

A new bacterial lysate protects by reducing infectious exacerbations in moderate to very severe COPD

A double-blind, randomized, placebo-controlled trial

Summary

Background. Infectious exacerbations are the most common complication in patients with chronic obstructive pulmonary disease (COPD). Some studies have shown that administering antigens extracted from a lysate of the most common bacterial species involved in respiratory exacerbation may reduce hospitalization rates and the global burden of the disease. However, at present time, the effectiveness of bacterial lysates in reducing the frequency of acute exacerbation of COPD (AECOPD) is debatable.

Purpose. The trial aimed to evaluate the clinical effectiveness of a new bacterial lysate (Ismigen®) in patients suffering from moderate to very severe chronic obstructive pulmonary disease. A subset of these patients were also affected with chronic *cor pulmonale*.

Methods. 178 patients were randomized into two different groups: one group was treated with a bacterial lysate (first 10 days of each month for three consecutive months) and the other with placebo. The trial was double blind. At the end of treatment, patients were followed for a further nine months.

Results. Selected clinical endpoints were seen to be significantly lower in the group treated with the lysate than in the placebo group. Ismigen® treatment led to a highly significant reduction in the frequency (215 vs 248 cases) and duration (10.6 vs 15.8 days) of exacerbations, as well as a decrease in antibiotic consumption (-270 doses) and hospitalization time (275 vs 590 days).

Conclusions. These results suggest that prophylaxis with Ismigen® can reduce the incidence, severity and duration of AECOPD episodes even in patients with severe COPD and comorbidities.

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Mario Cazzola

Pneumology and Allergology Unit and Respiratory
Clinical Pharmacology Section,
Department of Respiratory Medicine,
A. Cardarelli Hospital, Naples, Italy

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Mario Cazzola

Via del Parco Margherita, 24
80121 Naples, Italy
E-mail: mcazzola@qubisoft.it

Chronic obstructive pulmonary disease (COPD) is a condition characterized by airflow limitation which cannot be fully reversed. This airflow limitation is usually both progressive and associated with an abnormal inflammatory response of the lungs to noxious particles or gases. The most frequent cause of COPD is a long smoking history¹.

The Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines give a definition and provide a classification system for airway obstruction². Diagnosis of COPD is confirmed by a low forced expiratory volume in 1 second (FEV₁). There are five stages of COPD, ranging from stage 0 for patients 'at risk', to stage 4 for patients with 'very severe COPD'.

COPD is characterized by an accelerated decline in lung function as well as periods of acute exacerbation (AECOPD), particularly as the disease progresses³. Prospective studies indicate that patients with moderate to severe COPD experience an average of 1.5 to 3 exacerbations

per year^{4,5}. Most exacerbations are caused by a viral or bacterial infection of the tracheobronchial tree, and some may be caused by an increase in air pollution⁶⁻⁸. Infection of the lower respiratory tract has been suggested to account for up to 80% of AECOPD episodes. There are 3 classes of pathogens that are commonly implicated in exacerbations³: (1) respiratory viruses, with or without a superimposed bacterial infection, are associated with 30% of cases; the viral pathogens mainly include influenza, parainfluenza, and rhinoviruses; (2) atypical bacteria, mostly *Chlamydia pneumoniae*, are implicated in <10% of cases; (3) aerobic gram-positive and gram-negative bacteria occur in approximately 40% to 60% of cases.

AECOPD has negative effects on the health of patients, and its impact most likely increases with the frequency of episodes⁹. It has been shown that exacerbation frequency in particular is an important determinant of lung function decline in COPD¹⁰. Obviously, strategies for preventing COPD exacerbations may have an important impact on the natural course of this disease and on the morbidity and death rates of these patients. Sadly, despite the prevalence and seriousness of COPD, there is a widely-held belief that little can be done to treat the disease other than stop patients from smoking. A much more assertive and optimistic approach should be adopted, since there is clear evidence that the various treatments available improve the condition of patients in a variety of ways, although they are unable to cure the disease.

