ORIGINAL RESEARCH PAPER

Medicine

KEY WORDS: COPD, Neutrophil Lymphocyte Ratio, surrogate marker, mMRC dyspnea scale, BODE index, 6 minute walk test

A STUDY OF NEUTROPHIL TO LYMPHOCYTE RATIO AS MARKER OF DISEASE SEVERITY IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE - A HOSPITAL BASED STUDY

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ABSTRACT

BACKGROUND: Chronic Obstructive Pulmonary Disease or COPD is a progressive disease characterized by poorly reversible air flow limitation associated with an abnormal inflammatory response of lungs to noxious particles or gases, especially in people exposed to fumes and smoke. On the other hand, as a rapid and widely available systemic inflammatory marker Neutrophil lymphocyte ratio (NLR) in peripheral blood is gaining importance as an indirect indicator of disease severity and prognosis in COPD. Chronic inflammation in COPD causes the recruitment of both neutrophils and lymphocytes, which once activated, release neutrophil elastase, cathepsin G, proteinase 3, matrix metalloproteinase (MMP) 8, 9 myeloperoxidase (MPO) and human neutrophil lipocalin. These inflammatory mediators contribute to the pathological changes in COPD. Measurement of NLR in COPD patients could be a possible surrogate marker of severe disease. AIM AND OBJECTIVE: 1.To find out whether neutrophil to lymphocyte ratio can be used to assess the severity of disease by comparing with BODE score
2. To find out whether neutrophil to lymphocyte ratio is higher in COPD exacerbation compared to stable COPD **MATERIALS AND METHODS:** This study was undertaken at Silchar Medical College from June 2019 to May 2020. A total of 100 diagnosed COPD patients; either current or former smokers attending the indoor and outdoor departments of medicine in SMCH were included in the study after fulfillment of the inclusion and exclusion criteria. These patients were distributed into various groups according to different severity indices, and NLR was estimated for each group separately and subjected to different tests of significance. For the neutrophil to lymphocyte ratio, venous blood collected in EDTA vial was analyzed in Sysmex XN 550 auto analyzer. **RESULTS:** This study showed a higher neutrophil to lymphocyte ratio (NLR) in patients with a higher mMRC dyspnea scale (Spearman's rho value 0.821), a higher GOLD COPD severity score, and a higher BODE index (p value <0.0001). Conversely a higher NLR correlated inversely with a lower FEV1 (p value <0.0001), and a poor outcome in the 6 minute walk test (p value <0.001) Also the NLR was found to be higher in the COPD exacerbation group as compared to the COPD stable group (p value = o.001). **CONCLUSION:** From this study it is evident that in resource poor settings with high prevalence and morbidity and mortality rates of COPD, Neutrophil Lymphocyte Ratio can be readily used as an easily measurable, cost effective parameter to predict COPD exacerbations and prevent the same.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a progressive disease associated with obstruction of air flow with limited reversibility contributed by a dysregulated inflammatory response of lungs triggered by noxious particles or gases, especially in people exposed to fumes and smokes.[1] Although highly prevalent among smokers COPD is a heterogeneous condition, which is responsible for considerable and growing morbidity, mortality, and healthcare expenses world wide. [2,3] Chronic bronchitis, defined as the presence of cough and sputum production for at least 3 months in each of the two consecutive years, is more frequent type of COPD.[4,8]. Globally, there are around three million death annually from COPD or its complications.[8] the incidence of COPD is expected to rise over the next 40 years and by 2060 there may be over 5.4 million deaths annually from COPD and related condition.[9] Acute exacerbation of COPD are associated with increased risk of subsequent exacerbations, worsening of coexisting respiratory function and ultimately death[15]. About half of the acute exacerbation of COPD is precipitated by bacterial and viral infection; however non-infective factors such as environmental pollution can also contribute[16-19]. Moreover, an excessive inflammatory response against bacteria contributes to chronic inflammation[20-23] . Studies have shown that the absolute counts of major immune related cell populations in the peripheral blood and the ratios can adequately reflect chronic inflammatory conditions.[24-26]

In the recent years, the NLR has been investigated as diagnostic and prognostic marker in COPD. Chronic inflammation in COPD causes the recruitment of both the major white blood cell population, lymphocytes and neutrophils. Once activated, neutrophil elastase, cathepsin G, proteinase-3 , matrix metalloproteinase (MMP)-8 and MMP-9, myeloperoxidase (MPO) and human neutrophil lipocalin, and other inflammatory mediators participate actively in the pathophysiological mechanisms of emphysema and COPD. Both neutrophil elastase and MPO cause tissue destruction in COPD[36,37] .The mean range of neutrophil to lymphocyte ratio is 0.78 to 3.53. With this background we intend to investigate NLR as a putative marker of disease severity and prognosis.

