REVIEW ARTICLE

THE IMPACT OF NEUTROPHIL-TO-LYMPHOCYTE RATIO AND PLATELET-TO-LYMPHOCYTE RATIO IN CAROTID ARTERY DISEASE

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Abstract

Introduction: *Inflammation is a common underlying feature of atherosclerosis. Several inflammatory biomarkers have been reported to have prognostic value, in several areas, including in vascular surgery. The neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) may permit to identify patients at greater risk for cerebrovascular events, tailor patient management, improve preoperative status and possibly develop target anti-atherosclerotic therapy. However, studies reporting usefulness of these hematological biomarkers in the context of carotid artery disease are still scarce.*

The aim of this study was to review the literature concerning the prognostic ability of NLR and PLR in the subpopulation of vascular patients with carotid artery disease.

Methods: *A Medline search was performed in order to identify publications focused on the physiopathology of NLR and PLR and their impact in the management of patients with carotid artery disease.*

Results: *The study identified 18 articles with a total of 5339 patients. NLR is associated with carotid intima-media thickness, carotid plaques, carotid stenosis, symptomatic stenosis and intra-stent restenosis after carotid artery stenting and cognitive dysfunction after carotid endarterectomy. PLR is associated with carotid stenosis, symptomatic stenosis and predicts post-operative outcomes after carotid artery revascularization, including post-operative stroke, acute coronary syndrome and all-cause mortality.*

Conclusions: *The neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) have the ability to predict sub-clinic atherosclerosis, atherosclerosis progression in carotid artery disease and propensity for carotid stenosis to become symptomatic along with morbidity following CEA and carotid stenting. Consequently, these parameters may be considered to tailored therapy and improve patient management.*

INTRODUCTION

Carotid disease affects 3-4% of the general population with varying degrees according to age, gender and race.[1] In addition to cognitive decline, it is a major risk factor for ischemic stroke, with ipsilateral carotid atherosclerosis accounting for 20-30% of these events.[2] Patients with ≥50% carotid artery stenosis have an annual stroke risk of 0.34% and an annual transient ischemia attack (TIA) risk of 1.78%.[1] Despite the improved outcomes in recent years, stroke still poses a high burden of morbidity and mortality, leaving impaired half of those who survive.[3]

Thus, understanding carotid stenosis pathophysiology and progression and its crosstalk with sustained low-grade inflammation remains significant in identifying new disease predictors.[4-7] Several inflammation biomarkers have been reported, including new emerging complete blood count (CBC) related biomarkers, given its wide availability and prognostic value, which could allow closer monitoring of patients with expected negative outcomes, tailoring patient management or pre-operative status improvement and possibly the development of targeted anti-atherosclerotic therapy.[8,9] Platelets play a crucial role in the development and advance of atherosclerotic

disease by the secretion of proinflammatory cytokines.[10,11] Likewise, neutrophils promote plaque destabilization and subsequent rupture while stimulating thrombogenesis and increasing platelet aggregation.[12,13] Lymphopenia is associated with physiologic stress and immunodeficiency, cardiovascular complications, and mortality.[14,15] Two CBC markers with increasing relevance are the neutrophil-to--lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR), which have been suggested to predict outcomes in tumors, systemic lupus erythematosus, sepsis, and cardiovascular disease.[15-23] In the vascular field, these parameters have been associated with increased risk of death within two years of a major vascular surgery,[24] greater risk for progression or *de novo* chronic limb-threatening ischemia (CLTI)[25] and amputation rates in patients with CLTI,[26-28] poorer limb survival after embolectomy for acute limb ischemia,[29] higher risk of complications following surgical abdominal aortic aneurysm repair[30] and worse prognosis after acute mesenteric ischemia.[31,32] However, clinical relevance of these parameters in patients with carotid stenosis is scarcely described.

The aim of this study was to review the literature regarding the prognostic relevance of NLR and PLR in the subpopulation of vascular patients with carotid artery disease.

METHODS

A Medline search was performed in order to identify articles focused on these hematological parameters and their pathophysiology and effects on outcomes in carotid artery disease. Keywords used for research included "carotid disease", "neutrophil-to-lymphocyte ratio", "platelet--to-lymphocyte ratio", "hematological parameters," and "carotid endarterectomy". Additional articles of scientific interest for the purpose of this non-systematic review were included by cross-referencing. Primary endpoints were to describe association between serologic markers and carotid disease progression. Association between ratios and demographic features was also assessed.

RESULTS

The study identified 18 articles, including 5339 patients.

