Sputum Bacterial Spectrum and Predominant Inflammatory Cells in Acute Exacerbations of COPD

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ABSTRACT

This study was done to observe variation with seasons in sputum bacterial profile and predominant inflammatory cells in patients with AECOPD. Hundred sputum samples were cultured for bacteria and examined for type of inflammatory cells. Predominant bacteria and inflammatory cells and their variation with seasons were noted. Thirty six percent of sputum samples had bacterial growth. Bacterial growth was higher in summer and monsoon (43.3%) than in post- monsoon and winter period (21.2%) (p=0.031). Overall, Pseudomonas aeruginosa was the commonest organism cultured. In summer and post-monsoon, the commonest bacterium was Pseudomonas aeruginosa (16.6% and 15.4% respectively) and in monsoon, it was Klebsiella species (19.3%). Sputum neutrophilia (N>61%) was seen in 91% and sputum eosinophilia (E>3%) in 41% of the samples. There was no significant difference in the predominant inflammatory cells (N and N+ E) in sputum with seasons. Isolated sputum eosinophilia was higher in post- monsoon and winter than in summer and monsoon (12.1% v/s 7.5%, p=0.021). Length of hospital stay was less in patients with sputum eosinophilia than in patients with sputum neutrophilia (9.11v/s 10.12 days, p=0.023). Sputum neutrophilia was associated with higher sputum bacterial isolation. Eosinophilia in the sputum was likely to be associated with a sterile sputum. Bacterial isolation was higher in summer and monsoon than post- monsoon and winter. There was no significant difference in the predominant inflammatory cells with seasons. Sputum eosinophilia was associated with faster recovery from AECOPD than sputum neutrophilia.

KEY WORDS: acute exacerbations of COPD (AECOPD), bacterial growth, inflammatory cells, sputum eosinophilia, sputum neutrophilia, sterile sputum

INTRODUCTION:

Acute exacerbations of COPD (AECOPD) contribute significantly to morbidity and mortality. Pathogen infection-related inflammation is the major cause of AECOPD[1].

Both viruses and bacteria, either independently or in combination have been implicated in exacerbations. Bacteria causing AECOPD vary between geographical areas. Studies conducted in various countries showed the predominance of Streptococcus pneumonae, followed by Haemophillus influenzae, Moraxella catarrhalis, Stayphylococcus aureus and other gram negative bacteria whilst those in India showed predominance either of Streptococcus pneumonae or gram-negative bacteria eg. Pseudomonas aeruginosa, Klebsiella and E.coli [2-8].

Although COPD exacerbations are typically associated with increased neutrophilic inflammation, alterations in lower airway inflammation during exacerbations is not completely understood. Neutrophil predominance has been seen in sputum of patients with AECOPD caused by bacteria and eosinophil predominance in viral exacerbations[9-12].

The type of lower airway inflammation may relate to treatment response in AECOPD. Sputum eosinophilia is associated with corticosteroid responsiveness, whereas high bacterial load and sputum purulence with antibiotics[11-13].

This study was conducted to see the variation with seasons in bacterial profile and predominant inflammatory cells in sputum of patients with AECOPD in central India.

MATERIAL AND METHODS:

This was an observational (prospective, cross-sectional) study with a sample size of one

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hundred. The study was conducted after obtaining ethical clearance from Institutional Ethical Committee of People's College of Medical Sciences and Research Centre, Bhopal. One hundred and twenty six patients above the age of 40 years were evaluated for the study over a period of one year six months from January 2017 to June 2018. Twelve patients were excluded for not expectorating sputum and fourteen patients for giving inadequate sample.

Twelve patients were readmitted twice and one patient was readmitted thrice in one and half year of study duration. All patients aged 40 years and above, presenting to Pulmonary Medicine ward, and fulfilling the acute exacerbation of COPD diagnostic criteria according to GOLD 2015 were evaluated by medical interview and physical examination[14].

The inclusion criteria were: 1. Pre-diagnosed COPD patients aged > 40 years; 2. Patients aged > 40 years and newly diagnosed AECOPD. The exclusion criteria were: 1. Patients with Obstructive Pulmonary Disease other than COPD; 2. Patients associated with other lung diseases e.g. pneumothorax, lung carcinomas, active PTB, pleural effusion, pulmonary throm-boembolism; 3. Concurrent reason for worsening of COPD symptoms e.g. acute myocardial infarction, congestive cardiac failure; 4. Conditions associated with the inability to produce sputum e.g. dry cough, patients on ventilator support, unconscious patients; 5. Patients who received antibiotics and corticosteroids seven days prior to admission and 6. Uncooperative patients or those not willing to participate.

