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RESEARCH ARTICLE

Role of Immunological and Inflammatory markers in the Identification of Acute COPD Exacerbations with Infectious Etiology

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Abstract

Background: Better identification of immunological and inflammatory changes in chronic obstructive pulmonary disease (COPD) exacerbations will help identify exacerbations phenotypes associated with bacteria, viruses and sputum eosinophilia in order to predict prognosis, guide therapy and provide new therapeutic targets. This study aimed to identify systemic and local immunological and inflammatory changes during COPD exacerbations, and to compare clinical COPD exacerbation phenotypes especially those with infectious etiology.

Methods: This study included 30 patients with acute COPD exacerbations and 16 stable COPD patients as controls. Sputum samples from exacerbation patients were analyzed for bacterial and viral infections. IL-1β, IL-6, TNF-α, apolipoprotein A1, lipocalin-1 and CXCL-10 were assessed in serum and sputum samples from patients and controls.

Results: Acute COPD exacerbation patients had significantly higher levels of serum CXCL-10, and sputum IL-1β, IL-6, TNF-α, CXCL-10, apolipoprotein A1 and lipocalin-1 with areas under the receiver operating characteristic curves (AUC) of 1, 0.93, 1, 1, 0.766, 1 and 0.94, respectively. Bacterial exacerbation patients had significantly higher levels of sputum IL-6 and TNF-α with an AUC of 0.845, 0.839 respectively. Serum lipocaline-1 was significantly lower in patients with viral exacerbation compared to non-viral exacerbations with an AUC of 0.752. Peripheral percentage eosinophils were significantly higher in eosinophilic exacerbations with an AUC of 0.72.

Conclusion: Acute COPD exacerbations were associated with higher increase in the local inflammatory reaction rather than systemic inflammation. Sputum IL-6 and TNF-α can predict bacterial exacerbations while lipocalin-1 in blood can predict viral exacerbations. Peripheral percentage eosinophils are the best predictor for eosinophilic exacerbations.

Introduction

Acute exacerbations of chronic obstructive pulmonary disease (COPD) are associated with substantial morbidity and mortality (Rabe et al., 2007). COPD exacerbations are heterogeneous and their mechanisms are distinct, as they are associated with bacterial or viral infection or sputum eosinophilia. These exacerbation clinical phenotypes are likely to represent distinct pathophysiologic entities with specific immunological features. Therefore, several immunological markers can be used to identify specific clinical phenotypes during exacerbations of COPD

(Bafadhel et al., 2011). However most of these markers have not been fully validated and their diagnostic and prognostic value remains unclear (Nicholas et al., 2010).

Respiratory viral and bacterial infections have been implicated in causing most exacerbations (Sethi and Merphy, 2008). Markers of systemic inflammation, such as IL-6, IL-1β and TNF-α, are increased during mild to moderate COPD exacerbations. The increase in systemic inflammation seems to be limited to exacerbations caused by bacterial infections (Bafadhel et al., 2011, Saladias et al., 2012).

The application of clinical symptoms in combination with serum CXCL10 (IP-10) has been proposed as a possible marker for rhinovirus infection at exacerbation and a potential predictor of a virus-associated exacerbation (Bafadhel et al., 2011, Conway et al., 2010, Quint et al., 2010,).

The inflammatory profile of a COPD exacerbation is typically neutrophilic, but eosinophilic airway inflammation also exists (Bhowmik et al., 2000) and is associated with a favorable response to corticosteroid therapy (Brightling et al., 2000). The peripheral percentage eosinophils count was shown to be a sensitive biomarker of sputum eosinophilia. This finding raises the possibility that targeting corticosteroid therapy in a subgroup of exacerbations depending on the peripheral eosinophils count may reduce inappropriate use of systemic corticosteroids (Bafadhel et al., 2011).

Two immunological markers, apolipoprotein A1 and lipocalin-1, were also identified. Their concentrations in sputum were found to be reduced in COPD patients when compared to control smoking subjects who had no evidence of COPD, indicating their potential utility as biomarkers providing information about the pathophysiology of COPD (Nicholas et al., 2010). In a mouse model, apolipoprotein A1 has been shown to be protective against proinflammatory stimulus and sepsis (Jiao and Wu, 2008), possibly through sequestration of lipopolysaccharides. In pigs, plasma levels are reduced during sepsis (Carpintero et al., 2005).