NEUTROPHIL-TO-LYMPHOCYTE RATIO AND CAROTID ARTERY DISEASE

Clinical demographics

Higher values of NLR were associated with male gender (2.59 ± 2.03 *vs* 2.09 ± 0.86, P=0.009) in a retrospective study by Deser *et al*, which included 160 patients submitted to CEA for severe ICA stenosis and 201 patients without severe carotid stenosis,[33] a finding corroborated by another retrospective study enrolling 270 patients to CEA (P=0.012).[34] Additionally associated higher values of NLR with older age (P=0.006). Increased body mass index (BMI) was associated with NLR < 5 in a population of 432 patients that underwent CEA for high-grade carotid stenosis (both symptomatic and asymptomatic stenosis) (27.2 ± 4.6 *vs* 25.7 ± 3.5, P=0.01) [35].

Carotid intima-media thickness

In a cohort of patients with acute and subacute ischemic stroke, a significant positive correlation between NLR and increased carotid intima-media thickness (IMT) in male gender, after confounding adjustment (adjusted R2=0.185, root mean squared error [MSE]=0.152).[36] Likewise, in a study by Suarez-Cuenca *et al*, patients awaiting bariatric surgery with known cardiovascular risk factors had a positive correlation between NLR and IMT (P=0.05).[37] Both studies used carotid ultrasonography to evaluate IMT. Additionally, in patients with cardiac syndrome X and coronary artery disease, one also could verify association between NLR and IMT (P<0.05),[38] while patients with diabetes mellitus type 2 an increased NLR revealed higher media thickness (P=0.005 and odds ratio [OR], 140.89; 95% CI 1.71–11615.30, P=0.028, respectively).[39,40]

Carotid plaques

In order to evaluate subclinical atherosclerosis, Corriere *et al.*[41] recruited 384 patients ≥ 65 years who were submitted to carotid ultrasonography and found a statistically significant association between a higher value of NLR and the presence of carotid plaques, with NLR > 2.4 revealing a prevalence of 80% and NLR > 3.68 a prevalence of 97% of carotid plaques.

A prospective study enrolled 139 asymptomatic patients with intermediate carotid stenosis (50-70% stenosis), performing both carotid doppler ultrasonography and computed tomography angiography (CTA) to separate them in two groups: one with calcified plaques and another with non-calcified carotid plaques. This study found NLR to be significantly higher in the group with non-calcified plaques (OR, 5.686; 95% CI 2.498 – 12.944, P<0.001).[42]

Carotid artery stenosis

Examining 105 consecutive patients with digital subtraction angiography (DSA) presenting with ischemic stroke, transient ischemic stroke or suspected carotid stenosis in a health check-up, Hong Jiang *et al.*[43] found NLR was independently associated with both maximal extracranial carotid stenosis and accumulated extracranial carotid stenosis (sum of right and left maximal carotid artery stenosis) (adjusted R^2=0.179, P<0.001 and adjusted R^2=0.183, P<0.001, respectively). The suggested NLR cutoff of 1.89 was predictive of 50% maximal extracranial carotid stenosis or greater, with a 78.4% sensitivity and 77.4% specificity. Similarly, in the previously mentioned cohort by Deser *et al.*,[33] NLR was significantly higher in patients with 70-99% carotid artery stenosis vs 0-50% (P<0.01).

Symptomatic carotid artery stenosis

Retrospectively analyzing 270 patients who underwent carotid endarterectomy (CEA) and grouping them in 4 different categories concerning NLR and PLR values, Nicolas Massiot et al.[34] found that increases in both ratios were significantly associated with symptomatic ICA stenosis (P=0.005 and P=0.051, respectively). Erkan Köklü *et al*[44] found similar results, with NLR being independently associated with symptomatic carotid plaque in intermediate carotid stenosis (50-70%) after multivariate logistic regression (OR, 7.779; 95% CI 3.685-16.424, P=0.001) with an NLR cutoff value of ≥ 2.6 (73% sensitivity and 71% specificity).

Post-operative outcomes of carotid artery revascularization

Zhengze Dai *et al* in a study including 459 patients undergoing carotid stenting (164 for symptomatic carotid stenosis and 295 for asymptomatic stenosis), reported an overall rate of in-stent restenosis (ISR) of 15.7% at 14.6 ± 19.1 months follow-up. ISR rate among asymptomatic patients was 16,9% and, specifically in this subgroup, an association between ISR and a baseline NLR value ≥ 2.13 after multivariate Cox regression analysis (HR, 2.74; 95% CI, 1.46-5.14) was found.[45] This association was not observed in patients submitted to CAS for symptomatic stenosis. Xiang Bao *et al*,[46] who compared 108 patients submitted to CAS and had ISR *vs* 226 without IRS, also found a positive correlation with NLR and ISR (area under the curve [AUC]=0.703). A statistically significant association was not found for PLR.

