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The bacterial lysate Lantigen B reduces the number of acute episodes in patients with recurrent infections of the respiratory tract: The results of a double blind, placebo controlled, multicenter clinical trial



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ABSTRACT

Studies in the 1970s and 1980s reported that bacterial lysates (BL) had a prophylactic effect on recurrent respiratory tract infections (RRTI). However, controlled clinical study procedures have evolved substantially since then. We performed a trial using updated methods to evaluate the efficacy of Lantigen B[®], a chemical BL. This double blind, placebo controlled, multi-center clinical trial had the primary objective of assessing the capacity of Lantigen B to significantly reduce the total number of infectious episodes in patients with RRTI. Secondary aims were the RRTI duration, the frequency and the severity of the acute episodes, the use of drugs and the number of missed workdays. In the subgroup of allergic patients with RRTI, the number of allergic episodes (AE) and the use of anti-allergic drugs were also evaluated. One hundred and sixty patients, 79 allocated to the treated group (TG) and 81 to the placebo group (PG), were enrolled; 30 were lost during the study and 120 (79 females and 38 males) were evaluated. The PG had 1.43 episodes in the 8-months of follow-up while the TG had 0.86 episodes ($p = 0.036$). A similar result was observed in the allergic patients (1.80 and 0.86 episodes for the PG and the TG, respectively, $p = 0.047$).

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The use of antibiotics was reduced (mean 1.24 and 2.83 days of treatment for the TG and the PG). Logistic regression analysis indicated that the estimated risk of needing antibiotics and NSAIDs was reduced by 52.1 and 30.6%, respectively. With regard to the number of AE, no significant difference was observed between the two groups, but bronchodilators, antihistamines and local corticosteroids were reduced by 25.7%, 56.2% and 41.6%, respectively, in the TG. Lantigen B significantly reduced the number of infectious episodes in patients with RRTI. This finding suggests a first line use of this drug for the prophylaxis of infectious episodes in these patients.

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1. Introduction

Recurrent respiratory tract infections (RRTI) are less frequent in adults than in the children, but the frequency of patients affected remains significant [1]. The therapy is based on anti-inflammatory drugs and antibiotics, and only rarely is infection prophylaxis provided. Bacterial lysates (BL) are a family of “biological” drugs that were introduced in therapy many decades ago, but only in the last 10 years has their mechanism of action has been elucidated. Since the first in vivo studies, it was hypothesized that the clinical effects observed were related to the capacity of BL to elicit a specific immune-response against bacterial antigens. Along this line, since the mid 1970s, specific IgA directed against bacterial antigens have been observed in the salivary fluid of treated patients [1–4] following the oral administration of bacterial lysates. During the 1990s, the description of the toll-like receptor (TLR) family and its functions [5] within the larger context of the pattern recognition receptor (PRR) system explained how the innate immune system was alerted by the presence of different stimuli, mainly represented by bacterial derived structures. In that same period, the central role of dendritic cells (DC) in the generation of an efficient immune-response was described [6]. Starting from these lines of evidence, different bacterial lysates were shown to be able to induce significant activation and maturation of DC in vitro [7,8]. More recently, using a specific member of the bacterial lysate family (the Polyvalent Mechanical Bacterial Lysate – PMBL), other relevant effects were observed on B cells, T cells and NK cells [9,10] and these effects were also correlated with the clinical results [11,12]. Bacterial lysates are obtained by physical or chemical lysis. The former are in general composed of fragments of bacterial bodies. The latter may be constituted by either only low molecular weight proteins or a mixture of bacterial bodies and soluble proteins. Lantigen B belongs to the latter family, being a suspension of bacterial antigens obtained from *Streptococcus pneumoniae* type 3, *Streptococcus pyogenes* Group A, *Branhamella catarrhalis*, *Staphylococcus aureus*, *Hemophilus influenzae* type b and *Klebsiella pneumoniae*. The in vivo effects of Lantigen B were first described in the mid 1970s [11–13]. Since that time, few studies have been performed on this specific drug. Although the first studies were considered obsolete from a procedural point of view [14–16], the drug remained in the pharmacological armamentarium of both general practitioners and specialists. In this context, other studies even if not totally in line with modern clinical trial practice have been published [17–19], further demonstrating the clinical efficacy of Lantigen B. In particular, the main effect observed was the reduction of infections during the observation period. Indeed, by analyzing all the abovementioned studies, it can be calculated that a mean of 1.73 episodes/year were observed in treated patients vs. 2.63/year in the placebo group, corresponding to a reduction of 34% of the number of infectious episodes. In addition, in the same studies, the reduction of cough, of lost days and antibiotics use was also observed. Of note, only one non-positive study on the common cold [20] was published, while all the others demonstrated the efficacy of the treatment.