A systematic review of 9 randomized placebo-controlled trials concluded that regular administration of oral antibiotics reduced bronchitic exacerbation rates to a small but statistically significant degree ($P < 0.05$)¹¹. However, many experts believe that the widespread - and perhaps indiscriminate - prescription of antibiotics for respiratory infections has contributed towards the emergence of antibiotic-resistant strains among common bacterial pathogens¹².

Influenza immunization is arguably the single most effective way currently available of preventing severe COPD exacerbations¹³. Although the evidence is not as strong as for influenza immunization, pneumococcal vaccine may also have some protective effect against serious COPD exacerbations¹⁴. Specific immuniza-

tions against other viral and bacterial pathogens commonly found in the respiratory tract are not currently available, but several molecules are being developed. There are intriguing reports that the immune-modulating agent OM-85 (the alkaline proteolysis product of *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, *Klebsiella ozaenae*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus viridans*, and *Moraxella catarrhalis* lysates) may have substantial protective effects against COPD exacerbations and hospitalization^{15,16}. The trials available so far with this oral bacterial lysate were assessed in a recent meta-analysis¹⁷. When only the three most recent well designed trials were combined, the number needed in treatment to prevent an exacerbation was calculated to be ~15.4 (95% CI 5.5-∞)¹⁷. Overall, there are still very few studies in patients with well-defined COPD in advanced GOLD-stages, and a lack of studies longer than 6 months.

A new treatment has recently been introduced with a new type of bacterial lysate, fruit of a new technique based on mechanical lysis. Bacterial lysis using chemical substances (such as the chemical proteolysis used to obtain OM-85) may lead to structural alteration of antigenic macromolecules and consequently weaken the expected immune response. There is documentary evidence that several vaccines fail precisely because the vaccine molecules which induce the immune response are not suitably structurally conserved¹⁸.

The mechanical lysis of bacterial cells, on the other hand, prevents contamination with chemical substances potentially able to denature the antigenic structures they are used to obtain, with the advantage of producing a bacterial lysate containing structurally intact antigenic macromolecules which are therefore able to induce a more effective immune response. Ismigen[®] is a killed, freeze-dried bacterial lysate obtained by mechanical lysis of the eight most common respiratory pathogens (*S. aureus*, *S. pyogenes*, *S. viridans*, *K. ozaenae*, *H. influenzae*, *M. catarrhalis*, and six serotypes of *S. pneumoniae*). Ismigen[®] tablets are administered sublingually so that the antigenic molecules may easily spread in the upper respiratory tract mucous membrane and stimulate regional immunity, thus avoiding the gastric digestion of protein macromolecules which would occur if the tablet were swallowed.

Previous random controlled clinical trials have shown that bacterial lysates obtained by mechanical lysis have a greater protective effectiveness than traditional lysates against respiratory infection in patients with recurrent affections of the upper and lower respiratory tract^{19,20}.

The aim of this study was to examine whether ismigen[®] tablets administered sublingually repeatedly for 10 days a month for 3 consecutive months, with a follow-up of a further 9 months, could protect patients with moderate-very severe COPD complicated with cor pulmonale against acute exacerbations thought to be related primarily to respiratory tract infections.