Medical history was obtained using a questionnaire applied by a single investigator. Smoking history, place of residence either rural or urban and occupation of patients were taken at the time of admission. Seasons of sputum sample collection were classified according to Indian Meteorological Department: Winter- *January to February*, Summer - *March to May*, Monsoon- *June to September*, Post-monsoon- *October to December*[15].

Patients were requested to keep two to three ml. of sputum after coughing, as far as possible prior to starting antibiotics and corticosteroids. The sample was divided into two. One was subjected to Gram's stain and bacterial culture and sensitivity and another to total and differential leukocyte counts. Most purulent part of the sputum was taken for evaluation. Sputum was examined with light microscope in low magnification field[16]. A sputum sample was considered adequate when <10 epithelial cells and >25 leukocytes (pus cells) were present per low magnification field[17]. Gram's stain, culture and antibiotic sensitivity were done according to standard protocol[18-20].

For sputum cell count, mucus clumps were separated from salivary part of expectorate manually with forceps[1]. Sputum was mixed with WBC diluting fluid in a ratio of 1:1. Counting was done on Neuber's hemocytometer in low power field. For differential leukocyte count, smear was prepared from the sputum. After staining with Leishman's stain, counting of different cells (N, L, E, M) was done according to their morphological characteristics in 100 x field[21].

Based on previous published studies, following normal counts of cells in sputum was taken: TLC <5.5million/ml, Neutrophil <61%, Eosinophil <3%, Lymphocyte <4% and Macrophage <80%[1,11,22]. A sputum neutrophil count >61% was considered sputum neutrophilia and sputum eosinophil count >3% as sputum eosinophilia.

Data analysis was done using SPSS (Statistical Package for the Social Sciences) ver. 20. Quantitative data is expressed as mean whereas categorical data is expressed as number and percentage. Student t test and one way ANOVA was used for quantitative data where as chi Square test was used for categorical data. Level of significance was assessed at 5%.

RESULTS:

Hundred sputum samples were examined between January 2017 and June 2018. Twenty four samples were taken from 12 patients who were admitted twice and 3 samples were from one patient who was admitted thrice. Table 1 shows characteristics of patients. Two thirds of the samples were collected in summer (36%) and monsoon (31%) and the rest were collected in post-monsoon (13%) and winter (20%) seasons. Total duration of summer and monsoon was 11 months and of post- monsoon and winter was 7 months. Bacterial growth was present in 36% of the sputum samples.

Table 2 shows higher bacterial growth in summer and monsoon (43.3%) compared to winter and post- monsoon period (21.2%). Table 3 shows that the predominant bacterium isolated was Pseudomonas aeruginosa (14%), followed by Klebsiella species (10%), E. coli (7%) and Citrobacter (2%). The only Gram positive bacterium, Staphylococcus aureus, was grown in 3% of the patients.

Table 1: Characteristics of patients.

	Parameters		No of patients	Percentage
General Characteristics	Age	41 – 50	6	6
		51 – 60	24	24
		61 – 70	48	48
		71 – 80	21	21
		>80	1	1
	Gender	Male	89	89
		Female	11	11
	Residence	Rural	87	87
		Urban	13	13
	Occupation	Farmer	72	72
		Businessman	12	12
		Serviceman	12	12
		Other	4	4
	Smoking status	Current smoker	37	37
		Ex smoker	49	49
		Non smoker	14	14
	Exposure to form of smoke exposed	Bidi	70	70
		Cigarette	16	16
		Biomass	14	14
Sputum characteristics	Season of sputum collection	Summer	36	36
		Monsoon	31	31
		Post-monsoon	13	13
		Winter	20	20
	Growth present	No of patients	36	36
	Sterile	No of patients	64	64
	Cell count	TLC >5.5million/ml	81	81
	Sputum neutrophilia	Neutrophils >61%	91	91
	Sputum Eosinophilia	Eosinophils >3%	41	41

Table 2: Sputum bacterial growth and seasons.