In this study, using modern and effective clinical practice tools, we re-evaluated the efficacy of Lantigen B in a double

blind randomized placebo-controlled clinical trial in inducing a significant reduction in the number of infectious episodes in an unselected (real life-like) population of patients with RRTI. This type of study is needed not only to verify previous results using an up-dated methodology but also for the availability of drugs (such as third generation anti-histamine, local corticosteroids and modern bronchodilators) that have recently demonstrated a deep impact on the management of allergic patients who represent a significant fraction of patients with RRTI.

2. Material and methods

2.1. Definition of acute infectious episodes

Acute episodes of the upper respiratory airways were identified by the continuous presence of rhinorrhea (both sero-mucous and purulent), pharyngitis, and cough, lasting at least 48 h, with or without fever. Acute episodes of the lower respiratory airways were defined as the continuous presence of stridor, wheeze, crackles and rhonchi, an indrawing respiratory frequency > 50 cycles/min, or cyanosis lasting at least 48 h, with or without fever. Acute episodes of otitis were defined as the continuous presence of pain, erythema and a reduction in or loss of tympanic membrane mobility. Finally, an acute infectious episode was defined as new if at least 72 h had passed with a complete absence of symptoms from the resolution of the previous episode. The identification of an infectious event was based on patient's report (fever or cough or dyspnea or use of drugs) as well as on doctor's report (visits and therapeutic indications). The protocol provided that when an infectious episode occurs, the patient might contact to the primary care physician or the study center that enrolled her/him. In both cases, the diagnosis and the therapy were reported in the patient's diary and in the Case Report Form.

2.2. Study design

This was a national multi-center longitudinal, prospective randomized double blind versus placebo controlled clinical phase IV study, conducted on two parallel groups for an observation period of 8 months. The protocol EudraCT code was 2011-00-3229-76. The primary goal was the evaluation of the efficacy of the Lantigen B treatment on the number of infectious episodes (IE) in the group of treated patients. Secondary goals were (a) the total number of days with IE (in a 6 month follow-up); (b) the frequency and severity of episodes of allergy (AE), in particular asthma and rhino-conjunctivitis; (c) the disease free period evaluated as the time of occurrence of an IE after the end of one of the treatment cycles; (d) any other drug consumption in the 8 month period, including anti-infectious, anti-inflammatory and anti-allergic drugs; (e) the enumeration of days of absence from the community (school, work); (f) the safety and tolerability of the treatment.

eters as the second visit and to retire the remaining drug and the diary.

2.9. Patient consent withdrawal

Patients were allowed to withdraw from the treatment for any reason. When possible, the adverse reactions and the use of drugs were recorded for the retired patients and the personal diary was retired. Any relevant information was recorded in the CRF.

2.10. Patient compliance control

The compliance to the treatment was evaluated by comparing the registered doses and the amount of remaining drug in the bottles.

2.11. Safety assessments

The patients and the investigators were asked to monitor the possible occurrence of adverse reactions (ARs). These were classified as certain, likely, possible, unlikely, unrelated and not evaluable, according to the definition of the protocol. ARs were also classified as slight (if not interfering with daily activities), intermediate (if the AR interfered with the day activities) and severe (if the AR inhibited daily activities).

2.12. Exclusion of patients from the study

Patients were excluded from the study in the presence of a severe AR incompatible with the continuation of participation in the study.

2.13. Viro-epidemiological survey of the study period

The frequency of viral infections in the general population during the period of the study and the previous year was evaluated. The results of molecular biology-based research on influenza viruses, para-influenza viruses, bocaviruses, coronaviruses, Rhinoviruses, enteroviruses, adenoviruses and Respiratory Syncytial virus were recorded. The frequency of viral infections was based on the percentage of positive samples as described [10]. Thus, an increase in the percentages of positive samples was considered representative of an increased circulation of pathogens in a given period.

2.14. Statistical analysis

For the sample size calculation, a total of 190 patients (85 patients per group) were required to be recruited, by considering an expected drop out of 15% of the patients enrolled with the intention to treat (ITT). Thus, a total of 160 patients were expected to be evaluable. The primary efficacy analysis was based on the ITT. The efficacy of the treatment on the two groups was evaluated by an analysis of covariance (ANCOVA). For this analysis, the total number of infective episodes was the dependent variable, the treatment group was the factor and the number of previous year infective episodes was the covariate. In the ANCOVA model, the adjustment for baseline covariates was performed. The subgroup analyses were performed in compliance with the rules suggested in the European Medicine Agency Guideline on the investigation of subgroups in confirmatory clinical trials. Binary variables measuring the effects of treatment on the secondary objectives were analyzed by a χ^2 test. Finally, a logistic regression (multivariate) analysis was used to evaluate the probability of using drugs: antibiotics and NSAID, for the whole population and local corticosteroids, antihistamine drugs and bronchodilators for the subset of allergic patients. All

Table 2

Clinical characteristics of the patients, number of patients with a given diseases and number of episodes of disease in the period of the study (2012–2013).

