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Original Article

Rheumatoid Arthritis Disease Activity Assessment Using Neutrophil-To-Lymphocyte Ratio and Platelet-To- Lymphocyte Ratio

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Abstract

Introduction: *Rheumatoid Arthritis ,the most common chronic inflammatory polyarthritis affects 0.5-1%of population, commonly between the age of 30-50 years with a greater propensity to affect females. Diagnosis is based on clinical findings, immunological evidence in form of RA factor or AntiCCP antibodies and inflammatory markers like CRP or ESR levels supported by radiological investigations. Rheumatoid arthritis treatment includes currently the treat to target approach and includes anti-inflammatory drugs, DMARDS (biological and non biological), systemic corticosteroids, surgery and physical rehabilitation. Current treatments require frequent monitoring of disease activity therefore the importance of reliable and simple markers of RA disease activity like neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio, hence the study.*

Materials and Method: *50 patients of RA were screened clinically and subjected to evaluation to assess their disease activity using DAS-28(ESR) score as well as NLR and PLR was calculated. The results were compared with a group of age and gender matched healthy individuals as controls. In the patient group NLR and PLR was compared with DAS-28 score as well as ESR in an attempt to find whether NLR and PLR were effective in assessing disease activity.*

Results: *The NLR and PLR in the RA patient group was significantly higher than controls (2.69 v/s 1.64 and 137.63 v/s 79.04) with a p value of<0.001 and <0.01 respectively. Out of the two, NLR showed a positive correlation with DAS-28 (r=0.329) which was statistically significant (p=0.020) and PLR displayed a positive correlation with DAS-28 score (r=0.077 and p=0.595) which was not significant. Both NLR and PLR showed a positive correlation with ESR which was highly significant (p<0.01 in both).*

Conclusion: *NLR and PLR are significantly increased in RA patients of rheumatoid arthritis. NLR correlates significantly with DAS-28 score could be used to assess disease activity as an alternative marker. Both NLR and PLR correlate with ESR with high statistical significance in RA patients.*

Keywords: *Inflammatory Markers, Rheumatoid Arthritis Disease Activity, Neutrophil-Lymphocyte Ratio.*

Introduction

Rheumatoid Arthritis a chronic inflammatory multisystem disease of autoimmune basis and unknown etiology, affects 0.5-1% of adults, women 2-3 times more frequently than males[1].Synovitis and erosive destruction of joints

are the hallmark of RA which clinically presents with symmetrical inflammatory polyarthritis, constitutional symptoms and extra-articular involvement. Clinical evidence of synovitis, serological evidence like RA factor or anti CCP antibody and inflammatory markers like CRP and ESR[2] are diagnostic while synovial fluid analysis and radiological features may be used as supportive evidence. The traditional stepwise management of RA has evolved in recent times to the currently accepted Treat to Target approach which includes NSAIDS and systemic corticosteroids to control inflammation, biological and non-biological DMARDS to retard disease progression, surgery and physical rehabilitation. Regular assessment of disease activity enables clinicians to intensify treatment or introduce biological DMARDs, assess treatment response and in case of sustained remission ,to step down DMARDs. Some Scoring systems endorsed by American College of Rheumatology (ACR) for rheumatoid disease activity assessment are DAS-28 (Disease Activity Score 28 joints), SDAI (Simplified Disease Activity Index) and CDAI (Comprehensive Disease Activity Index) [3]. An effective disease activity assessment score must be simple, accurate, sensitive to small changes and able to grade the disease into high, moderate and low activity in clinical settings. The DAS/DAS 28 score which was developed by Dutch rheumatologists is an integral part of the Treat to Target approach especially when Biological anti TNF alpha agents are introduced[4,5,6]. However DAS 28 score has certain limitations namely representation of ESR /CRP as a logarithmic function (ESR is a highly variable parameter dependent on age, gender, presence of anemia etc), disproportionate representation of joint tenderness (a subjective parameter), inclusion of only 28 joints and inclusion of patients perception of his health using global health assessment scale which is again subject to patient perception. [5]

Rheumatoid arthritis being a chronic inflammatory disease is known to be associated with a variety of immune alterations. Neutrophil infiltration of synovial tissue with increased levels of inflammastory cytokines like TNF-alpha, IL-1, IL-6, oxidative damage[7-9], premature thymic aging and dysfunction, and lymphopenia with presence of abnormal senescent peripheral T cells due to selective destruction of some T cell subsets[10-14] , microcirculatory thrombosis and reactive thrombocytosis due to inflammation induced anemia and increased erythropoietin levels and neutrophil induced platelet activation[15,16] are recognized features of RA. Therefore neutrophilia, lymphopenia and thrombocytosis are known immune alterations in RA which may be reflected in altered relative proportions of these cells in the peripheral blood.

Neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio have come increasingly in focus as indicators of subclinical systemic inflammation which can be used to assess disease activity and prognosticate for a variety of infective, inflammatory, neoplastic and autoimmune conditions [17-21] including RA .The reference value of NLR is 2.8 (ref range 1.2-4.4) and PLR is 137(ref range 75-199).[22] This study aims to establish whether Neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) could be used as biomarkers of disease activity and to compare these simpler, reproducible and widely available estimates with the currently accepted biomarker i.e. DAS-28 score as these could be used as an alternative in the absence of clinicians, ESR levels and questionnaires to monitor RA patients and assist in clinical decision making.

Materials and Methods

Fifty patients diagnosed with Rheumatoid Arthritis or already under follow-up for the same were included .Their demographic details and history were recorded. A complete history and examination was conducted and investigations done as per performa. Disease activity were assessed by calculating DAS-28 (ESR) score using an online application.

DAS 28 is a composite score of 4 of the following criteria;

1. Count the no of swollen joints out of 28
2 .count the no of tender joints out of 28
3. The value of ESR or CRP
4. The patient is asked to make a global assessment of health on a scale of 1-10 where 1 is very good and 10 very bad.

The results are fed into a complex mathematical formula using a dedicated DAS-28 calculator to produce an overall disease activity score. In this study we used an online DAS-28 calculator using ESR. Thus results are interpreted as

DAS-28>5.1 implies active disease
DAS-28<3.2 implies low disease activity
DAS-28<2.6 implies remission

Neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) were calculated from the absolute Neutrophil count and absolute lymphocyte counts derived from complete and differential blood counts. A gender and age matched control group of 25 healthy persons was also subjected to same investigations and the results were analyzed statistically to compare NLR and PLR in the patient with the control group. In the patient group NLR and PLR were also correlated with DAS 28 score as well as with ESR in the patient group.

Results

The mean age of RA patients in our study group was 43.32 years (SD+/-11.67) with a minimum age of 22 years and maximum age of 70 years. There were 45 females and 5 males. The mean duration of symptoms was 3.53 years varying between a minimum duration of 1.5 months and 20 years.32 patients complained of morning stiffness lasting 1 hour and 7 suffered from joint deformities the commonest being swan neck deformity. The mean hemoglobin was 10.43 g/dl (SD+/- 1.38) with minimum hemoglobin of 6g/dl and a maximum of 15 mg/dl. The mean ESR was 46.14 mm (SD 30.28) and varied between 10 and 130 mm in the first hour. The RA factor was present in 28(56%) of the patients whereas CRP was raised in 34(68%) of patients. The most common co-morbidity was anemia in 14 (28%), hypertension in 9 (18%) and COPD in3 (6%).Only 12(24%)of our patients were taking regular treatment for RA in form of anti-inflammatory drugs, steroids, methotrexate and/or hydroxychloroquine with almost half the patients on irregular symptomatic treatment with NSAIDS. The mean NLR in the patient group was 2.69(SD+/- 1.27) as compared to control group having NLR of 1.64(SD +/- 0.569) with a p value of<0.001.The PLR in the patient group was 137.63(SD+/-20.70) and in the controls it was 79.04(SD+/-30.026) with a p value <0.01.The difference in the values of NLR and PLR between patient and control group were statistically highly significant. The mean DAS 28 score in the RA patient group was 5.30(SD+/- 1.26) .It was positively correlated with NLR with statistical significance (p= 0.020).The PLR in the RA patients was 137.63(SD+/- 20.70f) correlated positively with DAS-28 score but was not significant (p=0.595).Both NLR and PLR correlated positively with ESR and with high statistical significance (p=0.0001).

Table 1, NLR (Neutrophil Lymphocyte Ratio) in Patients and Controls.