	Total	Summer and Monsoon (11 months)	Post-monsoon and Winter (7 months)	Total	p value
No. of sputum samples analyzed	100	67 (67%)	33 (33%)	100	
No. of sputum samples with bacterial growth	36	29 (43.3%)	7 (21.2%)	36	0.031

Table 3: Sputum samples and bacterial spectrum.

Bacteria	No. of samples	Percentage of samples in which bacterial growth present
Citrobacter	2	5.5
Staph aureus	3	8.4
E coli	7	19.5
Klebsiella species	10	27.8
Pseudomonas aeruginosa	14	38.8
Total	36	36 (100)

Pseudomonas aeruginosa was the most frequently isolated bacterium in summer (16.6%) and post- monsoon (15.4%). In monsoon, it was Klebsiella species (19.3%). No predominance of any bacteria was seen in winter (Figure 1).

Table 4 shows that out of a total of 100 sputum samples 81 showed increased total leukocyte counts. Sputum neutrophilia was seen in 91 samples and

Table 4: Sputum samples with various sputum inflammatory cells.

Sputum with increased cells	No of sputum samples n, (%)	Total
TLC (>5.5 million/ml)	81 (81)	100
N (>61%)	91 (91)	100
E (>3%)	41 (41)	100
L (>4%)	11 (11)	100
M (>80%)	0	0

Table 5: Predominant sputum inflammatory cells according to seasons.

Inflammatory cells	Summer and Monsoon			Post Monsoon and Winter			
	Summer (6months) N=36 n (%)	Monsoon (5months) N=31 n (%)	Total n, (%)	Post- monsoon (3months) N=13 n (%)	Winter (4months) N=20 n (%)	Total n, (%)	p value
N >61%	22 (61.1)	18 (58.1)	40 (59.2)	6 (46.1)	13 (65)	19 (57.6)	0.728
N+E	10 (27.8)	12 (38.7)	22 (32.8)	6 (46.1)	4 (20)	10 (30.3)	0.458
E>3%	4 (11.1)	1 (3.2)	5 (7.5)	1 (7.7)	3 (15)	4 (12.1)	0.021

Table 6: Sputum inflammatory cells and mean hospital LOS of patients.

Increased sputum inflammatory cells	Mean length of hospital stay in no. of days (std deviation)	p value
N	10.12 (1.34)	0.023
E	9.11 (1.45)	

sputum eosinophilia in 41 samples.

Bacterial growth decreased progressively as neutrophils decreased and eosinophils increased in the sputum. Of the 59 neutrophil predominant samples, growth was seen in 25 (42%). Growth was less in samples containing both N+E (28%) and least in samples with predominant E (22%) (Figure 2).

Table 5 shows that there was no difference in the percentage distribution of isolated neutrophils and mixed population (N+E) in sputum with seasons. Percentage of samples with isolated sputum eosinophilia was higher in post-monsoon and winter than in summer and monsoon (p=0.021).

Table 6 shows that patients with predominant eosinophils in their sputum had shorter mean length of hospital stay (9.11 days) as compared to patients with predominant neutrophils in their sputum (10.12 days) (p=0.023).
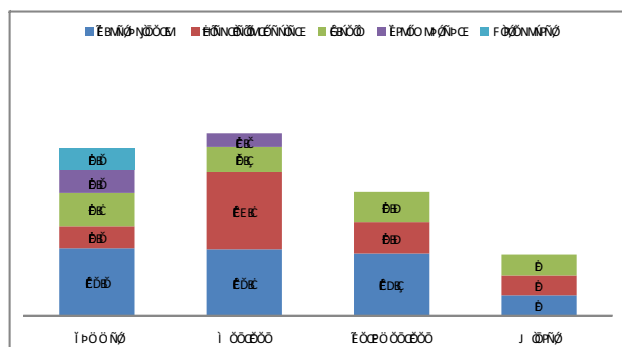

Figure 2: Predominant inflammatory cells and bacterial growth. .

Figure 1: Sputum bacterial spectrum according to seasons.

