Bacterial lysate in the prevention of acute exacerbation of COPD and in respiratory recurrent infections

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Abstract: Respiratory tract infections (RTIs) represent a serious problem because they are one of the most common cause of human death by infection. The search for the treatment of those diseases has therefore a great importance. In this study we provide an overview of the currently available treatments for RTIs with particular attention to chronic obstructive pulmonary diseases exacerbations and recurrent respiratory infections therapy and a description of bacterial lysate action, in particular making reference to the medical literature dealing with its clinical efficacy. Those studies are based on a very large number of clinical trials aimed to evaluate the effects of this drug in maintaining the immune system in a state of alert, and in increasing the defences against microbial infections. From this analysis it comes out that bacterial lysates have a protective effect, which induce a significant reduction of the symptoms related to respiratory infections. Those results could be very interesting also from an economic point of view, because they envisage a reduction in the number of acute exacerbations and a shorter duration of hospitalization. The use of bacterial lysate could therefore represent an important means to achieve an extension of life duration in patients affected by respiratory diseases.
Keywords: bacterial lysate, COPD, respiratory recurrent infections

Background and introduction

Respiratory tract infections (RTIs) represent one of the most common and important causes of human disease in terms of morbidity, mortality, and economic cost to society. RTIs are the most common, and potentially the most severe, infections treated by health care practitioners. Lower RTIs, along with influenza, are the most common cause of death by infection in the United States. Risk factors for pneumonia and other respiratory tract infections include: extremes of age (very young and elderly), smoking, alcoholism, immunosuppression, and comorbid conditions (File 2000). We can distinguish two main clinical manifestations of relevant impact: acute exacerbations of chronic bronchitis (AECB), and recurrent respiratory infections (RRI).

Recurrent respiratory infections (RRI) are characterized by at least three episodes of fever, locoregional inflammation, cough, asthma, wheezing without severe impairment of respiratory functions. This syndrome is frequent both in pediatric and in adult patients accounting for the principal cause of absence from school and work during winter time. RRI involve both upper and lower respiratory tract and are caused by a wide panel of microorganisms. In particular, viral infections, caused by influenza viruses, parainfluenza viruses, respiratory syncitial virus, adenovirus, rhinoviruses, are the original cause of the disease but recurrences are also caused by bacteria, including *Acinetobacter spp*., *Chlamydia pneumoniae*, *Enterobatteriacee*, *Hemophilous influenzae*, *Legionella pneumophila*, *Moraxella catarrhalis*, *Mycoplasma pneumoniae*, *Nocardia asteroids*, *Pasturella multocida*, *Pseudomonas aeruginosa*, *Staphylococcus*

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aureus, *Stenotrophomonas maltophilia*, *Streptococcus pneumoniae* and *Streptococcus pyogenes* (group A).

Chronic obstructive pulmonary disease (COPD) is a state characterized by airflow limitation that is not fully reversible. The airflow limitation is usually both progressive and associated with an abnormal inflammatory response of the lungs to noxious particles or gases. A diagnosis of COPD should be considered in any patient who presents with symptoms of cough, sputum production, or dyspnoea, and/or has a history of exposure to the risk factors for the disease. Clinical symptoms and signs, such as an abnormal shortness of breath and increased forced expiratory time, can be used to help with the diagnosis. Chronic cough and sputum production often precede the development of airflow limitation by many years, although not all individuals with cough and sputum production go on to develop COPD. The classification of Severity is shown in Table 2. The pathogenesis of COPD is characterized by chronic inflammation throughout the airways, parenchyma, and pulmonary vasculature. Macrophages, T lymphocytes (predominately CD8), and neutrophils are increased in various parts of the lung. Inflammation of the lungs is caused by exposure to inhaled noxious particles and gases. Cigarette smoke can induce inflammation and directly damage the lungs. Pathologic changes in the lungs lead to corresponding physiologic changes characteristic of the disease, including mucus hypersecretion, ciliary dysfunction, airflow limitation, pulmonary hyperinflation, gas exchange abnormalities, pulmonary hypertension, and cor pulmonale. The destruction of alveolar attachments, which inhibits the ability of the small airways to maintain patency, plays a smaller role. In advanced COPD, peripheral airways obstruction, parenchymal destruction, and pulmonary vascular abnormalities reduce the lung's capacity for gas exchange, producing hypoxemia and, later on, hypercapnia. Pulmonary hypertension, which develops late in the course of COPD (Stage III: Severe COPD), is the major cardiovascular complication of COPD and is associated with the development of cor pulmonale and a poor prognosis.

Patients with chronic obstructive pulmonary disease (COPD) are prone to exacerbations, which account for significant morbidity and mortality and are a key determinant of health-related quality of life. (Seemungal et al 1998) COPD exacerbations are defined as a change in the patient's chronic respiratory symptoms sufficient to warrant a change in management (Celli et al 2004; Global Initiative for Chronic Obstructive Lung Disease 2005)

The cardinal symptoms of COPD exacerbations are increase in dyspnoea, sputum volume and sputum purulence development (named the "Anthonisen's criteria") (Anthonisen NR 1987). There is considerable heterogeneity in the character, frequency, and time course of COPD exacerbations, which cannot be accounted for solely on the basis of degrees of airway obstruction or disease severity.

The lower airways of 25% to 50% of COPD patients are colonized by bacteria, especially noncapsulated Haemophilus influenzae, Streptococcus pneumoniae, and Moraxella catarrhalis (Anthonisen et al 1987; Cabello 1997).

The predominant bacteria recovered in the lower airways of patients with mild exacerbations are *H. influenzae*, *S. pneumoniae* and *M. catarrhalis*, whereas in severe COPD requiring mechanical ventilation gram negative bacilli, and *P. aeruginosa* are more frequent (Papi et al 2006). Lower airway bacterial colonization is increasingly recognized as an independent stimulus to airway inflammation (Sethi et al 2001) It can modulate the character and frequency of COPD exacerbations (Patel et al 2002).

A significant role in the aetiology of acute exacerbations of chronic bronchitis seems to be played also by viruses, responsible of almost 40% of the exacerbations. (Seemungal et al 2001).