Correlation of NLR between patient and control group				
		N	NLR Mean±SD	P Value
Group	Patient	50	2.69±1.27	<0.001(HS)
	Control	25	1.64±0.569	

Table 2 PLR(Platelet Lymphocyte ratio) in Patient and Control group.

		N	PLR Mean±SD	P Value
Group	Patient	50	137.63±20.70	<0.01(HS)
	Control	25	79.04±30.026	

Table 3 correlation of NLR and PLR with DAS-28 score in RA patients.

	Mean	Std. Deviation	N	correlation (r)	p value
NLR	2.69	1.27	50	0.329	0.020*
Das 28	5.38	1.26	50		

	Mean	Std. Deviation	N	correlation(r)	p value
PLR	137.63	20.70	50	0.077	0.595
Das 28	5.38	1.26	50		

*Significant at 5% level (P<0.05)

Table 4 Comparison of NLR and PLR with ESR.

	N	Mean±SD	P Value
NLR	50	2.69±1.27	<0.01(HS)
ESR	50	46.14±30.28	

	N	Mean±SD	P Value
PLR	50	137.63±20.70	<0.01(HS)
ESR	50	46.14±30.28	

Discussion

Our study included 50 patients of mean age 43.32 years with 45 females and 5 males. The mean NLR was 2.69 in the patient group as compared to 1.64 in the controls which was statistically significant (p=0.001). Zenghin et al [23] also found NLR to be 3.15 v/s 2.03 in the RA patients and control groups (p<0.01) and Mercan et al [24], Fawsy et al[25] (p<0.0002), Uslu et al[26] and Peng et[27] al also reported similar differences in NLR between patients and controls. These findings which are consistent in all similar studies reflect the central and multifactorial role of neutrophils in inflammatory and immune mediated diseases especially RA. Neutrophils infiltrate synovial tissues, release degradative enzymes, reactive oxygen species and cytokines, regulate cell-cell immune interactions and produce net intracellular traps(NET) producing autoantigens that act as neo-epitopes (e.g.citrullinated proteins) thus driving the inflammatory process from acute to chronic inflammation[7,8,9]. At the same time lymphopenia is recognized in RA patients with selective apoptosis of T cell subsets. Due to the increased levels of cytokines e.g.TNF-alpha, Interleukin1 and 6 lymphocytes are attracted to the synovial tissues where about 90% are anergised [10-13]. Animal studies have demonstrated that experimentally induced lymphopenia is associated with development of inflammatory autoimmunity due to the presence of IFN-gamma secreting proinflammatory T cells of subset TH-1.[14]

In our study the mean DAS-28 score of RA patients was 5.30(SD+/-1.26). NLR correlated positively with DAS-28 score and it was statistically significant (p=0.020). Fawzy et al[25] also reported a positive correlation between NLR and DAS-28 score(p<0.049) and Zeghin et al[23] found a significant difference in NLR between active disease and remission in RA (P<0.001) and Mercan et al[24] and Tekeglu et al[29] also reported a significant correlation between NLR and DAS-28 score.Ghang B et al[30] and Koiwa et al [31]while studying RA patients on biological DMARDS also found that NLR was significantly elevated in RA patients during flares and decreased with treatment concluding that NLR was a more reliable criteria than ESR or CRP to assess disease activity. In India Chandrashekhar et al [32] found that NLR is useful in prediction of sustained remission in RA disease along with patients' perception of pain. Uslu et al[27] concluded that there was a significant correlation between NLR and DAS-28 (r=0.345, p=/<0.0001).These findings reflect the immune alterations

In our study PLR was 137.63 which was significantly raised in RA patients as compared to controls 79.04 (p<0.01). Zenghin et al[23] also reported PLR between patients and controls 162.39v/s 131.23 (p<0.01) whereas Uslu also[27] reported statistically significant difference (p<0.001) in PLR. There was positive correlation of PLR with DAS -28 score in our patients but it was not statistically significant (r=0.77, p=0.595) which was similar to the observations of Fawzy et al[25] and Peng et al whereas Uslu et al [26] reported a significant positive correlation of PLR with DAS -28 score (r=0.352, p<0.0001).Peng et al [27]found PLR >115.7 to be significantly correlated with active RA disease. Zenghin et al[23] found a significant correlation of PLR with disease activity (p<0.001).It has been postulated by Klinger et al[32] that in chronic inflammation there is thrombocytosis due to cytokines particularly IL1 and IL-6, and due to anemia induced secretion of erythropoietin which is similar to thrombopoietin, bone marrow stress due to platelet consumption and sequestration in synovial tissues. Thus platelets apart from maintaining hemostasis play a critical role in inflammatory disorders. Hence the relative proportions of the neutrophils, lymphocytes and platelets are altered

in RA disease being reflected by NLR and PLR in direct proportion to disease activity.