Moreover, in a retrospective study, where 432 patients were submitted to CEA, NLR ≥ 5 was associated with a three-fold increased risk of cognitive dysfunction one day after surgery (OR, 3.38 [1.81-6.27], P<0.001).[35]

PLATELET-TO-LYMPHOCYTE RATIO AND CAROTID ARTERY DISEASE

Clinical demographics

Nicolas Massiot *et al*[34] studying patients submitted to CEA, found PLR increased with age (P<0.0001), and that it was also linked to a subpopulation with a higher proportion of non-smokers (P<0.001).

In a retrospective study enrolling 150 patients who underwent carotid CTA, higher levels of PLR were significantly associated with hypertension (P=0.022) [47].

Carotid artery stenosis

Higher PLR was significantly associated with a 90-99% CS (*vs* 0-50%, P<0.01).[33] Retrospectively analyzing 150 patients with carotid stenosis submitted to CTA, the PLR value was correlated with the degree of stenosis (P<0.017).[47] Ceyhun Varim *et al*[48] divided 140 patients who underwent carotid CTA in 2 groups, one with critical carotid stenosis (symptomatic patients with >50% carotid stenosis and asymptomatic patients with > 80% stenosis) and another one with non-critical carotid stenosis. The study found PLR values were higher in the group with critical carotid stenosis (P<0.0001), with a threshold of PLR > 117.1 (89% and 68% sensitivity and specificity, respectively [95% CI, 0.043-0.159; AUC 0.101 ± 0.03]).

Symptomatic carotid stenosis

In a mentioned series of 150 patients with carotid stenosis, Soylu *et al* identified a higher PLR as a predictor of symptomatic stenosis (OR 1.012; CI 1.001-1.024, P=0.008) with PLR > 119.43 as the best threshold with 85.9% and 60.1% sensitivity and specificity, respectively.[47] Furthermore, the retrospective analysis of 270 patients submitted to CEA suggested an association between higher PLR values and symptomatic ICA stenosis (P=0.051).[34]

Postoperative outcomes of carotid artery revascularization and mortality

Deser *et al* identified elevated PLR as an independent predictor of postoperative stroke for CEA (185.76 ± 23.72 *vs* 115.80 ± 43.39, P=0.035). The threshold of 145.304 provided a sensitivity of 83.3% and a specificity of 73.8%, with a C-statistic of 0.862 (95% CI, 0.802–0.921, P=0.002).[33]

In a cohort of 196 consecutive patients who underwent CEA, Aldo Bonaventura *et al*[49] unveiled a positive correlation between PLR and acute coronary syndrome at 18 months follow-up with the cut-off being 153.6 (HR, 1.44; 95% CI, 1.08-1.92).

In a retrospective study, which included 146 patients submitted to selective carotid angiography, after multivariate analysis, only higher PLR predicted all-cause mortality in this cohort of patients with coronary artery disease (OR, 0.010; 95% CI, 1.002-1.018, P=0.009).[50]

DISCUSSION

This review highlights the ongoing interest in the study of these hematological ratios and their ability to predict outcomes in patients with carotid artery disease. The neutrophil-to-lymphocyte ratio (NLR) and platelet--to-lymphocyte ratio (PLR) have the ability to predict sub-clinic atherosclerosis, atherosclerosis progression in carotid artery disease and propensity for carotid stenosis to become symptomatic along with morbidity following CEA and carotid stenting.

Atherosclerosis is a systemic vascular inflammatory disease, affecting medium and large-sized arteries, with inflammation playing an essential role in the initiation, progression, destabilization, and rupture of the atherosclerotic plaque, contributing to symptom onset and adverse outcomes. Plaques are composed of infiltrating inflammatory cells, namely monocytes, macrophages, T lymphocytes, and dendritic cells, in addition to smooth muscle cells, extracellular matrix proteins, lipids, and calcium deposits.[51-53]