Pharmacological treatments: systemic overview

The currently available treatments for COPD therapy include:

Bronchodilators (Evidence A)

Bronchodilator medications are central to the symptomatic management of COPD (Vathenen et al 1998; Gross et al 1989; Chrystyn et al 1988; Higgins et al 1991) (Evidence A). They are given either on an as-needed basis for relief of persistent or worsening symptoms, or on a regular basis to prevent or reduce symptoms. The side effects of bronchodilator therapy are pharmacologically predictable and dose dependent. Adverse effects are less likely, and resolve more rapidly after treatment withdrawal. All categories of bronchodilators have been shown to increase exercise capacity in COPD, without necessarily producing significant changes in FEV_1 (Ikeda et al 1995; Guyatt et al 1987a; Man et al 2004; O'Donnell et al 2004) (Evidence A). Regular treatment with long-acting bronchodilators is more effective and convenient than treatment with short-acting bronchodilators, but also more expensive (Mahler et al 1999; Dahl et al 2001; Oostenbrink et al 2002; Vincken et al 2002) (Evidence A).

Inhaled glucocorticosteroids

Regular treatment with inhaled glucocorticosteroids does not modify the longterm decline of FEV_1 in patients with COPD (Pauwels et al 1999; Vestbo et al 1999; Burge et al 2000; The Lung Health Study Research Group 2000). However, regular treatment with inhaled glucocorticosteroids is appropriate for symptomatic COPD patients with an FEV_1 <50% predicted (Stage III: Severe COPD and Stage IV: Very Severe COPD) and repeated exacerbations (for example, 3 in the last three years) (Mahler et al 2002; Szafranski et al 2002; Calverley et al 2003a; Jones et al 2003) (Evidence A). This treatment has been shown to reduce the frequency of exacerbations and thus to improve health status (Spencer et al 2004) (Evidence A). Withdrawal from treatment with inhaled glucocorticosteroids can lead to exacerbations in some patients. Inhaled glucocorticosteroid combined with a long-acting β_2-agonist is more effective than the individual components (Mahler et al 2002; Calverley et al 2003; Szafranski et al 2002; Hanania et al 2003; Calverley et al 2003b) (Evidence A).

Mucolytic (mucokinetic, mucoregulator) agents (ambroxol, erdosteine, carbocysteine, iodinated glycerol)

The regular use of mucolytics in COPD has been evaluated in a number of long-term studies with controversial results (Allegra et al 1996; Guyatt et al 1987b; Petty 1990). The majority of the studies showed no effect of mucolytics on lung function or symptoms, although some of them reported a reduction in the frequency of exacerbations. A Cochrane collaborative review performed a meta-analysis of all the available data, including those from a number of abstracts (Poole and Black 2003). A statistically significant reduction in the number of episodes of chronic bronchitis was found in patients treated with mucolytics compared to those receiving placebo. However, those data are not easy to interpret, as the follow-up ranged from 2 to 6 months and all the patients had an FEV_1 > 50% predicted. Although a few patients with viscous sputum may benefit from mucolytics (Siafakas et al 1995; American Thoracic Society 1986), the overall benefits seem to be very small. Therefore, the widespread use of these agents cannot be recommended on the basis of the present evidence (Evidence D).

Antioxidant agents

Antioxidants, in particular N-acetylcysteine, have been shown to reduce the frequency of exacerbations and could have a role in the treatment of patients with recurrent exacerbations (Boman et al 1983; British Thoracic Society Research Committee 1985; Rasmussen and Glennow 1988; Hansen et al 1994) (Evidence B). However, before their routine use can be recommended, the results of ongoing trials have to be carefully evaluated.

Antitussives

Cough, although it is sometimes a troublesome symptom in COPD, has a significant protective role (Irwin et al 1998). Thus the regular use of antitussives is contraindicated in stable COPD (Evidence D). For classification of evidences, see Table 1.

Table 1 Classification of evidence

Evidence	Category	Sources of evidence definition
A	Randomized control trial	Rich body of data. Evidence is from endpoints of well designed RCTs that provide a consistent pattern of findings in the population for which the recommendation is made. Category A requires substantial numbers of studies involving substantial numbers of participants.
B	Randomized control trial	Limited body of data. Evidence is from endpoints of intervention studies that include only a limited number of patients, post hoc or subgroup analysis of RCTs, or meta-analysis of RCTs. In general, Category B pertains when few randomized trials exist, they are small in size, or they were undertaken in a population that differs from the target population of the recommendation, or the results are somewhat inconsistent.
C	Nonrandomized trials	Observational studies. Evidence is from outcomes of uncontrolled or non randomized trials or from observational studies.
D	Panel	Consensus Judgment. This category is used only in cases where the provision of some guidance was deemed valuable but the clinical literature addressing the subject was deemed insufficient to justify placement in one of the other categories. The Panel Consensus is based on clinical experience or knowledge that does not meet the above listed criteria.

Table 2 Classification of severity according to GOLD guidelines. (http://goldcopd.com/ GOLD Global Iniative for Cronich Obstructive Lung Disease)

Stage	Characteristics
0: at risk	• Normal spirometry. • Chronic symptoms (cough, sputum production).
I: Mild COPD	• $FEV_1/FVC < 70\%$. • FEV_1 > or = 80% predicted. • With or without chronic symptoms (cough, sputum production),
II: Moderate COPD	• $FEV_1/FVC < 70\%$, • 50% < or = FEV_1 < 80% predicted. • With or without chronic symptoms (cough, sputum production).
III: Severe COPD	• $FEV_1/FVC < 70\%$. • 30% < or = FEV_1 < 50% predicted. • With or without chronic symptoms (cough, sputum production).
IV:Very Severe COPD	• $FEV_1/FVC < 70\%$. • FEV_1 < 30% predicted or FEV_1 < 50% predicted plus chronich respiratory failure.

Based on postbronchodilator FEV_1

Antibiotics

In several large-scale controlled studies (Francis and Spicer 1960; Francis et al 1961; Fletcher et al 1966), the prophylactic, continuous use of antibiotics was shown to have no effect on the frequency of exacerbations in COPD. Another study examined the efficacy of winter chemoprophylaxis over a period of 5 years and concluded that there was no benefit (Johnston et al 1969). Thus, on the present evidence, the use of antibiotics, other than for treating infectious exacerbations of COPD and other bacterial infections, is not recommended (Isada and Stoller 1994; Siafakas Bouros 1998) (Evidence A). In the case of an acute exacerbation, antibiotic therapy significantly shortens the duration of symptoms and can be cost-effective. Over the past 50 years, virtually all classes of antimicrobial agents have been studied in AECB. Important considerations include penetration into respiratory secretions, spectrum of activity and antimicrobial resistance. Those factors limit the usefulness of drugs such as amoxicillin, erythromycin and trimethoprim-sulfamethoxazole. Extended-spectrum oral cephalosporins, newer macrolides and doxycycline have demonstrated efficacy in clinical trials. Amoxicillin-clavulanate and flouoroquinolones should generally be reserved for patients with more severe disease. A number of investigational agents; including ketolides and newer quinolones; hold promise for treatment of AECB. (Dever et al 2002).

