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A Study to Predicit Role of Sputum Neutrophil Gelatinase - Associated Lipocalin as a Biomarker in Asthma - COPD Overlap Patients

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Abstract: ***Background: Asthma COPD overlap (ACO) is a consensus - based phenotype having characteristics of both COPD and asthma. Distinguishing ACO from other diseases is even more important as it is related to low health - related quality of life, augmented exacerbation rate and hospital admission, a rapid deterioration in lung function, and increased morbidity and mortality. But it cannot be diagnosed explicitly based on spirometry tests, patient demographics, radiology, or by - sputum cytology. There is an unmet need to develop biomarkers. Objectives: To assess the role of sputum neutrophil gelatinase - associated lipocalin (NGAL) as a biomarker of ACO. To find the correlation between sputum NGAL levels with forced expiratory volume 1 (FEV1) and exacerbation rate in ACO. To find the correlation between sputum NGAL level with sputum neutrophils and eosinophils in ACO. Materials and methods: In this comparative correlational study, 180 subjects were enrolled into four groups with 45 patients each with asthma, COPD, ACO, and healthy nonsmokers respectively. After taking detailed history and demographics, sputum was analyzed for the differential count and NGAL. Results: Asthma COPD overlap (ACO) cases had high sputum NGAL levels; the second was the COPD group, and the last in the case asthma group. Nonsmokers had notably lower readings than the diseased. Out of three, receiver operating characteristic (ROC) figures, the validity of NGAL was best in selecting patients of ACO than COPD and asthma. The area under curve (AUC) was highest for ACO and less than the acceptable limit for the remaining two. NGAL cut - off value of 2473 pg/mL had 80% sensitivity and 50% specificity for ACO. Conclusion: The present study investigated the sputum NGAL levels as a biomarker in ACO identified by the syndromic approach. Sputum NGAL, a biomarker associated with airway inflammation in airway diseases, was supportive of clinically differentiating ACO from asthma to COPD.***

Keywords: ACO, NGAL, COPD, FEV1

1. Introduction

Asthma COPD [chronic obstructive pulmonary disease] overlap (ACO) is defined as a condition with persistent airflow limitation and several clinical features that coincide with asthma and COPD.[1] An exact definition is not yet developed due to a lack of evidence about its clinical phenotypes and underlying mechanisms. A high number of patients who have chronic respiratory symptoms have a diagnosis and characteristics of both asthma and COPD.[2] Distinguishing between asthma and COPD is essential due to the distinct treatments available and the resulting clinical outcomes concerning illness and mortality rates.[3] While asthma and COPD differ in terms of their inflammation patterns, immunological mechanisms, and the degree of reversibility in airflow limitation, there is a substantial population of patients who experience symptoms and signs of both disorders.[4]

Initially "asthma – COPD overlap syndrome" was defined as the overlap of the symptoms of COPD and asthma in some patients. It was later revised to ACO from ACOs by Global Initiative for Asthma (GINA) since it covers a group of patients with variable intersection levels. This is most seen in asthmatic smokers. In COPD patients, it is very uncommon to find good postbronchodilator reversibility (BDR) in spirometry.[5] Most of the inflammatory pathways of asthma and COPD are markedly different; both are seen in ACO.

Sputum analysis showed both neutrophils and eosinophils in ACO. Asthma, COPD, and ACO can differ in their biomarker profile. Diagnosis of ACO cannot be made only based on lung function tests, patient demographics, sputum cell counts, or imaging of the lungs.[6–8] There is a prerequisite to developing novel diagnostic biomarkers for the clinical assessment of ACO. In ACO, there is increased neutrophilic airway inflammation and airway epithelial injury. So sputum neutrophil gelatinase associated lipocalin (NGAL), as a biomarker in ACO, together with the patient's clinical features, spirometry, and radiology, will be greatly helpful in diagnosing ACO patients.