	Patients with disease		Number of disease episodes	
	Placebo	Lantigen B	Placebo	Lantigen B
Otitis	2	1	2	1
Tonsillitis	5	2	7	2
Sinusitis	9	3	18	6
Pharyngotonsillitis	17	11	23	15
Rhinopharyngitis	20	18	32	21
Bronchitis	15	8	23	12
Pneumonia	0	1	0	1
Total	68	44	105	58

these statistics were carried out by using SPSS on data extracted from the CRFs.

3. Results

3.1. Study population

A total of 160 patients (79 in the treated group and 81 in the placebo group) were enrolled. The age of the placebo group was 42.4 ± 15.14 (mean \pm standard deviation) and the age of the treated group was 42.4 ± 13.94 years. One hundred and seventeen patients (58 in the treated and 59 in the placebo group) completed the study (Fig. 1). The drop out was observed both in the Lantigen B group (17 patients) and in the placebo group (23 patients). Some patients were lost for inadequate compliance or violation of the protocol (seven patients), three for adverse reactions, five were lost in the follow-up and 23 (corresponding to the 57.5%) for consent withdrawal. Finally, two were lost for other reasons. In patients who completed the study, the genders were equilibrated between the placebo (20 males and 38 females) and in the treated group (18 and 41, respectively) ($\chi^2 = 0.21$, $p = 0.65$). Table 2, in the lanes related to the reference period (2011–2012) shows the clinical characteristics of these patients, in line with the eligibility criteria.

3.2. Post-hoc power

The number of patients recruited and the number of drop-outs could have impacted the power of the study results. However, because the differences between the placebo and treatment were significant and the efficacy of the treatment was higher than could have been expected on the basis of the published literature, the post-hoc power of the study was confirmed. Indeed, a sample size of 58 in each group had an 84% power to detect a probability of 0.34 that an observation in the Lantigen B group was less than an observation in the placebo group (Wilcoxon Rank-sum test with a 0.05 two sided significance level). Of note, a power of 80% is considered acceptable by the scientific community for clinical studies. Thus, the original design of this study had a power suitable to achieve complete statistical significance even in the presence of a conspicuous drop-out of the enrolled patients.

3.3. The primary objective

The primary objective of the study was the reduction of the number of IE during an 8 months follow-up period. In the whole group of enrolled patients, the mean frequency of IE in the year preceding the study was 3.66/year (C.I. 3.38–3.93). The frequency was 3.34/year for males and slightly higher, 3.81/year for females, corresponding to a mean 2.56 IE in 8 months (2.5 and 2.9 in 8 months, respectively). The distribution of IE between the placebo

CONSORT 2010 Flow Diagram

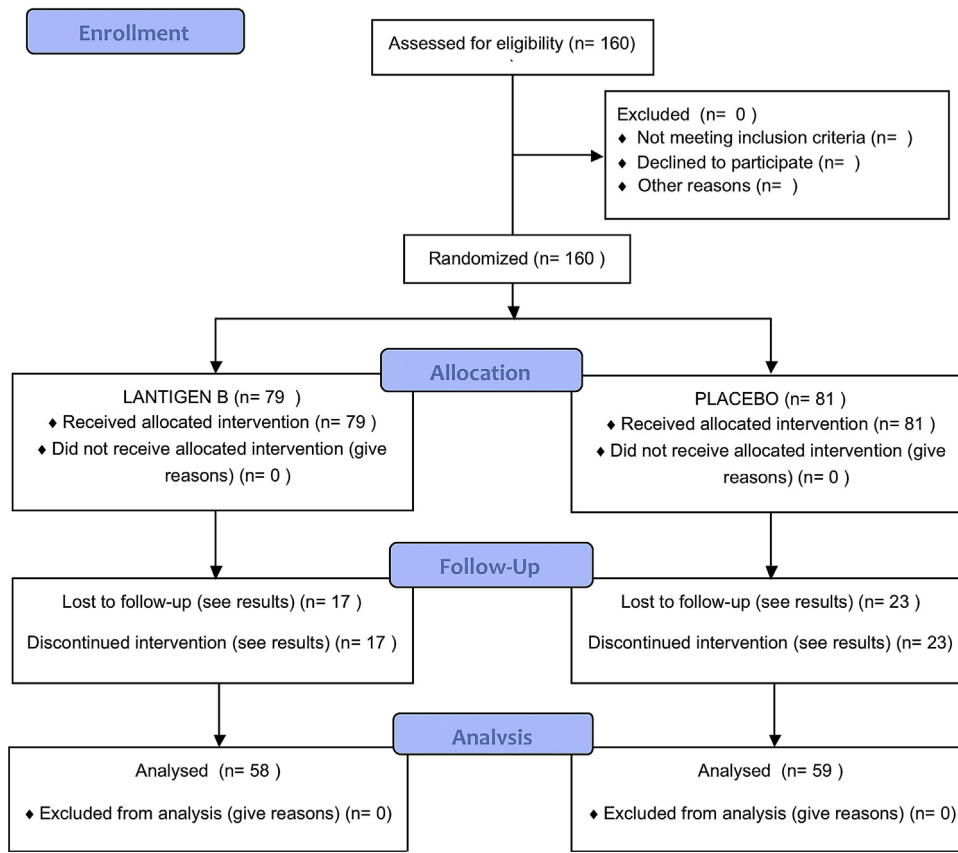


Fig. 1. The Consort 2010 flowchart.

