



# High Dose Inhaled Steroids and Herpes Simplex Virus-1 in Patients with an Exacerbation of COPD; Impact on Long Term Survival

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## Abstract

**Introduction:** COPD is a leading cause of hospitalization and death. Epidemic respiratory viruses have been associated with exacerbations of COPD. Recently it has been reported that HSV-1 can cause deterioration in COPD. In ventilated patients Herpes Simplex Virus-1 (HSV-1) is frequently identified and is associated with an increased mortality. We wished to establish if HSV-1 in COPD was associated with medication, disease severity and subsequent mortality.

**Methods:** Patients hospitalized with an exacerbation of COPD were recruited within 24 hours of admission. Their medication was documented and spirometry was performed. Sputum was obtained and lysed in dithiothreitol. Nucleic acids were extracted and specimens were tested for the presence and number of copies HSV-1 using real-time PCR. Chest radiographs were reported through the routine clinical service. Patients were followed up at a mean of 56 months. Cause of death was assessed by ICD code on the death certificate.

**Results:** One hundred and twelve patients with exacerbations of COPD were recruited. HSV-1 was detected in 21 patients (19%). This was associated with higher maintenance doses of inhaled steroids (1,000 mcg/day vs. 500 mcg/day,  $P < 0.005$ ) prior to admission. Radiographic features of pulmonary infiltrates were more frequent (38% vs. 15.6%,  $p < 0.05$ ). They had worse lung function with FEV<sub>1</sub> ( $0.54 \pm 0.23$  vs.  $0.92 \pm 0.48$  L,  $p < 0.05$ ). HSV-1 during exacerbations was associated with increased mortality on follow-up, hazard ratio 2.3 (1.3-4.0)  $p < 0.0001$ . The median survival was 2.08 (CI 1.00-3.16) years in the group with HSV-1 compared to 5.5 (CI 4.68-6.32) without the virus. There were significantly higher numbers of pulmonary related deaths in those with HSV-1 (72% vs. 41%,  $p < 0.05$ ).

**Conclusion:** HSV-1 is frequently detected in the sputum of COPD patients. It is more commonly found in patients taking higher doses of inhaled steroid. The presence of HSV-1 is associated with increased subsequent pulmonary mortality.

**Keywords:** COPD; Herpes Simplex Virus; Inhaled corticosteroids; Pulmonary infiltrates; Pneumonia; Mortality

## Introduction

COPD is the commonest fatal chronic lung disease in adults. It causes 2.75 million deaths annually [1] and is set to increase in the next decade [2]. In 2008 in the United States 822,500 patients were hospitalized with a primary diagnosis of COPD. Another 3.8 million hospital stays include COPD as a secondary, complicating condition – with over twenty percent having respiratory failure and a further fifth having pneumonia as the primary diagnosis. One in five people over the age of 40 in a hospital in the US has a diagnosis of COPD [3].

Inhaled steroids are widely used to treat COPD; they are usually prescribed in combination with long acting beta agonists [4]. These lead to improvements in symptoms, exacerbation rates

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and attenuation of the decline in FEV<sub>1</sub> [5]. The largest and longest study of combination inhaled steroid and long acting bronchodilator therapy revealed a 17% reduction in mortality [6]. This was argued to be of clinical relevance despite it not reaching the predetermined level of significance. Others have highlighted that this failure to reach statistical significance is compounded by the adverse effect of a high frequency of pneumonia [7]. In a separate randomized trial, pneumonia occurred more frequently in patients treated with combination inhaled corticosteroids than with tiotropium [8].

Most adults have been exposed to herpes simplex virus 1 (HSV-1). It sheds recurrently, often after a time of stress or with immune suppression. We have previously examined the airway secretions in ventilated Intensive Care Unit (ICU) patients. HSV-1 is frequently found in the lower airway and is associated with a worse outcome and significantly higher mortality [9]. A separate research group showed that critically ill patients who had HSV-1 are more likely to have more severe disease and develop ARDS [10].

We and other investigators have found that viral pathogens are commonly associated with exacerbations of COPD [11-13]. A recent report identified HSV-1 as a cause of recurrent exacerbations of COPD [14]. This prompted us to examine the outcome of the patients who had HSV-1 in their sputum. The aim of this study was to determine if HSV-1 in the sputum of patients hospitalized with an exacerbation of COPD had a different outcome to those without the virus. We analyzed their clinical and radiological features. We examined their medication, including inhaled steroid use, prior to admission. We recorded the interval to and cause of death.