Lipocalin-1 is an innate defense molecule that was shown to be expressed in the bronchial epithelium. Because of its structural similarity to other antimicrobial proteins in this superfamily and widespread distribution in the bronchial mucosa, its primary role is thought to be in epithelial defense in the respiratory tract. Its increase in healthy smokers compared with healthy nonsmoking subjects, was therefore not surprising, and the mechanism leading to this change could involve either direct effects of smoking or an increased susceptibility of smokers for bacterial infections (Nicholas et al., 2010, Redl et al., 1998).

During stable state, sputum eosinophilia is associated with corticosteroid responsiveness (Brightling et al., 2000), whereas the presence of a high bacterial load and sputum purulence has favorable outcomes with antibiotics (Ram et al., 2006). Identifying clinically important COPD exacerbation phenotypes is crucial because systemic corticosteroids and antibiotics have marginal efficacy and potential to cause adverse events in an already vulnerable population (Aaron et al., 2003, Rothberg et al., 2010), hence the importance of approaches that aim at the identification of COPD exacerbation phenotypes and recognition phenotype-specific markers to allow for better prognostic and therapeutic applications.

The aim of this study is to identify systemic and local immunological and inflammatory changes during COPD exacerbations and to compare clinical COPD exacerbation phenotypes especially those with bacterial and viral etiology.

Subjects and Methods

Subjects

This study is a case–control study that included 30 patients with acute COPD exacerbations; 19 (63.3%) males and 11 (36.7%) females, their age was 50.4 ± 6.5 years. Sixteen COPD patients in the stable state were included as controls; 10 (62.5%) males and 6 (37.5%) females; their age was 48.9 ± 5.6 years. The two groups were matched for age and sex. The patients and controls were collected from the Chest department, Assiut University Hospital during the period from May 2013 to April 2014. The patients were chosen for the study according to the following inclusion criteria: clinical and radiological diagnosis, post-bronchodilator FEV1/FVC (Forced Expiratory Volume in one second/Forced Vital Capacity) ratio of less than 0.7 as per global initiative for chronic obstructive lung disease (GOLD) criteria (Rabe et al., 2007), and age greater than 40 years. Exclusion criteria included a current or previous history of asthma, currently active pulmonary tuberculosis, lung diseases other than COPD and patients received prior corticosteroid or antibiotic therapy. Written informed consent was obtained from each participant. The study was approved by the local ethical committee. Exacerbations were defined in terms of increased dyspnea, sputum production, and sputum purulence (Rodriguez-Roisin, 2000). Patients were clinically assessed by chest radiograph, temperature and blood gas analysis, when indicated, to exclude other causes of breathlessness.

Methods

Sputum and serum samples were collected from each participant. Sputum samples were collected in two containers; the first container was used for bacterial culture. Serum samples and the second container of sputum samples were collected and stored in aliquots at -20°C within 1 hour of sampling. Sputum samples were analyzed for bacteria using standard routine culture and identification. Polymerase chain reaction (PCR) was used for diagnosis of viral and atypical bacterial infections.

Nucleic Acid Extraction and Amplification

RNA was extracted from all sputum samples using Total RNA Purification Kit (Jena Bioscience, Jena, Germany). QIAGEN OneStep RT-PCR Kit (Hilden, Germany) was used for reverse transcription and amplification of parainfluenza virus-2 (PIV-2), human metapneumovirus (hMPV) (Bharaj et al., 2009) and coronavirus (Moes et al., 2005). The primers used and the sizes of PCR product bands are shown in table (1). The primers were purchased from Metabion International AG (Martinsried, Germany).

Multiplex RT-PCR for *Mycoplasma pneumoniae, Chlamydophila pneumoniae,* respiratory syncytial virus (RSV), parainfluenza virus-1 (PIV-1), PIV-3, enterovirus, influenza A virus, influenza B virus and adenoviruses was performed according to the protocol of Grondahl et al. (1999), using QIAGEN OneStep RT-PCR Kit (Hilden, Germany). Positive controls for influenza A virus, PIV-1 and RSV were used to document the efficiency of the preparation procedure. Distilled H_2O was used as negative control. All PCR reactions were performed in TPersonal Thermocycler (Biometra, Goettingen Germany). Electrophoresis of 10 ml PCR products was performed on 2% agarose gel stained with ethidium bromide (Sigma, USA). PCR products were visualized by UV illumination. PCR bands were compared to 100 bp DNA Ladder H3 RTU that includes DNA fragments ranging from100-3000 base pairs (Nippon Genetics, Dueren, Germany).