and treated groups was 3.78 and 3.54, respectively. During the period of the study, the placebo group (58 patients) experienced a mean of 1.43 (C.I. 1.01–1.86) episodes in the 8 month study period, while 0.86 (C.I. 0.54–1.19) episodes were recorded in the 59 patients of the active group (t -test = 2.129, $p = 0.036$). To further support this difference, a non-parametric analysis of the number of infectious episodes showed a median value of 0 in the treated group and 1 in the placebo group. To better define this result, the difference in episodes in the single patients was also evaluated. For this, the frequency of the patients with 0, 1, 2 etc. IE was analyzed. Fig. 2 shows the frequency of IE in the pre-study population, in the Lantigen B treated group and in the placebo group. The placebo group had more IE/patients ($\chi^2 = 14.7$, $p = 0.023$) when compared with the active group. With regard to the diseases suffered by the patients, the frequency was 16% for pharyngotonsillitis (PT), 36% for rhinopharyngitis (RP) and 22% for bronchitis (B); the remaining 26% were otitis, tonsillitis, sinusitis and, rarely, pneumonia. After the treatment with Lantigen B, the total number of infections was significantly reduced, as stated before, but the relative frequency of different clinical pictures remained virtually unmodified (25% for PT, 41% for RP, 18% for B), while others were slightly reduced to 16% (Table 2). A subgroup analysis of those patients suffering from respiratory allergy (22 in the placebo and 22 in the treated group, according to the evidence collected during the eligibility visit) was also performed. Of these patients, only 24 had allergic episodes during the study period: 9 had oculorhinitis, 11 had asthma and 4 had both. Of note, the frequency of IE in those patients with allergy was slightly higher (3.80 IE/8 months) than in the non-allergic patients (3.58 IE/8 months) even if not significant ($t = -0.81$, $p = 0.42$). Like the original population, non-significantly different effects were observed when the gender (12 males and 32

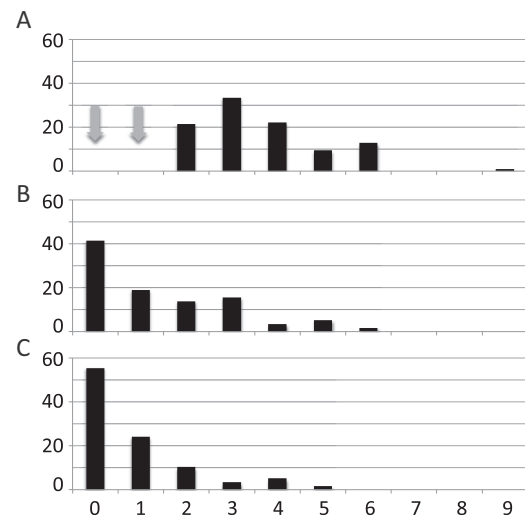


Fig. 2. Percentages of IE episodes (Y axis) distributed per number of episodes (from 0 to 9) (X axis) during the study period. Panel (A) Pre-study results. Panel (B) Placebo group results. Panel (C) Lantigen B group results. The arrows indicate the two zero values representing the eligibility criteria of the study (i.e., eligible patients had >2 episodes of respiratory airways infections in the previous year). Both 0 and 1 episodes were absent in the “pre-study” period because of the strict eligibility criteria. During the treatment, these two groups appeared.

females) of the allergic patients was evaluated. Similarly, no significant effects on the treatment results were observed when the smoking habits and the different clinical centers were evaluated (ANCOVA, $F = 1.473$, $p = 0.227$ and $F = 1.817$, $p = 0.087$, respectively).

Table 3a
Adverse reaction in the active group.

Patient	Adverse reaction	Seriousness	Intensity	Action	Relationships with the drug
2	Headache	Not serious	Light	None	None
2	Dry and burning mouth	Not serious	Intermediate	None	Possible
2	Dry and burning mouth	Not serious	Intermediate	None	Possible
31	Trauma, left ankle	Not serious	Light	None	None
32	Nasopharynx biopsy	Serious	Light	None	None
35	Persistent cough	Not serious	Light	None	None
57	Hypothyroidism	Not serious	Light	None	None
85	Vaginal candidiasis	Not serious	Light	None	None
85	Vaginal candidiasis	Not serious	Light	None	None
85	Dermatitis	Not serious	Intermediate	None	None
85	Urinary tract infection	Not serious	Intermediate	None	None
85	Urinary tract infection	Not serious	Intermediate	None	None
94	Arthralgia	Not serious	Intermediate	None	None
112	Lung cancer	Serious	Severe	Discontinuation of therapy	None
123	Headache	Not serious	Light	None	None
123	Backache	Not serious	Light	None	None
123	Nausea	Not serious	Light	None	None
123	Gastro-esophageal reflux	Not serious	Light	None	None
123	Backache	Not serious	Intermediate	None	None
123	Backache	Not serious	Intermediate	None	None