Additional evidence of the correlation of NLR and PLR was provided in our study by the significant correlation of these two markers of inflammation with ESR.

Conclusion

This study was conducted to compare Neutrophil-to lymphocyte ratio (NLR) and Platelet-to-lymphocyte ratio (PLR) between patients of Rheumatoid Arthritis and healthy controls and to find if NLR and PLR could be used as reliable markers of RA disease activity by correlating them with DAS-28 score in RA patients. The study established that RA patients have significantly higher NLR as well as PLR as compared to healthy controls. In our study NLR was found to correlate highly significantly with disease activity as evaluated by DAS-28 score in RA patients and PLR was also positively but was not significantly found to be correlated with DAS-28 score. Also both NLR and PLR were correlated with ESR with high statistical significance. We conclude that NLR and PLR may be important biomarkers of inflammation in RA disease and NLR may be a convenient and reliable indicator for disease activity assessment in RA which could be used to substitute or augment for DAS-28 score where the latter is not reliable i.e. in absence of records of previous scores, trained clinicians or in presence of severe anemia, to plan further management in cases of Rheumatoid Arthritis. Studies employing larger sample size are required to establish whether NLR and PLR could be used routinely to diagnose and /monitor patients of rheumatoid arthritis especially with the increasing use of biological and non biological DMARDS in these patients.

References

1. Silman AJ, Pearson AE. Epidemiology and genetics of rheumatoid arthritis. Arthritis Res.2002.vol4 (suppl 3):s265.
2. Wasserman AM. Diagnosis and Management of Rheumatoid Arthritis. Am Fam Physician.2011 Dec1; 84(11):1245-52.
3. Anderson J,Caplan L,Yazdany J et al.Rheumatoid Arthritis Disease Activity Measures.American College of Rheumtology Recommendations for use in clinical Practice. Arthritis Care and Research .Vol 64, No May 2012, pp 640-647.
4. Barczynska TA,Dura M,Blumfield E et al.DAS 28 score vs ultrasound exam for assessment of Rheumatoid Arthritis disease. Comparison and discussion of pros and cons.Rheumatologia.2015; 53(4):213-218.
5. Kiely P. The DAS 28 score .National Rheumatoid Arthritis Society.helpline@nras.org.uk.
6. van Riel PL.Development of the disease activity score (DAS) and disease activity score using 28 joint counts9DAS 28).Clin Exp Rheumatol.2014 Sep-Oct; 3295 suppl85) S-65-74.
7. Kaplan MJ.Role of Neutrophils in systemic autoimmune disease. Arthritis Res Ther.2013; 15(5):219.
8. Wright HL, Moots RJ.The multifactorial role of neutrophils in rheumatoid arthritis. Nature Reviews Rheumatol.2014; 10:593-6018.
9. Theiblemont N,Wright HL,Edwards SW.Human neutrophils in auto-immunity.Semin Immunol 2016 Apr;28(2):159-73.
10. Symmons DP, Farr M, Salmon M, Bacon PA. Lymphopenia in rheumatoid arthritis. J R Soc Med.1989; 82(8):462-463.
11. Goto M,Miyamato T,Nishioka K et al.Selective loss of suppressor T subsets in rheumatoid arthritis patients: analysis of peripheral blood lymphocytes by 2-D flow cytometry.J Rheumatol.1986 Oct;13(5): 853-7.
12. Timofeev VT,Shostak NA,Logoniva