Innate and adaptive immunity modulation in preventing and treating respiratory infection

The potentiation of both specific and non specific immunoresponse has been considered a central point in the treatment of recurrences in respiratory tract infections. Specific immunity against viruses (such as influenza virus) and bacteria (*Streptococcus pneumoniae*) can be raised using specific treatment with vaccines. This is the case of influenza vaccines and pneumococcal vaccine.

Influenza vaccines

Influenza vaccines can reduce serious illness (Wongsurakiat et al 2004) and death in COPD patients by about 50% (Nichol et al 1994; Wongsurakiat et al 2003) (Evidence A). Vaccines containing killed or live, inactivated viruses are recommended (Edwards et al 1994) as they are more effective in elderly patients with COPD (Hak et al 1998). The strains are adjusted each year for appropriate effectiveness and should be given once (in autumn) or twice (in autumn and winter) each year.

Pneumococcal vaccine

A pneumococcal vaccine containing 23 virulent serotypes has been used (Williams and Moser 1986; Davis 1987; Simberkoff et al 1996) (Evidence B).

The potentiation of nonspecific and specific immunoresponse against other bacteria (such as the bacteria shown as the cause of recurrent infection of the respiratory tract), can be obtained using polyvalent bacterial lysate. This category of drugs includes different kinds of bacterial extracts, and a different number of bacterial species (polyvalent extracts) obtained through different ways of lysis, both chemical or mechanical (PMBL, PCBL). We will further on discuss the effects of this kind of drugs, through an analysis of Randomized Controlled Trials published to date.

Characteristics of bacterial lysate

Bacterial lysate are constituted by a mixture of bacterial antigens derived from different bacterial species, according to the considered extract. The more often included species are: *Staphylococcus aureus*, *Streptococcus viridans*, *Streptococcus pneumoniae* (6 strains), *Streptococcus pyogenes*, *Klebsiella pneumoniae*, *Klebsiella ozenae*, *Moraxella catarrhalis*, *Hemophylous influenzae*. Each extract is prepared with billions of these bacteria. Antigens are obtained following a mass culture of reference bacterial strains, using a chemical or mechanical

lysis of cells and lyophilization. Different antigens are mixed and excipients are added in order to prepare the tablets.

Bacterial lysate is both a specific and non specific immunostimulating agent, indicated for the prevention and treatment of respiratory infections, including sequelae to common cold and influenza. In particular it is useful for the treatment of acute and chronic bronchitis, anginas, tonsillitis, pharyngitis, laryngitis, rhinitis, sinusitis, and otitis. It can also be used for infections resistant to common antibiotics and for the sequelae to bacterial and viral infections.

The more usual treatment schedule for PMBL is the following:

–acute events: one tablet per day to dissolve under the tongue, (for a minimum of ten days) until the symptoms disappear.

–long term treatment: one tablet per day to dissolve under the tongue for 10 consecutive days, followed by 20 days' rest. The cycle is repeated for three consecutive months.

Mode of action of bacterial lysate

The capacity of a virtually intact microbe to activate resting monocytic-macrophage cells is strictly linked to the presence of structures belonging to the bacterial cell wall (for example, protido-glycane or lipopolysaccharide) against which some receptor structures (such as the so called toll like receptors -TLR) are specific directed. TLR are expressed on the surface of monocyte membrane. The interaction between bacterial structures and TLR results in the activation of monocytes, their differentiation to immature dendritic cells and the following maturation to mature dendritic cells, able to be considered a suitable professional antigen presenting cell. The use of bacterial antigens obtained by mechanical or chemical lysis is thus able to activate monocytic-macrophagic cells of the submucosa, inducing the differentiation through immature dendritic cells and their maturation in mature dendritic cells. The activation of such a mechanism results in a suitable stimulation of the immune-response.

The presentation of bacterial antigens on mature dendritic cells results in the stimulation of the T cell compartment (with a consequent induction of a powerful helper function) and of the B cell compartment of the immune-response, with a following maturation to plasmacells and secretion of antibodies specifically directed to the administered. The administration of bacterial lysate is thus able to induce a T helper function and a maturation of B specific lymphocytes resulting in the production of IgA salivary antibodies directed to the administered mixture of antigens.

The secretion of antibodies directed to bacterial antigens has a positive function only in the case of these antibodies having the capability to opsonize living bacterial cells, thus favouring the phagocytosis and the killing mediated by professional phagocytes, such as granulocytes.

All these findings indicate that the maturation of dendritic cells, the specific activation of the T and B cell population of lymphocytes, and the resulting production of IgA in the respiratory mucosa specifically directed to the antigens administered, characterized by the capacity of opsonizing living bacteria thus allowing their engulfment and killing in phagocytes, represent the actual pathway for the potentiation of a both nonspecific (dendritic cells and phagocytes) and specific (T and B cells) immune-response, resulting in a prophylactic effect on recurrent infections of the respiratory tract.

Bacterial lysate clinical efficacy: literature review

The effects of bacterial lysate as an immunostimulatory agent has been debated in many clinical trials.

By performing a simple research on PubMed directory of the terms "bacterial lysate", and limiting the search only to randomized clinical trials, it is possible to find more than thirty papers. The aim of those studies, all published during the last twenty years, is to evaluate the effects of this class of drugs in maintaining the immune system in a state of alert, and in raising a defence against microbial infections, eventually leading to a reduction in their number.

Those studies are very different and heterogeneous. In fact the populations and samples of those trials are various, being representative of diverse diseases, such as: recurrent respiratory infections of upper and/or lower airways; chronic bronchitis; rhino-sinusitis and other ENT infections; chronic obstructive pulmonary disease. Even the age of patients included is different: many trials are conducted on pediatric patients (Braido et al).

We will briefly discuss the findings of these RCTs, analysing them separately, according to the characteristic of the patients included.

Pediatric trials

A pilot study involving 89 children pointed out that the administration of polivalent mechanical bacterial lysate could lead to a significant decrease in recurrent respiratory infections in patients treated with this drug, compared to the controls on the same children during the previous year; also information indexes were significantly lower in the treated

information group, and the values of B-lymphocytes were found to be increased (Rosaschino and Cattaneo 2004).