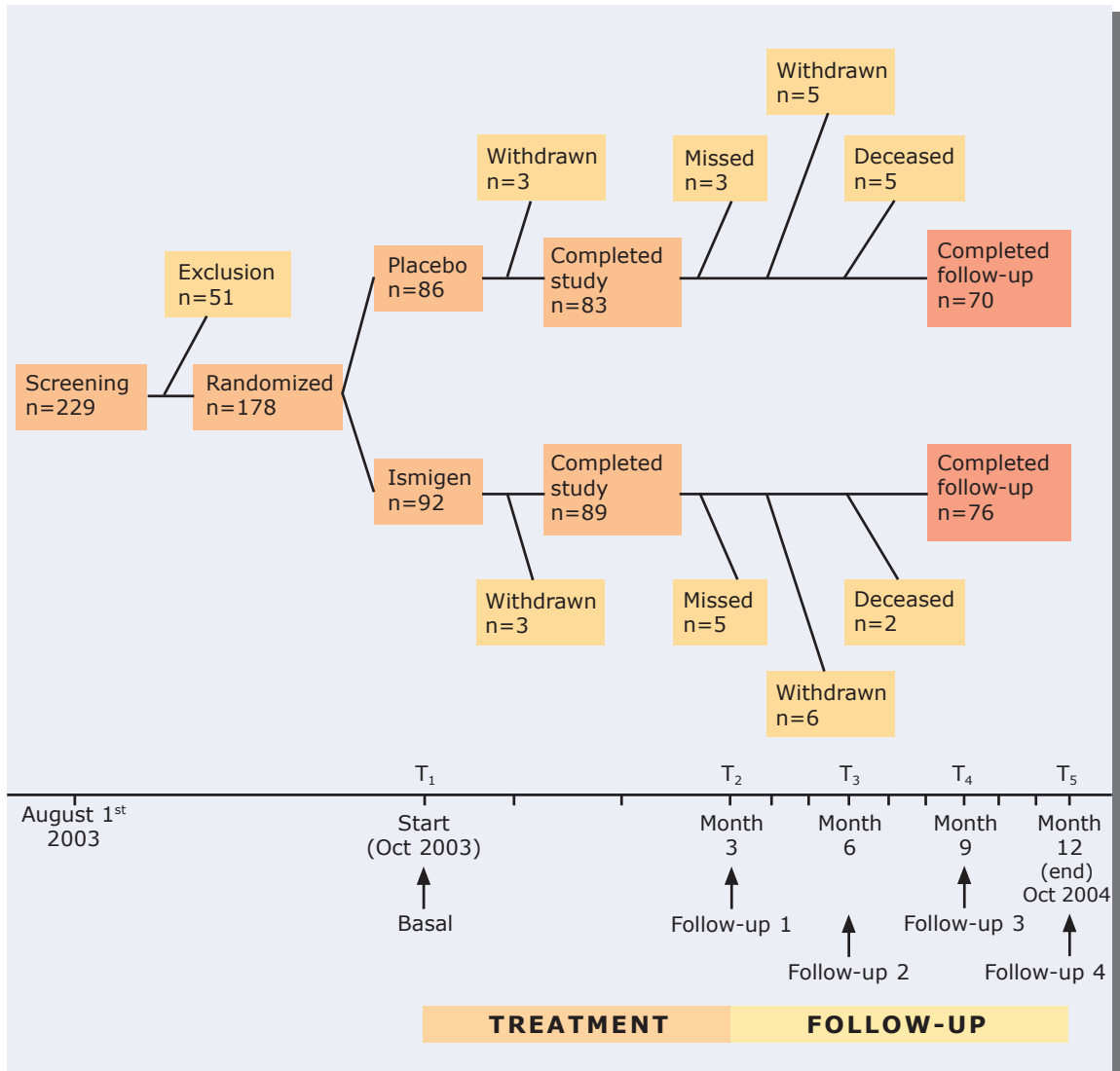
Patients and methods

This double-blind, randomized, placebo-controlled trial, started on August 2003 and finished on October 2004. The study protocol was approved by a local ethics committee, and signed informed consent was obtained from each patient before inclusion.

Population

A total of 229 outpatients suffering from moderate to very severe COPD according to the GOLD severity classification (II to IV)² were screened from August to October 2003. They had to be able to understand instructions given by medical staff, and be cooperative and

Figure 1. Study design and patient flow during the trial.



able to reach our centre regularly. Patients suspected of having lung cancer, those who needed continuous domiciliary oxygen therapy, and those with a body mass index (BMI) <19.5 and >30.0 were excluded. Patients taking or who took corticosteroids, azathioprine and other immunosuppressive drugs during the previous six months were also excluded, as were those who had had an episode of acute exacerbation treated with antibiotics within the previous month.