## Methods

### Subjects

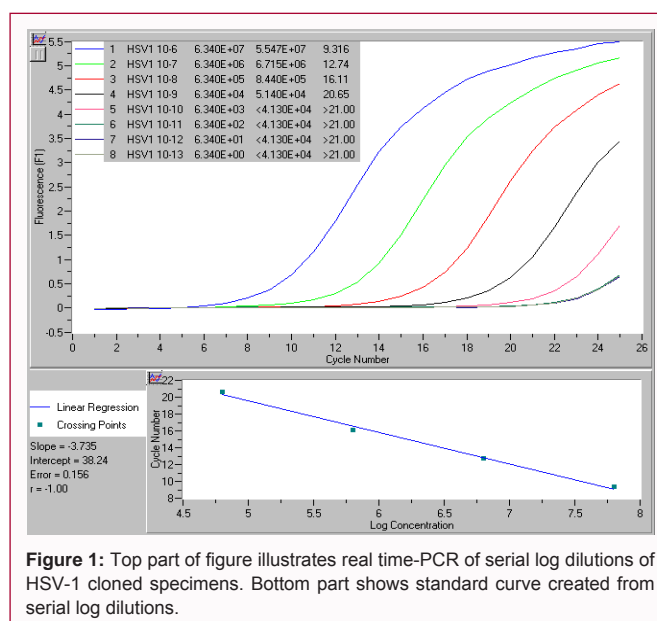
This study was approved by the Local Institutional Review Board. Written consent was obtained. Patients hospitalized with exacerbations of COPD were recruited within 24 hours of presentation over a two-year period from January 2003 to February 2005. An exacerbation of COPD was defined as two of the following three symptoms; increased dyspnoea, increased sputum production or increased sputum purulence [15]. COPD and assessment of severity was classified according to the GOLD criteria [16].

We documented the number of previous exacerbations requiring oral steroids and oral steroids prior to hospitalization both as acute treatment and as maintenance therapy. We recorded the usual inhaled medication. The dose of inhaled steroid was expressed as the beclomethasone dipropionate equivalent [17].

Spirometry was performed using a Vitalograph Spirometer (Vitalograph, Buckingham, England) and the best of 3 reproducible readings was taken [18]. Spirometry was repeated after nebulized beta agonist (albuterol 2.5 mg). Any patients with significant improvement in FEV<sub>1</sub> (>200 ml), bronchiectasis, cancer or other serious concomitant disease were excluded. The presence or absence of a visible oro-labial herpetic lesion was documented. The date and cause of mortality was recorded from the death certificate. Confirmation of patients being alive was made in each case by assessing clinical episodes either by the primary care team, the hospital care team or evidence of clinical activity on the laboratory computer up to September 2008.

### Sputum samples

Sputum was obtained by spontaneous expectoration. All samples were processed within two hours. Specimens were mixed with 4



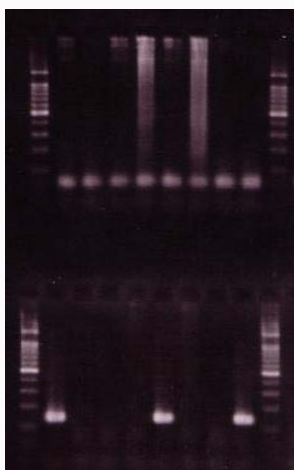
**Figure 1:** Top part of figure illustrates real time-PCR of serial log dilutions of HSV-1 cloned specimens. Bottom part shows standard curve created from serial log dilutions.

volumes 0.1% dithiothreitol (Sigma, Poole, UK) and shaken in an orbital incubator (Gallenkamp, Loughborough, England) for 15 minutes at 37°C followed by the addition of 4 volumes phosphate buffered saline and shaken for a further 5 minutes. The resulting suspension was then filtered through 50 micrometers Nylon Gauze (Locker tex, Warrington, England) and spun down at 1,000 g for 10 minutes. After removing the supernatant the cell pellet was re-suspended in Lysis Buffer (Qiagen, Crawley, England). Total nucleic acid extraction was performed on 200 microliters of sputum sample suspended in Lysis Buffer (QIAamp DNA Blood Mini Kit).