Even in patients from 3 to 6 years of age, affected by recurrent respiratory infections and IgG deficiency, a RCT proved the beneficial effect of bacterial lysate (Del-Rio-Navarro et al 2003).

Another RCT that included 232 children aged 3–5 years, showed that the treatment with PMBL significantly reduced the rate of upper respiratory tract infections, being this reduction higher in children affected more frequently by this kind of infections; the drug was also safe and well tolerated compared to placebo (Schaad et al 2002).

Another RCT involving 188 pediatric patients and lasting more than one year, put in evidence that the rate of infection was reduced of 50% in treated patients, and this was sustained for half a year after the end of drug administration; drug reactions were few, transient, expected and non serious (Ruah et al 2001).

A RCT lasting one year, including 54 children aged 1–12 years, evaluated for the two groups of patients (active/placebo) the number of acute respiratory tract infections and their duration, and also the number of antibiotic courses needed. The results showed a reduction in the number of infections, and a more significant reduction in antibiotic requirements and in the duration of the infection episodes in the treated group compared to placebo (Gutierrez-Tarango and Berber 2001).

56 young patients affected by subacute sinusitis were involved in a RCT lasting 6 months, evaluating the effects of bacterial lysate added to amoxicillin/clavulanate. The results pointed out that the cure and improvement of the patients in the active group were faster, and that these patients experienced a lower incidence of respiratory infections (Gomez Barreto et al 1998).

A bigger trial involving 423 children attending day-care centers (subjects with a higher risk of infection) showed that, during the period of treatment with bacterial lysate, treated children had a relative risk of 0.52 to present three or more episodes of upper respiratory infections (Collet et al 1993).

A much older study also revealed that, in children with rhinosinusitis, the treatment with bacterial lysate decreases the incidence and duration of infectious episodes and the number and duration of concomitant treatments; moreover, the clinical response showed a correlation with an increase in serum levels of IgA (Zagar and Lofler-Badzek 1989).

Quite a big trial involving 825 children in the treatment group and 327 in the placebo one, pointed out that the administration of a bacterial lysate applied intranasally for 6 months does not reduce the number of acute respiratory diseases compared to placebo (Sramek et al 1986).

Adult patient trials

Concerning the studies focused on samples of adult patients (Ahrens and Wiedenbach 1984; Keller 1984; Heintz et al 1989; Cvoriscec et al 1989; Debbas and Derenne 1990; Debelic and Eckenberger 1992; Orcel et al 1994; Collet et al 1997; Rutishauser et al 1998; Tielemans et al 1999; Li et al 2004; Steurer-Stey et al 2004; Tricarico et al 2004; Macchi and Vecchia 2005), we will discuss them separately, according to the disease considered (recurrent respiratory infections, chronic bronchitis, and COPD).

Recurrent respiratory infections

A recent study, a RCT involving 140 patients with a history of recurrent respiratory infections, compared the effects of bacterial lysate obtained through mechanical lysis (pts in the first group) to the effects of bacterial lysate obtained through chemical lysis (pts in the second group) and to the effects of no treatment at all (pts in the third group, control); the end points were: the number of upper respiratory tract infections, the number of patients free from disease, the duration of infectious episodes, the number of working days lost because of the disease, the need for antibiotic treatment. The results for each one of the end points showed the efficacy of the two treatments, but the best results were achieved with the treatment with the bacterial lysate obtained through mechanical lysis, and were significantly superior to those achieved with placebo and also with the bacterial lysate obtained through chemical lysis (Macchi and Vecchia 2005).

A particular study evaluated the efficacy of bacterial lysate in preventing upper respiratory tract infections in subjects belonging to a specific setting, that is a community of cloistered nuns. 47 nuns were allocated into two different groups, active treatment and placebo. The results showed a significant lower number in respiratory infections, and a shorter duration, in the active treatment group; moreover, a significant increase in serum Ig and salivary IgA was recorded in the active treatment group (Tricarico et al 2004).

Other trials have shown the efficacy of bacterial lysate in treating patients with recurrent respiratory infections (Ahrens and Wiedenbach 1984; Debelic and Eckenberger 1992; Rutishauser et al 1998), even in group of patients with an increased risk, such as patients in hemodialysis treatment (Tielemans 1999). A particular attention was also given to the investigation of the tolerability of such kind of treatments,

and the results were excellent (Ahrens and Wiedenbach 1984; Debelic and Eckenberger 1992).

One trial (Heintz et al 1989) was designed to test the efficacy of bacterial lysate in treating patients suffering from chronic purulent sinusitis. This study, carried over on 284 patients for a duration of six months, showed the efficacy of treatment in reducing cough, purulent nasal discharge and headache, on the basis of the score of symptoms.

Chronic bronchitis

Since acute bronchitis is a major source of morbidity in elderly patients, a RCT involving 354 patients living in institutions for elderly was designed, in order to assess the effects of treatment with bacterial lysate on the incidence of lower respiratory tract infections. A reduction of 28% was observed, and this was due to a 40% reduction in episodes of acute bronchitis, with no differences in the incidence of pneumonia in the two groups. During the 6 months of duration of the trial, a larger number of patients in the active treatment group didn't experience any episode of acute bronchitis, and a reduction in antibiotic prescription in the same group was recorded (Orcel et al 1994).

Another 104 patients affected by chronic bronchitis were involved in a RCT comparing bacterial lysate to placebo over a period of 6 months. The results showed a significant reduction of the duration of acute episodes and fever in the treatment group, with a concomitant sparing-effect on the use of antibiotics, an increase in serum IgA levels and in T-lymphocyte counts (Cvoriscec et al 1989).

A previous, much older study (Keller 1984), evaluated the effect of bacterial lysate in patients with chronic bronchitis. The conclusions on the effects of this treatment on clinical symptoms didn't reach a statistically significant benefit compared to placebo, and the authors advocated for further studies, with more patients, and with a longer period of observation.

COPD

One of the first RCT on the use of bacterial lysate in COPD patients (Debbas and Derenne 1990), lasting 6 months and involving 265 patients, demonstrated a statistically significant reduction of infectious events, and a concomitant reduction in the use of antibiotics.