NLR is linked to chronic low-grade inflammation, with high sensitivity C-reactive protein presenting a positive correlation with this ratio.[54] Although the role of NLR

is still not completely understood, evidence from a recent meta-analysis including 20 studies and 49097 patients suggested an association between IMT and serum levels of high sensitive C-reactive protein (hs-CRP), fibrinogen, and total leukocyte count.[55] Neutrophils have been implicated in cardiovascular inflammation and repair, as well as in atherosclerotic plaque invasion and instability through the production of proteolytic enzymes, arachidonic acid derivatives, and superoxide radicals.[56,57] Furthermore, neutrophils produce cytokines that have been suggested to increase plaque size and induce instability and rupture, such as IL-1b, IL-6, IL-18, and MCP-1.[58] These cytokines have been found in atherosclerotic plaques, including carotid atheromas,[59] and contribute with varying degrees to plaque formation and rupture. MCP-1 regulates migration and infiltration of monocytes/macrophages and the proliferation and migration of smooth muscle cells, migration of endothelial cells, and plaque neovascularization.[51]

The ratio PLR has been associated with cardiovascular disease and outcomes despite the incomplete understanding of the pathophysiology. Platelets have a central role in thrombus formation, although several molecular pathways that implicate them in atherogenesis have been suggested. Platelets induce a pro-inflammatory status through cytokine production and platelet-derived microvesicles, in addition to activating the complement system, an essential innate immunity component that contributes to vascular inflammation.[60,61] CCL5 secretion is one of the hallmarks of the initial inflammatory response to vascular injury, with platelets secreting a significant amount of this cytokine after their migration to the endothelial monolayer.[62] Platelets produce PDGF and MMPs, contributing to intimal thickening and atherosclerosis progression.[63]

Bochao D. Lin *et al*[64] have shown that NLR and, to an even a larger extent, PLR are influenced by heritability. Additionally, the authors revealed an association of both ratios with increased age, which was corroborated by others.[34] NLR has also been associated with an older population by other studies in the general population.[65,66] The authors argue that this correlation may be linked with underlying diseases present in the older population.[34,65] In fact, Basem Azab *et al*[66] reported higher values of NLR in patients with cardiovascular disease. Bochao D. Lin *et al*[64] also detected a higher mean level of NLR and PLR in men. Two other studies reported the same connection with higher NLR in men.[33,34] However, Bochao D. Lin *et al*[64] also had conflicting findings, describing a positive correlation with BMI, while Halazun *et al*[35] had a higher BMI in the cohort with NLR < 5. Nevertheless, what would be expected is a positive correlation as obesity generally reflects a chronic state of inflammation,[67] as described in previous studies.[65,66,68,69]

Interestingly, both NLR and PLR is positively correlated with carotid stenosis severity[33,43,47,48] and able to predict symptomatic stenosis.[34,44,47] Besides, it seems that both ratios are able to predict disease progression. Regarding NLR, it seems to provide a disease marker, being able to predict from higher IMT[36-40] to carotid plaques,[41,42] carotid stenosis[33,43] and symptomatic stenosis[34,44] while PLR is able to predict both stenosis severity[33,47,48] and symptomatic stenosis.[34,47] However, NLR seems a better predictor of sub-clinic atherosclerosis, given the positive correlation demonstrated in several studies with NLR and IMT,[36-40] a proven marker of sub-clinic atherosclerosis.[70]

Furthermore, both ratios have a positive correlation with the severity of the outcomes, as the higher the threshold is settled, the greater the severity of the predicted outcome: NLR > 1.89[43] is predictive of a carotid stenosis > 50% while NLR > 2.6 is predictive of a symptomatic carotid stenosis.[44] Similarly, PLR > 117.1 predicts critical stenosis,[48] PLR > 119.43 predicts a symptomatic stenosis[47] while higher thresholds predict worse outcomes after CEA, with PLR > 145.304 as a predictor of stroke[33] and PLR > 153.6 as a predictor of acute coronary syndrome.[49] Therefore, there is a positive correlation between the above mentioned ratios and the grade of inflammation and atherosclerosis severity, with clinical findings supporting this association. Recognizing this serologic link can provide valuable information, namely to support the decision of which asymptomatic carotid stenosis most benefit from revascularization, still one of the main challenging decisions concerning carotid artery disease.[44] Accordingly, Yuksel *et al*[42] studied patients with asymptomatic carotid stenosis between 50% and 70% and found that patients with non-calcified carotid plaques presented higher values of NLR, permitting to identify the subset of patients with increased risk for cerebrovascular events.[71]

Zhengze Dai *et al*[45] and Xiang Bao *et al*[46] linked higher levels of NLR to ISR after carotid stenting, which is associated with an increased risk of late cerebrovascular events.[72] Also, in coronary artery disease, higher levels of NLR have proven to be able to predict restenosis after stent implantation.[73,74]