Bacteria-associated exacerbations were defined as a positive bacterial pathogen on routine culture or a total aerobic CFU count greater than or equal to 10^7 cells (Ram et al., 2006). Virus-associated exacerbations were defined as a positive sputum viral PCR. Sputum eosinophils-associated exacerbations were defined as the presence of more than 3% nonsquamous cells (Bafadhel et al., 2011, Vandenplas et al., 2009).

Assessment of Inflammatory Markers

Inflammatory markers were assessed in sputum and serum samples. Semi-quantitative ELISA assays were used to assess IL-1β (Orgenium, Vantaa, Finland), IL-6 (Orgenium, Vantaa, Finland), TNF-α (Assaypro, Missouri, USA), apolipoprotein A1 (Assaypro, Missouri, USA), lipocalin-1 (Bioassay Technology laboratory, Shanghai, China), and CXCL-10 (WKEA Med Supplies, Changchun, China). The color change was read by Awareness Technologies Stat Fax 2100 Microplate reader (GMI, Ramsey, Minnesota, USA).

Statistical analysis:

Data were analyzed using SPSS version 16. Values were expressed as mean ± SE. Differences between values of each parameter in acute exacerbation and control patients as well as between acute exacerbations phenotypes were assessed by the student t-test. Receiver operating characteristic curves were done and area under the curve (AUC) was used to compare between markers. Sensitivity and specificity of different markers were determined from the data results of ROC curves. The best calculated point achieving the highest sum of sensitivity and specificity was used as a cutoff value. Results were considered statistically significant at $p \leq 0.05$.

Results

Out of 30 acute exacerbation patients, 11 (36.7%) were smokers and 19 (63.3%) were non-smokers, and out of the stable COPD patients, 6 (37.5%) were smokers and 10 (62.5%) were non-smokers. As both patients and control groups are matched for their states of smoking, so smoking will not be considered to affect the difference in the inflammatory markers pattern for the two groups.

In this study, bacterial infection was detected in 23/30 (76.7%) of acute COPD exacerbations. Twenty-five bacterial strains were isolated from exacerbation patients; 12 (48%) *Klebsiella pneumonia,* 7 (28%) *Staphylococcus aureus,* 3 (12%) *pseudomonas aeruginosa,* 2 (8%) *Streptococcus pyogenes*, 1 (4%) *Streptococcus pneumonia. Candida* spp. were isolated in 5 patients, all of them were associated with bacterial coinfection. Viral infection was detected in 7/30 (23.3%); adenoviruses were detected in three samples, RSV in two samples, influenza A virus in one sample and influenza B virus in one sample. Sputum eosinophilia was detected in 25/30 (83.3%). Viral and bacterial exacerbations were found in 6 patients, viral and eosinophilic in 6 patients, bacterial and eosinophilic in 19 patients. The three types were found in 5 patients. One patient in our study neither had bacterial or viral infections nor sputum eosinophilia, his inflammatory markers were low.

Table 1 Sequence of the primers used for amplification and sizes of PCR products

Genes	Primers	Sizes of genes (bp)	References
PIV-2 N gene	F 5-GATGACACTCCAGTACCTCTTG-3 R 5-GATTACTCATAGCTGCAGAAGG-3	197	Bharaj et al., 2009
hMPV N gene	F 5-AAGCATGCTATATTAAAAGAGTCTCA-3 R 5-ATTATGGGTGTGTCTGGTGCTGA-3	440	
Coronavirus	F 5'-ACWCARHTVAAYYTNAARTAYGC-3' R 5'-TCRCAYTTDGGRTARTCCCA-3'	251	Moes et al., 2005
RSV (F1 subunit of fusion glycoprotein gene)	F 5′-TGT TAT AGG CAT ATC ATT GA-3′ R 5′-TTA ACC AGC AAA GTG TTA GA-3′	239	
PIV-1 ((hemagglutinin-neuraminidase gene)	F 5′-CAC ATC CTT GAG TGA TTA AGT TTG ATG A-3′ R 5′-ATT TCT GGA GAT GTC CCG TAG GAG AAC-3′	179	
PIV-3 (5′ noncoding region of fusion protein gene)	F 5′-TAG CAG TAT TGA AGT TGG CA-3′ R 5′-AGA GGT CAA TAC CAA CAA CTA-3′	205	
M. pneumoniae (nucleotide sequence of 16S rRNA)	F 5′-AAG GAC CTG CAA GGG TTC GT-3′ R 5′-CTC TAG CCA TTA CCT GCT AA-3′	277	
C. pneumoniae (nucleotide sequence of 16S rRNA)	F 5′-TGA CAA CTG TAG AAA TAC AGC-3′ R 5′-CGC CTC TCT CCT ATA AAT-3′	463	Grondahl et al., 1999
Enterovirus (highly conserved noncoding region)	F 5′-ATT GTC ACC ATA AGC AGC CA-3′ R 5′-TCC TCC GGC CCC TGA ATG CG-3′),	154	
Influenza A virus (nonstructural protein gene)	F 5′-AAG GGC TTT CAC CGA AGA GG-3′ R 5′-CCC ATT CTC ATT ACT GCT TC-3′	190	
Influenza B virus (nonstructural protein gene)	F 5′-ATG GCC ATC GGA TCC TCA AC-3′ R 5′-TGT CAG CTA TTA TGG AGC TG-3′	249	
Adenoviruses (hexon gene)	F 5′-GCC GAG AAG GGC GTG CGC AGG TA-3′ R 5′-ATG ACT TTT GAG GTG GAT CCC ATG GA-3′	134	