### Polymerase chain reaction – herpes simplex virus-1

All specimens were tested for HSV-1 (Glycoprotein D gene) using published primers [19] and a SYBR green<sup>®</sup> based real-time PCR assay. Following nucleic acid extraction, 2 µl of each specimen was combined with 8 µl of mastermix containing primers specific to Herpes Simplex virus. First round mastermix was prepared using the commercial constituents as follows (Promega, Southampton, England). Each reaction mixture (8 µl) contained (final concentration) 4.5 µl Nuclease free water, 1 µl PCR buffer (1x), 1 µl nucleotides (0.2 mM), 1.4 µl MgSO<sub>4</sub> (2 mM), 0.01 µl of each primer (0.2 µM) and 0.05 µl of Taq (5 U/Test). Mastermix was prepared as detailed in Appendix A1.

PCR was performed on a Peltier Thermal Cycler (MJ Research, Essex, England) and 0.2 µl of the first round product was then used in combination with 9.8 µl of mastermix containing primers internal to the first round primers and SYBR green<sup>®</sup>. Each reaction mixture (9.8 µl) of the second round mastermix contained; 5.2 µl Nuclease free water, 1 µl Bovine serum albumin (0.2 µM), 1 µl PCR buffer (1x), 1 µl of nucleotides (0.2 mM), 1.4 µl MgCl<sub>2</sub> (3.5 mM), 0.02 µl of each primer (0.2 µM), 0.01 µl of SYBR green (1x) and 0.1 µl of Taq in storage buffer B (5 U/Test). Real-time PCR (25 cycles) was carried out using a Roche Light Cycler (Roche Diagnostics Corporation, Mannheim, Germany) with log dilutions of the cloned target sequence being used as calibrators thus enabling quantitation of copy numbers in unknown specimens (Figure 1). Melting curve analysis was employed for the HSV-1 assay to confirm the characteristic melting temperature of the target DNA. Melting curve characteristics were also used to exclude signal detection from non-specific amplicons. PCR cycling



**Figure 2:** Gel electrophoresis of clinical specimens for HSV-1. First round products run directly above second round products. Specimens flanked by molecular weight markers; positive control in lane 8.

conditions are listed in Appendix A2, primer and probe sequences are listed in Appendix A3. Coefficient of variation for these assays was calculated at between 5% and 13%. A positive result was recorded if a copy number greater than zero was obtained following completion of real-time PCR. All specimens were also tested for HSV-1 using a gel based nested PCR assay which was quality controlled and used for the routine diagnostic clinical use (Figure 2).

**Polymerase chain reaction – housekeeping gene assay**

A housekeeping gene (GAPDH) was included in the analysis to confirm that all specimens obtained cellular material using a published primer and probe set with a set of external nested primers [20]. Following nucleic acid extraction (Qiagen, QIAamp DNA Blood Mini Kit), 2 µl of each specimen was combined with 8 µl of mastermix containing primers specific to GAPDH mRNA. Each reaction mixture (8 µl) contained (final concentration) 4.9 µl Nuclease free water, 2 µl PCR buffer (1x), 0.2 µl nucleotides (0.2 mM), 0.4 µl MgSO<sub>4</sub> (2 mM), 0.05 µl of each primer (1.0 µM), 0.2 µl of AMV (1U/Test) and 0.2 µl of Tfl (1U/Test). Mastermix was prepared as detailed in Appendix A1. PCR was performed on a Peltier thermal cycler (MJ Research, Essex, England) and 0.2 µl of the first round product was then used in combination with 9.8 µl of mastermix containing primers and a TaqMan<sup>®</sup> (Qiagen, Crawley, England) probe (labeled with FAM and TAMRA dyes) internal to the first round primers. Each reaction mixture (9.8 µl) of the second round mastermix contained; 4.3 µl Nuclease free water, 1 µl Bovine serum albumin (0.2 µM), 1 µl PCR buffer (1x), 1 µl of nucleotides (0.2 mM), 1.4 µl MgCl<sub>2</sub> (3.5 mM), 0.4 µl of each primer (0.2 µM), 0.2 µl of probe (0.2 µM) and 0.1 µl of Taq in storage buffer B (0.25 U/Test). Real-time PCR (30 cycles) was carried out using a Roche Light Cycler (Roche Diagnostics Corporation, Mannheim, Germany) with log dilutions of the cloned target sequence being used as calibrators thus enabling quantitation of copy numbers in unknown specimens. PCR cycling conditions are listed in Appendix A2, primer sequences are listed in Appendix A3.

