the latter 5 years showing an increased incidence for patients presenting with more severe symptoms of CLI being offered angioplasty over open procedures. Despite this increase in CLI severity, mortality rates did not differ significantly. Prescribing practice of drug classes was consistent, with no significant differences identified over the time period; however, there was some variability in some of the preferred specific agents within the classes (i.e., simvastatin vs atorvastatin, etc).

**DISCUSSION**

Clinical decision making and planning for revascularization is difficult in patients presenting with CLI because of severe multiorgan comorbid disease associated with its presentation. Therefore, the identification and implementation of prognostic indicators is greatly beneficial to the clinician. Being able to identify patients who are at high
risk of death allows the clinician to use less invasive treat-
ments, such as endovascular intervention, for initial limb
salvage. This study found a significant relationship between
a high NLR and increased mortality at 1 year for patients
with CLI undergoing tibial angioplasty.

In the setting of coronary artery disease especially, the
NLR has emerged as a powerful predictor of outcome.
A high NLR has been shown to be predictive not only
for bare-metal stent restenosis and progression of athero-
硬化在冠状动脉18但，更明显地，
也死亡后经皮冠状动脉介入
和冠状动脉旁路移植
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NLR可能也与存在
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In the context of peripheral vascular disease, Bhutta
et al20 retrospectively analyzed 1021 patients who under-
gone major vascular surgery (open or endovascular aortic
aneurysm repair, lower limb revascularization, or carotid
endarterectomy) and found that a preprocedural NLR of
>5 identified patients at increased risk of death \( \equiv 2 \) years.
Furthermore, Kullar et al21 found that the postoperative
NLR was an independent predictor of graft failure in 126
patients.
Spark et al14 have been the only group to investigate
the value of the NLR in CLI that included endovascular
intervention. In their prospective study of 149 patients pre-
senting with CLI, they found that a preprocedural NLR
\( \equiv 5.25 \) was associated with higher mortality. Our
findings correlate with this and suggest that the NLR could also
be applied to infrapopliteal vessel angioplasty, with similar
correlations in regards to adverse outcomes.

In addition, our study has been the first to show that
lymphopenia is related to higher risks of death but not pri-
mary patency after intervention. In cardiac disease, a low
lymphocyte count has been shown to be an independent
prognostic marker in heart failure,22 with a suggestion
that patients with lower lymphocyte counts tend to be
older and have higher rates of comorbid disease.23

Fig 3. Overall patency is shown after infrapopliteal angioplasty for critical limb ischemia (CLI). Patency of vessels was
 adjudged by ultrasound imaging follow-up at 3 months. The standard error for calculated Kaplan-Meier curves (gray
area) remained <10% at the 12-month follow-up.

Fig 4. Survival stratified by a neutrophil-lymphocyte ratio (NLR) of <5.25 (dotted line) and \( \equiv 5.25 \) (solid line) in
patients after infrapopliteal angioplasty for critical limb ischemia (CLI) shows significance between a NLR \( \equiv 5.25 \) and
an increased risk of death. The standard error for calculated Kaplan-Meier curves (gray area) remained <10% at the 12-
month follow-up. CI, Confidence interval; HR, hazard ratio.
including diabetes mellitus, renal insufficiency, and atrial fibrillation. This burden of comorbid disease is also prevalent in our population of patients, with diabetes present in nearly 50% and periprocedural renal dysfunction in just over 60%. Furthermore, these factors have been previously identified as being associated with poor outcome after vascular intervention and thus may be reflected in a higher risk of death.

We believe that tibial disease may reflect a more aggressive disease process (ie, with greater oxidative free radicals, greater circulating proinflammatory cytokines, etc), with the relative lymphopenia a result of a greater cortisol-induced stress response, and although outside of the scope of this study, it would also be interesting to see whether this lymphopenia translates to an increased risk of other infections, respiratory or otherwise, in this group of patients.

No relationship was found between the absolute neutrophil count and primary patency or mortality. This contrasts with a number of studies that have demonstrated that neutrophils are a strong marker to predict adverse cardiovascular outcomes. Toor et al retrospectively studied 101 patients who underwent percutaneous transluminal angioplasty and found that preprocedural neutrophils could be used as a global risk factor for adverse outcomes after the procedure. Neutrophils mediate the inflammatory response after vascular injury and have been shown to accumulate at the sites of angioplasty #1 hour postprocedure. In animal models, this accumulation of neutrophils has been reported to promote intimal hyperplasia, with the localized inflammation resulting in subsequent thrombus formation, intimal hyperplasia development, and potential restenosis. Joviliano et al suggested that the value of the NLR is that it reflects the neutrophilia of inflammation and the relative lymphopenia of cortisol-induced stress. The results of our study do not dispute this but may suggest that lymphopenia may have a larger role in predicting adverse outcomes than first thought.

Our study achieved an AFS rate of 68% at 1 year, which is comparable to current standards reported to be between 75% and 100%. Similarly, our 1-year survival rate was 76%, which is also comparable to the 65% to 78% reported in the literature. Nair et al suggested that clinical success is often higher than long-term primary patency rates because healing of wounds can occur in 3 to 6 months and persist.
despite vessel reocclusion, which may be demonstrated by good AFs despite relatively poor primary patency.

If NLR is correlated with poorer outcomes, how should clinicians approach patients with ratios ≥5.25? Unfortunately, there is currently no evidence in the literature to guide how to directly affect this measure. NLR is a reflection of the systemic inflammatory burden created by the systemic disease state of atherosclerosis. We know that pharmacotherapy, such as with aspirin and statins, which are recommended for the treatment of atherosclerotic risk factors, also have pleiotropic anti-inflammatory properties that have been proposed as an adjuvant treatment for patients with sepsis. In atherosclerosis in particular, high-dose statin therapy has been demonstrated to exert anti-inflammatory effects, modulate endothelial function, and inhibit the thrombotic signaling cascade and has the potential to lead to plaque/disease regression. As an extension of this idea, one could speculate that maximizing secondary prevention with pharmacotherapy may also be likely to reduce NLR. However, further work is required to define and confirm this concept, which unfortunately falls outside the scope of this study.

Further limitations of this study were its retrospective design, and further prospective trials are required to further elucidate the value of the NLR in the setting of peripheral vascular disease. Furthermore, due the limited sample size, there may be type II statistical error when not having found correlations among NLR, lymphocytes, and neutrophils with patency.

Despite these limitations, NLR represents an attractive test for clinicians as a risk assessment model, especially given the simplicity and ready availability of the neutrophil and lymphocyte counts as a preoperative workup. We recommend that an NLR be calculated for all patients presenting with CLI. Those who are undergoing endovascular procedures and have a high preprocedural NLR or lymphopenia are at higher risk and may warrant aggressive preoperative optimization of risk factors and more diligent follow-up.

CONCLUSIONS

The preprocedural NLR may represent a valuable prognostic marker of increased risk of death after infrapopliteal angioplasty for CLI. Our retrospective study demonstrated an independent correlation between an increased NLR and lymphopenia with a greater risk of death. This study suggests that NLR and lymphocyte counts may be valid tools to risk stratify patients being considered for tibial angioplasty. However, further prospective trials are required to elucidate the validity of this measurement.

AUTHOR CONTRIBUTIONS

Conception and design: CC, PP Analysis and interpretation: CC, PP, SU, CD, IS Data collection: CC Writing the article: CC, PP, SU, CD, IS

Critical revision of the article: CC, PP, SU, CD, IS

Final approval of the article: CC, PP, SU, CD, IS

Statistical analysis: CC, SU

Obtained funding: Not applicable

Overall responsibility: IS

REFERENCES