PIV-1: parainfluenza virus-1; PIV-2: parainfluenza virus-2; PIV-3: parainfluenza virus-3; hMNV: human metapneumovirus

Acute COPD exacerbation patients had significantly higher levels of serum CXCL-10, and sputum IL-1β, IL-6, TNF-α, CXCL-10, apolipoprotein A1 and lipocalin-1 ($P \leq 0.001$). Serum levels of IL-1β, IL-6 and TNF-α were higher in exacerbations than in stable COPD but with no significant difference. Table (2) shows the inflammatory markers profiles of the two groups. Table (3) shows the areas under the receiver operating characteristic curves, cutoff value, sensitivity and specificity of inflammatory markers in patients with acute COPD exacerbations and stable COPD.

Bacterial exacerbation patients had significantly higher levels of sputum IL-6 and TNF-α (P = 0.033, 0.026 respectively) (table 4). The areas under the receiver operating characteristic curves of sputum IL-6 and sputum TNF-α in bacterial exacerbations were 0.845, 0.839 respectively (fig 1). At cutoff value 66 pg/ml, sputum IL-6 had sensitivity of 78.3% and specificity of 85.7%. At cutoff value 65.8 ng/ml, sputum TNF- α had sensitivity of 91.3% and specificity of 71.4%.

Serum lipocaline-1 had significantly lower levels in patients with viral exacerbation compared to non-viral exacerbations (P = 0.045), data are shown in table (5). The area under the receiver operating characteristic curve of serum lipocalin-1 in viral exacerbations was 0.752 (fig 2). At cutoff value 7.65 ng/ml, the sensitivity was 71.4% and the specificity was 65.2%.

Peripheral percentage eosinophils levels were significantly higher in eosinophilic exacerbations versus non-eosinophilic (P = 0.009). The area under the receiver operating characteristic curve for peripheral percentage eosinophils as a predictor for eosinophilic exacerbations was 0.72 (fig 3), at cutoff value 2.5% the sensitivity was 72% and the specificity was 60%. None of the other tested inflammatory markers could significantly predict eosinophilic COPD exacerbations.

Table 2 Inflammatory markers in acute exacerbation and stable COPD patients

	Acute Exacerbations (n=30)		Stable COPD (n=16)		
	Mean	SE	Mean	SE	*P* value
Serum IL-1β	35.5	13.3	24.9	4.4	NS
Serum IL-6	67.7	30	21.4	3.7	NS
Serum TNF-α	18.4	4.7	12.8	1.5	NS
Serum CXCL-10	26.6	2.9	4.9	0.5	< 0.001
Serum apolipoprotein A1	30.5	3.5	29.6	3.1	NS
Serum lipocalin-1	11.2	1.3	11.2	1.3	NS
Sputum IL-1β	50.5	10.3	9.4	1.4	< 0.001
Sputum IL-6	93.2	10.3	8.3	0.5	< 0.001
Sputum TNF-α	395.3	63.6	6.6	0.4	< 0.001
Sputum CXCL-10	4.1	0.2	2.9	0.3	0.001
Sputum apolipoprotein A1	37.2	1.9	5.2	0.7	< 0.001
Sputum lipocalin-1	4.3	0.3	2	0.2	< 0.001

Table 3 The areas under the receiver operating characteristic curve, cutoff value, sensitivity and specificity of inflammatory markers in patients with acute COPD exacerbations and stable COPD