METERIALS AND METHODS

The study was under taken hospital based cross sectional study, at Silchar Medical College from June 2019 to May 2020. A total of 100 diagnosed COPD patients, either current or former smokers attending the indoor and outdoor departments of medicine in SMCH were included in the study after fulfillment of the inclusion and exclusion criteria. These patients were then distributed into various groups according to different severity indices and NLR was estimated for each group separately and subjected to different tests of significance. For the neutrophil to lymphocyte ratio, venous blood collected in EDTA vial was analyzed in Sysmex XN 550 auto analyzer.

The results for each parameter for discrete data are represented in numbers, percentages and average (mean, standard deviation) are represented for continuous data which are represented in tables and figures are done with help of Student "t" test, Anova test. In the study, p value of less than 0.05 was taken as the indicator of statistical significance. Chi-square ($\chi 2$) test was used to identify the significance of

the relations, associations and interactions among various variables.

A written informed consent form was completed by all the participants and the ethical clearance has been obtained from the institution, Silchar Medical College and Hospital, Silchar which follows Helsinki Guidelines of research.

OBSERVATION AND RESULTS:

AGE DISTRIBUTION IN COPD

In our study including 100 cases,
15% cases{15}are between 41-50 years
27% cases{27} are between 51-60 years
40% cases{40} are between 61-70 years
13% cases{13} are between 71-80 years
5% cases{5} are between >80 years

SEX DISTRIBUTION

In our study it reveals that COPD is more common in male the result of our study are as follow:-
Males involved are-83 {83%}
Females involved are-17 {17%}

There is increased incidence of COPD in smoker. It is well documented in many studies and theories. Our study also clearly proves the fact of increased incidence in smokers.

In our study, all males were smokers and some of the females are also smokers.

SMOKING AND COPD

In our study 100 cases:-
- 87 cases {87%} were smoker
- 13 cases{13%} were non-smoker

In our study 87 cases out of 100 cases were smokers out of this:-
15 cases {17.2%} had smoking < 20 pack years
26 cases {29.9%} had smoking 20-29 pack years
21 cases {24.1%} had smoking 30-39 pack years
17 cases {19.5%} had smoking 40-49 pack years
8 cases {9.2%} had smoking >50 pack years

BMI AND COPD

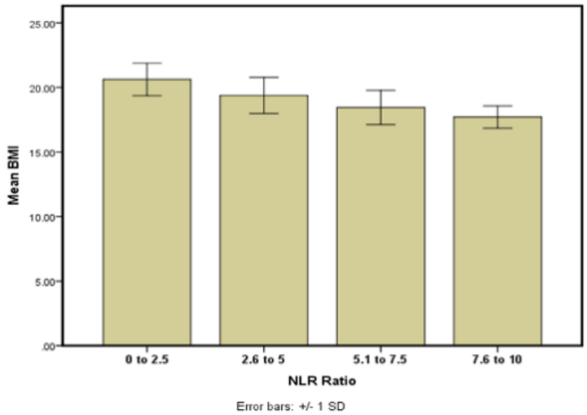
In our study: of 100 COPD patients

The relation between the mean BMI and NLR is also assessed in our study.

In our study as NLR increases, mean BMI decreases.

NLR is also associated with disease severity.

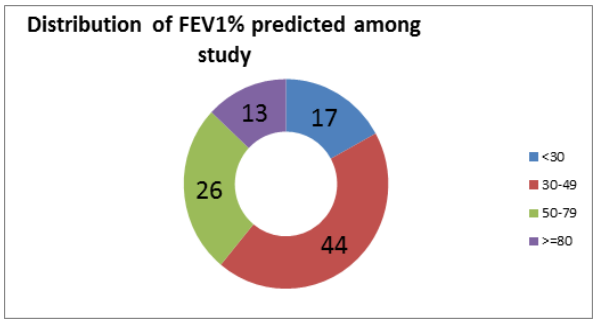
Hence, it can be inferred that lower BMI is associated with severe disease and poor prognosis. In this study the p value is <0.0001.