Anti-inflammatory therapy may emerge as an atherosclerotic treatment option and NLR and PLR can be used as inflammation markers and predictors of several postoperative outcomes.[33,35,49,50] There is a growing research interest, using hs-CRP as the control inflammatory biomarker, which correlates with NLR and PLR.[75-77] Anti-inflammatory therapies being tested include rosuvastatin[78] or canakinumab.[79]

This review has several limitations that warrant consideration. Firstly, it is possible that not all available data was captured, making publication bias possible. Additionally, most are single-center reports with limited populations. Finally, the interaction between serologic parameters and chronic atherosclerosis may have difficulty in establishing a causal relationship between serologic parameters and cerebrovascular events. Therefore, larger multicentric prospective, or ideally randomized controlled studies, should be pursued.

CONCLUSION

This study highlights the increasing interest in NLR and PLR as inflammation/ atherosclerosis markers. These ratios are easily available markers and predict sub-clinic

atherosclerosis and atherosclerosis progression in carotid artery disease. This has clinical implications as may permit to identify patients with greater propensity to become symptomatic and even with greater post-operative morbidity. These potential abilities would allow tailored therapy such as dual antiaggregant therapy, anti-inflammatory therapy, or the preference of eversion technique rather than conventional endarterectomy in order to attempt the extraction of the whole plaque burden[33] and improve outcomes.

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REFERENCES

1. Aday, A.W. and J.A. Beckman, Medical Management of Asymptomatic Carotid Artery Stenosis. Prog Cardiovasc Dis, 2017. 59(6): p. 585-590.
2. Bejot, Y., et al., Epidemiology of stroke in Europe and trends for the 21st century. Presse Med, 2016. 45(12 Pt 2): p. e391-e398.
3. Naylor, A.R., et al., Editor's Choice - Management of Atherosclerotic Carotid and Vertebral Artery Disease: 2017 Clinical Practice Guidelines of the European Society for Vascular Surgery (ESVS). Eur J Vasc Endovasc Surg, 2018. 55(1): p. 3-81.
4. Ross, R., Atherosclerosis--an inflammatory disease. N Engl J Med, 1999. 340(2): p. 115-26.
5. Taleb, S., Inflammation in atherosclerosis. Arch Cardiovasc Dis, 2016. 109(12): p. 708-715.
6. Geovanini, G.R. and P. Libby, Atherosclerosis and inflammation: overview and updates. Clin Sci (Lond), 2018. 132(12): p. 1243-1252.
7. Libby, P., Inflammation in atherosclerosis. Arterioscler Thromb Vasc Biol, 2012. 32(9): p. 2045-51.
8. Soeki, T. and M. Sata, Inflammatory Biomarkers and Atherosclerosis. Int Heart J, 2016. 57(2): p. 134-9.
9. Moriya, J., Critical roles of inflammation in atherosclerosis. J Cardiol, 2019. 73(1): p. 22-27.
10. Lievens, D. and P. von Hundelshausen, Platelets in atherosclerosis. Thromb Haemost, 2011. 106(5): p. 827-38.
11. Huo, Y. and K.F. Ley, Role of platelets in the development of atherosclerosis. Trends Cardiovasc Med, 2004. 14(1): p. 18-22.
12. Pircher, J., et al., Platelet-Neutrophil Crosstalk in Atherothrombosis. Thromb Haemost, 2019. 119(8): p. 1274-1282.
13. Tavora, F.R., et al., Monocytes and neutrophils expressing myeloperoxidase occur in fibrous caps and thrombi in unstable coronary plaques. BMC Cardiovasc Disord, 2009. 9: p. 27.
14. Ommen, S.R., et al., Usefulness of the lymphocyte concentration as a prognostic marker in coronary artery disease. Am J Cardiol, 1997. 79(6): p. 812-4.
15. Bressi, E., et al., Impact of Neutrophil-to-Lymphocyte Ratio and Platelet-to-Lymphocyte Ratio on 5-Year Clinical Outcomes of Patients with Stable Coronary Artery Disease Undergoing Elective Percutaneous Coronary Intervention. J Cardiovasc Transl Res, 2018. 11(6): p. 517-523.
16. Mungan, I., et al., Does the preoperative platelet-to-lymphocyte ratio and neutrophil-to-lymphocyte ratio predict morbidity after gastrectomy for gastric cancer? Mil Med Res, 2020. 7(1): p. 9.
17. Mady, M., et al., Neutrophil to lymphocyte ratio as a prognostic marker in metastatic gallbladder cancer. HPB (Oxford), 2020.
18. Vinal, D., et al., Prognostic value of neutrophil-to-lymphocyte ratio and other inflammatory markers in patients with high-risk soft tissue sarcomas. Clin Transl Oncol, 2020.
19. Ma, L., et al., Neutrophil to lymphocyte ratio and platelet to lymphocyte ratio in patients with systemic lupus erythematosus and their correlation with activity: A meta-analysis. Int Immunopharmacol, 2019. 76: p. 105949.
20. Huang, Z., et al., Prognostic value of neutrophil-to-lymphocyte ratio in sepsis: A meta-analysis. Am J Emerg Med, 2019.
21. Zhao, C., et al., Prognostic value of an inflammatory biomarker-based clinical algorithm in septic patients in the emergency department: An observational study. Int Immunopharmacol, 2020. 80: p. 106145.
22. Li, B., et al., Platelet-to-lymphocyte ratio in advanced Cancer: Review and meta-analysis. Clin Chim Acta, 2018. 483: p. 48-56.
23. Guo, W., et al., Prognostic value of neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio for breast cancer patients: An updated meta-analysis of 17079 individuals. Cancer Med, 2019. 8(9): p. 4135-4148.
24. Bhutta, H., et al., Neutrophil-lymphocyte ratio predicts medium-term survival following elective major vascular surgery: a cross-sectional study. Vasc Endovascular Surg, 2011. 45(3): p. 227-31.
25. Gary, T., et al., Platelet-to-lymphocyte ratio: a novel marker for critical limb ischemia in peripheral arterial occlusive disease patients. PLoS One, 2013. 8(7): p. e67688.
26. Gonzalez-Fajardo, J.A., et al., Prognostic significance of an elevated neutrophil-lymphocyte ratio in the amputation-free survival of patients with chronic critical limb ischemia. Ann Vasc Surg, 2014. 28(4): p. 999-1004.
27. Wang, Q., et al., Neutrophil-to-lymphocyte ratio is effective prognostic indicator for post-amputation patients with critical limb ischemia. Saudi Med J, 2017. 38(1): p. 24-29.
28. Tasoglu, I., et al., Neutrophil-lymphocyte ratio and the platelet-lymphocyte ratio predict the limb survival in critical limb ischemia. Clin Appl Thromb Hemost, 2014. 20(6): p. 645-50.
29. Tasoglu, I., et al., Usefulness of neutrophil/lymphocyte ratio as a predictor of amputation after embolectomy for acute limb ischemia. Ann Vasc Surg, 2014. 28(3): p. 606-13.
30. Lareyre, F., et al., Association of Platelet to Lymphocyte Ratio and Risk of 30-Day Postoperative Complications in Patients Undergoing Abdominal Aortic Surgical Repair. Vasc Endovascular Surg, 2019. 53(1): p. 5-11.
31. Wang, S., et al., Neutrophil-to-Lymphocyte Ratio and Platelet-to-Lymphocyte Ratio Are Effective Predictors of Prognosis in