DISCUSSION:

We did this study to observe the bacterial profile and the predominant inflammatory cell in sputum of patients with AECOPD in various seasons.
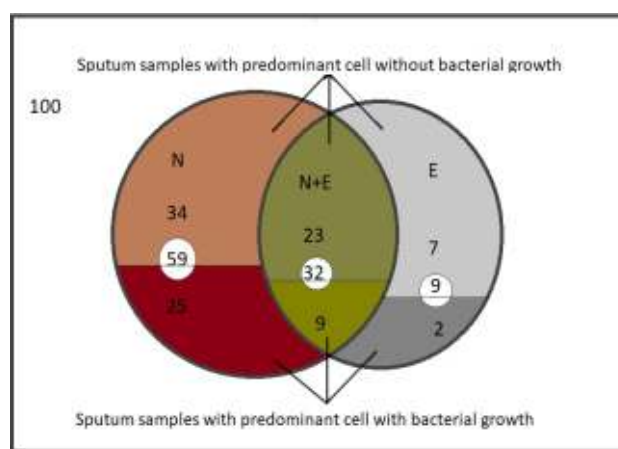
Bacteria causing AECOPD:

In the present study, bacterial growth was present in 36% of sputum samples. This was significantly lower than that reported by Groenewegen et al and Papi et al, who found bacterial growth in 50% to 55% in AECOPD[12,16]. However, Cukic et al reported that growth of pathogenic bacteria was slightly lower, at around 41%[23]. Dai et al observed 21% bacterial growth in their study on patients with AECOPD[24].

According to a systematic review by Uzun and colleagues, bacteria as the cause of AECOPD was reported 30% to 55% cases[25]. Prior use of antibiotics by patients within the past three months could be one of the reasons for culture negativity[26]. According to Kyo et al and Chawla et al, sputum samples collected at times other than morning may not have bacterial growth[27]. In our hospital-based study, patients were admitted any time during day or night and antibiotic treatment was given without any delay in such patients, sometimes before sputum collection.

Collection of morning sputum sample was also not possible every time.

Furthermore, the classical microbial culture techniques using standard conditions can culture only 30% of bacteria[28]. This may explain lower bacterial culture growth of 36% in our study.

The bacterial spectrum varies not only between geographical areas but also, from time to time, within the same geographical area. Studies conducted in various countries showed the predominance of Streptococcus pneumonae followed by Haemophillus influenzae, Moraxella catarrhalis, Staphylococcus aureus and other gram-negative bacteria[2-5]. Studies conducted in India showed predominance either of gram-negative bacteria eg. Pseudomonas aeruginosa, Klebsiella and E.coli or Streptococcus pneumonae[6-8]. In our study, predominance of Gram-negative bacteria was observed. The predominant organism that was isolated was Pseudomonas aeruginosa (14%). Other organisms were Klebsiella species (10%), E.coli (7%) and Citrobacter (2%). The only Gram-positive bacterium, Staphylococcus aureus, was grown in 3% of the sputum samples.

Bacterial spectrum with seasons:

Various cities have reported different hospital admission rates with seasons[29]. In London most of the exacerbations occurred in cold season, November-February[30], whereas in New Delhi, there was no statistically significant difference in admissions per month in winter- *November to February* v/s summer- *March to October*[31].

In our study, admissions with AECOPD were more in summer (36%) and monsoon (31%) than in winter (20%) and post-monsoon (13%). In study period of one and half year, the total duration of summer and monsoon was eleven months and of post-monsoon and winter season was seven months. Bacterial isolation was higher in summer and monsoon (43.3%) than in post-monsoon and winter (21.2%).

The low bacterial isolation in post monsoon and winter could be because of higher viral exacerbations of COPD and/or allergic cause of AECOPD. Previous studies by Donaldson et al and Wedzicha et al suggested that cold and humid environment might favour the occurrence of viral infections[30,32].

Papi et al reported that virus alone was responsible for about 25% of exacerbations of COPD[12]. Almost similar result was reported by Bafadhel et al (29%) in their study[11].

Allergens may increase respiratory symptoms and risk of COPD exacerbation[33,34]. According to Jamieson et al the allergic phenotype accounted for 25% and 30% in two different COPD cohorts and this phenotype was associated with increased risk of COPD exacerbations[35].

The predominant inflammatory cell in sputum in AECOPD:

Majority of the sputum samples (81%) had increased TLC (>5.5 million/ml). COPD exacerbation was associated with increased total leukocyte count in sputum. Nearly all sputum samples, 91%, had neutrophilia >61%. Forty one percent of sputum samples had eosinophilia >3%. Only 11% of the sputum samples had increased lymphocytes >4%. We found that the predominant cell that increased in sputum during exacerbations was neutrophils. Pure neutrophilia was seen in 59% of the samples. Sputum neutrophilia was associated with sputum eosinophilia in 32% of the samples. The two cells that were chiefly seen during exacerbations were neutrophils and eosinophils.