A well-built RCT (Collet et al 1997) recruited 381 patients with COPD and followed them for 6 months. This trial pointed out that the risk of having at least one exacerbation during the 6 months was similar in both groups. The most significant result showed a clear reduction in the total number of days of hospitalization for respiratory problems in the group treated with bacterial lysate (287) compared to the placebo group (642). Also the risk of being hospitalized was reduced for the same patients group (16.2% vs 23.2%). Moreover, the number of deaths observed was reduced in the treatment group (2 vs 6), but without statistical significance. These results showed that immunostimulating agents could be useful for treating patients with COPD, being able to reduce the likelihood of severe respiratory events possibly responsible for hospitalization.

Another, more recent, RCT (Li et al 2004) included 90 patients. The considered endpoints were: the frequency of acute exacerbations, symptom scores, lung function, all recorded for one year after the end of the treatment. The results showed, in the group treated with bacterial lysate compared to the placebo group: a decrease in incidence, duration, and severity of acute exacerbation; a reduction in the course of antibiotics; an improvement in symptom scored and a higher bacterial clearance rate in sputum cultures.

Recently, a systematic review and metanalysis was published (Steurer-Stey et al 2004), investigating the use of oral purified bacterial extracts in the treatment of patients affected by COPD. An extensive and systematic search for randomized clinical trials in all the electronic databases, biographies, and data from manufacturers was performed. Eventually, a total of 13 studies, corresponding to almost two thousands patients, were included in the analysis. Concerning the prevention of exacerbations, the results showed that the main treatment effect was found in the smaller studies, the ones with lower quality score. As a consequence, the combination of all the data results in high heterogeneity, and the difference between active extracts and placebo does not reach statistical significance. The metanalysis also showed a shorter duration of exacerbations in the active treatment groups. The improvement of symptoms, as assessed both by the patients and the observers; was in favour of bacterial extracts. Hospitalization admission resulted lower in the bacterial extracts group (but data were drawn only from one study) (Collet et al 1997). This systematic review clearly points out that a strong evidence for the prevention of exacerbation in COPD patients through the use of bacterial extracts is still missing; nonetheless, an improvement in symptoms has been underlined, as well as a shorter duration of exacerbation, and a reduction of hospitalization. Anyway, those conclusions are relevant as there could be some important economic consequences if a reduction of hospitalization can be prospected by the use of those relatively cheap drugs, which can be applied intermittently. Also a benefit on symptoms, as perceived by the patients, is important, as a reflection of a better quality of life.

Table 3 Pediatric trials

Pediatric trials					
Author	**Year**	**Disease**	**Patients**	**Preparation**	**Results**
Rosaschino	2004	Recurrent respiratory infections	89	PMBL	Infections, markers of inflammation, Lymph B
Del Rio Navarro	2003	Recurrent respiratory infections	40	OM85-BV	Clinical benefit
Schaad	2002	Upper respiratory tract infections	232	OM85	URTI rate, good safety and tolerance
Ruha	2001	Respiratory tract infections	188	LW50020	Infection rate, safety assessment
Gutierrez-Tarango	2001	Acute respiratory tract infections	54	OM85-BV	infections, antibiotics requirements, duration of infections
Gomez-Barreto	1998	Sub-acute sinusitis	56	OM85-BV	Effective, incidence of respiratory infections
Collet	1993	Upper respiratory infections	423	OM85-BV	Risk of presenting > or = 3 episodes of URI
Zagar	1998	Rhinosinusitis	51	BV	Infection number and duration, IgA levels
Sranek	1986	Acute respiratory diseases	1252	IRS19 (intranasal)	Not effective in ARD

Table 4 Adult trials

Adult trials					
Author	**Year**	**Disease**	**Patients**	**Preparation**	**Results**
Macchi	2005	Recurrent respiratory infections	140	PMBL/CLBL	Effectiveness of both treatments
Tricarico	2004	Recurrent respiratory infections	47 (closed community)	MLBL	Infections, duration of infections, Ig and salivary IgA
Rutischauser	1998	Recurrent respiratory infections		LW50020	Effective, treatment benefit
Debelic	1992	Recurrent respiratory infections	620		excellent tolerability, frequency and severity of infections
Ahrens	1984	Recurrent respiratory infections	230	BV	Significant protective effect
Tielemans	1999	Recurrent respiratory infections	40	IBE	Effectivness in pts in hemodialisys
Heintz	1989	Chronic purulent sinusitis	284	BV	Effectiveness of treatment
Orcel	1994	Chronic bronchitis	354 (living in institutions)	OM85-BV	Lower respiratory tract infections
Cvoriscec	1989	Chronic bronchitis	104	BV	Acute episodes, antibiotics utilization, IgA and T-Lymph
Keller	1984	Chronic bronchitis	75	BV	No significant differences
Debbas	1990	Chronic obstructive Pulmonary disease	265	OM85-BV	Infections, antibiotics intake,
Collet	1997	Chronic obstructive Pulmonary disease	381	OM85-BV	= risk of at least one acute exacerbation, days of hospitalization
Li	2004	Chronic obstructive Pulmonary disease	90	BV	Frequency of acute exacerbations, antibiotics need, symptom score

Discussion and conclusions

As we have previously reported, bacterial lysates are powerful inducers of a specific locoregional immune response that significantly enhance the concentration of antibodies directed to antigenic structures of bacteria most commonly observed during infections of the upper respiratory tract. Those antibodies have the capability of opsonizing living bacteria, thus allowing the engulfment and killing mediated by human phagocytes, such as granulocytes. This activity is linked to the capacity of inducing a significant reduction (or a complete disappearance) of signs and symptoms related to respiratory infections.

This effect comes out also from the systematic analysis of medical literature, and is reflected in the results of the randomized clinical trials. Those results underline an overall protective effect, with a different level of significance, depending on the study design, the disease considered, and the type of patients involved. Both in pediatric and adult trials, as reported in the Tables 3 and 4, we can find a positive trend in the results: a general reduction of infection rates, a reduction of their duration, a beneficial effect on symptoms, a reduction in the use of antibiotics.

Those effects also have, as a direct consequence, important economic implications. In the case of COPD, a reduction in the number of acute exacerbations, or a shorter duration of hospitalization, could represent an actual opportunity for curtailing costs of management of such patients. In fact, patients with a moderate-severe COPD can experience a mean of at least 2 AECB/year (Miravitlles et al 1999). The most serious patients, besides experiencing more frequent exacerbations, are more frequently hospitalized, and for longer periods (Donaldson et al 2002). We also have to consider that AECB represent an important cause of death. A trial reported that, in a sample of 1000 hospitalized patients affected by severe COPD, when dismissed, 20% of them die within two months, 33% within six years, and 43% within one year. (Hilleman et al 2000) The risk of death in COPD patients is strictly correlated with the frequency and gravity of exacerbations. As a consequence, it would be extremely useful to obtain a reduction of exacerbations, in order to extend the life duration of this kind of patients. Therefore, the use of bacterial lysate could represent an important means to achieve this prominent result.