Fifty-one patients did not meet the inclusion criteria and were excluded. One hundred and seventy eight patients were therefore randomly split into two parallel groups (mean age of 67 years). The enrolled population was composed of males and females (70% male) with previous smoking experience; about 43% of patients still smoked. Seventy-one patients suffered from stage IV COPD ($FEV_1/FVC < 70\%$; $FEV_1 < 30\%$) and 32 of them also suffered from *chronic cor pulmonale*. All randomized patients underwent renal and hepatic assessment laboratory tests and a chest X-ray before starting the treatment. The clinical data collected for the eligible patients revealed 249 episodes of AECOPD in the six months before randomization; thirty-nine of them required hospitalization, corresponding to a rate of 498 episodes of AECOPD/year with 78 cases of hospitalization/year.

Study design and endpoints

The trial aimed to evaluate the efficacy of three courses of Ismigen® in preventing AECOPD in susceptible patients. Patients were followed up for 12 months after the first course. The two primary study outcomes were the number of AECOPD events which occurred during the trial period and the follow-up, and the duration and severity of exacerbation episodes. Secondary outcomes included the rate and length of hospitalization due to AECOPD, and the use of antibiotics and other respiratory drugs.

Figure 1 shows the trial design and the patient flow during the course of treatment (1 cycle/month for 3 months) and follow-up (9 months). Five examinations were scheduled: upon randomization (T1), at the end of treatment (T2), and every three months during the follow-up until trial completion (T3-T5). Out of the 178 randomized patients, 83 patients from the placebo group and 89 patients from the lysate group completed the treatment protocol. At the end of the follow-up, 70 placebo and 76 lysate group patients were assessable.

Randomization and treatment groups

The eligible patients were randomized into two fairly uniform groups from the point of view of their demographic and baseline data (table 1). Patients had to take one Ismigen® (Zam-

Table 1. Demographic characteristics and clinical profile of patients included.

Parameter	Treated (N=92)	Placebo (N=86)
Sex (male/female)	60/32	59/27
Age (years)	66 (±2)	67 (±3)
Still smoking (pts)	41	37
Length of chronic bronchitis (years)	15.8 (±3.1)	16.6 (±2.4)
FEV_1 (% pred.)	46.2 (±13.2)	45.7 (±12.8)
FEV_1 (L)	1.0 (±0.41)	1.1 (±0.39)
FVC (% pred.)	62.4 (±13.3)	63.8 (±15.4)
FVC ₁ (L)	1.98	2.06
N° AECOPD during previous year	266	232
N° AECOPD/pts during previous year	2.9 (±0.6)	2.7 (±0.4)
N° hospitalizations during previous year	37	41
Stage II (Pts)	36	32
Stage III (Pts)	20	19
Stage IV (Pts)	21	18
Stage IV and cor pulmonale (Pts)	15	17

Table 2. Frequency of acute exacerbation cases occurred in each group broken down by COPD stage.

Stage	AECOPD cases	
	Lysate	Placebo
II	47	54
III	44	45
IV	67	69
IV + (CP) ^a	57	80
Overall	215	248

^aCP= Cor pulmonale

bon, Bresso, Italy) or placebo tablet sublingually in the morning, every day repeatedly for 10 consecutive days per month for 3 consecutive months. Each Ismigen[®] 50 mg tablet contained 7 mg of freeze-dried bacterial lysate. Upon randomization, many patients were receiving regular treatment for COPD and/or cardiovascular comorbidities. No previously prescribed treatments were suspended during the study period or follow-up.