Gao et al observed the predominance of neutrophils, eosinophils and macrophages in AECOPD. Based on the sputum inflammatory cell profile, they classified patients with AECOPD into neutrophilic, eosinophilic, mixed granulocytic and pauci-granulocytic groups[1].

In our study also, exacerbations could be classified as purely neutrophilic (59%), mixed neutrophilic and eosinophilic (32%) and purely eosinophilic (9%) (Figure 2). Papi and colleagues reported that sputum neutrophils were increased in all exacerba-tions of their sixty four patients (p <0.001)[12]. Bafadhel and colleagues found that a substantial number of exacerbations were associated with sputum eosinophilia (28% of 182 exacerbation events) and concluded that eosinophilic airway inflammation also existed in AECOPD[11].

Bacterial growth and predominant sputum inflammatory cells:

Sputum neutrophilia is associated with bacterial etiology of AECOPD[1]. In our study, out of 91 sputum samples which had neutrophilia, 34 (37.4%) had bacterial growth. Sputum neutrophilia was also present in sterile sputum. Papi et al observed that sputum neutrophils were increased in all exacerbation sub-groups: viral, bacterial, viral-bacterial co infection and non infectious[12].

Out of 41 sputum samples which had eosinophilia, bacterial growth was seen in only 11(26.8%) and there was no bacterial growth in 30 (73.2%). Sputum eosinophilia in AECOPD was less likely to be associated with bacterial growth. Similarly, Kolsum et al found that sputum eosinophil percentage was inversely related to bacterial load[10]. Papi and co-workers concluded that sputum eosinophilia was related to viral exacerbations in COPD[12].

Response to therapy and predominant sputum inflammatory cells:

We observed a lesser mean length of hospital stay in patients with sputum eosinophilia as compared to patients with sputum neutrophilia (9.11v/s10.12 days, p=0.023). This could be due to a prompt effect of corticosteroids in patients with allergic phenotype of AECOPD who presented with sputum eosinophilia.

Sputum inflammatory cells according to seasons:

Pure eosinophilic exacerbations were higher in post-monsoon and winter as compared to summer and monsoon (12.1%v/s7.5%, p=0.021). This could indicate an allergic or a viral etiology of exacerbations. Since sputum eosinophilia was associated with a lesser length of hospital stay, likely due to prompt response to steroid therapy, eosinophilic exacerbations were probably of allergic origin. It would be worthwhile to investigate whether patients with sputum eosinophilia could be treated with systemic steroids without antibiotics.

Although infections are a major cause of exacerbations in COPD, in our study the overall bacterial growth was less (36%) compared to 50-55% in other studies. This could be because many a time sputum samples could not be collected before antibiotic initiation. Patients either were not expectorating sputum at the time of admission or were admitted at night.

Furthermore, sterile sputum could be a result of a viral or an allergic exacerbation. Isolation of virus would have given us an idea as to what proportions of our patients had viral exacerbations. Since patients with sputum eosinophilia had a lower length of hospital stay than those with sputum neutrophilia, it could be interpreted that these patients had an allergic exacerbation that responded promptly to steroid therapy.

To facilitate differential cell count, sputum analysis for inflammatory cells is done after separation of sputum part from salivary part of the expectorate. Clumping of mucus is prevented by adding DTT (Dithiothretal) solution for sputolysis. Finally, the mixture is cytospined[36]. To cut down on the cost of the investigation the sputum in our study was examined after separating the sputum part from the saliva with the help of forceps, without adding DTT solution. This may not prevent the clumping of mucus. Despite this, results of our study are consistent with results of other studies where processing of sputum was done as described above[1,12,36].

CONCLUSIONS:

We have shown that AECOPD is associated with both sputum neutrophilia and sputum eosinophilia. Presence of neutrophils in sputum, in contrast to eosinophils, is more likely to be associated with bacterial exacerbations of COPD. The absence of bacterial growth in samples with eosinophilia could indicate an allergic exacerbation. It would be worthwhile to investigate whether patients with sputum eosinophilia could be treated with systemic steroids without antibiotics.

The principal reason why laboratories do not do sputum cell analysis, is its complexity. We have shown that direct smear method of sputum cell analysis can be done. However, to recommend direct sputum analysis, further studies are required to compare the various techniques of sputum processing.

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