In conclusion, even though the results of the analyzed studies are encouraging, it would be worthwhile to build up new trials. Those new trials should include a higher number of patients, selected according to the disease and its severity, and should be well-designed trials in term of blinding and randomization procedures. This could permit to get even stronger evidence of their beneficial effect, both on symptoms and on prevention of infections.

Disclosure of interest

The authors do not have any conflict of interest related to the pharmaceutical companies whose drugs are cited in this article.

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References

Ahrens J, Wiedenbach M. 1984. [Efficacy of the immunostimulant Broncho-Vaxom] *Schweiz Med Wochenschr,* 114(25):932–4.

Allegra L, Cordaro CI, Grassi C. 1996. Prevention of acute exacerbations of chronic obstructive bronchitis with carbocysteine lysine salt monohydrate: a multicenter, double-blind, placebo-controlled trial. *Respiration*, 63:174–80.

American Thoracic Society. Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. Adopted by the ATS Board of Directors, November 1986. *Am Rev Respir Dis,* 1987, 136:225–44.

Anthonisen NR, Manfreda J, Warren CP, et al. 1987. Antibiotic therapy in exacerbations of chronic obstructive pulmonary disease. *Ann Intern Med*, 106:196–204.

Boman G, Backer U, Larsson S, et al. 1983. Oral acetylcysteine reduces exacerbation rate in chronic bronchitis: report of a trial organized by the Swedish Society for Pulmonary Diseases. *Eur J Respir Dis*, 64:405–15.

British Thoracic Society Research Committee. 1985. Oral Nacetylcysteine and exacerbation rates in patients with chronic bronchitis and severe airways obstruction. *Thorax,* 40:832–5.

Burge PS, Calverley PM, Jones PW, et al. 2000. Randomised, double blind, placebo controlled study of fluticasone propionate in patients with moderate to severe chronic obstructive pulmonary disease: the ISOLDE trial. *BMJ*, 320:1297–303.

Cabello H, Torres A, Celis R, et al. 1997. Bacterial colonization of distal airways in healthy subjects and chronic lung disease: a bronchoscopic study. *Eur Respir J*, 10:1137–44.

Calverley P, Pauwels R, Vestbo J, et al. 2003. Combined salmeterol and fluticasone in the treatment of chronic obstructive pulmonary disease: a randomised controlled trial. *Lancet*, 361:449–56(a).

Calverley PM, Boonsawat W, Cseke Z, et al. 2003. Maintenance therapy with budesonide and formoterol in chronic obstructive pulmonary disease. *Eur Respir J*, 22(6):912–9 (b).

Celli BR, MacNee W. 2004. Standards for the diagnosis and treatment of patients with COPD: a summary of the ATS/ERS position paper. *Eur Respir J*, 23:932–46.

Chrystyn H, Mulley BA, Peake MD. 1988. Dose response relation to oral theophylline in severe chronic obstructive airways disease. *BMJ*, 297:1506–10.

Collet JP, Ducruet T, Kramer MS, et al. 1993. Stimulation of nonspecific immunity to reduce the risk of recurrent infections in children attending day-care centers. The Epicreche Research Group. *Pediatr Infect Dis J*, 12(8):648–52.

Collet JP, Shapiro P, Ernst P, et al. 1997. Effects of an immunostimulating agent on acute exacerbations and hospitalizations in patients with chronic obstructive pulmonary disease. The PARI-IS Study Steering Committee and Research Group. Prevention of Acute Respiratory Infection by an Immunostimulant. *Am J Respir Crit Care Med*, 156(6):1719–24.

Cvoriscec B, Ustar M, Pardon R, et al. 1989. Oral immunotherapy of chronic bronchitis: a double-blind placebo-controlled multicentre study. *Respiration*, 55(3):129–35.

Dahl R, Greefhorst LA, Nowak D, et al. 2001. Inhaled formoterol dry powder versus ipratropium bromide in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*, 164:778–84.

Davis AL, Aranda CP, Schiffman G, et al. 1987. Pneumococcal infection and immunologic response to pneumococcal vaccine in chronic obstructive pulmonary disease. A pilot study. *Chest*, 92:204–12.

Debbas N, Derenne JP. 1990. Preventive effects of an immunostimulating product on recurrent infections of chronic bronchitis in the elderly. *Lung*, 168(Suppl):737–40.

Del-Rio-Navarro BE, Luis Sienra-Monge JJ, Berber A, et al. 2003. Use of OM-85 BV in children suffering from recurrent respiratory tract infections and subnormal IgG subclass levels. *Allergol Immunopathol (Madr)*, 31(1):7–13.

Debelic M, Eckenberger HP. 1992. [Prevention of recurrent infection of the upper and lower airways. Multicenter, open study over three months] *Fortschr Med,* 110(30):565–70.

Dever LL, Shashikumar K, Johanson WG Jr. 2002. Antibiotics in the treatment of acute exacerbations of chronic bronchitis. *Expert Opin Investig Drugs*, 11(7):911–25.

Donaldson GC, Seemungal TAR, Bhowmik A, et al. 2002. Relationship between exacerbation frequency and lung function decline in chronic obstructive pulmonary disease. *Thorax,* 57:847–52.

Edwards KM, Dupont WD, Westrich MK, et al. 1994. A randomized controlled trial of cold-adapted and inactivated vaccines for the prevention of influenza A disease. *J Infect Dis,* 169:68–76.

File TM. 2000. The epidemiology of respiratory tract infections. *Semin Respir Infect,* 15(3):184–94.

Fletcher CM, Ball JD, Carstairs LW, et al. 1966. Value of chemoprophylaxis and chemotherapy in early chronic bronchitis. A report to the Medical Research Council by their Working Party on trials of chemotherpay in early chronic bronchitis. *BMJ*, 1(5499):1317–22.

Francis RS, Spicer CC. 1960. Chemotherapy in chronic bronchitis: influence of daily penicillin and teracycline on exacerbations and their cost. A report to the research committee of the British Tuberculosis Assoication by their Chronic Bronchitis subcommittee. *BMJ*, 1:297–303.