Evaluation

To optimize clinical evaluation, patients were allocated to five groups: an investigator and two nurses were assigned to each group. Patients were suitably trained to evaluate changes in respiratory function (dyspnoea), sputum production and purulence compared with baseline, and were taught how to record their use of medication and changes in dyspnoea and sputum with respect to baseline conditions on diary cards. All patients were asked to phone or report to their centre in the event of suspected exacerbation. An event was considered to be an episode of acute exacerbation when the patient met the following three conditions: (1) change in sputum characteristics; (2) occurrence of at least one of the following additional symptoms: breathlessness, coughing or fever; (3) evidence of the nontrivial nature of the episode (as determined either by an unscheduled medical examination and/or use of antibiotics). The investigator assigned to each group confirmed the diagnosis of AECOPD in 407/463 cases; 56 other episodes were diagnosed by the family doctor. Patients with AECOPD were treated at home with an orally administered antibiotic (ciprofloxacin for 7-10 days); the number of antibiotic tablets taken by each patient was recorded.

Statistical analysis

The Statistical Package for Social Sciences version 10.0 (SPSS Inc., Chicago, Illinois, USA) was used for all analyses. The comparisons between groups were made using appropriate statistical methods (Fisher exact test for categorical analyses, and Student's *t* test and the Mann-Whitney *U* test for continuous variables). $P < 0.05$ was considered significant.

Results

One hundred and forty-six out of the 178 randomized patients (82%) completed the trial. During the follow-up period 7 patients died: 5 receiving placebo (6%) and 2 receiving Ismigen[®] (2%). Moreover, 11 placebo patients and 14 lysate patients did not complete the trial due to poor protocol compliance. In line with the results of other studies, the incidence and severity of the AECOPD cases were strictly related to FEV₁. Two hundred and seventy-three of the 463 total cases occurred in the 71 stage IV patients (3.8 episodes/patient/year); 101 episodes took place in the 68 stage II patients (1.5 episodes/patient/year), and another 89 cases occurred in the 39 stage III patients (2.3 episodes/patient/year). The highest exacerbation rate was observed in the 32 stage IV patients with *chronic cor pulmonale*: 57 episodes in the 15 patients treated with lysate (3.8 episodes/patient/year) and 80 episodes in the 17 patients in the placebo group (4.7 episodes/

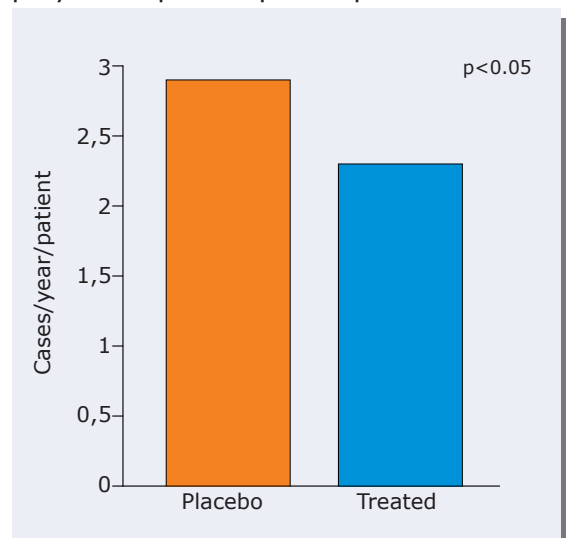
Figure 2. AECOPD episodes per treated patient per year compared to placebo patients.

Table 3. Respiratory symptoms and clinical success rates.

Parameter	Lysate (215 episodes)	Placebo (248 episodes)
Clinical success	192 (89.3%)	203 (81.8%)
Coughing ^a	14 (6.5%)	34 (13.7%)
Dyspnoea ^a	16 (7.4%)	31 (12.5%)
Mucopurulent sputum	23 (10.6%)	51 (20.5%)
Rales/Rhonchi ^a	11 (5.1%)	18 (7.2%)

^a=Moderate-severe

patient/year). Table 2 shows the number of AECOPD cases which occurred in each group broken down by COPD stage.