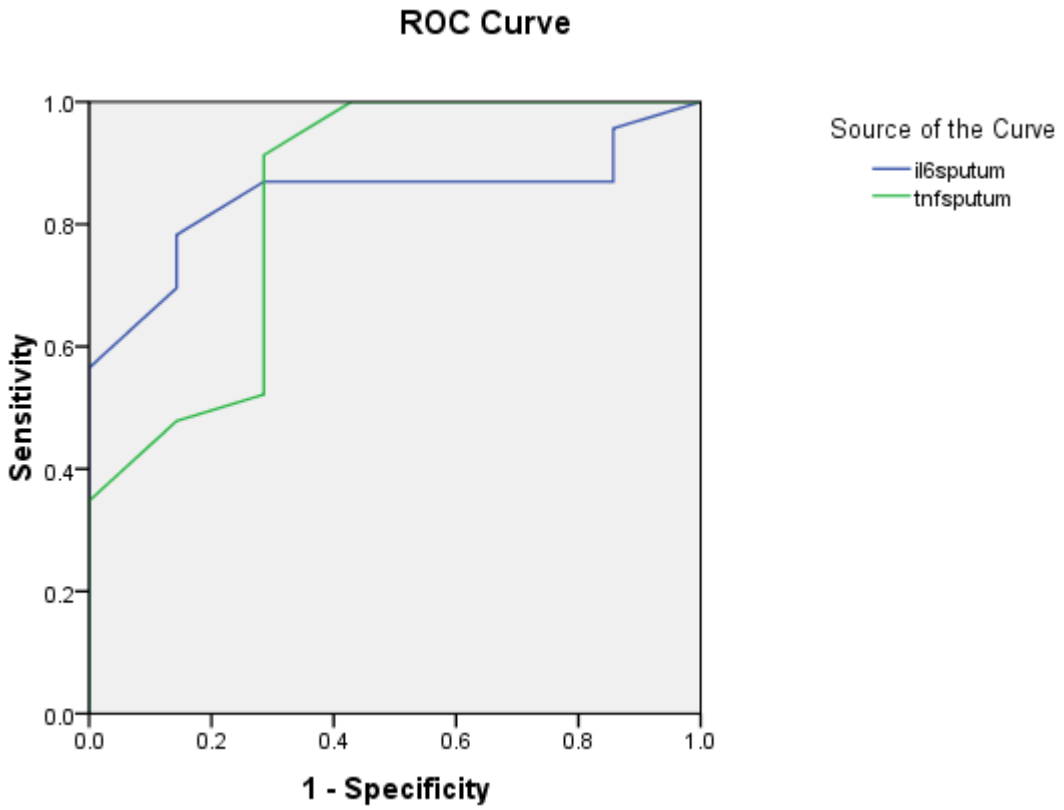
Parameter	AUC	Cutoff value	Sensitivity (%)	Specificity (%)
Serum CXCL-10	1	13.95 µg/ml	100	100
Sputum CXCL-10	0.766	3.9 µg/ml	63.3	75
Sputum IL-1β	0.93	22.75 pg/ml	76.7	100
Sputum IL-6	1	20.85 pg/ml	100	100
Sputum TNF-α	1	12.2 ng/ml	100	100
Sputum apolipoprotein A1	1	20 µg/ml	100	100
Sputum lipocalin-1	0.94	2.7 ng/ml	90	81.2