**Radiological findings**

All chest radiographs were reported through the routine clinical service. The presence or absence of infiltrates was documented.

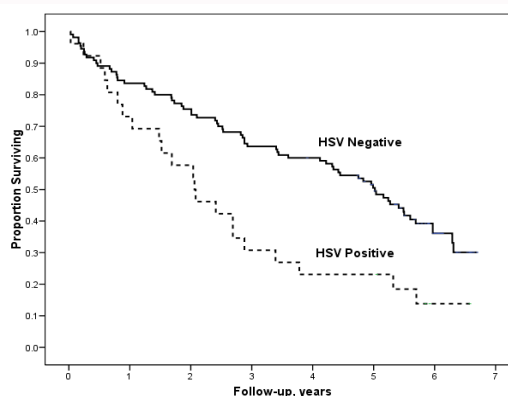
**Cause of death**

Patients were followed for a mean of 56 months (range 42-68

**Table 1:** Patient demographics.

Measurement	Patient Group	
	HSV-1 Negative	HSV-1 Positive
No. Patients	91 (81%)	21(19%)
Sex (M/F)	42/49	7/14
Age	69.7± 10.3	72.6 ± 7.5
FEV <sub>1</sub> – Liters	0.92 ± 0.48	0.54 ± 0.23
Smoking (Pack years)	46.3 ± 36.4	53.2 ± 45.8
X-ray Infiltrates	15.4%	38.1%
Inhaled Steroid BDP equivalent (mcg)	500 (0-1,000)	1,000 (400-2,000)
GAPDH Copy No. / epithelial cell (log)	4.12 (3.76-4.63)	4.27 (3.80-4.79)
HSV-1 Copy Number (log)	0	4.95 (3.68-5.32)

\*p<0.05



**Figure 3:** Kaplan-Meier Survival plot comparing HSV-1 positive and negative subjects.

months) following the close of recruitment. The date and causes of death were obtained from the General Register Office of Northern Ireland. The recorded cause of death certified was documented with International Classification of Diseases (ICD-10) codes [21] and the primary cause of death was noted to be pulmonary (J00-J99) or non-pulmonary on the ICD codes (actual coding term used in Northern Ireland is ‘respiratory’ or ‘non-respiratory’).

**Statistical analysis**

Normally distributed data were presented as mean ± standard deviation and non-parametric data were expressed as median and inter quartile ranges. Normally distributed continuous variables were compared by *t* test; otherwise the differences were assessed by the Mann-Whitney U test. For discrete variables frequencies and percentages were reported and groups compared using the chi squared test. Survival analysis was performed using a Kaplan-Meier plot and log rank comparison (Figure 3). These results are also presented as odds ratio and relative risk with 95% confidence intervals. A significance level of 5% was chosen. Statistical analysis was carried out using SPSS version 15.0 (Chicago, IL).

**Results**

One hundred and twelve patients were recruited into the study. Twenty one (19%) had HSV-1 detected in their sputum. The number of copies of virus DNA detected was expressed as the mean log 4.95 (3.68-5.32). This is 89,125 viruses per ml of sputum and is similar to the amount of virus seen in a rhinovirus infection [22]. The housekeeping gene GAPDH was present at log 4.12 (3.76-4.63) copies

**Table 2:** HSV status and use of inhaled steroid.

Inhaled Therapy	HSV Positive (%)	HSV Negative (%)
No inhaled steroid	3 (8%)	34 (92%)
Budesonide/Formoterol	1 (7%)	13 (93%)
Fluticasone/Salmeterol	3 (25%)	9 (75%)
Other inhaled steroid	14 (29%)†	35 (71%)

†p&lt;0.05

**Table 3:** HSV status and primary cause of death.

ICD 10 Coded primary cause of death	HSV Status	
	Negative	Positive
Pulmonary (J Codes)	41%	72%
Non-pulmonary	59%	28%

p&lt;0.05

in those without HSV and 4.27 (3.80-4.79) in those with the virus. No patients had visible oro-labial herpetic lesions.