Francis RS, May JR, Spicer CC. 1961. Chemotherapy of bronchitis: influence of penicillin and tetracylcline administered daily, or intermittently for exacerbations. *BMJ*, 2:979–85.

Global Initiative for Chronic Obstructive Lung Disease. Update 1 July 2005. A collaborative project of the National Health L, and Blood Institute, NIH and the World Health Organisation., ed. www.goldcopd.com: National Institutes of Health, National Heart Lund and Blood Institute, Bethesda, Md, 2005.

Gomez Barreto D, De la Torre C, Alvarez A, et al.1998. [Safety and efficacy of OM-85-BV plus amoxicillin/clavulanate in the treatment of subacute sinusitis and the prevention of recurrent infections in children*] Allergol Immunopathol (Madr),* 26(1):17–22.

Gross NJ, Petty TL, Friedman M, et al. 1989. Dose response to ipratropium as a nebulized solution in patients with chronic obstructive pulmonary disease. A three-center study. *Am Rev Respir Dis,* 139:1188–91.

Gutierrez-Tarango MD, Berber A. 2001. Safety and efficacy of two courses of OM-85 BV in the prevention of respiratory tract infections in children during 12 months. *Chest,* 119(6):1742–8.

Guyatt GH, Townsend M, Pugsley SO, et al. 1987. Bronchodilators in chronic air-flow limitation. Effects on airway function, exercise capacity, and quality of life. *Am Rev Respir Dis*, 135:1069–74(a).

Guyatt GH, Townsend M, Kazim F, et al. 1987. A controlled trial of ambroxol in chronic bronchitis. *Chest*, 92:618–20(b).

Hak E, van Essen GA, Buskens E, et al. 1998. Is immunising all patients with chronic lung disease in the community against influenza cost effective? Evidence from a general practice based clinical prospective cohort study in Utrecht, The Netherlands. *J Epidemiol Community Health,* 52:120–5.

Hanania NA, Darken P, Horstman D, et al. 2003. The efficacy and safety of fluticasone propionate(250microg)/salmeterol(50microg) combined in the Diskus inhaler for the treatment of COPD. *Chest,* 124(3):834–43.

Hansen NC, Skriver A, Brorsen-Riis L, et al. 1994. Orally administered N-acetylcysteine may improve general well-being in patients with mild chronic bronchitis. *Respir Med*, 88:531–5.

Heintz B, Schlenter WW, Kirsten R, et al. 1989. Clinical efficacy of Broncho-Vaxom in adult patients with chronic purulent sinusitis — a multi-centric, placebo-controlled, double-blind study. *Int J Clin Pharmacol Ther Toxicol*, 27(11):530–4.

Higgins BG, Powell RM, Cooper S, et al. 1991. Effect of salbutamol and ipratropium bromide on airway calibre and bronchial reactivity in asthma and chronic bronchitis. *Eur Respir J*, 4:415–20.

Hilleman DE, Dewan N, Maleskar M, et al. 2000. Pharmacoeconomic evaluation of COPD. *Chest,* 118:1278–85.

Ikeda A, Nishimura K, Koyama H, et al. 1995. Bronchodilating effects of combined therapy with clinical dosages of ipratropium bromide and salbutamol for stable COPD: comparison with ipratropium bromide alone. *Chest,* 107:401–5.

Irwin RS, Boulet LP, Cloutier MM, et al. 1998. Managing cough as a defense mechanism and as a symptom. A consensus panel report of the American College of Chest Physicians. *Chest,* 114:133S–181S.

Isada CM, Stoller JK. 1994. Chronic bronchitis: the role of antibiotics. In: Niederman MS, Sarosi GA, Glassroth J, eds. Respiratory infections: a scientific basis for management. London: WB Saunders, p.621–33.

Johnston RN, McNeill RS, Smith DH, et al. 1969. Five-year winter chemoprophylaxis for chronic bronchitis. *Br Med J*, 4:265–9.

Jones PW, Willits LR, Burge PS, et al. 2003. Disease severity and the effect of fluticasone propionate on chronic obstructive pulmonary disease exacerbations. *Eur Respir J*, 21:68–73.

Keller R. 1984. [Multicenter double-blind study of the action of Broncho-Vaxom in chronic bronchitis] *Schweiz Med Wochenschr*, 114(25):934–7.

The Lung Health Study Research Group. 2000. Effect of inhaled triamcinolone on the decline in pulmonary function in chronic obstructive pulmonary disease: Lung Health Study II. *N Engl J Med*, 343:1902–9.

Li J, Zheng JP, Yuan JP, et al. 2004. Protective effect of a bacterial extract against acute exacerbation in patients with chronic bronchitis accompanied by chronic obstructive pulmonary disease. *Chin Med J (Engl)*, 117(6):828–34.

Macchi A, Vecchia LD. 2005. Open comparative, randomized controlled clinical study of a new immunostimulating bacterial lysate in the prophylaxis of upper respiratory tract infections. *Arzneimittelforschung,* 55(5):276–81.

Mahler DA, Donohue JF, Barbee RA, et al. 1999. Efficacy of salmeterol xinafoate in the treatment of COPD. *Chest*, 115:957–65.

Mahler DA, Wire P, Horstman D, et al. 2002. Effectiveness of fluticasone propionate and salmeterol combination delivered via the Diskus device in the treatment of chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*, 166(8):1084–91.

Man WD, Mustfa N, Nikoletou D, et al. 2004. Effect of salmeterol on respiratory muscle activity during exercise in poorly reversible COPD. *Thorax*, 59(6):471–6.

Miravitlles M, Mayordomo C, Artés M, et al. 1999. Treatment of chronic obstructive pulmonary disease and its exacerbations in general practice. *Respir Med,* 93:173–9.

Nichol KL, Margolis KL, Wuorenma J, et al. 1994. The efficacy and cost effectiveness of vaccination against influenza among elderly persons living in the community. *N Engl J Med,* 331:778–84.

O'Donnell DE, Fluge T, Gerken F, et al. 2004. Effects of tiotropium on lung hyperinflation, dyspnoea and exercise tolerance in COPD. *Eur Respir J*, Jun; 23(6):832–40.

Oostenbrink JB, Rutten-van Molken MP, Al MJ, et al. 2004. One-year cost-effectiveness of tiotropium versus ipratropium to treat chronic obstructive pulmonary disease. *Eur Respir J*, 23(2):241–9.

Orcel B, Delclaux B, Baud M, et al. Oral immunization with bacterial extracts for protection against acute bronchitis in elderly institutionalized patients with chronic bronchitis. *Eur Respir J,* 7(3):446–52.