Relation of FEV1% and COPD

FEV1 < 30% -17 cases {17%}
FEV1 30-49% -44 cases {44%}
FEV1 50-79% -26 cases {26%}
FEV1 > 80% -13 cases {13%}

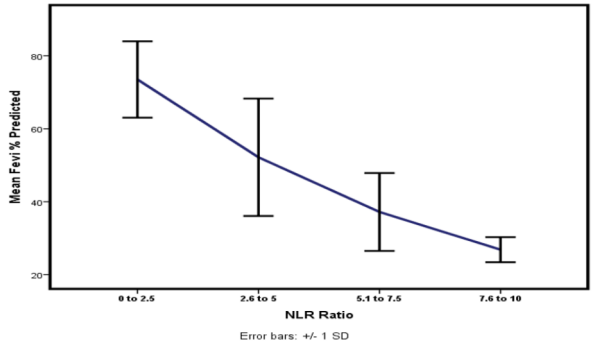
TABLE 4.9 SHOWING PERCENTAGE OF FEV1 AMONG COPD PATIENTS.



5.6 PIE DIAGRAM SHOWING PERCENTAGE OF FEV1 AMONG COPD PATIENTS.

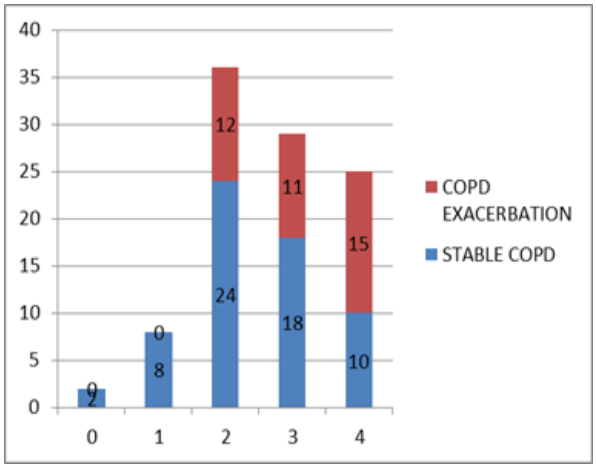
RELATION BETWEEN FEV1% AND NLR

The relation FEV1 and NLR shows significant p-value {.0001} using ANOVA test.In our study, it is seen that when FEV1 Decreases NLR increases.



5.7 LINE DIAGRAM SHOWING RELATION BETWEEN NLR AND FEV1.

Modified medical research council scale (mMRC) and COPD
In our study,
2cases (2%) had mMRC grade 0
8cases (8%) had mMRC grade 1
36cases (36%) had mMRC grade 2
29cases (29%) had mMRC grade 3
25cases (25%) had mMRC grade 4



5.9 BAR DIAGRAM SHOWING PERCENTAGE OF mMRC BETWEEN STABLE COPD AND COPD EXACERBATION.

Correlation between NLR and mMRC scale

Correlation between NL ratio grp and mMRC grade			r Value	Remarks
Spearman's rho	NLR Ration And mMRC GRADE	Correlation Coefficient	.821**	Positively correlated
		p value	<0.0001	

TABLE 4.13 SHOWING CORRELATION BETWEEN NLR AND mMRC SCALE.

In our study, the correlation coefficient between NLR and mMRC is .821.

Hence there is positive correlation between NLR and mMRC scale.

So,it can be inferred that as the mMRC grade increases NLR also increases.

6-MINUTEWALK DISTANCE AND STABLE COPD

Among stable COPD group 6-minute walk test was performed and the result were as follows:

6 Minute Walk (Distance (m)	Frequency	Percent
<149	4	6.5
150-249	8	12.9
250-349	28	45.2
>350	22	35.5
Total	62	100

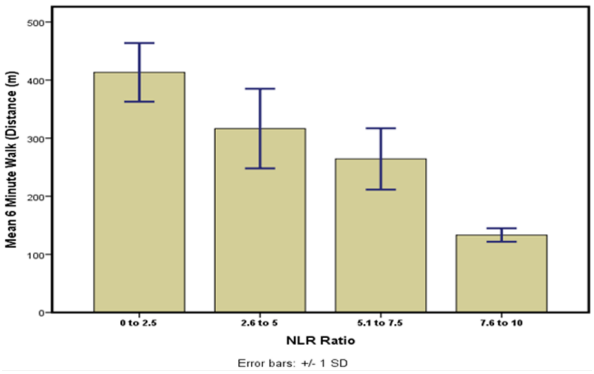
TABLE 4.14 SHOWING 6-MINUTE WALK DISTANCE AMONG STABLE COPD PATIENTS.