In Table 1, the characteristics of the patient subgroups with and without HSV-1 are shown. Compared to those without HSV-1, the 21 patients with HSV-1 were taking higher daily doses of BDP equivalent inhaled steroids with a median daily dose (range) 1,000 (400-2,000) mcg, v 500 (0-1,000), (p<0.05). The mean FEV<sub>1</sub> was 0.54 ± 0.23L vs. 0.92 ± 0.45L, p<0.05. They had 2.5 times the incidence of infiltrates reported on their chest radiograph (p<0.05). In Table 2, the frequency of HSV-1 is shown for each type of inhaled formulation used. In the HSV positive group there was a significantly increased use of inhaled steroid device other than in a combination formulation. Can this be expanded or abandoned.

There was no difference in oral steroids used as maintenance therapy in those without HSV (7/91 patients mean dose 12.1 mg/day) versus those with HSV (2/21 patients mean dose 10.0 mg/day) (p>0.05). There was no difference in the number of exacerbations in the previous year (p>0.05).

The date and certified cause of death were examined. In Figure 1, the Kaplan-Meier degradation curve is shown. The median survival was 5.5 (CI 4.7-6.3) years in those without HSV, however, in those with HSV it was 2.1 (CI 1.0-3.2) years (p<0.05). Using a Cox regression analysis HSV was associated with higher mortality OR 2.2 (1.03-3.38), p<0.05. This was also found for reduction in lung function (FEV<sub>1</sub> % predicted OR 0.98 (0.96-0.99), p<0.05), increasing age (OR 1.07 (1.03-1.1), p<0.005) and male gender (OR 2.49 (1.46-4.25), p<0.005). In Figure 2, the certified cause of death is shown as pulmonary (J Codes) and non-pulmonary for the two groups. Those without HSV-1 had 41% pulmonary cause of death. This is similar to the previously reported incidence of pulmonary cause of death [23]. However, those with the virus had a 72% pulmonary cause of death (p<0.05) (Table 3).

## Discussion

HSV-1 was found in the sputum of approximately 1 in 5 patients hospitalized with an exacerbation of COPD. There were visible no oro-labial herpetic lesions to account for this. The most significant feature in the group with HSV-1 was increased mortality in the months and years after discharge from hospital. This has not been previously described. The viral load (approximately 90,000 viruses per ml of sputum) is similar to that seen during an infection with rhinovirus. This indicates a viral infection rather than viral latency.

Lung function was poorer in the subjects with HSV-1. It could

be argued that patients with severe disease get herpes simplex as a result of being sicker and that the ensuing increased mortality was secondary to the disease. However, the regression analysis shows that the presence of HSV-1 is a substantially higher risk of death (Odds Ratio 2.2), than lower lung function (Odds Ratio 0.98). Furthermore, the higher incidence of radiographic infiltrates reported in the HSV group supports an active infection.

All patients were seen within 24 hours of hospitalization. Patients were seen first thing in the morning, often after a night time admission. The usual therapy for exacerbations of COPD is oral steroids, antibiotics and nebulized bronchodilators. We do not believe that the immunosuppression of a single dose of steroids could lead to high levels of herpes simplex in the sputum. The prior use of maintenance oral steroids was no different in either group. We found that higher doses of inhaled steroids were used by the patients with HSV-1 prior to coming into hospital.

Pulmonary infections with HSV-1 have been extensively described in patients who are immunosuppressed with HIV. But it also occurs in patient's immunosuppressed from chemotherapy [24], transplantation [25], acute high dose oral steroids [26] as well as maintenance steroid treatment of rheumatologic disorders [27]. It has even been described in early reports of high dose maintenance oral steroid therapy for COPD was complicated by HSV-1 infections [28]. Other diseases which cause a stress can result in reactivation of herpes simplex. We have identified increased viral shedding after the stress of oral surgery [29].

Furthermore, when patients are ventilated in ICU we have identified that 27% have detectable HSV-1 in throat and tracheal aspirates [10]. In a separate ICU cohort it was identified in the upper airway in 22% and lower airway in 16% [11]. A study examining bronchoalveolar lavage, found the virus in 32% of ICU patients [30]. In a specific study of ventilated COPD patients HSV-1, was present in tracheobronchial aspirates.

The presence of HSV-1 in these ICU studies is an important finding. It is associated with severe disease, progression to ARDS and prolonged hospitalization [11]. Moreover, mortality rises from 24% in uninfected to 41% in those with HSV-1 [10]. A similar excess mortality is seen in the BAL study where high numbers of virus (100,000 viruses) was a particular risk for early mortality (41% vs. 20%) [30].