3.4. Secondary objectives

The duration of the IE in the two groups was different (10.14 days in the placebo and 5.83 in the Lantigen B group), but a statistical significance was not achieved ($F = 2.724$, $p = 0.102$). The intervals between IE were evaluated at the end of the first and second treatment cycle. In the first interval, the differences in the number of events (eight in the placebo and five in the treated group) as well as the mean intervals (in days) were not significant. However, the cumulative hazard of suffering from an IE was 15% for the placebo and 9% for the Lantigen B group. Similar results, even if not statistically significant, were observed for the second interval (six EI for the placebo and two for the Lantigen B group). The relevant cumulative hazards of developing an IE in the second interval were 0.11 and 0.035 for placebo and Lantigen B groups. The concomitant drug use in the treated and in the control groups was also evaluated by a logistic regression analysis. For this, three families of drugs the use of antibiotics and NSAIDs was evaluated in the whole population, while anti-allergic drugs, which included bronchodilators, local corticosteroids and anti-histamine were evaluated in the subset of allergic patients. No significant differences were observed between the treated and control groups, even if a clear trend was evident: indeed, the treated patients were always treated less. Despite the logistic regression analysis demonstrated that the differences were not significant ($p = 0.421$, $p = 0.134$, $p = 0.283$, $p = 0.519$ and $p = 0.667$ for antibiotics, NSAID, anti-histamine, local corticosteroids and bronchodilators, respectively), the same statistics showed that probability (Exp(B), also called the odds-ratio) of using antibiotics was reduced by 52.1% (being Exp(B) = 0.479) in the treated patients, of NSAID by 30.2% (Exp(B) = 0.698), of anti-allergic drugs by 56.2% (Exp(B) = 0.438), of corticosteroids by 41.2% (Exp(B) = 0.588) and of bronchodilators by 25.7% (Exp(B) = 0.734) in the treated groups. Thus, not only the logistic regression showed that for all the drugs the trend was toward a reduction, but further confirmed the significance of the reduction of the number of IE ($p = 0.021$ and $p = 0.044$, for the whole group and the subset of allergic patients). Finally, in both groups, the mean days of absence from work were very low (0.79 for placebo and 0.74 days for Lantigen B, $p = 0.925$).

3.5. Adverse reactions (ARs)

With regard to the AR, 21 were recorded for Lantigen B and 23 for placebo. The ARs are described in detail in Tables 3a and 3b. Briefly,

the AR of the Lantigen B group occurred in 10 different patients, while to 17 different patients in the placebo group. Some AR could be related to the administration of Lantigen B and the placebo, such as a dry and burning mouth (two episodes in one patient of the group), stomatitis, odinophagia, and oropharynx burning (five episodes in the placebo group). The last three AR required the suspension of the therapy. Other AR included hypothyroidism, distortion of the left ankle, arthralgia, lung cancer, headache, urinary tract infection, vaginal candidiasis, viral gastroenteritis, oral herpes, hypertension, nausea and gastro-enteric reflux and were not related to the treatment. Only lung cancer required the suspension of the drug. The ARs were also classified according to the severity and the need for therapy interruption (TI). In the placebo group, the 23 ARs were divided into 11 slight (1 requiring TI), 10 intermediate (requiring TI) and 2 severe, both interrupted. In the Lantigen B group were 12, 7 and 1 (requiring TI), respectively.

3.6. Epidemiological surveys of the study period

During the study period (namely, September 2012–June 2013), the circulation of respiratory viruses (both in qualitative and quantitative terms) was largely superimposable on that of the previous year, used as the reference interval for the calculation of the number of IE for patient recruitment. Fig. 3 shows the results of this survey: the RSV and influenza viruses were clearly present in the December–April interval of the two different periods, while other viruses, such as adenoviruses and rhinoviruses were detected in the remaining period.

4. Discussion

In this report, the efficacy of a chemical bacterial lysate, Lantigen B, in the reduction of the number of IE in patients with RRTI has been clearly demonstrated using a rigorous scientific and clinical approach. Indeed, this phase IV study was conducted at 12 different centers, using a double blind, placebo controlled study protocol. The authors are aware that few studies on the effects of bacterial lysates have been conducted in the past, using controlled clinical trials in Good Clinical Practice (GCP) trained hospitals. For this study, the foreseen number of patients to be recruited was 190 and 30 were expected to be lost during the study. However, the clinical centers, even though strongly committed, were able to recruit 160 patients. Of these, more than 25% were lost and only 117 patients could be evaluated at the end of the study. The loss of one-fourth of

Table 3b
Adverse reaction in the placebo group.