Table 4 Inflammatory markers in bacterial versus non-bacterial exacerbations

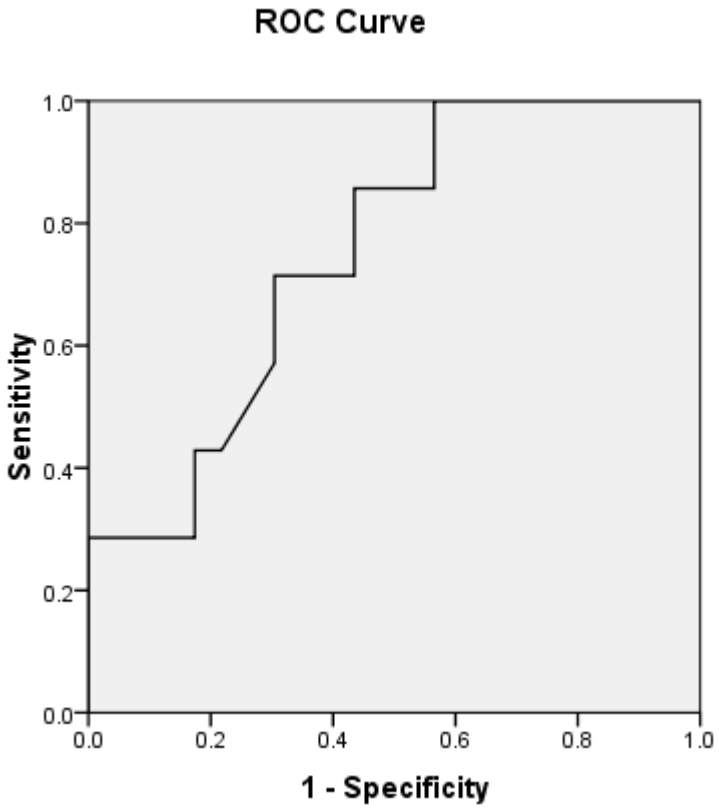
	Bacterial exacerbations (n=23)		Non-bacterial exacerbations (n=7)		*P* value
	Mean	SE	Mean	SE	
Serum IL-1β	43.8	17.1	8.2	3.3	NS
Serum IL-6	82.2	38.8	20.1	4	NS
Serum TNF-α	13.6	0.6	14.4	0.8	NS
Serum CXCL-10	28.1	3.7	21.4	0.6	NS
Serum apolipoprotein A1	28.9	4.3	35.7	5.9	NS
Serum lipocalin-1	11.4	1.4	10.3	3.4	NS
Sputum IL-1β	52.3	11	44.5	27.4	NS
Sputum IL-6	105.2	12.1	53.9	8.9	0.033
Sputum TNF-α	472.2	72.3	142.7	83.2	0.026
Sputum CXCL-10	4.2	0.2	3.8	0.4	NS
Sputum apolipoprotein A1	38	2.3	34.3	3.3	NS
Sputum lipocalin-1	4.3	0.3	4.2	0.6	NS
FEV	52.3	1.4	50.6	1.7	NS

Table 5 Inflammatory markers in viral versus non-viral exacerbations

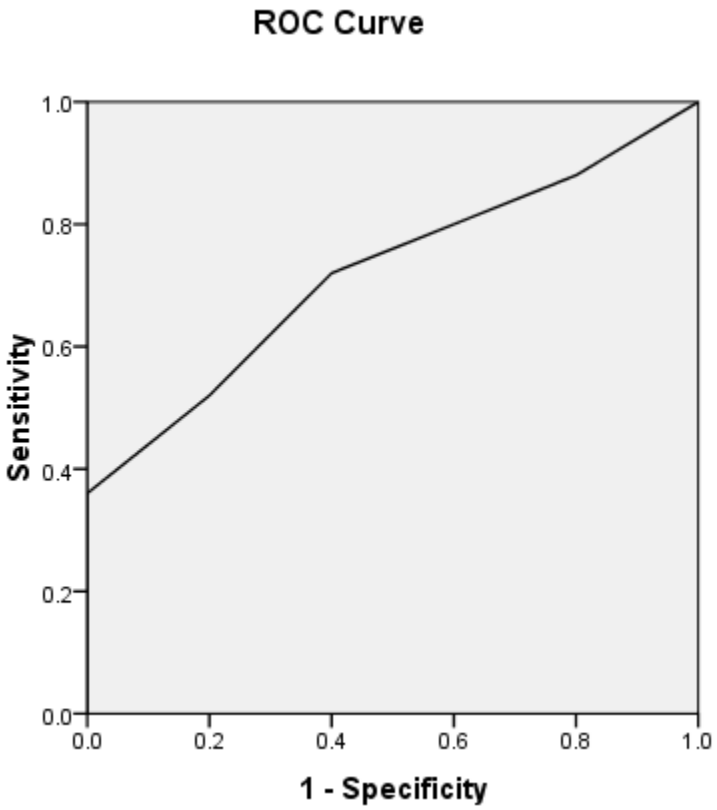
	Viral Exacerbations (n=7)		Non-viral Exacerbations (n=23)		*P* value
	Mean	SE	Mean	SE	NS
Serum IL-1β	37.7	27.8	34.9	15.6	NS
Serum IL-6	42.3	21.2	75.5	38.7	NS
Serum TNF-α	13.5	0.8	13.9	0.6	NS
Serum CXCL-10	22.3	0.4	27.9	3.7	NS
Serum apolipoprotein A1	32.6	5.6	29.9	4.3	NS
Serum lipocalin-1	6.5	1.7	12.6	1.5	0.045
Sputum IL-1β	66.6	25	45.6	11.3	NS
Sputum IL-6	94.8	8.3	92.8	13.3	NS
Sputum TNF-α	334	76.3	414	80	NS
Sputum CXCL-10	4.1	0.4	4.1	0.2	NS
Sputum apolipoprotein A1	36.3	3.6	37.4	2.2	NS
Sputum lipocalin-1	4.2	0.6	4.4	0.3	NS
FEV	51.9	2.5	51.9	1.3	NS