RELATION BETWEEN NLR AND 6-MINUTEWALKTEST

NLR Ratio	N	Mean 6 Minute Walk (Distance (m)	Minimum	Maximum	p value
0 to 2.5	15	413.33	320	500	<0.0001
2.6 to 5	23	316.52	190	440	
5.1 to 7.5	21	264.29	120	340	
7.6 to 10	3	133.33	120	140	
Total	62	313.39	120	500	

TABLE 4.15 SHOWING RELATION BETWEEN NLR AND 6-MINUTEWALKTEST

In our study, it shows significant p-value (.001) and it is seen that as NLR increases the 6-minute walking distance decreases. Thus, NLR is inversely co-related with 6minute walk distance'



5.11 BAR DIAGRAM SHOWING RELATION BETWEEN NLR AND 6-MINUTEWALKTEST.

BODE SCORE

In our study, the BODE score was calculated from the stable COPD group

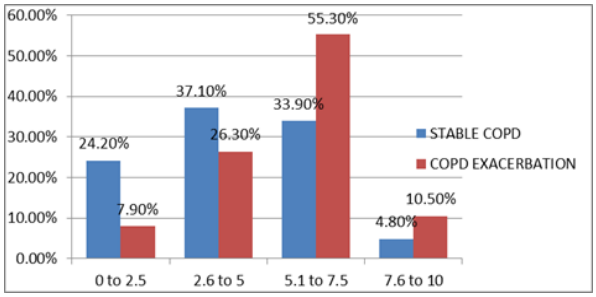
The result are as follows:

BODE Score	Frequency	Percent
0	4	6.5
1	4	6.5
2	6	9.7
3	9	14.5
4	4	6.5
5	9	14.5
6	11	17.7
7	9	14.5
8	2	3.2
9	0	0
10	4	6.5
Total	62	100

TABLE 4.16 SHOWING PERCENTAGE OF BODE SCORE AMONG STABLE COPD PATIENTS'

	STABLE COPD		COPD EXACERBATION		Total		P Value
NLR Ratio	n	%	n	%	n	%	0.047
0 to 2.5	15	24.20%	3	7.90%	18	18.00%	
2.6 to 5	23	37.10%	10	26.30%	33	33.00%	
5.1 to 7.5	21	33.90%	21	55.30%	42	42.00%	
7.6 to 10	3	4.80%	4	10.50%	7	7.00%	
Total	62	100.00%	38	100.00%	100	100.00%	

TABLE 4.17 SHOWING NLR RATIO BETWEEN STABLE COPD AND COPD EXACERBATION'

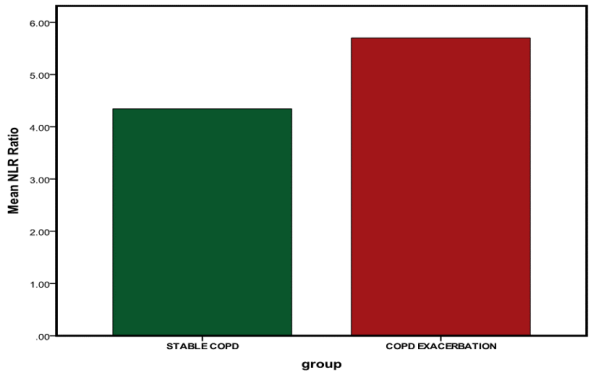


5.13 BAR DIAGRAM SHOWING NLR RATIO BETWEEN STABLE COPD AND COPD EXACERBATION.

In our study,
18% have NLR between 0 to 2.5
33% have NLR between 2.6 to 5
42% have NLR between 5.1 to 7.5
7% have NLR between 7.6 to 10.