Patients with Acute Mesenteric Arterial Embolism and Thrombosis. Ann Vasc Surg, 2018. 49: p. 115-122.
32. Augene, E., et al., Platelet to lymphocyte ratio as a predictive factor of 30-day mortality in patients with acute mesenteric ischemia. PLoS One, 2019. 14(7): p. e0219763.
33. Deser, S.B., et al., The association between platelet/lymphocyte ratio, neutrophil/lymphocyte ratio, and carotid artery stenosis and stroke following carotid endarterectomy. Vascular, 2019. 27(6): p. 604-611.
34. Massiot, N., et al., High Neutrophil to Lymphocyte Ratio and Platelet to Lymphocyte Ratio are Associated with Symptomatic Internal Carotid Artery Stenosis. J Stroke Cerebrovasc Dis, 2019. 28(1): p. 76-83.
35. Halazun, H.J., et al., Neutrophil-lymphocyte ratio as a predictor of cognitive dysfunction in carotid endarterectomy patients. J Vasc Surg, 2014. 59(3): p. 768-73.
36. Hyun, S., et al., Can the Neutrophil-to-Lymphocyte Ratio Appropriately Predict Carotid Artery Stenosis in Patients with Ischemic Stroke?-A Retrospective Study. J Stroke Cerebrovasc Dis, 2015. 24(11): p. 2646-51.
37. Suarez-Cuenca, J.A., et al., Neutrophil-to-lymphocyte ratio and its relation with pro-inflammatory mediators, visceral adiposity and carotid intima-media thickness in population with obesity. Eur J Clin Invest, 2019. 49(5): p. e13085.
38. Demirkol, S., et al., Neutrophils/lymphocytes ratio in patients with cardiac syndrome X and its association with carotid intima-media thickness. Clin Appl Thromb Hemost, 2014. 20(3): p. 250-5.
39. Kim, B.J., et al., The Combined Impact of Neutrophil-to--Lymphocyte Ratio and Type 2 Diabetic Mellitus on Significant Coronary Artery Disease and Carotid Artery Atherosclerosis. J Cardiovasc Ultrasound, 2016. 24(2): p. 115-22.
40. Li, X., et al., High neutrophil-to-lymphocyte ratio is associated with increased carotid artery intima-media thickness in type 2 diabetes. J Diabetes Investig, 2017. 8(1): p. 101-107.
41. Corriere, T., et al., Neutrophil-to-Lymphocyte Ratio is a strong predictor of atherosclerotic carotid plaques in older adults. Nutr Metab Cardiovasc Dis, 2018. 28(1): p. 23-27.
42. Yuksel, I.O., et al., Association of Neutrophil/Lymphocyte Ratio with Plaque Morphology in Patients with Asymptomatic Intermediate Carotid Artery Stenosis. Korean Circ J, 2016. 46(5): p. 699-705.
43. Jiang, H., et al., Neutrophil-to-Lymphocyte Ratio Correlates with Severity of Extracranial Carotid Stenosis-A Study Using Digital Subtraction Angiography. J Stroke Cerebrovasc Dis, 2017. 26(6): p. 1182-1190.
44. Koklu, E., et al., Is Elevated Neutrophil-to-Lymphocyte Ratio a Predictor of Stroke in Patients with Intermediate Carotid Artery Stenosis? J Stroke Cerebrovasc Dis, 2016. 25(3): p. 578-84.
45. Dai, Z., et al., Neutrophil to Lymphocyte Ratio as a Predictor of Restenosis After Angioplasty and Stenting for Asymptomatic Carotid Stenosis. Angiology, 2019. 70(2): p. 160-165.
46. Bao, X., et al., Neutrophil-to-lymphocyte ratio and platelet-to--lymphocyte ratio: novel markers for the diagnosis and prognosis in patients with restenosis following CAS. Biomark Med, 2020. 14(4): p. 271-282.