Papi A, Luppi F, Franco F, et al. 2006. Pathophysiology of exacerbations of chronic obstructive pulmonary disease. *Proc Am Thorac Soc*, 3(3):245–51.

Patel IS, Seemungal TAR, Wilks M, Lloy, et al. 2002. Relationship between bacterial colonisation and the frequency, character and severity of COPD exacerbations. *Thorax*, 57:759–64.

Pauwels RA, Lofdahl CG, Laitinen LA, et al. 1999. Long-term treatment with inhaled budesonide in persons with mild chronic obstructive pulmonary disease who continue smoking. European Respiratory Society Study on Chronic Obstructive Pulmonary Disease. *N Engl J Med*, 340:1948–53.

Petty TL. 1990. The National Mucolytic Study. Results of a randomized, double-blind, placebo-controlled study of iodinated glycerol in chronic obstructive bronchitis. *Chest,* 97:75–83.

Poole PJ, Black PN. 2003. Mucolytic agents for chronic bronchitis or chronic obstructive pulmonary disease. *Cochrane Database Syst Rev*, (2):CD001287.

Rasmussen JB, Glennow C. 1988. Reduction in days of illness after long-term treatment with N-acetylcysteine controlledrelease tablets in patients with chronic bronchitis. *Eur Respir J,* 1:351–5.

Rosaschino F, Cattaneo L. 2004. Strategies for optimizing compliance of paediatric patients for seasonal antibacterial vaccination with sublingually administered polyvalent mechanical bacterial lysates (PMBL). *Acta Biomed Ateneo Parmense*, 75(3):171–8.

Ruah SB, Ruah C, van Aubel A, et al. 2001. Efficacy of a polyvalent bacterial lysate in children with recurrent respiratory tract infections. *Adv Ther*, 18(4):151–62.

Rutishauser M, Pitzke P, Grevers G, et al. 1998. Use of a polyvalent bacterial lysate in patients with recurrent respiratory tract infections: results of a prospective, placebo-controlled, randomized, double-blind study. *Adv Ther*, 15(6):330–41.

Schaad UB, Mutterlein R, Goffin H; BV-Child Study Group. 2002. Immunostimulation with OM-85 in children with recurrent infections of the upper respiratory tract: a double-blind, placebo-controlled multicenter study. *Chest*, 122(6):2042–9.

Seemungal TAR, Donaldson GC, Paul EA, et al. 1998. Effect of exacerbation on quality of life in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*, 157:1418–22.

Seemungal T, Harper-Owen R, Bhowmik A, et al. 2001. Respiratory viruses, symptoms, and inflammatory markers in acute exacerbations and stable chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*, 164(9):1618–23.

Sethi S, Murphy TF. 2001. Bacterial infection in chronic obstructive pulmonary disease in 2000: a state of the art review. *Clin Microbiol Rev*, 14:336–63.

Siafakas NM, Vermeire P, Pride NB, et al. 1995. Optimal assessment and management of chronic obstructive pulmonary disease (COPD). The European Respiratory Society Task Force. *Eur Respir J,* 8:1398–420.

Siafakas NM, Bouros D. 1998. Management of acute exacerbation of chronic obstructive pulmonary disease. In: Postma DS, Siafakas NM, eds. Management of chronic obstructive pulmonary disease. Sheffield: ERS Monograph, p.264–77.

Simberkoff MS, Cross AP, Al-Ibrahim M, et al. 1986. Efficacy of pneumococcal vaccine in high-risk patients. Results of a Veterans Administration Cooperative Study. *N Engl J Med,* 315:1318–27.

Spencer S, Calverley PM, Burge PS, et al. 2004. Impact of preventing exacerbations on deterioration of health status in COPD. *Eur Respir J*, 23(5):698–702.

Sramek J, Josifko M, Helcl J, et al. 1986. Bacterial lysate (I.R.S. 19) applied intranasally in the prevention of acute respiratory diseases in children: a randomized double-blind study. *J Hyg Epidemiol Microbiol Immunol,* 30(4):377–85.

Steurer-Stey C, Bachmann LM, Steurer, et al. 2004. Oral purified bacterial extracts inchronic bronchitis and COPD: Systematic Review. *Chest*, 126;1645–55.

Szafranski W, Cukier A, Ramirez A, et al. 2002. Efficacy and safety of budesonide/formoterol in the management of chronic obstructive pulmonary disease. *Eur Respir J*, 21:74–81.

Tielemans C, Gastaldello K, Husson C, et al. 1999. Efficacy of oral immunotherapy on respiratory infections in hemodialysis patients: a double-blind, placebo-controlled study. *Clin Nephrol,* 51(3):153–60.

Tricarico D, Varricchio A, D'Ambrosio S, Ascione E, Motta G. 2004. Prevention of recurrent upper respiratory tract infections in a community of cloistered nuns using a new immunostimulating bacterial lysate. A randomized, double-blind clinical trial. *Arzneimittelforschung*, 54(1):57–63.

Vathenen AS, Britton JR, Ebden P, et al. 1998. High-dose inhaled albuterol in severe chronic airflow limitation. *Am Rev Respir Dis,* 138:850–5.

Vestbo J, Sorensen T, Lange P, et al. 1999. Long-term effect of inhaled budesonide in mild and moderate chronic obstructive pulmonary disease: a randomised controlled trial. *Lancet*, 353:1819–23.

Vincken W, van Noord JA, Greefhorst AP, et al. 2002. Improved health outcomes in patients with COPD during 1 yr's treatment with tiotropium. *Eur Respir J*, 19:209–16.

Wongsurakiat P, Lertakyamanee J, Maranetra KN, et al. 2003. Economic evaluation of influenza vaccination in Thai chronic obstructive pulmonary disease patients. *J Med Assoc Thai*, 86(6):497–508.

Wongsurakiat P, Maranetra KN, Wasi C, et al. 2004. Acute respiratory illness in patients with COPD and the effectiveness of influenza vaccination: a randomized controlled study. *Chest,* 125(6):2011–20.

Williams JH, J., Moser KM. 1986. Pneumococcal vaccine and patients with chronic lung disease. *Ann Intern Med,* 104:106–9.

Zagar S, Lofler-Badzek D. 1989. Broncho-Vaxom in children with rhinosinusitis: a double-blind clinical trial. *ORL J Otorhinolaryngol Relat Spec,* 50(6):397–404.