	N	Mean NLR Ratio	p value
STABLE COPD	62	4.3435	0.001
COPD EXACERBATION	38	5.7000	
Total	100	4.8590	

TABLE 4.18 SHOWING MEAN NLR RATIO BETWEEN STABLE COPD AND COPD EXACERBATION



5.14 BAR DIAGRAM SHOWING MEAN NLR RATIO BETWEEN STABLE COPD AND COPD EXACERBATION.

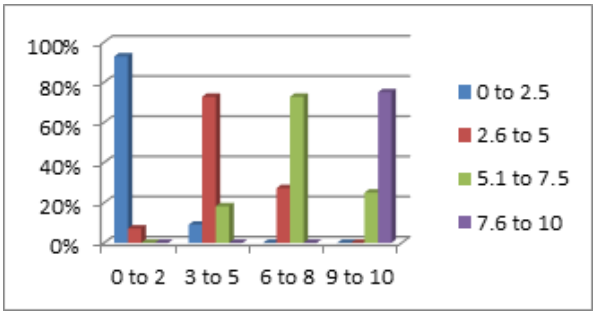
In our study comparison of NLR between stable and exacerbated COPD was done using the unpaired t-test and was found to be statistically significant. (p value=0.001) It is found that NLR is higher among COPD exacerbation group as

compared to stable COPD.

From this, it can be inferred that NLR can be used as a marker of COPD exacerbation.

NLR Ratio	BODE Score										p value
	0 to 2		3 to 5		6 to 8		9 to 10		Total		
	n	%	n	%	n	%	n	%	n	%	
0 to 2.5	13	93%	2	9%	0	0%	0	0%	15	24%	<0.0001
2.6 to 5	1	7%	16	73%	6	27%	0	0%	23	37%	
5.1 to 7.5	0	0%	4	18%	16	73%	1	25%	21	34%	
7.6 to 10	0	0%	0	0%	0	0%	3	75%	3	5%	
Total	14	100%	22	100%	22	100%	4	100%	62	100%	

TABLE 4.19 SHOWING RELATION BETWEEN NLR AND BODE SCORE



5.15 BAR DIAGRAM SHOWING RELATION BETWEEN NLR AND BODE SCORE

In this study it is seen that as NLR increases BODE score also increases.

It showed significant p value of 0.0001 using chi square test.Hence NLR can be used to assess disease severity as it correlates with BODE score.

DISCUSSION:

In this study, all patients developed COPD after age 40. Majority of the patients (40%) were between 61-70 years of age which was in concordance with previous studies conducted by Pearson et al and Sanket S et al[28] in which most of the patients were found to be elderly with mean age of 63.5+/-9.8 years and 56.93(+/-8.6) years respectively. Male patients (83%) outnumbered females due to increased prevalence of smoking among males across societies. This was echoed by earlier studies by Kumar et al and Janssens et al[30]. It was observed in our study that NLR increases with declining mean BMI of COPD patients. In the study conducted by Hallin et al[31] low BMI and weight changes were related to poor prognosis in COPD while other studies observed that low BMI is correlated with unplanned admission in COPD.(Pouw et al)[32]. The NLR parameter increase proportionally with decline in FEV1 in COPD (Yasar et al)[33]. Majority of our patients also had negative correlation between NLR and FEV1. Similarly the increase in NLR is associated with reduction in 6 minute walk test distance in our patients as well as observations from previous studies (Ryuko et al). BODE score was found to be useful in predicting the risk of death among COPD patients(Celli et al)[34]. Significant corerelation was noted between NLR parameter and BODE score, mMRC and 6- minute walk distance, whereas no correlation was documented between NLR with BMI and FEV1(Seung et al)[35]. In our study positive correlation was noted between NLR parameter with BODE score as well with mMRC and 6- minute walk distance.

NLR like CRP could be used as a cost effective marker of inflammation in acute exacerbation of COPD Kurtipek E et al[36]. In study conducted by Xiong W et al it is seen that exacerbation count of high NLR group was higher than that of low NLR group and the survival rate of high NLR group was lower than that of low NLR group. Persistently high NLR at the time of discharge is likely to be a predictor for increased medical utilisation and mortality risk ISAAC V et al[37]. NLR may be helpful for steroid and antibiotic management in COPD exacerbation with acute respiratory failure requiring ICU admission Cornelius PC[38]. Duman D et al[39] observed that NLR values with low CRP are associated with non-infectious COPD attack and they are useful biomarkers for guiding management of COPD exacerbation including steroid treatment. Increased NLR parameter was observed in COPD patients with severe airflow obstruction, high BODE score and severe emphysema. Furthermore the NLR was higher during exacerbation than in stable COPD patients . It was seen that NLR is a simple promising prognostic marker for assessing in hospital mortality in patient with acute exacerbation of COPD (Yao C Y et al)[40]. Our study has shown that NLR can be used not only as a marker of COPD exacerbation but also as a prognostic indicator and determinant of severity of COPD.