Acute exacerbations

A total of 463 episodes of AECOPD occurred during the study: 215 in lysate group patients (2.3 cases/patient/year) and 248 in placebo patients (2.9 cases/patient/year). The median case reduction averaged 21% per year per patient (figure 2). Since the trial lasted a total of 12 months, the observed exacerbation rate corresponded to an average of 2.3 (± 0.3) episodes/patient/year among the lysate group patients and 2.9 (± 0.4) episodes/patient/year in the placebo group, that is a reduction of 33 episodes per year in the lysate group ($p < 0.05$). In comparison with the AECOPD rates of the previous year (see table 1), the exacerbation rates measured were notably lower for the lysate group (-0.6 episode/year) and higher in the control group (+0.2 episodes/year).

Duration and severity of AECOPD

The mean duration of the 215 AECOPD cases which occurred in the lysate group patients was 10.6 days, whereas the mean duration observed in the placebo group was 15.8 days. The 5.2 day difference between the two groups was statistically significant ($p < 0.05$). This difference had a potential significant economic impact on the population: in fact, the 5.2-day decrease for each case which occurred multiplied by the 33-case difference between the placebo group (248 cases) and the lysate group (215 cases) implies a reduction in total hospitalization time of about 171 days for a small cohort of only 92 treated patients. Respiratory symptoms were also less severe in immunized patients (table 3). Lastly, lysate administration led to a greater rate of clinical success.

In our trial, clinical success was defined as the

complete remission of respiratory distress and resumption of the patient's everyday life activities. Under the same antibiotic regimen, clinical recovery was attained in 89.3% of the patients treated with lysate compared with 81.8% of the control patients ($p < 0.02$). Patients in the lysate group took fewer antibiotics (8.7 days of treatment) than placebo patients (12.8 days of treatment). More than 90% of the total episodes were treated with ciprofloxacin (500 mg twice daily). As a consequence 270 doses of this antibiotic were saved in the immunized group.

Hospitalization rates and length of stay

During the trial period, there were 87 cases of hospitalization due to respiratory diseases: 56 in the placebo group and 31 in the lysate group, amounting to a total of 865 days (average duration of 9.9 days ± 3.7). All deaths were associated with cardio-respiratory failure and all happened after an AECOPD episode. Although the difference in mortality rate between lysate treatment and placebo was not statistically significant, the figures suggest that active immunization with Ismigen[®] might reduce hospitalization rates and - perhaps - overall mortality in high risk patients. This trial only considered hospitalization related to respiratory diseases and did not take hospitalization due to other causes into account. Hospitalization mainly lasted 8.9 days (± 2.2) in the lysate group and 10.5 days (± 3.7) in the placebo group ($p < 0.05$).

Tolerability and adverse events

The lysate treatment was seen to be safe and well tolerated even though the population was elderly with moderate to very severe COPD and several comorbidities for which they were taking many respiratory and cardiovascular drugs. No clinically relevant differences were

noted between the groups concerning clinically significant abnormalities in physical assessments or clinical laboratory tests; no adverse events were reported by patients. No patients discontinued the treatment protocol due to unfavourable reactions. Twenty-five patients dropped out of the trial by failing to return for study examinations or due to noncompliance with the trial medication.

Discussion

Several studies have shown that bacterial extracts increase the expression of adhesion molecules at the surface of polymorphonuclear leucocytes, and enhance natural killer cell activity and the production of Tumour Necrosis Factor- α , interleukin (IL)-1, IL-2, and interferon γ by human peripheral blood mononuclear cells²¹⁻²⁴. It is not a surprise, therefore, that some trials have shown that active immunization with bacterial lysates administered with the same schedule as we used in this trial is able to reduce the incidence and duration of AECOPD^{16,25-28}. There are however differences in results between lysates obtained by chemical lysis administered by ingestion and the bacterial lysate obtained by mechanical lysis administered sublingually, presumably due to the different ways in which the immune response is induced.