TK.{Immunological aspects of early stage rheumatoid arthritis diagnosis.} Ter Arkh.2000; 72(5):19-21.
13. Blass S, Engel JM Burmester GR.[The immunologic homunculus in rheumatoid arthritis. A new viewpoint of immunopathogenesis in rheumatoid arthritis and therapeutic consequences].Z Rheumatol.2001 Feb; 60(1):1-16.g10.
14. Schulze-Koops H.Lymphopenia and autoimmune disease .Arthritis Res Ther.2004; 6:12
15. Farr M,Scott DL,Constable TJ.Thrombo-cytosis of active rheumatoid disease.Ann Rheum Dis.1983 Oct;42(5):545-549.
16. Hutchinson HM, Davis P, Jason MI.Thrombocytosis in rheumatoid arthritis. Ann Rheum Dis.1976 Apr; 35(2):138-142.
17. Pan L,Du J,Liao H.Platelet to lymphocyte ratio and Neutrophil-to –lymphocyte ratio association with disease activity in patients with Takayasu's Arteritis:a case control study.BMJ Open 2017.May4;□4)e014451.
18. Wu Y,Chen Y,Yang X.Neutrophil to lymphocyte ratio(NLR) and platelet to lymphocyte ratio(PLR) were associated with disease activity in patients with systemic lupus erythmatosis.Int Immunopharm.2016;26(3):372-6.
19. Yang W,Wang X,XangW et al.Neutrophil-to lymphocyte ratio and platelet –to – lymphocyte ratio are two new inflammatory markers associated with pulmonary involvement and disease activity in dermatomyositis.J Clin Chim. Acta.2017 Feb;465:11-16.
20. Prodromidou A,Andreakas P.Kazakas C. et al,The diagnostic efficacy of platelet to lymphocyte ratio and Neutrophil to lymphocyte ratio in ovarian cancer. Inflamm Res.2017 Jun;66(6):467-475.
21. Zahorec R.Ratio of Neutrophil to lymphocyte counts-rapid and parameter of systemic inflammation and stress in critically ill.Bratisl Lek Listy 2001; 102(1):5-14.
22. Alexander NI.Reference values of Neutrophil-lymphocyte ratio ,platelet–lymphocyte ratio and mean platelet volume in healthy adults of North Central Nigeria.J Blood and Lymph.2016.6:143.
23. Zenghin O., Onder M.E., Kelen A.et al .New inflammatory markers in early rheumatoid arthritis. Z. Rheumatol .2016 Sept 7. (Epub Ahead of print).
24. Mercan R., Bitik B., Tufan A. The association between Neutrophil /Lymphocyte Ratio and disease Activity in Rheumatoid Arthritis and Ankylosing Spondylosis. PMID: 26666737. Pubmed.
25. Fawzy RM,Said EA,Mansour AI. Association of neutrophil to lymphocyte ratio with disease activity indices and musculoskeletal ultrasonographic findings in recent onset rheumatoid arthritis. https://doi.org/10.1016/j.ejr 2017.05.001.
26. Uslu AU,Kucuk A,Sahin A et al.Two new inflammatory markers associated with Disease Activity Score-28 in patients of rheumatoid arthritis:neutrophil-to lymphocyte ratio and platelet-to lymphocyte ratio.Int J R heum Dis 2015 Sep;18(7):731-5.
27. Peng YF,Cao L ,Zeng YH.Platelet to lymphocyte ratio and Neutrophil to lymphocyte ratio in patients with rheumatoid arthritis. Open med (Wars) 2015.Apr 14; 10(1):249-253.
28. Tekeglu I., Gurol G., Harman H et al. Overlooked hematological markers of disease activity in rheumatoid arthritis. Int. J. Rheum. Dis. 2015 Dec1 .doi 1111/1756-185 X, 12805.Epub Ahead of publication.
29. Ghang B., Kwon O., Hong S. Neutrophil to lymphocyte ratio is a reliable marker of treatment response in rheumatoid arthritis patients using toclizumab therapy. Mod. Rheumatol. 2016. Aug 9:1-6.Epub Ahead of print.

30. Koiwa M., Goto s., Takahashi K. Neutrophil /Lymphocyte ratio in Patients with Rheumatoid Arthritis Treated with Biological Agents. J. Nippon Medical Sch.2016; 83(3):118-24.doi 10.1272/jnms.83.118.
31. Chandrasekhar S., Rajendran A., Bai Jaganath A.Neutrophil-to Lymphocyte ratio pain perception and disease activity score may serve as important predictive markers of sustained remission in rheumatoid arthritis.Rheumatismo.2015 Dec 30;67(3):109-15
32. Klinger MH,Jelkman W.Role of platelets in infection and inflammation.J Interferon Cytokine Res2002 Sep;22(9):913-22.