47. Idil Soylu, A., et al., The correlation of the platelet-to-lymphocyte ratio with the severity of stenosis and stroke in patients with carotid arterial disease. Vascular, 2017. 25(3): p. 299-306.
48. Varim, C., et al., Usefulness of the platelet-to-lymphocyte ratio in predicting the severity of carotid artery stenosis in patients undergoing carotid angiography. Kaohsiung J Med Sci, 2016. 32(2): p. 86-90.
49. Bonaventura, A., et al., Platelet-to-lymphocyte ratio at the time of carotid endarterectomy is associated with acute coronary syndrome occurrence. J Cardiovasc Med (Hagerstown), 2020. 21(1): p. 80-82.
50. Tek, M., et al., Platelet to lymphocyte ratio predicts all-cause mortality in patients with carotid arterial disease. Rom J Intern Med, 2019. 57(2): p. 159-165.
51. Koenig, W. and N. Khuseyinova, Biomarkers of atherosclerotic plaque instability and rupture. Arterioscler Thromb Vasc Biol, 2007. 27(1): p. 15-26.
52. Ammirati, E., et al., Markers of inflammation associated with plaque progression and instability in patients with carotid atherosclerosis. Mediators Inflamm, 2015. 2015: p. 718329.
53. Tabas, I., G. Garcia-Cardena, and G.K. Owens, Recent insights into the cellular biology of atherosclerosis. J Cell Biol, 2015. 209(1): p. 13-22.
54. Akpek, M., et al., Relation of neutrophil/lymphocyte ratio to coronary flow to in-hospital major adverse cardiac events in patients with ST-elevated myocardial infarction undergoing primary coronary intervention. Am J Cardiol, 2012. 110(5): p. 621-7.
55. Willeit, P., et al., Inflammatory markers and extent and progression of early atherosclerosis: Meta-analysis of individual-participant-data from 20 prospective studies of the PROG-IMT collaboration. Eur J Prev Cardiol, 2016. 23(2): p. 194-205.
56. Eriksson, E.E., et al., Direct viewing of atherosclerosis in vivo: plaque invasion by leukocytes is initiated by the endothelial selectins. FASEB J, 2001. 15(7): p. 1149-57.
57. Silvestre-Roig, C., et al., Neutrophils as regulators of cardiovascular inflammation. Nat Rev Cardiol, 2020.
58. Tecchio, C., A. Micheletti, and M.A. Cassatella, Neutrophil--derived cytokines: facts beyond expression. Front Immunol, 2014. 5: p. 508.
59. Alloza, I., et al., RNAseq based transcriptomics study of SMCs from carotid atherosclerotic plaque: BMP2 and IDs proteins are crucial regulators of plaque stability. Sci Rep, 2017. 7(1): p. 3470.
60. Kim, H. and E.M. Conway, Platelets and Complement Cross-Talk in Early Atherogenesis. Front Cardiovasc Med, 2019. 6: p. 131.
61. Rosinska, J., et al., Association of platelet-derived microvesicles and their phenotypes with carotid atherosclerosis and recurrent vascular events in patients after ischemic stroke. Thromb Res, 2019. 176: p. 18-26.
62. Suffee, N., et al., RANTES/CCL5-induced pro-angiogenic effects depend on CCR1, CCR5 and glycosaminoglycans. Angiogenesis, 2012. 15(4): p. 727-44.
63. Song, I.S., et al., Chebulinic acid inhibits smooth muscle cell migration by suppressing PDGF-Rbeta phosphorylation and inhibiting matrix metalloproteinase-2 expression. Sci Rep, 2017. 7(1): p. 11797.
64. Lin, B.D., et al., Causes of variation in the neutrophil-lymphocyte