(Fig 1): Receiver operating characteristic curves of sputum IL-6 and sputum TNF-α in bacterial exacerbations. The area under the curve is 0.845, 0.839 respectively.
At cutoff value 66, sputum IL-6 had sensitivity of 78.3% and specificity of 85.7%.
At cutoff value 65.8, sputum IL-6 had sensitivity of 91.3% and specificity of 71.4%.



(Fig 2): Receiver operating characteristic curve of serum lipocalin-1 in viral exacerbations. The area under the curve is 0.752.
At cutoff value 7.65 the sensitivity was 71.4% and the specificity was 65.2%.



(Fig 3): Receiver operating characteristic curve of peripheral blood eosinophils in eosinophilic exacerbations. The area under the curve is 0.72.
At cutoff value 2.5% the sensitivity was 72% and the specificity was 60%.

Discussion

In this study most exacerbations appeared to be multifactorial. Sputum eosinophilia was detected in 25/30 (83.3%) of acute COPD exacerbations. Viral infection was detected in 7/30 (23.3%) and bacterial infection was detected in 23/30 (76.7%). One patient in our study (3.3%) neither had bacterial or viral infections nor sputum eosinophilia, his inflammatory markers were low. Bafadhel et al. (2011) reported that 14% of acute COPD exacerbations are pauci-inflammatory and that the pauci-inflammatory exacerbations have limited changes in the inflammatory profile.

In the present study acute COPD exacerbation patients had significantly higher levels of serum CXCL-10, and sputum IL-1β, IL-6, TNF-α, CXCL-10, apolipoprotein A1 and lipocalin-1. The AUC for these biomarkers were 1, 0.93, 1, 1, 0.766, 1, and 0.94, respectively. Serum levels of IL-1β, IL-6 and TNF-α were higher in exacerbations than in stable COPD but with no significant difference. These findings indicate that the increase in the local inflammatory reaction was more prominent than systemic reaction in acute COPD exacerbation patients. Bafadhel et al. (2011) reported that serum TNF-α significantly increased during exacerbations, other inflammatory markers that increased during exacerbations were serum IL-6, TNF receptors I and II and sputum IL-1β, IL6, TNF-α, TNFRI, TNFRII. They also reported that no single inflammatory mediator had an AUC greater than 0.7 in determining an exacerbation from stable state.

Although there was no difference in serum apolipoprotein A1 and lipocalin-1 between exacerbations and stable COPD, the sputum levels of these two markers significantly increased in exacerbation patients, these findings indicate that serum and sputum levels of apolipoprotein A1 and lipocalin-1 are not related to each other. A study by Nicholas et al. (2010) found that sputum levels but not plasma levels of apolipoprotein A1 have significantly reduced in smokers with COPD than in healthy smokers and they concluded that the sputum levels of this protein are under independent control of the systemic amounts.

Bacterial Acute COPD Exacerbations

In the present study, 76.7% of exacerbations were bacterial. Bacterial exacerbation patients had significantly higher levels of sputum IL-6 and TNF-α with an AUC of 0.845, 0.839 respectively. In previous studies, Bacteria are considered to play a role in up to 50% of exacerbations (Sethi and Merphy, 2008). Bafadhel et al. (2011) reported that 55% of exacerbations were bacteria-associated and the most suitable inflammatory marker for determining bacterial exacerbations was sputum IL-1β with an AUC of 0.89. In concordance to this study, Bathoorn et al. (2009) reported that bacterial exacerbations showed a higher increase of IL-6 and TNF-α in sputum and IL-6 in serum when compared to non-bacterial exacerbations. Sputum TNF-α level during an exacerbation is a candidate marker for predicting airway bacterial infection as it has the best test characteristics to predict bacterial infection (Bathoorn et al., 2009). Saldias et al. (2012) reported that serum IL-6 had increased significantly during bacterial exacerbations compared to non-bacterial exacerbations.