CONCLUSION:

NLR value has been found to be associated with severity parameters of COPD. Increase in NLR is associated with increase in mMRC dyspnoea scale, BODE scale which correlated with exacerbations in COPD while FEV1 and 6 minute walk distance decreases. Thus NLR can be used as marker of inflammation in COAD which correlates with need for hospitalisation and can have profound influence on management in COPD patients. NLR can be an important, cost effective, inexpensive and easily measurable parameters and will be useful in predicting the exacerbation and outcome in COPD.

LIMITATIONS OF THE STUDY:

It is a cross-sectional study, hence control groups could not be involved. Sample size is relatively small. The BODE score could only be assessed in the stable COPD group.

AUTHOR CONTRIBUTION:

Bijush Difoesa: Designing, concept, manuscript modification; **Dibya Jyoti Sharma:** Concept, manuscript writing and modification; **Debraj Dey:** Data collection, manuscript writing, **Gaby Thomas Sam:** Manuscript modification

Conflict of Interest: None.

Funding: None declared.

REFERENCES:

1. Rabe KF, Hurd S, Anzueto A, Barnes PJ, Buist SA, Calverley P, et al. Global Initiative for Chronic Obstructive Lung Disease. Global Strategy for the diagnosis, management, and prevention of COPD: GOLD executive summary. Am J Respir Crit Care Med. 2007; 176:532-55.
2. Woodruff PG, Agusti A, Roche N, et al. Current concepts in targeting COPD pharmacotherapy: making progress towards personalized manage¬ment. Lancet. 2015;385(9979):1789–1798.
3. Wain LV, Shrine N, Miller S, et al; UK Brain Expression Consortium (UKBEC); OxGSK Consortium. Novel insights into the genetics of smoking behavior, lung function, and chronic obstructive pulmonary disease (UK BiLEVE): a genetic association study in UK Biobank. Lancet Respir Med. 2015;3(10):769–781.
4. American Lung association Epidemiology and Statistics Unit. Trends in COPD(Chronic Bronchitis and Emphysema) Morbidity and Mortality. 2013. https://www.lung.org/assets/documents/research/copd-trend-report.pdf (accessed 14 october 2019)
5. Kim V, Crapo J, Zhao, et al. Copmparison between an alternative and the classic definition of chronic bronchitis in COPDGene. Ann Am Thorac Sac 2015;12(3):332-9
6. Woodruff PG, Barr RG, Bleecker E, et al. Clinical Significance of Symptoms in Smokers with Preserved Pulmonary Function. N Engl J Med 2016, 374(19):1811-21
7. Regan EA, Lynch DA, Curran-Everett D, et al. Clinical and Radiological DiseaseIn Smokers with Normal Spirometry. JAMA Intern Med 2015; 175(9):1539-49.
8. Global Burden of Disease Study Collaborators. Global, regional, and national age- sex specific all cause and cause specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013.Lancet 2015;385 (9963):117-71
9. Lopez AD, Shibuya k, Rac, etal. COPD: Current burden and future projection Eur Respir J 2006; 27(2):397-412
10. Seemungal, Harper -Owen R, Bhowmick A etal. Respiratory viruses, symptoms and inflammatory markers in acute exacerbation and stable Chronic Obstructive Pulmonary disease. AmJ Respi crit care Med 2001;164;1618-1623
11. Wedzicha JA. Role of viruses in acute exacerbation of Chronic Obstructive Pulmonary Disease. Proc Am Thorac Sac 2004;0;115-120
13. Papi A , Luppi F , et al. Pathophysiology of exacerbation of chronic obstructive