Inhaled steroids are frequently used in combination with long acting beta agonists. The principal benefit of this treatment is reduced frequency of exacerbations and patients feel better. Many studies of inhaled steroids in COPD have been for periods of one year [5,31]. A longer study for three years compared fluticasone and salmeterol either alone or in combination in a placebo controlled trial [6]. The purpose of this study was to determine the effect of combination therapy on mortality. Again the findings were of reduced exacerbations and improved symptoms, however, despite these improvements it failed to improve survival by the predetermined level of significance. Mortality curves published in this study show that after 18 to 24 months the death rate rises in the group receiving inhaled steroid alone. This coupled with the reported increase in pneumonia in patients receiving either fluticasone as single treatment (18.3%) or in combination with salmeterol (19.6%) is of particular concern.

In our study we identified a subgroup where HSV-1 was present in the sputum (19%). It was detected at levels close to those identified

as being associated with high mortality in ICU patients [30]. There were increased infiltrates on chest radiograph. Death occurred at a mean of 2.1 years from the detection of the virus [31]. There was a high incidence of death from pulmonary causes. The group with HSV-1 was using significantly higher doses of inhaled steroids. This brings into focus the risk versus benefits of high dose inhaled steroids in COPD.

## References

- Lopez AD, Shibuya K, Rao C, Mathers CD, Hansell AL, Held LS, et al. Chronic obstructive pulmonary disease: current burden and future projections. *Eur Respir J*. 2006;27(2):397-412.
- Murray CJ, Lopez AD. Alternative projections of mortality and disability by cause 1990-2020: Global Burden of Disease Study. *Lancet*. 1997;349(9064):1498-504.
- Weir LM, Elixhauser A, Pfitner A, Au DH. 2011 Overview of hospitalizations among patients with COPD. 2008.
- Rabe KF, Hurd S, Anzueto A, Barnes PJ, Buist SA, Calverley P, et al. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. *Am J Respir Crit Care Med*. 2007;176(6):532-55.
- Calverley PM, Boonsawat W, Cseke Z, Zhong N, Peterson S, Olsson H. Maintenance therapy with budesonide and formoterol in chronic obstructive pulmonary disease. *Eur Respir J*. 2003;22(6):912-9.
- Calverley PM, Anderson JA, Celli B, Ferguson GT, Jenkins C, Jones PW, et al; TORCH investigators. Salmeterol and fluticasone propionate and survival in chronic obstructive pulmonary disease. *N Engl J Med*. 2007;356(8):778-89.
- Barnes PJ. Inhaled corticosteroids in COPD: a controversy. *Respiration*. 2010;80(2):89-95.
- Calverley PMA, Stockley RA, Seemungal TAR, Hagan G, Willits LR, Riley JH, et al. Reported pneumonia in patients with COPD: findings from the INSPIRE study. *Chest*. 2011;139(3):505-12.
- Ong GM, Lowry K, Mahajan S, Wyatt DE, Simpson C, O'Neill HJ, et al. Herpes simplex type 1 shedding is associated with reduced hospital survival in patients receiving assisted ventilation in a tertiary referral intensive care unit. *J Med Virol*. 2004;72(1):121-5.
- Bruynseels P, Jorens PG, Demey HE, Goossens H, Pattyn SR, Elseviers MM, et al. Herpes simplex virus in the respiratory tract of critical care patients: a prospective study. *Lancet*. 2003;362(9395):1536-41.
- McManus TE, Marley AM, Baxter N, Christie SN, O'Neill HJ, Elborn JS, et al. Respiratory viral infection in exacerbations of COPD. *Respir Med*. 2008;102(11):1575-80.
- Rohde G, Wiethage A, Borg I, Kauth M, Bauer TT, Gillissen A, et al. Respiratory viruses in exacerbations of chronic obstructive pulmonary disease requiring hospitalisation: a case-control study. *Thorax*. 2003;58(1):37-42.