and platelet-lymphocyte ratios: a twin-family study. Biomark Med, 2016. 10(10): p. 1061-1072.
65. Li, J., et al., Neutrophil-to-Lymphocyte Ratio Positively Correlates to Age in Healthy Population. J Clin Lab Anal, 2015. 29(6): p. 437-43.
66. Azab, B., M. Camacho-Rivera, and E. Taioli, Average values and racial differences of neutrophil lymphocyte ratio among a nationally representative sample of United States subjects. PLoS One, 2014. 9(11): p. e112361.
67. Cox, A.J., N.P. West, and A.W. Cripps, Obesity, inflammation, and the gut microbiota. Lancet Diabetes Endocrinol, 2015. 3(3): p. 207-15.
68. Aydin, M., et al., Neutrophil/lymphocyte ratio in obese adolescents. North Clin Istanb, 2015. 2(2): p. 87-91.
69. Bozkus, F., et al., Does the neutrophil-to-lymphocyte ratio have any importance between subjects with obstructive sleep apnea syndrome with obesity and without obesity? Tuberk Toraks, 2018. 66(1): p. 8-15.
70. Braicu, M.D., et al., The assessment of subclinic atherosclerosis objected through IMT in normal and dyslipidemic patients with various degrees of glucose tolerance. Rom J Intern Med, 2009. 47(3): p. 257-65.
71. Gorelick, P.B., Carotid endarterectomy : where do we draw the line? Stroke, 1999. 30(9): p. 1745-50.
72. Lal, B.K., et al., Restenosis after carotid artery stenting and endarterectomy: a secondary analysis of CREST, a randomised controlled trial. Lancet Neurol, 2012. 11(9): p. 755-63.
73. Bolca, O., et al., The neutrophil-to-lymphocyte ratio is associated with bare-metal stent restenosis in STEMI patients treated with primary PCI. Coron Artery Dis, 2015. 26(5): p. 402-8.
74. Turak, O., et al., Usefulness of the neutrophil-to-lymphocyte ratio to predict bare-metal stent restenosis. Am J Cardiol, 2012. 110(10): p. 1405-10.
75. Ozturk, Z.A., et al., Is increased neutrophil lymphocyte ratio remarking the inflammation in sarcopenia? Exp Gerontol, 2018. 110: p. 223-229.
76. Akboga, M.K., et al., Increased Platelet to Lymphocyte Ratio is Related to Slow Coronary Flow. Angiology, 2016. 67(1): p. 21-6.
77. Soliman, W.M., et al., Neutrophil to Lymphocyte and Platelet to Lymphocyte Ratios in Systemic Lupus Erythematosus: Relation With Disease Activity and Lupus Nephritis. Reumatol Clin, 2018.
78. Ridker, P.M., et al., Reduction in C-reactive protein and LDL cholesterol and cardiovascular event rates after initiation of rosuvastatin: a prospective study of the JUPITER trial. Lancet, 2009. 373(9670): p. 1175-82.
79. Ridker, P.M., et al., Antiinflammatory Therapy with Canakinumab for Atherosclerotic Disease. N Engl J Med, 2017. 377(12): p. 1119-1131.