The ability of lysates obtained by chemical lysis to reduce the frequency of exacerbation is much more debatable, although there is clear documentary evidence that bacterial extracts function by enhancing the postnatal maturation of Th1 function, which is normally driven by stimuli from the commensal gastrointestinal microflora. The Th1-stimulatory effects observed are likely to contribute to the clinical efficacy of bacterial lysates in enhancing resistance to infections²⁹. OM 85 BV was evaluated in a meta-analysis, which showed a 0.6 reduction in exacerbations per 6 months³⁰. In a more recent systematic review the relative risk of exacerbation was only modestly reduced by chemical bacterial lysates, but they improved symptoms in COPD patients¹⁷. Furthermore, a controlled and well conducted trial was unable to demonstrate that OM-85 BV administration could reduce the rate of exacerbation, although the likelihood of severe respiratory events leading to hospitalization was significantly lower among the ly-

sate group patients than in the placebo group¹⁶. It is apparent that, although there is no sufficiently strong evidence to suggest that bacterial extracts play a role in preventing exacerbations, there is clear evidence that they improve symptoms in patients suffering from COPD. This is an important conclusion that keeps interest in the topic alive³¹.

As regards the administration of the new bacterial lysate obtained by mechanical lysis, it should be emphasized that the significant decrease in incidence of upper and lower respiratory tract relapses, even in trials which involved patients in "extreme" environmental conditions (such as prisoners or secluded nuns), may be attributed to the greater immune response induced by sublingual administration of structurally intact antigenic macromolecules. Experimental immunology studies have shown that lysated macromolecules obtained by mechanical lysis are able to stimulate the maturation of dendritic cells, which are responsible for activating the two immunity branches, innate and acquired³². In particular, *in vivo* tests in man have shown that, thanks to this peculiar mechanism, administering the new bacterial lysate obtained by mechanical lysis significantly increases systemic IgG levels and above all secretory IgAs³³. Secretory IgAs are the most important arms in regional immune response when the respiratory mucous membrane is attacked by pathogens, also because immunization of the upper respiratory tract generally causes an intense immune reaction throughout the whole respiratory tract³⁴.

Bacteria clearance remains a debatable aspect in patients with COPD even after optimal antibiotic treatment³⁵. Several studies have documented the presence of the same bacterial strains as were isolated in previous episodes of AECOPD despite apparent microbiological eradication with an appropriate antibiotic regimen³⁶. This suggests that antibiotic agents may spread poorly into mucopurulent sputum or that, even when the concentrations are high enough, locally produced enzymes released during acute inflammation (i.e. elastase, metalloproteinase, etc.) may reduce the antibiotic effect. In this situation, even small clusters of bacteria which survive in elderly and/or immunocompromised patients may establish new centres of infection. Bacterial lysates can improve bacterial clearance by sti-

mulating the secretion of both local and systemic globulins (IgA, IgE and IgG). This mechanism has been observed in many experimental models^{37,38}.

The duration of our trial - at least regarding its follow-up - was longer than other published trials. Furthermore, an interim analysis six months after the beginning of the trial revealed a risk reduction (-41%) that appeared to be larger than the one recorded at the end of the follow-up (12 months). This result suggests that, although the benefits of immunization are long lasting, they are greater up to three-six months after the last cycle. This observation suggests that frequent immune-refreshing could be useful in preventing relapses, at least in elderly and/or immunocompromised patients. In any case, the duration of exacerbation and the hospitalization rates observed in this trial are in line with the results of other reports. This is an important finding because the majority of the enrolled patients were at high exacerbation risk, and a

sub-group of them was even affected with severe right ventricular haemodynamic failure.

Both the lower incidence and shorter duration of AECOPD cases allowed better allocation of economic resources, with notable cost savings in terms of drugs and hospitalization. In fact, 270 doses of an oral antibiotic and 315 hospitalization days (275 days in the lysate group *vs.* 590 days among control patients) were saved in one year on a small cohort of 92 patients. If we had treated a cohort of 1,000 patients with the same clinical profile as the sample we studied, the antibiotics saved would have averaged 2,930 doses a year.

In conclusion, on the basis of the important clinical results of this trial, i.e. lower incidence of exacerbations and greater efficacy of antibiotic treatment in patients immunized with bacterial lysates, it is possible to state that this therapeutic approach should always be considered in patients suffering from moderate to very severe COPD. **TiM**

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