Patient	Adverse reaction	Seriousness	Intensity	Action	Relationships with the drug
4	Dry and burning mouth	Not serious	Light	None	None
5	Dry and burning mouth	Not serious	Light	None	None
25	Candidiasis	Not serious	Intermediate	None	None
25	Surgery (lipoma removal)	Not serious	Intermediate	None	None
26	Hypothyroidism	Not serious	Intermediate	None	None
26	Pre-menopausal syndrome	Not serious	Light	None	None
42	Dental abscess	Not serious	Intermediate	None	None
43	Constipation	Not serious	Light	Discontinuation of therapy	Unlikely
65	Backache	Not serious	Light	None	None
65	Odinophagia	Not serious	Severe	Discontinuation of therapy	Possible
81	Dyspepsia	Not serious	Intermediate	None	None
81	Viral gastro-enteritis	Not serious	Intermediate	None	None
81	Dyspepsia	Not serious	Light	None	None
89	Hypertension	Not serious	Intermediate	None	None
97	Fip arthroplasty	Serious	Severe	Discontinuation of therapy	None
100	Oropharynx burning	Not serious	Intermediate	Discontinuation of therapy	Possible
100	Oropharynx burning	Not serious	Intermediate	Discontinuation of therapy	Possible
122	Stomatitis	Not serious	Intermediate	Discontinuation of therapy	Possible
133	Oral herpes	Not serious	Light	None	None
161	Thoracoalgia	Not serious	Light	None	None
165	Migraine	Not serious	Light	None	None
165	Migraine	Not serious	Light	None	None
168	Conjunctivitis	Not serious	Light	None	None

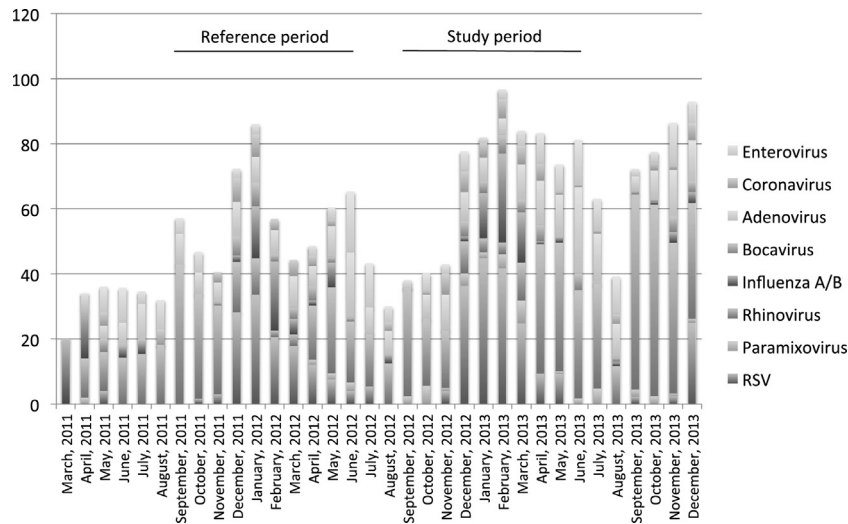


Fig. 3. The cumulative frequencies of virus isolation in the reference period (2011–2012) and in the study period (2012–2013) are represented as histograms. It is evident the increased frequency of virus infection in the late winter–early spring periods.

the patient cohort is a relevant phenomenon. Despite this reduction, the post-hoc analysis showed that the number of evaluable patients was still sufficient to maintain the statistical relevance of the study and thus the validity of the observed results. Moreover, the adverse reactions recorded as well as the causes of dropout were extremely heterogeneous and all virtually unrelated to the treatment. A significant reduction in the number of IE was observed in the treated group: an average of 0.86 episodes in the 8 month study period was observed in the Lantigen B treated patients, while in the placebo group the mean was 1.43 episodes. In this context, it should be noted that, for the population recruited, the average number of previous IE was 5.34/year (corresponding to 3.56/8 months). As stated before, in the period of the study, the mean number of IE in the placebo group was 1.43/8 months (females 1.24 IE, males 1.80 IE). This difference may have different explanations. Different weather and epidemic conditions in the two subsequent years, differences in winter and spring climates such as a warmer temperature or a less aggressive flu virus epidemic may reduce the number of respiratory tract infections, allowing the placebo

to mimic a pharmacologically positive effect. Moreover, a certain susceptibility to the effects of a placebo (more evident in females than in males) and a certain tendency to increase the number of IE in both groups when describing the severity of their conditions may have also influenced the study results. With regard to the different period epidemiology, it should be noted that the survey of virus infections in the study season, when compared with that of the previous “cold” season, showed virtually identical results, with the main viruses (influenza, rhinoviruses, adenoviruses and RSV) having the same prevalence in the two period. Therefore, this finding did not support the hypothesis of different “infectious” environments causing a different number of respiratory tract infections in the study population. The reduction in the number of IE in the placebo group may have depended on a well-known bias, the Hawthorne effect. This phenomenon, originally defined in an industrial setting [23] has been extended to the realm of medical research [24,25] and suggests that the subjects’ behavior or the study results may be altered by the subjects’ awareness that they are being studied or if they received additional attention.