- Seemungal T, Harper-Owen R, Bhowmik A, Moric I, Sanderson G, Message S, et al. Respiratory viruses, symptoms, and inflammatory markers in acute exacerbations and stable chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 2001;164(9):1618-23.
- Boland AC, Iveson EH, Elliott MW. An unusual exacerbation of chronic obstructive pulmonary disease (COPD) with herpes simplex tracheitis: case report. *J Med Case Rep*. 2007;1:91.
- Anthonisen NR, Manfreda J, Warren CP, Hershfield ES, Harding GK, Nelson NA. Antibiotic therapy in exacerbations of chronic obstructive pulmonary disease. *Ann Intern Med*. 1987;106(2):196-204.
- Pauwels RA, Buist AS, Calverley PM, Jenkins CR, Hurd SS. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease. NHLBI/WHO Global Initiative for Chronic Obstructive Lung Disease (GOLD) Workshop summary. *Am J Respir Crit Care Med*. 2001;163(5):1256-76.
- Wales D, Makker H, Kane J, McDowell P, O'Driscoll BR. Systemic bioavailability and potency of high-dose inhaled corticosteroids: a comparison of four inhaler devices and three drugs in healthy adult volunteers. *Chest*. 1999;115(5):1278-84.
- Brusasco V, Crapo R, Viegi G. Coming together: the ATS/ERS consensus on clinical pulmonary function testing. *Eur Respir J*. 2005;26(1):1-2.
- Aurelius E, Johansson B, Skoldenberg B, Staland A, Forsgren M. Rapid diagnosis of herpes simplex encephalitis by nested polymerase chain reaction assay of cerebrospinal fluid. *Lancet*. 1991;337(8735):189-92.
- Kasahara M, Takahashi Y, Nagata T, Asai S, Eguchi T, Ishii Y, et al. Thymidylate synthase expression correlates closely with E2F1 expression in colon cancer. *Clin Cancer Res*. 2000;6(7):2707-11.
- International Statistical Classification of Diseases and Health Related Problems, ICD-10 Second Edition. World Health Organization. 2005.
- Wright PF, Deatly AM, Karron RA, Belshe RB, Shi JR, Gruber WC, et al. Comparison of results of detection of rhinovirus by PCR and viral culture in human nasal wash specimens from subjects with and without clinical symptoms of respiratory illness. *J Clin Microbiol*. 2007;45(7):2126-9.
- Drummond MB, Wise RA, John M, Zvarich MT, McGarvey LP. Accuracy of death certificates in COPD: analysis from the TORCH trial. *COPD*. 2010;7(3):179-85.
- Ferrari A, Luppi M, Potenza L, Riva G, Morselli M, Imovilli A, et al. Herpes simplex virus pneumonia during standard induction therapy for acute leukemia: case report and review of the literature. *Leukemia*. 2005;19:2019-21.
- McGregor CG, Dark JH, Hilton CJ, Freeman R, Conacher ID, Corris PA. Early results of single lung transplantation in patients with end-stage pulmonary fibrosis. *J Thorac Cardiovasc Surg*. 1989;98(3):350-4.
- Shane SA, Wollman M, Claassen D. Herpes simplex dissemination following glucocorticoids for airway obstruction in an adolescent girl. *Pediatr Emerg Care*. 1994;10(3):160-2.
- Witt MN, Braun GS, Ihrler S, Schmid H. Occurrence of HSV-1 induced pneumonitis in patients patients under standard immunosuppressive therapy for rheumatic, vasculitic and connective tissue disease. *BMC Pulm Med*. 2009;18(9):22.
- Wiest PM, Flanigan T, Salata RA, Shlaes DM, Katzman M, Lederman MM. Serious infectious complications of corticosteroid therapy for COPD. *Chest*. 1989;95(6):1180-4.
- Hyland PL, Coulter WA, Abu-Ruman I, Fulton CR, O'Neill HJ, Coyle PV, et al. Asymptomatic shedding of HSV-1 in patients undergoing oral surgical procedures and attending for noninvasive treatment. *Oral Dis*. 2007;13(4):414-8.
- Linssen CF, Jacobs JA, Stelma FF, van Mook WN, Terporten P, Vink C, et al. Herpes simplex viral load in bronchoalveolar lavage is related to poor outcome in critically ill patients. *Intensive Care Med*. 2008;34(12):2202-9.
- Szafranski W, Cukier A, Ramirez A, Menga G, Sansores R, Nahabedian S, et al. Efficacy and safety of /formoterol in the management of chronic obstructive pulmonary disease. *Eur Respir J*. 2003;21(1):74-81.