2. Material and Methods

This comparative correlational study was done in the Department of medicine, in tertiary care hospital of Uttar Pradesh. Both male and female patients in the age - group of 40–75 years with stable asthma, COPD, and ACO on regular medications were included in the study. Diagnosis of asthma was based on GINA and defined as reversible airflow obstruction with a postbronchodilator forced expiratory volume 1 (FEV1) /FVC < 0.7, with an increase in FEV1 of ≥ 12% or 200 mL. COPD diagnosis was based on Global initiative for obstructive ling disease (GOLD) guidelines and defined by incompletely reversible airflow obstruction with a postbronchodilator FEV1/ FVC < 0.7.

ACO was identified by the features that it shares with asthma and COPD according to GINA guidelines and GOLD strategy—with more significant variability in airflow and airflow obstruction that is not fully reversible. Based on the medical history, self reported questionnaire data, standard guidelines, and inclusion criteria, 180 study subjects diagnosed with asthma, COPD, and ACO according to GINA, GOLD, and syndromic approach, respectively, were enrolled in the study and compared to healthy asymptomatic nonsmokers.

The sample size was calculated using the formula:
N = minimum sample size required. Z = critical ratio of the confidence interval at 5% error Z is 1.96. V = Variance of the area under the curve. D = Allowable margin of error in the estimation of sensitivity—taken as 10% of the area under curve (AUC) = 0.07

As more subjects reported and were eligible, 180 were enrolled in the study. Study subjects were enrolled into four groups— group I, asthma, n, 45; group II, COPD, n - 45; group III, ACO, n - 45; and group IV, control, n - 45. Patients with an exacerbation and use of oral corticosteroids in the past 4 weeks, active pulmonary tuberculosis, chronic lung diseases other than asthma, COPD and ACO, diabetes mellitus, malignancy, and renal diseases were excluded from the study. Informed written consent was taken from all subjects and ethical clearance was obtained from the Institutional Ethics Committee.

Baseline data of all study subjects were collected, including demographics, duration of symptoms, smoking history, history, treatment history, occupational history, biomass exposure, number of exacerbations in the last year, medication history, chest Xray findings, and spirometry. Baseline blood investigations were done, including complete blood count, renal function test, and absolute eosinophil count. To estimate sputum differential counts, sputum cytospin preparations were prepared by cytospin and centrifuged at 450 rpm for 6 minutes. Slides were stained with hematoxylin and eosin stains for cell differentiation. We assessed detailed cellular profiles in sputum consisting mainly of eosinophils and neutrophils. Induced sputum was collected for the differential count and NGAL estimation. Collected sputum was treated with dithioerythritol and then gently vortexed at room temperature for homogenization. The supernatant was stored at −80°C until the NGAL assay. NGAL was estimated using a human NGAL ELISA kit.

Statistical Analysis Data analysis was done using Statistical Package for the Social Sciences (SPSS.25). The data collected during the study were analyzed using descriptive statistics such as percentage, range, mean, standard deviation, correlation analysis, and inferential statistics.

Correlations of the sputum markers with demographics, lung function, and sputum cell profiles were calculated by Spearman's rank correlations. Multiple Linear regression analysis was done to evaluate the impact of variables like age, body mass index (BMI), FEV1/FEV ratio, and pack years of cigarette smoking on sputum NGAL value. Receiver operating characteristic (ROC) curve analysis was performed to assess the sensitivity, specificity, and diagnostic accuracy of the biomarker NGAL. Statistical significance was set at $p < 0.05$.

3. Results

Around 180 subjects were enrolled in the study in four groups after satisfying the inclusion and exclusion criteria. demonstrates the demographic and laboratory data of the study subjects. Sputum NGAL levels were significantly different among the four groups. Mean sputum NGAL levels in asthma patients were 2196.55 ± 1308.46 pg/mL, while in COPD patients, it was 2792.76 ± 1461.43 pg/mL; in ACO, it was 3746.68 ± 1376.17 pg/mL, and in the control group, it was 1625.79 ± 1484.96 pg/mL. There was a statistically significant difference between sputum NGAL levels in ACO patients compared to other groups ($p \leq 0.001$).