Viral Acute COPD Exacerbations

Viral exacerbations were detected in 7/30 (23.3%) patients. Serum lipocaline-1 had significant lower levels in patients with viral exacerbations compared to non-viral exacerbations with an AUC of 0.752. Bafadhel et al. (2011) reported that 29% of exacerbations were associated with viral infection, most commonly rhinovirus. They reported that the best marker for distinguishing the presence of a virus in exacerbation was serum CXCL10. It is worth to mention that in this study the viruses associated with COPD exacerbations - namely adenoviruses, RSV, influenza A and B viruses – did not include rhinoviruses.

Previous studies reported an increase in lipocalin-1 in healthy smokers compared to healthy non-smokers (Redl et al., 1998), whereas another study reported reduced sputum levels of lipocalin-1 in COPD compared to healthy smokers which may reflect deficiencies in the innate epithelial defense, this can explain the increased susceptibility of COPD patients to infectious exacerbations and indicate the potential role of lipocalin-1 in innate defense mechanisms (Nicholas et al., 2010). The increase in lipocali-1 due to smoking can be explained by its increased production as a part of inflammatory process in response to smoking, and its reduction in COPD patients can be explained by its consumption and lowering of local immunity in a chronic airway illness. Therefore the decreased levels of lipocalin-1 detected in the present study can be explained by either consumption of this molecule that has innate defense mechanism in cases of viral infections, or those patients originally have deficiency in innate immune reaction including lipocalin-1 that make them more susceptible to viral infection. To our knowledge, this is the first study that assesses lipocalin-1 as an inflammatory marker in viral COPD exacerbations.

Lipocalin-1 is also known as tear prealbumin due to its initial localization. It was initially believed to be secreted exclusively by the lacrimal glands into the tear fluid; it is now known that it is secreted by numerous exocrine glands, such as the lingual glands, sweat glands, prostate and secretory glands of the tracheobronchial tract, as well as the nasal mucosa (Redl, 2000). However, its role in the airways has not yet been carefully delineated. It is reported that lipocalin-1, similar to lipocalin-2, binds to different bacterial siderophores, and all major classes of fungal siderophores. Exogenous lipocalin-1 can inhibit bacterial and fungal growth under iron limiting conditions, suggesting that it might serve as a member of the innate immune system (Fluckinger et al., 2004). Lipocalin-1 has also been shown to bind to an unusually high number of hydrophobic ligands, in particular lipids, and to limit the harmful effects of lipid peroxidation products in vitro (Lechner et al., 2001). Lipocalins are another putative candidate for the development of novel drugs against airway inflammation (Dittrich et al., 2013).

Eosinophilic Acute COPD Exacerbations

Sputum eosinophilia was detected in the majority of acute COPD exacerbations (83.3%). In concordance to this finding, previous studies reported that COPD exacerbations are associated with sputum and bronchoscopic bronchial biopsy evidence of eosinophilic inflammation. Bronchial biopsies taken from patients during acute exacerbations and compared with stable COPD show a 30-fold increase in the total number of eosinophils (Saetta et al., 1994). The presence of high concentrations of the eosinophil products in induced sputum also supports a role of eosinophils in COPD exacerbations (Keatings and Barnes, 1997). On the other hand, other studies reported lower incidence of sputum eosinophilia in COPD exacerbations. Bafadhel et al. (2011) reported that sputum eosinophilia was observed in only 28% of exacerbations.

In the present study, peripheral percentage eosinophils levels were significantly higher in eosinophilic exacerbations versus non-eosinophilic with an AUC of 0.72. At cutoff value 2.5% the sensitivity was 72% and the specificity was 60%. Similarly, Bafadhel et al. (2011) reported that the most sensitive and specific measure to determine sputum eosinophilia in COPD exacerbations is the percentage peripheral blood eosinophil count with an AUC of 0.85. They reported that a cutoff of 2% peripheral blood eosinophils has a sensitivity of 90% and specificity of 60% for identifying sputum eosinophilia. A review study reported that peripheral blood eosinophil count is an important marker to direct therapy in eosinophilic COPD exacerbations (Brightling et al., 2013).

In conclusion, this study showed that acute COPD exacerbations were associated with higher increase in the local inflammatory reaction rather than systemic inflammation. It also revealed that bacterial exacerbations were associated with significantly higher levels of sputum IL-6 and TNF-α, therefore these immunological markers can predict bacterial infections in acute COPD exacerbations. Whereas significant lower levels of lipocalin-1 in blood in virus-associated exacerbations make this marker a candidate that help to predict viral infections in COPD exacerbations. Future studies are required to assess the local and the systemic role of lipocalin-1 in COPD exacerbations especially those associated with viral infections. Peripheral percentage eosinophils levels are shown to be an important predictor for eosinophilic exacerbations.

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