It should also be noted that recruitment was based on the number of respiratory tract infections in the previous year. Even if not generalized, a certain tendency of patients to (involuntary) increase their reported number of infectious episodes might have occurred, and this fact should at least in part explain the differences observed. However, to overcome these problems, double blind placebo controlled studies (like the present one) are mandatory to measure the differences between the treatment and placebo in the same period of the year and in the same locations. In this context, the finding that neither the doctors nor the patients really recognized the superior activity of the treatment is also intriguing. Of course, the positive effects of the placebo, as discussed above, had a certain role. But, more importantly, the differences observed were homogeneously distributed between the placebo and the treated groups. This clearly means that the positive effects of the administration of bacterial lysates could be observed at a population level while they were less evident at individual level. In addition, this can also explain why the medical community is sometimes skeptical about the use of these drugs. Indeed, just using personal daily experience and feelings, it is difficult to establish whether this family of drugs is active.

Some interesting, if inconclusive data can be derived from the analysis of the use of the drugs in the two groups. No statistically significant differences were observed between the placebo and the treated groups for antibiotics, NSAIDs, bronchodilators, antihistamine and local steroids. However, a clear trend was observed in the treated groups; the use of drugs was always reduced in this group. With regard to the small group of allergic patients, the first observation was that in the population of RRTI patients, patients with allergy symptoms represented 40% of the total. It was evident that the treatment with Lantigen B did not significantly alter the number of allergic episodes or the number of days with allergy. This finding was not expected even if the strict correlation between the allergy symptoms and respiratory infectious diseases could suggest a potential positive effect [20]. A certain effect was observed, as a trend, in the use of anti-allergy drugs: indeed, in allergic patients, logistic regression showed that the probability of using drugs specific for allergy was reduced. Some criticism can be raised on this study: (a) the number of patients recruited, even if statistically correct, is not very large; (b) during patient's recruitment and follow up, functional and laboratory tests were performed only if some IE or adverse reaction occurred; (c) concomitant diseases, potentially mimicking symptoms of the respiratory tract (such as unknown primary immune deficiency – undiagnosed defect however rare in a study cohort aged between 18 and 65 years) were not specifically considered; (d) the non-perfect synchronism of the recruitment (started in September and closed in December 2012) associated to the 8-month follow-up, that could at least in part dilute the effects of typical infections of the winter–spring period. However, strengths of the study are several. In particular, (a) the rigorous randomization homogeneously distributed the abovementioned biases in the two cohorts; (b) the statistical analyses (ANCOVA and logistic regression) not only documented the effects of Lantigen B on the number of IE, but also identified a clear trend toward reduction of the use of concomitant therapies in Lantigen B treated patients; (c) the real life-like enrollment of patients (as stated by the heterogeneity of adverse reactions not related to the administration of the drug) is representative of the population that has daily access to the offices of general practitioners and specialists: thus, the observed results are reliable.

In conclusion, the administration of Lantigen B, evaluated in a double blind controlled clinical study, resulted in a significant reduction in the number of IE in the treated group. In particular, not only was the frequency of IE reduced at the population level but also at the level of the individual, the number of episodes was lower in the treated than in the placebo group. The control

of respiratory tract infections, at least in the familial or work environment, results in a reduced spread of the pathogen and in a reduced use of drugs such as antibiotics, with evident advantages for the patient and the community. In conclusion, the described results, together with the significant reduction in concomitant therapies and the virtual absence of any adverse reactions, indicates that this drug may be used as an effective first line drug for the prophylaxis of infectious episodes in patients with RRTI. The capacity of controlling respiratory tract infections is a finding particularly relevant in this period, when not only a global increase of respiratory infections is recorded [24], but also new antibiotics will not be available on the market for the next years [25].

Conflicts of interest

G. Rinaldi, MD declares that he is full-time Scientific Director of Bruschetti S.r.l.; G. Melioli, MD is an advisor of Bruschetti S.r.l. for Research and Development. The authors report no other conflicts of interest in this work.