Even though there was an increasing trend of sputum NGAL values in ACO, it did not differ significantly between COPD and asthma. There was 1 weak negative correlation between sputum NGAL and FEV1 ($\rho = -0.336$) (Fig.2), FVC ($\rho = -0.150$) (Fig.3), and FEV1/FVC% ($\rho = -0.247$) (Fig.4), whereas it had a positive correlation with sputum neutrophils ($r = 0.443$, $p = 0.00$) (Fig.5) and sputum eosinophils ($r = 0.183$, $p = 0.014$). Sputum NGAL values were significantly higher in individuals having exacerbations of symptoms compared to those who did not (3225.23 vs.2219.33, $p < 0.001$) Sputum neutrophil gelatinase - associated lipocalin (NGAL) as a biomarker had maximum validity in identifying cases of ACO than COPD and asthma. The AUC was highest for ACO (0.731).

It was less than the acceptable limit for the remaining two (AUC Multiple linear regression analysis evaluated the impact of variables like age, Discussion for COPD = 0.471, AUC for asthma = 0.298). Sputum NGAL had the highest 80% sensitivity and 50% specificity at a cutoff value of 2473 pg/ mL for ACO, 51% sensitivity and 47% specificity at a cutoff value of 2644 pg/mL for COPD, and 40% sensitivity, and 40% specificity for asthma at NGAL concentration of 2593 pg/mL.

Multiple linear regression analysis evaluated the impact of variables like age, BMI, FEV1/FVC ratio, BDR, and pack - years of cigarette smoking on sputum NGAL value. Age, FEV1/FVC ratio, and smoking severity were significant risk factors for derangement in sputum NGAL value. As BMI and BDR were not significantly associated with the NGAL values, they were excluded to see the final predictor capacity of the remaining variables. Adjusted R2 for these variables of 0.556 indicates a 55.6% change in NGAL values could be explained by these variables. The regression equation for the model is, NGAL = 82.42 + 0.328 × age (in years) + 0.404 × smoking in pack years—0.665 × FEV1/FVC%.

Our study assessed the levels of sputum NGAL as a tool for discerning ACO from asthma to non - ACO COPD. A set of patients with both asthma and COPD features were represented by the global term ACO. More than a few criteria have been proposed for diagnosing ACO since 2008.

A consensus document was released by GOLD and GINA in 2016 for the diagnosis of ACO, recommending a syndromic method to stereotype ACO.2, 3 Because of the universal acceptance of GINA and GOLD, we implemented this tool in our study to estimate the size of ACO. Recent guidelines describe salient characteristics of ACO instead of providing a definite definition. With this syndromic approach, 16 asthma and 29 COPD patients in our study were diagnosed to have suffered from ACO. In our study, the prevalence of ACO in the asthmatic group was 35% lower than COPD group (64%). For ACO, four different pathways have been suggested. Early - life airflow limitation can last into adolescence and adulthood, and if other risk factors, like

smoking, are present, ACO is more likely than severe asthma.

Patients with COPD with significant histories of smoking or other exposures and late - onset asthmatic symptoms exhibit a second pathway. Adults with asymptomatic airway hyperresponsiveness who develop chronic airflow limitation consistent with a COPD diagnosis represent a third asthma dominant pathway. A fourth pathway is also known, which recognizes the connection between early life risk factors and small lungs and the likelihood of developing asthma and fixed airflow limitation.

As a result, from the molecular genetics perspective, ACO can represent a very diverse group of patients. Diagnosing ACO is essential for several reasons. Almost all the studies showed that ACO had a bad prognosis compared with asthma alone or COPD alone. They had more severe symptoms with impaired lung function, poor disease control, increased rate of aggravation of symptoms, and hospital admission, resulting in a more significant financial burden and worst outcomes.

NGAL (oncogene 24p3, lipocalin 2) is a 24 kDa glycoprotein, that was first purified from human neutrophils. NGAL is mainly expressed in activated neutrophils, respiratory and intestinal epithelial cells, endothelial cells, and renal tubules in response to inflammatory stimuli and during infection by myeloid and epithelial cells in response to toll - like receptor activation. NGAL is expressed within tracheal goblet cells and type II pneumocytes in normal human lungs.