pulmonary disease.Proac Am Thorac soc 2006;3:245-251
14. Peacock JL, Anderson HR, Bremner SA, etal. Outdoor air pollution and Respiratory health in patients with COPD. Thorax 2011;66;591-59
20. Sethi S, Murphy TF. Infection in the pathogenesis and course of Chronic Obstructive Pulmonary Disease. N EngL J Med 2008;359: 2355-2365 disease.
21. Nathan C. Points of control in Inflammation. Nature 2002; 420:846-852.
22. Ishii T, Kida K. Predictors of Chronic Obstructive Pulmonary disease, exacerbation s. Curr opin Pulm Med 2014;20;138-145.
23. Mantovani A, Cassatella MA, Costantini C, etal, Neutrophils in the activation and regulation of Innate and adaptive immunity. Nat Rev Immunol 2011;00;519-553.
24. Borregaard N. Neutrophils: from marrow to microbes. Immunity 2010; 33;657-670.
25. Nathan C. Neutrophils and immunity: Challenges and opportunities. Nat Rev Immuno 2006;6: 173-1822010;6:149-163.
26. Brusselle GG, Joos GF, Bracke KR. New insights into the immunology of chronic obstructive pulmonary disease.Lancet 2011;378:1015-1026.
27. Andelid K, Bake B, Rak S, et al. Myeloperoxidase as a marker of increasing systemic inflammation in smokerswithout severe airway symptoms. Respir Med 2007; 101:888–895.
28. Houghton AM, Rzymkiewicz DM, Ji H, et al. Neutrophil elastase mediated degradation of IRS-1 accelerates lung tumor growth. Nat Med 2010; 16: 219–223.
29. Pearson MG, Calverley PMA. Clinical andlaboratory assessment. In: Calverley P, PrideN, eds. *Chronic Obstructive PulmonaryDisease*. London: Chapman & Hall, 1996:310.
30. Jindal SK, Aggarwal AN, Gupta D, Agarwal R, Kumar R, Kaur T,et al. Indian study on epidemiology of asthma, respiratory symptoms and chronic bronchitis in adults (INSEARCH). Int JTuberc Lung Dis. 2012;(16): p. 1270-7.
31. Hallin R, Koivisto-Hursti UK, Lindberg E, Janson C. Nutritional status, dietary energy intake and the risk of exacerbations in patients with chronic obstructive pulmonary disease (COPD) Respir Med. 2006;100:561–567.
32. Pouw EM, Ten Velde GP, Croonen BH, Kester AD, Schols AM, Wouters EF. Early non-elective readmission for chronic obstructive pulmonary disease is associated with weight loss. *Clinical Nutrition.* 2000;**19**:95–9. [PubMed] [Google Scholar]
33. Yousef AM, Alkhiary W. Role of neutrophil to lymphocyte ratio in prediction of acute exacerbation of chronic obstructive pulmonary disease. Egyptian Journal of Chest Diseases and Tuberculosis. 2017;66(1):43–8.
34. Celli BR, Barnes PJ. Exacerbations of chronic obstructive pulmonary disease. Eur Resp J. 2007; 29: p. 1224-1238.
35. Lee SJ, Lee HR, Lee TW, Ju S, Lim S, Go S-I, et al. Usefulness of neutrophil to lymphocyte ratio in patients with chronic obstructive pulmonary disease: a prospective observational study. The Korean Journal of Internal Medicine. 2016;31(5):891–8.
36. Bulbul Y, Ozlu T, Aydin D, Tatar D, Kuyucu T, Erboy F, et al. Immunization status in chronic obstructive pulmonary disease: A multicenter study from Turkey. Annals of Thoracic Medicine. 2019;14(1):75.
37. Isaacson PG, Norton AJ, Addis BJ. The human thymus contains a novel population of B lymphocytes. Lancet. 1987;2(8574):1488-1491.
38. Bourne S, DeVos R, North M, Chauhan A, Green B, Brown T, et al. Online versus face-to-face pulmonary rehabilitation for patients with chronic obstructive pulmonary disease: randomised controlled trial. BMJ Open. 2017;7(7).
39. Duman D, Aksoy E, Agca MC, Durmus Kocak N, Ozmen I, Akturk UA, et al. The utility of inflammatory markers to predict readmissions and mortality in COPD cases with or without eosinophilia [Corrigendum]. International Journal of Chronic Obstructive Pulmonary Disease. 2016;:417.
40. Yao CY, Liu XL, Tang Z. Prognostic role of neutrophil–lymphocyte ratio and platelet–lymphocyte ratio for hospital mortality in patients with AECOPD. International Journal of Chronic Obstructive Pulmonary Disease. 2017;Volume 12:2285–90.