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References

- [1] Braido F, Schenone G, Palestini E, Reggiardo G, Cangemi G, Canonica GW, et al. The relationship between mucosal immunoresponse and clinical outcome in patients with recurrent upper respiratory tract infections treated with a mechanical bacterial lysate. *J Biol Regul Homeost Agents* 2011;25(July–September (3)):477–85.
- [2] Drake CH, Smith JE. Salivary antibody response to oral vaccine. *Lancet* 1975;(September):614–5.
- [3] Clancy RL, Cripps AW, Husband AJ, Buckley D. Specific immune response in the respiratory tract after administration of an oral polyvalent bacterial vaccine. *Infect Immun* 1983;39(February (2)):491–6.
- [4] Dirienzo W, Merli AL, Ciprandi G, Scordamaglia A, De Gugliemi A, Marugo A, et al. Risposta anticorpale salivare e sierica ad una vaccinoterapia orale. *Arch Med Intern* 1984;36(2):2–8.
- [5] Jannuzzi C, Campelli A, Taverna P, Piscopo R, Morandi N. Sulla validità delle vaccinazioni contro batteri della patologia del faringe e delle prime vie aeree. In: *Atti del convegno nazionale prevenzione, trattamento e riabilitazione dell'età evolutiva*. 1985.
- [6] Muzio M, Polentarutti N, Bosisio D, Prahaldan MK, Mantovani A. Toll-like receptors: a growing family of immune receptors that are differentially expressed and regulated by different leukocytes. *J Leukoc Biol* 2000;67(April (4)):450–6.
- [7] Banchereau J, Steinman RM. Dendritic cells and the control of immunity. *Nature* 1998;392(March (6673)):245–52.
- [8] Zelle-Rieser C, Ramoner R, Bartsch G, Thurnher M. A clinically approved oral vaccine against pneumotropic bacteria induces the terminal maturation of CD83+ immunostimulatory dendritic cells. *Immunol Lett* 2001;76(February (1)):63–7.
- [9] Morandi B, Agazzi A, D'Agostino A, Antonini F, Costa G, Sabatini F, et al. A mixture of bacterial mechanical lysates is more efficient than single strain lysate and of bacterial-derived soluble products for the induction of an activating phenotype in human dendritic cells. *Immunol Lett* 2011;138(July (1)):86–91.
- [10] Lanzilli G, Falchetti R, Cottarelli A, Macchi A, Ungheri D, Fuggetta MP. In vivo effect of an immunostimulating bacterial lysate on human B lymphocytes. *Int J Immunopathol Pharmacol* 2006;19(July–September (3)):551–9.
- [11] Lanzilli G, Traggiati E, Braido F, Garelli V, Folli C, Chiappori A, et al. Administration of a polyvalent mechanical bacterial lysate to elderly patients with COPD: effects on circulating T, B and NK cells. *Immunol Lett* 2013;149(January (1–2)):62–7.
- [12] Ricci R, Palmiero C, Bazurro G, Riccio AM, Garelli V, Di Marco E, et al. The administration of a polyvalent mechanical bacterial lysate in elderly patients with COPD results in serological signs of an efficient immune response associated with a reduced number of acute episodes. *Pulm Pharmacol Ther* 2014;27(February (1)):109–13.
- [13] Price HC, Henley G. Trial of an oral antigen in upper respiratory tract infections in two Bristol Schools. *Practitioner* 1974;213:720–6.
- [14] Price HC, Henley G. Trial of an oral antigen in upper respiratory tract infections: results in the second year. *Practitioner* 1976;216(March (1293)):341–6.
- [15] Meichen FW, Howell RW. Lantigen oral bacterial antigen in an industrial population. *Practitioner* 1981;225(February (1352)):225–7.

- [16] Mora R, Salzano FA, Mora E, Guastini L. Efficacy of a topical suspension of bacterial antigens for the management of chronic suppurative otitis media. *Eur Arch Otorhinolaryngol* 2012;269(June (6)):1593–7.
- [17] Pozzi E, Serra C. Efficacy of Lantigen B in the prevention of bacterial respiratory infections. *Monaldi Arch Chest Dis* 2004;61(January–March (1)):19–27.
- [18] Mora R, Bellussi L, Passali FM, Crippa B, Mora F, Cordone MP, et al. Efficacy of a topical suspension of bacterial antigens for the management of recurrent eczema in children. *Med Sci Monit* 2004;10(September (9)):PI99–103.
- [19] Chen AH, Chen RC, Zhang CQ, Chen D, Huang S, Lin Y, et al. Efficacy of sublingual polyvalent bacterial vaccine (Lantigen B) in children with recurrent respiratory infection: a randomized double-blind controlled clinical trial. *Zhonghua Er Ke Za Zhi* 2004;42(June (6)):463–4.
- [20] Tyrrell DAJ, Nolan PS, Reed S, Healy MJR. Trial of an oral bacterial antigen against common colds. *Br J Prev Soc Med* 1972;26:129–31.
- [23] Roethlisberger FJ, Dickson WJ. *Management and the worker*. Cambridge, MA: Harvard University Press; 1939.
- [24] Schluger NW, Koppaka R. Lung disease in a global context. A call for public health action. *Ann Am Thorac Soc* 2014;11(March (3)):407–16.
- [25] Bartlett JG. A call to arms: the imperative for antimicrobial stewardship. *Clin Infect Dis* 2011;53(August (Suppl. 1)):S4–7.