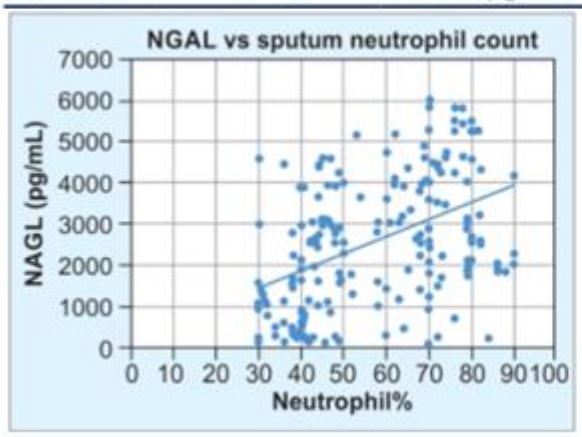
NGAL may be a marker of multiple upstream inflammatory pathways involved in the pathogenesis of COPD and ACO. Thus, the sputum NGAL level may be associated with neutrophilic inflammation and ongoing damage to the respiratory epithelium. This is the reason behind the analysis of sputum NGAL done in our study rather than serum NGAL. . So based on the properties of NGAL to reflect neutrophil activation, antibacterial, and matrix - degrading properties, it was hypothesized that NGAL could be a systemic marker of ACO.

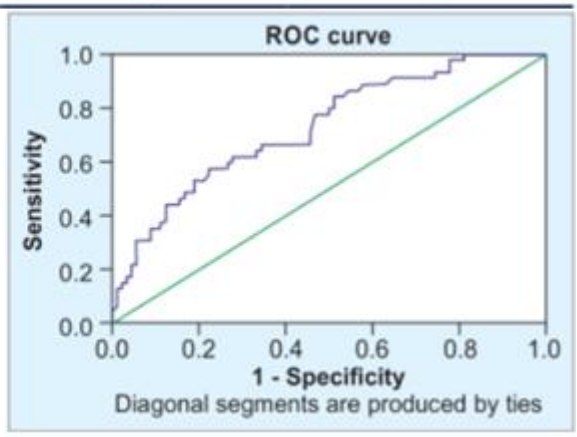
In our study, sputum NGAL values were significantly higher in individuals with exacerbations of symptoms compared to those who did not, like other studies, and positively correlated with sputum neutrophils. It supports the relationship between increased sputum neutrophils and NGAL in inflammation, especially in patients with ACO. Infections are one of the main reasons for the exacerbations in these patients. Therefore, the sputum NGAL levels can be associated with airway inflammation and lowgrade microbial colonization, making patients with ACO susceptible to acute viral and bacterial infections and exacerbating diseases.

Though this is statistically significant, inherently, the number of patients from each gender was different, to begin with. The difference observed could also be because of that. The mean age of study subjects suffering from ACO, asthma, and COPD differed significantly. The mean age of patients in each group is ACO = 55.96, asthma = 49.59, and COPD = 64.20. COPD and ACO were manifestations of a higher age than asthma. There are only very few studies to date on biomarkers that differentiate ACO from other chronic airway diseases.

NGAL is a novel biomarker in determining between ACO and non - ACO COPD; also, like in our study, using sputum as the sample might enhance the accuracy in diagnosing airway conditions, making it more effective in identifying specific respiratory diseases. This study showed that sputum NGAL is a promising biomarker in diagnosing ACO. Our study has a few limitations, like the small sample size and from a single center. Hence the results are required to be validated with more extensive cohort studies. Future studies are needed to divulge the bioinformatic differences in chronic airway diseases.

Parameters	*AUC*	*Cut off*	*Sensitivity (%)*	*Specificity (%)*
ACO	0.731	2473 (pg/mL)	80	50
COPD	0.471	2644 (pg/mL)	51	47
Asthma	0.298	2593 (pg/mL)	40	40





4. Conclusion

Our study shows that sputum NGAL can be used as a diagnostic tool in ACO and shows its role as a valuable biomarker in differentiating ACO from other chronic obstructive airway diseases. The sputum biomarker levels may reflect changes in cellular composition and lung function during disease progression.

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